Viridans Group Streptococci

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ABSTRACT

The viridans group streptococci (VGS) are part of normal flora in humans, colonized mainly in the oral cavity. Generally, they have been considered to be low virulence. However, in certain populations, VGS can cause invasive disease, such as infective endocarditis, meningitis and septicemia. Nowadays, there are increasing reports of infection due to VGS in both immune-competent and immune-compromised patients. The increase of resistance to various groups of antimicrobial agents such as β-lactams, macrolides, and quinolones, has raised concerns regarding treatment options in serious infections. The purpose of this article is to review the microbiology, currently accepted taxonomic classification, identification, clinical significance, and antimicrobial resistances of VGS. (J Infect Dis Antimicrob Agents 2013;30:37-46.)

Microbiology and Taxonomy

VGS are facultative anaerobic gram-positive spherical or ovoid shaped, non-spore-forming, non-motile and ferment carbohydrates with production of acid but not gas. On blood agar, most of them cause partial destruction of red blood cells, resulting in greenish discoloration (α, alpha-hemolytic) whereas the remaining has no effect on red blood cells (γ, gamma-hemolytic) or complete hemolysis of red blood cells (β, beta-hemolysis). Generally they do not react with Lancefield grouping (a serologic classification system based on cell wall carbohydrate) and are considered entirely non-groupable, except Streptococcus anginosus which are beta-hemolysis on blood agar and react with Lancefield group A, C, F or G antisera and Streptococcus mutan, which has a Lancefield D reaction. With biochemical test, they are leucine aminopeptidase positive, pyrrolidonylarylamidase negative, and do not grow in 6.5% NaCl, and almost all species are negative for growth on bile esculin agar except Streptococcus salivarius group.

In the view of taxonomic classification, they have several changes as in 1970s-Colman and William¹, 1977s-Facklam² and recently accepted with the molecular approaches based on genetic relatedness, 16S rRNA gene sequencing³, classifying them to 5 groups: the mitis group, the mutans group, the salivarius group, the sanguinis group, and the anginosus group as in Table 1.

Although 16S rRNA sequencing can differentiate VGS into species level but there are remaining problems in discrimination of S. mitis, S. oralis, and S. pneumoniae because of S. mitis, S. oralis possess more than 99% sequence homology to S. pneumoniae.³⁶ Advances in molecular technologies for studying a diversity of genetic characteristics, and target gene sequence analysis, have played a role

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to this discrimination. Target gene sequencing, namely rnpB gene\textsuperscript{7,8}, manganese-dependent sodA gene\textsuperscript{9,16S-23S intergenic spacer region\textsuperscript{10}}, D-alanine-D-alanine (ddl) gene\textsuperscript{11}, are all reasonably reliable techniques to discriminate \textit{S. mitis}, \textit{S. oralis}, and \textit{S. pneumoniae}. Groups of \textit{S. mitis} and \textit{S. sanguinis}, have also been difficult to discriminate by conventional test, and 16S rRNA gene sequencing.\textsuperscript{12-13} Kiratisin P, et al.\textsuperscript{14} reported use of the sequencing of 2 housekeeping genes, zwf (encoding for glucose-6-phosphate dehydrogenase) and gki (encoding for glucose kinase), have better discrimination when compared with 16S rRNA gene sequencing. Additionally, non-sequence-based methods such as mass spectroscopy, MALDI-TOF (matrix-associated laser desorption ionization-time of flight) which is a rapid and reliable technique to identify the microorganism into species and subspecies levels of both gram-positive bacteria and gram-negative bacteria but it also has limitations to discriminate \textit{S. mitis} group and \textit{S. pneumoniae}.\textsuperscript{15-16}

