Enterococcus faecalis; clinical significance & treatment considerations

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ABSTRACT

In the past two decades, research data has shown that, apical periodontitis occurring after root canal treatment presents a more complex etiologic & therapeutic situation than primary apical periodontitis. The long cherished goal of endodontic treatment has been to eliminate infectious agents or to substantially reduce the microbial load from the root canal and to prevent re-infection by root filling. E. faecalis is the most commonly implicated microorganism in asymptomatic persistent endodontic infection. The highly complex nature of the organism poses a great challenge to an endodontist. In this article, the clinical significance of this microbe and various considerations during treatment are emphasized. 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.

INTRODUCTION

In the past few decades research data has shown that apical periodontitis occurring after root canal treatment presents a more complex etiologic & therapeutic situation than primary apical periodontitis. There is a universal consensus that intra-radicular infection is an essential cause of primary as well as a major contributor of post treatment apical periodontitis. Enterococcus faecalis is the most commonly implicated microorganism in asymptomatic persistent infections. The highly complex nature of the organism poses a great challenge for endodontists [1, 2].

It was the purpose of this article to understand the clinical significance of E. faecalis in primary & secondary endodontic infections and to reinstate current approaches & treatment modalities towards combating E. faecalis from root canal system.

NATURE & CHARACTERISTICS

Enterococcus faecalis is Gram positive cocci that occur singly in pairs or in short chains [Table 1]. It is a facultative anaerobe present in small proportion of the flora of untreated canal as a part of polymicrobial flora. It is a predominant bacteria implicated in root canal failures & persistent infections [3, 4, 5]. In post treatment apical periodontitis the prevalence ranges from 24% to 77% [6, 7].

E. faecalis has an ability to survive harsh environments including extreme alkaline pH, salt concentrations. It resists bile salts, detergents, heavy metals, ethanol, azide & desiccation. It can survive a temperature of 60ÚC. The prevalence of E. faecalis in primary endodontic infection is 40% and in Persistent endodontic infection 24 to 77% [7, 8].
SURVIVAL & VIRULENCE FACTORS

E. faecalis endures prolonged period of nutritional deprivation. It binds to dentin and proficiently invades dentinal tubules [9]. It alters the host response and suppresses the action of lymphocytes. It possesses lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid [10]. It utilizes serum as the nutritional source. It resists intracanal medicaments i.e. calcium hydroxide by maintaining pH haemostasis.

Properties of dentin lessen the effect of sodium hypochlorite, chlorhexidine & iodine potassium iodide. It can colonize root canal and survive without the support of other bacteria and competes with other cells. It forms a biofilm that renders it more resistant to phagocytosis, antibodies & antimicrobial agents [11].

ANATOMIC CONSIDERATIONS

Enterococcus faecalis is known to colonize dentinal tubules, isthmus, rami, lateral & accessory canals. As shown by the LCSM (Laser Confocal Scanning Microscopy) & SEM (Scanning Electron Microscopy) analysis, Enterococcus faecalis penetrates the dentinal tubules to the depth of 1483.33 µm (nutrient rich aerobic condition) 1166.66 µm (nutrient rich anaerobic condition) 620 µm (nutrient deprived anaerobic condition). It is present as “mushroom shaped” micro colonies [10].

TREATMENT CONSIDERATIONS FOR ERADICATION OF E. FAECALIS

The long cherished goal of endodontic treatment has been to eliminate infectious agents or substantially reduce the microbial load from the root canal. [Fig. 1-3]

PRE-TREATMENT STEPS

The patient should rinse with chlorhexidine before we enter the root canal space.

Application of rubber dam is mandatory. The tooth & the rubber dam should be disinfected with chlorhexidine & sodium hypochlorite.

CLEANING & SHAPING

Current concepts focus to prepare the apical portion of the root canal to a larger instrument size which will facilitate removal of microorganisms, which otherwise will not be accessible by small MAFs (master apical file) [12]. Larger preparations facilitate removal of the innermost pulpal dentin which in turn removes intratubular dentin to allow antimicrobials to penetrate more effectively [13]. Apical patency of the foramen must be checked to allow irrigants to circulate & vent outwards of the canal [14]. In re-treatment cases, use of chloroform solvent & apical enlargement two sizes larger than original MAF showed significant reduction in cultivable microorganism [15].

ROOT CANAL IRRIGANTS
1. Sodium hypochlorite.

