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Is antimicrobial agents can considered as effective weapons against endodontic infections by *Enterococcus faecalis*?

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ABSTRACT

Endodontic infections, the infection of the dental root canal system, have a polymicrobial nature that characterized by mostly anaerobic and some facultative bacteria. Among them, Enterococcus faecalis is associated with a significant number of persistent endodontic infections. It has a large number of virulence factors that may be related with various stages of pathogenesis. Eradication of infectious agents or significantly reduction the microbial load is very important in treatment of endodontic infections. Although current treatment options are combination antibiotic therapies, E. faecalis can also have acquired antibiotic resistance to numerous classes of antibiotics due to its high genome plasticity. Treatment failures and resistance development have made therapy difficult. In this paper, we discuss the physiological features and ability of biofilm formation of E. faecalis, as well as pathogenesis and effective treatment of endodontic infections by E. faecalis. Knowledge about the pathogenesis of E. faecalis endodontic infections and mechanisms of E. faecalis antimicrobial resistance may help to prevent failures of endodontic treatment attributed to this organism.

Keywords: *Enterococcus faecalis*, endodontic infection, antibiotic resistant

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INTRODUCTION

Bacteria are considered as the main causative agents for dental pulp and dental periapical lesions due to production of toxins that led to dental pulp and dental periapical tissue damage [1]. Lack of suitable accessibility of immune system to dental root canal space results in incomplete removal of infection from infected root canal systems (i.e., endodontic infection) [2]. It's necessary to do endodontic treatment and the success rate of this procedure is critically dependent on management of pulpal space infection results in reduction of bacterial numbers [3]. Endodontic infections are polymicrobial and can be classified according to the anatomic location (intraradicular or extraradicular). Microorganisms colonizing the root canal system cause intraradicular infection, which can be classified as primary, secondry or persistent [4].

Microorganisms that initially invade and colonize the necrotic pulp tissue cause primary intraradicular infection. Persistent or secondary infections are caused by micro-organisms that were not present in the primary infection and are the major causes of endodontic treatment failure, which is characterized by persistence or appearance of apical periodontitis after treatment [5].

Enterococcus faecalis is found in 4 to 40% of endodontic infections [6]. It has high prevalence in root filled canal that associated with disease is that enters the canal in during or between treatment process and that allow it to survive in conditions that are commonly lethal for many other microorganisms [7]. Genus *Enterococcus* has been recognized by Thiercelin since 1899 [5, 6]. *Enterococcus* species have emerged, over the last decades, as very important opportunistic nosocomial pathogens, with patients having a high mortality rate of up to 61% [7].

There are now over 40 ecologically diverse species within the *Enterococcus* genus and only those that cause diseases in humans and animals have been studied [8]. Over the past two decades, with increasing antimicrobial resistance, the most important specie, *E. faecalis*, have been increasingly identified as causative agents of more than 90 percent of nosocomial infections in humans [9, 10]. Enterococci are frequent colonisers of the humans and animals gastrointestinal, genitourinary tracts and oral cavity in the root canals of teeth with failed endodontic treatment [11- 13].

Physiological features

E. faecalis is characterized as low GC Gram-positive bacteria and facultatively anaerobic oval cocci belonging to the phylum Firmicutes, catalase- negative, non-motile, non-sporulating and hydrolyze bile-esculin and L-pyrrolidonyl-B-naphthylamide (PYR) [14-17]. The size of its gene is around 150kb and encodes for 129 open reading frames (ORF) [18]. It's exceedingly hardy and can tolerate and adapt to adverse environmental conditions [19]. It can grow at 10°C and 45°C, in a wide range of pH (4.6–9.9), with the optimum being 7.5, in the presence of 40% (w/v) bile salts, in 6.5% NaCl broth, and even surviving at 60°C for as long as 30 minutes [19- 21]. The presence of a cation homeostasis is the pivotal role for resistance to these agents [22]. So withstand tough conditions allow it to colonize a wide range of niches, which could have relevance for their clinical importance.

