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The microbial community of persistent endodontic infection and the use of root canal disinfectants against E. faecalis: In vitro and in vivo review

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Abstract

In cases of unsuccessful endodontic treatment, E. faecalis is commonly detected and remains popular among the scientific community for in vitro and in vivo research. The nature of the bacteria's predominance is dependent on the difficulty in cleaning the apical portion of the tooth and E. faecalis' capacity to grow deep into the dentinal tubules. The purpose of this review is to gather current research on root canal disinfection against E. faecalis. In this literature review, four databases were searched: PubMed, EBSCOhost, ScienceDirect, and Google Scholar, with explicit inclusion and exclusion criteria. The compiled review shows that a predominant multi-microbial community within a persistent root canal infection include Porphyromonas gingivalis, Fusobacterium nucleatum, and Enterococcus faecalis. E. faecalis produces moderate biofilms and causes infection through its virulence factors such as esp, cylA, ace, gelE, asa, and efaA. Several disinfecting agents and approaches have been explored for their capacity to eradicate E. faecalis and penetrate the dentinal tubules. Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) gel eliminate E. faecalis and other resistant microbes such as Staphylococcus aureus, and Escherichia coli. Combing NaOCl irrigation with the following: Photon-induced photoacoustic streaming, passive ultrasonic irrigation, and diode laser irradiation, eradicates E. faecalis. The current research has shifted towards nanoparticles against E. faecalis because of its dissolution capacity, size, and antimicrobial efficacy. E. faecalis resists calcium hydroxide through its proton pump mechanism, but it is susceptible to Ca(OH)2 when applying a proton pump inhibitor or chlorhexidine.

Keywords: Enterococcus faecalis, root canal irrigants, apical periodontitis; nanoparticles, persistent infection, dentin

Introduction

Root canal treatment is carried out if the patient has primary or secondary/persistent infection within the tooth. The objective is to preserve the tooth and surrounding tissues and eradicate microbes to prevent reinfection. Primary root canal infection involves inflammatory pulp tissue of untreated teeth, whereas a secondary or persistent infection is a result of root canal treatment failure and microbial resistance [1]. The microbial invasion of the root canal and its virulence factors lead to the development of apical periodontitis. The reason is that the virulence factors allow the attachment of microbes to the walls of the root canal via surface proteins; it forms biofilms, releases toxins, and allows certain microbes to interact synergistically with others. Successful root canal treatment relies on substantial removal or complete eradication of microbes [2]. Persistent apical periodontitis is a common manifestation of endodontic failure. In such cases, the most prevalent bacteria species is Enterococcus faecalis, a Gram-positive cocci bacteria that is anaerobic as well as facultative [3]. It tolerates a high alkaline environment because of its proton

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pump and withstands low nutrient conditions [4]. E. faecalis can resist disinfection techniques and antimicrobial agents, resulting in incomplete elimination [5]. Conventional irrigation requires chemicals that eradicate organic and inorganic components such as debris and microbes. A perfect irrigant has a strong antiseptic effect on a multi-microbial community, gets rid of the smear layer, eliminates endotoxins, and is compatible with the host. For decades, sodium hypochlorite (NaOCl), chlorhexidine (CHX), calcium hydroxide and ethylenediaminetetraacetic acid have remained the common antimicrobials used in endodontics. Several studies explored natural alternatives to the standard irrigants because of cytotoxic effects, allergies, and other health issues, and these potential substitutes include bee glue, tea tree extract, turmeric, neem, and nanoparticles. Bee glue, known as Propolis, has been reported to have the same effect as sodium hypochlorite against E. faecalis but is inferior to chlorhexidine. Chitosan nanoparticles appear as a promising antimicrobial tool because microbes are less likely to develop resistance to it than standard antibiotics. Currently, zinc oxide nanoparticles have the potential to inhibit E. faecalis between the dentine and sealer boundary [6]. Additionally, silver nanoparticles have been reported to have the same antibacterial effect as chlorhexidine and sodium hypochlorite against E. faecalis. Moreover, research has shifted towards laser activation and photodynamic stimulation on root canal irrigants because of their efficacy [7]. Besides, the problem with using a standard irrigation system is that the irrigating tips are unable to reach the apical root canal region, and as a result, it poses challenges with disinfection. Moreover, needle, sonic and ultrasonic irrigation remains sufficient in cleansing the root canal depending on the irrigant used [8]. As E. faecalis develops resistance against disinfecting agents and approaches, exploring other options for eliminating the bacteria is crucial. This review article will explore the bacteria and resistance in failed root canal treatment. The virulent genes of E. faecalis will be observed as to how it correlates with biofilm formation and resistance. The purpose of this article is to summarize the latest developments in root canal disinfection against E. faecalis. The disinfecting components that will be discussed are sodium hypochlorite, chlorhexidine, nanoparticles and calcium hydroxide.

