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Review

Enterococcus faecalis, a nightmare to endodontist: A systematic review

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The main goal in endodontics is the prevention and treatment of diseases of the dental pulp and periapical tissues and it can be best achieved if preventive measures and treatment procedures are based on a thorough and detailed understanding of the etiology and pathogenesis of endodontic diseases. There are some cases in which the treatment has followed the highest technical standards and yet failure results. Scientific evidence indicates microbial factors which play an important role. In most cases, failure of endodontic treatment is a result of microorganisms persisting in the apical portion of the root canal system, even in well-treated teeth. *Enterococcus faecalis* is recognized as a pathogen in post-treatment endodontic infections and probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canal. Enterococci are also implicated in infections of the root canal system, however, they make up a small proportion of the initial flora which is dominated by Gram negative species. In contrast, it has been reported that enterococci are frequently isolated from obturated root canals of teeth that exhibit chronic periapical pathology. Eradication of *E. faecalis* from the root canal remains a challenge, while chlorhexidine and combinations of disinfectants show some promise. A better understanding of the role of the virulence factors of *E. faecalis* in endodontic infections may help in the development of new strategies to prevent or eliminate the infection by this species, thereby improving treatment results in endodontics.

Key words: *Enterococcus faecalis*, endodontics, periapical tissues, canal, infection.

INTRODUCTION

The main goal in endodontics is the prevention and treatment of diseases of the dental pulp and periapical tissues. This objective can be best achieved if preventive measures and treatment procedures are based on a thorough and detailed understanding of the etiology and pathogenesis of endodontic diseases (Markus et al., 2003). Root canal treatment usually fails when treatment fall short of acceptable standards (Seltzer et al., 1963). Undoubtedly, the major factors associated with endodontic failure are the persistence of microbial infection in the root canal system and the periradicular area (Nair et al., 1990). There are some cases in which the treatment has followed the highest technical standards and yet failure results. Scientific evidence indicates that some factors may be associated

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with the unsatisfactory outcome of well-treated cases. They include microbial factors, comprising extraradicular and intraradicular infections (Sjogren et al., 1996; Sundqvist et al., 1998; Lopes et al., 1999). In most cases, failure of endodontic treatment is a result of microorganisms persisting in the apical portion of the root canal system, even in well-treated teeth. To survive in the root-filled canal, microorganisms must withstand intracanal disinfecting measures and adapt to an environment in which there are few available nutrients. Therefore, the few microbial species that have such ability may be involved in the failure of root canal treatment. The ability to survive in such conditions is important for most bacteria because periods of starvation are commonly experienced. Several regulatory systems play an essential role in the ability of bacteria to withstand nutrient depletion. These systems are under the control of determined genes, whose transcription is activated under conditions of starvation. The microbiota associated with failed cases differs from that reported in untreated teeth (primary root canal infection). Whereas the latter is typically a mixed infection, in which Gram negative anaerobic rods are dominant, the former is usually composed of one or a few bacterial species, generally Gram positive bacteria, with no apparent predominance of facultatives or anaerobes.

Moller (1966), after examining failed cases, reported that *Enterococcus faecalis* was found in 29% of the cases. Sundqvist et al. (1998) observed a mean of 1.3 bacterial species per canal and 42% of the recovered strains were anaerobic bacteria. *E. faecalis* was detected in 38% of the infected root canals.

For many years, *Enterococcus* species were believed to be harmless to humans and considered unimportant medically. Because they produce bacteriocins, *Enterococcus* species have been used widely over the last decade in the food industry as probiotics or as starter cultures (Foulquie et al., 2006). Until the mid-1980s, enterococci were not allocated to a separate genus, even though their unique characteristics were recognized among streptococci. The basic observations on staining, cell shape and arrangement as well as lack of catalase placed enterococci in the genus Streptococcus. Enterococci are normal human commensals adapted to the nutrient-rich, oxygen-depleted, ecologically complex environments of the oral cavity, gastrointestinal tract, and vagina (Jett et al., 1994).

