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DNA Relatedness, Phenotypic Characteristics, and Antimicrobial Susceptibilities of *Globicatella sanguinis* Strains

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DNA-DNA reassociation was performed on 15 strains of *Globicatella sanguinis* to compare their taxonomic status with phenotypic characterization. All 15 strains selected for DNA-DNA reassociation readily met the criteria for species relatedness. The relative binding ratio was 81% or greater at the optimal temperature and 76% or greater at the stringent temperature, and the divergence was less than 3% for all strains hybridized with the type strain. These strains included nine strains from the Centers for Disease Control Streptococcus Laboratory culture collection that were previously included in comparative 16S rRNA gene sequencing studies as well as six additional phenotypically variant isolates. DNA-DNA relatedness was less than 18% at the optimal reassociation temperature to *Aerococcus viridans, Enterococcus avium*, and *Streptococcus uberis*, which are phenotypically similar to *G. sanguinis*. This study confirms these *Globicatella* strains were previously misidentified as *S. uberis* or *S. uberis*-like strains based on biochemical characteristics. The biochemical data from 28 strains was compiled to further define the phenotypic criteria for identification of this species. A revised description of the species should be variable reaction for pyrrolidonylarylamidase production (75% positive), positive reaction for the bile esculin test (100%), growth at 45°C (96%), variable reaction for acid production from arabinose (45% positive), and negative starch hydrolysis (0% positive). We also evaluated four rapid identification systems, the Biomerieux rapid ID32 STREP (ID32), the Crystal rapid gram-positive identification (Cry4), the BBL Crystal gram-positive identification (Cry24), and the Remel IDS RapID STR (IDS) systems for their ability to identify these strains.

In the 1977 summary of the identification of viridans streptococci isolated from human sources, 7 of 1,227 isolates were reported to be *Streptococcus uberis* (10). It was noted at that time that these isolates phenotypically resembled *Streptococcus mutans* based on biochemical reactions. However, four of the seven strains grew in 6.5% NaCl broth, which differentiated this species from all other viridans streptococci. Some of these strains, identified as *S. uberis*, were distributed to commercial manufacturers of kits designed to identify viridans streptococci (15, 16, 30). These strains had phenotypic characteristics similar to those described in 1977 and were identified as *S. uberis* (10) until 1990. With the advent of new tests to discriminate between the genera of gram-positive cocci, it became clear that our phenotypic criteria for identification of *S. uberis* was probably not valid.

In 1992, comparative 16S rRNA gene sequencing data for nine of these strains were analyzed to determine their phylogenetic position (4). In this study, the highest sequence homology was shown with the genus *Aerococcus* (91%), followed by the lactococci (88%), leuconostocs (86 to 89%), pediococci (89 to 90%), and streptococci (87 to 88%). Phylogenetic analysis showed an unknown line of descent within the lactic acid group of bacteria (4). Therefore, based on phenotypic similarities and 16S rRNA sequence analysis, these isolates were shown to represent a new genus and species for which the name *Globicatella sanguinis* (*sanguis*) was proposed (4). The species of the genus was later renamed from *sanguis* to *sanguinis* in order to conform with the rules of Latin grammar (27).

G. sanguinis closely resembles aerococci, streptococci, and enterococci phenotypically (9, 12, 14). The major differentiating characteristic between *Globicatella* and the aerococci is the cellular arrangement of the cells in the Gram stain. *Globicatella* forms chains while the aerococci form tetrads and clusters. The colonial morphology of *Globicatella* strains most closely resembles the viridans streptococci. However, these strains are readily distinguished with a negative leucine aminopeptidase reaction (LAP) and growth in the presence of 6.5% NaCl. The viridans streptococci are pyrridonylarylamidase (PYR) negative and LAP positive and do not grow in the presence of 6.5% NaCl. The enterococci are PYR and LAP positive and grow at 10°C. None of the *Globicatella* isolates grew at 10°C or gave positive LAP reactions.

The isolation of these strains from normally sterile body sites (21 of the isolates were from blood, 3 were from cerebrospinal fluid [CSF], and 4 were from urine) and that they were referred to the Centers for Disease Control and Prevention by 13 different state health departments and Canada indicates that this organism is clinically significant. The purpose of this communication is to report on DNA-DNA reassociation studies, modified phenotypic criteria for identification of this species, the evaluation of four commercial identification systems, and antimicrobial susceptibilities.

