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Bacteriophage Therapy Targeting Enterococcus Faecalis in Endodontics: A Systematic Review.

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KEYWORDS

Bacteriophage ·Biofilm ·Endodontics ·Enterococcus faecalis ·E.Faecalis ·Infection ·Novel ·Phage ·Root canals.

ABSTRACT:

The most prevalent bacteria encountered in secondary persistent endodontic infections is Enterococcus faecalis(E.Faecalis). The fundamental objective of any root canal treatment is to disinfect the root canal system in order to prevent microbial regrowth inside the root canal system. This makes E.Faecalis removal imperative. Because of the scarcity of therapeutic options for patients who are resistant to standard antimicrobials, there has been a renaissance of interest in (bacteriophage) treatment in recent years. The main aim of this review is to embark on a qualitative analysis of existing data on the antibacterial potential of bacteriophage therapy in the eradication of E. faecalis from the root canal system as a whole. Considering all the papers published till July 2023, a search of the databases PubMed, SCOPUS, and EBSCOhost was conducted. All of the English-language articles were included. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist guided the review process. After the data was extracted, the risk of bias was examined. Five Ex-vivo studies were included in the systematic review after assessing the distinguishing 46 studies according to the inclusion criteria.

Introduction

Phage therapy includes killing harmful microorganisms by using bacteriophages, viruses that solely target bacteria and are extremely host-specific.

Microbiologist Felix d'Herelle of the Institut Pasteur in Paris released a study in 1917 detailing the lysing of bacteria by an unnoticed microbe he called "bacteriophage." The first clinical use was seen in 1919 wherein a phage cocktail was given to a 12-year-old boy with sever dysentery. (1)

Nevertheless, phage therapy lost its popularity in western side due to reasons like improper storage and purification. The discovery of antibiotics transformed the way bacterial infections were treated and made them the accepted standard of care in a significant proportion of the world. (2)

Recent years have seen a resurgence in interest in (bacteriophage) treatment as a result of the dearth of therapeutic choices for patients who are resistant to traditional antimicrobials.(3)

Antibiotic effectiveness has reduced as a result of antimicrobial resistance and rising implanted device use, which increases the risk of biofilm-mediated infections.

Based on how they multiply virally, bacteriophages can be divided into two groups: lytic and lysogenic. The term lytic implies that when they infect a bacterial cell, they immediately take over the cell's replication machinery. The replicated phage particles accumulate within the host cell until they can no longer be harbored. The cell then ruptures, releasing straight away formed phages that can infect further bacterial cells.

Lysogenic bacteriophages have a distinct "lysogenic" life cycle from the lytic cycle. During the lysogenic cycle,

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phage DNA integrates into the genome of the host bacteria without triggering immediate cell lysis. A prophage is a phage with incorporated DNA. It integrates into the host cell's genome and replicates alongside host DNA during cell division. The phage remains inert or latent within the bacterial cell for an unknown amount of time in this integrated state. When exposed to stimuli such as UV radiation or chemicals, the prophage can become activated, extract itself from the host genome, and begin the lytic cycle. Lytic phages are to be used in bacteriophage therapy clinically. (4,5)

When a virus recognizes and binds permanently to a receptor (protein or sugar) on a bacterial cell's surface, lytic phage replication starts. In the cytoplasm of the bacterial cell, the phage deposits its genomic material. Proteins and genomes from the host are frequently repurposed to support phage replication. Redirecting host metabolism to the creation of new phage particles often marks the beginning of phage genome replication, transcription, and translation. After assembling new phage particles, the bacterial cell is lysed, allowing the newly reproduced phage particles to leave the cytoplasm and infect further bacteria that are vulnerable. (5-7)

Phage treatment has an assortment of perks over antibiotics; high specificity, ease of isolation, possibility of clinical improvement, single shot therapy, no residue left post the treatment, efficient biofilm destruction. (7)

Phage treatment is used to treat infections that are biofilm-mediated, multidrug-resistant, or both.(8) These conditions include recurring respiratory infections in patients with cystic fibrosis , osteomyelitis involving hardware, osteomyelitis of the skin and soft tissues, and chronic and recurrent infections like UTIs, rhinosinusitis, skin and soft tissue infections.(4) Phage treatment should generally only be used to treat infections if intolerance to or efficacy of antibiotic therapy has been established. Phage treatment need to be used primarily to treat infections that are accompanied with antibiotic resistance.

