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The Influence of enterococcus faecalis as A dental root canal pathogen on endodontic treatment- A review

Krishna Prasada L[1], Priyanka Yadav[2]

[1] HOD, Department of Conservative Dentistry and Endodontics, KVG Dental College and Hospital, SULLIA, Dakshina Kannada, Karnataka, India

[2] Department of Conservative Dentistry and Endodontics, KVG Dental College and Hospital, SULLIA, Dakshina Kannada, Karnataka, India

Abstract

Background: The main cause of endodontic failure is the persistence of microorganisms that cause an in traradicular or extratradicular infection and that become resistant to disinfection measures. The objective of this review is to identify the microbiota associated with endodontic failure, as well as the reasons why these microorganisms are capable of surviving basic disinfection measures.

Material and Methods: Search of scientific articles in the databases Google Scholar, PubMed with the following keywords "Endodontic Infections", "Endodontic Microbiology", "Endodontic Failure", "Enterococcus Faecalis", "Endodontics Retreatment" was carried out. Case reports and articles with publication date prior to 2009 were not included in this review.

Results: Most authors highlight *E. faecalis* as the main microorganism associated with endodontic failure, nevertheless there are recent studies that isolate, to a greater extent, other bacteria such as Fusobacterium nucleatum and Propionibacterium.

Discussion: These microorganisms have in common the following proprieties, which make them able to escape the disinfection measures: the ability to form a biofilm, to locate in areas unreachable to root canal instrumentation techniques, synergism, the ability to express survival genes and activate alternative metabolic pathways.

Keywords: Endodontic infections, endodontic microbiologic, endodontic failure, enterococcus faecalis, endodontic retreatment

Introduction

Enterococcus faecalis was formerly classified as part of the group D *Streptococcus* system– is Gram- positive, commensal bacterium inhabiting the gastrointestinal tracts of humans [1]. Like other species in the genus *Enterococcus*, *E. faecalis* is found in healthy humans and can be used as a probiotic. The probiotic strains such as Symbioflor1 and EF 2001 are characterized by the lack of specific genes related to drug resistance and pathogenesis [3]. As an opportunistic pathogen, *E. faecalis* can cause life-threatening infections, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity. *E. faecalis* has been frequently found in reinfected, root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases. Re-infected root canal-treated teeth are about nine times more likely to harbor *E. faecalis* than cases of primary infections [2].

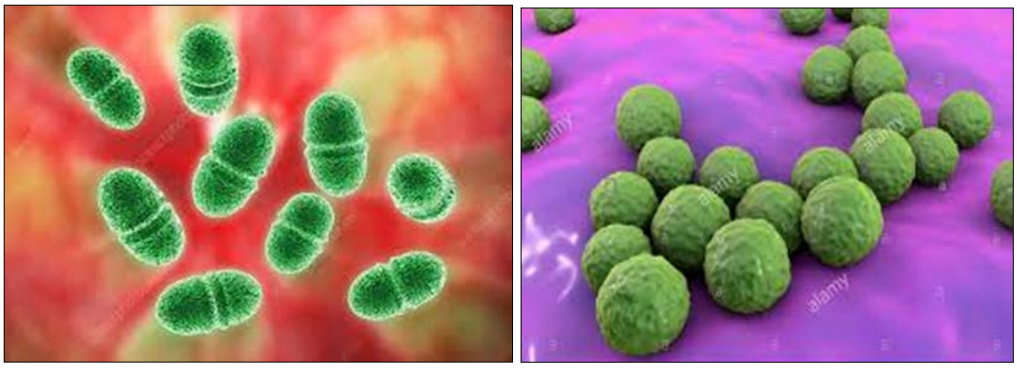

Fig 1

Factors that may contribute to a persistent periradicular infection after root canal treatment include intraradicular infection, extraradicular infection, foreign body reaction, and cysts containing cholesterol crystals [1]. It is generally believed that the major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth [1, 2]. Unlike primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms involved in secondary infections are composed of one or a few bacterial species [2].

Enterococcus faecalis is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora [1].

Enterococcus faecalis is an anaerobic gram-positive coccus that normally commences in the human oral cavity, gastrointestinal tract, and vagina because it has demonstrated good adaptation to such environments with rich nutrient and low oxygen levels and complex ecology. Several studies showed that E. faecalis was found more in cases of failed endodontic treatment than in cases with primary infections. Among all the reported cases with post-endodontic therapy pain and infection, it has been observed that E. faecalis is the most commonly found, with high prevalence values reaching up to 90%. Among all cases with primary endodontic infection, E. faecalis was more likely to be associated with asymptomatic cases than with symptomatic ones [3].

Enterococcus faecalis V583 Complete genome

E. faecalis is an extensively evaluated biological indicator. Several laboratory studies tested the susceptibility of E. faecalis to endodontic treatment, which showed high resistance of E. faecalis to antimicrobial agents. Furthermore, E. faecalis can survive in very harsh environments, with poor nutrient supply and high alkaline pH reaching up to 11.5. The capacity of E. faecalis for growing as a biofilm on root canal walls and as a mono-infection in treated canals without synergistic support from other bacteria makes high resistance to antimicrobial agents a very resistance pathogen to root canal treatment [2].

Many studies that were found to discuss the association between E. faecalis with different forms of periradicular diseases. However, few studies have investigated the E. faecalis associated with endodontic treatment. Finally, the aim of this review was to review all recently published studies concerning Enterococcus faecalis as a dental root canal pathogen that causes endodontic failure [5].

