What Happened to the Streptococci: Overview of Taxonomic and Nomenclature Changes

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INTRODUCTION

The purpose of this communication is to outline the changes in taxonomy and nomenclature of the *Streptococcus* genus that have occurred in the past 15 years. These changes are the result of the application of DNA-DNA reassociation, 16S rDNA gene sequencing, and other molecular techniques that help delineate differences in bacterial genera and species. The 1984 *Bergey's Manual of Systemic Bacteriology* listed only seven genera of facultatively anaerobic gram-positive cocci (GPC); *Aerococcus, Leuconostoc, Micrococcus, Pediococcus, Staphylococcus, Streptococcus*, and *Stomatococcus* (108). At present there are 17 different genera of GPC. The discussion is limited to the *Streptococcus* genus and closely related GPC that are catalase negative and display chains in the Gram stain. Technically, *Leuconostoc* bacteria fit into this category, but discussions on changes in this genus are not included, because iden-

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tification procedures and clinical relevance of the leuconostocs can be found in a review written in 1995 (52). Shortly before the publication of *Bergey's Manual* in 1986, the genus *Streptococcus* was split into three genera (*Enterococcus, Lactococcus,* and *Streptococcus*) (110, 111). Changes in the *Enterococcus* and *Lactococcus* genera are not detailed, but some changes are included to explain changes in the *Streptococcus* genus. Many new species of streptococci have been added to the genus, and six new genera of GPC that form chains have been established (*Abiotrophia* [35], *Granulicatella* [35], *Dolosicoccus* [34], *Facklamia* [29], *Globicatella* [28], and *Ignavigranum* [36]). The majority of these genera were split off the *Streptococcus* genus by genetic and phenotypic information.

The earliest attempt at differentiating the streptococci was probably made in 1903 by Shottmuller (118), who used blood agar to differentiate strains that were beta-hemolytic from those that were not. Before 1933, fermentation and tolerance tests were the only tests used for differentiating many of the streptococci. In 1933 Lancefield reported the technique of demonstrating specific carbohydrate "group" antigens associated with the beta-hemolytic strains (88). In 1937, Sherman proposed a scheme for placing the streptococci into four categories. These categories were organized by hemolytic reaction, group carbohydrate antigens, and phenotypic tests (primarily fermentation and tolerance tests) (116). Sherman's four divisions were the pyogenic division, the viridans division, the lactic division, and the enterococci. The pyogenic division included the beta-hemolytic strains with defined group antigens (A, B, C, E, F, and G). This division of the streptococci is not appreciably different from that of today's identification systems based on serogrouping. Sherman's viridans division included streptococcal species that were not beta-hemolytic, were not tolerant to high-pH growth conditions, were not salt tolerant, and did not grow at 10°C. This group is still known today as the viridans streptococci, and many more species have been added to this classification. Sherman's lactic division included strains that were associated primarily with the manufacture of dairy products. They were not associated with human infections. This group differed from the pyogenic group by not being beta-hemolytic, by having the capacity to grow at 10°C but not at 45°C, and by failing to grow in broth containing 6.5% NaCl. Sherman's lactic division was reclassified as the *Lactococcus* genus in the mid-1980s. *Lactococcus* species that have recently been isolated from human infections have phenotypic characteristics that are not the same as those described by Sherman (48, 54). Sherman's fourth division was termed the enterococci and included the four species known at that time. Although some of the enterococci were beta-hemolytic, other characteristics such as the capacity to grow in broths at high pH, high salt concentrations, and a wide temperature range (10 to 45°C) differentiated them from the other three divisions. The number of *Enterococcus* species has increased to more than 20. All of the new species have phenotypic characteristics similar to those described by Sherman (54).