Phage therapy has so far been characterized in few studies as an irrigating solution during root canal therapy and also in one study as an intracanal medication in ex-vivo trials.

Studies show bacteriophages isolated against oral cavity bacteria such enterococcus faecalis, pseudomonas aeruginosa, and streptococcus mutans. The reason for this being the occurrence to particular bacteria, namely S. Mutans in dental caries, staphylococcus aureus in apical periodontitis, P. aeruginosa in primary persistent infections, and E. faecalis in secondary persistent endodontic infections. (6-8)

The primary aim of any root canal treatment is to achieve disinfection of root canal system in order to avoid regrowth of microorganisms inside the root canal system and therefore to prevent the failure of root canal procedure. (9)

Preferably, endodontic treatment should attain a microbial free root canal system, but with the current protocols, this is doubtlessly unfeasible. The failure of root canal treatment is caused by the remaining microbial flora in root canal treated teeth. The reason is majorly due to the limitations of current procedures to battle persistent intracanal Enterococcus faecalis (E.Faecalis) infection. In spite of diligent mechanical preparation of the root canal, infection still remains in the root canal. (9-12)

Recently, a few ex-vivo studies that tested the utility of bacteriophages in treating bacteria frequently seen in endodontic infections have been published. Each study described a phage that was effective against a specific type of endodontic target bacteria. However, no literature summarizing all of this data for phage therapy targeting E.Faecalis in endodontic diseases has been published yet. In order to give a more comprehensive view of the advancement of phage therapy in ex-vivo research, this systematic review aims to compile all the studies that have focused on eradicating E.Faecalis in endodontic diseases. This review is intended to help in paving the way for further phage treatment clinical trials.

MATERIALS AND METHODS

This systematic review was performed according to the transparent reporting of systematic reviews and meta-analysis (PRISMA) (Figure 1) with PICO format. Population (P) included the extracted teeth. Intervention (I) done was bacteriophage therapy. Comparison (C) was

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with a control group. Outcome (O) was measured in terms of biofilm reduction. Two independent authors performed the data extraction after selecting the articles relevant to the review. Disagreements between the authors were resolved through meeting with a third reviewer. Data collected was organized in tabular form consisting of authors, study year, journal name, type of study done, groups specified, bacteriophages used, sources of bacteriophages and outcome of the study. The study has been registered in open science framework with DOI xxxxx

Search strategy

The laid-out literature search was conducted on electronic reference databases such as Pubmed, Scopus, Cochrane, Web Of Science, Cochrane Central Register Of Controlled Trials (Central), and open grey (www.opengrey.eu) until June 2023, and unpublished literature was searched on clinical trial register (www.clinicaltrial.gov.in). The selected papers' reference lists were also searched utilizing cross referencing. Enterococcus faecalis E.faecalis, biofilm, infection, root canals, novel, bacteriophage, phage and endodontics are the mesh terms.(Table 1) These MeSH terms were combined with the Boolean operators AND and OR to create a relevant search strategy that could be employed in the above-mentioned databases to find articles that are relevant to the review question. As a result, these MeSH terms were chosen from the top of the Mesh tree hierarchy in order to accommodate sub-headings.

Selection criteria

INCLUSION CRITERIA

- isolation of -bacteriophages targeting e. Faecalis
- human or animal root canal models
- articles involving endodontic applications
- ex-vivo human root canal model

EXCLUSION CRITERIA

- articles targeting
- articles not involving root canal model
- genetically engineered bacteriophages
- Scoping reviews
- articles involving usage of bacteriophage in periodontic clinical applications

