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Viridans Streptococci Isolated by Culture from Blood of Cancer Patients: Clinical and Microbiologic Analysis of 50 Cases

Xiang Y. Han,[1]* Mallika Kamana,[2] and Kenneth V. I. Rolston[2]

Section of Clinical Microbiology[1] and Infectious Diseases,[2] The University of Texas M. D. Anderson Cancer Center, Houston, Texas

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Clinical and microbiologic studies of 50 cases of viridans streptococcal bacteremia in cancer patients were performed. The bacteria were identified to species level by sequencing analysis of the 16S rRNA gene. At least nine *Streptococcus* spp. were found, including *S. mitis* (25 strains, 50.0% of 50); currently unnamed *Streptococcus* spp. (11 strains); *S. parasanguis* (five strains); *S. anginosus* (three strains); *S. salivarius* (two strains); and one strain each of *S. gordonii*, *S. sanguis*, *S. sobrinus*, and *S. vestibularis*. There were no *S. oralis* strains. Among 11 antibiotics of nine classes tested, no resistance to vancomycin, linezolid, or quinupristin-dalfopristin was seen. Resistance to penicillin (MIC, 4 to 12 μg/ml) was noted only among *S. mitis* strains (28.0%, 7/25) and not non-*S. mitis* strains (0/25) ($P = 0.004$). Significantly more *S. mitis* strains than non-*S. mitis* strains were resistant to fluoroquinolones and to ≥3 classes of antibiotics. Isolation of quinolone-resistant organisms was associated with the prior usage of quinolones ($P = 0.002$). Quantitative blood cultures showed that the strains resistant to levofloxacin or gatifloxacin were associated with higher colony counts than were their corresponding nonresistant strains. The young and elderly patients also had higher levels of bacteremia caused predominantly by *S. mitis*. Septic shock was present in 17 (34.0% of 50) patients, and 13 of those cases were caused by *S. mitis* ($P = 0.007$). These results suggest that *S. mitis* is the most common cause of viridans streptococcal bacteremia in cancer patients and is more resistant to antibiotics than other species.

Viridans streptococci represent a group of 24 currently described *Streptococcus* species that are nutritionally fastidious and mainly alpha-hemolytic on sheep blood agar (30). These gram-positive cocci are commensals of the oral cavity, upper airway, and gastrointestinal and genitourinary tracts. Despite the overall low virulence, they may cause infective endocarditis, contribute to polymicrobic abscess, and invade the bloodstream during the state of neutropenia.

The bloodstream infection usually occurs in cancer patients with mucositis and neutropenia due to antineoplastic chemotherapy-related toxicity. In these patients, studies have found that viridans streptococci are among the most common organisms isolated from the cultures of bacteremia samples (17). As cancer care has improved and intensified over recent decades and the patients survive longer, these and other infectious complications become more pronounced. A study from our institution showed that the rate of viridans streptococcal bacteremia increased from 1 per 10,000 admissions to 47 per 10,000 during the 13-year period from 1977 to 1989 (11). These bacteremias cause substantial morbidity, and mortality in these patients is at 6 to 12% (17, 29).

The most common viridans streptococci that cause neutropenic bacteremia have been *S. oralis*, *S. mitis*, and *S. salivarius* (4, 11, 15, 17). Identification of these species, however, was reached through traditional biochemical reactions that may be variable and overlapping among different as well as closely related species. Because of this limitation, most clinical microbiology laboratories rarely attempt to identify viridans streptococci to species level.

Sequencing analysis of the 16S rRNA gene has become an essential part of bacterial taxonomy, forming the backbone of a polyphasic approach for the description of new bacterial species (22). The method has also gained wide application for the identification of various unknown bacteria in research as well as clinical laboratory settings (6, 10, 13, 14). Most (if not all) *Streptococcus* species have been analyzed for the 16S rRNA gene sequences and their phylogenetic relationships delineated (18, 19, 35), thus establishing the basis for accurate identification of various streptococci by this method. In this study, we used a 16S rRNA gene sequencing method to identify to species level 50 strains of viridans streptococci that were isolated quantitatively from blood cultures of cancer patients. The antibiotic susceptibilities of these species were tested and analyzed. These results were correlated with clinical findings.

