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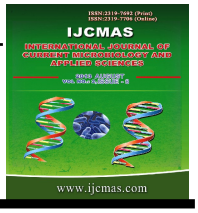

Review Article

Role of *Enterococci faecalis* in failure of Endodontic treatment

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A B S T R A C T

Enterococcus faecalis is assosciated with a significant number of refractory endodontic infections. Studies report a prevalence of *Enterococcus faecalis* ranging from 24% upto 77% in teeth with failed endodontic treatment. The origin of the micro-organism remains unclear, as enterococci do not belong to the normal oral microflora. The survival and virulence factors possessed by *Enterococcus faecalis* including its ability to compete with other microorganisms , invade dentinal tubules and resist nutritional deprivation. Oral *Enterococcus faecalis* may be a potential reservoir for the transferable elements of virulence and antimicrobial resistance. Future advances in endodontic microbiology will take us towards complete microbial elimination for successful root canal treatment.

Keywords

Enterococcus faecalis; virulence factors; root canal treatment.

Introduction

The resident microbial flora in the oral cavity typically contains 10^{10} bacteria (Mims *et al*., 2005). However, only 150 microbial species have been isolated and cultured from root canals. The endodontium is a sterile cavity and the invasion of oral microbes to establish infection is by the penetration to enamel and dentine and overcome the host responses (Bergenholtz, 1974). Although all the bacteria in the oral cavity can invade the root canal, only a few microbes have been identified in infected root canals (Miller, 1994; Sundqvist, 1994; Wilkins *et al.*, 2003). Endodontic infections with *Enterococcus faecalis* are probably not derived from patients own microflora, which indicates that in these infections *Enterococcus faecalis* is of exogenous origin (Vidana *et al.*, 2011).

Enterococci are normal human commensals adapted to the nutrient –rich, oxygen depleted and ecologically complex environment of the oral cavity, GIT and the vagina . Enterococci now rank among the top three nosocomial bacterial pathogens (Richards *et al*., 2000; Wisplinghoff *et al*., 2003). Up to 90% of enterococcal infections in humans are caused by *Enterococcus faecalis* (Kayaoglu and Orstavik, 2004).

The objective of this article is to describe characteristics inherent to *Enterococcus*

faecalis and as an etiology in failing root canal treatment.

Characteristics of *Enterococcus faecalis*

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. Enterococci can withstand harsh environmental conditions. Enterococci can grow at 10^0 C and 45^0 C at pH 9.6 in 6.5% NaCl broth and survive at 60^0C for 30 minutes (Sherman, 1937). This may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments.

There are currently 23 enterococci species and they are divided into five groups based on their interaction with mannitol, sorbose and arginine. Recently molecular methods PCR amplification assays have been used (Facklam RR *et al*., 2002). Random amplified polymorphic DNA (RAPD) analysis and pulse-field gel electrophoresis (PGGE) have been used to determine various *E.faecalis* subtypes (Dauttle *et al*., 2002).

Virulence factors

The factors most extensively studied are aggregation substance, surface adhesions, sex pheromones, lipoteichoic acid , extracellular superoxide, gelatinase, hyaluronidase and cytolysin (hemolysin).

Aggregation Substance (AS)

It is a pheromone –responsive, plasmid encoded bacterial adhesion that mediates efficient contact between donor and recipient bacterium, facilitating plasmid exchange(Zoletti *et al*., 2011). *E. faecalis* has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells and alter host responses (Love, 2001). As has frequently been detected in clinical isolates (Elsner *et al*., 2000) but is rarely found among clinical isolates from healthy volunteers (Coque *et al*., 1995) suggesting a possible role for AS in in human enterococcal infections. In a recent study of characterization of virulence factors and clonal diversity of *Enterococcus 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 (Toledo-Arana *et al*., 2001). Esp is associated with promotion of primary attachment and biofilm formation of *Enterococcus 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 (Nallapareddy *et al*., 2000a). The disruption of the ace gene impaired the conditional binding of *Enterococcus faecalis* to the extracellularmatrix proteins (Nallapareddy *et al*., 2000a). Serum invasion of dentinal tubules by *Enterococcus faecalis* was suggested while other test species *Streptococcus gordonni* DLI and *Streptococcus mutans* WG8 was inhibited.

