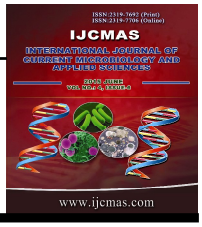
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 6 (2015) pp. 78-85**
http://www.ijcmas.com



Original Research Article

Resistance Patterns of Viridans Group of *Streptococci* isolated in transient bacteremia after third molar surgery

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Keywords

Resistance, Viridans group streptococci, Transient, Bacteremia, Third molar surgery

A B S T R A C T

The aims of this study were to investigate the prevalence of transient bacteremia due to Viridans group streptococci (VGS) after third molar impactions. To find the resistance patterns of the isolated viridians group of streptococci by antibiotic sensitivity studies and to perform virulence gene studies on the resistant strains of VGS isolated. These aims and objectives served as the basis for the current investigation. 100 generally healthy patients, who came to SRM Dental College, Ramapuram, Chennai, were taken for the study. All patients were scheduled to undergo third molar surgery. Blood samples were collected at baseline before extraction and after completion of the surgical procedure. No bacteria were isolated at baseline in all the patients the prevalence of bacteremia was 82% following third molar surgery. The most frequently isolated bacteria was VGS. 63 isolates (76.8%) of VGS were obtained from blood samples of patients who were positive for bacteremia. 36(57.1%) of the VGS isolates were found to be resistant to amoxicillin with *S. mitis* showing the highest resistance. Virulence gene studies performed on the 32 resistant isolates *S. mitis* detected *Mitilysin gene* 29(91%), *cbp 1* gene (66%), *amx_res_gene* 27(85%). Our findings suggest that the most important cause of transient bacteremia following third molar surgeries is VGS. *S.mitis* showed the highest prevalence rate and resistance to amoxicillin and a high percent of virulence genes were observed in the amoxicillin resistant *S. mitis* isolated.

Introduction

According to the Centers for Disease Control and Prevention (CDC), Blood Stream Infections can be defined as the presence of viable bacteria in the blood (i.e., bacteremia) which is documented by a positive blood culture result (Teresa C

Horann *et al.*,2008). Normal oral microbes can enter the bloodstream in relatively high numbers, during invasive dental procedures and circulate throughout the body, these bacteremias are however transient meaning that these microbes are usually eliminated by the reticuloendothelial system within a few minutes, and usually do not cause any clinical symptom. However a few bacteria may survive in the circulation. These persistent bacteria may evade the initial host immune response and to seed target organs. After a certain period of time, these bacteria start to multiply and can cause systemic infections. The most common complication due to transient bacteremia of dental origin is bacterial endocarditis (Harald Seifert, 2009). Bacteria can enter the bloodstream from the oral cavity by a number of mechanisms and by various routes. The bacterial biofilm in the gingival tissue is harmoniously balanced so a break or damage of this oral niche can cause spread of the oral flora into the bloodstream. Routine every day activities such as chewing, brushing, and flossing, can breakdown the barrier between the gingival tissues and oral biofilm. Most often in dentistry this disruption is due to tissue trauma caused by procedures such as scaling, probing and tooth extractions which can result in a breakage in capillaries or small blood vessels located near the plaque biofilms which allows the entry of bacteria into the systemic circulation (Parahitiyawa *et al.,* 2009).

The Viridians Group Streptococci (VGS)

They are a large group of commensal streptococcal bacteria species. They are γ – haemolytic and produce a green colour when grown on blood agar (the name "viridians" was derived from latin, which means green). The VGS are gram positive, facultative anerobes. They can be cultivated on blood agar. Many species in this group cause partial destruction of erythrocytes with resultant green discoloration on blood agar to produce α-haemolytic colonies. They do not produce catalase or coagulase. They can be differentiated from *Streptococcus pneumoniae* as they show resistance to optochin and are negative for bile solubility test. The viridans group streptococci are classified as:

Mitis group
Mutans group
Salivarius group
Anginosus group
Sanguis group

VGS can be present as normal commensals of the upper respiratory tract, the female genital tract, and the gastrointestinal tract, but are mainly prevalent in large amounts in the oral cavity. The presence and adherence of viridans streptococci to the dental and oral surfaces provides colonization resistance within the oral cavity to resist colonization of pathogenic bacteria. This is due to a fibronectin. The fibronectin adhesin on streptococci is lipoteichoic acid. Infective endocarditis is the most common clinical manifestation caused by viridans streptococci bacteremia and is mostly seen in patients with underlying valvular heart disease. Penicillin G remains the drug of choice in treatment of endocarditis due to viridans streptococci (Xiang Y Han, 2006).