The Bacterial Community and Resistance in Root Canals presenting Apical Periodontitis combined with Endodontic Treatment Failure

Through Polymerase chain reaction (PCR) amplification and 16S rRNA gene sequencing, the bacterial community in the case of persistent apical periodontitis has been identified to be diverse and region-dependent. Different species of bacteria tend to form biofilms in regions such as the intraradicular and extraradicular areas. The most prevalent bacterial phyla in both areas include Actinobacteria, Bacteroidetes, Proteobacteria, and Firmicutes, whereas the most common bacterial genera include Streptococcus, Fusobacteria, Morganella, Porphyromonas, and Bifidobacterium. The difference between intraradicular and extraradicular biofilms is that the extraradicular biofilm exhibits higher β-diversity and a higher bacterial richness than microbes present in the root canal. However, persistent apical periodontitis combined with a sinus tract influences the bacterial composition of both regions. Additionally, sinus tracts are channels in which fluid drains from the point of infection [9]. It is the connection of the infected apical site to the extraoral or intraoral region. Apical periodontitis combined with a sinus tract and persistent infection comprises higher bacterial levels of Prevotella, Tannerella, Treponema, Porphyromonas, Phocaeicola, and Eubacterium than in patients without a sinus tract. Enterococcus and Microbacterium were most abundant in cases without a sinus tract in both extraradicular and intraradicular regions [1]. Overall, the microbiology of each region is a multimicrobial community containing gram-positive and gram-negative bacteria. Apical periodontitis combined with endodontic failure accounts for Enterococcus faecalis, Porphyromonas gingivalis, and Fusobacteria nucleatum being the most resistant bacteria during reinfection and intraradicular biofilm formation [10]. E. faecalis is primarily identified in persistent infection because it is resistant to antibiotics and disinfecting approaches, and it has the capacity of occupying the dentinal tubules. Further, P. gingivalis, a Gram-negative anaerobic bacteria, is primarily found in pockets around the tooth with other bacteria such as Tannerella forsythia and Treponema denticola. When these bacteria combine with P. gingivalis, they can create aggregates upon bacterial interaction and become more pathogenic and resilient. Lastly, F. nucleatum is a Gram-negative anaerobic bacteria mainly found on the external surface of root ends. In addition, P. micra can interact with F. nucleatum and P. gingivalis, which creates a more pathogenic and resistant community [11,1]. Generally, bacteria invade the root canal and cause infection through microleakage in treated teeth [12]. However, root canal reinfection is also due to bacterial resistance to chemo-mechanical approaches. Barbosa-Ribeiro et al. used Nested PCR to examine the microbial community in root canals with persistent infection during various stages of endodontic retreatment. The results showed that Aggregatibacter actinomycetemcomitans, P. gingivalis, E. faecalis, and F. nucleatum were the most common bacteria detected in root canals before chemo-mechanical intervention. Upon chemo-mechanical intervention, P. gingivalis and E. faecalis bacterial counts were slightly reduced when applying treatments of NaOCl, EDTA, and sodium thiosulfate, whereas F. nucleatum and A. actinomycetemcomitans were moderately reduced. Additionally, intracanal medicine reduces the bacteria level further and is usually used following the chemo-mechanical technique. In a previously treated tooth with persistent apical periodontitis, chemo-mechanical intervention is sufficient in eradicating T. denticola, T. forsythia, Actinomyces israelii, Gemella morbillorum, and Actinomyces naeslundii. Although Porphyromonas endodontalis, Parvimonas micra, and Prevotella tannerae are also within the root canal, they are susceptible to intracanal medicine when combined with chemo-mechanical intervention. P. gingivalis, F. nucleatum, and E. faecalis also resist intracanal medicine such as Ca(OH)2 mixed with Chlorhexidine

