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# The potential use of bacteriophages as antibacterial agents in dental infection

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**Abstract**

Dental infections, such as apical Periodontitis, periodontitis, and peri-implantitis (PI), are closely associated with specific bacterial species, including *Streptococcus mutans* (*S. mutans*), *Porphyromonas gingivalis* (*P. gingivalis*), and *Fusobacterium nucleatum* (*F. nucleatum*), among others. Antibiotics are extensively utilized for prophylactic and therapeutic purposes in the treatment of dental infections and other dental-related issues. Unfortunately, the rapid emergence of antimicrobial resistance has accompanied the increased use of antibiotics in recent years. Specific bacterial pathogens have reached a critical stage of antibiotic resistance, characterized by the proliferation of pan-resistant strains and the scarcity of viable therapeutic alternatives. Therapeutic use of particular bacteriophage (phage) particles that target bacterial pathogens is one potential alternative to antibiotics that are now being seriously considered for treating bacterial illnesses. A kind of virus known as a phage is capable of infecting and eliminating bacteria. Because they can't infect cells in plants and animals, phages might be a harmless substitute for antibiotics. To control oral disorders including periodontitis and dental caries, several research have been conducted in this area to study and identify phages from human saliva and dental plaque. The capacity of these agents to disturb biofilms expands their effectiveness against dental plaque biofilms and oral pathogens in cases of periodontitis, PI, and apical periodontitis. This review summarizes the current antibacterial properties of phages used to treat a variety of dental infections, such as periodontitis, peri-implantitis, infected dentin, and apical periodontitis.

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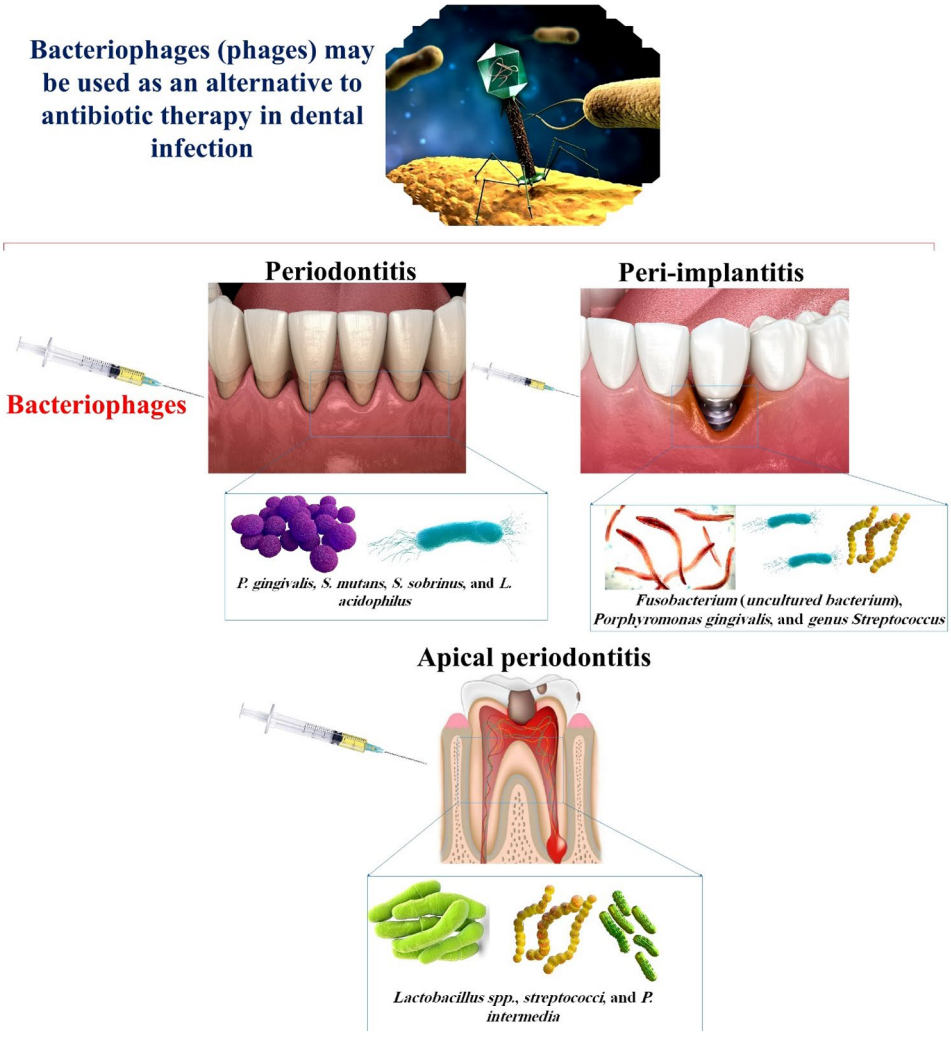
**Graphical Abstract**




**Keywords** Bacteriophage, Periodontitis, Peri-implant, Apical periodontitis, Antibiotics resistance

## Introduction

Antibiotics have been utilized to reduce infectious disease-related ailments and fatalities for over seven decades. A significant concern, the development of bacterial resistance to the most widely prescribed antibiotics, has the potential to claim the lives of a significant number of individuals worldwide. The renewed interest in bacteriophages (phages) results from this concern [1]. Phages, bacterial viruses designed to infect particular bacterial cells, are the most ubiquitous organisms on the planet, comprising over $10^{31}$ species [2]. Phages are a distinct group of viruses that exclusively infect bacteria, in contrast to plant and animal viruses which may undergo either a lytic or lysogenic life cycle. Candidates for phage therapy are lytic phages since they replicate swiftly into their host and lyse it. Phages have numerous potential advantages over conventional antibiotics. They exhibit remarkable specificity towards their intended hosts and are devoid of toxicity towards humans, as they do not disrupt eukaryotic cells [3, 4].

Despite possessing several advantages in comparison to antibiotics, the majority of Western European countries ceased industrial production of phages as a commercial product following the advent of antibiotics, in light of the escalating resistance to antibiotics and the diminished efficacy of antibiotics against bacterial biofilms, phage applications as potentially potent antibacterial agents are regaining international attention [3, 4]. Recently, the benefits of phages in managing multidrug-resistant bacteria have been gradually recognized, especially the wide-ranging proliferation of phages utilizing the host. Furthermore,

these organisms are generally non-intrusive to the microbial ecology and pose little threat to health [5].

Oral pathogenic biofilms are believed to be the cause of persistent oral infections, according to numerous studies. One of these conditions is periodontitis, which is frequently caused by plaque biofilm. Additionally, periimplantitis (PI) may result from the accumulation of bacteria around a dental implant, which can cause harm to the adjacent bone and periodontal tissue. Additionally, dental health is significantly compromised by bacterial biofilm contamination on the implant, which results in soft tissue irritation and adjacent bone resorption [6]. The microbes that cause dysbiosis in the periodontal tissues and the ensuing development of periodontal disease (PD) are intricately linked [7]. Diverse microflora (especially anaerobes) increasing in the cavities of teeth induces periodontitis by secreting toxins and enzymes and activating the immune system. Radicular dentinal tubule bacterial invasion may result from PD or infection in the root canal. In contrast, coronal dentinal tubule bacterial invasion may transpire from dentine exposure to the oral environment. Bacterial invasion can be influenced by both the composition and structure of dentinal tubules, with tubule patency being a critical factor. In cases of dentinal sclerosis, when bacterial invasion is limited due to more advanced sclerotic alterations in the apical radicular tubules, this helps explain why bacterial invasion can vary between regions [8, 9]. There is evidence to show that *Enterococcus* and *Streptococcus* are two of the most common genera of bacteria that first infiltrate dentinal tubules. Particularly enterococci may enter dentinal tubules with ease [10]. In dentistry, it has been shown that *Enterococcus* spp —specifically, *Enterococcus faecalis* (*E. faecalis*)—are linked to botched root canal operations that result in chronic apical periodontitis [11]. Results from an additional study show that Ace and serine protease facilitate *E. faecalis* binding to dentin [12]. This promotes the proliferation of bacteria inside the tubules. Specific interactions between invading *streptococci* and other oral bacteria may then promote the invasion of dentine by certain bacteria. Hence, it is crucial to understand the processes that govern inter-bacterial adhesion and invasion to aid the development of novel control techniques [8, 9].

Phage therapy represents a hopeful alternative strategy. Phages are essential to the predator-prey dynamic between bacteria and maintain a natural equilibrium; therefore, they have the potential to function as effective antibacterial agents. Plasmid-specific phages are exceptionally effective against biofilm and are simple to isolate and manipulate. Thus, similar to numerous other medical disciplines, phage therapy presents novel prospects for advancements in dentistry, encompassing both therapeutic applications and research [13]. Phages, which are highly selective, non-toxic, self-replicating, and capable of infiltrating biofilms, can be used as a novel alternative to traditional techniques of preventing biofilms. Phages of *Bacillus* spp., *Neisseria* spp., *Streptococcus* spp., *Veillonella* spp., *E. faecalis*, *Fusobacterium nucleatum* (*F. nucleatum*), and *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) have been identified and studied. Recombinant phage enzymes (lysins) have been shown to cause the breakdown of *Streptococcus* spp. and *Actinomyces naeslundii (A. naeslundii*) [14].

Furthermore, phage therapy may be a choice for managing biofilm formation and decreasing *Streptococcus mutans* (*S. mutans)* surface colonization [15]. The growth and biofilm formation of *S. mutans* were considerably reduced by adding ϕKSM96. When ϕKSM96 was present in cocultures of *S. mutans* with other bacterial species, the percentage of *S. mutans* dropped significantly. ϕKSM96 has specific anti-*S. mutans* action. For future genetic modification to produce more effective phages, the isolation of temperate phages is crucial [16]. Both periodontitis and PI may be treated with lytic phages. Specifically, it suggests the utilization of phages capable of destroying bacterial biofilm. The potential strategy involves the modification of phages to enhance their specificity against other bacterial strains and species or the use of compounds that contain phages that are active against the majority of known bacteria implicated in periodontitis/PI. The aforementioned mechanical therapy would be nearly particular to be necessary, as professional debridement is a component of every known periodontal treatment strategy. This is due to the high resistance of biofilm to external environments [17]. Eight μmol/L LysP53 mouthwash exhibited bactericidal activity against the primary cariogenic bacteria *S. mutans*, *Streptococcus sobrinus*, and *A. naeslundii*; however, it exhibited inadequate antibacterial activity against the common commensal bacteria *Streptococcus oralis*, *Streptococcus mitis*, and *Streptococcus sanguinis*. The biofilm structure was disintegrated and the number of viable bacteria was effectively reduced by applying LysP53 mouthwash to established biofilms. Good antibacterial activity, safety, and stability are all exhibited by LysP53 mouthwash. It has the potential to significantly prevent dental caries through routine oral hygiene practices [18]. Furthermore, the phage's capacity to combat dental plaque is demonstrated by its small size, which enables it to penetrate the biofilm layers with high penetration power [19]. Phage therapy might be an important therapeutic option for root canal infections resistant to traditional endodontic treatments [20].