MATERIALS AND METHODS

Study setting and cultures. The cases occurred sporadically from July 2002 to December 2003 at The University of Texas M. D. Anderson Cancer Center in Houston, Texas, a 500-bed comprehensive cancer center. They were identified among the approximately 45,000 blood cultures performed during the period. The bacteria were isolated from blood cultures using the Bactec 9240 automated culturing system (BD Diagnostic Systems, Sparks, MD) and Isolator lysis centrifugation tubes (Wampole Laboratories, Princeton, NJ). When an Isolator tube culture was positive, the number of bacterial colonies was quantitated from the 10 ml of blood cultured (31). Samples from children younger than 15 years of age were cultured using Pedi-Isolator (Wampole) with 1.5 ml blood drawn, and the final colony counts were adjusted to a 10-ml blood volume. All subcultures were plated on blood agar and chocolate agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubated aerobically at 35°C in 5% CO_2.

Identification of organisms. The organisms were all presumptively identified as viridans streptococci based on alpha-hemolysis, gram-positive reaction, coccus morphology in chains, negative catalase test, and exclusions of pneumococci and

* Corresponding author. Mailing address: Section of Clinical Microbiology, The University of Texas M. D. Anderson Cancer Center, Unit 84, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 792-3515. Fax: (713) 792-0936. E-mail: xhan@mdanderson.org.

TABLE 1. Reference *Streptococcus* species and their 16S rRNA gene sequences used in the study

Streptococcus species	GenBank no.	Strain	Reference
S. anginosus	AF104678	ATCC 33397^T = NCTC10713^T	16
S. gordonii	AF003931 and D38483	ATCC 10558^T = NCTC7865^T	Emler et al., 1997, unpublished; 18
S. mitis	AF003929 and D38482	ATCC 49456^T = NCTC12261^T	Emler et al., 1997, unpublished; 18
S. oralis[a]	AF003932 and X58308	ATCC 35037^T = NCTC11427^T	Emler et al., 1997, unpublished; 5
S. parasanguis	AF003933	ATCC 15912^T	Emler et al., 1997, unpublished
S. salivarius	AY188352	ATCC 7073^T = NCTC8618^T	Kiratisin et al., 2003, unpublished
S. sanguis	AF003928	ATCC 10556^T	Emler et al., 1997, unpublished
S. sobrinus	AY188349	ATCC 33478^T	Kiratisin et al., 2003, unpublished
S. vestibularis	AY188353	ATCC 49124^T = NCTC12166^T	Kiratisin et al., 2003, unpublished
Streptococcus sp. related to *S. mitis*	AY281086	ATCC 49296	Kiratisin et al., 2003, unpublished
Streptococcus sp. related to *S. mitis*	AY005045	Unspecified	27
Streptococcus sp. unrelated to *S. mitis*	AF432134	Unspecified	20
Streptococcus sp. unrelated to *S. mitis*	AY278634	Unspecified	25

[a] For differentiation from *S. mitis* only.

