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MINIREVIEW

It's Not Easy Being Green: the Viridans Group Streptococci, with a Focus on Pediatric Clinical Manifestations▽

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The viridans group streptococci (VGS) are a heterogeneous group of organisms that can be human commensals, colonizing the gastrointestinal and genitourinary tracts in addition to the oral mucosa. VGS are generally considered to be of low pathogenic potential in immunocompetent individuals. However, in certain patient populations, VGS can cause invasive disease, such as endocarditis, intra-abdominal infection, and shock. Within the VGS, the rates and patterns of antimicrobial resistance vary greatly depending upon the species identification and the patient population. In general, *Streptococcus mitis* group organisms are resistant to more antimicrobial agents than the other VGS species. This review addresses current VGS taxonomy, in addition to the current methodologies being used in clinical microbiology laboratories for identification of VGS. Automated systems struggle overall with species level identification and susceptibility testing for VGS. Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) identification is emerging as a potential alternative for organism identification. A review of recent pediatric-specific data regarding the clinical manifestations of VGS revealed that the *Streptococcus anginosus* group (SAG) organisms may be important pathogens in pediatric patients and that the VGS may contribute to disease in patients with cystic fibrosis. It also appears that rates of antimicrobial resistance in VGS in pediatric patients are surpassing those of the adult population.

TAXONOMY AND MICROBIOLOGY

The viridans group streptococci (VGS) are a heterogeneous group of organisms that can be both commensal flora and pathogens in humans. They are the "grab bag" that remains when the beta-hemolytic streptococci, enterococci, and pneumococci are excluded from the streptococci. The purpose of this review is to summarize the currently accepted taxonomic classification of this group of organisms, examine the state of the art for identification of the VGS, and then focus on the pediatric clinical manifestations associated with the VGS.

Perhaps the only consistency observed in discussions regarding the taxonomy of the VGS is a lack of consistency. The taxonomy is very controversial, and for many years, a standardized naming scheme or typing system for this group of organisms was lacking; therefore, discussions on this topic frequently include the phrase "poorly classified." There are now at least 30 recognized species of VGS (11, 14).

One of the first classifications of VGS was done in 1906 by Andrewes and Horder, who classified three species of nonhemolytic *Streptococcus* as the "*Streptococcus mitis* group" (14). This group has been poorly defined for many years because of the paucity of commonly queried substrates hydrolyzed by the group. So began the taxonomic problems with this group of organisms. Today, the VGS are classified into 6 major groups: the *S. mutans* group, *S. salivarius* group, *S. anginosus* group, *S. mitis* group, *S. sanguinis* group, and *S. bovis* group (14).

The *S. anginosus* group is one that has been the source of much controversy and confusion regarding taxonomy and classification. This group of organisms can be alpha-, beta- or nonhemolytic; it is the isolates lacking beta-hemolysis that are generally lumped into the VGS. The group includes *S. anginosus*, *S. constellatus*, and *S. intermedius*. However, each of these species has been known as *S. anginosus* or *S. milleri* at one time (11, 14). *S. milleri* has never been accepted as a confirmed taxonomic entity, although historically, it has been widely used interchangeably with the *S. anginosus* group.

IDENTIFICATION

Phenotypic and biochemical identification. Identification of VGS to the species level can be difficult, and phenotypic identification is not always accurate. The name "viridans" is somewhat of a misnomer, as many species do not produce any hemolysis on blood agar. From a classification perspective, it is not useful to try to differentiate alpha-hemolysis from a lack of hemolysis on blood agar plates (sometimes referred to as "gamma-hemolysis"); this feature can vary widely with the growth medium used to cultivate the organism, as well as the incubation temperature. Lack of alpha hemolysis does not seem to correlate with the clinical outcome or severity of disease; no enzymatic or toxigenic effect has ever been documented as a by-product of alpha hemolysis.

The VGS are a group of catalase-negative, Gram-positive cocci with a chaining morphology on microscopic examination. They are leucine aminopeptidase positive, pyrrolidonylaryl-

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amidase negative, and do not grow in 6.5% NaCl, and almost all species are negative for growth on bile esculin agar. They differ from pneumococci in that they are optochin resistant and are not bile soluble. However, Richter et al. examined misidentification of VGS submitted to antimicrobial surveillance programs as pneumococci and found that the distinction of *S. pneumoniae* from the VGS can be difficult, which is not surprising in light of the fact that *S. mitis* and *S. oralis* possess >99% sequence homology with *S. pneumoniae* (47). The authors also found that optochin disk testing did not perform as well as bile solubility testing for identification; in a survey of 1,733 isolates tested, bile solubility testing had higher sensitivity and specificity for differentiation of VGS from pneumococci (47).

***S. anginosus* group.** The *S. anginosus* group of organisms can be beta-, alpha- or nonhemolytic. The isolates lacking beta-hemolysis are generally those grouped with the VGS. *S. constellatus* is the most likely of this group to be beta-hemolytic. There is some evidence implicating beta-hemolytic *S. constellatus* subsp. *pharyngis* as a cause of pharyngitis (55). *S. intermedius* is the species in this group most commonly isolated from brain and liver abscesses. The *S. anginosus* group can possess Lancefield group antigens A, C, G, and F, although *S. intermedius* almost never possesses Lancefield group antigens. Isolates of the *S. anginosus* group have a characteristic "butterscotch" odor. Members of the group are universally positive for three biochemical reactions: acetoin production from glucose (positive Vogues-Proskauer reaction), arginine, and sorbitol. These are very useful for the differentiation of this group from other VGS.

