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Review Article

Anecdotal Evidences to Rise in Phage Therapy in Treatment of Oral Infections

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Abstract

Knowledge of microorganisms related to oral pathologies is necessary to develop a basic understanding of the disease mechanism and a sound rationale for the effective management of patients. The now spreading vast applicability of phage in therapeutic and preventive medicine has changed the previous view of its inability. The novel phages are being discovered and have proved their mantle in treating bacterial infections. Their ability to target planktonic as well as biofilm communities make them tiny weapons which will soon overcome multidrug resistance issue thriving due to bacterial evolution. The experiments so far been conducted have shown encouraging results and can be operative for various dental infections such as periodontitis and dental caries. The natural as well as engineered phages are non-toxic to mammalian cells, can be applied easily and also boost the host immune system. The phage related therapies involve use of endolysins and in synergism with antibiotics, can give intriguing benefits as they can access the vicinity of the target tissues. The better knowledge and application of phage therapy in emerging medical interventions may pose significant effect in reducing multidrug resistance curse in near future.

Keywords: *Phages; Biofilms; Dental Infections; Phage Therapy*

Introduction

Understanding microbial systems needs unbraiding the relationship that phages share with their hosts and environment. Bacterial viruses, intracellular oligoparasites are structurally simple with life cycles as short as 20 - 60 minutes. Bacteriophages are natural antibacterial, able to regulate bacterial populations by the induction of bacterial lysis. They are active against most bacteria, including Multi drug resistant bugs. The recent research on molecular biology has rekindled the original application of phage as therapeutics to treat human and animal infections. The recent renaissance has been triggered since the emergence of many antibiotic¬ resistant pathogens. Previously ignored, it is now becoming increasingly accepted that phages play a key role in oral dysbiosis. Fortunately, therapy can be developed for most of the infections as bacteriophages are found for almost all species of bacteria [1]. Oral cavity is one of the densely populated habitats comprising around 6 billion bacteria [2]. These bacteria along with saliva are major components of oral microbiology, having the capability to be harmful, but also performing beneficial and necessary roles in the immune system. These bacteria's have evolved to survive on tooth surface, gingival epithelium and oral cavity. Bacteria mostly live in complex communities called Biofilms. Oral biofilms that form on teeth produce acids, causing dental caries and biofilms that grow in the gingival sulcus contribute to the pathogenesis of periodontitis [3]. The marker to the antibiotic resistance is the toughest extracellular slime layer which the biofilms are surrounded off making indwelling bacteria almost 1000 times safer than the planktonic ones. The minimum inhibitory concentration of antibiotics for bacteria associated with biofilms is 1500 times the normal dose, which turns out to be lethal for human beings [4]. The two most common biofilm producing *Enterococci* species important in dental pathologies are *Enterococcus faecalis* and *Enterococcus faecium*, in which the former has life threatening implications [5]. *E. faecalis* has been frequently found in root canal treated teeth, in the prevalence values ranging from 30% - 90%. In the saliva surrounding human gums the ratio of phage to bacteria is 5:1 whereas it rises to 40:1 on the mucosal surfaces. E. fae-

calis has been observed creating phages to be used as weapons against closely related bacteria hence, given the name "Bacteria Warfare" [6]. Phage therapy may be an important alternative for the treatment of root canal infections refractory to the conventional or traditional treatment procedures, as they were non specific, mostly physical or chemical and removed desirable bacteria along with the target organisms. Now days, mouthwashes that contain a variety of bacteriostatic and bactericidal organic chemicals claim to reduce the pathogens in dental plaque. With the exception of fluorides and chlorhexidine, none of the currently available oral health measure is effective. Bacteriophages are much more specific than most antibiotics are in clinical use. Theoretically, phage therapy is harmless to the eukaryotic host undergoing therapy and it should not affect the beneficial normal flora of the host too. Though many believe that phages will never replace antibiotics but perhaps there combination with antibiotics may turn out to be a valuable approach [7]. Our knowledge of genetics, physiology, molecular and evolutionary biology of the phage is far better than it was 60 years ago. The quest for new drugs has made researchers to ponder on new interventions because of the growing concern over the failing antibiotic medicine discovery pipeline. So, there is a very good reason to believe that the success of treating and preventing dental diseases is based on development of phages. This review is an attempt to address the lacunae in our current knowledge of phages and its therapeutic and preventive applications relative to dentistry.

