DETECTION OF ACE (COLLAGEN BINDING PROTEIN) GENE FROM ENTEROCOCCUS FAECALIS IN PATIENTS WITH ENDODONTIC INFECTIONS

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ABSTRACT:

AIM AND OBJECTIVE:

To detect ace (collagen binding protein) gene from Enterococcus faecalis from patients with endodontic infections.

BACKGROUND:

The ace gene helps in the production of angiotensin converting enzyme which plays a major role in the regulation of blood pressure. Adherence of Enterococcus faecalis isolates to extracellular proteins such as collagen I and IV and laminin; identified an E.faecalis-specific gene, ace, whose encoded protein has characteristics of bacterial adhesion and implicated ace in binding to collagen type-I since Enterococcus faecalis has long been implicated in persistent root canal infections and endodontic infections.

MATERIALS AND METHODS:

A total of 20 samples were collected from patients undergoing endodontic treatment. The isolates were subjected to conventional tests and confirmed, DNA was extracted, quantified and identified sphectrophotometrically and electrophoretically. The ace (collagen binding protein) was amplified by PCR amplification technique, using specific primer and conditions.

RESULT:

Out of 20 isolates 2 isolates were shown to express ace (collagen binding protein) gene.

KEYWORDS: E.faecalis, ace gene, endodontic infections.

INTRODUCTION:

Enterococci inhabit the gastrointestinal tract, the oral cavity, and the vagina in humans as normal commensals. They can cause a wide variety of diseases in humans, infecting the urinary tract, bloodstream, endocardium, abdomen, biliary tract, burn wounds, and indwelling foreign devices.Enterococci now rank among the top three nosocomial bacterial pathogens and strains resistant to currently available antibiotics pose real therapeutic difficulties . Up to 90% of enterococcal infections in humans are caused by *Enterococcus faecalis* [1].The majority of the remainder is caused by *Enterococcus faecium*, and infections with the other species are quite rare. Enterococci have also been implicated in endodontic infections. Although they make up only a small proportion of the initial flora of untreated teeth with necrotic pulps , enterococci, particularly *E. faecalis*, have been frequently found in obturated root canals exhibiting signs of chronic apical periodontitis, isolated in 23- 70% of the positive cultures and often occur in monoculture. Moreover, *E. faecalis* was among a group of bacteria cultured from periapical lesions refractory to endodontic treatment[2].Many studies demonstrated the frequent presence of *E. faecalis* in association with a wide variety of aerobic and anaerobic bacterial species involved in various endodontic diseases and chronic apical periodontitis.Virulent factors of *E. faecalis* include adherence to host tissue, invasion and abscess formation, modulation of host inflammatory responses, secretion of various products which enhances biofilm formation.. Data on oral prevalence of *E. faecalis* and its virulence factors vary from one study to another.. Therefore, more investigation on potential virulence factors of *E. faecalis* would be useful in understanding their role in dental infections[3]. The existing knowledge of the factors that may influence the ability of enterococci to colonize host tissues, translocate across epithelial barriers, and survive in different host environments is rudimentary, but their in- creasing resistance to multiple antimicrobial drugs makes the study of pathogenesis of these organisms all the more important.[4,7,9]. About a dozen putative virulence factors have been identified in *E. faecalis*, but mechanisms of virulence remain not fully understood. These factors are involved in different steps of the infection process, such as attachment to host cells or extracellular matrix (ECM), macrophage resistance, tissue damage, and immune system evasion.For extracellular pathogens such as *E. faecalis* or *Staphylococcus aureus*, components of ECM or serum (i.e., collagen, fibronectin, and fibrinogen) are preferred targets for adhesion. Ace, an adhesin that binds collagen (types I and IV) and laminin and belongs to the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family, was identified in *E. faecalis* by sequence homology with the virulence factor Cna, a well characterized MSCRAMM in *S. aureus*.[5,6,8]. ACE is the key enzyme in the rennin-angiotensin system (RAS), which is a circulatory cascade primarily involved in the regulation of blood pressure and serum electrolytes . ACE converts angiotensin I to angiotensin II, the major effector of the RAS.Angiotensin II is a potent vasoconstrictor and activator of aldosterone, induces reabsorption of sodium and raises the blood pressure.[10].

This study is aimed to detect the presence of ace(collagen binding protein) gene from Enterococcus faecalis in patients with endodontic infections.

MATERIAL AND METHODS:

A total of 20 samples were collected from patients undergoing endodontic treatment. The samples were inoculated in Mac Conkey agar and Brain Heart infusion agar. Presumptive identification of Enterococci was done by Gram's stain, Catalase test and Heat tolerance test in which Enterococci are Gram positive cocci arranged in pairs, Catalase negative, tolerate the temperature of 60 degree C for 30 minutes respectively. On Mac Conkey agar they showed small Lactose fermenting colonies.

RESULT:

Genomic DNA used as template for polymerase chain reaction (PCR) amplification was prepared using conventional phenol-chloroform DNA extraction method. The oligonucleotideprimer pairs used to amplify the virulence genes and the expected amplicon size are as follows

Gene	Primer sequence	Product size
ace	F2 GAGCAAAAGTTCAATCGTTGAC R3 GTCTGTCTTTTCACTTGTTTCT	1003

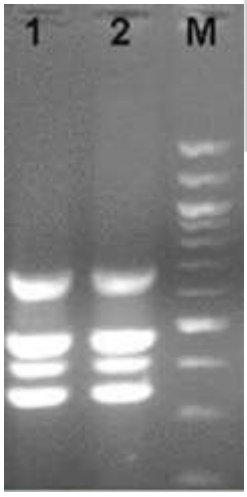
[Table:1]

The amplification of virulence genes was carried out as follows:
Initial denaturation at 95°C for 15 min followed by denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min.
The PCR amplification of the *ace* gene was carried out as follows:

Predenaturation at 95°C for 4 min followed by denaturation at 95°C for 30 cycles of 30 s each; 1 min for annealing at 52°C and elongation at 72°C for 1 min. Both positive control and negative control, consisting solely of the PCR reaction mixture without DNA template were included to check the validity of the technique utilized in the study.