enterococci by routine biochemical tests (optochin test, bile solubility, and PYR [*N*,*N*-dimethylaminocinnamaldehyde] test). The definitive species identification was achieved through sequence analysis of portions of the 16S rRNA gene described previously (14). Briefly, genomic DNA from pure culture colonies was extracted and subjected to amplification by PCR for a 593-bp fragment of the 16S rRNA gene. A set of universal bacterial primers, 5′-TGCCAGCAGCCGCGGTAATAC-3′ and 5′-CGCTCGTTGCGGGACTTAACC-3′ (positions 515 to 1107 of *Escherichia coli* J01859 or 517 to 1109 of sequence AF003929 of *S. mitis* ATCC 49456^T, respectively), was used for the amplification. To further differentiate the species between *S. mitis* and *S. oralis* (23 strains), the species among the *S. salivarius* group, and unclassified species (11 strains), a second set of primers was used for the amplification and sequencing of a 352-bp fragment (positions 7 to 358 of *S. mitis* sequence AF003929) that corresponds to the most variable region for various streptococci. These primers were 5′-GTTTGATCCTGGCTCAGAGCG-3′ and 5′-ACTGCTGCCTCCCGTAGGAG-3′. The amplicon was sequenced by the dye terminator method in an automated ABI sequencer (Applied Biosystems, Foster City, CA), and sequence analysis was performed through a query to the GenBank basic local alignment search tool (BLAST) (3). Species identification was reached by the best sequence match (99.2% to 100%) with the 16S rRNA gene of a type strain in GenBank. Generally, a sequence match of 99.0% or above corresponds to species identification (6, 10, 13); hence, the cutoff of 99.2% chosen here was slightly more stringent for the shorter and more variable sequenced region (18). The reference organisms and sequences used in this study are summarized in Table 1.

Antibiotic susceptibility. The antibiotic susceptibility tests were performed using Etest (AB Biodisk, Solna, Sweden), which is FDA approved, and the results correlate with results from the microdilution method. Briefly, the organism was plated on blood Mueller-Hinton agar and incubated at 35°C in ambient air (preferred), or with CO_2 if required for growth, for 20 to 24 h. Results for MIC (μg/ml) were interpreted according to the breakpoints set by the Clinical and Laboratory Standards Institute (formerly NCCLS) for *Streptococcus* spp. other than *Streptococcus pneumoniae* (7). For trimethoprim-sulfamethoxazole and ciprofloxacin, interpretive breakpoints for pneumococcus and enterococci were used, respectively.

Clinical information. The medical records were reviewed for clinical information, including demographics, underlying diagnosis of cancer, anticancer chemotherapy, dental procedures or periodontitis, status of stem cell transplantation, absolute neutrophil count (ANC; per cubic millimeter), and chemotherapy-induced oral and gastrointestinal toxicity such as mucositis, nausea, vomiting, dysphagia, epigastric pain, and diarrhea. Septic shock was defined as positive blood cultures with associated fever of ≥38.5°C, systolic blood pressure of <90 mm Hg, and heart rate of ≥110/minute (24).

Data analysis. Categorical data were analyzed for statistical significance using the χ^2 test. Quantitative culture data were first transformed by logarithm to normal distribution and then analyzed using the Student *t* test. Significant *P* values (≤0.05) or nearly significant ones were given.

RESULTS

Species and quantitative cultures. The 16S rRNA gene sequencing method allowed accurate species identification of the 50 streptococcal strains, with *S. mitis* and non-*S. mitis* each accounting for half (Table 2). The non-*S. mitis* strains included *Streptococcus* sp., *S. parasanguis* (also known as *S. parasanguinis*), *S. salivarius*, *S. anginosus*, *S. sanguis* (*S. sanguinis*), *S. gordonii*, *S. sobrinus*, and *S. vestibularis*. The strains of the *Streptococcus* sp. were diverse: six were closely related to *S. mitis* (98% to 99.1% matches within the ~850-bp sequenced regions), and five did not match well with any established

TABLE 2. Species and quantitative blood cultures of viridans streptococci

Streptococcus species	Total (%)	Distribution of no. of colonies per culture						Single species
		<10	10 to 49	50 to 99	100 to 199	≥200	Mean[a]	
S. mitis	25 (50.0)	9	4	3	2	7	38.8	22
All non-*S. mitis*	25 (50.0)	18	4	2	1		4.8	16
Streptococcus sp.	11 (22.0)	7	2	2				
S. parasanguis	5 (10.0)	5						
S. anginosus	3 (6.0)	2	1					
S. salivarius	2 (4.0)	1			1			
S. gordonii	1 (2.0)	1						
S. sanguis	1 (2.0)	1						
S. sobrinus	1 (2.0)	1						
S. vestibularis	1 (2.0)		1					
Total or *P* value	50 (100)	27	8	5	3	7	*P* = 0.001	*P* = 0.047

[a] Geometric mean.

