Molecular aspects of *Enterococcus faecalis* virulence

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ABSTRACT The *Enterococcus faecalis* (*E. Faecalis*) virulence factor plays an essential role in the persistence of root canal infection. Virulence factors of *Enterococcus faecalis* such as lipoteichoic acid, extracellular superoxide, gelatinase, hyaluronidase, and cytolysin are known to increase the ability of *Enterococcus faecalis* to induce inflammatory processes, colonization formation, and increase resistance. The virulence factor of *E. faecalis* is mediated by LTA, which has pattern recognition receptors for cytokine release, bone resorption and triggers apoptosis of osteoblasts, osteoclasts, periodontal connective tissue, macrophages, and neutrophils, which have implications for the occurrence of periradicular lesions. *Lipoteichoic acid* is also involved in producing D-alanine, which stimulates signals to other bacteria to form biofilms. The *E. faecalis* will change the balance of oxygen radical production in the periradicular lesion, fragment collagen. The fight host's defense mechanisms that cause periapical damage and worsening bone loss. Furthermore, cytolysin will respond to changes in oxygen conditions in the depleting root canals for the dominance of *E. faecalis* against other bacteria. The energy needs of *E. faecalis* that assisted by hyaluronidase, which degrades hyaluronan dentin. It has to produce disaccharide degradation products that can be transported and metabolized intracellularly. These materials hydrolyzing the substrate to obtain essential carbon for its growth. This article aims to describe the molecular aspect of *E. faecalis* virulence that is involved in root canal infections.

KEYWORDS: *Enterococcus faecalis*, cytolysin, extracellular superoxide, gelatinase, hyaluronidase, lipoteichoic acid

INTRODUCTION

One of the microflora that showing a high prevalence of persistent infection is *Enterococci*. From the 23 *Enterococci* species known, *Enterococcus faecalis* is known to play a significant role in cases of persistent periapical lesions with a prevalence of up to 77%. In fact, these bacteria are known to contribute 80-90% of root canal infections after treatment. The results of other studies also stated that 63% of root canal treatment failures experienced reinfection caused by *Enterococcus faecalis*.

The Enterococcus faecalis virulence factor plays an important role in the persistence of root canal infection. This virulence factor increases the ability of *Enterococcus faecalis* to form colonization, increases phenotypic tolerance to many disinfectants or physical agents, and is able to induce inflammatory processes. The ability of *E. faecalis* to adapt in root canals is an advantage for other species. This explains its survival in root canal infections, which are known to be poor in nutrition and limited access to avoid root canal medicaments. This places *Enterococcus faecalis* as a pathogenic bacterium and can lead to root canal treatment failure.

Based on these concerns, molecular understanding of the main *E. faecalis* virulence factors against persistent root canal infection strongly supports and enhances laboratory diagnosis and therapy of *E. faecalis*. This article aims to describe the molecular aspects of *E. faecalis* virulence in root canal infections.
DISCUSSION

Morphologi E. faecalis

The E. faecalis is a gram-positive, anaerobic, cocci form. These bacteria produce catalase and are involved in the hydrogen peroxide reaction. These bacteria are ovoid-shaped under the electron microscope with a diameter of 0.5-1 μm with short chains and are paired or single.11 The cell wall of E. faecalis is coated with peptidoglycan, which functions to maintain the cell surface and the influence of cytoplasmic osmotic pressure.12

Persistence of root canal infection

Enterococcus faecalis is commonly found in root canals and persists in them even after treatment.13-15 E. faecalis can survive at pH 4-11 and 10° C-45°C. The ability to survive in such an environment allows these bacteria to survive in the root canals to invade the dentinal tubules, leading to root canal treatment failure.15-17

