Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment

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Abstract

Enterococcus faecalis is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24% to 77%. This finding can be explained by various survival and virulence factors possessed by *E. faecalis*, including its ability to compete with other microorganisms, invade dentinal tubules, and resist nutritional deprivation. Use of good aseptic technique, increased apical preparation sizes, and inclusion of 2% chlorhexidine in combination with sodium hypochlorite are currently the most effective methods to combat *E. faecalis* within the root canal systems of teeth. 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. *(J Endod 2006;32:93–98)*

Key Words

Endodontic retreatment, *Enterococcus faecalis*

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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 (2–5). *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). The intent of this article is (a) to describe characteristics inherent to *E. faecalis*; (b) to cite studies that implicate *E. faecalis* as an etiology of failing root canal treatment; (c) to list the mechanisms that allow *E. faecalis* the ability to survive and cause persistent periradicular pathosis; and (d) to discuss current treatment modalities that are effective in eliminating *E. faecalis* from the root canal system.

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 (6, 7). *Enterococcus* species live in vast quantities [10^5 -10^8 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 (8). They catabolize a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine, and many α keto acids (6). *Enterococci* survive very harsh environments including extreme alkaline pH (9.6) and salt concentrations (6, 9). They resist bile salts, detergents, heavy metals, ethanol, azide, and desiccation (6). They can grow in the range of 10 to 45°C and survive a temperature of 60°C for 30 min (9). 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, 10). After establishing that the gram-positive coccus is a member of one of the five groups in the *Enterococcus* genus (Table 1) (10), several conventional tests are used to identify the specific species. In group 2, *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 (11). 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. Most of these methods are nucleic acid-based involving PCR amplification assays that are followed by electrophoretic analysis of the PCR products, probing, sequencing, or both (11). Random amplified polymorphic DNA (RAPD) analysis and pulse-field

TABLE 1. Categorization of *Enterococcus* species and two physiologically related gram-positive cocci based on phenotypic characteristics*

Group	Species
Group I (+) acid formation in mannitol broth (+) acid formation in sorbose broth (−) arginine hydrolysis	*E. avium* *E. gilvus* *E. malodoratus* *E. pallens* *E. pseudoavium* *E. raffinosus* *E. saccharolyticus*
Group II (+) acid formation in mannitol broth (−) acid formation in sorbose broth (+) arginine hydrolysis	***E. faecalis*** *E. faecium* *E. casseliflavus* *E. gallinarum* *E. mundtii* *Lactococcus sp.*
Group III (−) acid formation in mannitol broth (−) acid formation in sorbose broth (+) arginine hydrolysis	*E. dispar* *E. durans* *E. hirae* *E. porcinus* *(E. villorum)* *E. ratti*
Group IV (−) acid formation in mannitol broth (−) acid formation in sorbose broth (−) arginine hydrolysis	*E. asini* *E. cecorum* *E. sulfureus*
Group V (+) acid formation in mannitol broth (−) acid formation in sorbose broth (−) arginine hydrolysis	*E. columbae* *Vagococcus sp.*

*Adapted from Teixeira and Facklam (10).

gel electrophoresis (PFGE) are techniques that have been utilized to determine variations in DNA sequences and have been employed in determining various *E. faecalis* subtypes (12, 13). In fact, the Bacteriology Collection of the ATCC (American Type Culture Collection) currently lists 69 isolates of *E. faecalis* that are commercially available (14). These isolates each have a different ATCC number and designation. The biosafety level ranges from 1 to 2 and growth conditions differ among the subtypes. Sources for these isolates include sour milk (ATCC number 376™), meat involved in food poisoning (ATCC number 7080™), and the root canal of a pulpless tooth (ATCC number 4083™) (14).

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 (15). 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 (8). Studies have shown that nosocomial infections are not caused by the patient's own prehospitalization flora (16). 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 (17). 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 (18, 19). Enterococcal urinary tract and soft tissue infections are generally treated with single drug therapy, often with penicillin or vancomycin (20). 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 (21). Enterococcal strains, particularly those causing endocarditis, must be screened to define antimicrobial resistance patterns. Thirty-five vancomycin resistant *Enterococci* have demonstrated susceptibility to linezolid (antibiotic, oxazolidinone derivative), suggesting it may be the treatment of choice for multi-drug resistant enterococcal infections (22).