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gel [11]. For the most part, E. faecalis is generally found in the root canal of unsuccessful endodontic treatment cases, influenced by its genetic characteristics.

Virulence Genes of Enterococcus Faecalis in cases of Failed Endodontic Treatment

By enhancing its capacity to elude the immune system, bind to host tissue, and secrete substances that increase biofilm growth, E. faecalis virulence genes contribute to root canal-treated tooth infection. The most common virulent components detected in such a scenario include esp, cylA, ace, gelE, asa, and efaA [13,14]. Francisco et al. studied twenty-five failed root-canal treated teeth that manifested apical periodontitis. The results indicated that the most prominent virulent factor detected in all samples was cylA and efaA, followed by gelE in most samples. Furthermore, cylA is responsible for developing enterococcal cytolysin, which functions as a lantibiotic [15]. In contrast, efaA poses a significant health risk as an endocarditis antigen. GelE correlates with apical periodontitis, producing gelatinase, which causes bone resorption and biofilm production. For the bacteria to stick to the root canal walls, the esp gene produces surface proteins. Similarly, the ace gene functions the same as the esp gene, except it produces collagen-binding proteins. Lastly, asa (aggregation substance) increases bacterial stickiness to the extracellular matrix, such as dentine. Overall, E. faecalis virulence genes produces moderate biofilm formation [16]. The existence or lack of the CRISPR-cas loci is another aspect that affects the prevalence of E. faecalis. "Clustered regularly interspaced short palindromic repeats" convey prokaryotes' adaptive reaction to external factors. The bacteria use the CRISPR-cas loci to obtain a specific sequence, protecting it from mobile elements. Based on a study conducted by Tong et al., E. faecalis with CRISPR-cas loci were more frequent than without in cases of root canal reinfection. Furthermore, E. faecalis lacking CRISPR-cas loci will produce more biofilm and may exacerbate periapical lesions. It tends to develop resistance to root canal irrigants such as 2% CHX and MTAD than those with the CRISPR-cas loci. However, E. faecalis with or without CRISPR-cas is susceptible to 5.25% NaOCl, decreasing biofilm formation [17].

Irrigation with Sodium hypochlorite and Chlorhexidine against Enterococcus Faecalis

