

Importance of Enterococci (*Enterococcus faecalis*) for Dental Medicine – Microbiological Characterization, Prevalence and Resistance

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Abstract: Of all the 19 enterococci species present in genus *Enterococcus*, *E. faecalis* is the one that plays the most important role in dentistry. Although initially enterococci were considered to be non-virulent, they are now identified as one of the major causes of nosocomial infections worldwide. They are resistant to most antibiotics and can cause diseases such as periodontitis, peri-implantitis, pharyngitis, otitis, mastoidites, meningitis, endocarditis, urogenital tract infections and even septic conditions. As part of the normal intestinal flora, enterococci, together with *E. coli*, are used as a sanitary indicator for fecal contamination of drinking water. Of all the 19 enterococci species present in genus *Enterococcus*, *E. faecalis* is the one that plays the most important role in dentistry. *Enterococcus faecalis* is associated mostly with persistent infections that are difficult to treat in chronic, apical periodontitis, and recently in periimplantitis also. The assumption that the microorganisms, found in the root canal space, originate from those colonizing saliva and the oral cavity, is widespread. However, the data in the reviewed specialized literature is contradictory.

Keywords: enterococci, *Enterococcus faecalis*, oral bacteria, endodontic infection, endodontic retreatment

1. Introduction

Enterococci (genus *Enterococcus*, former group D streptococci), where *E. faecalis* and *E. faecium* belong, are diplococci most commonly found as commensals in the intestinal tract (gastrointestinal carriage), the oral cavity and the urogenital system in humans and animals (5, 40). Up to 10% of the nosocomial infections during the last decade are due to enterococci. Of all the 19 enterococci species present in genus *Enterococcus*, *E. faecalis* is the one that plays the most important role in dentistry. Their frequent incidence in foods of animal origin allows for a zoonotic route of transmission of *E. faecalis* to humans (26, 33). Transmission of enterococci through meat or raw milk to the human gastrointestinal tract has been suggested by Larsen et al. (2010) and Gelsomino et al. (2002) (23, 32).

Studies in recent years have shown that after eating cheese, enterococci from food can be integrated into the oral biofilm, *in vivo* (8, 11), which is confirmed by the *in vitro* studies of Thurnheer and Belibasakis (62). The finding that *E. faecalis* from food can be transmitted and find suitable conditions for development in the oral biofilm, raises the question as to whether the oral cavity serves as a reservoir for virulent and resistant *E. faecalis* strains (7, 8).

Biology and virulence

Enterococcus faecalis are Gram-positive cocci or ellipsoid microorganisms arranged in pairs or short chains, morphologically similar to *Streptococcus pneumoniae*. They grow in the temperature range of 10°C-45°C and propagate in a broth containing medium with pH = 9.6, and also in such that contains 6.5% NaCl or 40% bile (5). They have a cell wall with a group-specific antigen – group D glycerol teichoic acid. Their virulence is determined by their ability to adhere to host surfaces and form biofilms, as well as by their high antibiotic resistance (40).

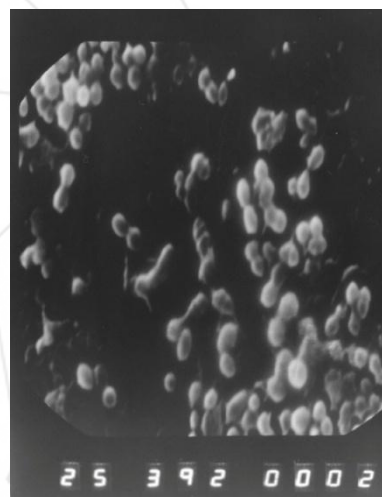


Figure 1: *Enterococcus faecalis* infected root canal dentin (magnification × 2420) (4)

Several virulence factors have been found to increase the capability of specific *E. faecalis* strains to cause a disease or worsen disease symptoms. It is known that the *enterococcal surface protein (esp)* increases adherence and colonization of cells and abiotic surfaces (43, 63).

Gelatinase (gelE) is an extracellular metalloprotease, able to hydrolyze gelatin, collagen and hemoglobin, which has been reported to also contribute to the bacterial adherence and biofilm formation (22, 29). *The aggregate substance (AS)* was also found to be involved in the microbial attachment and invasion of eukaryotic cells (31, 41, 61), as well as to contribute to biofilm formation (15).

Hyaluronidase (hyl) has been associated with virulence of enterococci in host tissue invasion (19, 29). It is also believed that the *E. faecalis endocarditis antigen A (efaA)* contributes to the adhesion of *E. faecalis* to heart cells in

endocarditis (55). And finally, *cytolysin* (*cyl*, *beta-hemolysin*) is a potent bacteriocin that exacerbates enterococcal infections in humans (64). It is capable of lysing many prokaryotic cells, as well as erythrocytes and other eukaryotic cells (65).

Besides the usual presence of various virulence and resistance genes, *E. faecalis* is highly proficient in the exchange and passing on of many of these genes through a horizontal gene transfer (36, 43). Over the past decade, a transfer of antibiotic-resistance genes between different strains of *E. faecium* as well as a transfer of vancomycin-resistance genes from *E. faecalis* to *Staphylococcus aureus* have been reported (34, 44, 71).