Enterococci frequently cause a wide variety of infections in humans and commonly infect the urinary tract (Felmingham et al., 1992), bloodstream, abdomen (Graninger and Ragette, 1992), endocardium (Eliopoulos, 1992), biliary tract (Khardori et al., 1991), burn wound, and *in situ* foreign devices. The source of the enterococci found in the root canal system is thus still unclear, but evidence shows an exogenous origin. Recently, enterococci have become one of the most common nosocomial pathogens, with patients having a high mortality rate of up to 61% (De Fatima et al., 2005). *E. faecalis* is responsible for 80 - 90% of human enterococcal infections.

In the past few years, *E. faecalis* has been the focus of increased interest both in medicine and dentistry. A recognized pathogen in post-treatment endodontic infections, *E. faecalis* is frequently isolated both in mixed flora and in monocultures. *E. faecalis* is probably the species that can best adapt to and tolerate the ecologically demanding conditions in the filled root canal. Enterococci are also implicated in infections of the root canal system, however, they make up a small proportion of the initial flora which is dominated by Gram-negative species. In contrast, it has been reported that enterococci are frequently isolated from obturated root canals of teeth that exhibit chronic periapical pathology (Molander et al., 1998)

This dramatic increase in resistance of *Enterococcus* species worldwide highlights the need for a greater understanding of this genus, including its ecology, epidemiology and virulence.

ENTEROCOCCI CHARACTERISTICS

In 1930's Lancefield serologically classified Enterococci as group D Streptococci. In 1937, Sherman proposed a classification scheme, in which he recommended that the term 'Enterococcus' should be used specifically for streptococci that grow at both 10 and 45°C, at pH 9.6 and in 6.5% NaCl, survive at 60°C for 30 min and have ability to split esculin. In 1980s, based on genetic differences, enterococci were removed from the genus Streptococcus and placed in their own genus, *Enterococcus*. The genus *Enterococcus* consists of Gram positive, catalas enegative, non-spore-forming, facultative anaerobic bacteria that can occur both as single cocci and in chains. Enterococci belong to a group of organisms known as lactic acid bacteria (LAB) that produce bacteriocins (Health Protection Agency, 2005). Endospores are not formed and some species can be motile by scanty flagella. They form creamy whitish colonies. Most enterococci are facultative anaerobes, but some species are strict aerobes. Enterococci do not normally reduce nitrate and do not digest pectin or cellulose. They are ubiquitous and potentially pathogenic species that are able to acquire an increased resistance or phenotypic tolerance to many disinfectants or physical agents. *E. faecalis* possess a group D carbohydrate cell wall antigen (Lancefield antigen), which is an intracellular glycerol teichoic acid associated with the cytoplasmic membrane. The cell wall contains a large amount of peptidoglycan and teichoic acid. The peptidoglycan (cross-linked peptide sugar), which is found in most of the bacterial cell walls, helps to maintain the microbe's shape and has a polysaccharide backbone of alternating N- acetylglucosamine (GlcNAc) and N-acetylmuramic acids (MurNAc). The chemical and

structural analyses of the capsular polysaccharides have shown glycerol teichoic acid-like molecules with a carbohydrate backbone structure and sialic acid. These polysaccharides are cross-linked with peptide bridges and contribute to the three-dimensional structure of peptidoglycan. Because of the location of the peptidoglycan on the outside of the cytoplasmic membrane and its specificity, the transglycosylation step has been indicated as a potential target for antibacterial medicaments.

They catabolize a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine, and many alpha keto acids. *Enterococcus* species live in vast quantities [105 -108 colony-forming units (cfu) per gram of feces] in the human intestinal lumen and under most circumstances cause no harm to their hosts. Enterococci can survive very harsh environments including extreme alkaline pH 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 (Flahaut et al., 1996).

VIRULENCE FACTORS

Aggregation substance (AS)