***S. mitis* group.** The *S. mitis* group of organisms contains several species and is biochemically very inert, which can make species level identification very challenging. The use of invalid species names has also been a particular problem with the *S. mitis* group. Isolates in this group are negative for acetoin production, arginine, esculin, and mannitol and are sorbitol fermentation negative (14). *S. pneumoniae* is a recently characterized member of the *S. mitis* group (1). As the organism is closely related to *S. pneumoniae* and other *S. mitis* group organisms, accurate identification can be difficult. *S. pseudopneumoniae* lacks the pneumococcal capsule and is resistant to optochin when incubated in an atmosphere with elevated CO_2 but is susceptible when incubated in ambient air. Bile solubility is a more specific test for *S. pseudopneumoniae* than optochin susceptibility, as the organism is not bile soluble (1).

***S. sanguinis* group.** The genetically heterogeneous *S. sanguinis* group was formerly known as *S. sanguinis*. Some taxonomists have lumped the *S. sanguinis* group in with the *S. mitis* group based on 16S rRNA gene sequence analysis, but *S. sanguinis* group organisms exhibit divergent phenotypic characteristics. Isolates in the *S. sanguinis* group are arginine and esculin positive. Like members of the *S. mitis* group, they are negative for acetoin production and mannitol and sorbitol fermentation. The *S. sanguinis* group includes *S. sanguinis*, *S. parasanguinis*, and *S. gordonii* (14).

***S. bovis* group.** The *S. bovis* group includes *S. equinus*, *S. gallolyticus*, *S. infantarius*, and other closely related species; they are the nonenterococcal group D streptococci. Members of this group are esculin positive and sorbitol negative and produce acetoin. Historically, *S. bovis* had been divided into three biotypes: I (mannitol fermentation positive), II/1 (mannitol negative and beta-glucuronidase negative), and II/2 (mannitol negative and beta-glucuronidase positive) (14). Further characterization demonstrated that biotype II/1 was *S. infantarius* subsp. *coli* and II/2 was *S. gallolyticus* subsp. *pasteruianus*. Isolates from the *S. bovis* group are most frequently encountered in blood cultures from patients with colon cancer. However, *S. bovis* group organisms (especially *S. gallolyticus* subsp. *gallolyticus* and *S. infantarius* subsp. *coli*) have been associated with endocarditis (3). Although infection with *S. bovis* group organisms occurs with higher frequency in adults than in pediatric patients, these organisms have been reported to cause neonatal sepsis and meningitis (20).

***S. salivarius* group.** The *S. salivarius* group is closely related to the *S. bovis* group. Species from this group that have been isolated from human infection include *S. salivarius* and *S. vestibularis*, as well as *S. thermophilus*, which has been identified from dairy products. *S. salivarius* group organisms are positive for acetoin production and are esculin positive but are negative for arginine hydrolysis and fermentation of mannitol and sorbitol.

***S. mutans* group.** Members of the *S. mutans* group are primarily isolated from the human oral cavity and includes several species that are phenotypically similar. *S. mutans* and *S. sobrinus* are the species within this group most commonly isolated from human infection. They do not hydrolyze arginine but are positive for acetoin production, esculin hydrolysis, and mannitol and sorbitol fermentation.

Automated biochemical methods for identification. For the VGS, the use of automated systems for identification has historically been reported as problematic, and this theme applies to multiple automated methodologies. One of the major factors affecting the quality of the identifications generated is that the systems may not have all species represented in their databases (32). In one investigation comparing the accuracy of the Vitek2 ID-GPC card to that of conventional agar-based biochemical methods for identification of a variety of Gram-positive and Gram-negative organisms, 72% of Gram-positive isolates were accurately identified by the Vitek2 system (21). Among the most problematic identifications (whether incorrectly identified or unresolved) were the VGS; *S. anginosus*, *S. mutans*, and *S. sanguinis* were misidentified as other VGS species and, in some instances, as *S. pneumoniae* (21).

An investigation into the ability of the BD Phoenix system SMIC/ID panel to identify *Streptococcus* spp. classified 97 consecutive clinical isolates of streptococci, including 34 isolates of VGS, with biochemical methods as the reference method (26). Ninety-one percent of the streptococcal isolates showed agreement between the Phoenix and the reference method. Of the 12 *S. mitis* group isolates tested, the Phoenix system correctly identified 7 isolates, with 2 discordant identifications, and there were 3 isolates for which the Phoenix system did not produce any identification. Of 22 *S. anginosus* group isolates, 18 were correctly identified and 4 were discordant. A second study evaluated the Phoenix SMIC/ID-2 panel using the API 20 Strep system as a comparator method (resolving discrepant results via 16S rRNA gene sequencing and amplification and sequencing of housekeeping genes) (5). For the VGS, 31 isolates were assayed, with only 53% concordance between the Phoenix and reference methods for *S. mitis* group organisms,

100% concordance for the *S. anginosus* group, and 75% concordance for *S. sanguinis* group organisms (5).

Sequence-based identification. Historically, DNA-DNA hybridization studies have been used to confirm species level identifications for the VGS. However, these procedures are not practical for clinical laboratories to use for identification of these organisms. Other sequence-based identification systems have subsequently been introduced for VGS species level identification. In general, 16S rRNA gene sequencing results in poor resolution to species level in the VGS. This is due to the high degree of 16S rRNA gene homology in this group of organisms; *S. mitis*, *S. oralis*, *S. pseudopneumoniae*, and *S. pneumoniae* almost always have >99% sequence homology in this gene. In light of the high degree of 16S rRNA gene sequence similarity, sequencing of alternative gene targets for reliable identification to the species level has been explored. One promising target, *rnpB*, was explored by Innings et al., who analyzed 2 variable regions of *rnpB* by pyrosequencing (28). Of the 43 species analyzed, all were identified to species level, except for 2 isolates: *S. anginosus*/*S. constellatus* and *S. infantis*/*S. peroris*. *rnpB* can be used for identification of VGS with high-level resolution. One other successful approach is sequence analysis of the manganese-dependent superoxide dismutase gene, described by Poyart et al. This technique was used to accurately differentiate over 29 streptococcal species, including 16 VGS species, with clear differentiation of *S. mitis*, *S. oralis*, and *S. pneumoniae* (44, 45). Other techniques that have been used, with various degrees of success, are sequence analysis of the 16S-23S intergenic spacer region, D-alanine-D-alanine ligase gene sequencing, and hyaluronate lyase gene sequencing.