Although the process is concentration- and time-dependent, using CHX and NaOCl solution as irrigants decreases the presence of E. faecalis in the root canal. Sun et al. created a model that resembled the dentinal tubules, inoculated it with E. faecalis, and tested the antimicrobial effect of NaOCl. A key finding was that as time progresses, the penetration depth and bacterial reduction increase when irrigating with either 1%, 2.5%, or 5% NaOCl solutions. It is essential to note the side effects of NaOCl and the treatment duration when selecting the ideal concentration. A greater concentration of NaOCl solution will dissolve in the root canal faster and achieve the most significant bacterial reduction. At about thirty minutes, 5% NaOCl showed the most extraordinary dissolution capacity and antimicrobial effect within the dentinal tubule model at a penetration depth of 500μm [18]. Another study revealed that E. faecalis resistance occurs when NaOCl solution is utilized at a concentration less than 3% [3]. Chlorhexidine irrigation has a significant potential for decreasing E. faecalis in the tooth. Dewiyani et al. cultured E. faecalis from endodontically treated teeth with persistent infection and tested the antimicrobial effect of CHX with a concentration of 1%, 0.2%, and 2% at varying durations. The most significant reduction of E. faecalis colonies occurred with 0.1% CHX; regardless of concentration, the ideal duration is between thirty minutes to an hour for a maximum antimicrobial effect. Additionally, the irrigant becomes ineffective in reducing E. faecalis over more extended periods [19]. Further, NaOCl and CHX solution delivered via an EndoVac irrigation system exhibits a greater capacity in eliminating microbes than conventional needle irrigation [20]. Another way for reducing E. faecalis biofilms is using NaOCl and CHX gel as an intracanal medicament. A recent experiment explored the role of a "Clorox bleach pen" and 2% CHX gel on E. faecalis by agar plating. The most significant inhibitory effect of E. faecalis is in plates with NaOCl gel rather than CHX gel, and in general, both gel types are potent antimicrobials. However, utilizing NaOCl gel has the drawback of potentially having cytotoxic effects, whilst using 2% CHX gel does not offer an adequate barrier in the intracanal region. Furthermore, the two gel types effectively combat resistant microbes including Staphylococcus aureus and Escherichia coli [21].

Potential Irrigation Methods with Sodium Hypochlorite against Enterococcus Faecalis

Laser-activated irrigation, diode laser irradiation, and passive ultrasonic irrigation all work to increase the antibacterial effect of NaOCl. Sodium hypochlorite and laser-activated irrigation may eradicate E. faecalis, notably when applying “photon-induced photoacoustic streaming” (PIPS). A study done in-vitro shows that laser-activated irrigation lessens E. faecalis biofilms in the apical, middle, and coronal areas more than conventional needle irrigation. In contrast to conventional irrigation paired with high NaOCl concentrations, Wen et al. showed that PIPS irrigation with 1% and 2% NaOCl safely removes E. faecalis better. Additionally, there was no notable difference in eradicating the bacteria between Laser activated 2% and 5.25% NaOCl. Although PIPS irrigation potentially removes E. faecalis biofilms safely with low concentrations of NaOCl (ideally with 2% solution), the limiting factor of this study was that Wen et al. used an artificial model lacking the dentinal tubule portion [22]. Another possible technique to effectively remove E. faecalis in an infected root canal is applying Diode laser irradiation followed by NaOCl irrigation. This combination decreases the bacteria by 99.7% on root canal surfaces. Dai et al. used this method on primary teeth inoculated with E. faecalis and examined the effects using scanning electron and confocal microscopy. The experiment demonstrates that Diode laser irradiation combined with NaOCl irrigation opens

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the dentinal tubules, removes the smear layer, and eradicates the bacteria. By reducing the smear layer, the dissolving ability of NaOCl increases, further enhancing the antibacterial effect. Although the diode laser tip could not reach the apical region, it had no effect on eradicating E. faecalis, where there were no differences observed between the apical, central, and coronal regions [23]. Furthermore, activating NaOCl gel or solution with passive ultrasonic irrigation (PUI) eliminates E. faecalis. Hasna et al. inoculated twenty lower premolars with E. faecalis and used PUI activation on 2.5% NaOCl solution and 3% NaOCl gel. The effect of passive ultrasonic irrigation on both irrigants significantly decreases E. faecalis biofilms and endotoxins such as LPS and LTA, which inhibits pathogenicity. Additionally, irrigating the root canal with 2.5% NaOCl solution (without PUI activation) does not entirely eradicate the endotoxins and biofilms of E. faecalis [24]. It is significant to notice that PUI improves the sodium hypochlorite gel and solution's dispersion into the dentinal tubules, improving the antibacterial efficacy. Another study measured CFUs and showed that activated 2.5% sodium hypochlorite solution with PUI eradicated E. faecalis in extracted human root canals [25]. Overall, NaOCl remains as the ideal irrigating solution for endodontic therapy.