Aggregation substance (AS) is a pheromone-responsive, plasmid- encoded bacterial adhesin that mediates efficient contact between donor and recipient bacterium, facilitating plasmid exchange AS is expressed by the donor cell, the bacterial conjugation process requires that 'binding substance' (BS, the chromosomally encoded cognate ligand for AS) be expressed on the surface of the recipient cell. AS was also found to mediate binding to extracellular matrix (ECM) proteins, including collagen type I. Binding to collagen type I by bacteria may be of particular importance with respect to endodontic infections, since this is the main organic component of the dentin (Linde A et al., 1993) .AS has been reported to promote direct, opsonin-independent binding of *E. faecalis* to human neutrophils via a complement receptor-mediated mechanism (Vanek et al., 1999). As a consequence of this special type of binding, *E. faecalis*-bearing AS was shown to be resistant to killing by human neutrophils, despite marked phagocytosis and neutrophil activation (Rakita et al 1999). phagosomal oxidant production by the neutrophils may be a possible contribution to tissue damage in case of infection with cells of *E. faecalis* expressing AS. Cell extracts of AS- and BS-positive *E. faecalis* were found to induce T-cell proliferation, with subsequent release of tumor necrosis factor beta and gamma interferon, and to activate macrophages to release tumor necrosis factor alpha. It also stimulates the production of the cytotoxic agent nitric oxide (NO) by a variety of cells, including macrophages and neutrophils, and may cause undesirable cell and tissue damage.

In a recent study of characterization of virulence factors and clonal diversity of *E. faecalis* isolates from treated dental root canals by phenotyping and Western blotting test, 45% had genes for AS (Archimbaud et al., 2002).

Surface adhesins (SA)

Enterococcal gene Esp., encoding the high molecular weight surface protein Esp., has been detected in abundance among bacterimia and endocarditis isolates. Esp. is associated with promotion of primary attachment and biofilm formation of *E. faecalis* on abiotic surfaces (Toledo-Arana et al., 2001). In a recent study 90% of virulence genes were efaA and ace genes detected by PCR from treated root canals of teeth. The disruption of the ace gene impaired the conditional binding of *E. faecalis* to the extracellularmatrix proteins (Nallapreddy et al., 2000).

Sex pheromones

Production of the sex pheromones by strains of *E. faecalis* and its bacterial clumping inducing effect was first described by (Dunny et al., 1978). It was subsequently shown that antibiotic resistance and other virulence traits, like cytolysin production can be passed in strains of *E. faecalis* by sex pheromone system (Clewell and Weaver, 1989). Some of *E. faecalis* sex pheromones were found to be chemotactic for human neutrophils (Sannomiya et al., 1990).

Gelatinase

E. faecalis posseses gelatinase (Hubble et al., 2003) which help it bind to dentin and gelatinase levels were elevated in oral rinses, crevicular fluid and whole saliva samples (Makela et al., 1994) and in gingival biopsy specimens (Soell et al., 2002), from periodontitis patients compared with healthy subjects. High gelatinase production has also been seen in epidemiologic studies with human clinical isolates (Kanemitsu et al., 2001).

Cytolysin

E. faecalis possess cytolysin or hemolysin as a virulence factor. Conflicting studies suggest the role of cytolysin as a possible virulence factor. Initial studies reported that approximately 60% of *E. faecalis* isolates derived from fecal specimens from healthy individuals. However recent studies show that the role of cytolysin as a virulence factor is small or negligible (Coque et al., 1995; Elsner et al., 2000).

Table 1. Studies investigating the prevalence of *E. faecalis* in root-filled teeth with an apical periodontitis.

Reference	Number of root filled teeth in study	Number of root filled teeth with bacterial growth	Prevalence of *E. faecalis* (%)	Method of detection
Engström (1964)	54	21	24	Culture
Möller (1966)	264	120	28	Culture
Molander et al. (1998)	100	68	47	Culture
Sundqvist et al. (1998)	54	24	38	Culture
Peciuliene et al. (2000)	35	20	70	Culture
Peciuliene et al. (2001)	40	33	64	Culture
Hancock et al. (2001)	54	33	33	Culture
Pinheiro et al. (2003)	60	51	53	Culture
Pinheiro et al. (2003)	30	24	46	Culture
Siqueira and Rocas (2004)	22	22	77	PCR
Gomes et al. (2004)	19	19	32	Culture
Rocas et al. (2004)	30	30	67	PCR
Asharf et al. (2005)	37	8	22	PCR
Chiara et al. (2007)	23	9	39.1	PCR
Xiaofei et al. (2010)	32	13	40.6	API20 Strep kits and 16S rRNA sequencing
Isabela et al. (2012)	29	11	38	Quantitative real time PCR

E. FAECALIS AND APICAL PERIODONTITIS

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. 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 (Rocas et al., 2004). Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% (Table 1)