Sodium hypochlorite is an effective irrigant for all presentations of E. faecalis including its existence as biofilm [16]. 0.5 % to full strength of sodium hypochlorite if used in adequate amounts & exchanged regularly has the capability to destroy E. faecalis [17]. Presence of increased amount of organic material increased resistance to medicaments at different physiological growth stages of E. faecalis. Organisms in stationary phase were found to be more resistant to medicaments than those in the growing phase. Cells in starvation phase exhibited maximum resistance to medicaments and root canal irrigants. Starved cells
survived maximum after challenge with chlorhexidine, sodium hypochlorite and calcium hydroxide [18].

2. Chlorhexidine
Chlorhexidine has been shown to be a potent broad spectrum antimicrobial that is effective against Gram +ve & Gram -ve organisms. 2% gel or liquid concentration is effective at reducing or completely eliminating E. faecalis from canal space & dentinal tubules (upto 100 µm) depth & contact of 15 secs [19].

3. MTAD [Tulsa Dentsply]
MTAD (a mixture of tetracycline isomer, acid and detergent) is a formulation of doxycycline, Tween- 80, and citric acid. Its effectiveness is attributed to its anticollagenase activity, low pH & ability to be gradually released over time. It is highly effective against E. faecalis, superior to NaOCl and beneficial for re-treatment. Doxycycline & Citric acid exhibits antimicrobial & acid etching properties [20, 21].

4. COMBINATIONS
Calcium hydroxide & camphorated paramonochlorophenol can completely eliminate E. faecalis. Metapex [Calcium hydroxide & 38% iodoform] provides effective disinfection than when calcium hydroxide used alone. 2% chlorhexidine & calcium hydroxide achieve pH of 12.8 and can completely eliminate E. faecalis. However, chlorhexidine alone is more superior to this combination. Tween-80 itself has limited antibacterial properties and is known to enhance antibacterial properties of other substances. However, it may neutralize antibacterial properties of chlorhexidine & Povidone Iodine.

5. MTAD & CHLORHEXIDINE DIGLUCONATE with / without CETRIMIDE
Disinfecting agents tested in vitro fail to do so in vivo due to different components of dentin as well as other substances in infected root canal can inhibit antimicrobial activity. MTAD contains Tween 80 which facilitates penetration of MTAD into bacterial cell membrane. Synergistic action of chlorhexidine & cetrixidine killed E. faecalis effectively & more immediately after contact than MTAD and chlorhexidine. Dentin delayed antibacterial property of chlorhexidine in presence of BSA (Bovine Serum Albumin)[22]. Chlorhexidine & Cetrimide killed E. faecalis rapidly than chlorhexidine alone and therefore synergistic action was observed. Tetracycline shows bacteriostatic effect by inhibition of cell wall synthesis. Chelating properties and low pH of citric acid in chasing E. faecalis is not known.

6. INTRACANAL MEDICAMENT: CALCIUM HYDROXIDE
E. faecalis is very resistant to calcium hydroxide because of two reasons. First is the “proton pump” which lowers internal pH of the cell & maintains it, thus pH haemostasis is maintained. Secondly at pH of 11.5, E. faecalis is unable to survive, but dentin exhibits inhibitory effect thereby buffering the pH. Currently calcium hydroxide utilization technique of delivery & maintenance needs to be looked upon [23].

SMEAR LAYER REMOVAL
EDTA [ethylene diamine tetraacetic acid] removes inorganic portion of smear layer allowing access of irrigants within dentinal tubules, but has no action against E. faecalis. 10% Citric acid removes smear layer but less effectively. 0.1% sodium benzoate & Citric acid can kill E. faecalis.
Chlorhexidine & sodium hypochlorite should not be used together. Chlorhexidine must be used as inter-appointment irrigant. Biopure MTAD has been suggested as final rinse [24]. Irrigants do not penetrate to more than 100 – 150 microns. Therefore the mesh of dentinal tubules harbouring E. faecalis is impossible to reach.

OTHER IRRIGANTS

Ozonated water & stannous fluoride has been shown to be effective at eliminating E. faecalis. Superoxidized water inactivates many organisms responsible for endodontic infections.

Dermacyn [super oxidized water] differs from others in that it has a neutral pH & shelf life of over 1 year. It is used in medicine as wound cleanser that has broad spectrum antibacterial properties and extremely compatible. Studies showed that it has no ability to prevent growth of E. faecalis. It could be used as cold sterile solution or surface disinfectant [24].

CHAIR-SIDE MONITORS

The possibility of monitoring the chair side bacterial activity is advantageous for the clinician beneficial for the total treatment outcome. Monitoring chair-side bacterial activity provides advantage for the clinician & beneficial for the treatment outcome.