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MATERIALS AND METHODS

Bacterial strains. A total of 28 strains were studied, including the 9 previously described strains that were determined to represent the genus and species based upon 16S rRNA sequencing data (4). All of the reference and test strains were obtained from the culture collection of the Streptococcus Laboratory, Centers for Disease Control and Prevention. The type or reference strains were received from the following: *Aerococcus viridans* (SS1251, ATCC 11563^T) was obtained from the American Type Culture Collection, Manassas, Va., *S. uberis* (SS842, 0100) was received from G. A. Cullen in England (5), and *Enterococcus avium* (SS817, NCTC 9938, NCDO 2369, ATCC14025^T) was received from the late W. Maxted, Streptococcus Reference Laboratory, Colindale, United Kingdom.

Phenotypic characterization. A total of 28 strains were compared for their phenotypic characteristics by performing conventional biochemical tests as described previously by Facklam et al. and Collins et al. (4, 11).

DNA reassociation studies. The cultures were grown in 1,000 ml of Todd-Hewitt broth for 18 to 24 h at 35°C on a rotary shaker (New Brunswick Scientific, Edison, N.J.). The bacterial cells were harvested by centrifugation and lysed as previously described (25). The bacterial cells were resuspended in 50 ml of TS buffer, which contains 50 mM Tris (pH 8.0)–12.5% sucrose, before the addition of 20 ml of lysozyme solution (Sigma, St. Louis, Mo.; 10 mg/ml), 500 μl of mutanolysin (Sigma; 2,000 U/ml), and 30 ml of EDTA (50 mM). After a 2-h incubation at 35°C on a rotary shaker, 500 μl of Proteinase K (25 mg/ml) was added and the suspension was incubated for an additional 1 h. The cells were lysed by the addition of 4 ml of a 25% sodium dodecyl sulfate solution. The techniques used in the purification and reassociation of DNA using the hydroxyapatite method were those previously described (3). The DNA from the type strain (1152-78, NCFB 2835) was labeled with [^{32}P]dCTP by using a nick translation kit (Gibco BRL, Life Technologies, Inc., Gaithersburg, Md.) as directed by the manufacturer. The temperatures used for DNA reassociation were 55°C (optimal conditions) and 70°C (stringent conditions). The percent divergence (%D) was calculated as a decrease of 1°C in thermal stability of a heterologous DNA duplex, which correlates to approximately 1% unpaired bases within related DNA.

Identification of *G. sanguinis* using commercial systems. The four systems evaluated for the identification of *G. sanguinis* were the rapid ID 32 STREP (ID32; Biomerieux, Inc., Hazelwood, Mo.) (17), the BBL Crystal Rapid Gram-Positive ID Kit (Cry24) and the BBL Crystal Gram-Positive ID Kit (Cry4; Crystal; BD Bioscience, Cockeysville, Md.) (28), and the RapID STR (IDS; Remel, Inc., Lenexa, Kaus.) (2, 18, 22). All systems were used according to the manufacturer's instructions provided in the package inserts. Each system generates a profile number which we transmitted to each manufacturer to obtain the reported identification.

Antimicrobial susceptibility testing. Antimicrobial susceptibility was determined using microdilution in customized panels containing lysed horse-blood-supplemented cation-adjusted Mueller-Hinton broth designed specifically for MIC testing of streptococci species (PML Microbiologicals, Wilson, Oreg.) and the methods described by NCCLS (21). The following antibiotics and ranges were tested: penicillin, 0.03 to 16.0 μg/ml; amoxicillin, 0.03 to 8.0 μg/ml; cefotaxime, 0.06 to 16.0 μg/ml; erythromycin, 0.06 to 16.0 μg/ml; cefuroxime, 0.25 to 32.0 μg/ml; trimethoprim-sulfamethoxazole, 0.12, 2.38 to 8.0, and 152.0 μg/ml; clindamycin, 0.06 to 2.0 μg/ml; chloramphenicol, 2.0 to 16.0 μg/ml; levofloxacin, 0.5 to 16.0 μg/ml; meropenem, 0.06 to 2.0 μg/ml; tetracycline, 2.0 to 8.0 μg/ml; and vancomycin, 0.12 to 2 μg/ml. The panels were incubated in ambient air at 35°C for 20 to 24 h and read visually with the aid of a mirror panel viewer. Quality control was performed as previously described with two control strains of *Streptococcus pneumoniae* (19, 20).