E. faecalis can increase resistance to various antibiotics such as penicillin, tetracycline, chloramphenicol, and vancomycin. E. faecalis resistance to antimicrobials is acquired intrinsically or acquired. Acquired resistance is obtained from mutations in DNA or new genes through the transfer of plasmids and transposons, while intrinsically acquired through gene transfer.6 Besides, maintaining the optimal cytoplasmic pH level makes these bacteria resistant to calcium hydroxide.11

Prolonged root canal infection allows bacteria to enter the entire root canal system, including ramifications and dentinal tubules. 63% of root canal treatment failures experienced reinfection are known to be caused by E. faecalis.4,5 The factors that cause E. faecalis to be able to survive in the root canal include: surviving the lack of nutrient availability, binds to dentin, invades the dentinal tubules, change the host response, suppress lymphocyte action, compete with other bacteria, form biofilms, and are resistant to calcium hydroxide administration.2

The E. faecalis virulence factor

The pathogenicity of E. faecalis to persistent root canal infection is related to its virulence factors. Virulence factor is the degree of ability of an opportunistic pathogen to cause disease to the host.18 Virulence factors are extracellular products secreted by bacterial cells and are involved in the pathogenesis of root canal infections.

Hyaluronidase

Hyaluronidase acts by depolymerization of connective tissue mucopolysaccharides. It is to facilitate the spread of bacteria and their toxins through the host tissue. Hyaluronidase facilitates the migration of other bacteria from the root canal to the periapical lesions to make it worse. Hyaluronidase triggers other bacteria to produce the toxins they have, which will increase the damage.26,27

1. Lipoteichoic Acid (LTA)

Of all the virulence factors of E. faecalis, LTA is a major etiological factor that is potentially pathogenic, where (1) LTA from E. faecalis triggers inflammatory responses and causes tissue damage19; (2) LTA E. faecalis increases bacterial resistance to antimicrobial peptides, antibiotics, or disinfectants.19 (3) LTA is a constituent of E. faecalis which acts as a receptor on receptor cells for aggregation which is an essential factor in biofilm formation.

2. Production of Extracellular superoxide (O2)

As many as 87 of 91 clinical isolates of E. faecalis have been reported to produce extracellular superoxide.20 Production of extracellular superoxide is known to increase the survival of E. faecalis.21 The main impact of cellular superoxide production is superoxide anion.22 Superoxide anion is a highly reactive oxygen radical that is known to damage cells and tissues. Superoxide anions can also exert destructive effects on various important compounds such as lipids, proteins, and nucleic acids.22

3. Gelatinase

Gelatinase is a metalloproteinase from E. faecalis that can hydrolyze gelatin, collagen, fibrinogen, casein, hemoglobin, insulin, and several other bioactive peptides.23 In pulp inflammation and periapical lesions, the amount of host gelatinase is known to be higher than that of healthy tissue.24 Gelatinase high levels are known to contribute to the degradation of the dentin organic matrix. 25

4. Hyaluronidase

As a degradative enzyme, hyaluronidase can cause tissue damage. Hyaluronidase acts by depolymerization of connective tissue mucopolysaccharides. It is to facilitate the spread of bacteria and their toxins through the host tissue. Hyaluronidase facilitates the migration of other bacteria from the root canal to the periapical lesions to make it worse. Hyaluronidase triggers other bacteria to produce the toxins they have, which will increase the damage.26,27
5. Cytolysin

Hemolysin of E. faecalis isolated in cytolysin expresses the toxins encoded by plasmids and chromosomes. Cytolysin target cells are erythrocytes and macrophages. PMNs and inflammatory cells were reported in the inflammatory process. They are cultured from human monocytes and leukocytes. Toll-like receptors (TLRs) receptors that play an essential role in the innate immune response. The LTA stimulates cells to express tumor necrosis factor-α (TNF-α) followed by activation of Nuclear factor-kB (NF-kB) and the p38 induced the mitogen-activated protein kinase (MAPK). They are involved in signaling the pathway of endodontic infections.