In addition, using phages to eliminate particular bacteria from the microbiome will enable us to investigate the function of their host in the microbiome and identify keystone pathogens in a variety of different infections. Therefore, the utilization of phages will be advantageous in acquiring knowledge regarding oral pathogens and

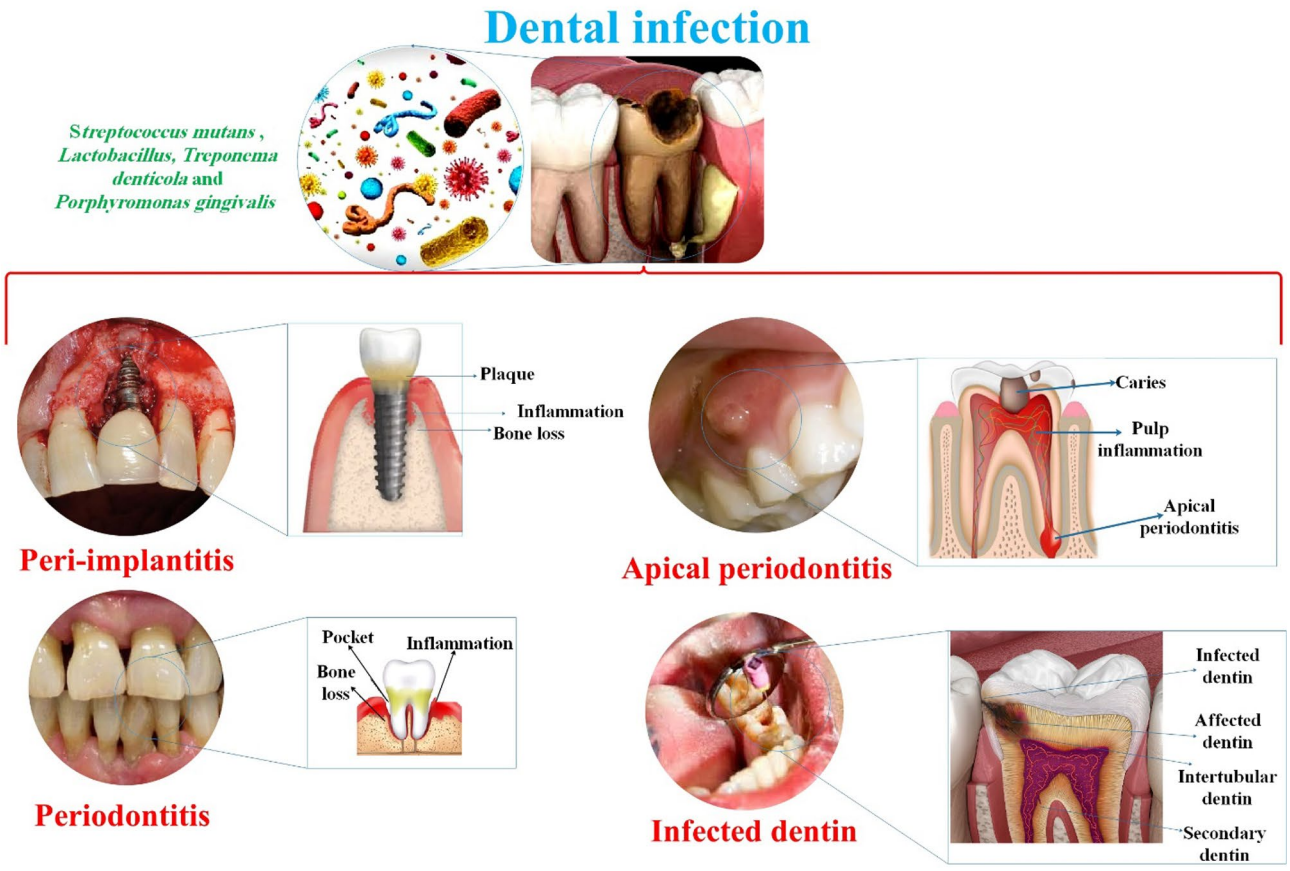



**Fig. 1** This figure illustrates the essential characteristics of the four types of dental infections: periodontitis, peri-implantitis, apical periodontitis, and infected dentin. In acute apical periodontitis, the dental pulp may be necrotic or retain vitality, resulting in tenderness and pain upon percussion

their successful removal. The development of "microbiome engineering" to prevent infections has the potential to result from the comprehension of the oral microbiome with the assistance of phages [21].

This study summarizes the current therapeutic effects of several phages against dental infectious diseases, such as periodontitis, PI, infected dentin, and reinfection apical periodontitis. In addition, we have discussed the advantages and disadvantages of phage therapy against infectious dental disease. The information could potentially be utilized to develop therapeutic alternatives based on phages to treat dental infections (Fig. 1).

## Characteristics of bacteriophages

Phages, first identified by Twort and d'Herelle in the early 1900s, have since emerged as a significant therapeutic agent against pathogenic bacteria in clinical settings, generating considerable interest and research in the field. Nevertheless, the inexorably increasing prevalence of bacterial antibiotic resistance has rendered phage applications (phage therapy) an unavoidable research topic. In various applications, phage particles have gained popularity as a biotechnological instrument and treatment for pathogenic bacteria [22]. Lytic phages and temperate phages are the two principal varieties of phages. The lytic life cycle is utilized by phages to replicate; lytic phages derive their name from the fact that they lyse the host bacterium as a typical process [23, 24]. Lysogenic phages, which are "temperate" or dormant phages, can transform into "prophages" through integration with the viral DNA located in the host's chromosome. They coexist with the host chromosome for generations, dividing and multiplying alongside it rather than lysing the host cell. The potential for exploiting the distinctive attributes of lysogenic or "temperate" phages has been illustrated in a system that reverses pathogen resistance to antibiotics, thereby restoring antibiotic efficiency. Unlike conventional phage therapy, this approach is not reliant on the phage's capacity to eliminate pathogens inside the infected host. Alternatively, it depends on the phage's capacity to transmit genetic constructions to the bacteria, rendering them susceptible to antibiotics before attacking the host [25]. The process of phage induction in model phages has been well studied. It involves DNA damage in bacteria due to unfavorable environmental circumstances, which then activates the bacterial "SOS

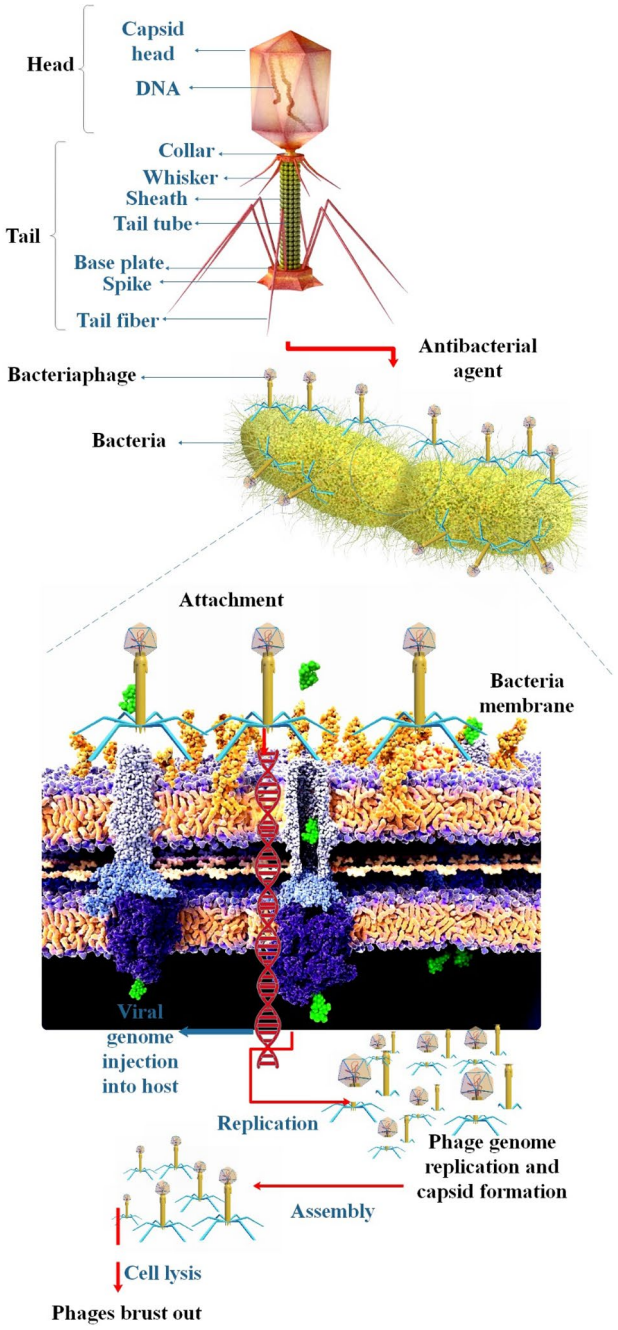



**Fig. 2** The structure and antibacterial action of phages. Only therapeutic research against bacterial infections uses virulent phages, also known as lytic phages, since they can grow in bacterial hosts, cause bacterial lysis during each infection cycle, and cannot lysogenizing their hosts. Clinical trials have used lytic phages that are either of the wild-type or modified varieties. When access to naturally occurring lytic phages is limited, engineered phages become necessary. When phages attach to specific receptors on host cells, such as lipopolysaccharides (LPS), teichoic acids, membrane proteins, or capsules, the lytic infection cycle starts. Following this, phage genetic material is translocated to the host, injected and used to hijack the host's replication machinery to make phage offspring. Bacteriolysis is caused by the activation of the phage-encoded protein, and the offspring then leave the bacterial cell to start the cycle all over again on new hosts synthesize [35]

response." The temperate phage protein CI, which acts as a suppressor of the lytic cycle and integrated phage genes, is broken down by bacterial proteins that are triggered as part of the SOS cascade response [26].