RESULTS AND DISCUSSION

Clinical data. The source, clinical diagnosis, and demographic data was provided with the 28 clinical isolates and is summarized in Table 1. This data suggests that *G. sanguinis* is capable of causing serious infection and is most likely an opportunistic pathogen. The majority of strains (22) were isolated from blood, and 2 isolates were from CSF. The remaining four strains were from urine. The ages of patients were given for 18 isolates, with infection occurring most often in the very young and very old. Five of the isolates were from children less than 3 years old, and 11 isolates were from patients over 65 (average age, 80.5 years old). The sex of the patient was known for 23 of the 28 isolates. At first glance it would appear that females are predominately more at risk; however, this is more likely due to the population in the age group rather than a propensity of the bacterium, since of the 7 patients under age 65, 3 were male and 4 were female.

TABLE 1. Source, clinical diagnosis, and demographic information on 28 clinical isolates of *G. sanguinis*

Strain[a]	Source	Patient characteristics[b]			
		Clinical diagnosis	Sex	Age	Locale
1152-78	Blood	Bacteremia	F	NA	Massachusetts
1104-78	Blood	Bacteremia	F	NA	New York
1713-79	Urine	Urinary tract infection	M	NA	Maine
1433-83	Blood	Bacteremia	NA	NA	Colorado
1434-83	Blood	NA	NA	NA	Colorado
1040-80	Urine	Urinary tract infection	M	69	Texas
399-83	Urine	Urinary tract infection	F	85	California
905-75	Blood	NA	NA	NA	Washington, D.C.
925-83	CSF	Meningitis	M	<1	Florida
2434-91	Blood	Sepsis	F	84	Texas
2161-93	Urine	NA	F	NA	Canada
135-94	Blood	Urosepsis	F	90	North Carolina
2504-96	Blood	NA	F	68	Georgia
162-97	Blood	NA	F	82	Canada
1415-93	Blood	NA	F	79	Canada
919-90	Blood	NA	NA	NA	Georgia
694-91	Blood	Septicemia	M	<1	South Carolina
1017-91	Blood	NA	NA	NA	Georgia
1702-93	Blood	Septicemia	M	58	South Carolina
2103-93	Blood	Septicemia	F	82	Texas
172-94	Blood	NA	F	2	Canada
1662-95	Blood	NA	F	92	Canada
1666-95	Blood	NA	F	NA	Canada
4867-96	Blood	Endocarditis	F	70	Virginia
163-97	CSF	NA	F	43	Canada
1577-97	Blood	NA	M	85	Canada
6728-99	Blood	NA	F	<1	Canada
28-00	Blood	Septicemia	F	3	South Carolina

[a] The number after the hyphen indicates the year the culture was received.
[b] NA, not available; F, female; M, male.

DNA-DNA reassociation. The type strain (1152-78) was labeled with ^{32}P in the DNA reassociation studies and hybridized with 14 biochemically similar strains of *G. sanguinis* (Table 2). The relative binding ratio at the optimal temperature (55°C) was 81% or greater and was 76% or greater at the stringent temperature (70°C), and the divergence was less than 3.0% for all *Globicatella* strains hybridized with the type strain. The three criteria for DNA-DNA relatedness at the species level include (i) relatedness greater than 70% under optimal conditions, (ii) relatedness greater than 60% under stringent conditions, and (iii) divergence less than 5% among the related sequences (29). Based on these criteria, these strains readily met the definition of species relatedness. In addition, the type strains of *E. avium* (SS817), *S. uberis* (SS842), and *A. viridans* (SS1251) were hybridized against *G. sanguinis* (1152-78) at the optimal temperature. The relative binding ratios were less than 18%, indicating that *Globicatella* are clearly genetically distinct from these phenotypically similar organisms.

