Enterococcus faecalis in Oral Infections

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Abstract

Enterococcus faecalis is a member of the normal microbiota; however, multidrug-resistant strains are important causes of nosocomial infections. Their ability to cause serious infections has been linked to variable traits that enhance their virulence. In the oral cavity, E. faecalis is commonly detected from root canals of teeth with post-treatment apical periodontitis or refractory/advanced marginal periodontitis. Isolates from oral infections have a genetic and virulence profile different from hospital-derived isolates. This Review discusses the occurrence of E. faecalis in oral infections, and the virulence factors that may contribute to the pathogenesis of post-treatment apical and marginal periodontitis. The susceptibility patterns of oral E. faecalis to various antibiotics of potential use in periodontal and endodontic therapy are also reviewed.

Keywords: Enterococcus faecalis; Endodontics; Periodontitis

Introduction

Enterococcus faecalis is a Gram-positive, facultative anaerobic coccus that can survive under harsh conditions, including high salt concentrations and temperatures > 45°C. It is a member of the mammalian gastrointestinal microbiota but multidrug-resistant strains have been considered relevant causes of hospital-acquired and community-related infections.

In the human oral cavity, E. faecalis has been frequently detected from patients with post-treatment apical periodontitis [1-17] or refractory marginal periodontitis [18-24]. Isolates from oral infections differ from clusters of hospital-derived isolates, as they do not present many mobile genetic elements. However, they usually carry virulence factors related to adhesion and biofilm formation, which may account for the colonization of different oral sites [15,17,25-29]. Moreover, oral strains may also carry certain antibiotic resistance determinants that have the potential to be transferred to other pathogenic bacteria in biofilm communities [23,26,28-33]. In this review, we discuss the occurrence, virulence factors and antimicrobial resistance of E. faecalis in oral infections.

Virulence factors of E. faecalis isolates from oral infections

The ability of E. faecalis to cause infections has been linked to variable traits that enhance its virulence. To date, only limited data are available on the virulence factors of oral enterococci in comparison with those of medical strains. Recently, it has been shown that E. faecalis isolates from endodontic infections have a genetic and virulence profile different from pathogenic clusters of hospitalized patients’ isolates [25]. In the latter, the genetic content of the E. faecalis pathogenicity island (PAI) was enriched among hospital-derived isolates and consisted of virulence determinants that are rare in endodontic isolates, including cyl (cytolysin production), gls24-like (general stress protein), nuc-1 (Staphylococcus homologue nuclease), and psaA (Streptococcus pneumoniae homologue metal-binding protein). In contrast, esp (enterococci surface protein) was a PAI gene frequently detected in both endodontic and medical isolates, which suggests that it may be relevant to E. faecalis adaptation in infected root canals [25].

Enterococcal surface protein (ESP) was highly associated with infection-derived isolates of E. faecium and E. faecalis [34]. Studies have shown that esp gene was detected in most strains isolated from endodontic [25,27], periodontal [28] and oral infections [29]. Since ESP has been associated to higher biofilm production of the strains, 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.

Several studies have attempted to identify additional genetic factors which influence biofilm formation in E. faecalis, including gelE (secretory metalloprotease gelatinase E) gene [34]. It has been shown a relationship of biofilm formation and gelE gene expression in Enterococcus faecalis recovered from root canal infections [17]. The production of gelatinase, a metalloprotease able to degrade collagen and fibrinogen, have been detected in 50% of E. faecalis isolates from endodontic [27] and periodontal [28] infections, which suggests a role for this factor in the pathogenesis of apical and marginal periodontitis.

Similarly, genes encoding adherence factors such as collagen adhesion protein (Ace), aggregation substance proteins (Agg), and antigen A (EfAA) have been frequently detected in isolates from both endodontic [15,26,27] and periodontal [28] infections. In this context, the expression of adhesion factors, such as Ace and Agg, may facilitate the colonization of dental root surfaces, since these factors can increase adhesion to collagen.

Capsule production is also an important mechanism of E. faecalis to circumvent the host’s innate immune response and establish infection. The presence of the capsule has been associated with the pathogenic lineages of E. faecalis isolated from hospitalized patients [35]. In endodontic infections, almost 40% of E. faecalis isolates from canals of root-filled teeth with apical periodontitis has been associated with capsule expression, which may account for their increased pathogenic potential [25,36].