1. Polymerase Chain Reaction is faster, more sensitive & accurate than culturing methods [25]. PCR-based detection methods enable rapid identification of both uncultivable and cultivable microbial species with high specificity and sensitivity. Real-time quantitative PCR (qPCR) and reverse transcription PCR (RT-PCR) are sensitive than traditional cultivation in detecting and quantifying E. faecalis in endodontic infections.

2. Optical spectroscopy detects chair side presence or absence of E. faecalis [26]. Optical spectroscopy in conjunction with specific enzyme-synthetic chromogenic substrate-based medium allows the early detection of E. faecalis activity quantitatively and qualitatively, without the need for additional laboratory based culturing and plating for cell counting.

OBTURATION OF ROOT CANAL

Long term survival of E. faecalis in obturated root canals depends mainly on the type of an endodontic sealer and the microbial gelatinase activity i.e. the virulence trait of the organism [27]. Chlorhexidine impregnated & Iodoform containing GP points have shown little inhibitory action against E. faecalis. AH plus, Grossman sealer, Roth’s 811 (ZnO based sealers) have shown greatest antimicrobial activity against E. faecalis.

Roeko seal [silicon based sealer] polymerizes without shrinkage and renders fluid impermeability either with cold lateral compaction or warm gutta percha. Initial antibacterial activity is short term, exhibits no antibacterial activity.

Real Seal [Resilon] is a synthetic polymer based root canal filling commercially available as Epiphany [Pentron, Sybron Endo] was developed to overcome the GP shortcoming of better sealability. Preliminary studies have shown optimistic and encouraging results when comparing bacterial leakage. However, there is no evidence to date of sealing abilities of Resilon in apical 1/3rd of canal when post space is prepared. There is no significant difference in bacterial leakage of E. faecalis after post space preparation in teeth filled.
### TABLE 1.
Categorization of Enterococcus species and two physiologically related gram positive cocci based on phenotypic characteristics*

<table>
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<th>GROUP</th>
<th>SPECIES</th>
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| I. (+) acid formation in mannitol broth  
(+ ) acid formation in sorbose broth  
(-) arginine hydrolysis | E. avium, E. raffinosus  
E. gilvus, E. saccharolyticus  
E. malodoratus, E. pallens |
| II. (+) acid formation in mannitol broth  
(-) acid formation in sorbose broth  
(+ ) arginine hydrolysis | E. faecalis,  
E. faecium, E. casseliflavus, E.gallinarum, E. mundtii |
| III. (-) acid formation in mannitol broth  
(-) acid formation in sorbose broth  
(+ ) arginine hydrolysis | E. dispar, E. durans, E. hirae,  
E. ratti |
| IV. (-) acid formation in mannitol broth  
(-) acid formation in sorbose broth  
(-) arginine hydrolysis | E. asini, E. cecorum, E. sulfureus |

*Adapted from Teixeira & Facklam [29].

**Fig 1**: A. Pre-operative radiograph showing persisting periapical infection and improper obturation. B. Post operative 6 month recall showing resolution of periapical radiolucency.

**Fig 2**: A. Pre-operative radiograph showing post core restoration with persistent periapical infection. B. Post-operative radiograph where canal is prepared to full apical extent and obturated. Plan for post and crown restoration.

**Fig 3**: Treatment regimens should be aimed at prevention and elimination of E. faecalis

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**CONCLUSION**

Current knowledge on the composition of root canal flora is based on microbial culture techniques. It is apparent that E. faecalis in persistent root canal infections remains unclear. Coaggregation interactions are involved in establishment and maintenance of biofilms which play a role in endodontic infections. Adequate asepsis, instrumentation & use of disinfectants and irrigants will optimize the chances of targeting E.
faecalis. Continued research awaits newer and more challenging measures to combat E. faecalis.

Molecular methods in endodontic microbiology has widened the taxonomic spectra of the endodontic flora and is about to herald a new era of great understanding of complex interaction among the microbial and host factors. Role of root canal microbes in apical periodontitis is well established. Emphasis of treatment procedures should be with management of problems associated with control and elimination of infection. Survey of publications in leading journals of Endodontology reveals a trend to focus on purely mechanical aspects of treating the disease. Although important are impressive technological advances, sound understanding of etiology & pathogenesis of the disease is necessary for effective, affordable clinical management of disease for ultimate benefit of endodontic patients.

REFERENCES


