



DETECTION OF ENHANCED EXPRESSION OF PHEROMONES AND QUORUM SENSING SYSTEM AMONG ENTEROCOCCUS FAECALIS FROM DENTAL CARIES BY PCR

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ABSTRACT

As, there has been a paucity in detecting such virulence markers among dental isolates of *Enterococcus faecalis* in Indian context, we have taken this objective to find out such factors to rule out the relationship between the pathogen and the disease establishment. It indicates that these genes may even play a cardinal role in causing dental caries by *E. faecalis*. A sum of 20 dental caries enterococcal isolates were detected for enhanced expression of pheromones and quorum sensing system genes by PCR. 4/20 (20%) were showed positive for enhanced expression of pheromone (*eep*) and 2/20 (10%) were found to have quorum sensing system (*fsr*) genes by PCR. It indicates that these genes may even play a cardinal role in causing dental caries by *E. faecalis*. However, it is important to include more number of isolates to validate the result.

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INTRODUCTION

The human dental cavity is colonised with large groups of aerobic and anaerobic bacterial species. *Enterococcus faecalis* as a nosocomial pathogen can cause serious infections that are frequently isolated (30-90%) from root canal treated patients [1]. *Enterococcus faecalis* is most commonly found in the faeces. The high prevalence of this species in root canal treated patients evidenced by culturing methods, and molecular detection tools suggested that it may be the reason for most of the endodontic treatment failures [2]. Considering virulence genes such as *eep* (enhanced expression of pheromone) and *fsr* (quorum sensing system) are playing its critical role in the pathogenesis of *E. faecalis* in dental infections [3]. The most common cause of root canal treatment failure is due to the microorganisms in the apical portion of the teeth [4, 5]. *Enterococcus* is the common bacteria that are found in the periapical lesion in the root canal treated teeth [6, 7]. Thus, this study planned to investigate the presence of enhanced expression of pheromones and quorum sensing system genes by PCR in *Enterococcus faecalis* isolates from dental caries.

MATERIALS AND METHODS

Clinical isolates

A total of 20 different non-repetitive dental caries isolates of *E. faecalis* were collected included in this study.

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These isolates were identified by standard biochemical parameters as described by elsewhere. Isolates were preserved in semi-solid brain heart infusion medium and stored at 4°C until further use.

Antimicrobial susceptibility test

Antibiotic susceptibility test was determined for these strains to routinely used antibiotics such as ampicillin (10µ), vancomycin (30µ), teicoplanin (30µ), erythromycin (15µ), ciprofloxacin (5µ), amikacin (200µ), gentamycin (10µ), tetracycline (30µ) and linezolid (30µ) (Hi Media, Mumbai) by kirby-bauer disc diffusion method [8].

Detection of enhanced expression of pheromones (*eep*) and quorum sensing system (*fsr*) among *Enterococcus faecalis* by PCR

E. faecalis isolates were detected for the presence of such genes by PCR analysis. Detection of the gene was carried out using primer as depicted in table 1. Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. The reaction mixture contained 1mM of MgCl₂, 0.2mM dNTP mix and 0.8µM of *eep* and *fsr* genes with 1U of Taq polymerase (New England Biolabs) in a 1x PCR buffered reaction. PCR amplification was carried out using thermal cycler (Eppendorf) with the following cycling condition.

Table 1 Gene sequencing of *eep* and *fsr* genes

Primer	Primer sequence	Product size
<i>eep</i>	5'-GAGCGGGTATTTTAGTTCGT-3' 5'-TACTCCAGCATTGGATGCT-3'	341 bp
<i>fsr</i>	5'-AACCAGAATCGACCAATGAAT-3' 5'-GCCCTCATAACTCAATACC-3'	511 bp

Initial denaturation at 98°C for 6 min and 35 cycles for 30s, 70°C for 30s and 69°C for 60s, followed by a final extension of 5 min at 75°C. PCR products were resolved in 1.5% agarose gel. A 100bp ladder was including in all the gel analysis [9].

RESULTS

Antibiotic susceptibility pattern

We found increased percentage of isolates were shown to be resistant to all the antibiotics used in this study. For ampicillin, amikacin, erythromycin, gentamicin, our isolates were found to resistant between 80-90%. Better sensitivity was observed in linezolid, teicoplanin and vancomycin antibiotics. The detailed results of antibiotic sensitivity patter of Enterococci was given in table 2.

Table 2 Results of antibiotic sensitivity patter of Enterococci

Antibiotics	Sensitivity	Intermediate	Resistance
Ampicillin	1(5%)	2(10%)	17(85%)
Vancomycin	15(75%)	1(5%)	4(20%)
Teicoplanin	12(60%)	3(15%)	5(25%)
Erythromycin	2(10%)	0	18(90%)
Ciprofloxacin	6(30%)	0	14(70%)
Amikacin	1(5%)	1(5%)	18(90%)
Gentamycin	2(10%)	2(10%)	16(80%)
Tetracycline	4(20%)	4(20%)	12(60%)
Linezolid	18(90%)	1(5%)	1(5%)

Results for enhanced expression of pheromone (eep) and have quorum sensing system (fsr) genes

Of the 20 dental isolates of *Enterococcus faecalis*, 4/20 (20%) were showed positive for enhanced expression of pheromone (eep) and 2/20 (10%) were found to have quorum sensing system (fsr) genes by PCR.

DISCUSSION

With increasing resistance to some routinely used antibiotics, enterococcal infections pose a big threat as a nosocomial pathogen. *E. faecalis* was appeared to cause 90% of the enterococcal infections in humans, and it was frequently found in obturated root canals exhibiting symptoms of chronic apical periodontitis, particularly in post dental monocultures [10, 11]. Study conducted by Prashanth and co-workers in 2016, observed different virulence factors in enterococcal isolates from dental caries. Wherein, 48% of the isolates were found to have eep gene indicates that bacterial pheromone secretion is necessary for inducing conjugation, and confirming its role in different dental conditions. It was also reported that eep also involved in biofilm formation [12] and it also provides lysozyme resistance to the host. 81% of isolates also had fsr gene which encodes for pheromone synthesis of this bacteria. In our study, we have observed 20% and 10% of our isolates were found to have eep and fsr gene that codes for enhanced expression of pheromones and quorum sensing system respectively.

CONCLUSION

As, there has been a paucity in detecting such virulence markers among dental isolates of *Enterococcus faecalis* in Indian context, we have taken this objective to find out such factors to rule out the relationship between the pathogen and the disease establishment. It indicates that these genes may even play a cardinal role in causing dental caries by *E. faecalis*. However, it is important to include more number of isolates to validate the result.

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