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DETECTION OF *FIM*A GENE FROM VIRIDANS GROUP STREPTOCOCCI (VGS) ISOLATED FROM ORAL CAVITY OF HEALTHY POPULATION

R. SANDHIYA*, S. SENTHIL KUMAR AND THANGAM MENON

Department of Microbiology, Dr.A.L.M. Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamilnadu, India

ABSTRACT

Viridans group streptococci (VGS) are commensals of human oral cavity. They are the major cause of native valve endocarditis in immunocompetent individuals. Among the factors contributing to its virulence, its ability to adhere to host cells is considered to be important. FimA is an important virulence factor which promotes the adherence of bacteria to fibrin in vegetations of infective endocarditis (IE) patients. FimA is an adhesin protein encoded by *fim*A gene. The aim of the present study was to detect *fim*A gene among viridans group streptococci by Polymerase Chain Reaction (PCR). Viridans group streptococci including *S. mitis* (11), *S. salivarius*(11), *S. oralis*(1), *S. sanguinis*(2)and *S. parasanguinus*(1) isolated from oral cavity of healthy population were included in the study. The *fim*A gene was detected by PCR. Of the strains, only one (3.8%) was positive for *fim*A gene and it belonged to species *S. parasanguinus.* None of the other species were positive for *fim*A gene. Although *fim*A gene is considered as a major virulence factor and associated with IE, our data suggests that it may also be present in *S. parasanguinus* strains isolated from oral cavity of healthy population.

KEYWORDS - Viridans group streptococci, Polymerase Chain Reaction, infective endocarditis, *fim*A gene.

R. SANDHIYA
Department of Microbiology, Dr.A.L.M. Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamilnadu, India

INTRODUCTION

Viridans group streptococci (VGS) constitute normal flora of human oral cavity, gastrointestinal tract and female genital tract. These endogenous strains gain access to normally sterile sites through breaks in the mucous membranes and can cause septicaemia and endocarditis[1]. Adherence to damaged heart valves is a crucial event in the pathogenesis of infective endocarditis (IE). The genes for several adhesins have been identified which include *fimA, cshA, cshB, abpA, fnbp*and *gtf*[2]. In patients with valve lesions, low grade bacteraemia (due to tooth brushing) may lead to IE. Even in healthy individuals (without preexisting valvular pathology) exposure to dental manipulations, invasive upper respiratory tract, gastrointestinal and genitourinary diagnostic and surgical procedures predisposes to IE[3]. The gene *fimA* encodes an adhesin protein FimA. It is a 36-kDa surface associated protein of *S. parasanguinus*, the primary colonizer of dental plaque and has got major role to play in IE. In the oral cavity this protein mediates adherence to salivary pellicle[4]. FimA acts as an important virulence determinant in *S.parasanguinus* endocarditis and is implicated in promoting bacterial adherence to fibrin in sterile vegetations[3]. The present study was aimed to investigate the presence of *fimA* gene among VGS isolated from oral cavity of healthy population.

MATERIALS AND METHODS

Throat swabs were collected from fifty one healthy subjects using sterile cotton swabs and transported to the lab in an icebox and processed immediately. Throat swabs were subcultured on blood agar plates and incubated at 37ºc with 5% CO_2 for 24 hours. After 24 hours the plates were observed for the presence of alpha/ non-hemolytic, pinpoint to small colonies characteristic of VGS. Colonies which were gram positive cocci in chains and catalase negative were characterised and identified by standard procedures[5]. For the molecular biology study the DNA was extracted from VGS using alkaline lysis method[6]. Briefly, a single colony of VGS was suspended in 100 µl of 50mM sodium hydroxide. Suspension was incubated at 94ºc for two minutes (by keeping it in a water bath) and immediately kept at 4ºc for five minutes (by keeping it in the refrigerator). It was then neutralized with 16 µl of 1 M Tris - HCl (pH 8.0). After centrifugation for two minutes at 8000 rpm supernatant were collected and used for PCR amplification. The *fimA* gene was detected by amplifying template DNA using *fimA* specific primer and cycling conditions as described by Viscount *et al*[3]. The primer sequence, cycling condition and master mix condition are given in Tables 1, 2 and 3 respectively.