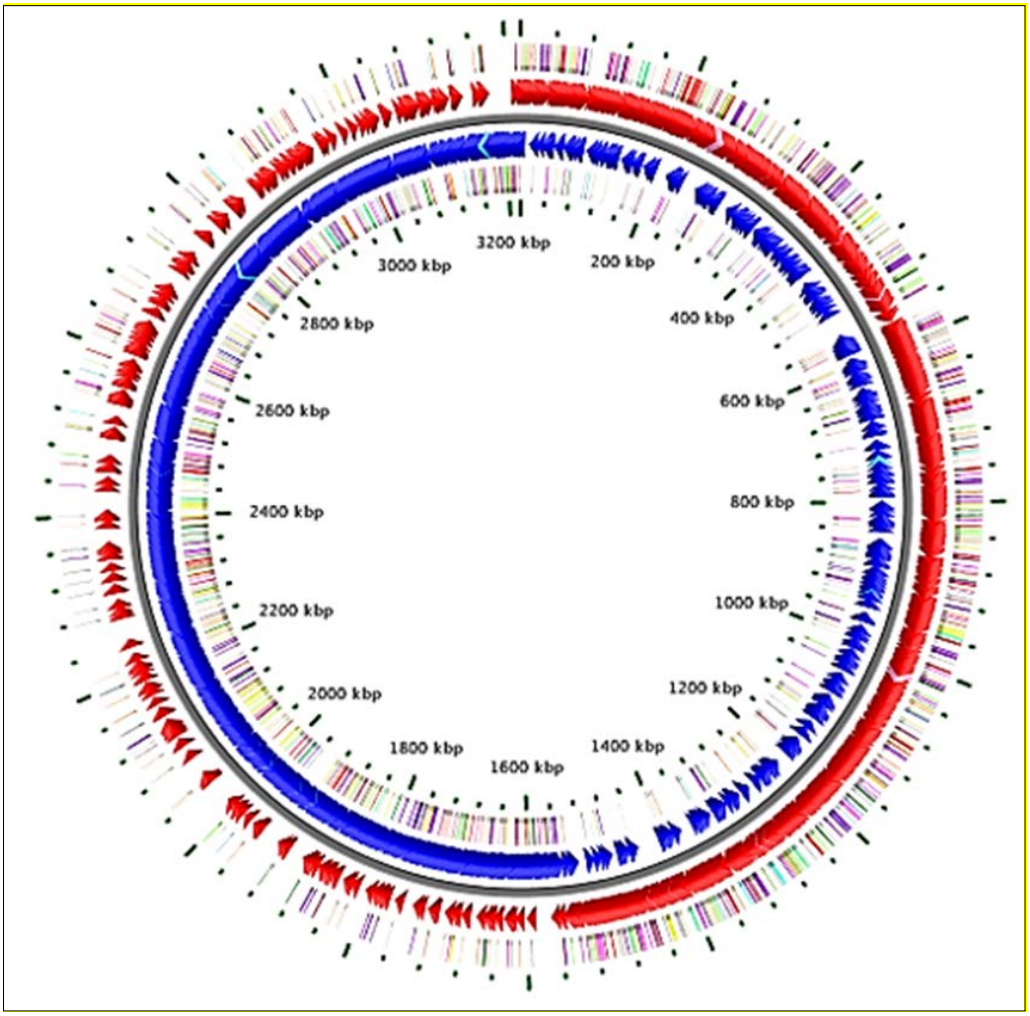

Fig 2

E. faecalis Characteristics and Strains

Enterococci are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen [5]. *Enterococcus* species live in vast quantities, 108 colony-forming units (cfu) per gram of feces] in the human intestinal lumen and under most circumstances cause no harm to their hosts. They are also present in human female genital tracts and the oral cavity in lesser numbers. They catabolize a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine, and many keto acids. *Enterococci* survive very harsh environments including extreme alkaline pH (9.6) and salt concentrations. They resist bile salts, detergents, heavy

metals, ethanol, azide, and desiccation. They can grow in the range of 10 to 45°C and survive a temperature of 60°C for 30 min [6].

There are currently 23 *Enterococci* species and these are divided into five groups based on their interaction with mannitol, sorbose, and arginine. *E. faecalis* belongs to the same group as *E. faecium*, *E. casseliflavus*, *E. mundtii*, and *E. gallinarum.* These five species form acid in mannitol broth and hydrolyze arginine; however, they fail to form acid in sorbose broth [6, 7].

E. faecalis can normally be identified by further testing with arabinose, tellurite, and pyruvate. *E. faecalis* is arabinose negative and except for some atypical variants, is the only member of the group to utilize pyruvate and to tolerate tellurite. More recently, molecular techniques have been developed that have the capability to rapidly and accurately identify the *Enterococcus* species. Techniques involving DNA-DNA hybridization, sequencing of the 16S rRNA genes, whole-cell protein (WCP) analysis and gas liquid chromatography of fatty acids have been used for taxonomic purposes(7).

Attention has been turned towards *Enterococci* since the 1970s when they were recognized as major nosocomial pathogens causing bacteremia, endocarditis, bacterial meningitis, urinary tract, and various other infections. Sources of the bacteria in these infections have been reported as originating from the hands of health care workers, from clinical instruments, or from patient to patient. Studies have shown that nosocomial infections are not caused by the patient's own prehospitalization flora. Enterococcal infections now account for roughly 12% of nosocomial infections in the United States with the majority of those being caused by *E. faecalis* (greater than 80%) and *E. faecium* being responsible for the majority of the remaining infections. Studies show *E. faecalis* is able to translocate from the root canal system to the submandibular lymph nodes of germ-free mice, suggesting this route of infection may play a role in the pathogenesis of opportunistic infections in patients [8] . Enterococcal urinary tract and soft tissue infections are generally treated with single drug therapy, often with penicillin or vancomycin. There is emerging evidence of vancomycin resistance among *Enterococcus* species and routine use of previously standard recommendations for treatment of enterococcal infections can no longer be expected to provide optimal results [7, 8].

Table 1

Author	Study Design	Year	Conclusion
Schirrmeister JF et al	*In-Vitro*	2009	In all teeth with Parvimonas micra and Dialister invisus, F.nucleatum and S.moorei were found. Moreover, members of additional different genera were detected delivering bacterial compositions that have been not described yet.
Narayanan LL et al	*In-Vitro*	2010	The well filled root canal offers the microbial flora of small, dry nutritionally limited space. Thus, we should obtain a better understanding of the characteristic and properties of bacteria and their biofilms along with the environmental changes to enhance success
Rocas IN et al	*In-Vitro*	2012	The findings call into questions the status of E.faecalis as the main pathogen and suggest that other species can be candidate pathogens associated with persistent or secondary endodontic infections.
Endo M et al	*In-Vitro*	2013	The great majority of taxa found in post-treatment samples were gram positive bacteria
Del Fabbro M et al	*In-Vitro*	2014	The picture emerging from the review is that extraradicular infections is likely a multi factorial disease that requires further systematic investigation using standard techniques
Jhajharia K et al	*In-Vitro*	2015	The most common endodontic infection is caused by the surface associated growth of microorganisms
Ran S et al	In-Vitro	2015	A number of the regulated genes may be useful candidates for the development of new therapeutic approaches for the treatment of E.faecalis infections
Pereira RS et al	In-Vitro	2017	Further studies are necessary to elucidate the role of these microorganisms in endodontic treatment failures
OH Donmez	In-Vitro	2019	The data of the induced and non-induced fabclavine promoter exchange mutans clearly show the fabclavine derivative are bioactive compounds responsible for the bactericidal effect.
Lee D et al	In-Vitro	2019	These results suggest that phage HEF13 has the characteristics of a lytic phase and is a potential therapeutic agent for the treatment or prevention of E.faecalis associated infectious diseases.
Ghorbanzadeh	In-Vitro	2020	All three disinfection methods were effective for the partial elimination of E.faecalis biofilm, but convention chemomechanical debridement and light activated disinfection was significantly more efficacious in decreasing both mature and immature biofilms

Enterococcal Invasion of the Root Canal During or After Treatment

Few data exist in the literature to support or contradict the theory that enterococci could enter the root canal system during or even after endodontic treatment. It has been surmised from longitudinal studies that culture reversals with the sudden occurrence of enterococci after the initial treatment session could be due to leakage through the temporary filling (Sjogren et al. 1991, Sundqvist et al. 1998). However, again these observations were too infrequent to allow generalization. The only study so far that has addressed the correlation between the clinical occurrence of enterococci and other enteric bacteria and the root canal seal was based on samples that were sent in by private practitioners, accompanied by a questionnaire regarding the treatment steps that had been performed (Siren et al. 1997) [5, 6]. As it turned out, there was a significant positive correlation between the occurrence of the target species and the number of visits, as well as leaving the canal unsealed between treatment sessions. Studies on the

occurrence of enterococci in root filled teeth (Engstrom 1964, Molander et al. 1998, Kaufman et al. 2005) also suggest that these bacteria could have entered the canal system after the root filling procedure [6].