The classification and identification of streptococci was severely hampered by a hierarchical dichotomous approach relying on a very limited number of complex characters (colony size, hemolysis, and group carbohydrate antigens) that resulted in species definitions that are often qualified by a number of

exceptions. Moreover, because these organisms grow slowly and may require additional factors for isolation and characterization, as well as the somewhat cumbersome classification system, identification of streptococci to the species level is rarely performed in time to be relevant to the treatment of the patient from whom the organism was isolated. One of the most useful tools applied to the revision of the classification system for the *Streptococcus* genus is the application of 16S rRNA gene sequencing. Figure 1 depicts a phylogenetic tree of the currently available sequences for the species included in the *Streptococcus* genus. I have chosen to present the identification schemes listed in the following tables based strictly on phenotypic characteristics because I feel that the clinical microbiologist can easily place the species into groups that are phenotypically related with only a few microbiologic tests. These phenotypic groups do not necessarily correlate with the groups shown in the genetic tree in Fig. 1.

BETA-HEMOLYTIC STREPTOCOCCI

For clinical laboratories as well as taxonomists, one of the most useful phenotypic characteristics of streptococci is the reaction of the bacteria on blood agar plates. Hemolysis is used as a guide for managing patients as well as an aid in classification of the bacterium to the species level. J. H. Brown in 1919 first defined the reactions of streptococci on blood agar plates (23). This monograph is no longer available, but Brown's definitions are accurately shown in reference 122. It is very important that clinical and physician office laboratories accurately identify the beta-hemolytic reactions of the streptococci.

Table 1 lists all beta-hemolytic streptococci known to date. There is one exception included in this table; *S. dysgalactiae* subsp. *dysgalactiae* is not beta-hemolytic but is included for taxonomic reasons. Column 1 in Table 1 lists all the species and subspecies of beta-hemolytic streptococci. Column 2 lists the Lancefield group antigens that are associated with each species. Columns 3 to 12 list 10 phenotypic characteristics that can be used to help identify the streptococci to the species level when hemolysis and identification of group carbohydrate antigen fail to do so. The last column gives the most common natural host of the species. With the exception of *S. dysgalactiae* subsp. *dysgalactiae, S. equi* subsp. *equi,* and two recently described species, *S. phocae* (119) and *S. didelphis* (106), all the species listed in Table 1 have been isolated from human infections. Table 1 was constructed based on the molecular and phenotypic characteristics described by Schleifer and Kilpper-Balz (111), Vandamme et al. (131), Vieira et al. (136), and our own unpublished results of testing all reference strains for each of the species. Investigators used a variety of techniques including DNA-DNA reassociation, 16S rDNA sequencing, whole-cell protein analysis, multilocus enzyme electrophoresis, and phenotypic characteristics to help establish the species and subspecies included in this table.