Prevalence in Secondary Root Canal 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 (23). *E. faecalis* is associated with different forms of periradicular disease including primary endodontic infections and persistent infections (7). 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 (7). 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 (7). Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% (3–5, 7, 24–31). The wide range of *E. faecalis* prevalence among studies may be attributed to different identification techniques, geographic differences, or sample size (32, 33). In some cases, *E. faecalis* has been found as the only organism (pure culture) present in root-filled teeth with periradicular lesions (4, 28). 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* (34, 35). This method proves to be faster, more sensitive, and more accurate than culturing methods (35). It has enabled researchers to detect bacteria that were difficult, and in some cases impossible, to detect (35). 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 (7). An optical spectroscopy-based method has also been studied as a way to detect *E. faecalis* activity (36). It is possible that this detection system could be used chairside to rapidly monitor the presence or absence of *E. faecalis* in the root canal system (36). Table 2 provides a list of studies that report on the occurrence of *E. faecalis* in root filled teeth with apical periodontitis.

Survival and Virulence Factors

E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid (7). It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses (7, 37). *E. faecalis* is able to suppress the action of lymphocytes, potentially contributing to endodontic failure (38). *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 (15). 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

TABLE 2. Studies investigating the prevalence of *E. faecalis* in root-filled teeth with an apical periodontitis

Author/year	Number of Root-filled Teeth in Study	Number of Root-filled Teeth with Bacterial Growth	Prevalence of *E. faecalis*	Method of Detection
Engström 1964 (24)	54	21	5/21 = **24**%	Culture
Möller 1966 (25)	264	120	34/120 = 28%	Culture
Molander et al. 1998 (3)	100	68	32/68 = 47%	Culture
Sundqvist et al. 1998 (4)	54	24	9/24 = 38%	Culture
Peciuliene et al. 2000 (26)	25	20	14/20 = 70%	Culture
Peciuliene et al. 2001 (27)	40	33	21/33 = 64%	Culture
Hancock et al. 2001 (5)	54	33	10/33 = 33%	Culture
Pinheiro et al. 2001 (28)	60	51	27/51 = 53%	Culture
Pinheiro et al. 2003 (29)	30	24	11/24 = 46%	Culture
Siqueira & Rôças 2004 (30)	22	22	17/22 = **77**%	PCR
Gomes et al. 2004 (31)	19	19	6/19 = 32%	Culture
Rôças et al. 2004 (7)	30	30	20/30 = 67%	PCR

Adapted from Rôças et al. (7).

widespread genetic polymorphisms (23). It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which help it bind to dentin (39). It is small enough to proficiently invade and live within dentinal tubules (37). It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available (40). Once available, the starved cells are able to recover by utilizing serum as a nutritional source (40). Serum, which originates from alveolar bone and the periodontal ligament, also helps *E. faecalis* bind to type I collagen (37). *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days (41, 42). *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 nonbiofilm producing organisms (43).

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 (42, 44–46). The following reasons have been proposed to explain why *E. faecalis* is able to survive intracanal treatment with calcium hydroxide: (a) *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. (b) *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. (c) At a pH of 11.5 or greater, *E. faecalis* is unable to survive (1, 45). 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 (46). 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 (47, 48). Diverse components of dentin including dentin matrix, type-I collagen, hydroxyapatite, and serum are responsible for altering the antibacterial effects of these medicaments (49). Table 3 summarizes the survival and virulence factors associated with *E. faecalis*.

