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REVIEW ARTICLE

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Extreme resistance of *Enterococcus faecalis* and its role in endodontic treatment failure

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ABSTRACT

A successful root canal treatment aims at complete elimination of microorganisms from the root canal space, thus preventing chances of reinfection. In spite of the relatively high success rate of endodontic procedures, failures occur. The high incidence of failure is attributed to microbial reinfection by facultative anaerobic microorganisms. The predominant microorganisms considered in secondary infections are *Enterococcus faecalis*. The objective of this review article is to provide a sound understanding of etiology and pathogenesis of the fundamental microbial pathogen. This article also provides an update on the virulence factors of *E. faecalis*, clinical significance, and treatment modalities to combat persistent endodontic infections. In the changing face of community oral care, continued research on *E. faecalis* and its elimination is of crucial importance. It is also likely that health-care professionals will benefit from this review and attain a deeper insight to deal with the highly complex nature of this organism.

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Introduction

Root canal treatment is executed to maintain our natural teeth as long as possible in the oral cavity. This is achieved by thorough debridement and cleaning of the root canal system followed by placement of an inert material. The primary goal of endodontic treatment is to eliminate the infected pulp tissue in order to achieve healing of the periradicular tissues and minimize any possibility of reinfection. Although endodontic treatment has a good success rate of 86%–98%, there is a tremendous impact of multiple variables on the causes for its failure [1].

The occurrence of root canal treatment failure is multifactorial. The common factors associated with failure are persistence of bacteria, inadequate obturation of the canal, overextensions of root filling materials, improper coronal seal, and procedural errors such as poor access cavity design, ledges, perforations, separated instruments, untreated accessory canals, and missed canals [2]. Amongst all these causes of endodontic failure, one of the principal causes is persistent microbiological infection [3]. 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. The predominant microorganisms considered in secondary infections are *Enterococcus faecalis*. This group of bacteria was considered as the most prevalent microorganism isolated in chronic apical periodontitis and periapical lesions refractory to endodontic treatment [4].

Enterococcus faecalis is Gram-positive, catalase-negative, fermentative, non-spore-forming, facultative anaerobic bacteria. Their cells are ovoid and are about 0.5–1 μm in diameter. They occur singly, in pairs, or in short chains and most strains are nonhemolytic and nonmotile [5]. They are considered as a normal commensal member of the gut microbiota, oral cavity, and vaginal vault. Enterococci were traditionally regarded as low-grade pathogens, but their ability of surviving in

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hostile environments and multidrug resistance highlights the need for a greater understanding of this genus. Hence, this review article focuses on the development of virulence factors of the organism, their clinical significance in root canal treatment failures, and novel treatment strategies to eliminate *E. faecalis*.

Factors Contributing to High Virulence and Antimicrobial Resistance of *E. faecalis*

The virulence traits of *E. faecalis* are cell surface-associated protein, namely, enterococcal surface protein (ESP), secreted toxins such as cytolysin, haemolysin, gelatinase, aggregation substance (AS), serine protease, and cell wall polysaccharide. These virulence traits are attributed to pathogenicity islands which are virulence coding genes present on the genome. These genes encode for transposases, transcriptional regulators, and proteins which are known to have potential roles in enhancing virulence [6].

Enterococcal surface protein

ESP is a cell wall-associated protein that enhances the persistence of *E. faecalis*. The high prevalence of ESP within oral isolates suggests that this surface protein may be a potential virulence trait that participates in colonization of different niches of the oral cavity. It promotes biofilm production and helps the organism to adhere to epithelium through mucin or uroplakin [7].

Aggregation substance

It is a pheromone-inducible surface protein of *E. faecalis* which promotes mating aggregate formation during bacterial conjugation. It mediates enterococcal donor–recipient contact to facilitate plasmid transfer. AS promotes adhesion to host cells, increases cell surface hydrophobicity, and resists phagocytosis. Its surface adhesion helps to form a biofilm, which in turn resists alkalinity of chemical disinfectants [8].

Haemolysin

It is a cytolytic protein that causes lysis of human, horse, and rabbit erythrocytes. *Enterococcus faecalis* produces haemolysin, thereby increasing the severity of infection and enhancing virulence [9].

Gelatinase

It is a metalloprotease that is capable of hydrolyzing gelatin, collagen, casein, haemoglobin, and other peptides. *Enterococcus faecalis* contributes to high virulence in endocarditis and escalates mortality rate among patients with bacteremia due to the production of gelatinase. Gelatinase has been detected in endodontic and periodontal infections. Gelatinase helps in the degradation of collagen and fibrinogen, thereby plays a role in the pathogenesis of apical and marginal periodontitis. It also has the ability to produce other collagen-binding proteins such as serine protease and specific gene Ace [9].