RESISTANCE OFFERED BY *E. FAECALIS*

Enterococci can withstand harsh environmental conditions. As originally defined by Sherman (1937), enterococci can grow at 10 and 45°C, at pH 9.6, in 6.5% NaCl broth, and survive at 60°C for 30 min. *E. faecalis* can adapt to adverse conditions: Following pre-exposure to sublethal stress conditions, *E. faecalis* becomes less sensitive to normally lethal levels of sodium dodecyl sulfate, bile salts, hyperosmolarity, heat, ethanol, hydrogen peroxide, acidity, and alkalinity. Furthermore, 'cross-protection' is pronounced against diverse challenges. Starving *E. faecalis* cells maintain their viability for extended periods and become resistant to UV irradiation, heat, sodium hypochlorite, hydrogen peroxide, ethanol, and acid (Giard et al., 1996; Hartke et al., 1998). Moreover, *E. faecalis* can enter the viable but non-cultivable (VBNC) state, a survival mechanism adopted by a group of bacteria when exposed to environmental stress, and resuscitate upon returning to favorable conditions (Lleò et al., 2001). The ability of *E. faecalis* to tolerate or adapt to harsh environmental conditions may act as an advantage over other species. It may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments.

E. faecalis can adhere to root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become 1,000 times more resistant to phagocytosis, antibodies, and antimicrobials than non- bio film-producing organisms (Stuart et al., 2006). Upon contamination of the root canal with the bacterium, it can colonize the dentinal walls, adhering to the mineral part, probably through lipoteichoic acids (LTA), and to the collagen through Aggregation substance (AS) and other surface adhesins. These surface adhesins 'Ace', which is expressed by the bacterium under disease conditions and particularly under stress (Rich et al., 1999). Bacteria face a variety of stressful conditions in the root canal, such as nutrient deficiency, toxins of other bacteria, and endodontic medicaments. These conditions may modulate the adhesion expression of the bacterium. In addition, leakage of serum into the root canal can induce the expression of aggregation

substance (AS) and other carbohydrate moieties, thereby increasing the adhesiveness of the bacterium. Adhesion to dentin and penetration along dentinal tubules by *E. faecalis* may serve as a means of protection from endodontic medicaments.

Another mechanism by which *E. faecalis* survives may be through Lipoteichoic acids (LTA), which has been associated with resistance of the bacterium against a variety of lethal conditions. Since *E. faecalis* suppresses the growth of other bacteria with its cytolysin, AS-48 (Aggregation substance), and other bacteriocins, among the target cells of cytolysin are the erythrocytes, PMNs and macrophages, and a broad range of Gram-positive, but not Gram-negative organisms (Jackson RW (1971). It has been hypothesized that if the bacteriocin effect of cytolysin of *E. faecalis* favors colonization of the Gram-negatives, there could be a shift to a bacterial flora usually associated with periodontal disease (Jett and Gilmore, 1990)

The root canal is hardly a nutrient-rich medium, but *E. faecalis* may derive the energy it needs from the hyaluronan present in the dentin through degradation by hyaluronidase. *E. faecalis* may also feed on serum components present in the fluid in the dentinal tubules. Moreover, an inadequate apical seal of root fillings may allow serum to flow into the root canal. Therefore, it seems that, even in a well-debrided and coronally well-sealed root canal, remaining or arriving cells of E. faecalis may still grow and utilize local sources of energy and nutrients. Production of extracellular superoxide and release of the lytic enzymes gelatinase and hyaluronidase and the toxin cytolysin by *E. faecalis* can cause direct damage in the dentinal as well as in the periapical tissues. In contrast, *E. faecalis* can also induce host-mediated tissue damage in the periradicular tissues. Since cells of *E. faecalis* in the dentinal tubules cannot be reached and eliminated by the cells of the host defense system, they may elicit a permanent provocative effect on these cells. PMN leukocytes, lymphocytes, monocytes, and macrophages are stimulated by a group of virulence factors of *E. faecalis*, which will contribute to the periradicular damage.

