REVIEW ARTICLE

International Research Journal of Pharmaceutical and Biosciences

Pri -ISSN: 2394 - 5826

http://www.irjpbs.com **e-ISSN: 2394 - 5834**

Enterococcus faecalis: An Enigma in Root Canal Infections

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Article info

Article history:
Received 22 JAN 2016
Accepted 31 JAN 2016

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Abstract

Enterococci are considered opportunistic human pathogens. The two most important species; *E. faecalis* and *E. faecium*, which are considered part of the normal intestinal flora, are among the leading causes of root canal treatment failures as well as nosocomial infections. These infections are often difficult to treat due to the increased antibiotic resistance associated with this microorganism. The recent increase of vancomycin-resistant strains in clinical isolates is especially a cause of serious concern, because this glycopeptide-type antibiotic often remains the last treatment available in life-threatening infections. The dramatic increase in antibiotic resistance of *Enterococcus* species worldwide highlights the need for a greater understanding of this genus, including its ecology, epidemiology and virulence.

Key words: antibiotic resistance, *Enterococcus faecalis*, endodontic treatment, root canal infections, virulence factors.

INTRODUCTION

For many years *Enterococcus* species were believed to be harmless to humans and considered unimportant medically. However, more recently *Enterococcus* species have become one of the most common nosocomial pathogens, with patients having a high mortality rate of up to 61% [1]. These infections are often difficult to treat due to the increased antibiotic resistance associated with this microorganism [2]. Resistance rates of *Enterococcus* species have reached endemic or epidemic proportions in North America and Europe [3].

Studies have shown a significant association of *Enteroccocus faecalis* with the incidence of root canal treatment failures. 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 [4]. It is generally believed that the major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth [5]. 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 [6]. *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. The purpose of this review article is to discuss about the characteristics features, virulence factors and the eradication methods of *Enterococcus faecalis*.

TAXONOMY

The genus *Enterococcus* consists of Gram-positive, catalase negative, non-spore forming, facultative anaerobic bacteria that can occur both as single cocci and in chains. *Enterococci* belong to a group of microorganisms known as lactic acid bacteria (LAB) that produce bacteriocins [7]. In 1937, Sherman classified *Streptococcus* species into four subgroups: faecal S*treptococci (Enterococci)*, dairy S*treptococci*, viridans group and pyogenous S*treptococci*. In 1984, through the use of DNA hybridization and 16S rRNA sequencing, it was established that the species *Streptococcus faecium* and *Streptococcus faecalis* were sufficiently distinct from the other S*treptococci* group and were designated under another genus: *Enterococcus* [8].

PHYSIOLOGY

Enterococcus species will grow at a wide range of temperatures from 5 to 50 ^{0}C. The optimum, minimum and maximum temperatures, according to the Rosso model, are 42.7, 6.5 and 47.8 ^{0}C, respectively, on brain heart infusion (BHI) agar. Trypticase soy agar or Columbia agar with 5% (v/v) defibrinated sheep blood may be used to assess the haemolysis produced by E*nterococci*. If human or horse blood is used, haemolysis is based on cytolysin activity and causes a β-haemolytic reaction [9]. *E. faecalis* and *E. faecium* will grow in a wide range of pH (4.6–9.9), with the optimum being 7.5. They will also tolerate and grow in presence of 40% (w/v) bile salts. *E. faecalis* is able to grow in 6.5% NaCl and has a cation homeostasis which is thought to contribute to its high resistance to pH, salt, metals and even desiccation [10].

The resistance of *E. faecalis* to a range of pH values is thought to be due to its membrane durability and impermeabilty to acid and alkali, although some studies have suggested that it may be associated with membrane bound H^+-ATPase activity [11]. Temperature resistance is also associated with membrane structure and has been related to lipid and fatty acid content. The membrane has been demonstrated to be more stable near the minimal temperature for growth, which is a specific mechanism associated with E*nterococci* [12]. At higher temperatures E*nterococci* are less resilient, with the membrane fatty acid content increasing and the saturated fatty acid levels decreasing. The heat resistance of E*nterococci* is dependent not only on the temperature but also the phase of growth [10].

ANTIBIOTIC RESISTANCE

The antibiotic resistance of *Enterococcus* is well documented. Bacteria may show resistance to glycopeptides such as vancomycin, teicoplanin and aminoglycosides [13]. Antibiotic resistance has been of growing concern for a number of years. It has been reported that if glycopeptides resistant enterococci are present in an infected patient rather than an antibiotic-susceptible strain, clinical treatment failure is increased by 20% and mortality is increased from 27% to 52% [14].

When assessing the studies on E*nterococcal* antibiotic resistance, the pattern that is emerging is the possible occurrence of multidrug resistant strains. Glycopeptide resistance in E*nterococci* involves a two component system where the cell wall composition is altered from the peptidoglycan precurser D-Ala-D-Ala (vancomycin-susceptible) to D-Ala-D-lactate. The latter has 1000 times less affinity for vancomycin, while DAla- D-Ser has a sevenfold decrease in affinity for vancomycin, thus removing the susceptible target. The genes involved in this two-component system are vanS/vanR. The VanS sensor kinase is activated in response to vancomycin, resulting in the activation of DLac or D-Ser peptidoglycan precursor and the repression of D-Ala-D-Ala [15].