History

Although due to financial and technical constraints, bacteriophage research had come to a standstill but bacterial infections were being treated with bacteriophages in some parts of the world. The bacterial activity of phage was recognized by Hankin in 1896 [8] but Felix d'Herelle was credited for the discovery and evaluation of therapeutic potentials of phages in 1917. He coined the term Bacteriophage: bacteria + phagein (Greek: to eat or nibble) [9]. The first case was treated by Richard Bruyoghe and Joesph Maisin by directly injecting the phage to Staphylococcal skin infection [10]. The Bacteriophage Institute of Tbilisi, Georgia established in 1923, is still doing research and providing phages for treatment modalities. Eli Lilly, a pharmaceutical company started commercialization of the phages in US during 1940's. These preparations consisted of phage-lysed, bacteriologically sterile broth cultures of the targeted bacteria (e.g., Colo-lysate) or the same preparations in a water-soluble jelly base (e.g., Colo-jel) used to treat various infections [11]. D'Herelle's commercial laboratory in Paris produced five phage preparations, marketed by a French company which later became the L'Oreal [9]. The development of antibiotics had led to the temporary setback on the phage therapy in 1930's but now the interest has been renewed. Recently even Phage treatment via inhalation is being used at the Eliava Institute [11]. All these discoveries have invigorated researchers to find an alternative treatment mode for various emerging diseases.

Phages in Natural Environment

The human oral cavity is the perfect repository for the bacteria and viruses. There is a vast diversity of phages and bacteria in oral ecology due to their co-evolutionary behavior. Their interactions play vital role in equilibrating the microbial ecosystem. The accelerated rate of mutation in phages and their respective hosts for evolution pooled the oral virome diversity. They benefit the host bacteria making them able to combat the potent antibiotics through lysogenic conversion, empowering bacterial heterogeneity. On the contrary, the lytic phage can benefit human by killing the specific host bacteria which are etiological agents of various dental infections. Oral phage community is very stable and undergoes dysbiosis during bacterial invasion. The oral phage community becomes homogeneous in structure in periodontal disease [12]. The eukaryotic viruses in oral virome are very limited as compared to phage. Some metagenomic techniques are necessary to discern the oral micro biome. Metagenomic analysis to study oral phage-bacteria interaction throws light through its Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) tool [13]. The CRISPR arrays are composed of short repeats and intervening sequences derived from foreign invaders. CRISPR-arrays as an integral part of bacterial genome gives useful investigation of how many phages have encountered a particular bacterial genome. CRISPR defends bacteria and helps them adapting to enemy viruses by keeping genetic memory of past incursions. In simple terms, it is suggestive of the folds of infection a bacterium faces, through their direct repeats and phage spacers in the bacterial genome. By CRISPR based PCR approach followed by comparative sequence analysis with phage whole genome sequence explains the infection network of that specific phage. The majority of oral phages followed one to one infection model e.g. *Actinomyces* phage contig possessed same protospacer as was found in CRISPR of one species of *Actinomyces* [14]. Some phage followed one to many infection model e.g. Cyanophage can attack *Synechococcus* than its normal prey *Prochlorococcus* [15].