E. FAECALIS AND BIOFILIM FORMATION

Many microorganisms are able to form surface-attached microbial communities, known as biofilms. An immature biofilm of *E. faecalis* 12 h on cellulose filters showed variation in the number of viable cells eluted from the biofilm, whereas a pseudo-steady state was developed and maintained from 12 to 96 h. During this time period, the number of cells attached to the biofilm and those shed to the perfusates was constant (Foley and Gilbert, 2001). In contrast Lima et al. (2001) found that 3-day-old biofilms lost more cells than the number of adhering cells. Anaerobic conditions or the presence of 5% CO_2 did not have an effect on the adhesion of enterococci to the microtiter polystyrene plates, with the exception of *Enterococcus hirae*. However, the presence of carbohydrates in the medium would strongly increase the biofilm formation of *E. faecalis. E. faecalis* had a greater ability to adhere to the microtiter polystyrene plates and form a biofilm than *E. faecium* (Baldassarri et al., 2001). With maturation, biofilms on cellulose filters showed a decreased susceptibility to antibiotics and a reduced growth rate than planktonic cultures. Further, biofilms were resistant to vancomycin in a concentration of 4x minimum inhibitory concentration (MIC).

Lima et al. (2001) tested the effect of different chlorhexidine- or antibiotic-containing medicaments on 1or 3 day biofilms on cellulose nitrate membrane filters of *E. faecalis*. In the presence of clindamycin or clindamycin combined with metronidazole, the number of cells was reduced in the 1 day biofilm. Furthermore, chlorhexidine-containing medicaments were able to reduce strongly the number of bacterial cells of *E. faecalis* in the 1 and 3 day biofilm. Spratt et al. (2001) showed that 2.25% NaOCl was the most effective medicament on a 2 day old *E. faecalis* biofilm, whereas 10% povidone iodine (Betadine) required 60 min to eliminate 100% of the cells, and in the presence of 0.2% chlorhexidine gluconate most of the cells survived after 60 min.

Quorum sensing occurs when a bacterial population produces a signal via an auto inducing peptide (AIP), regulated by a two-component system. AIP then accumulates in the environment by increased expression of the communication signal, or by increased numbers of cells producing the signal. Once the AIP reaches a threshold concentration, it interacts with a cell-surface receptor or reenters the cell and causes a cascade of transcriptional regulation (Alksne and Projan, 2000).

ADHESION OF *E. FAECALIS* TO DENTINAL TUBULES

Although the mechanism of bacterial invasion is not completely understood, bacterial adhesion to dentinal tubule walls (TWs) is a logical early step in the process. Adherence is considered to be the first step for bacterial colonization of host tissue, including tubule invasion, and is mediated by bacterial specific cell-surface components (adhesins) (Patti et al., 1994). Collagen is widely considered to be the primary substrate for specific binding of *E. faecalis* to dentine, and the collagen binding protein of *E. faecalis* (Ace) and a serine protease (Spr) have been proposed to play significant roles in binding to the root canal wall (Nallapareddy et al., 2000; Hubble et al., 2003). Ace also promotes E. faecalis binding to collagen type I and in vitro ace gene expression at 37C was enhanced in the presence of collagen (Nallapareddy and Murray, 2006). The ability of *E. faecalis* to grow as chains has been suggested as another explanation for the moderate to high extent of tubule invasion. After initial attachment to

the poorly non mineralized pre-dentine at the tubule orifices, deeper penetration may not require specific binding as invasion may result from intratubular cell growth (Love and Jenkinson, 2002).

STEPS THAT CAN BE CONSIDERED FOR THE ELIMINATION AND PREVENTION *OF E. FAECALIS*

Treatment regimens should aim at prevention and elimination of *E. faecalis* during treatment in between appointments and after completion of treatment. We can prevent its re-entry by following certain norms. That includes, ensuring that the patient rinses with 0.2% chlorhexidine prior to the 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. A study by Sukawat et al. (2002) suggested a combination of calcium hydroxide mixed with camphorated paramonochlorophenol completely eliminated *E. faecalis* within dentinal tubules. Mickel et al. (2003) showed the addition of stannous fluoride to calcium hydroxide is also more effective than calcium hydroxide by itself .Gomes et al. (2003) concluded that Two percent chlorhexidine gel combined with calcium hydroxide achieves a pH of 12.8 and can completely eliminate *E. faecalis* within dentinal tubules. Shur et al. (2003) chlorhexidine-impregnated and iodoform-containing gutta-percha points have shown little inhibitory action against *E. faecalis*. In Nagayoshi et al. (2004), ozonated water has been shown to have the same antimicrobial efficacy as 2.5% sodium Hypochlorite. In Mickel et al. (2003), 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* as compared to other sealers. Saleh et al. (2004) concluded that AH Plus and Grossman's sealer are effective in killing *E. faecalis* within infected dentinal tubules.