Traditionally Viridans streptococci has been considered to be uniformly susceptible to penicillin. The emergence of penicillin resistant strains of Viridans streptococci which are intermediately resistant or highly resistant to penicillin is on the rise, worldwide, and this issue has complicated the antibiotic therapy for bacteremia due to VGS. Amoxicillin is still the most widely used antibiotic in routine dental practice in

India and most parts of the world. However, several recent studies indicate that the VGS present in the oral cavity as part of the normal flora to have become increasingly resistant to amoxicillin (Chitra, 2014). VGS can act as a reservoir of antimicrobial resistance genes, transferring different resistance traits to more pathogenic organisms like *Streptococcus pneumoniae* and *Streptococcus pyogenes* (Whatmore *et al.,* 2000). Only a few isolated studies have investigated the susceptibilities of viridans streptococci isolated from blood cultures, to various antimicrobial agents and the number of studies on viridans streptococci causing bacteremia which originate from the normal oral flora are even more less. In the present investigation virulence gene studies were conducted for the species of VGS which showed highest resistance to amoxicillin, as it is the routinely prescribed antibiotic for dental procedures.

Materials and Methods

Ethical approval

This study has been approved by the Ethical committee of SRM University. The purpose of the study was explained and a written consent was obtained from all patients included in the study before collection of sample from the patients. This study was carried out in the Department of Microbiology, SRM Dental College and Hospital – Ramapuram, SRM University, Chennai, India. The study comprised of 100 generally healthy patients who came to the Dept. of Oral and Maxillofacial Surgery, SRM Dental College and Hospital. All the patients were scheduled to undergo third molar surgery and none had taken any pre-prophylactic antibiotic. Blood samples were collected at baseline, and after the surgical procedure.

Blood collection and processing

Before sampling, the patient's skin was cleaned with antiseptic – povidone iodine and 70% iso propyl alcohol and allowed to dry before collection of blood sample – to avoid risk of contamination. Each blood sample (10 ml) was collected using an IV cannula (BD Venflon – Becton Dickinson India (P) Ltd) placed in the antecubital vein (Tomas *et al.,* 2008). Before local anesthetic injection was given, the baseline blood sample was collected and the second sample was collected after the surgical procedure. Blood samples were inoculated into the Aerobic culture media bottles (Hi media Mumbai, India - BHI broth with SPS) and Anaerobic culture media bottles(Hi media Mumbai, India - BHI and RCM broth) (Chitra and Mangayarkarasi, 2015).

Microbiological analysis

The blood culture bottles were transferred to the Department of Microbiology, SRM University within 10 minutes of collection. The blood culture bottles were incubated separately for aerobic and anaerobic cultivation. Blood samples which were collected were incubated for 48 hours at 37˚C. Culture bottles were examined for turbidity and subcultures were done. For aerobic culture, positive blood cultures were subcultured on blood agar and chocolate agar plates and incubated aerobically in a CO_2 incubator at 37˚C with 5%-10% CO_2 for 24-72 hours and observed for growth of bacterial colonies. For anaerobic cultures, positive blood cultures were subcultured onto blood agar and brucella agar plates (Rajasuo *et al.,* 2004) supplemented with haemin and vitamin K and incubated in anaerobic jar with gaspak (Hi-media Ltd). On plates with growth, the colonies were isolated and subcultures were made.

Table.1 Primers used for subtyping of VGS group

VGS	Primer used	Annealing Temp
S. mitis	FP-5'-TCGCGAAAAAGATAAACAAACA-3' 5'-GCCCCTTCACAGTTGGTTAG-3'	Annealing at 58°C for 30s
S. mutans	5'-ATT CCC GCC GTT GGA CCA TTC C-3' 5'-CCG ACA AAG ACC ATT CCA TCT C-3'	Annealing at 60°C for 30s
S. sangius	F1 (5′-GATTGACCAAGAACGCCGGGCT-3′) R3 (5′-CGCATGATATCAGAGATGCAACCC-3′)	Annealing at 60°C for 30s
S. salivarius	5'-CAAGGAATTGATTCAGCAACAGTGC-3' 5-CTTCTCAACAAGCATTGGCAGATGC-3'	Annealing at 54°C for 30s
S. anginosus	5'-ACA GTT TAT ACC GTA GCT TGC TAC ACC AT-3' 5'-CGT AGT TAG CCG TCC CTT TCT GG-3'	Annealing at 57°C for 30s