EMERGING TECHNOLOGIES

MALDI-TOF. Matrix-associated laser desorption ionization–time of flight (MALDI-TOF) is gaining momentum as a rapid and cost-effective means of identification of pure cultures of bacteria, as well as direct identification of microorganisms from positive blood culture broth. In 2007, Friedrichs and colleagues assessed the utility of MALDI-TOF for identifying VGS from relevant clinical samples, following a preliminary identification based on phenotypic analysis (17). MALDI-TOF identification was compared to biochemical analysis using the RAPID Strep assay, and discrepant results were resolved using 16S rRNA gene sequencing and organism-specific PCR assays. A series of reference strains was used to produce a spectrum database with the Bruker Daltonics MALDI-TOF platform. Ninety-nine clinical isolates (isolated from blood cultures, peritonsillar abscesses, other abscesses, wounds, catheter tips, and cerebrospinal fluid [CSF]) were analyzed, and 10 different VGS species were included in the analysis, although the majority of them were *S. mitis* group and *S. anginosus* group members. The analysis revealed that the spectra of more closely related organisms (such as those within the *S. anginosus* group) were more similar than those that were more antigenically distinct (such as *S. salivarius* and *S. mutans*). Twenty-three samples were identified as *S. mitis* or *S. oralis* by species-specific PCR and 16S rRNA gene sequence analysis; in all but two cases, these were identified correctly by MALDI-TOF (17). In general, MALDI-TOF appeared to be a rapid and accurate mode of VGS identification.

Studies are also emerging that examine the ability of MALDI-TOF to identify VGS directly from positive blood culture broth. Overall, the preliminary results are promising, but MALDI-TOF may suffer from the same limitation as 16S rRNA gene sequence-based identification—poor resolution of *S. mitis* group organisms and *S. pneumoniae* to the species level (9, 16).

ANTIMICROBIAL SUSCEPTIBILITIES OF VIRIDANS GROUP STREPTOCOCCI

Automated systems. Just as automated systems struggle with accurate identification of VGS, they may be less than ideal at performing accurate susceptibility testing for these organisms. A 2007 study by Richter et al. queried the ability of the BD Phoenix system to accurately provide antimicrobial susceptibility results for 2,013 streptococcal isolates tested against 13 antimicrobial agents (48). Broth microdilution was used as the reference method. For the 369 VGS isolates, the minor error rate was >10% for some of the antimicrobial agents tested. The very major error rate was up to 3%, and very major errors occurred with 7 of the antimicrobial agents. Categorical agreement was below 90% for penicillin. The VGS had the lowest rate of concordance between the Phoenix and the reference method of all the streptococci queried in the study. However, the average time to results was approximately 9 h, making automated testing a very enticing option. A second, smaller study (32 VGS isolates) evaluating the Phoenix SMIC-ID2 panel (compared to Etest as a reference method) was more favorable, with no major, minor, or very major errors observed with the 8 antimicrobial agents tested (6).

Epidemiology of resistance in children. In 1949, a published series describing 57 cases of subacute endocarditis identified a single fatality due to a highly penicillin-resistant VGS (24). Sixty years later, penicillin is still an important antibiotic in treating VGS infections, and resistance continues to increase in the face of its use. A recent survey of Gram-positive cocci isolated in North America showed that of 182 VGS, 28.6% were nonsusceptible to penicillin, with only 4.9% being fully resistant (8). These findings are consistent with those of others in Europe and Asia. The profile for clindamycin is somewhat similar in that 6.6% of isolates were resistant; however there were no intermediately resistant isolates, as 93.4% were susceptible. VGS in this study were most resistant to the macrolide erythromycin, with nearly 50% being fully resistant. This study provides a nice overview of the VGS resistance picture, but the epidemiology is too complex to be summarized as a group. VGS resistance rates vary widely between species and even within different patient populations. Some have even shown that resistance appears to be more prevalent in the pediatric versus adult population (25, 39).

In pediatric and adult populations alike, those most at risk for resistant VGS infections are the immunocompromised. Not coincidentally, this is also a population that receives frequent antibiotic treatment. The mantra that antibiotic usage drives resistance holds true for VGS, and there are numerous studies that have shown direct correlations be-

tween both penicillin and macrolide usage and the development of resistance in VGS.

In 2001, Kastner and Guggenbichler conducted a randomized controlled study in which the development of macrolide resistance was assessed in children being treated for upper respiratory tract infections (31). Patients were excluded from the study if any macrolide-resistant organisms were found in initial pretreatment cultures. After only 1 week of treatment, over 60% of the patients in both the azithromycin and clarithromycin groups had at least one macrolide-resistant organism; 6 weeks following antibiotic treatment, 87% of the patients in the azithromycin group were colonized with macrolide-resistant VGS. Clarithromycin-treated patients fared slightly better at 6 weeks posttreatment, but 60% still had macrolide-resistant VGS colonizing their oral mucosa.

Malhotra-Kumar et al. showed similar trends in their 2007 study in healthy nonpediatric volunteers. Their study demonstrated an effect on colonizing resistant VGS 6 months posttherapy (35). As in the Kastner and Guggenbichler study, they found that azithromycin was more likely than clarithromycin to select for macrolide resistance initially and to maintain that resistance over extended periods of time. They also showed that azithromycin was more likely to select for resistance than clarithromycin. Both azithromycin and clarithromycin select for VGS possessing the *ermB* gene, which can be carried on the same mobile genetic element as the tetracycline resistance gene, *tet*(*M*). This has obvious implications for the selection of multiclass resistance. The Castanheira et al. data may support this hypothesis, as macrolide resistance rates in their study were similar to those for tetracycline (8). With respect to penicillin, the story is the same. Studies have shown that VGS penicillin resistance rates are significantly higher in those patients receiving penicillin than in those who are not (23).