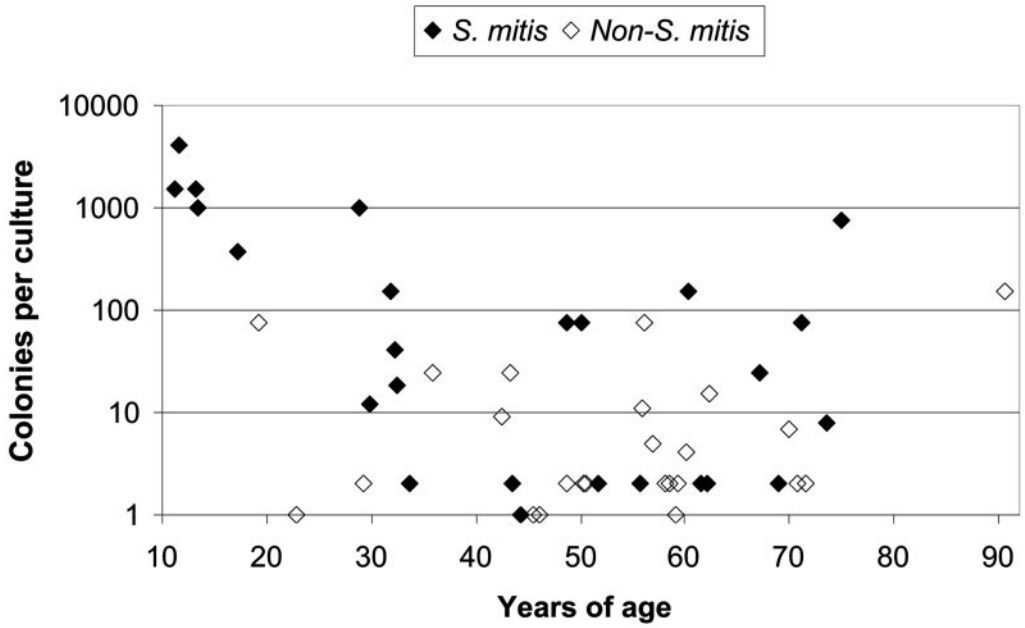

FIG. 1. Analysis of the number of colonies, patient age, and *Streptococcus* species.

species (<98% matches). Phenotypically, these 11 strains were indistinguishable from other established species. There were no *S. oralis* isolates.

Quantitative cultures showed that the *S. mitis* strains caused significantly higher levels of bacteremia than the non-*S. mitis* organisms did (geometric mean, 38.8 colonies per culture versus 4.8; $t = 3.45$; $P = 0.001$) (Table 2; Fig. 1). By category, 7 of 25 *S. mitis* strains had ≥200 colonies per culture in contrast to none of 25 non-*S. mitis* strains ($P = 0.004$). More *S. mitis* strains than non-*S. mitis* strains caused monomicrobic infection (22/25 versus 16/25, $P = 0.047$).

The ages of patients also affected the colony counts. High numbers of colonies were seen mainly in the young and elderly (Fig. 1), particularly children. All five children (11 to 17 years of age) had high-level *S. mitis* bacteremia (≥375 colonies).

Antibiotic susceptibility. Eleven antibiotics of nine classes were tested against the organisms (Table 3). During the testing process, most (23 of 25, 92.0%) *S. mitis* strains grew well in the ambient air without the requirement of CO_2, whereas 17 of 25 (68.0%) non-*S. mitis* strains grew this way ($P = 0.034$). Thus, *S. mitis* was less capnophilic.