Latest Developments of Chlorhexidine against Enterococcus Faecalis

Recently chlorhexidine has been developed into a nanoparticle or nanoemulsion root canal disinfectant. It appears to be an effective endodontic irrigant against E. faecalis because of its particle size and disinfection properties, making it a better antibacterial agent than the standard Chx. HCl solution [26]. Abdelmonem et al. inoculated twelve single-rooted adult teeth (maxillary, mandibular incisors, and premolars) with E. faecalis and then irrigated the samples with 1.6% Chx. HCl nanoemulsion. This nanoemulsion formulation exhibits an excellent drug delivery rate (about 2 minutes), a short emulsification rate, and a particle size of 12.18nm, allowing the substance to enter the dentinal tubules quickly. Activated 1.6 % Chx. HCl nanoemulsion via passive ultrasonic irrigation demonstrates a more effective removal or eradication of E. faecalis than the standard chlorhexidine solution. Additionally, it will result in a lower debris surface area within the root canal [27]. Furthermore, PLGA submicron particles have been discovered to carry chlorhexidine into the dentinal tubules. The particles are usually loaded with chlorhexidine, calcium, and phosphorus (PCaPC). An in vitro experiment revealed that PLGA submicron particles containing chlorhexidine had no inhibitory effect on osteoblastic cells such as MC3T3-E1 cells. The particles can safely infiltrate the dentinal tubules via an ultrasonic activator, making disinfection successful. Chlorhexidine positively affects PLGA submicron particles by shrinking the diameter and increasing the zeta potential. As a result, a positive zeta potential improves the probability of PCaPC infiltration to the dentinal tubules. A CFU test revealed that PCaPC exhibited an excellent antibacterial effect against E. faecalis [28]. The advantage of using PCaPC is that the release of calcium and phosphorus improves dentine integrity compared to common intracanal irrigants such as EDTA, which does the opposite. Another experiment found that chlorhexidine packed in PLGA nanoparticles exhibits low cytotoxicity, an effective antimicrobial effect, and excellent drug delivery in dentinal models and extracted teeth [29]. Root canal disinfection with PLGA (filled with calcium, chlorhexidine, and phosphorus or just chlorhexidine) seems promising; nevertheless, much more research is needed on activating the particle by methods other than ultrasonic cleaning.

Developments in Nanoparticle Disinfectants against Enterococcus Faecalis

By generating reactive oxygen species and harming lipids, proteins, DNA, and RNA, nanoparticles kill resistant microbes by penetrating the dentinal tubules [30]. Firstly, the antibacterial and anti-inflammatory qualities of ethanolic propolis particles make them useful. It comprises many antioxidants such as phenolic acids and flavonoids [31]. Propolis nanoparticles are usually created by propolis ultrasonication and are characterized by good dentinal penetration and high levels of quercetin, kaempferol, and pinocembrin flavonoids. These flavonoids have antiseptic effects in which it destroys cell wall and membrane structures. Moreover, an in vitro assessment with CFU and confocal laser scanning microscopy found that propolis nanoparticles were most efficient in eliminating E. faecalis biofilms for ten minutes at a dentinal depth of 200µm and 400µm. The results demonstrate that propolis nanoparticles of 100 µg /ml and 300 µg /ml are as equivalent to 2% CHX, 6% NaOCl and superior to saline in reducing resistant E. faecalis strains [32]. Moreover, chitosan is a cationic substance with antimicrobial properties, and lately, chitosan and propolis were combined as a medicament. The chitosan-propolis nanoparticle is characterized by high zeta potentials, small particle size, and high encapsulation efficacy. According to in-vitro study done by Parolia et al., chitosan-propolis nanoparticles (CPN) used as an intracanal medicament are just as effective as Ca(OH)2 and chlorhexidine in eliminating E. faecalis. As with most root canal disinfectants, chitosan-propolis nanoparticles are concentration and time-dependent. Parolia et al. conducted a CFU assessment on 240 inoculated teeth (resistant E. faecalis strains) and compared different intracanal medications at a dentinal depth of 200µm and 400µm. The experiment confirmed that within 24 and 72 hours, a high concentration of CPN (250 µg/ml) is superior in removing E. faecalis to 2% chlorhexidine gel, varying concentrations of propolis and chitosan alone, calcium hydroxide, and saline solution. However, within a week, the results showed that a lower concentration of CPN and 2% chlorhexidine gel were just as effective as using high concentrations of CPN [33]. Propolis and chitosan-propolis nanoparticles have an overall cumulative antibacterial effect and should be thought of as an intracanal medicament between visits [34]. Selenium nanoparticles exhibit a negative zeta potential, resulting in an exceptional distribution in the root canal and an antimicrobial effect on positively charged