### Clinical significance

**Infective endocarditis (IE)**

VGS are the most common cause of native valve endocarditis\textsuperscript{17}, and late on set prosthetic valve endocarditis.\textsuperscript{18} Annual incidence of IE caused by VGS from a study of population-based survey in Olmsted county, Minnesota over 3 decades from 1970 to 2000\textsuperscript{19}, was 1.7 to 3.5 cases per 100,000 person-years and mitral valve prolapse was the underlying heart disease that was found to be increasing when compared with rheumatic heart disease. And in an extended study from 2001 to 2006\textsuperscript{20}, the annual incidence of IE caused by VGS was 3.57 cases per 100,000 person-years, and \textit{Staphylococcus aureus} had trends to increase over VGS (with incidence 3.81 cases per 100,000 person-years), but not significantly. \textit{Streptococcus sanguinis} is the most common species of VGS IE.\textsuperscript{21-23} Clinical manifestation of VGS IE typically is presented with subacute courses. Cardiac murmurs are detected in more than 90%, and splenomegaly is found in up to half of the cases. Immune complex manifestation such as Osler’s nodes, petechiae, and splinter hemorrhage may also occur (28% of cases).

Recommended regimens of treatment of streptococcal endocarditis are based largely on clinical observations and studies of antibiotic efficacy in animal models.\textsuperscript{24} Penicillin G is the

### Table 1. Classification of viridans group streptococci.

<table>
<thead>
<tr>
<th>Streptococcal Group</th>
<th>Hemolysis on Blood agar</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitis Group</strong></td>
<td>α</td>
<td>\textit{S. cristatus, S. infantis, S. mitis, S. oralis, S. peroris, S. orinis, S. pneumoniae}</td>
</tr>
<tr>
<td><strong>Mutans Group</strong></td>
<td>Atypical colony, α</td>
<td>\textit{S. cricetus, S. downei, S. ferus, S. hyovaginalis, S. macaccae, S. mutans, S. ratti, S. sobrinus}</td>
</tr>
<tr>
<td><strong>Salivarius Group</strong></td>
<td>α, γ</td>
<td>\textit{S. alactolyticus, S. hyointestinalis, S. infantarius, S. salivarius, S. thermophilus, S. vestibularis}</td>
</tr>
<tr>
<td><strong>Sanguinis Group</strong></td>
<td>A</td>
<td>\textit{S. gordonii, S. parasanguinis, S. sanguinis}</td>
</tr>
<tr>
<td><strong>Anginosus Group</strong></td>
<td>Small: α, γ, β</td>
<td>\textit{S. intermedius, S. constellatus, S. anginosus}</td>
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drug of choice. Antibiotic regimens that are currently recommended by the American Heart Association\textsuperscript{25} are summarized in Table 2. For infective endocarditis caused by VGS, regimens of treatment are dependant on penicillin MIC (minimal inhibitory concentration) for 3 groups: highly susceptible (MIC ≤ 0.12 μg/mL), relatively resistance (MIC > 0.12 but ≤ 0.5 μg/mL) and resistance (MIC > 0.5 μg/mL). First group (highly susceptible VGS) can be treated with penicillin or ceftriaxone alone for 4 weeks or combination penicillin or ceftriaxone plus gentamicin for 2 weeks. In the second group (relatively resistance VGS) can treat with combination of penicillin or ampicillin plus gentamicin for 4-6 weeks. And the third group (resistance VGS) treated as enterococci, with a combination of penicillin or ampicillin plus gentamicin for 4-6 weeks. For patients who have immediate-type hypersensitivity to penicillin, vancomycin is an alternative regimen.

Recently there are many reports of penicillin resistant VGS IE but have paucity of data on management of penicillin-resistant VGS IE.\textsuperscript{26-29} Knoll et al.\textsuperscript{28} reported from the largest and retrospective cohort study of penicillin-resistant VGS IE from the Mayo clinic (Rochester, MN) between January 1967 and April 2006, 29 patients with IE due to either relatively resistant or resistant

Table 2. Antimicrobial therapy for adult native valve caused by VGS.