Table 1 Search strategy applied to current review

Database	Search strategy
PubMed	(((((((enterococcus faecalis) OR (E.faecalis)) OR (biofilm)) OR (infection)) OR (root canals)) OR (novel)) AND (bacteriophage)) OR (phage)) AND (endodontics)
Scopus	(((((((enterococcus faecalis) OR (E.faecalis)) OR (biofilm)) OR (infection)) OR (root canals)) OR (novel)) AND (bacteriophage)) OR (phage)) AND (endodontics)
EBSCOhost	(((((((enterococcus faecalis) OR (E.faecalis)) OR (biofilm)) OR (infection)) OR (root canals)) OR (novel)) AND (bacteriophage)) OR (phage)) AND (endodontics)

Screening and selection:

The search strategy's findings were uploaded into the online screening tool Rayyan, which allowed the writers to screen the articles based on title and abstract. The selection criteria were Ex-vivo studies that included isolation of bacteriophages targeting enterococcus faecalis and ex-vivo root canal models. The studies that included periodontal application, genetically engineered bacteriophages, scoping reviews, targeting bacteria other than enterococcus faecalis, in-vitro studies were excluded from this review. To identify any papers that may have been missed during the preceding processes, reference lists of relevant articles and gray literature (OpenGrey) were searched.

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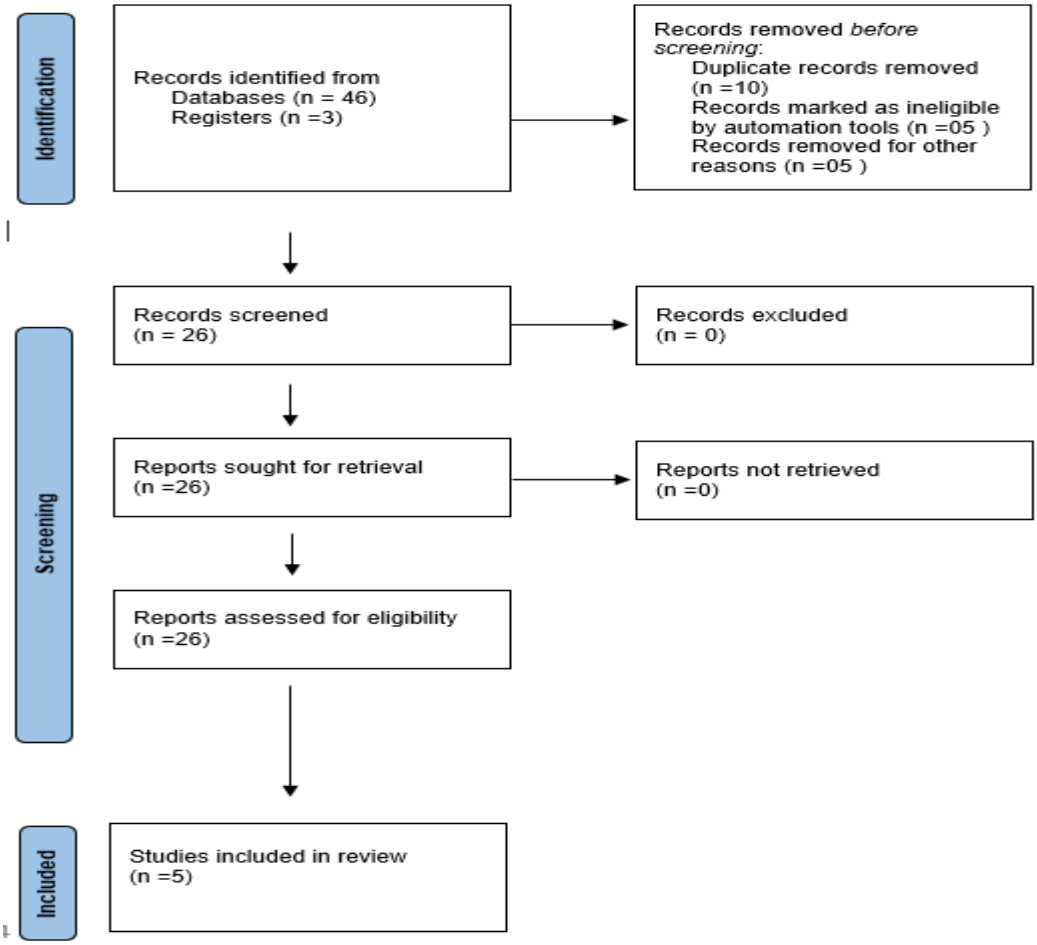
Figure 1 - Prisma flow diagram 2020 depicting the flow from identification to screening and selecting the articles for systematic review



Aims and objectives

This paper provides a comprehensive view of the advancement of phage therapy in ex-vivo research. We aim to compile all the studies that have focused on eradicating E.Faecalis in endodontic diseases.