All 50 strains were susceptible to vancomycin, and no resistance to linezolid or quinupristin-dalfopristin was seen either. Resistance to other antibiotics varied with species. Seven of the 25 (28.0%) *S. mitis* strains were resistant to penicillin (MIC, 4 to 12 μg/ml) in contrast to none of the 25 non-*S. mitis* strains

TABLE 3. Antibiotic susceptibilities of blood isolates of viridans streptococci[a]

Antibiotic	No. of strains							
	S. mitis ($n = 25$)	All non-*S. mitis* ($n = 25$)	*Streptococcus* sp. ($n = 11$)	*S. parasanguis* ($n = 5$)	*S. salivarius*, *S. vestibularis* ($n = 3$)	*S. anginosus* ($n = 3$)	*S. gordonii*, *S. sanguis*, *S. sobrinus* ($n = 3$)	Resistance comparison[b]
Vancomycin	S25	S25	S11	S5	S3	S3	S3	NS
Linezolid	S25	S23/I2	S11	S4/I1	S3	S2/I1	S3	NS
Quin-dalfo	S24/I1	S23/I2	S11	S4/I1	S3	S2/I1	S3	NS
Ceftriaxone	S18/I4/R3	S23/I1/R1	S9/I1/R1	S5	S3	S3	S3	NS
TMP-SMZ	S14/I3/R8	S20/I1/R4	S9/I1/R1	S4/R1	S3	S2/R1	S2/R1	NS
Tetracycline	S15/I1/R9	S15/I2/R8	S6/I1/R4	S1/I1/R3	S3	S2/R1	S3	NS
Azithromycin	S9/I3/R13	S16/R9	S8/R3	R5	S2/R1	S3	S3	NS
Penicillin	S11/I7/R7	S14/I11	S7/I4	I5	S1/I2	S3	S3	$P = 0.004$
Gatifloxacin	S9/I2/R14	S16/I1/R8	S5/R6	S3/I1/R1	S2/R1	S3	S3	NS[c]
Levofloxacin	S8/I1/R16	S16/R9	S5/R6	S3/R2	S2/R1	S3	S3	$P = 0.048$
Ciprofloxacin	S4/I2/R19	S9/I3/R13	I2/R9	S2/I1/R2	S2/R1	S3	S2/R1	$P = 0.077$
R to ≥3 classes	14	3	2	1				$P = 0.001$

[a] Abbreviations: S, susceptible; I, intermediate; R, resistant; NS, not significant; Quin-dalfo, quinupristin-dalfopristin; TMP-SMZ, trimethoprim-sulfamethoxazole.
[b] *S. mitis* strains versus all non-*S. mitis* strains.
[c] $P = 0.048$ when susceptible strains were compared (9/25 versus 16/25).

TABLE 4. Clinical features of viridans streptococcal bacteremia in cancer patients

Feature	No. of patients			
	S. mitis, $n = 25$	Non-*S. mitis*, $n = 25$	Both, $n = 50$ (%)	*P* value[b]
Women/men	10/15	11/14	21/29	NS
Age <18 yrs	5	0	5 (10.0)	0.018
Hematologic cancers	21	16	37 (74.0)	NS
Anticancer treatment	21	20	41 (82.0)	NS
Mucosal/GI[a] toxicity	17	14	31 (62.0)	NS
Prior use of quinolone	20	16	36 (72.0)	NS
Use of corticosteroids	6	8	14 (28.0)	NS
ANC <500 for >10 days	18	9	27 (54.0)	0.011
ANC <100	10	3	13 (26.0)	0.024
Fever	25	21	46 (92.0)	NS
Septic shock	13	4	17 (34.0)	0.007
Cause of death	1	0	1 (2.0)	NS

[a] GI, gastrointestinal.
[b] By *t* test. NS, nonsignificant.