<table>
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<tr>
<th>MIC of penicillin (μg/mL)</th>
<th>Antibiotic regimen Dosage</th>
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<tbody>
<tr>
<td>&lt; 0.12</td>
<td>1. Penicillin 12-18 mu IV(divided in four/six doses) for 4 weeks</td>
</tr>
<tr>
<td></td>
<td>2. Ceftriaxone 2 g IV for 4 weeks</td>
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<tr>
<td></td>
<td>3. Penicillin/ ceftriaxone (dose as above) 2 weeks with gentamicin 3 mg/kg IV, IM daily in one dose for 2 weeks</td>
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<tr>
<td></td>
<td>4. Vancomycin (if unable to tolerate penicillin/ ceftriaxone) 30 mg/kg daily, divided to 2 equal doses, not to exceed 2 g/day for 4 weeks</td>
</tr>
<tr>
<td>&gt; 0.12 and &lt; 0.5</td>
<td>1. Penicillin 24 mu IV daily/Ceftriaxone 2 g IV daily for 4 weeks plus gentamicin 3 mg/kg IV, IM for 2 weeks</td>
</tr>
<tr>
<td></td>
<td>2. Vancomycin (if unable to tolerate penicillin/ ceftriaxone) 30 mg/kg daily, divided into 2 equal doses, not to exceed 2 g/day for 4 weeks</td>
</tr>
<tr>
<td>&gt; 0.5</td>
<td>1. Penicillin 18-30 mu IV daily/ampicillin 12 g IV in 6 divided doses for 4-6 weeks plus gentamicin 3 mg/kg IV, IM in 3 divided doses for 4-6 weeks</td>
</tr>
<tr>
<td></td>
<td>2. Vancomycin (if unable to tolerate penicillin/ ceftriaxone) 30 mg/kg daily, divided into 2 equal doses, not to exceed 2 g/day for 6 weeks plus</td>
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</table>
VGS during 39-year period, 9 with infecting strains were relatively resistant VGS, and 20 strains were resistant, prevalence of penicillin-resistant VGS IE was 8 cases per decade.

*Streptococcus sanguinis* was the most common identified species (but the limitation of this study was identifying VGS into species levels in only 40% of patients), 16 of 19 patients with native valve endocarditis received a combination of a β-lactam agent and an aminoglycoside; 17 of 19 were cured. Ten of the patients had prosthetic valve endocarditis and 9 of 10 received a combination of a β-lactam agent plus an aminoglycoside (often streptomycin). All 10 with prosthetic valve endocarditis were cured; 1 had multiple relapses before cure. Overall mortality rate of IE was 6.9% (2 of 29 patients). From this data, they concluded that such a combination of β-lactam-aminoglycoside is likely to be curative.

Fujitani et al. reported a case of a 56-year-old man who presented with *Streptococcus parasanguinis* IE, with MIC to penicillin G 4 μg/mL, also treated successfully combination of a β-lactam agent plus an aminoglycoside (vancomycin-gentamicin 10 days, then ceftriaxone-gentamycin 8 days with surgery due to congestive heart failure on day 18 and then vancomycin monotherapy for 38 days). Appiwattanakul et al. reported a case of penicillin-resistant VGS IE from Thailand, 16-year-old Thai girl with congenital cyanotic heart disease (ventricular septal defect, pulmonary atresia, and major aortopulmonary collateral arteries) presented with *Streptococcus mitis* IE, MIC to penicillin 1.5 μg/mL, treated with vancomycin and developed an unusual reaction to vancomycin (due to both immunological and non-immunological reactions), a skin rash by 18 hours, an intermittent high fever by 48 hours, decreasing peripheral leukocytes and platelet counts, and hypotension development on day 8, then all abnormalities returned to normal after vancomycin was discontinued and oral prednisolone was given. Another regimen for treatment in this patient was considered; based on 2 previous studies which ceftriaxone with or without aminoglycoside and ceftriaxone plus gentamicin was added to treatment recommendations for penicillin resistant VGS endocarditis. This patient had been treated with single-drug therapy with cefotaxime (which mentioned in this article that it has similar antimicrobial spectrum to ceftriaxone) for 4 weeks with successful results, and follow up 5 month echocardiogram did not reveal any vegetations and she remained well for 12 months.