Lysins of phages are a new way to fight germs resistant to antibiotics. At first, it was thought that these lytic enzymes would work best against the exposed cell walls of Gram-positive bacteria because they cut through the bacterial cell wall peptidoglycan. On the other hand, lysin's use as an enzymatic antibiotic was expanded to Gram-negative bacteria by finding ways to get past the outer membrane (OM) these bacteria cells have. Several lysins that target *Staphylococcus aureus* (*S. aureus*) are in different stages of human studies right now [27]. There is something called phage–antibiotic synergy (PAS) that happens when two things work together to kill germs more effectively than either one would alone. There are several possible ways to understand the behavior of PAS, including (1) antibiotics cause cells to get longer and thinner; (2) antibiotics increase plaque size, which speeds up phage amplification and boosts burst size; (3) the appearance of phage and/or antibiotic-resistant mutants decreases; (4) antibiotics become more effective when phages are present; (5) the minimum inhibitory concentration (MIC) of antibiotics drops when phages are added to an antibiotic; and (6) phage enzymes (glycan depolymerase) break down bacterial polysaccharides, which makes antibiotics more efficacious [28].

Moreover, phages whose genomes contain exopolysaccharide depolymerase can use exopolysaccharides as main targets and cut polymer links until they reach the cell membrane. This helps break down biofilm and attack bacteria already there [29]. Several studies have shown that people who received phage treatment had much more robust immune systems with fewer side effects. When all antibiotics and other drugs have failed to treat multidrug-resistant (MDR) *Acinetobacter baumannii*, phage treatment is very helpful. This proves that using phages for medical purposes would be an excellent alternative way to treat MDR bacterial illnesses. However, the practical use of phage therapy for treating MDR bacterial infections has been limited due to its inherent disadvantages (such as phage tolerance, limited range of hosts, symbiosis instead of lysis, and insufficient knowledge about the ongoing competition between phages and bacteria, among other factors). Recently, changes in phage host range-determining regions (HRDRs) have been used as an efficient way to prevent *Escherichia coli* (*E. coli*) infection. These alterations allow bacteria to resist the phage [30–34]. The success of this endeavor provides validation for the creation of genetically modified phages and the design of treatments that surpass the inherent constraints of phages, resulting in the production of antimicrobial agents for future use [30–34] (Fig. 2).

## Antibiotic resident in dental infections

Globally, dental caries (caused by oral bacteria) is the most prevalent infectious disease. *S. mutans* is one of the principal pathogens that has a significant impact on the development of dental caries. Gram-positive *S. mutans* is an acidogenic and aciduric bacterium that inhabits the buccal cavity by nature. A clear correlation exists between the capacity of bacteria to develop biofilms and dental caries. During this phase, the bacteria become impermeable to antibacterial agents and the immune system of the body. Biofilms composed of cariogenic bacteria can be easily formed on the surface of the tooth, leading to the rapid production of lactic acid and subsequent dental caries [21, 36, 37]. Furthermore, a diverse range of bacteria that produce exopolysaccharides may be found in the oral cavity. The oral biofilm is mainly composed of these extracellular polysaccharides. They make up the biofilm matrix, combined with extracellular proteins, DNA, and lipids to support bacterial colonization, biofilm formation and maintenance, and pathogenicity [38]. Many oral infections, like tooth caries, gingivitis, periodontitis, periapical periodontitis, and PI, are made worse by this biofilm. In biofilms' life cycle, bacteria connect first, which can be a changeable attachment. Then, they colonize, which is a permanent attachment, grow and mature by making more EPS, and finally, they spread out in the environment [39, 40].

10% of all outpatient antibiotic prescriptions are written by dentists, which amounts to over 25.7 million prescriptions annually. The American Dental Society states that although many patients take antibiotics as a preventative measure before getting prosthetic joints, this is not always the best course of action [41]. Tetracyclines, penicillins, macrolides, quinolones, cephalosporins, and nitroimidazole chemicals are some of the most popular antibacterial drugs doctors recommend. These drugs work in different ways, and they can be given to people who have a wide range of sensitive germs, even ones that are resistant to antibiotics. These drugs can also be given independently or together to make them more beneficial [42, 43].

PI is additionally linked to the pathogenic species affiliated with periodontitis, including *Fusobacterium* spp, *A. actinomycetemcomitans*, and *Porphyromonas gingivalis* (*P. gingivalis*). PI is most prevalent during the initial twelve months following implantation. Patients with poor oral health, smoking, or implants coated with calcium phosphate or roughened surfaces have an increased risk of developing this condition. While the clinical effectiveness of biomaterial treatments using beads, gels, and fibers to provide antibiotics has not been well demonstrated, they have been used to treat PI [44, 45].

At this time, antibiotics are advised as adjuvants for the treatment of suppurative orofacial infections only in cases where drainage and elimination of causative factors are inadequate or when systemic involvement is present. Antibiotics should be reserved for specific patients in endodontics as apical periodontitis to prevent the danger of bacteremia, without affecting the success rate of treatments or ameliorating clinical signs and symptoms. Identical principles may be applied to oral surgery, where routine tooth extractions can be performed on healthy patients without antibiotics. Additionally, it is feasible to formulate a preoperative strategy or prolong the therapy for a few days after the procedure. The duration of surgery and the need for osteotomy, which are often linked to bacterial contamination and surgical stress, should be considered as crucial factors. Oral surgery must consider bacterial burden and atraumatic procedures when performing regenerative and implantological procedures to reduce the likelihood of contamination and failure. Patients who are in good periodontal health and have minimal bacterial burdens may be candidates for a brief apical periodontitis before undergoing implant and/or regenerative procedures. When prescribed correctly, antiseptic medicines like chlorhexidine may effectively reduce bacterial load for short periods. The following recommendations clarify the necessity of decreasing antibiotic usage while maintaining clinical efficacy; the objective is to decrease antimicrobial resistance (AMR) and its potentially fatal ramifications [46]. The emergence of AMR is an inherent consequence of microbial evolution. Nevertheless, resistance development and dissemination have been expedited due to anthropogenic activities. Antibiotic resistance is augmented by the improper and inappropriate application of antibiotics. Dental infections must be universally acknowledged by all healthcare professionals as a critical factor in averting the development and dissemination of resistance. Potentially facilitating rational dental care would be incorporating primary oral health care into primary health care [47, 48]. (Fig. 3)

## Bacteriophages in dental diseases

An increasing number of potential general applications of phage therapy have been proposed in the buccal cavity. Phages exhibit activity against both planktonic bacteria and bacteria organized in biofilms, which is of greater significance in the context of oral and dental treatments. Nevertheless, phages and bacteria can coexist due to the formation of anti-phage refuges by bacteria within biofilms. To target biofilm-embedded cells ubiquitous in their ecosystem, phages may evolve and adapt. Viruses can infiltrate dense biofilm and propagate across the densely packed adjacent cells, damaging the entire structure. Additionally, some phages penetrate a bacterial capsule or biofilm matrix by employing a variety of depolymerases [49]. Phages and their enzymatic counterparts have demonstrated efficacy against planktonic and