Dental Plaque and Biofilm

Dental plaque is defined as "a specific but highly variable structural entity consisting of microorganisms and their products embedded in a highly organized intercellular matrix". The architecture and function of dental plaque is very person specific like fingerprints, although the bacterial species may be same. Biofilm is implicated as chief culprit in the etio-pathogenesis of dental caries and periodontal diseases. Uncalcified biofilms can be removed by routine oral hygiene aids or professional dental instruments, but once calcified into dental calculus, the removal poses a great challenge to the dentist in controlling and eradicating biofilm-associated diseases. Biofilm exhibit an altered phenotype with respect to growth rate and gene transcription [4] and is completely different in carious and periodontal disease regions [12]. The supragingival areas are continuously subjected to the environment and exposed to acidogenic and aciduric bacteria as we eat resulting in caries. E.g. *Streptococcus* spp. The microorganisms inhibiting the oral ecosystem may render any bacterial species, pathogenic due synergistic or antagonistic relationship [16]. Subgingival areas have desquamated epithelium and gingival crevicular fluid in it. Due to its anatomy and stagnant environment it does not get cleansed by itself harboring motile bacteria. In acute phase, *Actinobacillus actinomycetemcomitans, P. gingivalis , Bacteroids , Spirochetes* etc. level increases dramatically [17,18]. The antibiotic resistance of plaque biofilm led to the notion that bacteria in biofilm express an entirely different set of genes from that of planktonic [19]. These behavioral changes and the unique phenotype present in healthy and diseased biofilms, have led to various researches plaque-related diseases. Biofilm-associated bacteria show an important distinctive property of Quorum sensing [20]. This involves the regulation of expression of specific genes through the accumulation of signaling compounds that mediate intercellular communication. E.g. Expression of genes for antibiotic resistance at high cell densities may provide protection encouraging the growth of beneficial species [21]. *P. gingivalis* the main periodontal pathogen shows that AI (auto inducer)-2 has some role in controlling its virulence [22]. Gene transfer is another important property of biofilms through which bacteria communicate. In *S. mutans*, quorum sensing is mediated by competence stimulating peptide, whereas genes are responsible for biofilm formation, competence and acid tolerance [23]. When exposed to saliva *S. gordonii* induces some genes that mediate host surface binding and co-aggregation with *P. gingivalis* and *Actinomyces*. Similarly, genes encoding glucan and fructan synthesis are differentially regulated in biofilm associated *S. mutans* [24]. The presence of phages with slight diversity difference in both supra and sub gingival plaque, have been demonstrated using metagenomic techniques [12,25,26].

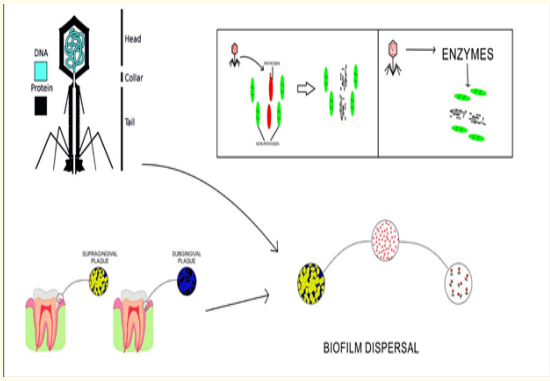
Formation of Dental Biofilm

Within few minutes of cleansing, the oral ecosystem is conditioned with the saliva which provides nutrients and several proteins which contribute to the complimentary fit for the binding of the cells [27]. The irreversible attachment (covalent and hydrogen bonds) confers the adhesion in which some bacteria bind to pellicle receptor with the help of special surface molecules (adhesions) becoming the "primary colonizers". *Streptococcus oralis, S. mitis, S. salivarus* and *S. vestibularus* are the instant colonizers four hours post insertion as studied through an enamel chip model [27]. The primary colonizers form a biofilm by auto aggregation (attraction between same species) and co-aggregation (attraction between different species). The protein expressed on *S. gordonii* DL1 is exemplary in expressing CshA [28], SspA and SspB [29]. These multifunctional proteins harbor the interaction between bacteria and host surface proteins. Other oral bacteria like *A. naeslundii* [30], *F. nucleatum* [31] also possess adhesins for dental plaque development. Co-aggregation results in a functional organization of plaque bacteria resulting in corncob appearance where coccoid cells such as *Streptococci* attach to a long rod such as *Fusobacterium* or *S. sanguis* [32]. An increase in thickness of the plaque biofilm creates nutritional and atmospheric gradients reducing the oxygen levels and allowing growth of anaerobes. The microenvironment thus changes from aerobic to facultative anaerobic. Microcolonies form complex groups and the plaque starts maturing. After one day, organization starts taking place within the biofilm. The thickness of the final dental plaque increases to 20 to 30 μm after three days. Bacteria within the biofilm are protected from phagocytic cells (PMN) and against exogenous bactericidal agents. In the course of time, gram-negative cocci as well as gram-positive and gram-negative rods and the first filamentous forms begin to colonize [33]. *Fusobacterium nucleatum, Treponema denticola* and *Bacteroides forsythus* are microorganisms which are thought to appear in more advanced stages of plaque formations. Oral putative pathogens, such as *Porphyromonas gingivalis*, need the presence of a mature biofilm in order to be able to colonize the gingival [34]. Consortium within the biofilm exhibits metabolic relationships which helps them surviving the fluctuating conditions in the oral cavity which they could not be able to planktonically.