Pathogenesis of endodontic infections

E. faecalis is a frequent cause of a wide diversity of infections in humans such as urinary tract infection (UTI), bacteremia, endocarditis, abdomen, biliary tract, endodontic infection, wound infections, intraabdominal and pelvic infections and indwelling foreign devices (like intravascular catheters). Also, it has isolated from a range of oral conditions including carious lesions, chronic periodontitis, and endodontic infections and has been associated with persistent apical periodontitis [6, 10, 11, 15, 21].

E. faecalis have been shown to possess a range of virulence factors that regulated by virulence coding genes present on the genome in special regions which are termed pathogenicity islands (PAI) [22]. The most important among virulence factors being haemolysin, gelatinase (*GelE*), hyaluronidase, enterococcal surface protein (*Esp*), *E. faecalis* regulator (*Fsr*), aggregation substance (*AS*), Microbial surface component recognizing adhesive matrix molecule adhesin of collagen from Enterococci (MSCRAMM *Ace*), serine protease (*SprE*), sex pheromones, *BopD, EfaA,* second collegen adhesin of *E. faecalis* (*Scm*), lipoteichoic acid (LTA), capsule, cell wall polysaccharide and extracellular superoxide that help these pathogens with colonization, immunoevasion, and immunosuppression of their hosts and often responsible for causing disease [15, 21, 22, 24]. Each of them may be associated with various stages of an endodontic infection as well as with periapical inflammation [25]. The role of virulence factors is shown in Table 1.

Antimicrobial resistance

The objectives for endodontic treatment are removal of the microbes, their byproducts, and pulpal debris from the infected tooth root canal system. Since 1928, antibiotics revolutionized health care treatment of bacterial infections including those of endodontic origin. Because endodontic infections are polymicrobial, and antibiotic selection is empirical based on the organisms usually involved in endodontic infections, no single antibiotic is likely effective against all the strains of infecting bacteria [26].

Penicillin is the most frequently used antimicrobial agent for endodontic infections because it is high susceptibility, low toxicity, and low cost. In penicillin allergy subjects, clindamycin is usually the alternative drug for severe oral infections [27]. Amoxicillin and clavulanate may be used for serious odontogenic infections because have a longer half life and higher, broad spectrum, more sustained serum levels and the highest sensitivity to endodontic infections [28]. Metronidazole is bactericidal to anaerobes and is effective when used in combination with penicillin or clindamycin. Appropriate use of antibiotics and implementation of infection control practices diminished the risk of these infections [29]. But over time, they were resistant to some of these antibiotics. Like other major nosocomial agents [30-34], multidrug-resistant enterococci are currently a problem in around the world and are well documented.

Several mechanisms are involved in the development of antibiotic resistance. Among them, there are mechaisms for the transfer of resistance genes such as exchanging plasmids via conjugation, as well as exchanging resistance genes on transposons or via bacteriophages [35]. Another mechanism is the appearance of innate and acquired resistance in bacteria. These bacteria display intrinsic resistance (universally found within the genome of the species) to several antibiotics, also an ability to rapidly acquire antibiotic resistance (through acquisition of new genetic material or through sporadic mutations to intrinsic genes) [12, 36]. Enterococci exhibit intrinsic resistance to certain classes of beta-lactam antibiotics (e.g. cephalosporins), low-level resistance to aminoglycosides, lincosamides, streptogramins (in case of *E. faecalis*) and monobactams. Acquired resistance can be seen to aminoglycosides, penicillines,

chloramphenicol, quinolones, nitrofurantoin especially ampicillin, tetracyclines and vancomycin [15, 37]. Nevertheless, some of these antibiotics are in compounds that are used for the treatment of endodontic infections.

Biofilm formation as a challenge for management of endodontic infections

Biofilm formation begins with the attachment of complex group of microbial cells to biotic and abiotic surfaces [38, 39]. Bacterial biofilms is a common cause of persistent infections that lead to resistance to antibiotics. Endodontic disease is a biofilm-mediated infection, and *E. faecalis* is an important organism in it that is capable to produce biofilms [40]. Factors influencing biofilm production are shown in table 1.