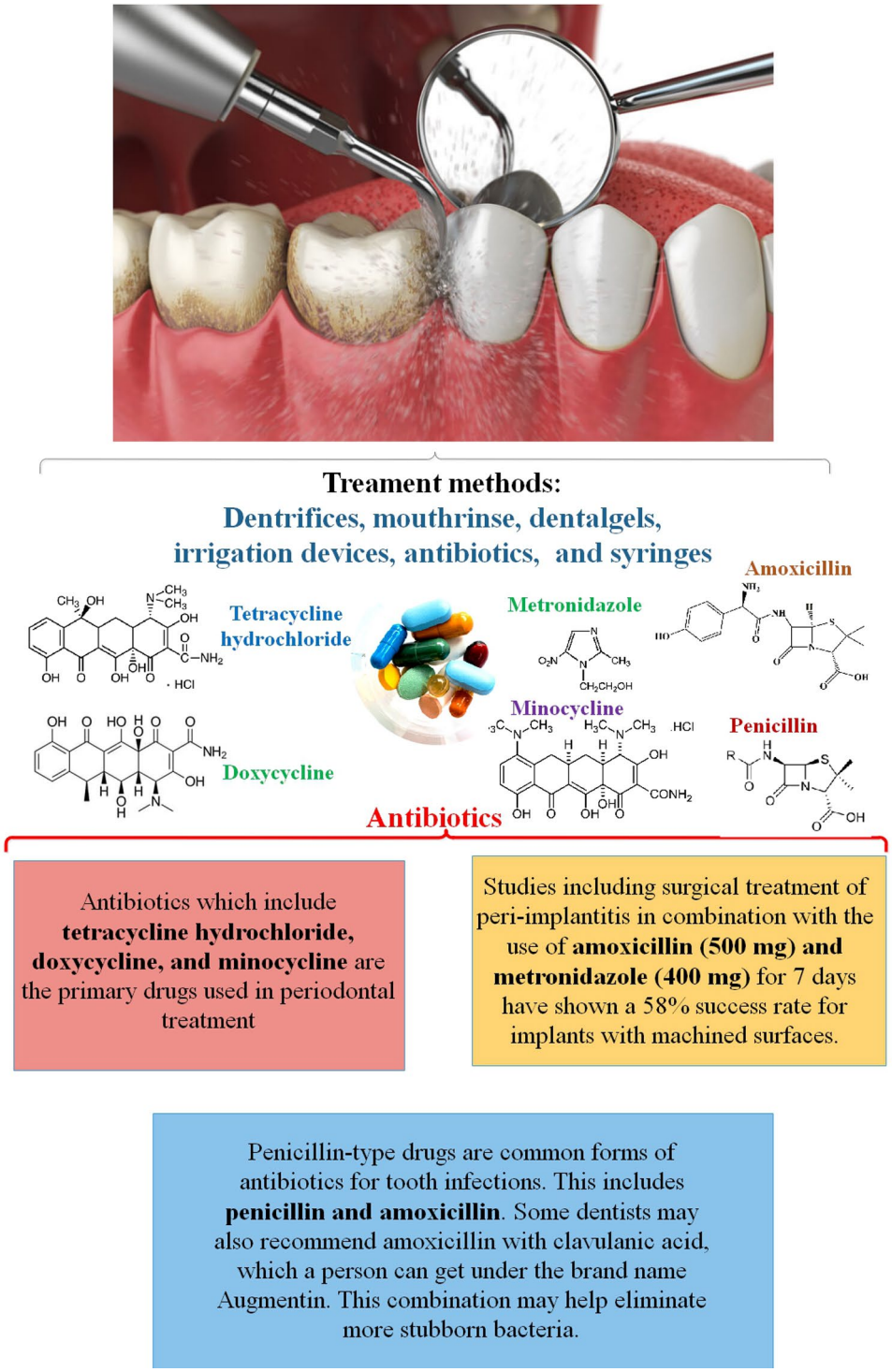



**Fig. 3** Several antibiotics are used for periodontitis, peri-implantitis, and dental infection

biofilm-dwelling oral bacteria. Phages that target *A. actinomycetemcomitans*, *A. naeslundii*, *Veillonella* spp., *Lactobacillus* spp., *Neisseria* spp., and *E. faecalis* have been documented. Recombinant phage enzymes exhibit activity against *Streptococcus* spp. and *A. naeslundii*. However, isolation and characterization of a mere fraction of the available phages has occurred. Phage activity in a multispecies context, animal models, and in combination with other antimicrobials must be the subject of additional research. To be evaluated is the application of phages or their enzymes to oral microbiome engineering [14].

The overarching objective would be the creation of accessible, economically viable phage-based therapeutics and preventatives for oral health. The application of enterococcal phages has effectively inhibited the growth of *E. faecalis* on the surface of human dental roots. An infection multiplicity of 0.1 for phages was adequate to reduce the capability of *E. faecalis* [50]. *S. aureus* and *E. faecalis*, both known to cause dental caries. These bacteria showed resistance to the majority of the antibiotics that were tested. To tackle this difficulty, two lytic phages were isolated, identified, and used to impede the growth of *S. aureus* and *E. faecalis*, respectively: vB_SauM-EG-AE3 and vB_EfaP-EF01. Both phages exhibited extraordinary lytic activity, notable stability, and a restricted range of hosts. Phosphates exhibited blast sizes of 78.87 and 113.55 PFU/cell on the one-step growth curve, while latent periods of 25 and 30 min were recorded for *S. aureus* phage and *E. faecalis* phage, respectively. Complete inhibition of bacterial growth was attained by incubating phages with MOIs of $10^3$, $10^2$, and 10 at 37 °C for 1, 3, 5, and 24 h. This study suggests that the phages that were isolated have the potential to serve as effective biocontrol agents against antibiotic-resistant dental caries bacteria, thereby paving the way for the development of novel alternatives to antibiotics [37].

The comprehensive sequencing of the genome of the phage BAG1, which specifically targets an endodontic clinical strain of *E. faecalis* (K3), is documented in a research article. BAG1 was exceptionally effective at eradicating K3 bacteria from dentine slabs infected with the endodontic K3 clinical strain, as determined by planktonic broth and biofilm fraction analysis. Researchers demonstrated that the lytic properties of anti-*E. faecalis* phage BAG1 effectively eradicates *E. faecalis* K3 biofilm on dentine slabs. This is attributed to the absence of lysogenic genes, rendering BAG1 a viable alternative to adjunctive anti-*E. faecalis* therapy [51].

*S. mutans* is crucial to the development and progression of caries because it may make extracellular polysaccharides, and glucosyltransferases, and promote bacterial adhesion and aggregation. As a result, biofilms are formed in which the bacteria break down dietary carbohydrates to create acids. Therefore, it is essential to develop efficient methods to reduce *S. mutans* biofilm production to avoid dental caries and promote oral health [52]. The ϕAPCM01 phage, which is novel, was obtained from a sample of human sputum. After 24 h of contact with phage ϕAPCM01, the metabolic activity of an *S. mutans* biofilm was observed to be diminished. This reduction persisted for a minimum of 48 h, and the number of viable cells within the biofilm decreased by a minimum of 5 log CFU/ml [53]. Dental caries are caused by strains of *S. mutans* of serotype C, which is specifically targeted by the lytic phage M102. The whole genomic sequence of M102 was discovered in an investigation. The genome has 41 open reading frames (ORFs) and is 31,147 bp in size. Most ORFs encoding potential phage structural proteins are comparable to those found in *Streptococcus thermophilus* phages. A holin and two lytic enzymes are encoded by a unique lysis cassette found in the M102 genome, according to a bioinformatic study [54]. The researchers of a study document the successful extraction of the temperate phage 4KSM96 from *S. mutans*. The circular DNA of 4KSM96 is 39,820 base pairs long and reveals the morphology of *Siphoviridae*. 4KSM96 exhibits a diverse array of susceptibilities to *S. mutans* strains containing various serotypes. Significant inhibition of *S. mutans* growth and biofilm formation was observed upon adding 4KSM96. 4KSM96 resulted in a substantial decrease in the proportion of *S. mutans* in cocultures with other bacterial species. To summarize, 4KSM96 exhibits anti-*S. mutans* activity that is selective [55].

Understanding of the possible function of phages in the formation, control, and management of pathogenic microbiomes of the periodontium and other oral locations has improved due to metagenomic profiling of oral biofilms. A single phage has been linked to the oral phyla *Synergistetes* and *Saccharibacteria* (previously known as TM7). The oral cavity is also home to *Lactobacillus* phages that are prevalent in other settings as well as phages of harmful invaders (such as *E. Coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*). Researchers have not found any phages of the phyla *Chlamydiae*, Absconditabacteria (previously known as SR1), or Chloroflexi in the oral cavity [56].

To get a better grasp of the function viruses play in the intricate oral ecology, researchers examined 2,267,695 virome reads from viral particles and compared them with 2,63,516 bacterial 16 S rRNA gene sequences from the saliva of five healthy human individuals over two to three months. Researchers' discovery of 122 728 homologs for virulence factors raises the possibility that salivary viruses operate as repositories for harmful gene activity in the mouth cavity. Given that phages make up the great majority of human oral viruses and that several of their proposed gene functions indicate they play a significant part in lysogeny, these viruses may have a considerable influence on the microbial diversity found in the human mouth cavity [57].

Recent research has examined the antimicrobial characteristics of oral phages and phage enzymes. The experimental *F. nucleatum* biofilms were disrupted by the *Fusobacterium* phage FNU1, as shown by confocal microscopy and crystal violet staining. Oral phages that resemble Haemophilus phiKZ may have therapeutic effects on *Pasteurellacae* infections. In endodontics, the multi-antibiotic-resistant *E. faecalis* bacterium is

a severe problem. A lysogenicity-deficient genetically modified *Enterococcus* phage ϕEf11 variant showed a broader host range and may decrease the amount of *E. faecalis* in human dentin specimens by up to 100 times. It was shown that a refined lysin derived from the ϕEf11 phage had activity against 73 out of 103 strains of *E. faecalis* and could significantly damage *E. faecalis* biofilms [56]. In vitro and in an ex vivo dental model, researchers examined the efficacy of ClyR, a chimeric lysin with an expanded streptococcal lytic spectrum, against planktonic and sessile *E. faecalis* cells. It was shown by researchers that ClyR had decisive and quick lytic action against many strains of *E. faecalis*, eliminating over 90% of planktonic cells in under one minute at a 50 μg/mL concentration. When 50 μg/mL of ClyR is added to *E. faecalis* biofilms for one hour, the biochemical studies and microscopy analysis show that ClyR destroys the biofilm with great effectiveness and, in dose-dependent, lowering the survival rate to less than 40%. Using a low dose of 50 μg/mL, ClyR demonstrated a significant biofilm removal efficacy in the ex vivo dental model, killing over 90% of viable bacteria within biofilms. This is comparable to calcium hydroxide, a commonly used intracanal medication for treating dental traumatology and endodontics, and far superior to ampicillin. The promise of ClyR in treating endodontic infections caused by *E. faecalis* is suggested by its intense action against both planktonic and sessile forms of bacteria [58].

The SMHBZ8 phage, which targets *S. mutans*, was recently identified and described. Researchers aimed to assess SMHBZ8's ability to prevent dental caries utilizing both in vitro and in vivo caries models. In a mouse caries model, SMHBZ8 phage suspension subsequently inhibited the formation of carious lesions in vivo. Caries lesions were examined radiographically and clinically using μCT scans in both animals. Investigators demonstrated how in vitro and in vivo mice models, using SMHBZ8 phage treatment targeting *S. mutans*, may function as an effective caries-prevention strategy whether used in suspension or with a sustained-release delivery mechanism [59].

Investigators GH12 at 64 mg/liter was selected for subsequent in vitro and in vivo testing because it demonstrated the most efficient reduction of lactic acid generation, EPS synthesis, pH drop, and biofilm integrity of human dental plaque-derived multispecies biofilms in vitro. 64 mg/liter of GH12 controlled the microbiota of dental plaque in the rat caries model fed a diet that promoted dental caries, resulting in an increase in commensal bacteria and a reduction in caries-associated bacteria. Furthermore, in every site, 64 mg/liter GH12 dramatically lowered the caries scores for both smooth surface and sulcal caries. Finally, the researchers showed that GH12 controlled the dysbiotic microbial ecology and halted the development of caries under cariogenic circumstances, inhibiting the cariogenic qualities of dental plaque without affecting the microbiota of dental plaque in healthy persons [60].