To date six gene clusters associated with glycopeptide resistance have been identified in *Enterococcus* species: vanA to vanG. The three main types of resistance are those encoded by the vanC, vanA and vanB clusters. Intrinsic vanC resistance is specific to *E. gallinarum, E. casseliflavus* and *E. flavescens*, and the vanC operon is chromosomally located and is not transferable. The vanA resistance operon comprises seven genes (vanH, vanA, vanX, vanR, vanS, vanY and vanZ) and is acquired through the Tn1546 transposon. The transfer of vanB resistance occurs through the exchange of transposon Tn1547 and/or Tn5382. Both vanA and vanB are present on the chromosome but can also be carried on a plasmid [16].

SURVIVAL AND VIRULENCE FACTORS

E. faecalis possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid [17]. It has been shown to adhere to host cells,

express proteins that allow it to compete with other bacterial cells, and alter host responses [18]. *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 [19].

The aggregation substance (Agg) on the surface of *E. faecalis*, has been shown in vivo to form large aggregates and hence may contribute to pathogenesis. The presence of Agg increases the hydrophobicity of the E*nterococcal* cell surface. This induces localization of cholesterol to the phagosomes and is thought to delay or prevent fusion with lysosomal vesicles [20]. Agg is a pheromone-inducible surface glycoprotein and mediates aggregate formation during conjugation, thus aiding in plasmid transfer as well as adhesion to an array of eukaryotic surfaces. Another cell-surface protein present in *E. faecalis* is Ace. This is a collagen-binding protein, belonging to the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family. Ace may play a role in the pathogenesis of endocarditis.

Extracellular surface protein (Esp) is a cell-wall-associated protein first described in *Enterococcus* species by Shankar *et al*. It is thought to promote adhesion, colonization and evasion of the immune system, and to play some role in antibiotic resistance and biofilm formation. *E. faecalis* strains that carry the gene esp have higher conjugation rates than strains that do not possess this gene. They also demonstrate higher resistance to ampicillin, ciprofloxacin and imipenem [8].

The ability of E*nterococci* to produce biofilms is fundamental in causing endodontic and urinary tract infections, as well as endocarditis. The formation of pili by E*nterococci* is necessary for biofilm formation, the gene cluster associated with this being ebp (endocarditis- and biofilm-associated pili). The ebp operon consists of ebpA, ebpB, ebpC and an associated srtC (encoding sortase C) gene [21]. A non-piliated mutant of *E. faecalis* was unable to produce a biofilm. *Enterococcal* pili are heterotrimetric and the pilus shaft contains two minor pilins [22].

Cytolysin is a bacterial toxin, the genes for the production of which are located on pheromone-responsive plasmids. Cytolysin has a β-haemolytic action in humans and is bactericidal against other Gram-positive bacteria [23]. Cytolysin is regulated by a quorum-sensing mechanism involving a two-component system. A group of hydrolytic enzymes including hyaluronidases, gelatinase and serine protease are involved in the virulence of *Enterococcus* species. Hyaluronidase acts on hyaluronic acid and is a degradative enzyme which is associated with tissue damage. Hyaluronidase depolymerizes mucopolysaccharide moiety of connective tissue, thus facilitating spread of E*nterococci* as well as their toxins through host tissue [24].

ENTEROCOCCUS FAECALIS IN ROOT CANAL INFECTIONS

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 [17]. Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% [25].

E. faecalis overcomes the challenges of survival within the root canal system in several ways. It has been shown to exhibit widespread genetic polymorphisms [26]. It possesses serine protease, gelatinase, and collagen-binding protein, which help it bind to dentin [27]. It is small enough to proficiently invade and live within dentinal tubules [18]. 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. *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days [28]. *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.

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. The following reasons have been proposed to explain why *E. faecalis* is able to survive intracanal treatment with calcium hydroxide[29]:

(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. 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 [28]. Diverse components of dentin including dentin matrix, type-I collagen, hydroxyapatite, and serum are responsible for altering the antibacterial effects of these medicaments.

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. 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 [30]. 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 [31]. 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 [32]. 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*.

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 [33]. 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. 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.

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 [34]. A 2 min rinse of 2% chlorhexidine liquid can be used to remove *E. faecalis* from the superficial layers of dentinal tubules. Two percent chlorhexidine gel is effective at completely eliminating *E. faecalis* from dentinal tubules for up to 15 days [25]. This may be in part attributed to its substantive antimicrobial activity.

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 [35]. 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 [36]. 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.

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 [34]. 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 [35].

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 [37]. Other possibilities may include using an obturating system that can provide a more effective seal. Newer obturation systems such as Epiphany (Pentron Corp., Wallingford, CA) have been designed to bond to the root canal walls and thus prevent bacterial leakage. Although research is still needed, a preliminary pilot study shows that this system is better at preventing microleakage of *E. faecalis* than gutta-percha filled canals. A well-sealed coronal restoration and root canal filling are important steps in preventing bacteria from entering the canal space provides steps that can be used to eliminate *E. faecalis* during endodontic retreatment.

CONCLUSION

Studies indicate that the prevalence of *E. faecalis* is low in primary endodontic infections and high in persistent infections. Our challenge as endodontic specialists is to implement methods to effectively eliminate this microorganism during and after root canal treatment. 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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