Their frequent incidence in food and livestock could allow for a zoonotic route of transmission of *E. faecalis* to humans (26, 33). The transmission of *E. faecalis* from meat products of porcine origin or from raw milk to the human gastrointestinal tract has been suggested by Larsen et al. (2010) and Gelsomino et al. (2002) (23, 32).

Other authors have demonstrated that after eating cheese, food-borne enterococci can integrate into the oral biofilm *in vivo* (8), and recently Thurnheer and Belibasakis (2015) confirmed that *E. faecalis* is able to colonize an *in vitro* established six-species oral biofilm in high numbers (62). The scientific results showing that *E. faecalis* from food can incorporate into the oral biofilm and is prevalent in dental endodontic disorders, raises the question as to whether the oral cavity and saliva may serve as a reservoir for virulent and resistant *E. faecalis* strains (7, 8).

Characteristics of cultures/Diagnostics

Microorganism identification methods using cultures allow for the bacterial susceptibility to antimicrobial agents – antibiotics – to be determined, and an antibiogram to be made. The drawbacks of these methods are connected with the detection of low levels of microorganisms and the difficulty to cultivate some species. Diagnosing always starts with a microscope examination of Gram-stained preparations, with crystal-violet. Enterococci are Gram-positive and cannot be clearly differentiated from *Streptococcus pneumoniae* on microscope preparations. If cocci are present in the Gram preparation and there is no growth in the plating, the presence of anaerobic cocci should be suspected (2). Streptococci are in chains on the preparations.

Enterococcus faecalis is easily incubated in ordinary non-selective media, growing under the conditions created for streptococci (2). It develops in the temperature range of 10°C - 45°C and propagates in a broth containing medium with pH = 9.6, and also in such that contains 6.5% NaCl (unlike streptococci) or 40% bile (2, 5). It causes a cloudy coloring of liquid nutrient media. In solid media, it grows as smooth, small, protruding, opaque or white colonies with dry consistency (resembling staphylococcal colonies); on blood agars it is non-hemolytic or displays an α -hemolysis. Some strains may display a β -hemolysis. It reduces 0.1% methylene blue in milk. It hydrolyzes the polysaccharide esculin and is resistant to optochin. *E. Faecium* is arabinose-positive, and *E. faecalis* is arabinose-negative (5). It is

differentiated from related microorganisms by simple tests: it is catalase-negative; L-pyrrolidonyl arylamidase-positive, bile and optochin resistant (2, 5, 40). It does not produce exotoxins and hydrolytic enzymes but it produces bacteriocins. It has adhesins – proteins and carbohydrates, with which it colonizes specifically the colon and, less often, the vagina.

Resistance

Up to 10% of the nosocomial infections during the last decades are due to enterococci, which is a consequence of the emergence of polyresistant strains to the commonly used antibiotics. Enterococci have natural resistance to cephalosporins, penicillinase-resistant penicillins and monobactams. They are 10 to 1000 times less susceptible to penicillin and ampicillin than streptococci (2). They may show susceptibility to sulfonamides *in vitro*, and are not susceptible *in vivo*. The usual concentrations of antibiotics behave towards them as bacteriostatical agents, not as bactericidal agents. For this reason, it is recommended to use a combination of penicillin (ampicillin or vancomycin) and aminoglycoside in the treatment of acute enterococcal infections. The therapy is best to be based on the antibiogram data. β -lactam-, aminoglycoside- and vancomycin-resistant strains represent a serious problem. Resistance is plasmid-induced and can be transmitted to *S. aureus* (2, 5).

Radeva et al. (2012) investigated the antimicrobial resistance of *Enterococcus faecalis* isolated from infected root canals (4). The results show 100% resistance to penicillins and 75% to ampicillin. In 87.5%, enterococci are resistant to amoxicillin/clavulanic acid. In the study carried out, enterococci retained their susceptibility to glycopeptides to 100% (vancomycin, teicoplanin). Clinical isolates were sensitive to gentamycin to 68.9% and a high level of resistance to aminoglycosides was not established (Kanamycin) (4).

Disorders

Enterococcus faecalis colonizes the gastrointestinal tract in humans and animals. It spreads on other mucosal surfaces also if a prior application of broad spectrum antibiotics has eliminated the normal local microflora. The structure of its cell wall, which is typical of gram-positive bacteria, allows it to survive on surrounding surfaces for prolonged periods of time.

Most of the infections, which they cause, are endogenous (from the patient's own intestinal microflora), but they can also be exogenous, and some are due to patient-to-patient transmission. Diseases include urinary tract infections, peritonitis (usually polymicrobial), wound infections, and bacteremia with or without endocarditis. Patients with an increased risk of developing a nosocomial infection from *Enterococcus faecalis* are those who are hospitalized for an extended period of time and are treated with broad-spectrum antibiotics (especially cephalosporins to which enterococci are naturally resistant) (2, 40). They can cause sepsis in patients with urethral and intravenous catheters. In newborns, they can cause meningitis (2).