One patient population at particular risk for developing resistant VGS infection is pediatric oncology patients. Recent analyses of VGS bacteremia isolates in this population have shown anywhere from 21 to 37% to be resistant to penicillin. Penicillin resistance is often accompanied by resistance to other beta-lactams, such as ceftriaxone and cefepime, as well as other classes of antibiotics (7).

Another population at risk of harboring drug-resistant VGS is the cystic fibrosis (CF) population. Evidence, discussed below, is starting to suggest that *S. anginosus* group organisms may be important pathogens in this population whereas other VGS colonize the cystic fibrosis lung in a nonpathogenic capacity. These patients receive many antibiotics which likely have poor penetration into the lung and provide an ideal environment for the development of resistance. To compound the matter, CF patients are commonly treated with azithromycin for its anti-inflammatory effect and with the hope that it will interrupt quorum-sensing mechanisms employed by *Pseudomonas aeruginosa*. Altogether, the CF patient is at very high risk for colonization with drug-resistant VGS. In one study where macrolide resistance was measured for VGS isolated from respiratory cultures of CF patients, 95% of VGS were macrolide nonsusceptible and 80% were fully resistant (52).

Among viridans group streptococci, *S. mitis* group organisms are the most likely to become resistant to beta lactam and macrolide antibiotics. Within one study, 56% of *S. mitis* isolates were resistant to 3 classes of antibiotics (39). This is in contrast to the *S. anginosus*, *S. sanguinis*, *S. salivarius*, and *S. bovis* groups, which remain relatively susceptible to penicillin (49). Of the non-*S. mitis* VGS in this study, only 9% of *S. salivarius* strains demonstrated high penicillin resistance. No resistance was observed in the other non-*S. mitis* VGS. The implications of *S. mitis* resistance go beyond infection with *S. mitis* itself. As was discussed above, the *S. mitis*/*S. oralis* species are closely related to *S. pneumoniae*, and through recombinatorial events, they are thought to have played a crucial role in the development of pneumococcal resistance.

Although VGS resistance to penicillin and the macrolide-lincosamide-streptogramin B (MLS) drugs is an evolving problem, several drugs remain uniformly active against the VGS. Thus far, VGS resistance to vancomycin, linezolid, and daptomycin remains extremely rare (8).

CLINICAL SIGNIFICANCE

VGS are considered to be normal flora of the oropharyngeal, urogenital, and gastrointestinal microbiota. They are not typically thought of as organisms with high pathogenic potential; however, VGS infection can be associated with significant morbidity and mortality. Although VGS disease can occur in healthy hosts, it most commonly manifests in those with underlying conditions, such as being immunocompromised or having cardiac abnormalities. Below, we review the specific manifestations of pediatric VGS disease, focusing on recent publications and highlighting new disease associations.

Infective endocarditis. Infective endocarditis (IE) occurs less frequently in children than it does in adults, at a rate of 1 per 1,280 pediatric admissions. In a 2004 retrospective review of 2,071 pediatric cardiology consultations, only 4.4% were for suspicion of subacute endocarditis. Of that 4.4%, in only 3 cases (3.3%) was a culture-confirmed diagnosis of infective endocarditis made. Two of the three episodes were due to VGS; the third was due to *Staphylococcus aureus* (22). Although pediatric infective endocarditis is rare, when it does occur, a significant percentage of episodes are due to viridans group streptococci. Ferrieri et al. conducted a review of the literature and found that VGS was the most common cause of IE in children. The studies reviewed found VGS to be the cause of between 32 and 43% of cases (15, 30, 36, 51). In addition, 27 to 33% of IE was caused by *S. aureus*, which was the second most common etiologic agent. 2000 and 2003 data presented by Day et al. suggest a slightly different epidemiology, with VGS and *S. aureus* causing 20 and 57% of cases, respectively (12). Overall, Day et al. found the mortality of IE in children to be relatively low at 5.3%. Of the 14 noninfant children who died from IE, *S. aureus* was the causative agent in 93% of the cases. They also concluded that VGS are particularly important causes of IE after the first year of life and are more frequently associated with native valve endocarditis. VGS IE is more likely to occur more than 60 days after surgery, consistent with the subacute nature of VGS IE (15). Most commonly, VGS endocarditis presents with an indolent course involving prolonged low-grade fever and a variety of somatic complaints, such as arthralgias, myalgias, weight loss, rigors, fatigue, weakness, and diaphoresis. As in adults, it is very common for these children to have continuous bacteremia.