Table 1. An Overview of the virulence factors of E. *faecalis* and their functions and roles

Virulence factors	Function and roles
Haemolysin	- A bacterial toxin
	-Lysing human, horse and rabbit erythrocytes, macrophages and polymorphonuclear neutrophils
GelE	- Hydrolyzing gelatin, collagen, casein, hemoglobin and other peptides
	- Damage to the host tissues
	- Regulating of the host immune response
	- Formation of a biofilm
	- Regulated by two-component regulatory system via quorum sensing mechanism
	- Role in increasing dissemination of bacteria in high density environments
Hyaluronidase	- Cleave hyaluronan, which is a constituent of the extracellular matrix of connective tissues
	- Increasing the magnitude of the damage
	- Supply nutrients for the bacteria
Esp	- Promoting adhesion to mucin or uroplakin, colonization and evasion of the immune system
	- Formation of a biofilm
	- Lead to resistance to antibiotics and environmental stresses
Fsr	- An important regulator with both positive and negative effects
	- Regulating *gelE, sprE* and *bopD* expression that are important for biofilm formation
AS	- Adhesion and colonization
	- Promoting cell-cell contact
	- Increasing the cell surface hydrophobicity
	- Promoting direct, opsonin-independent binding *E. faecalis* to human neutrophils
	- Protecting *E. faecalis* from killing the polymorphonuclear leukocytes
MSCRAMM Ace	- Expressing in the early stages of infection
	- Connecting to the components of the host extracellular matrix
	- Mediating binding to immobilized collagen type I, collagen type IV and mouse laminin
SprE	- Providing nutrients to the bacteria by degrading host tissue
	- Formation of a biofilm
Sex pheromone	- Dissemination the antibiotic resistance and other virulence traits, such as cytolysin production among strains of *E.faecalis*
	- Chemotactic for human neutrophils
BopD	- Formation of a biofilm in the presence of specific carbohydrates
EfaA	- Adhesion
	- A solute binding-protein receptor for manganese transport system
Scm	- Binding to collagen type V and fibrinogen
LTA	- Affording resistance to complement-mediated opsonophagocytosis
Capsule	- Contributes to host immune evasion
Extracellular superoxide	- Lysing of red blood cells
	- Being in various phases of the inflammatory response

Strategies for biofilm control

Biofilms are not easily removed by antimicrobiocidal factors, quarternary ammonium compounds, halogens and halogen release agents [41]. In recent decades it has been shown that bacteriophages (phages) are potential and biological control agents for eliminating of biofilms [42]. Phages have the best performance in particular dilution because the reason for this issue is that phages tend to bind to their receptors in varoius form in different dilutions [41]. Bacterial growth rate causes that the number of bacteria is more than of phages in low concentrations and phages will not be able to remove all bacteria [43]. Therefor, appropriate techniques should be used to eliminate

biofilm formation via *E. faecalis*. One notable feature of *E. faecalis* is that can colonize root canal and survive and form a biofilm that renders it more resistant to phagocytosis, antibodies and antimicrobial agents [44].

The long treasured goal of endodontic treatment depends on the eradication of infectious agents or significantly reduction the microbial load from the root canal. Its success relies on the combination of proper instrumentation, irrigation, and obturation of the root canal [45-48]. Irrigation of the root canal is the most important determinant in the healing of the periapical tissues [49]. An irrigant should be able to disinfect and penetrate dentin and its tubules, offer long-term antibacterial effect (substantivity), remove the smear layer, microorganisms, tissue remnants, and dentin chips from the root canal through a flushing mechanism [50]. Recently, Photodynamic therapy (PDT) was introduced to endodontics to improve the results acquired by traditional techniques. Different wavelengths have been shown to be effective in reducing bacteria in root canal system. PDT uses light of a specific wavelength to activate a nontoxic photoactive dye to producing reactive oxygen species (ROS), including free radicals and singlet oxygen that mediate bactericidal and fungicidal activities [51-53].

CONCLUSION

E. faecalis is commensal organisms well suited to survival in oral cavity and establishes an endodontic infection and maintains a periradicular inflammation due to its virulence factors. This microorganism has the capability to maintain root canal infection and periradicular lesion because it has high genome plasticity. Clinicians stated that a mature biofilm can tolerate antibiotics at high concentrations. Biofilm develops slowly but has great effects both clinically and economically on treatment. In order to improve the treatment results, newer antimicrobial strategies such as photochemotherapy must be used to prevent or eliminate the infection and biofilm formation.

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