The role of *Enterococcus faecalis* for endodontic infections

The role of *Enterococcus faecalis* in the oral cavity has not yet been elucidated. *Enterococcus faecalis*, although not usually considered to be part of the normal oral microflora (6, 52, 68), has been found in common dental diseases such as: periodontitis, periimplantitis and dental caries (16, 20, 30, 47). *Enterococcus faecalis* has been found primarily in secondary endodontic infections with a prevalence of 24% to 70%, that is, in previously filled root canals, where it can also form a biofilm (9, 10, 24, 35, 38, 48, 58, 67). The presence of *Enterococcus faecalis* is associated with both a primary and a persistent endodontic infection. *Enterococcus faecalis* is isolated in 10% of the cases of a primary endodontic infection. According to some authors' investigations, it is more often found in asymptomatic cases than in symptomatic cases (21, 49, 57, 59) Other studies have shown that *Enterococcus faecalis* is more often isolated in teeth with failed treatment within the range of between 30% and 90% (45, 49, 60). In many of the studies, culture methods for isolation of enterococci were used, and in these cases the isolates were 24% – 70%. Using a PCR results in a higher isolation rate of 67% – 77% (37).

In the oral cavity, with respect to dental pulp diseases, the path of infection penetration is important for the type of the predominant bacterial strains in the infected root canal. Dental pulp can be irreversibly impaired most often through a carious lesion, through an open pulp in a trauma or fracture, through micro cracks, through lateral root canals, after a tooth preparation and restoration (toxic effects of the medications and materials used) as well as through defects in these restorations (13, 18, 28, 69). The microbial count of bacterial cells in an infected root canal is from 10^3 to 10^8 CFU (50, 66). When the endodontic space communicates with the oral cavity, the number of bacteria involved in the pathological processes in the endodontium increases. This why it is important not to leave the pulp chamber open in order to avoid the greater invasion by bacteria. The opened endodontium may cause a delay in the healing process in the periapex, which is not beneficial to the healing process.

The bacterial penetration in the dentin is proven in the presence of a pulp necrosis. Bacteria can very easily enter the necrotic pulp because of a lack of protective factors in the root canal also. Predentin is usually more easily infected, whereas calcified dentin makes this more difficult. Colonized microorganisms along the root canal wall often penetrate into the dentinal tubules – from 150 μ m to 300-700 μ m. The maximum penetration depth of *S. sanguis* and *E. faecalis* is 880 μ m and 770 μ m after a 60 day incubation (1). When *Enterococcus faecalis* invades the micro-channel system of the dentin, it becomes difficult to treat and after the other microbes are removed, it seems to be making use of the possibilities to grow in a nutrient-poor environment such as the environment of the treated canal (35). Because of these features, *E. faecalis* is a microorganism commonly used in *in vitro* studies. *E. faecalis* fulfills the necessary conditions for pathogenicity of the microorganisms, which are the following for the endodontic conditions:

- They must be present in a sufficient quantity in order to initiate a periapical inflammation;

- They must possess great virulence;
- They should be located in the space of the root canal system in such a way as to be able to gain access to the periapical tissues.

The root canal environment allows the survival and growth of microorganisms (oxy-reduction processes are greatly reduced or absent and the environment is poor in oxygen) (28, 56, 70).

Enterococci are difficult to remove after root canal preparation and medication.

A number of authors have examined the effect of chlorhexidine as an endodontic irrigant and an intracanal medicament against *E. faecalis* and have found that it has an antibacterial activity comparable to that of NaOCl and is effective against some calcium hydroxide resistant strains (14, 25, 46, 53, 54). In our previous *in vitro* studies on the effectiveness of intracanal medicaments against *E. faecalis*, chlorhexidine showed slightly higher values of antibacterial activity compared to sodium hypochlorite (3). Other authors have also demonstrated in *in vitro* studies a higher antimicrobial activity of chlorhexidine compared to sodium hypochlorite in the treatment of *E. faecalis* (42, 53).

In a clinical study, Ercan et al. (2004) found that 2% chlorhexidine was more effective as an endodontic irrigant than 5.25% sodium hypochlorite (17). In our *in vitro* studies, using an agar-diffusion method, chlorhexidine showed a slightly higher antimicrobial activity compared to sodium hypochlorite (4).

2. Conclusion

The two species, *Enterococcus faecalis* and *Enterococcus faecium*, the first being the predominant one, have gained importance in the recent decades as leading opportunistic pathogens causing nosocomial infections (26, 27, 39, 65). They are associated with various infections, including urinary tract infections, bacteraemia, meningitis, wound infections and neonatal infections. More recently, biofilm-associated infections from artificial medical devices have been attributed to enterococci (12, 43, 51). These are difficult to treat due to their increased antibiotic resistance. Several virulence factors have been found to increase the capability of specific *Enterococcus faecalis* strains to cause a disease or worsen the disease symptoms. These and other facts explain and justify why *Enterococcus faecalis* is an interesting subject for future studies by specialists.

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