Enterococci are able to induce and maintain an apical lesion as monoinfectants (Fabricius et al. 1982a, Ferrari et al. 2005). On the other hand, they have been found more frequently in filled canals without a radiographic lesion compared with counterparts with a lesion (Kaufman et al. 2005). It appears rather unlikely that enterococci from a primary infection survived treatment and root filling procedures only in the coronal aspect of the canal and not in the apical portion (which would, with a high degree of certainty, result in a lesion). Hence, it may be assumed from everything that we know at this point that enterococci in filled root canals without apical rarefaction are likely to have entered after the root was filled. In this context, it should be stated that almost one general shortcoming in endodontic articles is the lack of information regarding the restoration and the history of the teeth under investigation. Depending on the coronal restoration, filled root canal systems may invariably have been exposed to the oral cavity at one point during treatment. This is especially the case with teeth that receive an indirect restoration which undergo a phase of temporization. However, clinical studies that investigated microbial leakage around temporary fillings or crowns are few, and the involved microorganisms have not been identified (Beach et al. 1996). Taken together, the little evidence currently available points in the direction that enterococci enter the root canal system at some time after the root canal treatment has been initiated. The source of infection for pulpless root canals appears to be the oral cavity with its currently more than 700 identified bacterial species or phylotypes [6, 7].

Survival and Virulence Factors

E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses. *E. faecalis* is able to suppress the action of lymphocytes, potentially contributing to endodontic failure.

E. faecalis is not limited to its possession of various virulence factors. It is also able to share these virulence traits among species, further contributing to its survival and ability to cause disease. These factors may or may not contribute to the innate characteristics of *E. faecalis* to cause disease. Because *E. faecalis* is less dependent upon virulence factors, it relies more upon its ability to survive and persist as a pathogen in the root canals of teeth [7].

E. faecalis overcomes the challenges of survival within the root canal system in several ways. It has been shown to exhibit widespread genetic polymorphisms. It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which help it bind to dentin. It is small enough to proficiently invade and live within dentinal tubules. It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available. Once available, the starved cells are able to recover by utilizing serum as a nutritional source. Serum, which originates from alveolar bone and the periodontal ligament, also helps *E. faecalis* bind to type I collagen [8]. *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days. *E. faecalis* is able to form a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non biofilm producing organisms [9].

Calcium hydroxide, a commonly used intracanal medicament, has been shown to be ineffective at killing *E. faecalis* on its own, especially when a high pH is not maintained. *E. faecalis* is able to survive intracanal treatment with calcium hydroxide because *E. faecalis* passively maintains pH homeostasis. This occurs as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity. Also *E. faecalis* has a proton pump that provides an additional means of maintaining PH homeostasis. This is accomplished by "pumping" protons into the cell to lower the internal pH [10].

At a pH of 11.5 or greater, *E. faecalis* is unable to survive. However, as a result of the buffering capacity of dentin, it is very unlikely that a pH of 11.5 can be maintained in the dentinal tubules with current calcium hydroxide utilization techniques. Studies using the dentin powder model have shown that the presence of dentin has an inhibitory effect on various concentrations of root canal medicaments including calcium hydroxide, sodium hypochlorite, chlorhexidine, and iodine potassium iodide. Diverse components of dentin including dentin matrix, type-I collagen, hydroxyapatite, and serum are responsible for altering the antibacterial effects of these medicaments [11].

The capability of E. faecalis to survive in up to 6.5% concentrated sodium hypochlorite (NaOCl), sodium dodecylsulfate, hydrogen peroxide, heat, hyperosmolarity, ethanol, and both acidity and alkalinity. Adding to these characteristics, E. faecalis can establish extra radicular infection by secreting toxins directly or through the induction of inflammation indirectly. Additionally, it can gain and transfer extrachromosomal elements and encoding virulence traits, which help colonize and compete with other bacteria, resist host defense mechanisms, and produce pathological changes. Furthermore, E. faecalis can make a well-organized biofilm that can resist the healing process. It can induce hydroxyapatite precipitation in a mature biofilm to form a calcified biofilm [12].

In a study done in 2006 by Stuart C *et al*., they added to the characteristics of E. faecalis the capacity of this microorganism to use serum from dentin and the periodontal ligament (PDL) as a source of nutrition. As a result, this serum ensures the survival of E. faecalis and allows the bacteria to adhere to and invade the dentinal tubules the virulence factors of E. faecalis to establish endodontic infection and the induction of periradicular inflammatory response [12, 13]. The most important virulence factors are aggregation substance, bacterial surface adhesins, sex pheromones, lipoteichoic acid, the production of extracellular superoxide, and the release of two important lytic enzymes (gelatinase and hyaluronidase). The aggregation substances are plasmid-encoded adhesive substances, which support the exchange of the plasmid between the donor and recipient bacteria during the conjugation process between the bacteria. They also promote the binding between E. faecalis and various eukaryotic cells, enforce the adhesion of the bacteria to types I and IV collagens, and act as a protective factor against host neutrophils [13]. Possessing these aggregation substances makes microorganisms such as E.

faecalis qualified enough to stimulate the release of α tumor necrosis factors (TNF-α) by macrophages and the release of γ interferon (INF-γ) and β tumor necrosis factors (TNF-β) as a result of the proliferation of T cells induced by the bacteria. As a consequence, the release of TNF will lead to bone resorption while the release of INF-γ will stimulate the secretion of more hydrogen peroxide and superoxide anions, resulting in damage to the cells and tissues. On the other hand, surface adhesins add to the virulence of E. faecalis by adhering the bacteria to many essential substances, such as abiotic surfaces (to form biofilm), other species of bacteria (for exchanging genes and nutrients), collagen fibers, human serum, and dentinal tissues [14].

E Faecalis in Other Dental Infections

E. faecalis is a normal inhabitant of the oral cavity. The prevalence of *E. faecalis* is increased in oral rinse samples from patients receiving initial endodontic treatment, those midway through treatment, and patients receiving endodontic retreatment when compared to those with no endodontic history. *E. faecalis* is associated with different forms of periradicular disease including primary endodontic infections and persistent infections. In the category of primary endodontic infections, *E. faecalis* is associated with asymptomatic chronic Periradicular lesions significantly more often than with acute periradicular periodontitis or acute periradicular abscesses. *E. faecalis* is found in 4 to 40% of primary endodontic infections [6]. The frequency of *E. faecalis* found in persistent periradicular lesions has been shown to be much higher. In fact, failed root canal treatment cases are nine times more likely to contain *E. faecalis* than primary endodontic infections. Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% [11]. The wide range of *E. faecalis* prevalence among studies may be attributed to different identification techniques, geographic differences, or sample size .In some cases, *E. faecalis* has been found as the only organism (pure culture) present in rootfilled teeth with periradicular lesions. The majority of these studies have been carried out using culturing techniques; however, polymerase chain reaction (PCR) is currently a more predictable method for detection of *E. faecalis*. This method proves to be faster, more sensitive, and more accurate than culturing methods. It has enabled researchers to detect bacteria that were difficult, and in some cases impossible, to detect. When compared to detection of *E. faecalis* by culturing (24-70%), *E. faecalis* has been found at consistently higher percentages (67-77%) when a PCR detection method is used. An optical spectroscopy-based method has also been studied as a way to detect *E. faecalis* activity [14, 15].