Data extraction and quality assessment

The data was extracted and collected in a well-designed format as shown in (Table 2), which included the following: authors, study year, journal name, type of study done, groups specified, bacteriophages used, sources of bacteriophages and outcome of the study. Furthermore, the study parameters were recorded as follows: Phage morphology, Phage lytic activity in biofilm, Sensitivity of bacteria to antibiotics, phage genome sequencing and phage growth characteristics (burst size and latent period) shown in (Table 3).

RESULTS

The initial database search yielded 46 studies. Following a review of the titles and abstracts, 41 studies were eliminated. Following the exclusion of duplicates, three reviewers eliminated studies and reviews that were incapable of meeting the eligibility criteria. The obscurity in the studies was settled through discussion among the reviewers. This systematic review included the remaining five studies (14-18) which solely examined the efficacy of bacteriophage therapy on E. faecalis eradication in the tooth models.

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Table 2 : Characteristic features of the individual studies

YEAR	JOURNAL	TYPE OF STUDY	GROUPS	BACTERIOPHAGES USED	SOURCES	OUTCOME
2015	Applied and Environmental Microbiology	Ex – vivo study	Group 1 – untreated Group 2 – phage treated	EFDG1	Sewage water	EFDG1 was found to be an efficient killer of E. faecalis in an ex vivo model of root canal infection, where it reduced significantly the levels of E. faecalis.
2021	Microorganisms	Ex – vivo study	80 extracted teeth 4 groups Group A: saline irrigation as a negative control Group B: 200 µL of phage (108 PFU/mL) irrigation Group C: 2.5% NaOCl and 200 µL of phage (108 PFU/mL) irrigation Group D: 2.5% NaOCl and 17% EDTA irrigation	vB_ZEFP	Sewage water	Results of turbidity measurement revealed a greater ability of vB_ZEFP phage to reduce bacterial leakage from the root apex compared to other treatments. The enumeration of bacterial counts after 72 h confirmed the reduction in viable bacteria for the phage treated groups compared to conventional hypochlorite treatment. Moreover, vB_ZEFP phage proved to be effective when used in combination with hypochlorite allowing for the use of dual therapies.

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S.No	AUTHOR
1.	Khalifa et al
2.	Mohamed El-Telbany et al

TYPE OF STUDY	GROUPS	BACTERIO PHAGES USED	SOURCES	OUTCOME
Ex – vivo study	Group 1- phage treated Group 2- untreated	vB_EfaS_HEf13	Sewage water	Phage HEf13 appeared to be highly stable to a broad range of temperatures (4–60◦C) and pH values (3–12). Phage HEf13 is relatively more stable to high temperatures and strongly basic conditions than other phages. Comparative genome analysis revealed that the genome sequence of phage HEf13 is highly conserved with those of other tested Sap6virus phages. The host specificity of HEf13 against E. faecalis appears to be associated with the PIPEF.
Ex-vivo study	4 groups Group 1 : phosphate buffered saline (PBS) Group 2 :E.Feacalis Group 3 :phage only Group 4 :heat killed phage	SHEF2, -4, -5, -6, and -7, SDS-PAGE	Sewage water	Isolated phages (SHEF2, -4, -5, -6, and -7) possessed different host ranges, with SHEF2 having the broadest. It is showed that SHEF2 can significantly reduce biofilm formation of a range of sensitive

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S.No	AUTHOR	YEAR	JOURNAL
	Lee et al	2019	Frontiers in Microbiology
	Al-Zubidi et al	2019	Infection and Immunity