**Meningitis**

The frequency of meningitis caused by viridans group streptococci is 0.3-10%. They are an uncommon cause of acute bacterial meningitis which the most common causes are *Streptococcal pneumoniae, Haemophilus influenzae* and *Neisseria meningitides*. Clinical manifestations are composed of fever, headache, meningeal irritation, and signs of cerebral dysfunction such as confusion, and decreased level of consciousness, are typical for acute bacterial meningitis. The routes of entry in most cases are otopharyngeal route, followed by endocarditis, primary extracranial infection, and head trauma or neurosurgery. Recently, it has been reported that meningitis occurred the most after dural puncture such as spinal anesthesia or lumbar puncture. Drug of choice for treatment is Penicillin G 24 million units/day. Although there is few reports of penicillin resistance of VGS in CSF isolates, which treatment with vancomycin plus third-generations cephalosporins may be considered depends on full susceptibility data. The complication of acute meningitis caused by VGS had been reported as high incidence focal suppuration and cerebral vasculitis.
Bacteremia

VGS account for 2.6% of positive blood cultures reported from clinical laboratories. Of these, only 21% are thought to be clinically significant. These may be explained by their low virulence, and the transient nature of positive cultures. In immune-compromised patients such as neutropenic patients from treatment of hematologic malignancy or cancer patients, they are the important causes of bacteremia and sepsis. A study from M. D. Anderson Cancer Center, reported the incidence of VGS bacteremia was 1 case per 10,000 admissions in 1972 and increased to 47 cases per 10,000 admissions in 1989. In another study, from Switzerland (during 1988-1991), VGS accounted for 30% of all episodes of bacteremia in hospitalized neutropenic patients. In a group of bone marrow transplantation patients, VGS were recovered from the blood of 17.5% of autologous stem cell transplantation patients, with bacteremia occurring at a median of 6 days after transplantations. Risk factor of VGS bacteremia in neutropenic patients were severe neutropenia (<100 neutrophils/mm³) mucositis especially oral mucositis, cytomegalovirus, prophylactic antibiotic treatments with quinolone or cotrimoxazole. S. mitis, S. oralis and S. salivarius were common species of VGS in neutropenic patients. Toxic shock-like syndrome (characterized by hypotension, rash, palmar desquamation, and acute respiratory distress syndrome) had been observed in 25% of neutropenic patients with VGS bacteremia, and in 13-21% of children after bone marrow transplantation. Streptococcus mitis was the cause in most cases. Despite early initiation of broad-spectrum antibiotics, the mortality rate of viridans streptococcal bacteremia is approximately 6 to 12% and increases to 60-100% in viridans streptococcal shock syndrome. In immune-competent patients, there also have emerged reports of toxic shock-like syndrome associated with VGS. Lu HZ et al. reported an outbreak of toxic shock-like syndrome caused by S. mitis strain in China and had identified a 34-kda exoprotein that may possess superantigen-like activity. Madhusudhan T et al. reported case of a previously healthy woman, 33 years old presenting with severe continuous pain at right upper limb with progressive erythematous rash with diagnosis of necrotizing fasciitis, drowsiness, respiratory failure, DIC with acute kidney injury and hemoculture positive for S. mitis, which had been treated successfully with antibiotics (imipenam with clindamycin) and intensive care support.