Human dental caries is caused mainly by *S. mutans*. Glucosyltransferases (Gtfs), one of its main virulence factors, use sucrose to produce EPS, which causes dental plaque biofilm to develop. To prevent the creation of biofilms, researchers created a unique self-targeting gene editing technique that specifically targeted gtfs. The CRISPR-Cas system, which consists of a clustered, regularly interspaced short palindromic repeat and CRISPR-associated proteins, has been extensively developed for genomic engineering and offers sequence-specific defense against foreign genetic material in bacteria and archaea. The first goal of researchers was to determine if the *S mutans* UA159 CRISPR-Cas9 system's components were required for defense against foreign DNA. According to the research, the *S mutans* CRISPR-Cas9 system requires an appropriate PAM site, tracrRNA, Cas9, and RNase III to operate normally. The researchers created self-targeting CRISPR arrays with spacer sequences that identify with gtfB and cloned them onto plasmids based on their findings. To get the required mutants, researchers next converted the plasmids and editing templates into UA159 (self-targeting). Data demonstrated the technology's effectiveness and ability to modify the gtfB or gtfBgtfC genes. This led to a significant decrease in EPS production and could disrupt the development of biofilms, making it a potentially helpful tool for dental offices to stop *S. mutans* biofilms from forming in the future [61].

In conclusion, research must determine the most effective way to use phages in clinical settings and the efficacy of oral phage treatment, even if phage safety has been confirmed. Potential advancements in the production of commercial phage products might be facilitated by using modern synthetic biology. Along with good manufacturing practices, commercial phage therapy must also handle several clinical, manufacturing, and regulatory obstacles. In the future, studies may focus on phage encapsulation technology, which is utilized to control the gut microbiota and release phages intelligently in response to variations in salivary pH. phage therapy also faces challenges from the quick acquisition of phage resistance, the evolution of clinical bacterial strains, the variety and unpredictability of immune responses, and the incapacity of separated phages to handle all the complicated and unique clinical instances. Fortunately, researchers could to understand the genetic characteristics of phages better and alter them to suit therapeutic needs thanks to advancements in viral metagenomics and phage genome engineering [56, 62].

### Antibacterial effect of bacteriophage on infected dentin and apical periodontitis

This research project aimed to evaluate the efficacy of a genetically engineered phage in eradicating antibiotic-resistant strains of *E. faecalis* from dentin. Following a 7-day incubation period at 37 °C, a suspension of ΫEf11/ŔFL1C(Δ36)PnisA, a genetically modified phage, was introduced into the root canal of every infected dentin segment. The duration of the incubation phase was prolonged by an extra 72 h. An analysis was conducted on the dentin composition of the walls of each root canal to quantify the presence of *E. faecalis* cells that persisted. The E. *faecalis* titer in the JH2-2 infected rats decreased by 18%, but in the V583 infected models, it decreased by 99%. Utilizing phage ϕEf11/ϕFL1C(Δ36)PnisA on dentin infected with *E. faecalis* consistently resulted in a decrease in the remaining bacterial population, including both vancomycin-sensitive and resistant strains [63].

When microorganisms access the root canal system, they can penetrate the root canal dentin. A reservoir of these bacteria could potentially serve as a source of root canal reinfection both during and after endodontic treatment [64]. The objective of the researchers' endeavor was to isolate and characterize lytic phages that specifically target *E. faecalis* that were obtained from root canal infections at institutions affiliated with the Faculty of Dentistry in Ismalia, Egypt. vB_ZEFP, a phage, was isolated from a hospital effluent that had been concentrated. The phage is classified as a member of the Podoviridae family, as determined by morphological and genomic analysis. Its linear double-stranded DNA genome comprises 18,454 nucleotides and 32.8% G+C. With plating efficiencies greater than 0.5, host range analysis revealed the phage could infect ten of thirteen *E. faecalis* isolates harboring a variety of antibiotic resistances recovered from infected root canals. This phage has a latent period of 10 min and an explosion size of 110 PFU per infected cell, according to one-step growth curves. The lytic activity exhibited by this phage against *E. faecalis* biofilms demonstrated its capacity to inhibit *E. faecalis* growth in vitro. Also capable of preventing ex-vivo *E. faecalis* root canal infection was phage vB_ZEFP. Phage vB_ZEFP can be utilized in phage therapy, specifically to prevent infection following root canal treatment, according to these findings [65].

In a root canal model, investigators investigated the potential application of phage therapy against *Pseudomonas aeruginosa* strain PA14 biofilms. Two phages (JBD4 and JBD44a) with putative biofilm-degrading activities were identified. Applying these phages to PA14 biofilms resulted in a substantial decrease in the average biomass percentage after 24 h and 96 h. Phage treatment did not result in a statistically significant difference in the quantity of colony-forming units between 24-hour and 96-hour PA14 biofilms in a root canal model. The biomass of 24-hour and 96-hour PA14 biofilms grown on microplates was significantly diminished by phage application, whereas the biomass of 24-hour and 96-hour PA14 biofilms grown in the extracted tooth model remained unaffected [66]. Two newly discovered highly pathogenic phages, vB_Efa29212_2e and vB_Efa29212_3e, were obtained and studied from urban wastewater. For 21 days, the *E. faecalis* biofilm was established on 15 bovine molars. This observation provided evidence of the phage's ability to cause lysis in *E. faecalis*. The phage demonstrated its effectiveness against the isolates by reducing the *E. faecalis* biofilm by 54.6% in the ex vivo experiment. The researchers' results strongly support the hypothesis that phage therapy has true therapeutic promise for preventing and treating *E. faecalis*-associated illnesses [67].

Resistance to *E. faecalis* infection was examined in vitro and in vivo using the phage PEf771, which selectively infects and lyses pathogenic *E. faecalis* YN771 in patients with refractory periapical periodontitis. Compared to 10 commonly used therapeutic antibiotics, PEf771 showed the most bacteriostatic activity in less than 72 h. Within 72 h, PEf771 showed a more significant antibacterial impact on removed teeth thantraditional root canal disinfectants, including formaldehyde cresol solution, camphorated phenol, and $Ca(OH)_2$. Sprague Dawley (SD) rats were used to create intraperitoneal and periapical infection models using *E. faecalis*. According to researchers' findings, every SD rodent injected with either $9.6\times10^{11}$ CFU/mL *E. faecalis* YN771 or $2.9\times10^{11}$ CFU/mL *E. faecalis* RYN771 perished in 8 h. Furthermore, after receiving an antibiotic course and YN771 injection, every SD rat died in 72 h. The pathological anatomy of the RYN771-inoculated, antibiotic-treated SD rats revealed purulent discharge, multiple pus- and blood-filled ascites, and large liver abscesses despite the rats' 72-hour survival. Remarkably, the pathological anatomy of the YN771 and RYN771 rats treated with PEf771 and RPEf771, respectively, revealed normal liver, kidneys, intestines, and mesenteries after the rats had lived for 72 h. Even though the experimental teeth infected with YN771 had a standard root canal therapy, a computed tomography study of SD rats infected with periapical periodontitis revealed pathological alterations in those teeth. On the other hand, after receiving PEf771 therapy, no experimental tooth showed signs of root periapical inflammation. The experimental teeth showed a gap between the periodontal ligament and the cementum when stained with hematoxylin and eosin; in contrast, the teeth treated with PEf771 showed normal findings [68].

Using 16 S rDNA and biochemical methods, two bacterial isolates, *E. faecalis* A.R.A.01 (ON797462.1) and *E.*

*faecalis* A.R.A.02, were discovered. Specific phages were isolated from these isolates using them as hosts. Two phages were discovered genetically by RNA ligase of *Enterococcus* phage vB_EfaS_HEf13 sequencing and PCR amplification. BLAST analysis revealed that researchers' phages were 97.2% similar to *Enterococcus* phage vB-EfaS-HEf13. Furthermore, using in silico analysis and annotations of the genomes of the two phages, it was discovered that 69 ORFs were engaged in a range of tasks related to integration excision, replication recombination, repair, stability, and defense. During phage optimization, the two isolated phages showed a high specific host range with *E. faecalis* among six different bacterial hosts. The concentration of *E. faecalis*_phage-01 was 115.76 PFU/mL with a latent period of 30 min, while the concentration of *E. faecalis*_phage-02 was 80.6 PFU/mL with a latent period of 25 min. Furthermore, they exhibited stability across a wide pH (4–11) and temperature (10–60 °C) range, as well as little cytotoxicity on the oral epithelial cell line at different doses (1000–31.25 PFU/mL). The data highlight the promise of phage treatment in dentistry, offering a unique way to enhance patient outcomes and fight antibiotic resistance [69].

The primary species identified in secondary persistent infections that result from the failure of root canal therapy is *E. faecalis*. *E. faecalis* is a primary cause of periapical lesions, as it can withstand fluctuations in pH, temperature, and osmotic pressure in the pharynx as a result of its robust tolerance and the formation of biofilm. In conclusion, the researchers endorsed that phages can be easily isolated and characterized. They also suggested that phage therapy is a promising complementary strategy to conventional antibiotic treatment, particularly when treatment fails, such as biofilm and multidrug resistance strains, when used with caution. Nevertheless, none employed a clinical strain of *E. faecalis* that was directly isolated from a root canal infection. phages are recognized for their ability to infiltrate biofilms through water channels or to demolish the biofilm matrix by generating depolymerizes. Lysins, derived from phages, are also effective in disrupting biofilms by disintegrating the bacterial cell wall. Therefore, additional research is required to determine the precise mechanism by which the phage isolated in this study achieves its anti-biofilm effect [70, 71] (Table 1).