NF-kB is transcription, an important factor that promotes the rapid release of cytokines. Another study also suggested the involvement of NF-kB and the MAPK signaling pathway in the production of TNF-α. The interaction of LTA with TLR2 can activate MyD88, MAPKs, and other transcription factors, including NF-kB, to express inflammatory cytokines.

TNF-α cytokines are known to be involved in bone resorption. These TNF-α cytokines are found periapical. It causes apoptosis in osteoblasts, osteoclasts, periodontal ligamentous connective tissue, macrophages, and neutrophils to cause periodontal lesions. On the other hand, the dlt gene in LTA is also involved in producing D-alanine, which will trigger other bacteria to form biofilms.

Neutrophil superoxide and phagocytic cells are the antibody cells involved in eliminating E. faecalis. Superoxide anions facilitate this activity to produce neutrophil chemotactic factors. This causes an altered balance of oxygen radical production in periapical lesions, consequently increasing the periapical damage and bone loss that occurs in chronic apical periodontitis.

Superoxide anions are also produced by osteoclasts, which are involved in bone resorption.

Apart from being produced by the host’s cells, bacteria can also produce superoxide anions. In addition to causing tissue damage at the site of inflammation, this extracellular superoxide will fragment collagen. This process is facilitated by Aggregation substance (AS) and surface adhesin, allowing E. faecalis to adhere to collagen.

Consequently, collagen fragmentation will attract monocytes, macrophages, and neutrophils to the site of damage. Here, the mechanisms for damaged tissue and bone resorption will continue to occur. Furthermore, collagen will continue to stimulate the release of destructive reactive oxygen. The collagen also released superoxide anions and lytic enzymes (gelatinase). The E. faecalis gelatinase will hydrolyze collagen for the role in periapical inflammation. Gelatinase can also be produced by inflammatory cells and osteoclasts. The increase in gelatinase from both sides will accelerate the rate of bone resorption.

Aggregation substance can function against the host’s defense mechanism by keeping neutrophils bound so that E. faecalis remains alive even though the active phagocytosis mechanism occurs. Other studies have also shown that the cylLL and cylLS genes in cytolysin E faecalis can increase the survival rate of E. faecalis. As it is known, E. faecalis plays an essential role in forming biofilms. Even though it forms colonies and biofilms, Enterococcus faecalis is the dominant microorganism in root canals. Cytolysin is known to inhibit the growth of other bacteria. The cylLL and cylLS genes in cytolysin E faecalis encode structural subunits of cytolysin. They are to produce the cytolysin under anaerobic conditions. It also to changes the response of oxygen conditions in root canals.

The supplied energy of E. faecalis facilitated by hyaluronidase. They working degrades hyaluronic dentin to produce saccharides. It has transported and metabolized intracellularly. Also, the E. faecalis can obtain essential carbon for its growth through substrate hydrolysis. It explains the low number of other bacteria in up-persistent root canal infections so that Enterococcus faecalis becomes the dominant microorganism in the root canal.
CONCLUSION

The *E. faecalis* virulence factor is mediated by lipoteichoic acid, which has pattern recognition receptors to respond to cytokine release and bone resorption. It triggers apoptosis of osteoblasts, osteoclasts, periodontal connective tissue, macrophages, and neutrophils that cause periradicular lesions. Lipoteichoic acid is also involved in producing D-alanine, which stimulates signals to other bacteria to form biofilms. The *E. faecalis* will change the balance of oxygen radical production in the periapical lesion. It process to fragmenting collagen, fighting host defense mechanisms, so cause periapical damage and worsening bone loss. Furthermore, cytolysin will respond to changes in oxygen conditions in the depleting root canals for the dominance of *E. faecalis* against other bacteria. The energy needs of *E. faecalis* will be assisted by hyaluronidase, which degrades hyaluronan dentin to produce saccharide degradation products that can be transported and metabolized intracellularly and by hydrolyzing the substrate to obtain essential carbon for its growth.

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