Taken together, the latter studies have shown that most oral E. faecalis possess virulence factors related to adhesion and biofilm
formation. Moreover, some strains can also produce an anti-phagocytic capsule that may help to evade the immune system and sustain successful long-term infection. In heavily infected sites, these virulence factors may contribute to the pathogenesis of post-treatment apical and marginal periodontitis.

**Occurrence of E. faecalis in oral infections**

*E. faecalis* in *post-treatment apical periodontitis*: Post-treatment apical periodontitis is an inflammatory disease of apical tissues that persists or develops after endodontic treatment as a consequence of persistent or secondary intra-radicular infection. In these cases, *E. faecalis* has been the most frequently detected species [1-17] (Table 1). Since this species is more prevalent in persistent/secondary endodontic infections than in primary infections [7,12,13], it has been suggested that *E. faecalis* may play a role in the etiology of post-treatment apical periodontitis. As shown in Table 1, there is a wide variability in the reported prevalence of *E. faecalis* in persistent/secondary endodontic infections. The differences among studies could be explained by factors such as the sensitivity of the microbiological method, diagnosis criteria in patient selection, geographic location, and sample size.

Target directed molecular methods usually exhibit greater sensitivity for *E. faecalis* detection from root canal samples than culture based methods. Moreover, molecular methods can detect this bacterium in the viable but non-culturable (VBNC) state. By using reverse Transcriptase-PCR (RT-PCR), a RNA- based molecular method, VBNC *E. faecalis* could be clinically detected in root canal samples which were negative by culture [13]. Thus, several studies reported an increased sensitivity of molecular methods over culture for detecting *E. faecalis* in endodontic infections [8,10,12].

Using culture analyses, its prevalence ranged from 20% to 70% of root-filled canals with detectable bacteria [1,4,15-17,37-40]. However, one study failed to detect *E. faecalis* in cases of post-treatment apical periodontitis using culture assays [41]. Species-specific polymerase chain reaction (PCR) assays have revealed a higher prevalence of *E. faecalis* than culture-based studies. Apart from the findings of two studies [42,43], *E. faecalis* was the most prevalent species detected in root-filled teeth with apical periodontitis by standard PCR, with a prevalence ranging from 47% to 78% of the cases [5-11]. The use of more sensitive molecular methods like quantitative polymerase chain reaction (qPCR) indicated that this species was present in up to 89.6% of the root-filled canals [12].

Although *E. faecalis* is considered one of the most prevalent species in root-filled canals with apical periodontitis, it is usually not the main component of the mixed infections [44]. Moreover, studies using non-target molecular methods, such as pyrosequencing or 16S rRNA cloning and sequencing, have revealed a high degree of microbial diversity in root-filled teeth with apical periodontitis, and new candidate pathogens associated with persistent/secondary endodontic infections have been suggested [45-50].

Besides the microbiological methods, the divergence of the findings regarding the prevalence of *E. faecalis* in root canal infections may be also dependent on the patient selection. Usually, studies that have included only restored teeth [9,13,37,38,44] have shown lower prevalence of *E. faecalis* in root-filled canals than those that have also included non-restored teeth, considering the same detection method [4,10,11] (Table 1). Therefore, the presence of coronal leakage by defective coronal restorations, old temporary restorative materials, or non-restored teeth may have influenced the microbial findings of the latter studies [4,10,11]. These findings support the hypothesis that *E. faecalis* may enter the root-filled canal via coronal leakage during or after root-canal treatment as secondary invaders [51]. However, as many studies did not mention the quality of restoration, it is not possible to determine whether *E. faecalis* positive samples resulted from its persistence to prior root-canal treatment (persistent infections) or were originated after root-canal treatment from invading *E. faecalis* into the root-filling, via coronal leakage (secondary infections).

One possible source of *E. faecalis* is contamination of the unsealed necrotic or root-filled canal by food-borne strains, which are usually transient in the oral cavity but may become colonizers of the root canal system [25,51]. It has been shown that *E. faecalis* was detected more often in oral rinse samples from patients receiving endodontic treatment than from dental students with no history of endodontic treatment [52].