So, the following treatment protocol can be followed to eliminate *E. faecalis* from root canal: Adequate apical preparation; use of canal irrigants such as (6% sodium hypochlorite, 17% EDTA and 2% chlorhexidine); use of intracanal medicaments such as (2% chlorhexidine gel or 2% chlorhexidine gel + calcium hydroxide); Considering AH plus or Grossman's sealer and Proper coronal seal are given.

CALCIUM HYDROXIDE, AN INACTIVE INTRACANAL MEDICAMENT AGAINST *E. FAECALIS*

Calcium hydroxide, a commonly used intracanal medicament, has been shown to be ineffective in killing *E. faecalis* on its own, especially when a high pH is not maintained (Lin et al., 2003; Tronstad et al., 1981). 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 (McHugh et al., 2004). 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 (Portenier et al., 2001).

NEWER STRATEGIES OF TREATMENT AGAINST *E. FAECALIS*

MTAD. Bio PureMTAD

MTAD. Bio PureMTAD (Dentsply, Tulsa, OK) is a mixture of a tetracycline isomer, an acetic acid, and Tween 80 detergent (MTAD) was designed to be used as a final root canal rinse before obturation (Torabinejad et al., 2003). MTAD mixture is effective against *E. faecalis*, and it is also less cytotoxic than a range of endodontic medicaments, including eugenol, hydrogen peroxide (3%), EDTA, and calcium hydroxide paste. Newberry et al. (2007) determined the antimicrobial effect of MTAD as a final irrigant on eight strains of *E. faecalis*. After irrigating with 1.3% NaOCl, the root canal and the external surfaces were exposed to MTAD for 5 min. Roots or dentin shavings were cultured to determine the growth of *E. faecalis*. The results showed that this treatment regimen was effective in completely eliminating growth in seven or eight strains of *E. faecalis* (Zhang et al., 2003).

Tetraclean

Tetraclean (Ogna Laboratori Farmaceutici, Muggio (Mi), Italy), like MTAD, is a mixture of an antibiotic, an acid and a detergent. However, the concentration of the antibiotic, doxycycline (50 mg/mL), and the type of detergent (polypropylene glycol) differ from those of MTAD (Giardino et al., 2006). In another study, they compared the antimicrobial efficacy of 5.25% NaOCl, MTAD and Tetraclean against an *E. faecalis* biofilm generated on cellulose nitrate membrane filters. Only the NaOCl could

disaggregate and remove the biofilm at every time interval tested although treatment with Tetraclean caused a high degree of biofilm disaggregation at each time interval when compared with MTAD (Giardino et al., 2007).

QMiX

QMiX is a novel endodontic irrigant for smear layer removal with added antimicrobial agents. It contains EDTA, CHX and a detergent. QMiX is a clear solution, ready to use with no chair-side mixing. Mixing EDTA and CHX is known to produce a white precipitat. In QMiX, this is avoided because of its chemical design. Another recent concern in endodontic irrigation is a potentially carcinogenic precipitate between sodium hypochlorite and CHX. A recent study by Ma et al. (2011) showed QMiX to be as effective as 6% sodium hypochlorite against *E. faecalis* in dentinal tubules.

Electrochemically activated solutions

Electrochemically activated (ECA) solutions are produced from tap water and low-concentrated salt solutions. The ECA technology represents a new scientific paradigm developed by Russian scientists at the All-Russian Institute for Medical Engineering (Moscow, Russia, CIS). Principle of ECA is transferring liquids into a metastable state via an electrochemical unipolar (anode or cathode) action through the use of an element/reactor ("Flow-through Electrolytic Module" or FEM). ECA is showing promising results due to ease of removal of debris and smear layer, nontoxic and efficient in apical one third of canal. It has a potential to be an efficient root canal irrigant (Solovyeva and Dummer, 2000).