***S. anginosus* group.** Members of the *S. anginosus* group of organisms are well recognized for their predilection for abscess formation. In contrast to other species of VGS, where disease typically occurs in hosts with underlying conditions, SAG organisms commonly cause disease in healthy hosts. In a 2002 retrospective analysis of pediatric *S. anginosus* group infection, Belko et al. found that only 18% of patients had a risk factor for infection (4). Of those with risk factors, congenital abnormality was the most common. In the 180 patients who developed *S. anginosus* disease, only 12 (7%) had a congenital abnormality while 146 (82%) had no identifiable risk factor. Although the specific disease manifestation was not stated in the study, the most common abnormalities were cyanotic heart disease ($n = 4$) and myelomeningocele ($n = 2$). As is true in the adult population, the abdomen was the primary site of infection, followed by superficial skin infections. Over the 6-year period of the Belko study, 29% of all central nervous system (CNS) cultures that were positive for a bacterial agent were positive for an *S. anginosus* group organism. Interestingly, in this study of 213 SAG isolates, no *S. anginosus* sensu stricto isolates were identified. However, 40% of the SAG organisms were identified only as group F streptococci and could have been *S. anginosus*. At least one other study has found a similar result in children, where 14 of 16 isolates that were identified to the species level were *S. intermedius* and no *S. anginosus* isolates were identified (29). This is in contrast to data presented by others who have found *S. anginosus* to be the predominant disease-causing SAG organism. However, the patient populations in these studies were not defined as pediatric (2, 10, 53, 54). It is possible that *S. intermedius* plays a more significant role in pediatric infection than does *S. anginosus*, but more data are needed to make a firm association. Several studies have suggested a correlation between SAG species and the site of infection. Although not all the studies were conducted in pediatric populations, *S. intermedius* seems to have a predilection for the CNS, while *S. constellatus* may be more often involved in pulmonary infection (53). Overall, the mortality for SAG pediatric infections is low. Of the 213 SAG organisms isolated in the Belko study, there was no mortality. This included 11 episodes of CNS disease.

An emerging body of evidence suggests that SAG is a significant pathogen in patients with CF, but thus far, few studies have been conducted in the pediatric CF population. Parkins et al. published a series of 10 cases of SAG disease in adults with CF. In 7 of the 10 cases, bronchopulmonary symptoms were attributed to SAG, as they were the predominant organism and patients responded both clinically and bacteriologically to SAG-specific treatment (43). Whether SAG is the sole cause of disease in these patients remains to be seen. Parkins and others have found that SAG disease in CF patients is almost always associated with *P. aeruginosa* cocolonization (50). This is not surprising, as a high percentage of CF patients are colonized with *P. aeruginosa* by early adulthood. However, there is evidence that *P. aeruginosa* and SAG may work synergistically to cause disease (13). In most published cases of SAG disease, patients responded to SAG-specific therapy that would not have had activity against *P. aeruginosa.* This suggests that at the very least SAG is a critical component of disease in these cases. More studies in the CF population will be needed to determine whether SAG is the sole cause of disease or is working in conjunction with *P. aeruginosa*.

Viridans group streptococci in pediatric cancer patients. The VGS are common causes of bacteremia in pediatric cancer patients, specifically those that are febrile and neutropenic. In the late 1980s, an increase in VGS bacteremia in this patient population was thought to be due to an increase in central venous catheter use. Since that time, anywhere from 11 to 30% of blood cultures from febrile, neutropenic cancer or stem cell transplant patients have been positive for VGS in various series. In a 4-year retrospective study, Husain et al. found 33 episodes of bacteremia in this patient population. Of these, 58% were due to *S. mitis* and 21% were due to *S. oralis* (27). The predominance of *S. mitis* bacteremia may be explained by studies showing that in children undergoing allogeneic bone marrow transplants, the overall bacterial flora decreases but *S. mitis* becomes the dominant VGS species (34). Although numerous risk factors have been identified for VGS bacteremia, the last finding is consistent with others that suggest that reducing competing flora increases the risk of becoming bacteremic with a VGS. Through selective pressure, antibiotics that target Gram-negative organisms, such as quinolones, may promote VGS expansion and increase the risk of a patient becoming bacteremic (41, 46). Although quinolones are generally avoided in pediatrics, they may present an appealing oral option for prophylaxis of febrile and neutropenic pediatric cancer patients (41). Mucositis is another important risk factor for VGS bacteremia, specifically *S. mitis* bacteremia. Of those patients who had mucositis-associated bacteremia, 66% was due to *S. mitis*. Not only is *S. mitis* the most frequent VGS species causing bacteremia, it is also the most likely to be penicillin resistant. Despite the relatively high rate of penicillin resistance in *S. mitis*, there was no association between resistance and the risk for developing complications. However, resistance was correlated with fever lasting more than 10 days (27). Other studies have found that there is in fact a correlation between increased resistance and mortality (40).

Mucositis is the most common route of entry for these organisms; however, in the Hussain study, nearly half the cases of bacteremia (45%) occurred in patients without mucositis, suggesting that other mechanisms of entry are also important. In those that become bacteremic in the absence of mucositis, gastrointestinal toxicity was implicated as a potential risk factor (18). *S. acidominimus* was implicated in 12% of the cases. This species is part of the normal flora of the vagina, and all cases occurred in female patients, suggesting genitourinary entry. Other important risk factors for developing VGS bacteremia are treatment with high dose 1-β-D-arabinofuranosylcytosine (ara-C) (HiDAC) and severe neutropenia (<500 cells/mm^3) (27).

VGS bacteremia often results in complications, the most serious of which is viridans streptococcal shock syndrome (VSSS), with published mortality rates in children ranging between 40 and 100% (19, 37). In contrast to adult cancer patients, the pediatric population may be particularly prone to developing VGS bacteremia and subsequent VSSS. As many as 25% of children with VGS bacteremia may go on to develop VSSS. *S. mitis* is the most common VGS species associated with VSSS. Attempts to identify the mechanism by which *S. mitis* might cause sepsis have led to mixed results. Ohuoba et

al. published a 2006 report in which they failed to identify any *S. mitis* superantigens that were either novel or homologous to those of group A *Streptococcus* (42). This is in contrast to other studies which have analyzed *S. mitis* supernatants and found them to be capable of stimulating T-cell proliferation responses, suggesting the production of a superantigen (38). Lu et al. later found that *S. mitis* produces a 34-kDa exoprotein that may possess superantigen-like activity (33). Additional studies are required to more clearly define the mechanism by which VGS cause VSSS.