Biochemical reactions. The biochemical reactions for the 28 *G. sanguinis* strains tested are shown in Table 3. All strains

TABLE 2. Relative reassociation DNA-DNA binding within *G. sanguinis*

Source of unlabeled DNA	Results with labeled DNA from *G. sanguinis* (1152-78)[a]		
	RBR at 55°C	% D	RBR at 70°C
G. sanguinis 1152-78^T (reference strain)	100	0.0	100
G. sanguinis 1104-78 (reference strain)	91	1.0	88
G. sanguinis 1713-79 (reference strain)	100	0.0	92
G. sanguinis 1434-83 (reference strain)	92	0.5	92
G. sanguinis 905-75	90	1.5	88
G. sanguinis 1040-80	90	1.0	90
G. sanguinis 399-83	92	1.5	88
G. sanguinis 925-83	86	2.5	81
G. sanguinis 1433-83	86	1.0	76
G. sanguinis 2434-91	88	2.0	83
G. sanguinis 2161-93	81	3.0	76
G. sanguinis 135-94	82	2.0	77
G. sanguinis 2504-96	87	1.5	85
G. sanguinis 162-97	90	1.5	88
G. sanguinis 1415-97	90	1.5	87
A. viridans SS1251^T	9	ND	ND
S. uberis SS842	8	ND	ND
E. avium SS817^T	18	ND	ND

[a] Calculations of %D assumed that a 1% decrease in thermal stability of a heterologous DNA duplex compared with that of the homologous duplex was caused by 1% unpaired bases. ND, not determined. RBR, or relative binding ratio, is determined by the following equation: (percent DNA bound to hydroxylapatite in heterologous reactions)/(percent DNA bound in homologous reactions) × 100.

were nonmotile, gram-positive ovoid cocci in short chains, produced alpha hemolysis on tryptic soy agar (TSA) supplemented with 5% defibrinated sheep blood, and were susceptible to vancomycin. All strains were positive for the hydrolysis of esculin and hippurate and growth in 6.5% NaCl. None of the strains produced gas in *Lactobacillus* Mann-Rogosa-Sharpe broth, produced LAP, or grew at 10°C. In contrast to the previous description of the type strain of the species (4), all strains hydrolyzed esculin in the presence of bile, the majority grew at 45°C, and all were negative for starch hydrolysis and were variable in the tellurite (0.4%) tolerance test. Biochemical testing of additional *G. sanguinis* strains (confirmed by DNA-DNA reassociation) revealed that the production of PYR and acid production from arabinose, ribose, and sorbitol are variable reactions for the species. As previously described, none of the strains produced acid from glycerol or sorbose, and all were negative in the reactions for urea, pyruvate, and Voges-Proskauer. Acid was produced from inulin, lactose, maltose, mannitol, melibiose, raffinose, sucrose, and trehelose as previously described.

This present study indicates that the original phenotypic description of *G. sanguinis* should be broadened for identification of this species. These variable phenotypic reactions make it even more difficult to distinguish *Globicatella* from other phenotypically similar bacteria (Table 3). The biochemical characteristics of the most recently identified strains of *A. viridans* (28 strains), *E. avium* (28 strains), and *S. uberis* (9 strains, all nonhuman) were compared to the 28 strains of *G. sanguinus*. *A. viridans* is biochemically very similar to *G. sanguinis*. The colony morphologies are very similar on TSA sup-

TABLE 3. Phenotypic differences between *G. sanguinis, A. viridans, S. uberis*, and *E. avium*[a]

Test	Results for:			
	G. sanguinis (28 strains)	*A. viridans* (28 strains)	*E. avium* (28 strains)	*S. uberis* (9 strains)
Gram stain	Pairs, short chains	clusters, tetrads	short chains	short chains
PYR	V (75)	+ (100)	+ (95)	+ (100)
LAP	− (0)	− (0)	+ (89)	+ (100)
Bile esculin	+ (100)	V (79)	+ (100)	− (0)
Growth in 6.5% NaCl	+ (100)	+ (96)	+ (100)	+ (90)
Growth at 10°C	− (0)	− (0)	V (68)	+ (90)
Growth at 45°C	+ (96)	V (25)	+ (100)	+ (90)
Esculin	+ (100)	V (82)	+ (100)	+ (100)
Hippurate	+ (100)	V (70)	V (30)	+ (100)
Pyruvate	− (0)	− (0)	+ (86)	− (0)
Tellurite	V (71)	V (20)	− (4)	− (0)
Acid production from:				
Arabinose	V (42)	− (14)	+ (100)	− (0)
Inulin	+ (93, 11% w+)	− (7)	− (7)	+ (100)
Lactose	+ (93, 11% w+)	V (54)	+ (100)	+ (100)
Maltose	+ (100, 19% w+)	V (50)	+ (95)	+ (100)
Mannitol	+ (96, 11% w+)	V (25)	+ (100)	+ (100)
Melibiose	+ (100, 19% w+)	V (22)	V (50)	V (30)
Raffinose	+ (100, 15% w+)	V (18)	− (0)	V (80)
Ribose	V (75, 19% w+)	V (13)	+ (94)	+ (90)
Sorbitol	V (82, 11% w+)	− (14)	+ (100)	+ (100)
Sorbose	− (0)	− (7)	+ (100)	− (0)
Sucrose	+ (100, 15% w+)	V (68)	+ (100, 4% w+)	+ (100)
Trehelose	+ (100, 15% w+)	V (54)	+ (100)	+ (100)