Capsular polysaccharide and cell wall carbohydrate

Capsular polysaccharide is a surface-exposed carbohydrate. It consists of glycerol phosphate, glucose, and galactose residues. An operon encoding biosynthesis of capsular polysaccharide is commonly expressed in clinical isolates of *E. faecalis*. This cell wall carbohydrate yielded *E. faecalis* with enhanced susceptibility to phagocytic killing [10].

Other virulence traits

Other variable traits of *E. faecalis* are that it can withstand high temperatures and undergo viable but non-cultivable change under stress and can revert to cultivable form in favorable conditions. This transformation makes *E. faecalis* less sensitive to toxic dosages of heat, acidity, and alkalinity of chemical substances. They also have ability to produce superoxide that enhances its survival in mixed infection. Most cytolytic strains are reactive oxygen species (ROS) associated with superoxide that synergistically destroys mammal tissues. *Enterococcus faecalis* facilitates immune evasion by encoding bacterial surface ESP gene. The ESP gene structure consists of a central core of repeating units. It is hypothesized that the central repeat region acts as a retractable arm and may actually assist in immune evasion [11].

Antimicrobial resistance

Enterococcus faecalis has emerged posing a therapeutic challenge to physicians due to the ease of acquiring and transferring antimicrobial drug resistance. Resistance can be either intrinsic or acquired. Resistance of *E. faecalis* is attributed to the production of inactivating enzymes which is ribosomally mediated or plasmid mediated. They also acquire resistance via mutations in existing DNA or through the acquisition of new DNA. The high-level resistance to most beta-lactam antibiotics is because of its low affinity to penicillin binding proteins (PBPs). *Enterococcus faecalis* produces at least five PBPs, and the expression of the enzyme

and mutations in amino acid sequence have been implicated in higher levels of resistance. These proteins enable them to synthesize cell wall components even in the presence of modest concentration of most beta-lactam antibiotics.

Enterococcus faecalis also shows resistance to cephalosporins which is mediated by CroRS, a two-component signaling pathway that is postulated to alter transcription via a DNA binding domain. These microbes also show resistance to glycopeptides, vancomycin, and teicoplanin which are mediated by the *van* operons. In general, they consist of genes that encode two-component signal transduction systems, which activate the genes responsible for antimicrobial resistance [12].

Clinical Significance of *E. faecalis* in Endodontic Failure

Enterococcus faecalis is the most isolated species from oral infections including marginal periodontitis, infected root canals, and periradicular abscesses. Principal cause for *E. faecalis* to be associated with endodontic failure is its ability to invade dentinal tubules and strongly get adhered to collagen, which is abundantly present in root dentin and cementum. A confocal laser scanning microscope showed that the depth of viable *E. faecalis* ranges from 100 to 400 μm into dentinal tubules, thus resisting eradication and leading to secondary infection [13].

In root-filled teeth, these microorganisms get entombed and change the microenvironment and create favorable conditions for infection. Adhesive moieties such as AS, surface carbohydrates facilitate adherence of organism to type I host collagen, and extracellular matrix proteins present in the dentin. Other corresponding moieties such as gelatinase contribute to bone resorption and degradation of dentinal organic matrix. Hyaluronidase enzyme helps in the degradation of hyaluronan, present in the dentin, to dissacharides and provides energy to the organism. Lipoteichoic acid, superoxide production, and peptide inhibitors each may modulate local inflammatory process by stimulating leukocytes to release several mediators like tumor necrosis factor, interleukins, and prostaglandins. All of which play an important role in the pathogenesis of periapical inflammation [14].

Enterococcus faecalis exists in the nutrient deficit ecological conditions of the root-filled teeth by forming biofilms. Biofilms are more resistant to antibacterial agents, phagocytosis, and antibodies than non-biofilm-producing bacteria. Its ability to produce biofilm under stress promotes *E. faecalis* to survive in variety of adverse environments and withstand antimicrobial effect of intracanal medicaments. One of the most commonly used intracanal medicament is calcium hydroxide. The surface adhesion and biofilm formation resist alkalinity of calcium hydroxide. *Enterococcus faecalis* also has a proton pump mechanism by which it maintains optimal cytoplasmic pH levels and resists antimicrobial effect of calcium hydroxide [15].