The detection rate of E. faecalis in saliva ranges from 18.8%– 40.5%. Some researchers think that the prevalence of E. faecalis in root canals referred for endodontic retreatment is associated with the presence of E. faecalis in saliva. E. faecalis is more prevalent in persistent infections than in primary chronic periapical periodontitis reminds us that incautious root canal treatment might be the cause of persistent infections of E. faecalis. Theorganism may enter the root canal system at some time after the root canal treatment has been initiated [12, 15]. It is the most common enterococcal species found in the oral cavity occasionally, and patients with periodontitis appear to harbor more E. faecalis than their counterparts with a healthy periodontium. Therefore, the oral cavity might be the origin of E. faecalis entering root canal systems, and the use of a rubber dam to insure good isolation is necessary to prevent the introduction of E. faecalis during endodontic treatment. Proper periodontal treatment or tooth cleaning before root canal therapy in order to control periodontal inflammation may reduce the amount of E. faecalis. E. faecalis is ubiquitous in many food products, such as cheese and milk derivatives. Niches such as root canal lumens and dentinal tubules may favour their survival and long-standing local infection. In this way, for patients with good oral hygiene, bacteria inside the root canal could be the consequence of a coronal colonization after contaminated food ingestion so a good coronal sealing is also important in the prevention of E. faecalis [12]. Vidana *et al* also think E. faecalis found in persistent endodontic infections are probably not derived from the patient's own normal microbiota, which indicates an exogenous origin. Consequently, the use of the aseptic technique including the disinfection of obturation materials might be critical in preventing the introduction of E. faecalis. It is reported that 19.4% of gutta-percha cones removed from their packaging contained bacteria on the surface, 5.25% sodium hypochlorite and 2% chlorhexidine are the most common disinfectants for gutta-percha cones. Povidone-iodine and peracetic acid have also been studied for rapid disinfection [10, 16].

There appears to be a consensus that the primary causes of pulpal necrosis and the subsequent occurrence of apical periodontitis are dental caries and its sequelae. The progressive infection of dentine eventually leads to microabscesses in the pulp and tissue breakdown mediated by proteolytic enzymes (Langeland 1987, Gusman *et al*. 2002, McLachlan et al. 2004). It has been known for some time that, as a carious lesion progresses into the dentine close to the pulp, the microbial infiltrate therein resembles the one in primary root canal infections (Edwardsson 1974). This was recently reconfirmed in a study using contemporary molecular biology methods (Martin et al. 2002) [18].

Enterococci in Primary Root Canal Infections

It has been reported that 20% of the samples isolated from primary teeth with primary root canal infections. Although these teeth presented primary infections without a retreatment evaluation, it is important to highlight that the presence of E. faecalis in primary teeth of children will add knowledge concerning the early presence of this pathogen in the oral cavity. Only a few authors have investigated the microorganisms present in root canals of primary teeth, and the available records are from studies using microbial culture and molecular methods. da Silva et al. and Ruviere et al. evaluated primary teeth using culture and checkerboard DNA–DNA hybridisation, respectively, and did not find E. faecalis in their studies. In his study, study, E. faecalis was investigated in primary teeth with primary infection because the role of this pathogen is unclear. Only two studies have been carried out to evaluate the presence of E. faecalis in paediatric patients with necrotic pulp. Cogulu et al. using culture and PCR methods, reported that E. faecalis was present in 18% of primary (from 45 samples) and 26% of permanent teeth (from 38 samples). The authors showed that both culture and PCR methods are sensitive to detect E. faecalis in root canals. In 2008, using PCR, these authors found that this pathogen was the most prevalent

species (20%) in 79 primary teeth with primary infection [18]. Even if not present in caries, enterococci may theoretically still enter the necrotizing pulp space at an early stage. Not all primary endodontic infections are caused by caries (Abbott 2004). Many teeth contain cracks (Ratcliff et al. 2001), and thus it is conceivable that enterococci found in primary root canal infections entered via that route rather than dentinal tubules in carious teeth. Furthermore, inadequate coronal restorations with open margins have been linked to the occurrence of apical periodontitis (Kirkevang et al. 2007). However, the issue of studying primary root canal infections is difficult, especially when trying to determine how and when the microorganisms that eventually lead to pulpal breakdown entered the endododontic system. One of the most prominent problems in this context is again contamination from saliva or plaque from the outer tooth surface (Mo¨ller 1966) [13].

Because root canal infections are usually polymicrobial (Sundqvist 1994), and the most common invaders of the endodontium can be found in other sites of the oral cavity, it is impossible in the laboratory to discern between the microorganisms that were actually present in the root canal at the time of sampling and contaminants. As already highlighted by Engstrom, many of the teeth that harbour enterococci in the root canal system also show positive enterococcal growth on outer tooth surfaces (Engstrom 1964). False positive results are even more likely when PCR is used to detect specific DNA sequences of microorganisms suspected to be present in the root canal (Nair 2007) [11]. Hence, a meticulous sampling technique including disinfection of the tooth and the access cavity with the respective sterility checks from both sites is a prerequisite to yield meaningful results. Whilst such protocols have been validated and published for both culture and molecular methods (Moller 1966, Ng et al. 2003), relatively few studies have complied with these guidelines. In studies on the culturable microbiota of primary endodontic infections with proper sterility checks of the access cavity, enterococci have usually been found in a rather low proportion of infected canals or not at all (for review, see Portenier et al. 2003). This is in contrast to the high frequency of enterococci encountered in filled root canal systems associated with treatment failure (Molander et al. 1998, Sundqvist et al. 1998, Peciuliene et al. 2000, Hancock et al. 2001). On the other hand, studies employing DNA-DNA hybridization or PCR techniques (without checking the access cavity) found a somewhat higher occurrence of E. faecalis in primary root canal infections as compared to those investigations, which were performed using culture techniques (Siqueira et al. 2002, Pirani et al. 2008). Nevertheless, the occurrence of E. faecalis in a PCR assay was still significantly lower in teeth containing necrotic pulps compared with root filled counterparts with apical lesions (Pirani et al. 2008). Contrary to these findings, a relatively high occurence of E. faecalis in primary root canal infections has been reported when nested PCR was used to identify this taxon and was compared with a conventional culture technique: E. faecalis was identified in 82% vs. 4%, respectively (Gomes et al. 2006) [17].

The authors concluded that E. faecalis could be present in numbers below the detection level for culturing or that they could be in a viable but non-culturable state. On the other hand, the authors also conceded that 49/50 of their sampled teeth had coronal leakage. Consequently, proper decontamination of the access for PCR was a difficult, if not impossible task, and enterococcal DNA could thus have originated from sources outside the root canal [15].

Furthermore, nested PCR is notoriously difficult to perform; the paper describes only sequencing a single band from the gels – this would afford little confidence in the band identification. Moreover, it is well-known that E. faecalis is one of the easiest species to culture, and it does not enter a viable but non-culturable state (Bogosian et al. 1998). As already suspected from the low or non-existing occurrence of enterococci in caries, members of the genus Enterococcus probably exist relatively rarely in primary root canal infections. To the best of our knowledge, only one study so far has specifically targeted the difference in the microbiota recovered from necrotic root canals between teeth with an exposed and counterparts with a non-exposed pulp space. However, in that investigation, cracks were not taken into consideration. Furthermore, the incidence of E. faecalis was merely 0/45 vs. 2/43 in pulps from teeth that had a visible contact to the oral cavity compared to those that did not, respectively (Chu et al. 2005) [17]. These numbers are again too low to allow any conclusions. The low occurrence of enterococci in primary root canal infections makes one possible pathway for the colonization of necrotic pulp tissue that cannot be terminally excluded unlikely: anachoresis, i.e. the transfection of microorganisms via the blood stream (Tunnicliff & Hammond 1937). Animal experiments with high numbers of microorganisms injected in the blood stream have shown that a colonization of necrotizing pulp tissue is possible (Gier & Mitchell 1968, Tziagas 1989). In the case of enterococci, it is conceivable that these bacteria could enter the bloodstream from the large intestine and then enter necrotic areas of a tooth with terminal pulpitis. However, as indicated above, the low occurrence of enterococci in primary root canal infections and the low likelihood of any bacterium to colonize necrotic pulp tissue via the blood stream make the pathway of direct oral entry more likely [17, 18].