Sex Pheromones

Production of the sex pheromones by strains of *Enterococcus faecalis* and its bacterial clumping inducing effect was

first described by (Danny *et al*., 1978) . It was subsequently shown that antibiotic resistance and other virulence traits, like cytolysin production can be passed in strains of *Enterococcus faecalis* by sex pheromone system (Clewell and Weaver, 1989). Some of *Enterococcus faecalis* sex pheromones were found to be chemotactic for human neutrophils (Sannomiya *et al*., 1990). A strong assosciation between gingival crevicular fluid neutrophilic lysosomal enzymes and chronic periodontal disease have been found (Buchmann *et al* ., 2002). In a study on oral enterococci response to pheromones in *Enterococcus faecalis* culture filtrate was seen (Sedgley *et al*. , 2004).

Gelatinase

Enterococcus faecalis posseses gelatinase (Hubble TS et al,2003) which help it bind to dentin and gelatinase levels were elevated in oral rinses, crevicular fluid and whole saliva samples (Makela Met al,1994) and in gingival biopsy specimens(Soell M 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 K et al ,2001).

Cytolysin

Enterococcus faecalis posses cytolysin or hemolysin as a virulence factor. Conflicting studies suggesting the role of cytolysin as a possible virulence factor. Initial studies reported that approximately 60% of *Enterococcus 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 and Elsner *et al*., 2000).

Survival of *Enterococcus faecalis*

E. faecalis is less dependent upon virulence factors, it relies more upon its ability to survive and persist as a pathogen in root canals of teeth (Rocas *et al.*, 2000). It exhibits antibiotic resistance of genes from other microbes or by spontaneous mutation thereby making these microbes recalcitrant to the usual root canal treatments (Mundy *et al.*, 2000). The presence of serine protease and collagen binding protein help in the invasion of *E.faecalis* into the dentinal tubules (Hubble *et al*., 2003).

E. faecalis is also known to possess alkaline tolerance due to cell wall associated proton pump [31] which makes it resistant to the antimicrobial effect of Ca OH (Fabricus *et al*., 1982; Tansiverdi *et al*., 1997). *E. faecalis* is able to form a biofilm that helps it resist destruction by enabling the bacteria to become 1000 times more resistance to phagocytosis, antibodies and antimicrobials than non-biofilm producing organisms (Chavez De Paz Le *et al*., 2003).

Eradication of *Enterococcus faecalis*

Sodium hypochlorite is an effective irrigant for all presentations of *E.faecalis* including its existence as a biofilm (Distel *et al.*, 2002).

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 its preliminary studies (Abdullah M et al,2005). Calcium hydroxide is relatively ineffective against *E.faecalis* because of considerations mentioned previously.

Combination of irrigants to eliminate *E.faecalis* have been used. Erythromycin mixed with Ca OH seems to be a valuable option against monoinfections of enterococci (Shabahang and Torabinejab, 2003). Chlorhexidine has shown to provide a better antimicrobial action against *E. faecalis* (Basrani *et al*., 2002).

The antimicrobial activity against *E. faecalis* of various sealers has also been studied. Roth 811(Roth International Ltd, Chicago) has shown to exhibit the greatest antimicrobial activity against *E. faecalis* as compared to other sealers(Mickel, 2003). Recently antimicrobial efficacy was assessed against clinical isolates of enterococci from persisting root canal infections by using nanometric bioactive glass 45s5, the killing efficacy was higher (Waltimo *et al*., 2007).

A better understanding of the role of the virulence factors of *E.faecalis* in endodontic infections, survival mechanisms that enable it to cause persistent endodontic infections and continued research on *E.faecalis* and its elimination from the dental apparatus will improve treatment results in endocarditis.

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