($P = 0.004$), making the overall penicillin (high-level) resistance 14.0% (7 of 50). The *S. mitis* strains also showed higher rates of resistance to each of the three fluoroquinolones tested than did the non-*S. mitis* strains, but the overall resistance (4 to >32 μg/ml) for all 50 strains was high: 22 strains (44.0%) for gatifloxacin, 25 strains (50.0%) for levofloxacin, and 32 strains (64.0%) for ciprofloxacin. In particular, the resistance to levofloxacin was at a high level for all 25 strains (MIC, ≥32 μg/ml), and the resistant strains were also associated with higher colony counts in blood cultures than the sensitive strains (geometric mean, 27.5 versus 6.8, respectively; $t = 2.15$; $P = 0.037$). Similarly, gatifloxacin-resistant strains also had higher colony counts than nonresistant strains (36.3 versus 6.4, respectively; $t = 2.73$; $P = 0.009$). For all antibiotics, 14 (56.0%) of the 25 *S. mitis* strains were resistant to ≥3 classes of antibiotics compared to 3 (12.0%) of 25 non-*S. mitis* strains ($P = 0.001$). Therefore, *S. mitis* was the most resistant viridans streptococcus.

Clinical features of infection. The clinical features of patients are shown in Table 4. The patients included 21 women and 29 men with a mean age of 48 years. Hematologic cancers were predominant (74.0%, 37 of 50). The majority (82.0%) of patients had undergone anticancer chemotherapy within 3 weeks with frequent (62.0%) occurrence of mucositis and gastrointestinal symptoms. Severe neutropenia (ANC of <500/mm^3) was common (66.0%), and it lasted significantly longer and was more pronounced in patients with *S. mitis* than in those with non-*S. mitis* strains.

Prophylactic use of antibiotics was common, particularly fluoroquinolones (mainly levofloxacin) (72.0%, 36 of 50). Among the 25 cases with levofloxacin-resistant streptococci, 23 (92.0%) cases had prophylactic use or treatment of this drug or another quinolone within a week of the positive culture. In contrast, prophylaxis with a quinolone was present only for 13 (52.0%) of the 25 cases that did not have levofloxacin-resistant organisms ($P = 0.002$). Thus, the emergence or selection of a quinolone-resistant strain was associated with prior usage.

Fever was present in 46 (92.0%) patients. Seventeen (34.0%) patients manifested septic shock, and *S. mitis* caused 13 of those cases, far more cases than those caused by non-*S. mitis* organisms ($P = 0.007$). In addition, all the *S. mitis* cases were monomicrobic, whereas two of the four non-*S. mitis* cases also involved methicillin-resistant *Staphylococcus aureus* and *Enterobacter aerogenes*, respectively. Ten (58.8%) of the 17 shock cases had colony counts of ≥50 per culture, significantly more than the nonshock cases (15.2%, 5/33) ($P = 0.001$). One patient died as a consequence of the streptococcal bacteremia; all others responded to specific treatment that usually consisted of vancomycin and another antibiotic(s).

***Streptococcus parasanguis*.** In view of the recent species status of *S. parasanguis* and limited clinical experience with it (9, 12, 21, 35), attention was paid to the clinical and microbiologic features of the five *S. parasanguis* cases. Four patients presented with fever, while the only afebrile one was on steroids. The colony counts were all below 10 per culture. Two cases were monomicrobic with *S. parasanguis* only. All infections resolved with treatment. Notably, all five strains were resistant to azithromycin, in contrast to 18 of 45 (40.0%) strains of other streptococci ($P = 0.011$). These strains were also intermediately resistant to penicillin (MIC range, 0.25 to 2 μg/ml).