### Bacteriophage in peri-implantitis

PI is an infectious disease specific to a particular site and induces inflammation in the soft tissues surrounding a functional osseointegrated implant, leading to bone loss [73]. Dental implants are being inserted into a growing proportion of patients. PI and mucositis are prevalent microbial-biofilm-associated conditions that impact the tissues surrounding the dental implant. The microbiome

**Table 1** Bacteriophage as an antibacterial agent in dental diseases

| Bacteriophage | Bacteria | Antibacterial effects | Ref |
|---|---|---|---|
| ϕAPCM01 phage | *S. mutans* biofilm | This reduction persisted for a minimum of 48 h, and the number of viable cells within the biofilm decreased by a minimum of 5 log CFU/ml. | [53] |
| vB_SauM-EGAE3 and vB_EfaP-EGAE1 | *S. aureus* and *E. faecalis* | Complete inhibition of bacterial growth was attained by incubating phages with MOIs of $10^3$, $10^2$, and 10 at 37 °C for 1, 3, 5, and 24 h. | [37] |
| Phage BAG1 | *E. faecalis* (K3) | The BAG1 phage was evidenced by the complete eradication of the K3 strain within 180 min during the killing test. | [51] |
| T4-like coliphage cocktail | *Escherichia coli* | Significant variation in the composition of fecal microbiota was also observed in 71 pediatric diarrhea patients who solely received oral rehydration therapy and in 38 patients who received coliphage preparations or placebo when their samples were collected 1.2 or 4 days apart, respectively. | [72] |
| Bacteriophage M102 | *S. mutans* | Dental caries is caused by strains of *S. mutans* of serotype C, specifically targeted by the lytic phage Bacteriophage M102. | [54] |
| Bacteriophage 4KSM96 | *S. mutans* | 4KSM96 resulted in a significant decrease in the proportion of *S. mutans* in cocultures with other bacterial species. | [55] |
| ϒEf11/ŕFL1C(Δ36)PnisA | *E. faecalis* | For the JH2-2 infected models, the recovered *E. faecalis* titer was diminished by 18%, while for the V583 infected models, it was reduced by 99%. | [63] |
| Phage lysate | *E. faecalis* | Significant inhibition of bacterial growth was observed with phage lysate incubation at multiplicities of infection of 1.0, 10.0, and 0.1. | [20] |
| vB_ZEFP | *E. faecalis* | With plating efficiencies more significant 0.5, host range analysis revealed the phage could infect ten of thirteen *E. faecalis* isolates harboring a variety of antibiotic resistances recovered from infected root canals. | [65] |
| BD4 and JBD44a | *Pseudomonas aeruginosa* strain PA14 | Applying these phages to PA14 biofilms resulted in a substantial decrease in the average biomass percentage after 24 h and 96 h. | [66] |
| vB_Efa29212_2e, and vB_Efa29212_3e | *E. faecalis* | A reduction of 54.6% in the *E. faecalis* biofilm in the ex vivo model confirmed the bacteriophage's efficacy against the isolates. | [67] |

of PI is distinct to each site, as the microbiomes of healthy implants were more comparable to those of healthy sites on the opposite side. In contrast, mucositis had a higher presence of *F. nucleatum*, which played a crucial role as a primary colonizer. Microbiome-based machine learning showed diagnostic solid and predictive capabilities for peri-implant illnesses. Strain-level profiling successfully discovered a previously unknown subspecies of *F. nucleatum* that had a substantial association with the condition [74].

A peptide phage display system was employed to ascertain the location of a zirconia-binding peptide motif. As the objective, zirconia crystals and discs stabilized with yttria were utilized. A quartz crystal microbalance was employed to observe the phage-zirconia binding process. Investigators repeated cycles of biopanning against zirconia beads, beginning with a library of phages displaying random sequences of 12-mer peptides. Upon completing four rounds of biopanning, phage clone Φ#17 was successfully isolated. Scholars discovered that Φ#17 exhibited a binding affinity for zirconia discs 300-fold more significant than that of phages lacking the peptide. A rapid increase in energy dissipation was observed in the quartz crystal microbalance assay from Φ#17 phages but not from the control phages. This finding suggests that Φ#17 attaches to the surface of zirconia through its exhibited peptide. The discovery of a phage that attaches to the zirconia well surface implies the potential presence of phages capable of disrupting PI-causing biofilms. Nevertheless, clinical testing of this hypothesis is premature at this time [75].

Phage therapy presents itself as a prospective alternative approach to managing peri-prosthetic joint infection (PJI), especially when the availability of efficacious antibiotics is limited. Using a clinically relevant model of *S. aureus*-induced PJI, researchers initiated preclinical trials to determine the therapeutic efficacy of a phage cocktail, both alone and combined with vancomycin, to reduce bacterial populations within the infected joint. Twenty-one days after surgery, infected animals were randomized into one of four treatment groups: phage alone, vancomycin alone, phage and vancomycin, or placebo. Animals were euthanized on day 28 following surgery to conduct microbiological and immunological evaluations of the implanted joints. The administration of phage or vancomycin in isolation resulted in bacterial burden reductions of 6.2 and 5 times, respectively, in peri-implant tissue when compared to animals that were sham-treated. In conjunction with vancomycin, phage-treated animals exhibited a 22.5-fold decrease in the burden of *S. aureus* in joint tissue compared to sham-treated animals. This reduction in *S. aureus* burden coincided with a decrease in knee-implanted edema. Animals undergoing phage therapy will exhibit a diminished burden of *S. aureus* in peri-implant tissue. Furthermore, the concurrent application of phage and vancomycin will further decrease the bacterial load. The microbiological data were corroborated by observations of reduced joint inflammation in animals that received a combination of vancomycin and phage, as opposed to treated with placebo agents. Investigators' results offer additional evidence in favor of phage therapy as a viable and productive supplementary treatment for PJI [76].

PI is believed to be caused by specific pathogens, such as *S. epidermidis*, *P. gingivalis*, and *Tannerella forsythia*, which create an environment conducive to tissue degradation [77]. From wastewater, a new phage, CUB-EPI_14, exclusive to the bacterial species *S. epidermidis*, was isolated, and its genomic and phenotypic properties were examined. Its genome has 46,098 nucleotides overall and 63 putative genes. A subset of these genes have been linked to structural proteins, proteins involved in DNA and metabolism, and proteins involved in packing and lysis. The phage genome did not include any known virulence proteins or lysogeny-associated proteins. Although CUB-EPI_14's host range against *S. epidermidis* was restricted, it exhibited stability across various temperature conditions (ranging from −20 °C to 50 °C) and pH levels (pH 3–pH 12). The phage demonstrated antibacterial solid and antibiofilm properties when tested against a highly vulnerable bacterial sample. Researchers' findings are encouraging and open new treatment avenues in the fight against resistant biofilm-associated illnesses caused by *S. epidermidis* [78]. Compared to planktonic bacteria in the exponential phase, *S. epidermidis* biofilms and stationary growth phase bacteria exhibit more excellent resistance to phage K lysis. This research demonstrates the variations in *S. epidermidis* sensitivity to phage K killing depend on whether the bacteria is cultured in planktonic or biofilm phenotypes [79]. The bactericidal and virucidal efficacy of the denture disinfectant against T1 phage and methicillin-resistant *Staphylococcus aureus* (MRSA) was evaluated by researchers at a concentration of 10 ppm of ozone. The number of bacteria was $3.1\times10^3$ CFU/mL at the beginning of the experiment when the ozone supply was activated for the bactericidal activity test. This number decreased to 1.0×100 CFU/mL 10 min later and subsequently reduced to 1.0×100 CFU/mL or lower. Conversely, the ozone supply was interrupted, resulting in a decrease in the number of bacteria from $3.4\times10^3$ CFU/mL at the beginning of the experiment to $3.0\times10^3$ CFU/mL 60 min later (no statistically significant change). This was due to the sole presence of an air bubble. The quantity of phages in the virucidal activity assay was $1.2\times10^6$ PFU/mL before ozone treatment. This quantity decreased to approximately 1/10 of the original value 10 min later, reaching 6.1×100 PFU/mL 40 min later [80].

Gram-negative *T. forsythia* bacterium is closely linked to the development of PD. *T. forsythia* secretes a recently discovered metalloprotease-like enzyme called karilysin. Karilysin is a promising therapeutic target since it alters the host's immune response. Through phage display, peptides specific to the catalytic domain of Karilysin (Kly18) were chosen for this investigation. The linear peptides have modest binding affinities at the micromolar level. Peptides 14 and 15, the two most potent binders, have the consensus sequence XWFPXXXGGG. Peptide 15 was fused to the N-terminus of maltose binding protein (MBP) to create a peptide-15 fusion. Kly18-specific binding was confirmed using the purified fusion protein, produced from the expression of peptide15-MBP in *E. coli*. Peptide 15 (SWFPLRSGGG), which was chemically synthesized, has the potential to suppress the enzymatic activity of intact Karilysin (Kly48) as well as Kly18. Peptide15 may also slow down intact Kly48's autoprocessing into Kly18. The N-terminal serine was also necessary for Kly18 inhibition, as shown by a truncation analysis, and the WFP motif was significant for inhibition. Like the intact peptide, the SWFP peptide's Ki value was in the low micromolar range15. To sum up researchers indicated SWFP is the first known inhibitor of Karilysin and is a helpful tool for studying the structure-function of the drug [81].

Researchers showed that individuals with peri-implantitis (PI), *P. gingivalis*, and *F. nucleatum* mostly showed excellent resistance to erythromycin, metronidazole, and tetracycline. *A. actinomycetemcomitans* also showed a significant level of resistance to doxycycline and clindamycin. Substantial levels of antibiotic resistance were also demonstrated by additional bacteria, including *Prevotella intermedia*/*nigrescens*, *Parvimonas micra*, and *Tannerella forsythia*, to amoxicillin, azithromycin, and moxifloxacin. The majority of bacteria, however, did not exhibit resistance to the amoxicillin-metronidazole combination. Researchers noted that relevant bacteria were resistant to the antibiotics used to treat PI, which is often recommended in dentistry. However, the management of supplementary antimicrobials in the treatment of PI is contentious. Clinicians should consider the public health implications of antibiotic resistance that have been shown in the treatment of PI patients [82].

Furthermore, phages can increase the number of treatment options available in austere environments at a relatively low cost and with minimal effort, thereby enabling the affected warfighter to return to duty quickly and in better health [83]. Consequently, basic practical investigations on the therapeutic effects of phages should be conducted by researchers to overcome the lack of research and provide an alternative therapy approach for bacterial resistance in PI.