Targeting the Oral Biofilms

Biofilm is the landmark for the commencement of a new micro community which endows the intergenic and intragenic bacteria with multi drug resistance. Intracanal biofilm infection is a reservoir of chronic infections as they easily escape the present tools to combat. Human oral phages co-evolve with bacteria limiting the ability for other phages to enter the community. The oral mucosa provides an extensive surface area for horizontal gene transfers and adhesion of phage. Siphoviruses have been mostly found in saliva, sub gingival and supragingival plaque suggestive of lysogenic conversions of bacteria. Phage nucleic acids have been found in abundance in mucosa and also blood of some immunocompromised patients, suggesting its role in limiting the access of phage into the bloodstream [35]. Mostly phages reach the bloodstream via gastrointestinal tract but oral mucosa can also lead phages to the bloodstream. The primary means by which host and phage interact is by receptor proteins. The phages bind to these receptors and modify them leading to inability of oral bacteria to form biofilms. There are at least four mechanisms with which phages target bacteria.

1. Bacteriophages replicate in their host cells, resulting in release of the infectious progeny into the biofilm. The particles then destroy the bacteria.
2. Bacteriophages either carry or express depolymerizing enzymes that disrupt the extracellular polymeric substance of bacteria.
3. Bacteriophages can induce the depolymerizing enzymes within the host genome that disrupts the EPS.
4. Bacterial communities form dormant, antibiotic tolerant cells called Persister cells. These cells are not mutants, but phenotypic variants of the wild type. Bacteriophage can infect these cells; remain within these bacteria until they reactivate and then start a productive infection, which then destroys the cells [36] (Figure 1).



Naturally occurring bacteriophages can penetrate biofilms even when they do not produce depolymerases but some researchers are of the believe that EPS degrading enzymes are necessary for biofilm degradation [37]. A mixture of three bacteriophages could completely eliminate a single species biofilm [38] but mixed biofilms have been reported to reduce the efficacy of bacteriophages [39]. Even seven-day-old mature biofilm can be targeted effectively using bacteriophage [40].

The mode of action of phages against biofilm differs from antibiotics. The pharmacokinetics too differs from that of antibiotics. The critical parameters involved are clearance rate, adsorption rate, burst size, latent period and initial dose [41]. Clearance of phage takes place via reticuloendothelial system. After administration the phage appears in the human bloodstream within 2 - 4 hours and in the internal organs like spleen in 10 hours, staying in the body for upto several days [42]. Majority of the therapeutic phages destroy the target bacteria by replicating inside and lysing the host cell, undergoing a lytic cycle. The oral virome predominantly entails the bacteriophage communities which are individual, specific and remains stable for a long period of time [43].

The effect of viability of *Enterococcus faecalis* ATCC 29212 in human dental roots was assessed by Paisano., *et al* [44]. Lysozyme-like enzymes were isolated from bacteriophages capable of killing cariogenic bacteria and other periodontal disease-causing organisms. It was concluded that the phage therapy could be used as an alternative to conventional therapies. A phage that affected *A. actinomycetemcomitans* was isolated in the early 1980's and the studies positively correlated the bacteria with Rapidly Destructive Periodontitis [45,46]. Earlier studies made use of electron microscopes to detect particles in dental plaque [47]. But now a day's single PCR and in situ hybridization can detect phages easily within the single host cell [48]. Recently, researchers have isolated a *S. mutans* phage, APCM01, belonging to the *Siphoviridae* family. This phage has the ability to reduce *S. mutans* growth and biofilm formation. It can be commercially used in combination with other phages and antimicrobial agents for various treatments [49].

Phages in Action

Studies have been conducting to isolate new phages for definite remission of periodontal pathogens. A lytic bacteriophage (belonging to *Cystoviridae* family) specific to dental plaque forming *Streptococcus salivarius* revealed its potential in phage therapy. However, there is no report of whether viruses of *Cystoviridae* could attack gram positive bacteria [50]. A lytic phage, EFDG1 belonging to *Myoviridae* family was isolated from sewage had bactericidal activity specific for E. *faecalis* and E. *faecium*. The EFDG1 phage was proved to be efficient that 100 PFU/ml were sufficient to eliminate 109 CFU/ml of E. *faecalis*. It might be used against this bacterium after root canal treatment. Various studies have shown that biofilms conf A lytic phage, EFDG1 and the phage has the credibility to disperse a two weeks old biofilm of E. *faecalis* significantly up to 600μm width [5].