E. faecalis And Diabetes

Endodontic medicine has evolved with an increasing number of reports describing the association between periapical inflammation and systemic diseases. Studies suggest an association between apical periodontitis (AP), root canal treatment (RCT), and systemic conditions such as diabetes mellitus (DM), tobacco smoking, hypertension, coronary heart disease (CHD), osteoporosis, bleeding disorders, chronic liver disorders, etc., Several studies have reported a higher prevalence of periapical lesions, delayed periapical repair, greater size of osteolytic lesions, greater likelihood of asymptomatic infections, and poorer prognosis for root-filled teeth in diabetic patients [19].

Patients with diabetes have documented alterations in immune functions and may also have pathogenic endodontic microbial flora which makes them susceptible to more severe periradicular disease. DM was found to be associated with significantly reduced endodontic treatment outcome of teeth with preoperative infections, suggesting that diabetes may serve as a disease modifier. It predisposes to chronic inflammation, diminishes tissue repair capacity, and causes a greater susceptibility to infections. The relationship between oral health and diabetes has been extensively reported in the literature. The success rate of RCT is 95% which is reduced to 68% in immuno compromised patients.

Diabetes Mellitus (DM) compromises the immune response aggravating periapical chronic inflammation and impairing bone turnover and wound healing, increasing the prevalence of persistent Apical Periodontitis, greater size of the osteolytic lesions, greater likelihood of asymptomatic infections, and worse prognosis for root filled teeth. Diabetics have reduced likelihood of success (10%–20%) of endodontic treatment in cases with preoperative periradicular lesions [18, 19].

On the other hand, recent studies have found that a poorer periapical status correlates with higher HbA1c levels and poor glycemic control in type 2 diabetic patients. *E. faecalis* is found in high prevalence, levels and proportions in infected root canals, and from nonhealing cases in DM. This is the most common species recovered in over one-third of the canals of root-filled teeth with persisting periapical lesion. Regardless of the thorough chemome chanical preparation and three dimensional obturation, bacteria can persist in the complex anatomy of root canal space. Thus, the ability of intracanal medicament to restrain or eliminate residual bacteria and prevent reinfection may play an increasingly important role in achieving and maintaining a higher success rate of RCT [18]. Calcium hydroxide is the most commonly used and studied intracanal medication which was introduced in dentistry by Herman in 1920. Its antimicrobial effect is chiefly related to the release and diffusion of hydroxyl ions (OH^-) which injures cytoplasmic membrane and interferes cell metabolism. Propolis, a resinous product rich in flavonoid, is ten times less cytotoxic than calcium hydroxide and has a distinguished antibacterial, antifungal, antiviral, immunomodulatory, and antioxidant effect. Recent studies have reported that propolis is more effective against resistant microorganisms and is biocompatible. Moxifloxacin is a new fluoroquinolone with expended spectrum of activity, including anaerobes and Gram-positive organisms, especially the multiresistant ones. Moxifloxacin has been found to be one of the most active antibiotics against *E. faecalis* with the lowest MIC50 and MIC90. Triple antibiotic paste (TAP) containing metronidazole, ciprofloxacin, and minocycline has been reported to be a successful regimen in controlling the root canal pathogens. Till date, no *in vivo* study has been done to check the combined efficacy of propolis with moxifloxacin as intracanal medicament and to compare the results with that of TAP and calcium hydroxide against *Streptococcus* spp. and *E. faecalis* in type II DM patients with chronic AP. Therefore, the current study proposes to compare and evaluate the antimicrobial efficacy of TAP, propolis with moxifloxacin, and calcium hydroxide as intracanal medicaments against *E. faecalis* and *Streptococcus* spp. in chronic AP patients with Type II DM [19].

E. faecalis in Infected Root Canals

PDT is more efficient in eliminating *E. faecalis* from infected root canals compared to traditional endodontic treatment regimes (instrumentation and irrigation). Results from nearly 70% of the showed that PDT is more efficient in eliminating *E. faecalis* in infected root canals than conventional endodontic therapy. It is pertinent to men tion that there was an inconsistency in the methodology and laser parameters used in these studies. For example, studies by Soukos et al. Nagayoshi et al. and Schlafer et al, showed that PDT kills significantly more *E. faecalis* compared to conventional instrumentation/irrigation. However, the diode laser wavelengths (635 nm, 805 nm and 628 nm respectively), diameter of fiber used for laser delivery (500 _m,400 _m and 0.4 cm respectively), power output (1 W, 1 Wand 5 W) and type of PS used (MB, indocyanine green and TBO respectively) were erratic [20].

Also, the duration of irradiation varied between studies. For example, in studies by Bago et al. and Rios et al. durations of irradiation were 60 s (S) and 30 S respectively, however Foschi et al. and Pagonis et al. irradiated the root canals for5 min and 10 min correspondingly. In this regard, it was found that demanding to precisely contemplate the laser parameters that would be most effective in eliminating *E. fecalis* from infected root canals in a clinical scenario. Concentration of PS has been reported to influence the overall bactericidal efficacy of PDT. Nearly 80% of the studies included in this review that reported PDT to be effective in killing *E. faecalis* used either MB and/or TBO as photosensitizers. Studies have shown that MB (a synthetic non-porphyrin compound [phenoth-iazine]) is compatible with wavelengths of visible light (up to685 nm) and has a high rate of generation of reactive species. In addition, studies have reported that MB when used in concentrations of 100 g/mL minimizes the chances of dental discoloration, while safeguarding its photo-bactericidal properties studies [19, 20].

TBO is structurally similar to MB and exhibits bactericidal effects similar to those of MB. From the literature reviewed, we observed an inconsistency in the concentration of the photosensitizers used. In these studies, concentrations of MB concentrations ranged from 6.25 g/mL to 25g/mL whereas TBO concentrations ranged from 15g/mL to 12.5 mg/mL. However, it has also been reported that PDT with either MB or TBO does not have a significant additional effect to the chemomechanical preparation using 2.5% NaOCl as an irrigant in the reduction of *E. faecalis* counts. An explanation in this regard may be the presence of low concentration of available oxygen in the canals, particularly in irregularities and in dentinal tubules. Under such circumstances, formation of cytotoxic oxygen derivatives may be either be blocked or minimized. In clinical scenar ios, the PS may be unable to diffuse well into irregular canals and dentinal tubules or even through possible bacterial biofilms persisting on untouched canal walls. These factors may compromise the outcome of PDT in infected root canals. Further studies using standardized laser parameters and PS concentration/s are warranted to assess the efficacy of PDT in eliminating *E. faecalis* from infected root canal. Sassone et al. reported that 1.0-5.0% NaOCl is most effective in an *in vitro* test on agar plates and presence of residual NaOCl inside the dentinal tubules is critical for effective disinfection. It is pertinent to mention that in all studies, initial instrumentation and irrigation with 1-6% NaOCl and 17% EDTA was performed prior to PDT. Therefore, it is tempting to speculate that residual NaOCl in the dentinal tubules played a role in the overall canal disinfection process [21].