Table 1
Primer sequence for fimA gene

Gene	Primer sequence	Expected amplicon size
fimA	5'- GCTGGGGATAAGATCGAGCTCCACAG -3' 5'- TTCATCATGCTGTAGTAGCTATCGCC -3'	930 bp

Table 2
Cycling conditions for fimA PCR

28 cycles	Denaturation at 94ºc for 30 sec
	Primer annealing at 55ºc for 20 sec
	Extension at 72ºc for 45 sec

Final extension at 72ºc for 7 minutes.

Table 3
Master mix conditions

Constituents	Amount
Buffer	5 µl
dNTP	1 µl
Forward primer	3 µl
Reverse primer	3 µl
Taq polymerase 5 U/µl	1 µl
Template DNA	5 µl
Mq water	32 µl

The PCR products were resolved in 1.5% agarose gel and stained with ethidium bromide. 100 bp ladder was used as marker and electrophoresis was done for one hour at 100 volts. After electrophoresis the gels were visualised under gel documentation system (Bio Rad) and was documented (Figure 1).

Gel documentation of the Viridans group streptococci (VGS) analyzed for the presence of fimA gene by Polymerase Chain Reaction (PCR)

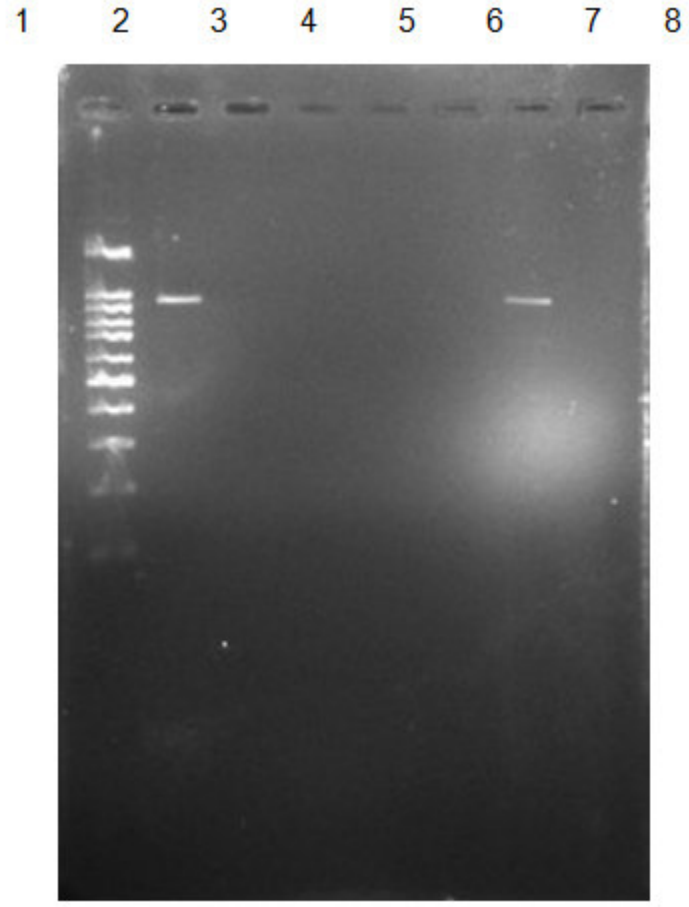

Figure 1
Legend: Lane 1- 100bp DNA ladder, Lane 2- The fimA gene in S. parasanguinus, Lane 7- fimA gene positive control

RESULTS

A total of 26 VGS including *S. salivarius* (11), *S. mitis* (11), *S. oralis* (1), *S.sanguinis* (2), *S. parasanguinus* (1) were isolated from oral cavity of healthy population. Of all the 26VGS analyzed for the presence of *fimA* gene by PCR, 1/26 (3.8%) revealed positive for *fimA* gene and belonged to the species *S. parasanguinus*. None of the other species were positive for *fimA* gene (Table 4).