Fusobacterium nucleatum, a gram negative anaerobe is predominantly found in successive community of dental plaque. A novel bacteriophage isolated from human saliva sample, FnpΦ02 was found to be highly sensitive to *Fusobacterium nucleatum* subsp. *Polymorphum* and moderately sensitive to *Fusobacterium nucleatum* subsp. *Nucleatum* and subsp. *Vincentii*. This phage belongs to *Siphoviridae* family and the genome size of its dsDNA is approximately 59kbp [58]. There are many bacteriophages commercially being sold in disciplines other than dentistry. In 2006 FDA approved LMP-102 bacteriophage (Intralytic, USA) against *Listeria monocytogenes* in meat products. ECP-100TM effectiveness against *E. coli* O157:H7 made its use commercial. BioPhage-PA (AmpliPhi Biosciences Corp., USA) is being used for chronic ear disease against *Pseudomonas aeruginosa*. Viridax Company (USA) has developed phage preparation named Viridax™ against *Staphylococcus aureus* in respiratory, systemic, topical and wound infection. There are also available phages which infect multiple host called as Cross Infection Phages [14]. Launched in 2011, BacWashTM (Omnilytics, USA) has infection ability against *Salmonella* and *E. coli O157:H7* and being used directly on animals without any toxic effect. Oral *Campylobacter* spp. is associated with periodontal diseases [51]. A negative correlation between *Campylobacter jejuni* and *Campylobacter*-specific bacteriophage was studied and significant ($P < 0.001$) reduction in count of *Campylobacteria jejuni* was seen in broiler chicken ceca [52]. The reduction in *Campylobacter jejuni* number in chicken broilers when subjected to two different phages as therapeutic treatment [53]. Split skin grafts in guinea-pigs by *Pseudomonas aeruginosa* 3719 were successfully treated with lytic bacteriophage BS24 [54]. *In-vitro* maintained mycobacteriophage delivered by non-virulent mycobacterium killed *Mycobacterium avium* and *Mycobacterium tuberculosis* intracellularly.

In Georgia, bacteriophage Sb-1 was found to be quite effective in eliminating MRSA infections, in a patient of cystic fibrosis infected with MRSA [55]. Recently human clinical trials have been initiated by various companies. Phage therapy proved its success rate of 85% against antibiotic resistant septicemia in 94 patients. In another study 20 cancer patients were treated for their bacterial infections by administering oral doses of phage thrice daily, and the result suggested that infection was cured in all cases. Hence, when further research is being undertaken, definitely consider phage therapy as a successful treatment option [56].

Mode of Administration

Phages cannot diffuse across membranes; therefore methods of delivering phages to target bacteria need to be devised. Some researchers have even proposed that non-pathogenic species of bacteria can be used to bring phage to target. Recently, proposals have been made that phages can be included in nebulizers and sprays as respirable powders to treat various pulmonary infections [57,58].

Advantages of Phage Therapy

Phages outnumber bacteria and grow along with their target and they too die rendering the host safe after killing pathogen. The phages are available in almost all possible forms rendering their administration very easy. Intravenous administration omits the need of repetition. Phages are more host specific than antibiotics making them less toxic so, less or no chances of collateral damage. Even gut flora remains unchanged, reducing the chances of opportunistic secondary infections by organisms such as *Clostridium difficile, Candida albicans* etc. [1,7]. Bacterial resistance to phages is less of a concern as phages synthesize enzymes that break the biofilm and if resistance develops, it is easier to discover phage than antibiotics. Phages mutate at higher rates then bacteria, so easily adapt to phage resistant bacteria. Phages are cheaper than antibiotics and have deep seated action. Only few side effects have been reported with phage therapy because of the extensive liberation of endotoxins [1]. Phages are omnipresent living organisms found in soil, water, plants and humans and this could be the reason that no case of allergy towards phage has been reported yet, making them an useful alternative for patients allergic to antibiotics. Since selection of active phages is a natural process, evolutionary arguments support the idea that active phage can be selected against every resistant bacterium, by an ever ongoing process of natural selection. This selective advantage of using phages over other commercial interventions increases its future prospects.