DISCUSSION

It is well known that, in most centers, isolates of viridans streptococci from blood cultures are more likely to be contaminants than true pathogens (33, 34). In cancer patients with profound neutropenia, however, this dogma probably does not apply, i.e., they are more likely pathogens than contaminants. The cases and strains in this study were consecutive and unselected, yet 92% of patients were febrile and those nonfebrile ones might have had other reasons for being nonfebrile. Various risk factors for viridans streptococcal bacteremia have been identified and reviewed (11, 23, 28, 29, 32), including profound neutropenia, oral mucositis, prophylactic use of a fluoroquinolone or trimethoprim-sulfamethoxazole, exposure to high-dose chemotherapy (particularly cytosine arabinoside), stem cell transplantation, age of <18 years, and others. The importance of such bacteremia among pediatric patients with cancer has also been appreciated (1, 17, 26). The clinical findings from this study are consistent with these general features. The focus of this study, however, is microbiological, i.e., quantitative blood cultures, accurate identification of the streptococcal species, and antibiotic susceptibility.

Of the 50 strains sequenced, at least nine species were identified, more diverse than findings from previous studies that are based on biochemical tests. The new finding of five (10%) cases caused by *S. parasanguis* contributes to the knowledge about this relatively new species, and they were all intermediately resistant to penicillin and resistant to azithromycin. While the finding of the predominance of *S. mitis* is consistent with most previous studies, the lack of *S. oralis*, however, is somewhat surprising. A number of earlier phenotype studies have found that *S. oralis* was either the predominant species or a significant part, ranging from 32% to 61%, of the bacteremia-causing viridans streptococci (4, 11, 29). Biochemically, *S. oralis* and *S. mitis* are closely related, sharing several overlapping reactions. Their 16S rRNA gene sequences, however, differ overall by 13 nucleotides (of 1,453 bp or 99.1% identity) (18), 12 of which are within the beginning ~300-bp region that was determined in this study. Thus, our differentiation between

these two species was confident. Similar to our finding, another sequence-based study of six cases showed that the strains all turned out to be *S. mitis* (28). Two recent phenotype-based studies of viridans streptococci (2, 23), both from Spain, also found no *S. oralis* strains, in contrast to *S. mitis* as the vast majority (77.9 to 81.8%, 60/77 and 72/88, respectively). Therefore, the wide variation in the presence of *S. oralis* hints at the need for a study to compare the biochemical tests and sequencing method for uniformity.

In addition to the 24 established viridans streptococcal species, several other yet-to-be-described oral streptococci have been noted by culture-independent studies (20, 27). In this study, we found that 11 strains did not match established streptococcal species, some close to *S. mitis*, some distant. It is possible that these organisms represent novel species. The medical significance of these organisms may warrant further microbiologic studies and extensive sequencing analysis.

Resistance to penicillin among viridans streptococci is commonly seen at present: 13.4 to 23.4% with a MIC of ≥4 μg/ml (high level) (2, 8) and 16.9 to 42.9% with a MIC of 0.25 to 2 μg/ml (intermediate) (2, 8, 23). Our data further showed that high-level resistance was present only among *S. mitis* strains (28.0%, 7/25), not non-*S. mitis* strains. The *S. mitis* strains were also more resistant to fluoroquinolones and to ≥3 classes of antibiotics. This finding is consistent with the results of Doern et al. (8). Selection of quinolone-resistant *S. mitis* by prophylactic use of these drugs has been reported in a study of six cases (28). Among our 50 cases, statistical analysis showed that isolation of a levofloxacin-resistant organism was linked to prophylactic use of quinolones. In addition, strains resistant to levofloxacin or gatifloxacin were associated with higher colony counts on blood cultures than were their corresponding nonresistant strains.

A recent study has suggested that *S. mitis* causes more septic shock than non-*S. mitis* organisms (23). In another report (28), half of the six cases with quinolone-resistant *S. mitis* also presented with septic shock. Our data are consistent with these findings, and they further show that such septic shock was usually associated with high-level (≥100 colonies) bacteremia on quantitative blood cultures. The *S. mitis* strains caused higher levels of bacteremia than the non-*S. mitis* strains (Table 2; Fig. 1), which were seen more often in the young and elderly. Together, these data suggest that *S. mitis* is a more pathogenic viridans streptococcus.

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