E. faecalis in Retreatment Cases

Disinfection of root canals with root canal irrigants show antimicrobial activity by killing microorganism when in direct contact. However, due to the complex root canal anatomy of none of the available irrigation solutions is sufficient to penetrate into depth areas where bacteria may

remain and survive. Therefore, irrigation agitation methods can help irrigation solutions to penetrate into deeper areas and eliminate bacteria from these complex canal systems. Manual and machine-assisted agitation techniques are commonly used as disinfection methods. Chemomechanical preparation along with irrigation of 2.5% NaOCl is effective in reducing the initial bacterial load regardless of the disinfection methods. Ferrer-Luque *et al*. reported that reduction of E. faecalis from infected root canals by mechanical debridement along with irrigation is ranged from 95.9 to 100% by using nickel titanium rotary files [28].

PUI is one of the most preferred irrigation activation technique to improve removal of bacteria, smear layer, and calcium hydroxide from root canal walls. Studies have shown that PUI did not significantly succeeded in reduction of E. faecalis after chemomechanical preparation. This reduction in bacterial counts was statistically lower than chemomechanical preparation (65%). Sampling method may be the one of the reasons for insignificant improvement in disinfection after PUI, because it only provides information about the bacterial load of the main canal. In the present study to standardize the potential effect of chemomechanical preparation on the bacterial reduction, root canals were prepared up to size #30at the WL in all experimental groups. It may be concluded that this apical enlargement did not allow enough space for free displacement amplitude of the ultrasonic instrument [29].

MDA depends on the up and down movements of a well-fitting gutta-percha point inside the root canal. Several studies reported the effectiveness of MDA in cleaning of the root canal walls. However, its effectiveness in elimination of bacteria has not been studied yet. Due to the lack of studies evaluating the effectiveness of MDA on bacterial elimination, the results of many in-vivo study were compared with the findings of in-vitro studies investigating its cleaning efficiency of root canal walls. Studies have revealed that MDA technique did not significantly reduce E. faecalis counts after chemomechanical preparation. The percentage reduction was 19% in MDA group as obtained in PUI group. Data from ex-vivo studies evaluating the cleaning effectiveness of MDA have been inconclusive [28, 29].

In a previous reports, MDA showed better results than conventional needle irrigation in terms of cleaning root canal. In another previous research, no significant differences were found between manual dynamic agitation and PUI in terms of penetration of sodium hypochlorite into dentinal tubules. The activation time and total volume of irrigant and root morphology may have led to the differences in effectiveness of MDA shown in these studies. According to the results of intragroup analysis of various studies, PDT was the only disinfection methods that was significantly succeeded in reduction of bacterial counts after chemo-mechanical preparation [28, 30]. The protocol for PDT application was based on the reaction of photosensitizer agent with molecular oxygen. The production of reactive oxygen species that formed at the end of reaction induces death of E. faecalis. In several in-vitro studies, wavelengths of diode laser were ranged between 625 and 805 nm. However, recently, an 810 nm diode laser was used in an in-vivo study by Asnaashari *et al*. They reported that PDT with diode laser 810 nm could evidently decrease the amount of E. faecalis in root canals. PDT has been reported as a promising approach and excellent bactericidal potential against E. faecalis in several studies. Garcez *et al*. reported that E. faecalis was significantly reduced following chemomechanical treatment with adjunct PDT compared to chemomechanical treatment alone. All techniques provided reduction in E. faecalis load, however any of these techniques were not able to eliminate E. faecalis completely. These results revealed that bacteria were still in root canal even though disinfection approaches were used. However, it is important to point out that to achieve an optimal outcome, maximal reduction of the bacterial load is necessary to be compatible with periradicular tissue healing [30].

Methods of Eradication of E. faecalis

Antibiotic medications were widely used for the treatment and prevention of E. faecalis caused by many pathogenic bacteria. In a study conducted by Donmez Ozkan H et al., in 2019, they found that fabclavine is an antimicrobial agent with a strong antibacterial effect against E. faecalis. In this study, they found that fabclavine-rich supernatant was highly effective against broad strains of E. faecalis with multidrug resistance when used as an intracanal medicament. Other studies discussed novel treatment techniques using newly isolated phages for targeting E. faecalis strains from the oral cavity. Lee D et al. found that phage HEf13 has high lytic activity against human dentin, which suggests the effectiveness of using phage HEf13 as a dental therapeutic agent against E. faecalis-related apical periodontitis (15). A recent study conducted by Ghorbanzadeh A *et al*. in 2020 found three effective disinfection methods for the partial elimination of E. faecalis biofilm [21].

Furthermore, E. faecalis has specific characteristics that enable it to escape chemomechanical instrumentation during root endodontic treatment. These characteristics can be outlined as the following: ability to form biofilms and colonize in remote unreachable areas away from the main canals, such as accessory canals, apical deltas, and isthmuses, to be protected by residual tissue, dentinal tissues, human serum, and dead cells that reduce the effect of antimicrobial means. In addition, E. faecalis uses different mechanisms to survive in harsh environments. Those mechanisms include activating some survival genes, using alternative metabolic pathways, living in an area with high sources of a nutrient, and possessing bacterial synergism and aggregation capacity [21, 22].

Several studies have been directed towards finding an effective way to eradicate and/or prevent *E. faecalis* from gaining access to the root canal space. *E. faecalis* can gain entry into the root canal system during treatment, between appointments, or even after the treatment has been completed. Therefore, it is important to consider treatment regimens aimed at eliminating or preventing the infection of *E. faecalis* during each of these phases. Preparing the apical portion of the root canal to a larger instrument size will help eliminate intracanal microorganisms by reaching areas not normally accessible by smaller master apical files [16, 17]. In addition, larger apical preparation sizes facilitate removal of the innermost (pulpal) dentin. This provides the potential to remove intratubular bacteria and open the dentinal tubules to allow antimicrobials to penetrate more effectively [20].

Three percent to full strength sodium hypochlorite, if used in adequate amounts and exchanged regularly, has the capability to destroy *E. faecalis* in the root canal. Sodium hypochlorite is an effective irrigant for all presentations of

E. faecalis including its existence as a biofilm. EDTA has little antibacterial activity, but is important in its ability to remove the inorganic portion of the smear layer thus allowing other irrigants access to the dentinal tubules. A 10% citric acid solution will remove the smear layer and, like EDTA, has little effect against *E. faecalis*. A 0.1% sodium benzoate solution added to 10% citric acid will increase the chances of killing *E. faecalis* [17]. MTAD, a new root canal irrigant consisting of a mixture of a tetracycline isomer, an acid, and a detergent has shown success in its ability to destroy *E. faecalis* in preliminary studies. Its effectiveness is attributed to its anticollagenase activity, low pH, and ability to be released gradually over time. The effects of MTAD are enhanced when 1.3% sodium hypochlorite is used as an irrigant during instrumentation [19, 21].

Calcium hydroxide is relatively ineffective against *E. faecalis* because of considerations mentioned previously. Iodine potassium iodide may be a more effective intracanal agent than calcium hydroxide [17, 18].

