DETECTION OF efaA (ENDOCARDITIS ASSOCIATED ANTIGEN) GENE FROM ENTEROCOCCUS FAECALIS ISOLATED FROM PATIENTS WITH ENDODONTIC INFECTIONS

TYPE OF MANUSCRIPT- Research Article

RUNNING TITLE: Detection of efaA (Endocarditis associated antigen) gene from Enterococcus Faecalis isolated from patients with endodontic infections.

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ABSTRACT:

AIM AND OBJECTIVE:
To detect efaA (endocarditis associated antigen) gene from Enterococcus faecalis isolated from patients with endodontic infections.

METHOD:
A total of 20 isolates of Enterococcus faecalis were collected from patients undergoing endodontic treatment. The samples were inoculated in Mac Conkey agar and Brain infusion agar. DNA was extracted, quantified and identified spectrometrically and electrophoretically. The gene EfaA (endocarditis associated antigen) was amplified by PCR amplification technique using specific primer and conditions.

RESULT: Among 20 isolates, 3 isolates were detected with efaA gene.

Keywords: endodontic infection, efaA gene, antigen

INTRODUCTION:

Enterococci are normal commensals in the gastrointestinal tract, oral cavity, vagina etc.They are organisms of low virulence, but are known to cause various clinical infections. 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. 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].

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 infection[2]. *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[3]. *E. faecalis* endocarditis antigen (efaA) was first identified from the antiserum of a patient with *E. faecalis* endocarditis. The amino acid sequence of the associated protein efaA revealed 55–60% homology to a group of streptococci proteins known as adhesins. Hemolysin, gelatinase, ace, and esp also function as putative virulence factors of endocarditis-causing *E. faecalis*. *E. faecalis* strains derived from different sources such as endocarditis, urinary tract infections, and even endodontic infections have been shown to possess distinct patterns of 'virulence factors'[4]. Hence the aim of our study was to detect the presence of putative *E. faecalis* virulence factor efaA in root canals of therapy-resistant endodontic infections using the PCR method.

MATERIALS AND METHOD:

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

DNA EXTRACTION:

Genomic DNA used as template for polymerase chain reaction (PCR) amplification was prepared using conventional phenol-chloroform DNA extraction method.

AMPLIFICATION:

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.

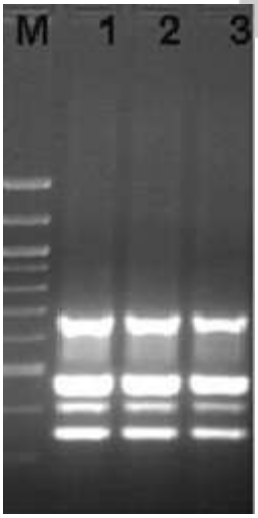
The PCR amplification of the efaA gene was carried out as follows: Predenaturation at 95°C for 4 min followed by denaturation at 95°C for 30 cycles of 30 s each; 1 min for annealing at 52°C and elongation at 72°C for 1 min. Both positive control and negative control, consisting solely of the PCR reaction mixture without DNA template were included to check the validity of the technique utilized.

DNA ANALYSIS:
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

RESULT:

Efa gene was detected in 3 out 20 isolates.

Gene	Primer sequence	Product size
efaA	EFA 1 GACAGACCCTCACGAATA EFA 2 AGTTCATCATGCTGTAGTA	705



DISCUSSION:

Enterococci are gram positive cocci that can occur singly, in pairs or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. Enterococci can withstand harsh environmental conditions. There are currently 23 enterococci species and they are divided into five groups based on their interaction with mannitol, sorbose and arginine [5] .*E.*

faecalis can survive extreme challenges. Its pathogenicity ranges from life-threatening diseases in compromised individuals such as bacteraemia, septicaemia, endocarditis, and urinary tract infections to less severe conditions, such as infection of obturated root canals with chronic apical periodontitis.The persistence of E.faecalis might be due to the virulence factors ace and esp .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.[6] Serum plays an important role in the invasion. The expression of virulence factor efaA is expressed when E.faecalis is grown in a medium that contains serum.[7] The extra cellular matrix of our tissue consists of glycoproteins like collagen,laminin, fibronectin and proteoglycans. These can be exploited by micro-organisms for colonization and initiation. The ability of a microorganism to adhere to collagen play an important role in the pathogenesis of endocarditis.[8] The dentinal tissue and the heart tissues share common proteins, it is believed that efaA should be facilitated through bacterial adhesion to collagen and extracellular matrix relevant in endodontic infections.[9]

About 3 out of 20 (almost 15%) isolates were detected with efaA gene. 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.

A similar recent molecular-based study by Randa Salah indicated that virulence determinants endocarditis antigen (efaA) genes and ace genes has been found in *E. faecalis* isolates of oral rinse samples of patients suffering from dental diseases[10].

In a study conducted by Creti *et al*. showed that *E.faecalis* strains derived from different sources such as endocarditis, urinary tract infections possessed distinct patterns of virulence factors ace, efaA, and gelE genes. These were found to be the most common virulence factors.

Thomas Preethee conducted a study in which 32 contaminated root canal samples were analysed in which 15 of them were positive for E.faecalis. Out of the 15 positive samples, efaA gene was identified in 11 samples (almost 73%).[11]

CONCLUSION:

E.faecalis though a commencal bacteria in Genito urinary tract it is gaining importance as in nosocomial pathogen and in endodontic infections. From this study it can be concluded that efaA, a potent *E. faecalis* virulence gene can be found in strains obtained endodontic infections. Hence this virulence gene can be correlated with the pathogenicity of E.faecalis. Further studies should aim at obtaining blood samples from patients immediately after endodontic retreatment procedure to analyse the role of the efaA gene in causing bacteremia.

REFERENCES:

1. Suchitra U,Kundabala.M, Enterococcus faecalis : An endodontic pathogen
2. Love RM. Enterococcus faecalis--a mechanism for its role in endodontic failure. Int Endod J. 2001;34:399–405.
3. Arias-Moliz MT, Ferrer-Luque CM, Espigares-García M, Baca P. Enterococcus faecalis biofilms eradication by root canal irrigants. J Endod. 2009;35:711–4.
4. Sedgley CM, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, et al. Virulence, phenotype and genotype characteristics of endodontic enterococcus spp. Oral Microbiol Immunol. 2005;20:10–9
5. Charles H. Stuart, DDS, Scott A. Schwartz, DDS, Thomas J. Beeson, DDS, and Christopher B. Owatz, DMD, Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment.
6. Kayaoglu G, Ørstavik D. Virulence factors of enterococcus faecalis: Relationship to endodontic disease. Critical Rev Oral Biol Med. 2004;15:308–20.
7. Lowe AM, Lambert PA, Smith AW. Cloning of an Enterococcus faecalis endocarditis antigen: Homology with adhesins from some oral streptococci. Infect Immun. 1995;63:703–6.
8. Creti R, Imperi M, Bertuccini L, Fabretti F, Orefici G, Di Rosa R, et al. Survey for virulence determinants among enterococcus faecalis isolated from different sources. J Med Microbiol. 2004;53:13–20
9. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB, Owatz Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32:93–8
10. Randa Salah, Najla Dar-Odeh, Osama, Osam Abu Hammad, Shahabi Asem A. Prevalence of putative virulence factors and antimicrobial susceptibility of enterococcus faecalis isolates from patients with dental diseases. Biomedical Oral Health. 2008;8:1–7
11. Thomas Preethee, Deivanayagam Kandaswamy, and Rosaline Hannah, Molecular identification of an *Enterococcus faecalis* endocarditis antigen efaA in root canals of therapy-resistant endodontic infections, Journal of conservative dentistry.2012 Oct-Dec.