After root canal invasion, *E. faecalis* seems to fit to the ecological condition of the root-filled canal being able to survive in an environment with scant available nutrients [1]. Even in low levels, *E. faecalis* may establish infections that are difficult to treat due its resistance to disinfection procedures during endodontic re-treatment of root-filled canals, especially when forming a biofilm [53].

*faecalis* in marginal periodontitis: Periodontitis is an inflammatory disease characterized by the destruction of connective tissues and alveolar bone, in response to the subgingival biofilm. The microbial shift from a healthy periodontium to chronic periodontitis is characterized by increased proportions of Gram-negative anaerobic rods and spirochetes, including the recognized periodontopathogenic species *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, and a decrease in the proportion of beneficial species belonging to the genus *Actinomycodes* and *Streptococcus*. Although *E. faecalis* is not considered a periodontopathogen, this species has been more frequently detected in subgingival samples from patients with periodontitis than from periodontally health subjects, suggesting that the local conditions in periodontitis may favor its colonization [18-24].

The prevalence of *E. faecalis* in sub-gingival samples of periodontitis patients ranges from as little as 1% to almost 50%, depending on the microbiological method used in the studies [18,19,21-23]. Culture-based studies have reported a low rate of *E. faecalis* isolated from periodontitis, with prevalence ranging from 1% to 5% [18,23]. In contrast, a PCR-based study has showed that approximately 48% of periodontitis patients carried *E. faecalis* in subgingival sites [21].

The frequency of *E. faecalis* in patients with periodontitis may also be influenced by the severity and type of periodontitis. *E. faecalis* was detected in subgingival samples of 20.6% periodontitis patients, mostly from sites with probing depths ≥6 mm (57%), indicating its association with severe destruction [24]. Moreover, *E. faecalis* was detected in 21.4% of patients with refractory periodontitis (patients that failed to respond to the periodontal treatment) [19].

Similarly, systemic health conditions of the patients may also affect the detection rate of *E. faecalis* in supra- and sub-gingival biofilm [25,54]. *E. faecalis* was detected in higher prevalence in dental biofilm of hemodialysis patients when compared to the healthy group [54]. Furthermore, a higher prevalence of *E. faecalis* has been reported in patients positive for human immunodeficiency virus (HIV) infection when compared to HIV-seronegative subjects [22]. Other data confirmed that *E. faecalis* is frequently associated with necrotizing gingival lesions in the HIV-infected patients [55].

The presence of *E. faecalis* in periodontitis lesions may have an
E. faecalis possesses intrinsic mechanisms of resistance to several antimicrobial agents and additional resistance may also be conferred by acquisition of genetic determinants by horizontal transfer [34,35]. In this review, we discuss susceptibility patterns of oral E. faecalis to various antibiotics of potential use in periodontal and endodontic therapy. For a more detailed discussion on the mechanisms of antimicrobial resistances and their dissemination by the mobile genetic elements in E. faecalis, the reader is directed to other reviews [34,36].

Penicillins are the most frequently used antimicrobial agents in dentistry. Important classes of penicillins of potential use in odontogenic infections include amoxicillin and its association with beta-lactamase inhibitors, such as clavulinate. The development of enterococcal resistance to beta-lactams can be mediated by alterations in the expression or binding affinities of penicillin-binding proteins. Additionally, resistance has been associated with the production of beta-lactamas. In this context, in vitro antibiotic susceptibility studies have shown that oral E. faecalis hardly ever produced beta-lactamase enzymes. Moreover, it has been reported that amoxicillin or ampicillin resistances are rare in E. faecalis isolates from endodontic [26,30,31], periodontal [23,28,32,33] and deep oral infections [29].

In penicillin allergy subjects, clindamycin is usually the alternative drug for severe oral infections. However, since E. faecalis has intrinsic resistance to clindamycin, this drug is not clinically effective for enterococcal infections. This finding was confirmed by in vitro study testing clindamycin against oral E. faecalis isolates [29].