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cell components. In addition, selenium nanoparticles also reduce protein and carbohydrate constituents of E. faecalis. An antibiofilm test found that biosynthesized selenium nanoparticles exhibited the most significant reduction of E. faecalis biofilms, subsequently 5.25 % sodium hypochlorite, 2% chlorhexidine, and calcium hydroxide [35]. Moreover, Afkhami et al. performed a CFU test and confirmed that silver nanoparticles' activation with “passive ultrasonic irrigation and photon-induced photoacoustic streaming,” increases its ability to eradicate E. faecalis. The antimicrobial impact of silver nanoparticles on E. faecalis is similar to that of 5.25% NaOCl and equal to calcium hydroxide [36]. These positively-charged nanoparticles function by inhibiting the "transcription and expression of ribosomes and inducing cell lysis [37]. "Lastly, copper nanoparticles was found to kill both resistant aerobic and anaerobic bacteria such as Pseudomonas putida, Cutibacterium acnes and E. faecalis, possibly by contact via the discharge of ions in a localized area. Sacoto-Figueroa et al. confirmed that copper nanoparticles exhibit low values of “minimum inhibitory concentration and minimum bactericidal concentration” against resistant strains of E. faecalis. Because of their spontaneous effect, copper nanoparticles may be an alternative to antimicrobial agents in treating chronic root canal disease [38].

Advances with Calcium Hydroxide as an Intracanal Medicament

Calcium hydroxide, which has disinfectant and mineralizing qualities, is typically administered between appointments as a root canal medicament. By producing a high pH environment, it destroys microbes, however it has been shown that Candida albicans and Enterococcus faecalis are resistant to calcium hydroxide [2]. Currently, there are several potential formulations or adjuvants with calcium hydroxide that may be able to eradicate resistant microbes. Using a timekill kinetics test, Sy et al. verified that calcium hydroxide paste combined with low doses of chlorhexidine had the ability to destroy resistant E. faecalis strains without changing the physical features of Ca(OH)2. Additionally, Nunes et al. revealed how the addition of calcium hydroxide and 2% chlorhexidine on a chitosan base greatly decreased E. faecalis and Staphylococcus aureus biofilms on an agar diffusion test. It is important to note that these combinations are compatible because of the “increase in chitosan crystallinity by calcium hydroxide and presentations of pseudoplastic behavior” [39]. Another key finding of this study was that metallic salts, chitosanmetalloid, and metalloid nanoparticles, particularly copper, zinc, and silver, fail to exhibit a sufficient antibacterial effect against resistant E. faecalis strains when combined with calcium hydroxide [40]. Also, Fahim et al. reported that Ca(OH)2 nanoparticles are equally effective as Ca(OH)2 medicaments in eliminating resistant E. faecalis strains, except that the Ca(OH)2 nanoparticles will exhibit greater pain control in patients with persistent root canal infections [36]. Moreover, E. faecalis has a proton pump mechanism that enables the bacteria to resist Ca(OH)2 close to a pH of 11.1 but becomes susceptible when paired with a proton pump inhibitor [4]. A few in vitro studies investigated the effects of calcium hydroxide supplemented with proton pump inhibitors such as pantoprazole, lansoprazole, and omeprazole on E. faecalis. These inhibitors are commonly used in treating gastric diseases and may have the potential to function as an intracanal medicament. Divakar et al. conducted a CFU test and confirmed that calcium hydroxide formulated with pantoprazole exhibited more significant E. faecalis reduction than calcium hydroxide alone [41]. Anija et al. discovered that lansoprazole combined with chlorhexidine and calcium hydroxide would significantly eliminate E. faecalis more than calcium hydroxide alone, chlorhexidine mixed with calcium hydroxide or lansoprazole combined with calcium hydroxide [42]. Lastly, omeprazole and calcium hydroxide mixtures considerably eliminate E. faecalis but were shown to be inferior to a triple antibiotic paste [43]. Overall, this favorable outcome is because proton pump inhibitors may permanently affect the proton pump mechanism in E. faecalis, creating a high pH within the bacteria. This mechanism allows either calcium hydroxide or chlorhexidine to attack the bacteria directly. The antimicrobial efficacy of the various types of inhibitors when paired with calcium hydroxide and the safety of using proton inhibitors during chronic root canal infection requires much more research. The studies above used low concentrations of proton inhibitors, which may be ideal if they were to be used in clinical trials.