Antimicrobial Resistance

Penicillin is the drug of choice for treatment of infections caused by VGS such as endocarditis or bacteremia. In the 1970s, VGS were universally sensitive to β-lactam agents, with 90 to 99% sensitivity to erythromycin and clindamycin. During the period 1993 to 1994, among 352 blood culture isolates of VGS from 43 USA medical centers, only 44% were fully susceptible to penicillin by using penicillin MIC ≤ 0.125 μg/mL; defined by the National Committee for Clinical Laboratory Standards. Some strains of VGS exhibit high levels of resistance to penicillin, defined as penicillin MIC ≥ 4 μg/mL (13.4%) and more common among strains were S. mitis (16%) and S. salivarius (17%); for 42.9% of the strains, penicillin MICs were 0.25 to 2.0 μg/mL (intermediate resistance). Traub et al. (1997) reported from Germany, his collection of 116 VGS isolates from patients and 162 from healthy adults: all isolates were susceptible to vancomycin and teicoplanin, and susceptibility to penicillin was 79.1%. Study from the United Kingdom (2001), resistance to β-lactam
agents and macrolides were mainly found in *S. mitis*. Chayakul P et al. (2002)\(^5\) reported from Thailand, among 57 isolates of VGS from gum-tooth margin swabs of 3 groups of patients; group 1- prosthetic heart valve patients who did not receive antimicrobial agent within 3 months, group 2- known case rheumatic heart disease who regularly receive penicillin prophylaxis, and group 3- infective endocarditis patients caused by VGS with dental swab performed before and on the third day of treatment, found that *S. mitis* was common isolates (49% from group 1, 2 and all in group 3) and 49% of isolates were penicillin-susceptible with the remaining were intermediate-resistant penicillin. Like penicillin resistant *S. pneumoniae*, penicillin resistant VGS are due to altered penicillin-binding proteins\(^57-58\), which is associated with point mutations of the PBP 1a and 2b, 2x genes.\(^58-60\) VGS are still susceptible to glycopeptides or linezolid but increasing resistance to erythromycin\(^61\) has been reported. Uh et al. (2004)\(^62\) reported from South Korea with erythromycin resistant VGS from blood cultures were 33.9%. Brown et al. (2008)\(^61\) reported from the United Kingdom that erythromycin resistant VGS from blood cultures were 31.3%.

Two major mechanisms of macrolide resistance in VGS: First, it is post-transcriptional target modification of 23S) rRNA by methylation, that encoded by the erythromycin ribosome methylase (erm B) gene which lead to high-level cross resistance to macrolides, lincosamides and streptogramin B, MLS\(_B\) phenotype.\(^63\) Another mechanism is an active efflux pump encoded by *mef* (A/E) gene which lead to low-level resistance to 14, 15 membered macrolides, such as erythromycin and azithromycin, known as M phenotype.\(^64\) Also, there have been more evidences of fluoroquinolone resistant VGS isolates from blood cultures were 64% for ciprofloxacin, 50% for levofloxacin. Mutations at the quinolone resistance determining regions (QRDRs) in DNA gyrase and DNA topoisomerase IV are major mechanisms of fluoroquinolone resistance of VGS. DNA gyrase (so called DNA topoisomerase II) is composed of A\(_2B_2\) subunits encoded by the *gyrA* gene and *gyrB* gene\(^66\), which is necessary for the supercoiling of chromosomal DNA in bacteria to accomplish efficient cell division. DNA topoisomerase IV is composed of C\(_2E_2\) subunits encoded by the *parC* and *parE* gene, which is required for segregation of chromosomes into two daughter cells during cell division. ParC is the primary and GyrA is the secondary target of ciprofloxacin, which mutations of both of ParC and GyrA lead to its high-level resistances.\(^67-68\)

In conclusion, VGS are now increasing in virulence and antimicrobial resistance patterns. This will be recognized as invasive viridans group streptococci, to be aware of severe infection caused by these organisms and can be managed with optimal uses of all antimicrobial agents.

References
4. Kawamura Y, Hou XG, Sultana F, Miura H, Ezaki T. *Determination of 16S rRNA sequences of Streptococcus mitis* and *Streptococcus gordonii* and


21. van der Meer JT, van Vianen W, Hu E, et al. Distribution, antibiotic susceptibility and tolerance of


38. Swenson FJ, Rubin SJ. Clinical significance of viridans streptococci isolated from blood cultures. J Clin...