SUMMARY

In summary, the VGS are important pathogens in the pediatric population. In fact, evidence suggests that children may be at greater risk of VGS infection than adults. A new disease association has been made for SAG organisms in CF patients. However, more studies within the pediatric CF population are needed to better understand the role of SAG disease in children. The evolution of resistance marches on in VGS. Indiscriminate use of azithromycin to treat respiratory illness and its use as a prophylactic agent in the CF and oncology populations are thought to exacerbate this problem. Penicillin resistance is also a prominent problem in pediatric oncology patients, a population at great risk of developing serious VGS infection. Historically, VGS have been troublesome organisms to accurately characterize and identify. Recently, MALDI-TOF technology has been adapted to bacterial identification and shows promise as a potential replacement for existing techniques. Rapid turnaround time and low cost make this an appealing new option. VGS are emerging as important pathogens, especially in immunosuppressed individuals. As our understanding of the disease manifestations and antimicrobial susceptibility profiles associated with different VGS species continues to expand, accurate species level identification of VGS may become increasingly important.

REFERENCES

1. **Arbique, J. C., C. Poyart, P. Trieu-Cuot, G. Quesne, G. Carvalho Mda, A. G. Steigerwalt, R. E. Morey, D. Jackson, R. J. Davidson, and R. R. Facklam.** 2004. Accuracy of phenotypic and genotypic testing for identification of *Streptococcus pneumoniae* and description of *Streptococcus pseudopneumoniae* sp. nov. J. Clin. Microbiol. **42:**4686–4696.
2. **Bantar, C., L. Fernandez Canigia, S. Relloso, A. Lanza, H. Bianchini, and J. Smayevsky.** 1996. Species belonging to the "*Streptococcus milleri*" group: antimicrobial susceptibility and comparative prevalence in significant clinical specimens. J. Clin. Microbiol. **34:**2020–2022.
3. **Beck, M., R. Frodl, and G. Funke.** 2008. Comprehensive study of strains previously designated *Streptococcus bovis* consecutively isolated from human blood cultures and emended description of *Streptococcus gallolyticus* and *Streptococcus infantarius* subsp. *coli.* J. Clin. Microbiol. **46:**2966–2972.
4. **Belko, J., D. A. Goldmann, A. Macone, and A. K. Zaidi.** 2002. Clinically significant infections with organisms of the *Streptococcus milleri* group. Pediatr. Infect. Dis. J. **21:**715–723.
5. **Brigante, G., F. Luzzaro, A. Bettaccini, G. Lombardi, F. Meacci, B. Pini, S. Stefani, and A. Toniolo.** 2006. Use of the Phoenix automated system for identification of *Streptococcus* and *Enterococcus* spp. J. Clin. Microbiol. **44:** 3263–3267.
6. **Brigante, G. R., F. A. Luzzaro, B. Pini, G. Lombardi, G. Sokeng, and A. Q. Toniolo.** 2007. Drug susceptibility testing of clinical isolates of streptococci and enterococci by the Phoenix automated microbiology system. BMC Microbiol. **7:**46.
7. **Bruckner, L. B., D. N. Korones, T. Karnauchow, D. J. Hardy, and F. Gigliotti.** 2002. High incidence of penicillin resistance among alpha-hemolytic streptococci isolated from the blood of children with cancer. J. Pediatr. **140:**20–26.
8. **Castanheira, M., R. N. Jones, and H. S. Sader.** 2008. Update of the *in vitro* activity of daptomycin tested against 6710 Gram-positive cocci isolated in North America (2006). Diagn. Microbiol. Infect. Dis. **61:**235–239.
9. **Christner, M., H. Rohde, M. Wolters, I. Sobottka, K. Wegscheider, and M. Aepfelbacher.** 2010. Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption-ionization time of flight mass spectrometry fingerprinting. J. Clin. Microbiol. **48:**1584–1591.
10. **Claridge, J. E., III, S. Attorri, D. M. Musher, J. Hebert, and S. Dunbar.** 2001. *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* ("*Streptococcus milleri* group") are of different clinical importance and are not equally associated with abscess. Clin. Infect. Dis. **32:**1511–1515.
11. **Coykendall, A. L.** 1989. Classification and identification of the viridans streptococci. Clin. Microbiol. Rev. **2:**315–328.
12. **Day, M. D., K. Gauvreau, S. Shulman, and J. W. Newburger.** 2009. Characteristics of children hospitalized with infective endocarditis. Circulation **119:** 865–870.
13. **Duan, K., C. Dammel, J. Stein, H. Rabin, and M. G. Surette.** 2003. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. Mol. Microbiol. **50:**1477–1491.