**Bacteriophages in periodontitis**

Periodontitis, an inflammatory disease of the gums that obliterates the support structure of teeth, is further characterized by the development of periodontal pockets, the deterioration of periodontal ligaments (PDLs), and the resorption of alveolar bone. Pockets are susceptible to periodontitis infection due to the proliferation of microflora, particularly anaerobes that generate toxins, and enzymes, and stimulate the immune system [84]. From the available evidence, the host's reaction to a bacterial challenge appears to be the most significant factor in determining susceptibility. Plaque accumulation, poor oral hygiene, and possibly occlusal trauma were deemed adequate to induce periodontitis, according to historical beliefs, which held that all individuals were equally susceptible to developing the disease. On the contrary, over the last forty years, it has become widely acknowledged that PD is exclusively brought on by particular bacterial infections and that neither these infections nor the harm they inflict are universally transmissible to individuals [85, 86]. As the principal keystone pathogen of the periodontal biofilm, *P. gingivalis* induces immune responses in the host that result in gingival tissue injury and bone resorption [87]. The oral microbiome has been the subject of extensive research, encompassing healthy and diseased individuals. The oral cavity is inhabited by bacterial species from thirteen distinct *phyla*, including *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Spirochaetes*, *Synergistetes*, *Tenericutes*, and two unnamed *phyla*, SR1 and TM7 [88]. The accumulation of *spirochetal* debris in subgingival plaque is proportional to the clinical severity of PD. The precise number of distinct spirochetal species that inhabit the plaque is unknown; however, spirochetes can be classified as small, medium-sized, or large based solely on their size. It has been demonstrated that *T. denticola*, one of four cultivable species of small spirochetes, contains proteolytic and keratinolytic enzymes in addition to factors or mechanisms that inhibit fibroblast and polymorphonuclear leukocyte (PMNL) function and suppress lymphocyte blastogenesis. Each of these characteristics may potentially contribute to the damage of periodontal tissue [89]. The genetic analysis of the genome of Siphoviridae_29632, an innovative siphovirus identified through viral metagenomics, is presented. The virus was obtained from a patient diagnosed with periodontitis. Although there is some distant homology among viral morphogenesis proteins, the viral genes encoding structural proteins were found to be distinct from their counterparts in other viruses among the 43 predicted ORFs in the genome. Twenty-eight predicted coding sequences exhibited substantial homology with other well-documented phage ORF sequences. Furthermore, it is worth noting that the incidence of Siphoviridae_29632 was notably elevated at

41.67% in a cohort of patients diagnosed with chronic periodontitis, in contrast to the healthy group. This finding implies that both the virus and its hosts potentially contribute to the ecological milieu that promotes the development of chronic periodontitis [90].

Temperate phage ϕEf11 is capable of infecting various genotypes of *E. faecalis*. Before this, its properties were altered through genetic engineering and sequencing to render it effective as a therapeutic agent in phage therapy. Phage ϕEf11/ϕFL1C(Δ36)PnisA was generated through additional genetic modification of the phage in an investigation. The objective of this research was to assess the effectiveness of phage ϕEf11/ϕFL1C(Δ36) PnisA in disrupting biofilms formed by two strains of *E. faecalis*: V583 (which is resistant to vancomycin) and JH2-2 (which is vancomycin-sensitive). After phage treatment, viable cells (CFU/biofilm) decreased by a factor of 10 to 100, consistent with comparisons of treated and untreated biofilm images as determined by the maximum projections of the Z-series [91].

A newly isolated phage (FnpΦ02) was identified as a specific pathogen for this bacterium. As determined by transmission electron microscopy, the virion has a segmented tail and an icosahedral head. It was approximated that the phage genome comprised 59 kbp of double-stranded DNA. Genetic and morphological characteristics indicate that FnpΦ02 is a member of the Siphoviridae family. The latent period, burst size, and adsorption rate were determined to be 15 h, 100 infectious units per cell, and $7.5\times10^{-10}$ ml $min^{-1}$, respectively, through one-step growth and adsorption experiments. Following cloning and sequencing, a minute segment of phage DNA was identified, revealing 93% nucleotide identity with the phage PA6 of Propionibacterium acnes and amino acid identity with fragments of two phage proteins (Gp3 and Gp4). FnpΦ02, to the best of the researcher's understanding, is the initial phage to infect *F. nucleatum* and lays the groundwork for subsequent investigations into the potential of phages to manage PD [92].

Five phages' physical, biological, genetic, and functional traits were investigated. JD-Fnp1 through JD-Fnp5 myoviruses were all found using transmission electron microscopy. JD-Fnp1–JD-Fnp5 had genomes of 180,066 bp for JD-Fnp1, 41,329 bp for JD-Fnp2, 38,962 bp for JD-Fnp3, 180,231 bp for JD-Fnp4, and 41,353 bp for JD-Fnp5, in that order. The biological traits of JD-Fnp1–JD-Fnp5, including host range, growth properties, pH, and heat stability, showed clear trends. Of these, JD-Fnp4 is thought to have the most potential for clinical use. The characterization and identification of JD-Fnp1–JD-Fnp5 provide the groundwork for further research into potential treatment approaches for disorders linked to *F. nucleatum* [93].

The identification of phage infection in the subgingival plaque of patients diagnosed with swiftly destructive forms of periodontitis associated with *A. actinomycetemcomitans* prompted the speculation that such an infection could potentially enhance the virulence of this bacterium. 68 subjects from the Netherlands and Switzerland with localized juvenile periodontitis, rapidly progressing periodontitis, or adult periodontitis were used to isolate *A. actinomycetemcomitans*, which was examined using the overlay plate technique for the presence of temperate phages. It was discovered that over 50% of the *A. actinomycetemcomitans* strains could release phages that developed into individual plaques on indicator strains. The electron microscopy analysis of virions isolated from seven strains unveiled the characteristic features of double-stranded DNA phages, including an icosahedral head and a contractile tail. No correlation was observed between the carriage of temperate phage by *A. actinomycetemcomitans* and either the clinical manifestation of PD or the composition of the subgingival microflora. As determined by several clinical parameters, destructive PD in patients infected with phage-carrying *A. actinomycetemcomitans* was not more severe than in patients infected with phage-free *A. actinomycetemcomitans*. Adult subjects with periodontitis infected with phage-carrying *A. actinomycetemcomitans* exhibited considerably reduced pocket depth and attachment loss [94] (Table 2).

*A. actinomycetemcomitans*, linked to the genesis of both juvenile and adult periodontitis, may be less virulent due to lysogeny. 185 *A. actinomycetemcomitans* strains from two well-characterized collections contained DNA sequences linked to phage Aa~\23. The researchers have connected these results to the population genetic structure of the collections. In Southern blot hybridization assays, two cloned Aa~\23–specific DNA probes were employed to identify homologous sequences in the strains' whole-cell DNA. Of the 185 strains, 65 (about 35%) had DNA that hybridized to one of the DNA probes. Phage Aa~\23 was detected in 74% of the hybridizing strains, which had the same hybridization pattern. The whole-cell DNA of the remaining hybridizing strains exhibited distinct patterns of hybridization with the probes, suggesting the presence of DNA sequences in these strains connected to phage Aa~\23 but distinct from it. Serotype a strain comprised the majority (81%) of strains harboring phage Aa~\23, whereas serotype d strains seemed resistant to infection with this phage. Based on earlier population genetic investigations of *A. actinomycetemcomitans*, researchers found a strong link between hybridization patterns and genetic subdivisions. The absence of a significant correlation was observed between the periodontal status of the patients from whom the isolates were obtained and the presence of

**Table 2** Bacteriophage antibacterial effects against periodontitis

| Bacteriophage | Peri-implantitis bacteria | Antibacterial effects | Ref |
|---|---|---|---|
| Temperate phage ϕEf11 | *E. faecalis* | After phage treatment, viable cells (CFU/biofilm) decreased by a factor of 10 to 100, consistent with comparisons of treated and untreated biofilm images as determined by the maximum projections of the Z-series. | [91] |
| vB_EfaS-SRH2 | *E. faecalis* | The genome did not contain any harmful genes, including those associated with antibiotic resistance, lysogenic dependence, or virulence factors. Given its physiological properties and genomic characteristics, this phage exhibits potential as a viable option for root canal infection and periodontitis phage therapy. | [99] |
| Phage PEf771 | *E. faecalis* YN771 | Rats subjected to treatment with calcium hydroxide preparation, phage PEf771, and 2% chlorhexidine gel exhibited a considerably reduced area of bone degradation compared to the untreated group. | [100] |
| FnpΦ02 | *F. nucleatum* | Following cloning and sequencing, a minute segment of phage DNA was identified, revealing 93% nucleotide identity with the phage PA6 of Propionibacterium acnes and amino acid identity with fragments of two phage proteins (Gp3 and Gp4). | [92] |
| Temperate bacteriophage | *A. actinomycetemcomitans* | As determined by several clinical parameters, destructive periodontal disease in patients infected with phage-carrying *A. actinomycetemcomitans* was not more severe than in patients infected with phage-free *A. actinomycetemcomitans*. Adult subjects with periodontitis infected with phage-carrying *A. actinomycetemcomitans* exhibited considerably reduced pocket depth and attachment loss. | [94] |

Aa~\23 among *A. actinomycetemcomitans* strains. This suggests that the virulence of *A. actinomycetemcomitans* is not significantly affected by this phage [95].

Researchers looked at the in vitro antibacterial activity of new phages and the resistance of bacteria to phage infections. Standard strains of *E. coli* (ATCC 25927), *P. aeruginosa* (ATCC 27853), *E. faecalis* (ATCC 29212), and *S. aureus* (ATCC 6538) were used to evaluate lytic phages. In further exposure, 20 µL of every phage was exposed to $10^6$ CFU/mL of *P. aeruginosa*, *E. coli*, *E. faecalis*, and *S. aureus*. Thirdly, phages were introduced to robust colonies that had been formed. Diffusion disks were used to examine the antibiotic sensitivity of bacteria that showed resistance to phage infection after the third exposure. Scientists measured the diameters of inhibitory halos. Furthermore, fourteen phage cocktails were created to assess the resistance of bacteria against phage infections. *S. aureus*, *E. coli*, or *E. faecalis* were not infected by Phages; only *P. aeruginosa* was. There were phage-resistant bacteria in each of the three exposures. Furthermore, no phage could infect another phage when titrated at $10^8$ PFU/mL, four at $10^{10}$, and one at $10^{13}$. In every one of the examined phage mixtures (~ $10^8$ PFU/mL, ~$10^{10}$ PFU/mL, and ~ $10^{13}$ PFU/mL), bacterial resistance to phage infection was confirmed. The antibiotic susceptibility of *P. aeruginosa* was unaffected by these results. Colonies of *P. aeruginosa* developed resistance to phage infection after many exposures. Nevertheless, the formation of resistant colonies was inhibited by specific phages at titers of around $10^{10}$ and $10^{13}$ PFU/mL. *P. aeruginosa*'s antibiotic sensitivity did not change despite resistance. It was discovered that *P. aeruginosa* variations resistant to phage cocktail could withstand infection at any viral titer. Resistant colonies were halted by specific combinations with $10^{10}$ and $10^{13}$ PFU/mL titers. Even though bacteria that are resistant to phages have emerged, further research on phages in infection control is crucial [96].