GROUPS	BACTERIOPHAGES USED	SOURCES	OUTCOME
			E. faecalis strains (3- to 10-fold) that were preformed (24 h) on polystyrene surfaces (mimicking catheters for example), as well as on a novel in vitro tooth cross-section biofilm mode. Systemic phage treatment after infection with E. faecalis dramatically decreased the mortality of zebrafish embryos and greatly improved their health during infection
Test group (n=9) Positive group(n=3) Negative group (n=3)	vB_Efa2921 2_2e and vB_Efa2921 2_3e	Sewage water	In the initial stage of the study, a higher potential of the 3e phage than the 2e phage against E. faecalis biofilms was observed. Despite the administration of a single dose of the lysate for 48 h, over a 50% reduction in the number of viable bacterial cells in the biofilm was observed. SEM analysis revealed the destruction of the treated biofilm.

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S.No	AUTHOR	YEAR	JOURNAL	TYPE OF STUDY
	Moryl et al	2022	Journal of clinical medicine	Ex – vivo study

GROUPS	BACTERIOPHAGE	SOURCE	OUTCOME
			In the initial stage of the study, a higher potential of the 3e phage than the 2e phage against E. faecalis biofilms was observed. Despite the administration of a single dose of the lysate for 48 h, over a 50% reduction in the number of viable bacterial cells in the biofilm was observed. SEM analysis revealed the destruction of the treated biofilm. One dose of the phages administered for 48 h mimicking clinical situations to maintain phages in between the clinical visits. Among all the investigated chemical agents, sodium hypochlorite influenced the phages the most. The investigated phages were quite stable in a wide spectrum of temperatures. Phages were stable at up to 50C, and their titers were similar to the positive control values. Phages were stable at up to 50C, and their titers were similar to the positive control values. Among all the investigated chemical agents, sodium hypochlorite influenced the phages the most

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S. No	AUTHOR	YEAR	JOURNAL	TYPE OF STUDY

Table 3 :Characteristic features of individual studies

Moryl et al	Al-Zubidi et al
Family : Herelleviridae Phage 2e head size 1897.83 ± 172.17 nm2 and phage 3e head size 4102.10 ± 757.82 nm2 . The head shape of phage 2e was rounder, and its diameter was 51.38 ± 4.01 nm. The tail was 195.20 ± 7.45 nm in length, while its width in half of the length was 8.50 ± 1.25 nm. The capsid of the 3e phage had a diameter of 75.50 ± 10.15 nm. Capsid of 3e had a length and width of 95.49 ± 15.39 nm and 18.75 ± 2.29 nm	Family : Siphoviridae Order : Caudovirales Polyhedral head shapes and noncontractile long tails ranging from 200 to 250 nm and polyhedral heads with diameters between 41 and 46 nm
From the untreated biofilms (positive control), an average of 1.4 × 107 CFU/mL was obtained, while in the bacteriophagetreated biofilms an average of 6.36 × 106 CFU/mL was found, resulting in a reduction of 54.6% of E. faecalis forming biofilms after treatment with phage 3e for 48 h	A significant reduction in bacterial numbers, as indicated by a reduction in detectable metabolic activity ; P 0.0001 [resazurin assay]) to approximately 7 103 bacteria