14. **Facklam, R.** 2002. What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin. Microbiol. Rev. **15:**613–630.
15. **Ferrieri, P., M. H. Gewitz, M. A. Gerber, J. W. Newburger, A. S. Dajani, S. T. Shulman, W. Wilson, A. F. Bolger, A. Bayer, M. E. Levison, T. J. Pallasch, T. W. Gage, and K. A. Taubert.** 2002. Unique features of infective endocarditis in childhood. Pediatrics **109:**931–943.
16. **Ferroni, A., S. Suarez, J. L. Beretti, B. Dauphin, E. Bille, J. Meyer, M. E. Bougnoux, A. Alanio, P. Berche, and X. Nassif.** 2010. Real-time identification of bacteria and Candida species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **48:**1542–1548.
17. **Friedrichs, C., A. C. Rodloff, G. S. Chhatwal, W. Schellenberger, and K. Eschrich.** 2007. Rapid identification of viridans streptococci by mass spectrometric discrimination. J. Clin. Microbiol. **45:**2392–2397.
18. **Gamis, A. S., W. B. Howells, J. DeSwarte-Wallace, J. H. Feusner, J. D. Buckley, and W. G. Woods.** 2000. Alpha hemolytic streptococcal infection during intensive treatment for acute myeloid leukemia: a report from the Children's Cancer Group study CCG-2891. J. Clin. Oncol. **18:**1845–1855.
19. **Gassas, A., R. Grant, S. Richardson, L. L. Dupuis, J. Doyle, U. Allen, O. Abla, and L. Sung.** 2004. Predictors of viridans streptococcal shock syndrome in bacteremic children with cancer and stem-cell transplant recipients. J. Clin. Oncol. **22:**1222–1227.
20. **Gavin, P. J., R. B. Thomson, Jr., S. J. Horng, and R. Yogev.** 2003. Neonatal sepsis caused by *Streptococcus bovis* variant (biotype II/2): report of a case and review. J. Clin. Microbiol. **41:**3433–3435.
21. **Gavin, P. J., J. R. Warren, A. A. Obias, S. M. Collins, and L. R. Peterson.** 2002. Evaluation of the Vitek 2 system for rapid identification of clinical isolates of gram-negative bacilli and members of the family Streptococcaceae. Eur. J. Clin. Microbiol. Infect. Dis. **21:**869–874.
22. **Geggel, R. L.** 2004. Conditions leading to pediatric cardiology consultation in a tertiary academic hospital. Pediatrics **114:**e409–e417.
23. **Ghaffar, F., I. R. Friedland, K. Katz, L. S. Muniz, J. L. Smith, P. Davis, J. Reynolds, and G. H. McCracken, Jr.** 1999. Increased carriage of resistant non-pneumococcal alpha-hemolytic streptococci after antibiotic therapy. J. Pediatr. **135:**618–623.
24. **Griffith, G. C., and D. C. Levinson.** 1949. Subacute bacterial endocarditis; a report on 57 patients treated with massive doses of penicillin. Calif. Med. **71:**403–408.
25. **Guiot, H. F., J. W. van der Meer, P. J. van den Broek, R. Willemze, and R. van Furth.** 1992. Prevention of viridans-group streptococcal septicemia in oncohematologic patients: a controlled comparative study on the effect of penicillin G and cotrimoxazole. Ann. Hematol. **64:**260–265.
26. **Hirakata, Y., J. Matsuda, M. Nakano, T. Hayashi, S. Tozaka, T. Takezawa, H. Takahashi, Y. Higashiyama, Y. Miyazaki, S. Kamihira, and S. Kohno.** 2005. Evaluation of the BD Phoenix Automated Microbiology System SMIC/ID panel for identification and antimicrobial susceptibility testing of *Streptococcus* spp. Diagn. Microbiol. Infect. Dis. **53:**169–173.
27. **Husain, E., S. Whitehead, A. Castell, E. E. Thomas, and D. P. Speert.** 2005. Viridans streptococci bacteremia in children with malignancy: relevance of species identification and penicillin susceptibility. Pediatr. Infect. Dis. J. **24:**563–566.
28. **Innings, A., M. Krabbe, M. Ullberg, and B. Herrmann.** 2005. Identification of 43 *Streptococcus* species by pyrosequencing analysis of the *rnpB* gene. J. Clin. Microbiol. **43:**5983–5991.
29. **Jackson, D. S., D. F. Welch, D. A. Pickett, G. M. Mukwaya, and T. L. Kuhls.** 1995. Suppurative infections in children caused by non-beta-hemolytic members of the *Streptococcus milleri* group. Pediatr. Infect. Dis. J. **14:**80–82.
30. **Johnson, D. H., A. Rosenthal, and A. S. Nadas.** 1975. A forty-year review of bacterial endocarditis in infancy and childhood. Circulation **51:**581–588.
31. **Kastner, U., and J. P. Guggenbichler.** 2001. Influence of macrolide antibiotics on promotion of resistance in the oral flora of children. Infection **29:**251–256.
32. **Kikuchi, K., T. Enari, K. Totsuka, and K. Shimizu.** 1995. Comparison of phenotypic characteristics, DNA-DNA hybridization results, and results with a commercial rapid biochemical and enzymatic reaction system for identification of viridans group streptococci. J. Clin. Microbiol. **33:**1215–1222.