[a] +, 85% or more of the strains tested positive; −, 15% or less of the strains tested positive; V, variable reaction (16 to 84% of the strains tested positive). The first value in each parenthesis indicates the total number of strains giving positive reactions; where a second value is shown, it is the number of strains giving weakly positive (w+) reactions (values are percentages). Biochemicals were incubated at 37°C for 14 days.

TABLE 4. Comparison of rapid identification systems for *G. sanguinis*

Test kit	No. of tests[a]	No. positive[b]	No. negative[b]	No. variable[b]	Useful tests (%)[c]	No. of profiles	Proportion of correct IDs[d]	ID(s) by manufacturer (no. identified)
Cry24	29	8	7	14	52	20	8/28	*G. sanguinis* (8), *E. raffinosus* (1), *S. cricetus* (1), *S. bovis* (2), no ID (16)
Cry4	29	13	6	10	66	22	8/28	*A. viridans* (7), *E. avium* (1), *E. raffinosus* (12), no ID (8)
ID32	32	16	6	10	69	25	28/28	Unacceptable ID (28)
IDS	14	3	6	5	64	13	21/28	*S. mutans* (5), *E. casseliflavus* (1), *E. maloderatus* (1), no choice (21)

[a] Number of tests included in each kit.
[b] Number positive, ≥95% of strains tested positive; number negative, ≤5% strains tested positive; number variable, >5% strains tested positive and <95% of strains tested negative.
[c] Useful tests, the number of positive and negative tests divided by total number of tests, indicates the usefulness of the tests used for formulating an identification scheme for the species.
[d] ID, identification.

plemented with 5% sheep blood agar, and both are LAP negative and grow in 6.5% NaCl. The cellular arrangement from growth in broth can be used to differentiate the two, as *A. viridans* is arranged in clusters and tetrads and *G. sanguinis* is arranged in short chains. Fermentation of inulin can be a useful test, as the majority of *G. sanguinis* strains are positive and *A. viridans* strains are negative. The other key reactions for bile esculin, esculin, and hippurate are of limited value in separating these two species, since the PYR test yields a variable result for *G. sanguinis* and the bile esculin, esculin, and hippurate tests yield variable reactions for *A. viridans*.

The LAP test is also useful in distinguishing enterococci from *Globicatella* strains. *E. avium* is further identified by positive pyruvate and sorbose tests and negative inulin and raffinose tests. *G. sanguinis* has the reverse reactions. The two species are phenotypically similar in their reactions to PYR and bile esculin, their growth in 6.5% NaCl and at 45°C their hydrolysis of esculin and hippurate reactions, and their acid production from lactose, maltose, mannitol, sorbitol, sucrose, and trehelose.

S. uberis has been included in the identification scheme with viridans streptococci for a number of years (10, 24). The incorporation of the PYR and LAP tests in the identification scheme as well as molecular studies have confirmed that many of the strains that are now *Globicatella* were previously reported as *S. uberis*-like. The key test for differentiation of the streptococci from *Globicatella* is the positive LAP reaction. *S. uberis* is also negative for esculin hydrolysis in the presence of bile, and it is positive for growth at 10°C. The majority of strains for both species are PYR positive, grow in 6.5% NaCl, hydrolyze esculin and hippurate, and produce acid from inulin, lactose, maltose, mannitol, raffinose, ribose, sorbitol, sucrose, and trehelose. Acid is not produced from glycerol and sorbose for both species.