Streptococcus pyogenes

S. pyogenes is also known as beta-hemolytic group A streptococcus or Lancefield's group A strep (GAS). It is the most pathogenic bacterium in the genus *Streptococcus.* All clinicians should be aware that GAS is the agent that causes bacterial
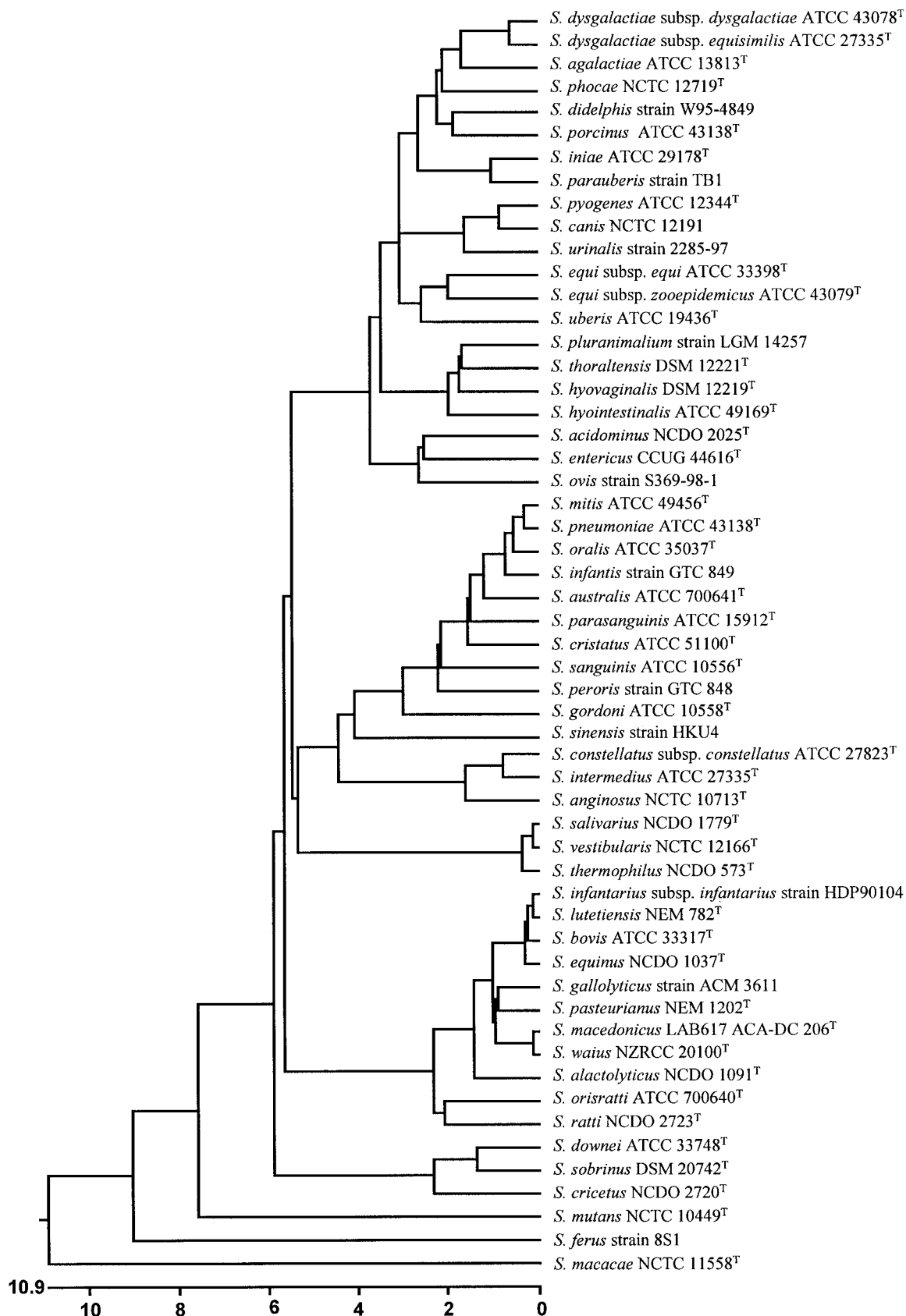
FIG. 1. Phylogenetic relationship among 55 *Streptococcus* species based on analysis of 16S rRNA gene sequences. The dendrogram was constructed by the clustal method using the DNASTAR program. The units at the bottom of the tree indicate distance between sequence pairs.

TABLE 1. Identification of the beta-hemolytic streptococci[a]