Table.2 Virulent genes detected in *S. mitis* and primers used

S.No	VIRULENCE GENE	PRIMERS USED
1.	Mitilysin [Cytolysin from *Strep mitis*] –***Mly gene***	Fp: ACCCTCGAAATTGAAATGCTTCC Rp: CAATAATGGACTGGCCGCCT
2.	*S. mitis* Cell binding protein - ***cbp 1 gene***	Fp: GAATACCACAGGTGGCCGAT Rp: GCCATCTACGGTCGTATTCACT
3.	*Streptococcus mitis* amoxicillin resistant gene 12 – ***amx_res_gene* 12**	Fp: CCGGTCAGCATCTACACACA Rp: GATTGTCAGCAACGGGCTTC

Table.3 Antibiotic sensitivity patterns of Viridans group streptococci (n=63)

Antibiotic	Content Disc/µg	Interpretation (Disk Difffusion method)					
		Sensitive (mm)	No.of isolates	Intermediate (mm)	No. of isolates	Resistant (mm)	No.of isolates
Amoxicillin	20	(≥22mm)	20	(19-21mm)	07	(≤18mm)	36
Azithromycin	15	(≥18mm)	20	(14-17mm)	18	(≤13mm)	25
Clindamycin	2	(≥21mm)	28	(15-20mm)	20	(≤14mm)	15
Moxifloxacin	5	(≥18mm)	60	(15-17mm)	3	(≤14mm)	-
Doxycycline	30	(≥16mm)	49	(13-15mm)	08	(≤12mm)	6
Ceftriaxone	30	(≥27mm)	46	(25-26mm)	12	(≤24mm)	5
Cefdinir	5	41	41	16	16	6	6
Amoxyclav	20	(≥18mm)	34	(14-17mm)	25	(≤13mm)	4
Levofloxacin	5	(≤13mm)	58	(14-16mm)	5	(17mm)	0

Table.4 Antibiotic resistance of the different species of Viridans group streptococci (n=63) to amoxicillin

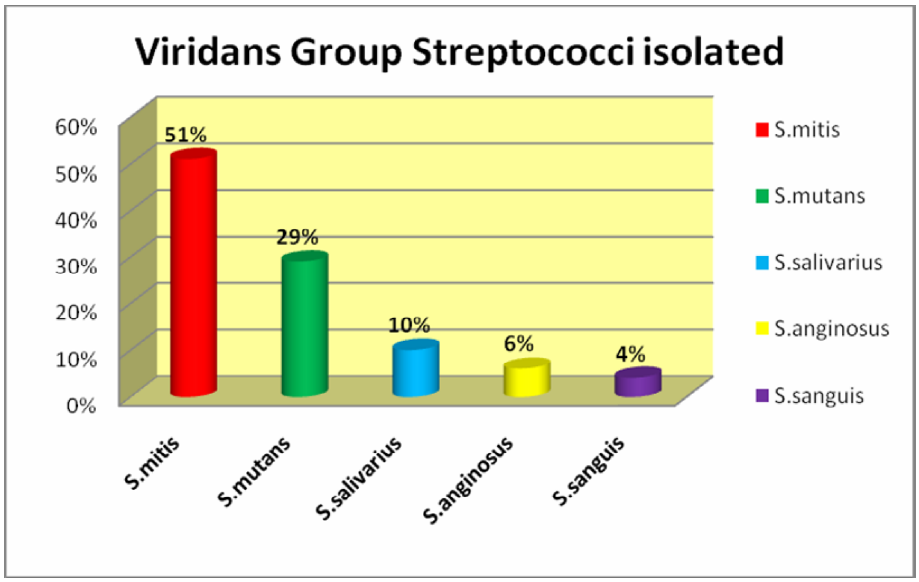
S.no	VGS species	No. isolated	No.Susceptible	No.Resistant
1.	*S.mitis*	32	0	32(100%)

2.	*S.mutans*	18	18(100%)	0
3.	*S.anginosus*	3	1(33%)	2(67%)
4.	*S.salivarius*	6	4(67%)	2(33%)
5.	*S.sanguis*	4	4(100%)	0

Table.5 Virulent genes detected in *Streptococcus mitis* strains isolated(n=32)

S.no	Name of virulent gene	No. of *S.mitis* strains positive
1.	*amx_res_gene 12*	27(85%)
2.	*Mly gene*	29(91%)
3.	*Cbp1 gene*	21(66%)

Figure.1 ***S****treptococcus viridans* species isolated after third molar surgery (n=63)



Results and Discussion

No bacteria was isolated from samples collected at baseline. Bacteria was isolated from 82(82%) of the 100 subjects included in the study, in blood samples which were collected after the surgical procedure. The most frequently isolated bacteria after third molar surgery was *Streptococcus viridians* (76.8%). Other predominant bacteria isolated were *Moraxella catarrhalis* (43.9%) and *Staphylococcus epidermidis* (34.1%). Antibiotic sensitivity studies showed that 36(57.1%) isolates of VGS are resistant to amoxicillin (Table 3).