Identification of *G. sanguinis* using commercial systems. Of the four commercial identification systems, only Cry24 has *G. sanguinis* in its data bank. Therefore, the correct identifications should be *G. sanguinis* for the Cry24 system, "unacceptable identification" for the Cry4 and ID32 systems, and "no choice" for the IDS system. In each system the tests are scored to give a profile number. These profile numbers were transmitted to the manufacturer of each product, and their identification was reported back to us. No recommendation was given for the probability cutoff for an acceptable identification using the Cry24 and Cry4 systems. We considered any identification with a probability of less than 0.9 to be an unacceptable identification. A summary of these identifications is shown in Table 4. In each of the test systems, a test was considered positive when greater than 94% of the strains tested positive and were considered negative when less than 6% of the strains tested positive. A test was considered variable when greater than 5% strains tested positive and less than 95% of the strains tested negative.

Cry24. The Cry24 system is comprised of 29 different tests. The 28 strains of *G. sanguinis* generated 20 different profile numbers, with results from 8 of 29 tests yielding positive results, 7 of 29 yielding negative results, and 14 yielding variable results (Table 4). This correlates to 52% of the tests described as being useful in devising a profile. Using the manufacturers current database and our cutoff value of 0.9 probability, the Cry24 system identified 8 (29%) strains as *G. sanguinis*, 2 strains (7%) as *Streptococccus bovis*, 1 strain (4%) as *Streptococcus cricetus*, and 1 strain (4%) as *Enterococcus raffinosus*. The other 16 strains (57%) were not identified. The incorporation of one key test, the LAP reaction, would have excluded these misidentifications. The 16 other strains that were unidentified or identified with a probability of less than 0.9 could most likely be correctly identified with the incorporation of the additional profiles generated from our strains into their database. The fluorescent and colorimetric reactions in this panel as well as in the Cry4 system were somewhat difficult to interpret with these strains.

Cry4. The Cry4 system also contains 29 tests. Twenty-two different profile numbers were generated with this system, yielding 12 positive test results, 7 negative test results, and 10 variable test results. This correlates to 66% of the tests being useful in establishing a profile. The Cry4 system identified 12 strains (43%) as *E. raffinosus*, 7 strains (25%) as *A. viridans*, and 1 strain (4%) as *E. avium*. The profiles of the remaining 8 strains (29%) were "no identification" profiles or "low indeterminate" identifications. As mentioned earlier, the incorporation of the LAP reaction would eliminate the majority of enterococcus misidentifications. However, the seven profiles that showed a high match with *A. viridans* are more problematic. As we noted earlier, these two species are very similar at the genus level in tests using conventional biochemicals. The only conventional clear-cut test to distinguish these two species is the Gram stain. The cellular arrangement of *G. sanguinis* is pairs and short chains, whereas *Aerococcus* is arranged in clusters and tetrads.

ID32. The 28 strains of *G. sanguinis* tested with this kit generated 25 different profile numbers. All these profiles were

correctly identified as "unacceptable profile," shown in Table 4. Of the 32 tests in this panel, 16 were positive, 6 were negative, and 10 were variable. This kit showed the greatest potential for use, since there were 22 clear-cut test results resulting in 69% useful tests. The manufacturer should have to include only the profiles we generated in their database to identify this species. This kit was relatively easy to set up and interpret, although there was some difficulty in reading some of the colorimetric reactions with these strains.

IDS. The IDS system is comprised of 14 different tests. The 28 test strains generated 13 different profiles, with 3 positive, 6 negative, and 5 variable tests (Table 4). Sixty-four percent of the tests could be used in establishing a profile for the identification of *Globicatella*. Seven strains (25%) were misidentified as *S. mutans* (5 strains), *Enterococcus casseliflavus* (1 strain), or *Enterococcus malorderatus* (1 strain). *Globicatella* strains could be distinguished from these genera with the incorporation of the LAP reaction. As previously mentioned, *Globicatella* strains are LAP negative. Twenty-one strains (75%) gave inadequate profiles for identification. These results are promising, since the incorporation of the profiles generated and incorporation of the LAP reaction should identify the majority of strains.