Al-Zubidi et al		Khalifa et al	Mohamed El-Telbany et al	Lee et al
The genomes of phages SHEF2,-4, and-5 revealed similar sizes of 41.7, 41.1, and 41.6 kbp, respectively. Each genome was assembled into one large contig with low read mapping coverage at the 5= and 3= ends and no clear edges at the ends of the contigs, suggesting circularity of terminally redundant permuted genomes. A high identity was found for both DNA and primary amino acid level of between 77 and 94%No putative integrase encoding genes were detected in the genome sequences, suggesting that the SHEF phages are likely to be lytic in nature.	**Phage morphology**	Family: Myoviridae Subfamily :Spounavirinae EFDG1 has a hexagonal head with a measured diameter of 98.71 8.88 nm and tail length of 118.05 6.87 nm	Family : podoviridae vB_ZEFP phage has icosahedral heads with short non-contractile tail. Phage vB_ZEFP had mean head dimensions of 43.4 ± 2.1 × 41.1 ± 1.8 nm while tail length was calculated to be 20 ± 0.5 nm	Family : Siphoviridae Order : Caudovirales Phage HEf13 has a prolate head with a long non-contractile tail. Head diameter is 38.4 ± 2.4 nm with a length of 95.6 ± 1.7 nm, and tail length is 135.2 ± 4.7 nm
A latent period of only 30 min (Fig. 4A), and a burst size of 9.3 PFU for this strain	**Phage lytic activity in biofilm**	EFDG1 reduced significantly and dispersed a 2-week-old 600-mm-width E. faecalis biofilm. Viable counts showed a 5-log reduction after exposure to EFDG1	The phage preparation significantly reduced the crystal violet stainable biofilm content compared to control ($p < 0.003$). The highest MOI of 10 produced the greatest reductions, exhibiting a significantly greater reduction compared to MOIs 0.1 and 1 ($p < 0.004$)	Phage HEf13 showed strong lytic activity against E.faecalis where clear-plaque formation was observed in the previous spotting assay, but not the strains where turbid-plaque formation was observed.

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	Khalifa et al	Mohamed El-Telbany et al	Lee et al	Moryl et al
phage genome sequencing	The largest and most significant contig contained 149,589 bp, assembled from 186,686 reads (96% of the reads), with a pairwise identity of 99% and a mean coverage of 295 81, which was predicted to be circular. The EFDG1 genome is AT-rich with a GC content of 37.1%, similar to that of its host E. faecalis (37.5%).	linear genomes of 18,454 bp (Genbank accession MT747434) with G + C contents of 32.8%.The genome sequence contained 28 open reading frames, of which 15 could be ascribed putative functions whilst 13 remained as hypothetical proteins.	Whole genome sequence analysis showed 57,811 bp in length with 95 predicted ORFs and one tRNA gene, a GC content of 40.03%, and the following nucleotide composition: G (10,737 bp, 18.57%), C (12,405 bp, 21.45%), A (15,765 bp, 27.26%), and T (18,904 bp, 32.69%).Average gene length was 532 bp, with a range of 116–3,992 nucleotides, and gene coding percentage was 87.4%. Among the 95 putative ORFs, 15 ORFs were on the positive strand while the other 80 ORFs were on the negative strand.	The phage vB_Efa29212_2e genome consisted of 41,351 nucleotides with a % G + C content of 34.83 and was assembled in a single contig. The phage encoded 75 putative open reading frames (ORFs) and zero tRNAs. The phage vB_Efa29212_3e genome was determined to be 141,162 bp in length with a 35.83% G + C content and was assembled in two contigs. The phage is predicted to encode 237 proteins and 9 tRNA genes.
phage growth characteristics (burst size and latent period)		Phage vB_ZEFP has a burst size of 110 per infected cell and a latent period of 10 min	Phage HEf13 had a short latent period (25 min) with a large burst size (approximately 352 virions per infected cell).	The latency period was calculated as 25 min for the 2e phage and 55 min for the 3e phage. The burst size was similar for both the 2e and 3e phages and was 138 and 127 viruses per bacterial cell, respectively.

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	Lee et al	Moryl et al	Al-Zubidi et al
	The lytic activity of phage HEf13 against E. faecalis on human dentin slice was examined by scanning electron microscope (SEM) analysis. When E.faecalis on dentin slice infected with phage HEf13 was visualized using a SEM, E.faecalis was completely eradicated.	The lytic activity of phages against E.faecalis examined using UV light visual analysis of the biofilms on bovine incisors. From the untreated biofilms (positive control), an average of 1.4 107 CFU/mL was obtained, while in the bacteriophage treated biofilms an average of 6.36 106 CFU/mL was found. SEM demonstrated a clear disruption of the bacterial cells after the phage therapy	The lytic activity of phage against E. faecalis on human root slices estimated using standard curves of emission.. A significant reduction in bacterial numbers, as indicated by a reduction in detectable metabolic activity (P< 0.0001 [resazurin assay]) to approximately 7 103 bacteria.