Chlorhexidine, in a 2% gel or liquid concentration, is effective at reducing or completely eliminating *E. faecalis* from the root canal space and dentinal tubules. A 2-min rinse of 2% chlorhexidine liquid can be used to remove *E. faecalis* from the superficial layers of dentinal tubules up to 100 mm. Two percent chlorhexidine gel is effective at completely eliminating *E.faecalis* from dentinal tubules for up to 15 days. This may be in part attributed to its substantive antimicrobial activity. It is questionable as to whether 0.12% chlorhexidine is more effective than calcium hydroxide. Some studies suggest it is more effective, yet neither will completely eradicate *E. faecalis*. Another study suggests 10% calcium hydroxide alone is more effective. When heated to 46°C, both 0.12% chlorhexidine and 10% calcium hydroxide have greater antimicrobial effects against *E. faecalis* than at normal body temperature [18, 19, 21].

Other irrigants that may be effective at eliminating *E. faecalis* include ozonated water and stannous fluoride. Ozonated water has been shown to have the same antimicrobial efficacy as 2.5% sodium hypochlorite. Stannous fluoride demonstrated greater antimicrobial effectiveness against *E. faecalis* than calcium hydroxide. Combinations of irrigants to eliminate *E. faecalis* have also been studied. In one study, a combination of calcium hydroxide mixed with camphorated paramonochlorophenol completely eliminated *E. faecalis* within dentinal tubules. Metapex, a silicone oil-based calcium hydroxide paste containing 38% iodoform, more effectively disinfected dentinal tubules infected with *E. faecalis* than calcium hydroxide alone. The addition of stannous fluoride to calcium hydroxide is also more effective than calcium hydroxide by itself [19]. Concentrations of 1 to 2% chlorhexidine combined with calcium hydroxide have also demonstrated efficacy at killing *E. faecalis*. Chlorhexidine combined with calcium hydroxide will result in a greater ability to kill *E. faecalis* than calcium hydroxide mixed with water. Two percent chlorhexidine gel combined with calcium hydroxide achieves a pH of 12.8 and can completely eliminate *E. faecalis* within dentinal tubules. It is important to note, however, that chlorhexidine alone has been shown to provide as good, or even better, antimicrobial action against *E. faecalis* than calcium hydroxide/chlorhexidine combinations. Until further studies have been conducted, an intracanal dressing of 2% chlorhexidine placed for 7 days may be the best way to eradicate *E. faecalis* from dentinal tubules and the root canal space. In some studies, chlorhexidine-impregnated and iodoform-containing gutta-percha points have shown little inhibitory action against *E. faecalis*. In another study, 5% chlorhexidine in a slow release device (Activ Point, Roeko, Langenau, Germany) completely eliminated *E. faecalis* in dentinal tubules upto 500 mm. The antimicrobial activity against *E. faecalis* of various sealers has also been studied. Roth 811 (Roth International Ltd., Chicago, IL), a zinc-oxide eugenol based sealer, has been shown to exhibit the greatest antimicrobial activity against *E. faecalis* when compared to other sealers [20].

AH Plus epoxy-resin based sealer(Dentsply, DeTrey, Konstanz, Germany) and Sultan zinc oxide-eugenol based sealer (Sultan Chemists, Inc., Englewood, NJ) both exhibit good antibacterial effects against *E. faecalis* using agar diffusion and direct-contact tests. AH Plus and Grossman's sealer are effective in killing *E. faecalis* within infected dentinal tubules. Based on these studies it can be concluded that a combination of adequate instrumentation, and appropriate use of irrigants, medicaments, and sealer will optimize the chances of eradicating *E. faecalis* during retreatment of failed root canal cases [21, 22].

Additional steps should be taken to prevent *E. faecalis* from re-entering the root canal space. These include having the patient rinse with chlorhexidine before treatment, disinfecting the tooth and rubber dam with chlorhexidine or sodium hypochlorite, and disinfecting gutta-percha points with sodium hypochlorite before insertion in the canal [23]. Other possibilities may include using an obturating system that can provide a more effective seal. Newer obturation systems such as Epiphany (Pentron Corp, Wallingford, CT) have been designed to bond to the root canal walls and thus prevent bacterial leakage. Although research is still needed, a preliminary study shows that this system is better at preventing microleakage of *E. faecalis* than gutta-percha filled canals [20, 21]. A well-sealed coronal restoration and root canal filling are important steps in preventing bacteria from entering the canal space [24].

PDT causes a reduction of bacterial viability in both primary endodontic infection and retreated root canals infected with E. faecalis. Treating root canals with sodium hypochlorite (NaOCl), PDT or a combination of NaOCl irrigation and PDT caused a significant reduction of E. faecalis in the root canals. In the cases of primary infections, the combination of NaOCl irrigation and PDT. NaOCl irrigation achieved the highest number of culturenegative root canals (90%), NaOCl irrigation alone achieved culture-negative root canals in 80%, whereas after treatment using PDT only one specimen was culture-negative. For secondary infections, NaOCl irrigation achieved the highest number of culture-negative root canals, whereas after treatment using PDT all the specimens were culturepositive. Garcez et al. [24] investigated the effect of PDT in endodontic retreatments in vivo. They found that PDT as an adjuvant to conventional endodontic treatment leads to a significant further reduction of bacterial load afterirrigation using NaOCl, hydrogen peroxide and EDTA and is effective against multi-drug resistant bacteria [25].

Concluding Remarks and Call For Future Studies

Enterococci, especially E. faecalis strains, appear to adapt to the habitat of a treated root canal better than other taxa.

From the information available, it may be concluded that these bacteria are not amongst the early invaders of the necrotizing root canal system. They may enter the root canal at any point in time during or after treatment if the coronal seal is inadequate. Their source is most likely food. In individuals with an adequate level of oral hygiene, they do not colonize the oral cavity [31].

However, they may enter the unsealed root canal system, where they find a habitat that allows their growth and survival. Future studies should be directed at several issues. First, virulence factors that favour the occurrence of enterococci in filled root canals should be identified. This work has already started and has yielded some interesting results (Sedgley 2007). Gelatinase production is one of the virulence factors that may be associated with the survival of E. faecalis in filled root canals [32]. The E. faecalis strains producing gelatinase were termed S. faecalis var. liquefaciens in early studies and were frequently recovered from root canal systems (Guthof 1953, Winkler & van Amerongen 1959, Engstro¨m 1964, Meja`re 1975) [32, 33]. Interestingly and perhaps obviously, gelatinase production is also a factor that promotes the presence of enterococci in fermented food products (Franz et al. 2003). The origin of enterococci in root canals should be further identified by comparing the scheme of highly preserved genes between clones found in root canals and counterparts from food products, preferably from a similar area (Ruiz-Garbajosa et al. 2006) [34]. On a less sophisticated level, contamination of different enterococcal species in typical regional foods could be compared with the recovery of these species from root canals in a given country or area. Last but not least, it has been known for a long time that the healthy microbiota of the oral cavity can defend against potential pathogens (Deyloff & Sanders 1980). It would be interesting to identify the mechanisms preventing the colonization of the oral cavity by enterococci [35, 36].

Conclusion

Studies indicate that the prevalence of *E. faecalis* is low in primary endodontic infections and high in persistent infections. *E. faecalis* is also more commonly associated with asymptomatic cases than with symptomatic ones. Although *E. faecalis* possesses several virulence factors, its ability to cause periradicular disease stems from its ability to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth. Our challenge as endodontic specialists is to implement methods to effectively eliminate this microorganism during and after root canal treatment.