Drug resistance in oral bacteria may have been brought on by the widespread use of antibiotics. Since there aren't many new medicines being developed, alternative medicine is becoming more and more popular as a means of treating and controlling periodontal infections worldwide. Researchers discussed the functions of phages as substitutes for the available treatments. While many benefits are linked to alternative therapies, these methods also have some drawbacks. Phage treatment has a significant disadvantage in that it may lead to the growth of bacteria resistant to the phage. Phage-resistant bacteria have been seen to arise in many animal and human research, according to experimental data. Since phage-derived lysins are naturally proteins, they may elicit humoral immune responses in addition to their anti-periodontium solid pathogen activity [97]. Phage therapy has been effectively employed lately to treat antibiotic-resistant systemic illnesses since phages exclusively infect bacterial cells. This gives them a vast potential for application as a therapeutic approach. The range of action against periodontal pathogens and dental plaque biofilms in periodontitis is increased by their capacity to destroy biofilms. New directions in periodontal treatment may be enabled by future studies concentrating on the oral phageome and the safety and efficacy of phage therapy [98].

## Advantages and disadvantages

Dental diseases are arguably the most common diseases associated with infections in the human population. Almost every infectious disease that compromises oral health is associated with biofilm, including caries, PD, gingivitis, endodontic infections, and PI. Existing treatments for oral infections caused by biofilms exhibit a deficiency in sensitivity; they target both pathogenic and commensal species, which serve as a protective barrier against the development of pathogenic biofilms. Furthermore, antibiotics are virtually ineffective against biofilm and are rarely prescribed for oral diseases. Phage therapy represents a hopeful alternative strategy. Phages are essential to the predator-prey dynamic between bacteria and maintain a natural equilibrium; therefore, they have the potential to function as effective antibacterial agents. Plasmid-specific phages are exceptionally effective against biofilm and are simple to isolate and manipulate. Thus, similar to numerous other medical disciplines, phage therapy presents novel prospects for advancements in dentistry, encompassing both therapeutic applications and research [13]. Phages have been isolated and characterized for the following microorganisms: *A. actinomycetemcomitans*, *Actinomyces* spp, *E. faecalis*, *F. nucleatum*, *Lactobacillus* spp, *Neisseria* spp, *Streptococcus* spp, and *Veillonella* spp. Phages may be regarded as prospective therapeutic instruments for eradicating oral cavity diseases such as periodontitis and caries [101].

In recent decades, phage therapy has become a prospective therapeutic approach for recalcitrant infections, owing to the growing demand for alternative or supplementary antimicrobials to traditional antibiotics. Numerous uncontrolled case studies have documented favorable clinical outcomes. Clinical failures, on the other hand, are probably underreported, and the limited number of randomized controlled trials that have been undertaken have been ineffective in demonstrating benefit. Therefore, it is not possible to endorse the routine clinical application of phage therapy under any circumstances; there is considerable uncertainty regarding the effectiveness of phage therapy and possible factors contributing to its failure, including but not limited to dosing, frequency, duration, routes of administration, interactions with antibiotics and other phages, emergence of phage resistance, inadequate phage delivery, and super host immune response Although further clinical research studies are required, the purpose of this document is to provide clinicians with an overview of the most important considerations when evaluating phage therapy for experimental clinical use. The extensive range of possible clinical applications, the safety and tolerability of phages, the prerequisites for secure phage therapy administration, the existing regulatory routes for increased access, and the absence of standardized assays for phage-stimulated tests (PST) and phage quantification techniques are emphasized. Recognizing the need to develop standardized, accurate methods for lytic activity confirmation before treatment, whenever practicable, and emphasizing the significance of individualized therapy are both emphasized. It is emphasized that synergies may occur when antibiotics are combined with phage therapy. While phage therapy is presently employed only as a salvage measure, its potential utility may enable its implementation earlier in the course of an infection, thereby potentially conserving antibiotics by reducing their initial usage. Although not exhaustive or conclusive, this document is intended to establish a foundation for logical phage selection and dosing as further investigation is conducted to deepen comprehension of the intricate dynamics between bacteria, humans, and phages [102].

Phage isolation from *S. mutans* is a potential therapeutic application for this ailment. It has not yet been implemented, however, in clinical practice. The correlation between periodontal health and disease and the diversity of phage communities, with healthier individuals harboring a more diverse phage community, implies that therapeutic interventions may be conceivable by leveraging these communities. Research examining the impact of phages on endodontic lesions has primarily focused on *E. faecalis*. The presence of a varied microbial community within endodontic biofilms implies that additional research in this domain may be warranted. The discovery of a phage that attaches to the zirconia well surface implies the potential presence of phages capable of disrupting peri-implants-causing biofilms. Nevertheless, clinical testing of this hypothesis is premature [49]. While some research has examined the use of phages in treating dental infections, a thorough and effective investigation into the therapeutic benefits of phages against PI has not yet been carried out. In this instance, investigations should be carried out by researchers to develop a different course of therapy to address the issue of antibiotic resistance in PI patients. It is still too early to test it therapeutically. Still, discovering a phage that binds to the surface of zirconia well implies the presence of phages that may interfere with biofilms that cause PI [1].

Oral bacteria in planktonic and biofilm forms have been effectively targeted by phages and their enzymes. Recombinant phage enzymes have activity against *Streptococcus* species and *A. naeslundii*. Only a tiny portion of the accessible phages have been identified and studied, nevertheless. Phage action must be investigated in multispecies settings, in animal models, and conjunction with other antimicrobials. It is necessary to assess the use of phages or their enzymes for oral microbiota engineering. Creating accessible, affordable phage-based preventatives and treatments for oral healthcare would be the ultimate objective [14].

There are five primary methods for combating the biofilm matrix. (1) Phage therapy is a method that involves the use of the entire organism to eliminate bacterial biofilm by destroying the bacteria hosts from within. This is achieved through the initial penetration of the matrix by depolymerase, which is followed by the lytic cycle. (2) Phage-derived depolymerase, which functions by degrading the EPS, capsular polysaccharide (CPS), and glycocalyx, could be used as a free enzyme or tail-spike protein (TSP). (3) Endolysins derived from phages that infiltrate the EPS structure and externally combat the local bacteria. (4) A combination therapy that involves the application of phage and other antimicrobial compounds to promote the complete eradication of both the matrix and latent bacteria, as well as a reduction in resistance to phages. (5) The host-species interaction range is expanded by genetically engineered phages, which modify the proteins implicated in the phage attachment and/or insertion. The future of phage therapy is centered on expanding the scope of phage and its derived enzymes. This can be accomplished through the further investigation of (i) combinational therapy with phage and antibiotics; (ii) genetically engineered phages; and (iii) genetically engineered proteins, such as artilysins and chimeolysins, that overcome the limitations that allow endolysins to target gram-negative bacteria [103].

Regrettably, bacteria can also evolve resistance to phages via mechanisms such as phage DNA degradation induction, binding site mutation, adsorption inhibition, or DNA penetration block. Therefore, researchers hypothesized that the concurrent use of phages and antibiotics or natural products could be a practical solution to this issue. For instance, an *S. aureus* infection was more effectively treated with a combination of rifampicin and a phage than with a combination of rifampicin and alternative antibiotics, such as azithromycin and vancomycin. Similarly, when meropenem and a phage were combined, the survival rate of *Galleria mellonella* infected with *B. cepacia* was increased by a minimum of 36 times compared to using either antibiotic or phage alone. The collaboration of bacterial killers with distinct antibacterial mechanisms makes the combination of antibiotics or natural products and phages promising; nevertheless, it is necessary to clarify the extent to which this combination enhances antibacterial efficiency and how these distinct mechanisms mutually benefit [104–107].

Concerning the dynamics of phage colonization in oral microbiota, much remains to be clarified. It is essential to highlight the complete dissimilarity in pharmacokinetics between phages and antibiotics: phage amplification on target bacterial strains increases the number of active phage particles after phage administration, whereas the concentration of white antibiotics diminishes over time. Therefore, determining the optimal therapeutic phage dosage is challenging. The prospective efficacy of phage therapy in PD is determined by various factors, including phage interactions with oral microbiota, phage pharmacokinetics development, host immune response to administered phages, and so forth. Particularly, anaerobic bacteria, constituents of the red complex of periopathogens, are challenging to cultivate. This complicates the research and restricts the ability to identify and isolate phages specific to these pathogens. Furthermore, it is crucial to consider the polymicrobial nature of PD during the development of phage preparations designed for the prevention or treatment of periodontitis [17, 108–110].

Specific phages may be used to target bacteria in dentistry because of the infectious character of several illnesses, including caries, PDs, periapical diseases, inflammatory disorders of the oral mucosa, and infections brought on by implant treatments. Phages can eliminate biofilm or restrict its development, which might lessen the severity of illnesses or manage their acute stages. One of the drawbacks of phage therapy is that each patient's course of treatment must be tailored to their particular bacterial state. Since it only targets the bacteria that cause the illness, this might be a benefit. Researchers anticipate that in the future, more rational methods to avoid antibiotic resistance and improve patient immunity will be developed since isolating a phage is inexpensive and simple [1].

## Conclusion

Phage therapy, which was recently effective in managing antibiotic-resistant systemic infections, is just one example. Phages have been demonstrated to exert a significant antibacterial influence on various dental infectious diseases, such as periodontitis, PI, infected dentin, and root canal reinfection. Phage therapy may have some limitations, including the limited antibacterial spectra of phages, the activation of host immunity against phages, and horizontal gene transfer to bacterial populations, in addition to its numerous potential benefits. Nevertheless, by utilizing cutting-edge techniques in synthetic biology—such as phage and phage product engineering and the creation of phage genome resources—it is possible to circumvent these drawbacks of phage therapy. Researchers shall be able to utilize phages as a substitute or supplementary treatment for dental maladies by enhancing clinical trial data, including dose determination.

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## Publisher's note