Ozone

Ozone is a very powerful bactericide that can kill micro-organisms effectively. It is an unstable gas, capable of oxidizing any biological entity. It was reported that ozone at low concentration, 0.1 ppm, is sufficient to inactivate bacterial cells including their spores (Broadwater et al., 1973).

Photon-activated disinfection

The use of photodynamic therapy (PDT) for the inactivation of microorganisms was first shown by Oscar Raab who reported the lethal effect of acridine hydrochloride on Paramecia caudatum (Raab, 1900). PDT is based on the concept that nontoxic photosensitizers can be preferentially localized in certain tissues and subsequently activated by light of the appropriate wavelength to generate singlet oxygen and free radicals that are cytotoxic to cells of the target tissue (Dougherty et al., 1998). Methylene blue (MB) is a well-established photosensitizer that has been used in PDT for targeting various gram-positive and gram-negative oral bacteria and was previously used to study the effect of PDT on endodontic disinfection (Harris et al., 2005). Several studies have shown incomplete destruction of oral biofilms using MB-mediated PDT due to reduced penetration of the photosensitizer. Soukos et al. (2003) used the combined effect of MB and red light (665 nm) exhibited up to 97% reduction of bacterial viability. Pagonis et al. (2010) studied the in vitro effects of poly (lacticcoglycolic acid) (PLGA) nanoparticles loaded with the photosensitizer methylene blue (MB) and light against *E. faecalis* (ATCC, 29212). The study showed that utilization of PLGA nanoparticles encapsulated with photoactive drugs may be a promising adjunct in antimicrobial endodontic treatment.

HERBAL IRRIGANTS AGAINST *E. FAECALIS*

A wide variety of synthetic antimicrobial agents have been used over the years as endodontic irrigants against *E. faecalis*. Because of the increased antibiotic resistance to these antimicrobial agents, toxic and harmful side effects of few common antibacterial agents, there is a need for alternative agents which are affordable, non-toxic and effective. It has been found that natural plant extracts could be used as effective endodontic irrigants (Sharad et al. (n.d.). The major advantages of herbal irrigants are safety, easy availability, increased shelf life, cost effectiveness and lack of microbial resistance so far. The *in vitro* studies conducted so far have shown that herbs can have a promising role as root canal irrigants.

Propolis

This is prepared from resins collected by bees from trees of poplars and conifers or from flowers of genera clusia. It also contains viscidone. It is used as intracanal medicaments, root canal irrigant. In a study conducted by Al-Qathami and Al-Madi (2003), the antimicrobial efficacy of propolis, sodium hypochlorite and saline as endodontic irrigants was compared and it was found that propolis showed antimicrobial activity equal to that of sodium hypochlorite. Another study by Sudha and Deepak (2012) confirmed the antibacterial action of propolis against *E. faecalis*.

Morinda citrifolia (noni)

M. citrifolia also known as Indian mulberry has a wide range of uses due to its biocompatibility, and anti-bacterial, anti-inflammatory, anti-viral, anti-oxidant and

analgesic effects. It is one of the first herbal alternatives given for an intra-canal irrigant. In a study conducted by Prabhakar et al (2013), *M. citrifolia* was compared with Chlorhexidine as anti-microbial endodontic irrigants. From this study, *M. citrifolia* was found to have significant antibacterial activity which is attributed to its contents of alizarin, scopoletin, aucubin and asperuloside. *M. citrifolia* juice and $Ca(OH)_2$ has been compared on *E. faecalis* infected root canal dentin at two different depths and three intervals. It was concluded that it was effective against *E. faecalis* in dentin on extracted teeth.

Acacia nilotica (Babool)

A.nilotica also known as the gum Arabic tree, possesses good anti-microbial anti-oxidant, anti-fungal, anti-viral and antibiotic activity. It has been shown by Rosina et al. (2009) that this tree possesses anti-bacterial activity against *Streptococcus mutans* and *E. faecalis*. In another study by Dhanya and Preena (2010), extracts of liquorice, clove, cinnamon, babool were investigated for their anti-microbial activity. It was shown that babool at a concentration of 50% had the highest activity against *E. faecalis*.