Table 4
Prevalence of fimA gene among Viridans group streptococci (VGS)

VGS Species	Number tested	Number positive for *fimA* gene (%)
S. salivarius	11	0
S. mitis	11	0
S. oralis	1	0
S.sanguinis	2	0
S. parasanguinus	1	1 (3.8%)

DISCUSSION

VGS are the major cause of native valve endocarditis in immunocompetent individuals. They account for nearly 60% of infections[4]. Streptococcal adhesins play a role in pathogenesis of endocarditis by enhancing adherence to vegetations. Adherence of microorganisms to damaged cardiac tissue is an important event in the pathogenesis of IE. Fibrin and platelets are deposited at the site of endothelial cell injury and they give rise to sterile vegetation. During bacteraemia, these bacteria will adhere and colonize these sterile vegetations. Then multiplication of these microorganisms take place giving rise to formation of bigger sized vegetation. This subsequently leads to mechanical dysfunction of heart valves and release of infected thrombi. FimA a surface protein is a major virulence factor associated with *S. parasanguinus* endocarditis. FimA is a 36 kDa protein, belonging to lipoprotein receptor antigen I (Lra I) family of proteins[7]. In the oral cavity FimA facilitates adherence to the salivary pellicle which is crucial for colonization because the salivary flow can dislodge the microorganisms if they are not firmly attached[8]. It has been shown that FimA mediates colonization of *S. parasanguinus* in experimental endocarditis in rats[9]. Dental manipulations precede nearly one third of streptococcal viridans IE[10]. Following invasive dental procedures, streptococci enter into the blood circulation and colonize cardiac valves even without pre-existing abnormality leading to development of IE[4]. So prophylactic antibiotics are to be given which target these pathogens. Only about 50% of patients with IE have recognisable predisposing cardiac valve abnormalities and IE associated with invasive healthcare (diagnostic and surgical) procedures constitute only a minority of cases. This indicates that only a small percentage of IE cases may be preventable with antibiotic prophylactic coverage[11]. Vaccines or artificial peptides directed against specific bacterial adhesins could interfere with colonization of valves. Some experimental successes have been achieved with vaccination against the streptococcal FimA protein[12]. FimA is a virulence factor in rat model of endocarditis. Its ability to act as a vaccinogen has been tested[4]. FimA immunized animals were found to be less susceptible to subsequent challenge with *S. parasanguinus* than the non-immunized ones. Antibodies hinder the microbial attachment to vegetations[13].

The degree of conservation of gene encoding FimA among several species of streptococci was examined to evaluate the reliability of FimA as a vaccine for streptococcal endocarditis[3]. The results from southern hybridization and PCR analysis of genomic DNA demonstrate that fimA homolog is common in other species of oral streptococci. DNA from *S. mutants*, *S. oralis* and *S.*

salivarius strongly hybridized with *fimA* probe. This indicates the presence of closely related genes in these species[3]. Also results from western blot analysis demonstrate the expression of FimA antigen by a majority of streptococcal strains which were isolated from blood culture of bacteraemic patients with infective endocarditis. A total of 81% of the blood isolates tested expressed proteins that were reactive with anti- FimA serum. Proteins comigrating with FimA were widely detected. Hence FimA protein is an ideal target for a preventive vaccine[3]. Viscount *et al*., (3) have reported that majority of streptococcal strains associated with infective endocarditis have a gene that encode FimA-like proteins. But, in our study 3.8% of VGS isolated from healthy subjects were positive for *fimA* gene. It was also reported that genes which exhibit close similarity with *fimA* are common in other species of oral streptococci. The presence of *fimA* gene in *S. parasanguinus* was demonstrated by Curley *et al*. (4). In our study only *S.parasanguinus* revealed *fim*A gene while the other species were negative for this gene.

CONCLUSION

Of the total 26 VGS from oral cavity of healthy population, 1/26 (3.8%) revealed positive for *fimA* gene and belonged to the species *S. parasanguinus*. None of the other species were positive for *fimA* gene. Although *fimA* gene is regarded as a most important virulence factor associated with infective endocarditis, our data suggests that it may also be present in *S. parasanguinus* strains isolated from healthy population.

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