Methods of Eradication

Many 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 (7). 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 (50). 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. 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 (51). Sodium hypochlorite is an effective irrigant for all presentations of *E. faecalis* including its existence as a biofilm (52). 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 (53, 54). 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* (55). 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 (53, 56). Its effectiveness is attributed to its anticollagenase activity, low pH, and ability to be released gradually over time (56). The effects of MTAD are enhanced when 1.3% sodium hypochlorite is used as an irrigant during instrumentation (57). Calcium hydroxide is relatively ineffective against *E. faecalis* because of considerations mentioned previously (1, 41). Iodine potassium iodide may be a more effective intracanal agent than calcium hydroxide (58).

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 (59–61). 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 μm (59). Two

TABLE 3. Survival and virulence factors of *E. faecalis*

- Endures prolonged periods of nutritional deprivation
- Binds to dentin and proficiently invades dentinal tubules
- Alters host responses
- Suppresses the action of lymphocytes
- Possesses lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid
- Utilizes serum as a nutritional source
- Resists intracanal medicaments (i.e. $Ca(OH)_2$)
  - -Maintains pH homeostasis
  - -Properties of dentin lessen the effect of calcium hydroxide
- Competes with other cells
- Forms a biofilm

percent chlorhexidine gel is effective at completely eliminating *E. faecalis* from dentinal tubules for up to 15 days (60). This may be in part attributed to its substantive antimicrobial activity (62). 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* (44, 63). Another study suggests 10% calcium hydroxide alone is more effective (64). 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 (65).

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 (66). Stannous fluoride demonstrated greater antimicrobial effectiveness against *E. faecalis* than calcium hydroxide (67).

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 (68). Metapex, a silicone oil-based calcium hydroxide paste containing 38% iodoform, more effectively disinfected dentinal tubules infected with *E. faecalis* than calcium hydroxide alone (69). The addition of stannous fluoride to calcium hydroxide is also more effective than calcium hydroxide by itself (67). Concentrations of 1 to 2% chlorhexidine combined with calcium hydroxide have also demonstrated efficacy at killing *E. faecalis* (60, 68, 70). Chlorhexidine combined with calcium hydroxide will result in a greater ability to kill *E. faecalis* than calcium hydroxide mixed with water (70). Two percent chlorhexidine gel combined with calcium hydroxide achieves a pH of 12.8 and can completely eliminate *E. faecalis* within dentinal tubules (60). 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 (60, 61). 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 (60, 61). In some studies, chlorhexidine-impregnated and iodoform-containing gutta-percha points have shown little inhibitory action against *E. faecalis* (71, 72). In another study, 5% chlorhexidine in a slow release device (Activ Point, Roeko, Langenau, Germany) completely eliminated *E. faecalis* in dentinal tubules up to 500 μm (73).

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 (74). 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 (75). AH Plus and Grossman's sealer are effective in killing *E. faecalis* within infected dentinal tubules (76). 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.

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 (77). 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 (78). A well-sealed coronal restoration and root canal filling are important steps in preventing bacteria from entering the canal space (79). Figure 1 provides steps that can be used to eliminate *E. faecalis* during endodontic retreatment.

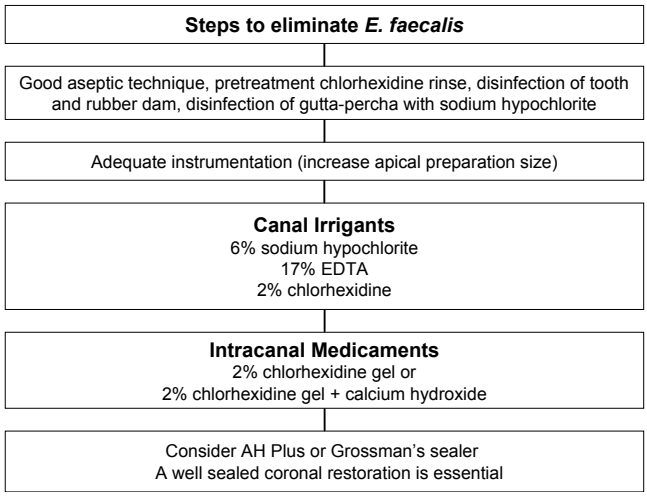

Figure 1. Steps to eliminate *E. faecalis* during endodontic retreatment (2 appointments).

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.

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