Curcuma longa (turmeric)

Curcumin, a member of a ginger family possesses anti-inflammatory, anti-oxidant, anti-microbial and anti-cancer activity. In *an in vitro* study conducted by Prasanna et al. (2011), it was shown that curcumin has significant anti-bacterial activity against *E. faecalis* and can be used as an alternative to sodium hypochlorite for root canal irrigation. Thus, this herb can be used especially in endodontics for root canal failure cases.

Azadirachta indica (neem)

Neem's anti-viral, anti-fungal, anti-bacterial and anti-carcinogenic activity makes it a potential agent for root canal irrigation. Neem leaf extract is also used to treat dental plaque and gingivitis. Being a bio-compatible anti-oxidant, use of neem is advantageous as it is not likely to cause the severe harms to patients that might occur through sodium hypochlorite accidents. Aarati et al. (2011) observed that ethanolic extract of neem had significant anti-microbial activity against *E. faecalis*.

Aloe vera (*Aloe barbadensis* miller)

Aloe vera possesses good anti-bacterial and anti-fungal activity. In a study conducted by Sureshchandra and Kumar (n.d.), anti-microbial effect of water, alcohol, chloroform extracts of aloe vera gel were investigated and it was found that chloroform extract of aloe vera had significant anti-microbial effect against *E. faecalis*. It also has been found to be effective against the resistant microorganisms commonly found in the pulp.

Triphala and green tea polyphenols

Triphala's fruit is rich in citric acid which helps in removing the smear layer. Its chelating property makes it an effective alternative to sodium hypochlorite for root canal irrigation. Green tea polyphenols have significant anti-oxidant, anti-cariogenic, anti-inflammatory, thermogenic, probiotic and anti-microbial properties. Triphala and Green tea polyphenols are preferred over the traditional root canals irrigants due to their curative properties such as anti-oxidant, anti-inflammatory and radical scavenging activities. In an *in vitro* study conducted by Prabhakar et al. (2010), Triphala and green tea polyphenols were found to have significant anti-microbial activity against *E. faecalis* biofilm formed in tooth substrate. In another study by Madhu et al. (2011), antimicrobial efficiency of triphala, gren tea polyphenols and 3% sodium hypochlorite were compared against and *E. faecalis* and it was observed that triphala and green tea polyphenols showed significantly better antibacterial activity against 2 week biofilm.

German chamomile and tea tree oil

German chamomile has anti-inflammatory, analgesic and anti-microbial properties. Tea tree oil also has many properties such as being an antiseptic, anti-fungal agent, anti-bacterial and a mild solvent. The active component in tea tree oil is terpinen-4-ol which is responsible for the above properties. In a SEM study conducted to overcome the undesirable effects of sodium hypochlorite, it was observed that chamomile when used as an irrigant was more effective in removing smear layer when compared to sodium hypochlorite used alone but less effective than sodium hypochlorite combined with EDTA (Milind and Nitin, 2006). In another study by Uday et al. (2013), anti-bacterial efficacy of tea tree oil was compared with 3% sodium hypochlorite and 2% chlorhexidine *against E. faecalis*. It was found that maximum anti-microbial activity was shown by chlorhexidine followed by tea tree oil and then sodium hypochlorite.

Aroeira-da-praia and Quixabeira

In an *in vitro* study conducted by Edja (2012), anti-microbial activity and root canal cleaning potential of Aroeira-da-praia and Quixabeira against *E. faecalis* was

evaluated. It was concluded that Aroeira-da-praia showed anti-microbial activity at all concentrations tested whereas Quixabeira was effective only at 100 and 50% concentrations.

Spilanthes calva DC

S. calva DC is an important herb for oral health care. It is nontoxic to human beings and has anti-bacterial and anti-fungal activities. Moulshree (2013) compared the anti-bacterial efficacy of methanolic extract of *S. calva* DC roots with 2% chlorhexidine, 3% sodium hypochlorite and doxicycline at different concentrations against *E. faecalis*. From the study, it was concluded that *S. calva* DC root extract had comparable anti-bacterial activity to sodium hypochlorite. Thus, it may have potential as a root canal irrigant.

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. Eradication of *E. faecalis* from the root canal remains a challenge, while chlorhexidine and combinations of disinfectants show some promise. A better understanding of the role of the virulence factors of *E. faecalis* in endodontic infections may help in the development of new strategies to prevent or eliminate the infection by this species thereby improving treatment results in endodontics.

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