Current Research and Potential Problems

There is a still a long way to go before the therapy can be applied on a widespread basis and researchers are working to overcome their limitations. The main issue is that most of the experiments are done *in vitro* and the results need to be deduced *in-vivo* [59]. Transfer of bacterial toxin gene can be overcome by selection of phages that do not have specialized transduction abilities. Genetically modified mutant phages can also be used against such phages [60]. Bacteriophages are viruses and have the tendency of swapping genes with each other and any organisms they come in contact with. This creates chance of spread of antibiotic resistance in bacteria. Resistance can also be caused by changes in the receptor molecules in gram-negative bacteria [1,61]. The issue can be avoided by using cocktails of slightly different phages; to target the plasmid protected bacteria or even those with mutations. Mutation may not always be bad since resistance affects the fitness of bacteria as loss of receptor decreases the virulence of bacteria specifically [47]. Currently the quorum sensing issues is being researched upon. It improves the defense mechanism by avoiding infection during growth in competitive conditions. A study reflected that *E. coli* cells avoided infections by phages to a higher degree when grown in cultures containing AHL's. The AHL treatment reduced the phage adsorption rate and allowed growth of bacterial cells [62]. Phages cannot survive at a low pH so protection from gastric acids can be done by polymer microencapsulation that enhances the efficacy of the phage [1]. When administered intravenously they need to be cleared out by the human immune system as human body will recognize phage as a foreign body even before their target is accomplished. To overcome the issue scientists have proposed a natural selection strategy [63]. Topical application needs continuity and has the disadvantage of interfering with the immune system. The phages need to be devoid of toxins so ion exchange chromatography, high speed centrifugation and other modern purification techniques can be employed [42]. The strongly specific nature can be a disadvantage over the broad spectrum antibiotics as the exact bacteria should be known to make it a target. This limitation can also be overcome using polyvalent phage cocktails which lyse the majority of etiologic agent strains. The development of phage neutralizing antibodies is another potential problem affecting its effectiveness [64]. The Conventional Phage Therapy administers the phages which occur naturally in the environment. Phages are being modified and are under clinical trials for last decades because of its safety, reduced or null side effects and better efficacy to combat the infections in controlled manner. Modification of the phage confers protection of phage in extreme living environment (temperature, pH, enzymes) and increase the residence time on the site. A nanoencapsulation system has been successfully developed increasing the survival efficacy of *Salmonella* bacteriophage felix-O1 captured in chitosan-alginate-CaCl2 microspheres [65]. A non-scientific downside to phage therapy is that it would not be patentable due to the Intellectual property law and its current use in public. The pharmaceutical companies would not produce as there is no patent protection. There are various limitations to phage therapy and we have tried summarizing them along with the required approaches.

Enzymatic Concepts of Bacterial Lysis

Enzybiotic is a term first coined taking bacteriophage encoded enzymes in consideration. The enzyme important to phage therapy concept is endolysin containing muralytic activities. The endolysins are designed to attack one of the four major bonds in the peptido-