Subtyping was done by PCR for *Streptococcus viridians*. 5 subtypes were isolated S. *mitis, S. mutans, S. salivarius, S. sanguis and S. anginosus* (Figure 1). *S. mitis* showed the highest prevalence rate (50.7%) and also showed high resistance to Amoxicillin (Table 4).

The VGS was the most frequently isolated bacteria in the present study (76.8%), similar results were obtained by Tomas *et al.* (2008), where Viridans Group of

Streptococci was the most frequently isolated bacteria which showed a prevalence of 87.9%. VGS is the predominant bacteria which has been isolated in a majority of similar studies conducted worldwide (Xiang Y Han, 2006; Rafael Poveda *et al.,* 2008).

In a study done by Diz Dios *et al.* (2006), blood samples were collected from healthy adults, in a similar manner to our study, before and after tooth extractions to evaluate the comparative efficacies of three antibiotics – Amoxicillin, Clindamycin, Moxifloxacin similar to our study where studies were done to find the prophylactic efficacy of Amoxicillin.

Matsuda *et al.* (2012) found oral streptococci which were highly resistant to amoxicillin, in a similar study conducted on healthy Japanese children and adolescents. The bacterial strains were identified as major oral streptococci species. Similar to our study these findings indicate that oral streptococci with high resistance to amoxicillin can exist in small populations in healthy individuals.

In the present study *S. mitis* was isolated in high numbers after third molar surgery and showed the highest resistance to amoxicillin. In our study, genes coding for the virulence and resistance of the bacteria isolated in high numbers was done to see if the isolated commensal bacteria are virulent and can be potentially pathogenic. Only a few isolated studies on virulence genes have been conducted by other researchers who have done similar studies on bacteremia after dental procedures (Dowson *et al.,* 1997)

In the present study the *mly gene* was isolated in 29(91%) of the 32 resistant isolates of *S. mitis* isolated in this study. The results obtained in our study show a slightly higher number of *S.mitis* strains with *mly gene* but is in accordance with studies conducted by Johanna Jefferies *et al.* (2007), where a high incidence of *mly gene* was detected in VGS isolates. The *cbp1 gene* which code for the CBP's of the *S.mitis* strains was isolated from 21 (66%) of the 32 *S.mitis* strains tested in the present study. According to Madhour *et al.* (2011) the cell surface proteins of *S.mitis* play a central role in the interaction with host cells. This 'virulence factor' or proteins implicated in adhesion and attachment to host cells is present in commensal species like *S. mitis* and thus should be considered as factors essential for host interaction independent on the pathogenicity potential of the bacteria. Lee-Jene Teng *et al.* (1998) and Helena Seppälä *et al.* (2003) have conducted studies on penicillin resistance, genes in the VGS group. In the present study as the *S. mitis* strains showed a high resistance to amoxicillin. The *amx_res_gene* was detected in 27 (85%) of the 32 of the amoxicillin resistant *S. mitis* strains obtained in this study (Table 5).

S. mitis showed the highest prevalence rate and also showed high resistance to amoxicillin and since a high percent of virulence genes were observed in the *S. mitis* strains isolated, it is concluded that *S. mitis* can cause high levels of bacteremia when compared to non-*S. mitis* strains and is the most pathogenic species among the Viridians streptococci. Alternative drugs such as ceftriaxone, levofloxacin and moxifloxacin showed good activity against Viridans group streptococci and can be prescribed as alternatives to amoxicillin. Moxifloxacin showed most promising results and can be considered as the best alternative to amoxicillin for chemoprophylaxis after surgical procedures like third molar surgery. In conclusion, the data from our study reveals that the gene

coding for cell binding proteins – *cbp 1 gene* were present in 66% of the *S. mitis* strains isolated. This 'virulence factor' or proteins implicated in adhesion and attachment to host cells is present in commensal species like *S. mitis* and thus should be considered as factors essential for host interaction independent on the pathogenicity potential of the bacteria. *Mly gene* similar to the cytolysin gene of *S.aureus* was found in 91% of the *S. mitis* strains isolated, which seems to be a major virulence factor. The gene coding for resistance to amoxicillin *amx_res_gene 12* was found in 85% of the isolated strains, this gene could be a major gene contributing in *S. mitis* to resist the action of amoxicillin.