	Khalifa et al	Mohamed El-Telbany et al
Ex-vivo tooth model	Ex vivo two chamber bacterial leakage model of human teeth was used. No turbidity was observed in the phage-treated samples. Confocal laser scanning microscopy images of horizontal root sections showed no stained bacteria were seen in the phage-treated teeth.	Ex vivo two chamber bacterial leakage model of human teeth was used. The efficacy of their irrigant was evaluated by measuring the turbidity in LB. Viable counts recorded for phage treated groups: phage only (2 102CFU/mL), phage with NaOCl (3 104CFU/mL), and NaOCl and EDTA (3 106CFU/mL).

Assessment of risk of bias

The findings of the risk of bias evaluation are shown in Table 4. The evaluation was based on a set of criteria that included whether the experimental groups used extracted human teeth, bacterial inoculation verification, the presence of a control group, bacteriophage identification verified with genomic sequencing, and the presence of any conflict of interest. All of the studies revealed a low risk of bias.

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Table 4: Assessment of risk of bias

Risk of bias	Low	Low	Low	Low	Low
Was there any conflict of interest?	No	No	No	No	No
Was the bacteriophages identification verified with genomic sequencing?	Yes	Yes	Yes	Yes	Yes
Was a control group present ?	Yes	Yes	Yes	Yes	Yes
Was bacterial inoculation verified?	Yes	Yes	Yes	Yes	Yes
Were tooth cleaned and shaped before irrigation?	Yes	Yes	Yes	Yes	No
Were human teeth model used in the study?	Yes	Yes	Yes	No	Yes
	Khalifa et al	Mohamed El-Telbany et al	Lee et al	Moryl et al	Al-Zubidi et al

DISCUSSION

Antibiotic resistant E.faecalis strains have become a limitation for the use of antibiotics in clinical scenarios, paving a path for bacteriophage therapy as an alternative approach in the current scenario. However, there is limited literature on this topic. Studies have been conducted depicting isolation of different phages, characterizing them and evaluating their lytic activity against different bacteria and their strains.

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Bacteriophages isolated against the bacteria commonly encountered in dental field is again less in number. In this study, we reviewed 5 such studies wherein bacteriophages are isolated against Enterococcus Faecalis. E.Faecalis is a commonly encountered bacteria in persistent endodontic infections. We aimed to review these articles which have used ex-vivo tooth models as a method to simulate the similar oral environment.

Bacteriophages have been isolated from a variety of sources like sewage water, gut, saliva and bodily fluids of humans and animals, bioreactors as mentioned in numerous research thus far.(13) However, sewage water is the most often used and a source that is easier to extract. All the five studies mentioned in this review have a common source for bacteriophage isolation which is sewage water. The results of the studies have been assessed using a variety of parameters such as phage morphology, lytic activity, genome sequencing, and growth characteristics. Phage morphology indicated head diameters ranging from 38 to 52 nm and tail diameters ranging from 20 to 192 nm. (14-18)

A bacteriophage's ability to specifically target specific bacterial species is fundamental to its effectiveness. This host specificity guarantees the phage's survival and growth in a hostile microbiological environment. Bacterial populations are not homogenous; they are made up of a multitude of strains, each of which has a distinct genetic make-up. Herein lies the intriguing twist: a bacteriophage may exhibit a broader host range within the same bacterial species, capable of infecting multiple strains. The phage's striking capacity to change along with its bacterial hosts is demonstrated by this flexibility that is termed as host range specificity. The isolated bacteriophages were tested against different strains of E.faecalis for determining their host range. Lee et al trial showed the broadest host range with 12 strains of E.faecalis and 14 clinical isolates.(16) Whereas other trials like Al Zubidi et al (17) trial showed 9 of 13 strains tested, Telbany et al (15) trial 10 out of 13 strains of E.faecalis, Khalifa et al (14) trial shows bacteriophage potential against 11 E.faecalis strains. There is one trial (Moryl et al trial), where phages were tested against monospecies of E.faecalis making it a limitation. (18)