Analysis of DNA by agarose gel electrophoresis:

Twenty-five microliters of respective amplified products were loaded into the wells and electrophoresed at a constant current of 50V for about 45 min using 1.5% agarose gel. A 100 bp DNA ladder marker was included as the standard molecular weight marker. The electrophoresed gel was later subjected to ethidium bromide staining and photographed under UV transillumination.



DISCUSSION:

Diversity of ace, a gene encoding a microbial surface component recognizing adhesive matrix molecules, from different strains of enterococcus faecalis. Enterococci are Gram-positive facultative anaerobic bacteria which can cause a variety of diseases in humans, including septicaemia, endocarditis, urinary tract infections, wound infections, and meningitis.Ace function as putative virulence factors of endocarditis-causing *E. faecalis*. A potential explanation for the persistence of *E. faecalis* in the root canal might be an expression of adherence (virulence) factors like ace.Virulence factors of *E. faecalis* enable adherence to host tissue, invasion and modulation of host inflammatory response, and secretion of various products which enhance biofilm formation.

Love *et al*. suggested the role of serum in the invasion of dentinal tubules by *E. faecalis*. Serum, which originates from alveolar bone and periodontal ligament, facilitates enhanced dental invasion and collagen adhesion of *E. faecalis*. This

synergistic effect between serum and enterococci cells promotes binding and allows invasion of the dentine to occur in the presence of serum.The extracellular matrix of all mammalian tissues consists of glycoproteins (e.g. collagen, laminin, fibronectin) and proteoglycans that can be exploited by micro-organisms for colonization and initiation. The ability of a bacterium to adhere to collagen has been shown to play an important role in the pathogenesis of endocarditis. As dentinal tissues share common proteins with the heart tissues, a role for ace should facilitate bacterial adhesion to collagen and extracellular matrix relevant in endodontic infections.

The result of this study illustrated that out of 20 isolates 2 were shown to express ace (collagen binding protein) gene.E.faecalis is a microorganism that can survive extreme challenges. Its pathogenicity ranges from life-threatening diseases in compromised individuals such as bacteremia, septicemia, endocarditis and urinary tract infections to less severe conditions, such as infection of obturated root canals with chronic apical periodontitis.A potential explanation for the persistence of E.faecalis in the root canal might be an expression of adherence (virulence) factors like ace. In the past few years, *Enterococcus faecalis* has been mentioned with increased frequency with regard to teeth with asymptomatic persistent endodontic infections, predominantly in therapy-resistant endodontic infections.

CONCLUSION:

E.faecalis have been able to form biofilms in root canals, and this ability can be important for bacterial resistance and persistence after endodontic procedures. Virulence factors like collagen binding protein (ace) plays a significant role in enterococcal adhesion to collagen mix.From this study, it is evident that ace, a potent E,faecalis virulence gene can be found in the E.faecalis strains obtained from the patients with endodontic infections which is responsible for its pathogenecity leading to the failure of root canal treatments.

REFERENCES:

1. Güven Kayaoglu, Dag Ørstavik .Virulence factors of Enterococcus faecalis:A relationship to Endodontic diseases. *Crit Rev Oral Biol Med 15(5):308-320 (2004).*

2. Gunnar Dahle´ n*, Susanne Blomqvist, Annica Almsta˚hl and Anette Carle´n.Virulence factors and antibiotic susceptibility in enterococci isolated from oral mucosal and deep infections. Journal of Oral Microbiology 2012, 4: 10855.

3. Salah, Randa, et al. "Prevalence of putative virulence factors and antimicrobial susceptibility of Enterococcus faecalis isolates from patients with dental Diseases." *BMC Oral Health* 8.1 (2008): 17.

4. Vairaktaris, Eleftherios, et al. "Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with increased risk for oral cancer." *Acta oncologica* 46.8 (2007): 1097-1102.

5. Lebreton, Francois, et al. "ace, which encodes an adhesin in Enterococcus faecalis, is regulated by Ers and is involved in virulence." *Infection and immunity* 77.7 (2009): 2832-2839.

6. Nallapareddy, Sreedhar R., et al. "Diversity of ace, a gene encoding a microbial surface component recognizing adhesive matrix molecules, from different strains of Enterococcus faecalis and evidence for production of ace during human infections." *Infection and immunity* 68.9 (2000): 5210-5217.

7. Kowalski, William J., et al. "Enterococcus faecalis adhesin, Ace, mediates attachment to particulate dentin." *Journal of endodontics* 32.7 (2006): 634-637.

8. Nallapareddy, Sreedhar R., and Barbara E. Murray. "Ligand-signaled upregulation of Enterococcus faecalis ace transcription, a mechanism for modulating host-E. faecalis interaction." *Infection and immunity* 74.9 (2006): 4982-4989.

9. Mohamed JA, Huang DB. Biofilm formation by enterococci. J Med Microbiol 2007; 56: 1581 8.

10. Kayaoglu G, O rstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Crit Rev Oral Biol Med 2004; 15: 308 20.