Concluding Remarks

A multi-microbial community exists in cases of persistent apical periodontitis combined with endodontic failure. Both intraradicular and extraradicular biofilms differ in their bacterial makeup, with extraradicular biofilms having a higher bacterial richness. The presence of a sinus tract affects both biofilm regions; Enterococcus and Microbacterium were primarily present in cases without a sinus tract. The most resistant bacteria are Enterococcus faecalis, Porphyromonas gingivalis, and Fusobacterium nucleatum, possibly due to bacterial interaction and pathogenic genes. Many studies consider E. faecalis the most resistant bacterial species detected in a failed root canal treatment combined with apical periodontitis. E. faecalis colonizes deep areas of the dentinal tubules, which poses a problem when disinfecting the root canal. The virulence factors of E. faecalis impact secondary root canal infections and enable the bacteria to form moderate biofilms. The use of sodium hypochlorite solutions ranging from 3% to 5.25% has still been successful in eliminating Enterococcus faecalis in the root canal. It is imperative to consider options because of their cytotoxic effects at higher doses. Treatment with chlorhexidine solution appears to be time-dependent and effective against E. faecalis in low doses. These two irrigants administered in the form of a gel may increase their antibacterial efficacy. So far, photon-induced photoacoustic streaming and passive ultrasonic irrigation appear to increase the antibacterial effect of sodium hypochlorite by causing the complete elimination of E. faecalis biofilms. Based

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on in vitro studies, nanoparticles appear successful in getting rid of E. faecalis due to their optimal disinfecting qualities, including their particle size penetrating deeply into the dentinal tubules, excellent drug delivery rate, and biocompatibility. To overcome E. faecalis resistance against calcium hydroxide medicament, adjuvants such as chlorhexidine, chitosan bases, and proton inhibitors increase the potential of eradicating E. faecalis in persistent root canal infections. Future studies are needed to evaluate E. faecalis` virulence factors or genetics responsible for endodontic failure combined with apical periodontitis. The limiting factor of this review article is that some of the in vitro studies tested disinfectants against cultured non-resistant E. faecalis, whereas the others used resistant strains. Additionally, there are limitations to using E. faecalis as a baseline when testing potential antimicrobials against resistant bacteria. Such studies need to evaluate antimicrobials against a multi-microbial community. Exploring alternatives to the standard irrigants will combat resistant microbes and help avoid root canal treatment failure. Nanoparticles appear highly effective in eliminating resistant bacteria and should be evaluated through clinical trials in cases of root canal retreatment.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

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Ethical approval

Ethics committee approval is not required.

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