33. **Lu, H. Z., X. H. Weng, B. Zhu, H. Li, Y. K. Yin, Y. X. Zhang, D. W. Haas, and Y. W. Tang.** 2003. Major outbreak of toxic shock-like syndrome caused by *Streptococcus mitis*. J. Clin. Microbiol. **41:**3051–3055.
34. **Lucas, V. S., D. Beighton, G. J. Roberts, and S. J. Challacombe.** 1997. Changes in the oral streptococcal flora of children undergoing allogeneic bone marrow transplantation. J. Infect. **35:**135–141.
35. **Malhotra-Kumar, S., C. Lammens, S. Coenen, K. Van Herck, and H. Goossens.** 2007. Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. Lancet **369:**482–490.
36. **Martin, J. M., W. H. Neches, and E. R. Wald.** 1997. Infective endocarditis: 35 years of experience at a children's hospital. Clin. Infect. Dis. **24:**669–675.
37. **Martino, R., M. Subira, R. Manteiga, I. Badell, B. Argiles, A. Sureda, and S. Brunet.** 1995. Viridans streptococcal bacteremia and viridans streptococcal shock syndrome in neutropenic patients: comparison between children and adults receiving chemotherapy or undergoing bone marrow transplantation. Clin. Infect. Dis. **20:**476–477.
38. **Matsushita, K., W. Fujimaki, H. Kato, T. Uchiyama, H. Igarashi, H. Ohkuni, S. Nagaoka, M. Kawagoe, S. Kotani, and H. Takada.** 1995. Immunopathological activities of extracellular products of *Streptococcus mitis*, particularly a superantigenic fraction. Infect. Immun. **63:**785–793.
39. **Mogi, A., J. I. Nishi, M. Yoshinaga, H. Harada, S. Narahara, K. Kawakami, and I. Maruyama.** 1997. Increased prevalence of penicillin-resistant viridans group streptococci in Japanese children with upper respiratory infection treated by beta-lactam agents and in those with oncohematologic diseases. Pediatr. Infect. Dis. J. **16:**1140–1144.
40. **Mrazova, M., A. Docze, E. Buckova, L. Bucko, M. Kacmarikova, E. Grey, J. Korcova, J. Koprnova, Z. Saboova, P. Beno, M. Karvaj, I. Svetlansky, A. Ondrus, J. Benca, M. Taziarova, B. Rudinsky, and V. Krcmery, Jr.** 2005. Prospective national survey of viridans streptococcal bacteraemia risk factors, antibacterial susceptibility and outcome of 120 episodes. Scand. J. Infect. Dis. **37:**637–641.
41. **Mullen, C. A., D. Petropoulos, W. M. Roberts, M. Rytting, T. Zipf, K. W. Chan, S. J. Culbert, M. Danielson, S. S. Jeha, J. F. Kuttesch, and K. V. Rolston.** 1999. Outpatient treatment of fever and neutropenia for low risk pediatric cancer patients. Cancer **86:**126–134.
42. **Ohuoba, E. F., R. G. Kansal, R. T. Hayden, M. Kotb, and E. E. Adderson.** 2006. Failure of viridans group streptococci causing bacteremia in pediatric oncology patients to express superantigens. J. Pediatr. Hematol. Oncol. **28:**627–629.
43. **Parkins, M. D., C. D. Sibley, M. G. Surette, and H. R. Rabin.** 2008. The *Streptococcus milleri* group—an unrecognized cause of disease in cystic fibrosis: a case series and literature review. Pediatr. Pulmonol. **43:**490–497.
44. **Poyart, C., G. Quesne, S. Coulon, P. Berche, and P. Trieu-Cuot.** 1998. Identification of streptococci to species level by sequencing the gene encoding the manganese-dependent superoxide dismutase. J. Clin. Microbiol. **36:** 41–47.
45. **Poyart, C., G. Quesne, and P. Trieu-Cuot.** 2002. Taxonomic dissection of the *Streptococcus bovis* group by analysis of manganese-dependent superoxide dismutase gene (*sodA*) sequences: reclassification of '*Streptococcus infantarius* subsp. *coli*' as *Streptococcus lutetiensis* sp. nov. and of *Streptococcus bovis* biotype 11.2 as *Streptococcus pasteurianus* sp. nov. Int. J. Syst. Evol. Microbiol. **52:**1247–1255.
46. **Razonable, R. R., M. R. Litzow, Y. Khaliq, K. E. Piper, M. S. Rouse, and R. Patel.** 2002. Bacteremia due to viridans group streptococci with diminished susceptibility to levofloxacin among neutropenic patients receiving levofloxacin prophylaxis. Clin. Infect. Dis. **34:**1469–1474.
47. **Richter, S. S., K. P. Heilmann, C. L. Dohrn, F. Riahi, S. E. Beekmann, and G. V. Doern.** 2008. Accuracy of phenotypic methods for identification of *Streptococcus pneumoniae* isolates included in surveillance programs. J. Clin. Microbiol. **46:**2184–2188.
48. **Richter, S. S., W. J. Howard, M. P. Weinstein, D. A. Bruckner, J. F. Hindler, M. Saubolle, and G. V. Doern.** 2007. Multicenter evaluation of the BD Phoenix Automated Microbiology System for antimicrobial susceptibility testing of *Streptococcus* species. J. Clin. Microbiol. **45:**2863–2871.
49. **Rodríguez-Avial, I., C. Rodriguez-Avial, E. Culebras, and J. J. Picazo.** 2005. In vitro activity of telithromycin against viridans group streptococci and *Streptococcus bovis* isolated from blood: antimicrobial susceptibility patterns in different groups of species. Antimicrob. Agents Chemother. **49:**820–823.
50. **Sibley, C. D., M. D. Parkins, H. R. Rabin, K. Duan, J. C. Norgaard, and M. G. Surette.** 2008. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. Proc. Natl. Acad. Sci. U. S. A. **105:**15070–15075.
51. **Stockheim, J. A., E. G. Chadwick, S. Kessler, M. Amer, N. Abdel-Haq, A. S. Dajani, and S. T. Shulman.** 1998. Are the Duke criteria superior to the Beth Israel criteria for the diagnosis of infective endocarditis in children? Clin. Infect. Dis. **27:**1451–1456.
52. **Tazumi, A., Y. Maeda, C. E. Goldsmith, W. A. Coulter, C. Mason, B. C. Millar, M. McCalmont, J. Rendall, J. S. Elborn, M. Matsuda, and J. E. Moore.** 2009. Molecular characterization of macrolide resistance determinants [*erm*(*B*) and *mef*(*A*)] in *Streptococcus pneumoniae* and viridans group streptococci (VGS) isolated from adult patients with cystic fibrosis (CF). J. Antimicrob. Chemother. **64:**501–506.
53. **Whiley, R. A., D. Beighton, T. G. Winstanley, H. Y. Fraser, and J. M. Hardie.** 1992. *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. J. Clin. Microbiol. **30:**243–244.
54. **Whiley, R. A., H. Fraser, J. M. Hardie, and D. Beighton.** 1990. Phenotypic differentiation of *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* strains within the "*Streptococcus milleri* group". J. Clin. Microbiol. **28:**1497–1501.
55. **Whiley, R. A., L. M. Hall, J. M. Hardie, and D. Beighton.** 1999. A study of small-colony, beta-haemolytic, Lancefield group C streptococci within the *anginosus* group: description of *Streptococcus constellatus* subsp. *pharyngis* subsp. nov., associated with the human throat and pharyngitis. Int. J. Syst. Bacteriol. **49:**1443–1449.