Temperature is a crucial element in order to maintain phage stability. Phages are often adapted to thrive at specific temperature ranges, typically mirroring the conditions of their natural bacterial hosts. The highest temperature range was seen in Al Zubidi et al trial, the phage was inactivated when phage suspension was treated at 80°C for a time duration of 45 minutes. They observed a 7-log fold decrease in PFU/ml. (17) In Moryl et al trial, 3e phage was not detected after heating to a temperature 80°C whereas phage 2e had a titre of 65 PFU/ml even after incubation at 80°C. (18) Lee at al trial showed bacteriophage HEf13 had temperature range of only 4-60°C. 16 On the other hand, pH is an important consideration. Phage adaptation to pH is similar to adaptation to temperature in typical bacterial settings. The pH scale spans the spectrum of extremely acidic to extremely alkaline. The pH range of these studies did not differ much ranging from 3-12, making it clear that the bacteriophages isolated are stable in extreme acidic and alkaline environments they can encounter in oral cavity. (14-18)

Due to the immediate implications for dental operations and patient care, it is imperative to comprehend how irrigating solutions used in endodontics affect bacteriophages. There has been some controversy over this in relation to two studies (Moryl et al (18) and Telbany et al (15)). It is essential to make sense of how these substances interact with bacteriophages since it affects their viability and efficacy of phage-based treatments for tooth infections. In Moryl et al trial, the influence of commonly used irrigating solutions on bacteriophages was determined. They found that concentrations of sodium hypochlorite above 3% kills bacteriophages. Whilst for EDTA and CHX also had negative impact on the bacteriophages, it appeared as a drop in the titres depending on the time of action and agent type. Moryl et al recommended to use phage therapy after the conventional disinfection protocol is completed. (18) In contrast, Telbany et al trial studied two groups which had sodium hypochlorite along with phage as a part of the irrigating solution and found that there was decrease in the biofilm concluding the presence of bacteriophages despite the combination. (15)

The effectiveness of bacteriophages to target and eliminate bacterial populations inside the root canal system can be carefully evaluated using an ex-vivo tooth model. In order to evaluate bacterial eradication, the model can help in optimizing treatment protocols, particularly phage concentration, contact time, and

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administration strategies since it simulates oral environment. Two of the studies reviewed, used tooth model as a two-chamber bacterial leakage model for assessing the activity of bacteriophages (Khalifa et al and Telbany et al) and found reduction in the turbidity indicating bacterial lysis.(14,15) Two others of the studies (Lee at al (16), Moryl et al(18), Al Zubidi et al(17)), checked the lytic activity of phages using SEM analysis wherein the bacterial eradication was evident. In one study (Al Zubidi et al), it was determined using standard curves of emission (17). The biofilm assays showed a range of 5-7-fold log reduction in the studies reviewed. (14-18)

These studies indicate the future of bacteriophage as a potential agent to replace or use as an adjunct along with antibiotics in clinical dentistry. Bacteriophages have been discovered to have a substantial impact on a variety of microbiological habitats. Phage therapy provides a number of advantages compared to antibiotics (19). The persistence of E.Faecalis bacteria in the root canal during therapy indicates the need for superior defense against them.(20) Several studies have shown that bacteriophage therapy on endodontic biofilms can help eradicate even vancomycin-resistant E. faecalis.(21-23) However, there are certain limitations when it comes to the clinical use of bacteriophages. There is lack of standardization for the dosage and also lack of consistency for phage susceptibility testing. This is extremely needed to ensure bacteriophages are against bacterial pathogen only. More clinical trials are needed to acquire enough data and knowledge to determine its standardization.

CONCLUSION

In accordance to the extensive data acquired from these studies, bacteriophages can be utilized as an alternative or in conjunction to traditional antibiotics. Their potency, however, is regulated by a variety of biological (burst size, latency time, host range) and physical parameters (sensitivity to pH, temperature, and other irritants). Due to growing need for alternative or supplemental anti-infectives in addition to conventional antibiotics, phage therapy has reemerged as an effective option for refractory infections in recent years. It is essential to standardize dosage, frequency of dosing, and duration for therapy. When contemplating bacteriophage for clinical application, there is an insufficient understanding regarding interactions with antibiotics, interactions with other varieties of phages, and the emergence of phage resistance.

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