With the exception of the ID32 system, it is apparent the *G. sanguinis* strains have the potential of being misidentified by using these rapid commercial systems. Incorporation of the generated profiles into the database of the ID32 system should identify the majority of strains of *G. sanguinis*. The products of manufacturers of the Cry24, Cry4, and IDS systems may require supplemental tests in order to obtain an accurate identification, since some strains gave identical profiles with phenotypically similar species. This updated description should improve the identification of *G. sanguinis*.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on 27 of the 28 *G. sanguinis* clinical isolates in the study. One strain (2434-91) was not viable in the medium, and MICs were not determined for this isolate. The MIC range and the MICs at which 50 and 90% of the isolates tested are inhibited of 12 antimicrobial agents are shown in Table 5. The NCCLS MIC interpretive standards for *Streptococcus* spp. (viridans) other than *S. pneumoniae* were used for penicillin, cefotaxime, meropenem, erythromycin, clindamycin, chloramphenicol, levofloxacin, and vancomycin (21). The NCCLS MIC interpretive standards for *S. pneumoniae* were used for amoxicillin, cefuroxime, and trimethoprim-sulfamethoxazole. Resistance varied significantly among the 12 agents assayed. All isolates were susceptible to 2 of the 12 antimicrobial agents, amoxicillin and vancomycin. The penicillin MICs for two of the isolates were in the intermediate susceptibility range as defined by NCCLS (21). Of isolates tested with the remaining antimicrobial agents, 48% were resistant to cefotaxime, 74% were resistant to cefuroxime, 37% were resistant to meropenem, 48% were resistant to erythromycin, 52% were resistant to trimethoprim-sulfamethoxazole, 30% were resistant to clindamycin, and 52% were resistant to vancomycin. Twenty-six of the isolates were readily susceptible to levofloxacin; only one isolate was resistant. This strain was a blood isolate and showed resistance upon repeat testing. Four percent of the strains were resistant to chloramphenicol; however, 15% were intermediately resistant. Similar susceptibility patterns have been shown in the *Facklamia* sp. and viridans streptococci in recent studies (23, 26).

TABLE 5. In vitro susceptibilities of 27 clinical isolates of *G. sanguinis*

Antimicrobial agent	MIC (μg/ml)[a]			Resistance	
	Range	50%	90%	MIC[b]	%
Penicillin	≤0.03–0.50	0.06	0.12	≥4.0	0
Amoxicillin	≤0.03–0.03	≤0.03	≤0.03	≥8.0[c]	0
Cefotaxime	0.06–4.0	1.0	4.0	≥2.0	48
Cefuroxime	0.12–16.0	2.0	8.0	≥2.0[c]	74
Meropenem	0.06–2.0	0.5	1.0	>0.5	37
Erythromycin	0.06–≥16.0	0.5	>16.0	≥1.0	48
Trimethoprim-sulfamethoxazole	0.12–≥8.0	8.0	>8.0	≥4.0[c]	52
Clindamycin	0.06–2.0	0.5	1.0	≥1.0	30
Chloramphenicol	2.0–16.0	4.0	8.0	≥16.0	4
Levofloxacin	0.50–8.0	≤0.5	≤0.5	≥8.0	4
Vancomycin	≤0.12	≤0.12	≤0.12	>1.0	0
Tetracycline	1.0–≥8.0	>8.0	>8.0	≥8.0	52

[a] Range of MICs determined from isolates; 50% and 90%, MICs at which 50% and 90% of isolates, respectively, were inhibited.
[b] NCCLS MIC interpretive standards for *Streptococcus* spp. (viridans) other than *S. pneumoniae* were used unless otherwise indicated.
[c] NCCLS interpretive standards for *S. pneumoniae* were used.

G. sanguinis isolates are clinically significant microorganisms. Based on colonial morphology on 5% sheep blood agar, they are easily confused with viridans streptococci but are clearly distinguished by the negative LAP reaction and growth in 6.5% NaCl. The negative LAP reaction also separates the genus from the enterococci. This genus is very difficult to distinguish from the aerococci. The acidification of inulin and the Gram stain (the only clear-cut test) are most useful. The aerococci are arranged in tetrads and clusters and are inulin negative. *G. sanguinis* is arranged in pairs and short chains and is inulin positive. With the increasing incidence of drug-resistant microorganisms, accurate identification and susceptibility testing is becoming more important (8). Previous studies of antimicrobial susceptibilities of viridans streptococci may have included some misidentified *G. sanguinis* strains. Since this bacterium shows some intermediate resistance to penicillin, it is important to accurately identify and monitor drug resistance.

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