Species	Lancefield group	Bac	PYR	Cam	VP	Hip	Arg	Esc	Str	Sbl	Tre	Rib	Origin
S. pyogenes	A	+	+	−	−	−	+	v	−	−	NA	−	Human
S. agalactiae	B	−	−	+	−	+	+	−	−	−	NA	NA	Human, bovine
S. dysgalactiae													
subsp. *dysgalactiae*[b]	C	−	−	−	−	−	+	v	−	v	+	+	Animals
subsp. *equisimilis*[c]	A, C, G, L	−	−	−	−	−	+	+	−	−	+	+	Human, animals
S. equi													
subsp. *equi*	C	−	−	−	−	−	+	v	+	−	−	NA	Animals
subsp. *zooepidemicus*	C	−	−	−	−	−	+	v	+	+	v	NA	Animals, human
S. canis[c]	G	−	−	+	−	−	+	+	−	−	v	NA	Dog, human
S. anginosus (group)[d]	A, C, G, F, None	−	−	−	+	−	+	+	−	−	+	NA	Human
S. constellatus subsp. *pharyngis*	C	−	−	−	+	−	+	+	−	−	+	NA	Human
S. porcinus	E, P, U, V, none, new	−	+	+	+	v	+	+	−	+	+	NA	Swine, human
S. iniae	None	−	+	+	−	−	−	+	+	−	NA	NA	Dolphin, fish, human
S. phocae	C, F	+	−	−	−	−	−	−	−	−	NA	NA	Seal
S. didelphis	None	−	−	−	−	−	+	−	−	−	+	NA	Opossum

[a] Abbreviations: Group, group carbohydrate antigen; Bac, S, susceptible, R, resistant to bacitracin; Pyr, pyrrolidonylarylamidase; Cam, CAMP reaction; VP, Voges-Proskauer reaction; Hip, hydrolysis of hippurate; Arg, deamination of arginine; Esc, hydrolysis of esculin; Str, hydrolysis of starch; Sbl, Tre, and Rib, production of acid in sorbitol, trehalose, and ribose broth, respectively. +, positive reaction >95%; −, negative reaction >95%; v, variable reaction 6 to 94% positive; NA, not applicable.

[b] *S. dysgalactiae* subsp. *dysgalactiae* strains are not beta-hemolytic but are included in this table for taxomonic reasons.

[c] To differentiate between the group G *S. canis* and group G *S. dysgalactiae* subsp. *equisimilis*, testing of α-galactosidase, β-galactosidase, and β-glucoronidase needs to be performed. *S. canis* is positive for α- and β-galactosidase and negative for β-glucoronidase; *S. dysgalactiae* subsp. *equisimilis* gives the opposite reactions.

[d] The *S. anginosus* group includes beta-hemolytic strains of *S. anginosus*, *S. constellatus*, and *S. intermedius*. There are insufficient data to know the percentage of each of these beta-hemolytic species that contain carbohydrate antigens.

pharyngitis, impetigo, and a host of other infections including severe invasive diseases. The recent review by Cunningham is an excellent source of information for interested investigators to familiarize themselves with the severity and diversity of diseases caused by this bacterium (39). The major (but not exclusive) virulence factor associated with GAS is the M-protein antigen. This surface antigen is the antigen that allows the GAS to avoid phagocytosis and to survive in the human host. The hypervariable N-terminal portion of this protein dictates the type specificity of each antigen. Lancefield prepared type-specific antisera to many of these antigens and used the antisera in a capillary precipitin test to subtype the GAS (87). This system is still in use in some laboratories after more than 60 years. In recent years, an alternative system called *emm* typing has been developed, which uses the sequence of the gene that encodes the M-protein hyperviable region. Correlation between the serologic and *emm* typing systems is very good, and in most cases the *emm* type reflects the M-protein serologic type. The *emm* typing system can be accessed through the CDC web site http://www.cdc.gov/ncidod/biotech/infotech_hp.html. Methods for *emm* typing and the sequences of more than 120 *emm*-types of GAS are described in references 7 and 8.

From Table 1 the reader can determine that *S. pyogenes* is not the only *Streptococcus* that may possess the group A antigen. The true incidence of non-*S. pyogenes* GAS strains found in human infections is unknown, but from the information available to us at the Centers for Disease Control and Prevention (CDC) Streptococcus laboratory, these strains are not common. More information regarding the two species *S. dysgalactiae* subsp. *equisimilis* and *S. anginosus* is given below where these two species are discussed. *S. pyogenes* is best identified by demonstration of the group A antigen on the cell. Presumptive identification can be made by bacitracin susceptibility or pyrrolidonylarylamidase activity. *S. pyogenes* strains

are the only beta-hemolytic streptococci that are positive in both of these tests. Some of the other beta-hemolytic streptococci can also be positive in one or the other, but not both, of these tests; therefore, these tests alone are not 100% specific for *S. pyogenes* (Table 1).