Currently, use of good aseptic technique, increased apical preparation sizes, and inclusion of full strength sodium hypochlorite and 2% chlorhexidine irrigants are the most effective methods to eliminate *E. faecalis*. Recent studies have helped us better understand *E. faecalis* and the mechanisms that enable it to cause persistent endodontic infections. In the changing face of dental care, continued research on *E. faecalis* and its elimination from the dental apparatus may well define the future of the endodontic specialty.

References

1. Quantitative and qualitative analysis of microorganisms in root-filled teeth with persistent infection: monitoring of the endodontic retreatment. Endo MS, Ferraz CCR, Zaia AA, Almeida JFA, Gomes B. *Eur J Dent*,2013:7:302–309.
2. Endodontic microbiology. Narayanan LL, Vaishnavi C. *J Conserv Dent*,2010:13:233–239.
3. Williams JM, Trope M, Caplan DJ, Shugars DC. Detection and quantitation of E. faecalis by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. Journal of endodontics,2016:32(8):715-21.
4. Johnson EM, Flannagan SE, Sedgley CM. Coaggregation interactions between oral and endodontic Enterococcus faecalis and bacterial species isolated from persistent apical periodontitis. Journal of endodontics,2016:32(10):946-50.
5. Koch S, Hufnagel M, Theilacker C, Huebner J. Enterococcal infections: host response, therapeutic, and prophylactic possibilities. Vaccine,2017:22:822–30.
6. Microbiology of endodontic infections. Singh H. https://scientonline.org/open-access/microbiology-of-endodontic-infections.pdf*J Dent Oral Hyg*,2016:2:1–4.
7. Tendolkar PM, Baghdayan AS, Shankar N. Pathogenic *Enterococci*: new developments in the 21st century. Cell Mol Life Sci,2019:60:2622–36.
8. Efficacy of sodium hypochlorite and chlorhexidine against Enterococcus faecalis--a systematic review. Estrela C, Silva JA, de Alencar AH, Leles CR, Decurcio DA. *J Appl Oral Sci*,2009:16:364–368.
9. Characterization of microbiota of root canal-treated teeth with posttreatment disease. Roças IN, Siqueira JF. *J Clin Microbiol*,2012:50:1721–1724.
10. Ex vivo comparison of antibacterial efficacy of conventional chemomechanical debridement alone and in combination with light-activated disinfection and laser irradiation against Enterococcus faecalis biofilm.
11. Ghorbanzadeh A, Bahador A, Sarraf P, Ayar R, Fekrazad R, Asefi S. *Photodiagnosis Photodyn Ther*,2020:29:101648
12. Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. International endodontic journal, 2009:42(4):277-87. Teixeira LM, Facklam RR. *Enterococcus*. In: Murray PR, ed. Manual of clinical microbiology, 8th ed. Washington: ASM Press, 2020, 422–33.
13. Biofilm in endodontics: a review. Jhajharia K, Parolia A, Shetty KV, Mehta LK. *J Int Soc Prev Community Dent*,2015:5:1–12.
14. John G, Kumar KP, Gopal SS, Kumari S, Reddy BK. Enterococcus faecalis, a nightmare to endodontist: A systematic review. African journal of microbiology research,2015:9(13):898-908.
15. Analysis of the secondary endodontic lesions focusing on the extraradicular microorganisms: an overview. Del Fabbro M, Samaranayake LP, Lolato A, Weinstein T, Taschieri S. *J Investig Clin Dent*,2014:5:245–254.
16. ASM Press, 2002:1–54.1. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J,2002:35:221–8.
17. Zhang C, Du J, Peng Z. Correlation between Enterococcus faecalis and persistent intraradicular infection compared with primary intraradicular infection: a systematic review. Journal of endodontics,2015:41(8):1207-13. Baumgartner JC,

Falkler WA. Bacteria in the apical 5 mm of infected root canals. J
18. Cancio V, Carvalho Ferreira DD, Cavalcante FS, Rosado AS, Teixeira LM, Braga Oliveira Q, *et al*. Can the Enterococcus faecalis identified in the root canals of primary teeth be a cause of failure of endodontic treatment?. Acta Odontologica Scandinavica, 2017:18:75(6):423-8.
19. Transcriptome analysis of Enterococcus faecalis in response to alkaline stress. Ran S, Liu B, Jiang W, Sun Z, Liang J. Front Microbiol,2015:6:795
20. Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N, *et al*. Evaluationof the bactericidal effect of Nd: Yag, Er: Yag, Er, Cr:YSGG laserradiation, and antimicrobial photodynamic therapy (aPDT) inexperimentally infected root canals. Lasers In Surgery AndMedicine,2012:44:824-31.
21. Bago I, Plecko V, Gabric Panduric D, Schauperl Z, Baraba A,Anic I. Antimicrobial efficacy of a high-power diode laser,photo-activated disinfection, conventional and sonic activatedirrigation during root canal treatment.
22. Tirukkolluru C, Thakur S. Comparative evaluation of triple antibiotic paste, propolis with moxifloxacin, and calcium hydroxide as intracanal medicaments against Streptococcus spp. and Enterococcus faecalis in type II diabetes mellitus patients: A randomized clinical trial. Contemporary clinical dentistry,2019:10(2):191.
23. Love RM. Enterococcus faecalis–a mechanism for its role in endodontic failure. International endodontic journal,2001:34(5):399-405.
24. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg
25. Nematode-associated bacteria: production of antimicrobial agent as a presumptive nominee for curing endodontic infections caused by Enterococcus faecalis.
26. Donmez Ozkan H, Cimen H, Ulug D, et al. *Front Microbiol*,2019:10:2672.
27. Oral Med Oral Pathol Oral Radiol Endod,1998:85:86 – 93.
28. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North Am population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod,2010:91:579–86.
29. Gilmore MS. The *Enterococci*: pathogenesis, molecular biology, and antibiotic resistance.
30. Bilgin B, Türker S, Aslan M, Saglam B, Koçak S, Koçak M, Bodrumlu E. Entrococcus faecalis elimination in retreatment cases using passive ultrasonic irrigation, manual dynamic activation and photodynamic therapy: a randomized clinical trial. Tanta Dental Journal,2020:17(1):31-.
31. Supotngarmkul A, Panichuttra A, Ratisoontorn C, Nawachinda M, Matangkasombut O. Antibacterial property of chitosan against E. faecalis standard strain and clinical isolates. Dental materials journal,2020:39(3):456-63.
32. Virdee SS, Seymour DW, Farnell D, Bhamra G, Bhakta S. Efficacy of irrigant activation techniques in removing intracanal smear layer and debris from mature permanent teeth: a systematic review and meta-analysis. International endodontic journal,2018:51(6):605-21.
33. Clinical implications and microbiology of bacterial persistence after treatment procedures. Siqueira JF Jr, Rocas IN. J Endod,2008:34:1291–1301.
34. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics,2015:32(2):93-8.
35. Alghamdi F, Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment: A systematic review. Cureus, 2020, 12(3).
36. Sadık B, Arıkan S, Beldüz N, Yaşa Y, Karasoy D, Cehreli M. Effects of laser treatment on endodontic pathogen Enterococcus faecalis: a systematic review. Photomedicine and laser surgery,2013:31(5):192-200.
37. Rocas IN, Siqueira Jr JF, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. Journal of endodontics, 2004:30(5):315-20.