References

Chitra, N. 2014. Amoxicillin resistance in transient bacteremia after third molar surgeries. *J. Pharm. Res.*, 8(3): 277–280.

Chitra, N., Mangayarkarasi, V. 2015. Isolation of aerobic and anaerobic bacteria in transient bacteremia. *Int. J. Pharm. Bio Sci.*, 6(1): (B): 245–251.

CLSI, 2012. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, twenty second informational supplement. CLSI document M100-S22. CLSI, Wayne, PA 19087, USA.

Diz Dios, P., Tomas Carmona, I., Limeras Posse, J. 2006. Comparative efficacies of amoxicillin, clindamycin moxifloxacin in prevention of bacteremia following dental extractions. *Antimicrob. Agents Chemother*., 50(9): 2996–3002.

Dowson, C.G., Barcus, V., King, S., Pickerill, P., Whatmore, A., Yeo, M. 1997. Horizontal gene transfer and the evolution of resistance and virulence determinants in Streptococcus. *Soc. Appl. Bacteriol. Symp. Ser.*, 26: 42S–51S.

Harald Seifert, 2009. The clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. *Clin. Infect. Dis*., 48: S238–45.

Helena Seppälä, Marjo Haanperä, Mohammad Al-Juhaish, Helinä Järvinen, Jari Jalava, 2003. Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from normal flora. *J. Antimicrob. Chemother.*, 52: 636–644.

Johanna Jefferies, Leena Nieminen, Lea-Ann Kirkham, Calum Johnston, Andrew Smith, Tim J. Mitchell, 2007. Identification of a Secreted Cholesterol-dependent Cytolysin (Mitilysin) from *Streptococcus miti. J. Bacteriol.*, 189(2): 627–632.

Lee-Jene Teng, Po-Ren Hsueh, Yu-Chi chen, Shen-Wu Ho., Kwen-Tay Luh, 1998. Antimicrobial susceptibility of viridans group streptococci in Taiwan with an emphasis on the high rates of resistance to penicillin and macrolides in *Streptococcus oralis. J. Antimicrob. Chemother*., 41: 621–627.

Madhour, A., Maurer, P., Hakenbeck, R. 2011. Cell surface proteins in *S. pneumoniae*, *S. mitis* and *S. oralis. Iran. J. Microbiol.*, 3(2): 58–67.

Parahitiyawa, N.B. 2009. Microbiology of odontogenic bacteremia: beyond endocarditis. *Clin. Microbiol. Rev.*, 22(1): 46–64

Rafael Poveda Roda, Yolanda Jimenez, Enrique Carbonell, Carmen Gavalda, Maria Margaix Munoz, 2008. Bacteremia originating in the oral cavity. Med. Oral Patol. Oral Cir. Bucal., 13(6): E355–62.

Rajasuo, A., Perkki, K., Nyfors, S., Jousimies-Somer, H., Meurman, J.H. 2004. Bacteremia following surgical dental extraction with an emphasis on anaerobic strains, *J. Dent. Res.*, 83: 170–174.

Teresa C. Horan., Mary Andrus, Margaret A., Dudeek, M.P.H. 2008.CDC/NHSN Surveillance definition of healthcare-associated infection and criteria for specific types of infections in the acute care settings. *Am. J. Infect. Control*, 36(5): 309–332.

Tomas F. Pereira., Llucian, R., Poveda, R., Diz, P., Bagan, J. 2008. Prevalence of bacteremia following third molar surgery. *Oral Diseases*, 14: 89–94.

Whatmore, A.M., Efstratiou, A., Pickerill, A.P., Broughton, K., Woodard, G., Sturgeon, D., George, R., Dowson, C.G. 2000. Genetic relationships between clinical isolates of *Streptococcus pneumoniae*, Streptococcus oralis, and *Streptococcus mitis*: characterization of "atypical" pneumococci and organisms allied to *S. mitis* harboring *S. pneumoniae* virulence factor-encoding genes. *Infect. Immun.*, 68: 1374–1382.

Xiang Y. Han, 2006. Viridans Streptococci isolated by culture from blood. *J. Clin. Microbiol*., 44(1): 160–165.