Novel Treatment Protocol to Eradicate *Enterococcus faecalis*

A combination of adequate instrumentation, appropriate use of irrigants, medicaments, and sealer will optimize the chances of eradicating *E. faecalis* in endodontic failed cases. Mechanical instrumentation is often the first means of bacterial reduction during endodontic treatment of infected root canals. Several new instrumentation systems with advanced instrument designs, different cross sections, and varying tapers have been developed for root canal preparation. A recent study supports the contention that instruments with a greater taper can play an important role in maximizing the effectiveness of reducing bacterial numbers in the root canals [16]. Novel techniques using single-file systems as well as reciprocating instrumentation have also proven to be effective in reducing microorganisms within the root canal system. Although these techniques involve the use of only one file to perform the root canal therapy, it has been considered effective in reducing the *E. faecalis* biofilm [17].

Recently, a study concluded that there was no significant difference in bacterial count reduction amongst the manual, rotary, and reciprocating techniques and that all systems reduced bacterial counts to a similar level [18]. However, *E. faecalis* can invade dentinal tubules, thus elimination of bacteria can be accomplished by a combination of mechanical instrumentation, irrigants, and antibacterial medicaments.

An array of irrigants has been mentioned in the literature amongst which sodium hypochlorite most frequently used. They have the advantage of pulpal dissolution and antimicrobial effect. Other irrigants such as chlorhexidine has a unique property of substantivity thus persistent antimicrobial effect [19]. Broad spectrum antimicrobial agent such as triclosan, ozonated water, and phytotherapuetic agents such as green tea, curcumin, morindacitrifolia, propolis, azadirachtaindica

(neem leaf extract), and *Acacia nilotica* have also been used [20]. Chelating agents such as ethylenediaminetetraacetic acid, citric acid, and mixture of Doxycycline, citric acid and a detergent that is a mixture of 3% doxycycline, 4.25% citric acid, and detergent, maleic acid, 1-hydroxyethylidene-1, 1-bisphosphonate, and tetraclean have been used to chemically soften the root canal dentine and dissolve the smear layer. These chelating agents increase dentine permeability, thereby facilitating the penetration of the medicaments into the dentinal tubules [21].

Recent advances in bionanotechnology encourage the use of nanoparticles (NPs) in endodontics. Antibacterial NPs show a broad spectrum of antimicrobial activity. The mechanism of action is attributed to the binding of NPs to the targeted bacterial cell membrane through electrostatic forces, causing an alteration in the membrane potential and eventually loss of membrane integrity. NPs also enhance the production of oxygen free radicals such as ROS that can influence survival of the bacterial cell by blocking the protein function, destroying DNA, and resulting in excess radical production [22]. Literature shows the use of metallic NPs such as titanium, gold, zinc, and copper. Non-metallic NPs such as chitosan which is polycationic and is derived from the chitin exoskeletons of arthropods is also used [23].

Gold NPs (GNPs) are self-therapeutics nanomaterials. The interaction between these metallic NPs and the target cells occurs in an extremely short time because of the large surface-area-to-volume ratio. The conjugation of functional ligands onto the surface allows for direct multivalent interactions [24]. Such an approach could negate the drawbacks of the encapsulation of current antimicrobials due to its low toxicity and, hence, prevent the development of bacterial resistance. Nano-photothermal therapy mediated by GNPs revealed promising results against *E. faecalis* [25].

Other treatment modalities include light amplification by stimulated emission of radiation (lasers). They help to clean and disinfect root canals system while eliminating highly resistant species such as *E. faecalis*. Erbium-doped yttrium aluminium garnet laser has been suggested as an effective laser in canal disinfection since it has the highest absorption level in water. Neodymium-doped yttrium aluminum garnet lasers have shown to be able to eliminate 99.16% of *E. faecalis* bacterial population. Diode laser with 810 and 980 nm wavelength was effective in sealing dentinal tubules and eliminate bacteria that have penetrated to a depth of 500 microns in dentin [26].

Another promising method is phage therapy. It uses bacteriophages against pathogenic bacteria. A bacteriophage or virus phage is used to target and destroy disease-causing bacteria by invading bacterial cells, disrupting their metabolism, and causing lysis. Biofilm destruction by phages is much more efficient when compared with antibiotics. Phage therapy was tested on *E. faecalis* biofilms and in post-treated root canal infections. It revealed that in retreatment cases, there was a dramatic reduction of bacterial load. Confocal microscopy images demonstrated that dead bacteria was evident in the dentinal tubules of phage-treated teeth [27].

Conclusion

Enterococcus faecalis possesses several virulence factors and is the prime cause for endodontic failures. 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. Adequate asepsis, instrumentation, use of antibacterial agents will optimize the chances of targeting *E. faecalis*. Consequently, the combinations of therapeutic agents and advanced technology can benefit the host by reducing the chances of recurrent infections. Continued research awaits newer challenging measures to combat *E. faecalis*.

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