Macrolides are also alternative regimens recommended for dental procedures when patients are allergic to penicillin. However, erythromycin seems to be of limited value against oral enterococci [26,30,31,32,52]. Recently, Rams et al. [33] have shown that only 19% of periodontal E. faecalis clinical isolates were susceptible to erythromycin, and most of the isolates (55%) showed an intermediate pattern. These findings are similar to previous evaluations of endodontic E. faecalis strains [26,30,31,52]. Moreover, the genetic determinant of macrolide resistance (ermB) has been detected in approximately 60% of endodontic E. faecalis isolates [25].

Tetracyclines are broad-spectrum antibiotics, but bacterial resistance has reduced their clinical usefulness in oral infections. This antibiotic has exerted poor in vitro activity against periodontal E. faecalis, and tetracycline resistance was detected in over 50% of the E. faecalis periodontal isolates [23,28,32,33]. Moreover, a high prevalence of the genetic determinant of tetracycline resistance (tetM) has been recently detected in endodontic isolates [25]. Interestingly, in the latter study, approximately 50% isolates of endodontic origin carried both the ermB and tetM genes. The occurrence of multiple resistances to erythromycin and tetracycline is probably associated with the presence of conjugal transposons Tn916 family-Tn545, which carry ermB and tetM genes. It has been suggested that this mobile genetic element may have contributed to the dissemination of erythromycin and tetracycline resistance within the oral microbiota [23].

In summary, clinical isolates of E. faecalis recovered from root canal and periodontal infections can demonstrate antimicrobial resistance to conventional treatment regimens recommended for dental procedures, especially to tetracycline and erythromycin. On the other hand, studies have shown that the oral isolates are susceptible to antibiotics used to treat serious infections of hospitalized patients, such as vancomycin. Likewise, high-level gentamicin resistance was rarely found in oral isolates [25,28]. Therefore, oral E. faecalis might represent a reservoir of resistance to tetracycline and erythromycin, but not to vancomycin and gentamicin.

<table>
<thead>
<tr>
<th>Detection Method</th>
<th>% of canals with E. faecalis</th>
<th>Country</th>
<th>No. of canals analyzed</th>
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<td>Anderson et al. [48]</td>
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Table 1: Prevalence of E. faecalis in Root-Filled Canals with Apical Periodontitis Detected by Different Different Microbial Methods

ND: not detected or detected as low abundant microorganism (< 1%).

*Percent of canals with bacteria.

Studies that have used more than one technique to detect E. faecalis. Results of the most sensitive technique were reported here.

*Percent of teeth with and without apical periodontitis.

*Number of positive samples using the 16s rRNA cloning technique.

impact on the patient’s response to periodontal therapy. Since E. faecalis may be more resistant to antimicrobial chemotherapy and mechanical debridement, its presence may increase the probability of treatment failure [33].

Antibiotic resistances and determinants of E. faecalis isolates from oral infections

E. faecalis possesses intrinsic mechanisms of resistance to several antimicrobial agents.
Concluding Remarks

The oral cavity may serve as a reservoir for bacterial pathogens of medical importance such as enterococci in systemically healthy or diseased subjects. Oral *E. faecalis* possess virulence factors that may contribute to the pathogenesis of apical or marginal periodontitis. Differing from nosocomial infections isolates, usually oral *E. faecalis* do not carry multiple antimicrobial resistance determinants. However, tetracycline and erythromycin resistance genes have been frequently detected in isolates from root canal and periodontal infections, and *E. faecalis* selection is expected in oral sites after their usage.

Recent studies have shown that there is no specific virulent cluster associated with oral diseases, but the oral *E. faecalis* usually carry genes that encode surface proteins related to adhesion and biofilm formation. Apical and marginal periodontitis are biofilm-induced diseases, thus their treatment is mainly the mechanical debridement concurrent with chemical agents for disinfection. The biofilm organization and/or its inaccessibility, associated with the high fitness of *E. faecalis* to stressful conditions such as low nutrient sources and use of chemical agents, may result in bacterial persistence in the root canals or subgingival biofilm after endodontic or periodontal treatment, respectively. These findings may explain the high detection frequency of *E. faecalis* in cases that do not properly respond to either endodontic or periodontal treatment, and additional strategies may be needed for a successful treatment in these *E. faecalis* infected oral sites.

References