glycan. Two are hydrolases: N-acetylmuramidases (lysozymes) and N-acetyl-β-D-glucosaminidases (glycosidases) hydrolyzing the β-1-4 glycosidic bond in the sugar moiety of the cell wall. Third is the amidase : N-acetylmuramoyl-L-alanine amidases, which cleave the amide bond connecting the sugar and peptide moieties of the bacterial cell wall and last one is peptidase: L-alanoyl-D-glutamate endopeptidases and interpeptide bridge-specific endopeptidases, which attack the peptide moiety of the cell wall peptidoglycan. Of the phage lytic enzymes that have been reported thus far, the great majority are amidases. The protein holin helps lysin gaining access to the peptidoglycan layer by forming holes in the bacterial cell wall disrupting the osmotic potential of the bacterium. Hence, the cytoplasmic material gets extruded out of the cell causing cell death. Lysin if administered alone exogenously, can itself gain access and lyse the bacterial cell wall. Lysin activity is usually found against gram positive bacteria due to lack of outer membrane. The endolysins are being cloned, expressed in a controlled way and purified to explore its antibacterial activity. Nature has culminated the phages with lysins to get one step ahead of the bacteria. They can kill the bacteria within seconds of the contact [66]. E.g Nanogram quantities of lysin could reduce 10^7 *S. pyogenes* by >6 logs seconds after enzyme addition [67]. The effectiveness of lysin produced by bacteriophage C1 against group A *Streptococcus* (GAS) infections has been widely studied [68,69,70]. In another instance, different lysins were combined instead of phage cocktail and found all the 50 virulent strains of *Clostridium perfringens* killed [71,72]. This multilysin approach can reduce bacterial resistance effectively. Researchers have supported the idea of non-toxicity of lysin in mammalian cells in mice models [7,73,74] and its pre clinical trials have proved safe [75]. However, its little reduced activity was clearly seen in some studies with *Bacillus anthracis* and *S. pyogens* lysin treatment [76] because lysins were not recognized safe by the immune system. The PEGylation of lysostaphin, lysine against *Staphylococcus aureus* although reduced its activity but increased its resistance to antibody reaction making it important therapeutically [77]. Lysins were evaluated clinically for their efficacy against drug resistant bacteria.E.g., LysK lysin of staphylococcus phage K against MRSA and VRSA [78-80]. *Enterococcus faecalis* phage lysin PlyV12 has broad spectrum potential to eradicate Vancomycin Resistant *Enterococcus*, *Staphylococcus* and Group A, B and C *Streptococci* [81]. Unlike antibiotics, lysins are less likely to induce resistance in bacteria. This has been confirmed by intermittent lysin exposure and mutagenesis. No lysin resistant bacteria have been found so far [66].

Compliment to Antibiotics

The study on antibiotic-phage combination treatment has not been widely discussed. About half a century back, penicillin was combined with phages against Staphylococcus by Himmelweit. But research was more diverted to discovering new antibiotics and this area remained dormant. Now regaining the focus, synergistic effect of the antibiotic-phage combination has been examined in various studies. The temperate Phage σ-1 of family *Siphoviridae* and Ceftriaxone had shown synergistic effect against *P. aeruginosa* ATCC 9027 [82]. In another study, T4 phage and Cefotaxime synergy was examined for *in vitro* removal of biofilm produced by *E. coli* 11303 [83]. Pharmacological characteristics of the antibiotic determine whether it is effective with phage combination or not. There is unexplained but consistent finding that cell elongation by the antibiotic is necessary for synergism. Antibiotics can induce the phage lytic phase in the host bacterial cell [84]. E.g., A defined concentration of cefotaxime triggered the ΦMPF phage induction in *E. coli* cells. This synergistic relation can be proved effective in regulating the microbial community in oral biofilms where antibiotics alone cannot deter.

Future of Phage Therapy

The possible novel use of phages has been seen in various applications like agriculture and aquaculture. In fact the phage therapy in aquaculture is already in practice successfully today. Various companies make products from phages which help in improving food, water safety and even prepare defense against biological weapons. Researchers have been working upon the use the protein of phage rather than the whole phage. Lysins can be administered alone if isolated from phages. These peptides are specific at the sub species level and it has been found that if bacteria mutate to resist the lysins, it results in the death of bacteria. Omnilytics is a company, making products from phages to be used in agriculture e.g. they received permission from USDA to treat poultry with *Salmonella* contamination [85]. People's ideological beliefs do not support the medical evidences for the safety of phages like vaccines. The virtue of phage therapy should be realized for its acceptable practicality.

Conclusion

Phages can rightly be called the knight in shining armor, against the bacterial infections. Phages are self limiting as they die immediately after killing the target bacteria; have a specific nature and therefore do not harm humans or plants. They are rapidly modifiable to combat the newer bacterial threats. The isolation and identification of new bacteriophages capable to eliminate dental plaque colonizers can be considered a powerful approach for phage therapy of oral pathogenic bacteria in dentistry. As suggested by researchers, phage might prove suitable for controlling dental biofilms but still insufficient evidences are in this support. Various studies are being conducted to understand the cell-cell and genome-genome interaction which can help developing new strategies to combat the oral infections. The multidrug resistance eventually made researchers to ponder on the new interventions and phages in comparison to antibiotics that display a greater diversity in their mechanism of actions. Human body and all its parts are fragile. When a part of our body is damaged and destroyed, you cannot return it to the pre-accident status. Prevention is always better than treatment, and preventing most oral diseases is possible today and this is our expectation with the phage therapy.

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