Streptococcus agalactiae

S. agalactiae, or Lancefield's group B streptococcus (GBS) is the most common cause of neonatal sepsis (113). Reference 113 is an excellent starting point for those interested in learning more about the epidemiology and types of diseases caused by this organism. Two major avenues of investigation have been explored for the prevention of GBS disease: the development of vaccines (115) and the screening procedures for the presence of GBS in anal, cervical or vaginal carriers and subsequent antimicrobial management (112). *S. agalactiae* is the only *Streptococcus* species that has the group B antigen. Some other streptococcal species have recently been identified however, that cross-react with commercial slide agglutination tests (see the discussion of *S. porcinus*, below). GBS can also be presumptively identified by the CAMP and hippurate reactions. Together with the unique hemolytic reaction (very small zone of lysis), these two presumptive tests are very accurate in the identification.

Streptococcus dysgalactiae subsp. *dysgalactiae*

The exact composition of the taxon *Streptococcus dysgalactiae* has been in a state of flux for the past few years. *S. dysgalactiae* subsp. *dysgalactiae* is the only species listed on Table 1 that is not beta-hemolytic. This species is included because of the other subspecies included in this discussion. Isolation of this bacterium from human infections has not been

documented. The organism has virulence factors similar to those of *S. pyogenes*, including M-like proteins (133). Identification by determining the characteristics in Table 1 should be considered presumptive. Determining that an alpha-hemolytic streptococcus has group C antigen is insufficient for identification; other non-beta-hemolytic streptococci, e.g., viridans streptococcal species, may also have group C antigen (50). Additional phenotypic or genetic characteristics should be used to identify this bacterium (61).

Streptococcus dysgalactiae subsp. equisimilis

S. dysgalactiae subsp. *equisimilis* is the revised taxonomic epithet for what was previously termed *S. equisimilis* or Lancefield's group C *Streptococcus*. Genetic investigations indicated that Lancefield's group C strain (also known as group C human strain or *S. equisimilis*), Lancefield's group G and L strains, and the species known as *S. dysgalactiae* were all genetically similar and should be included in one taxon. Since *S. dysgalactiae* was the oldest officially recognized species, all of these entities were placed in the *S. dysgalactiae* classification (55, 80). Later investigations (131, 136) indicated that Lancefield group C strain (*S. equisimilis*) and Lancefield's group G and L strains should be grouped into one category and that *S. dysgalactiae* should be placed in different category, thus creating two separate subspecies, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis.* More recently, investigators have shown that some strains of *S. dysgalactiae* subsp. *equisimilis* possess the group A antigen (15, 20). Group A, C, G, and L *S. dysgalactiae* subsp. *equisimilis* strains are found in human infections. The true incidence figures are difficult to estimate but in our experience at the CDC, group C and G strains are found much more commonly in human infections than are group A and L strains of this species. Like *S. dysgalactiae* subsp. *dysgalactiae*, the strains possessing different group antigens of *S. dysgalactiae* subsp. *equisimilis* also have virulence factors similar to *S. pyogenes*, including *emm* gene homologs (17, 27) (see the CDC web site above).

The group antigen can be used only as an aid in species identification. The phenotypic tests in Table 1 should be used, together with hemolytic reaction and group antigen, to identify the species possessing Lancefield's group C antigen. A total of six different beta-hemolytic streptococcal species or subspecies can have the group C antigen.

Streptococcus equi subsp. equi

Streptococcus equi subsp. *equi* is a beta-hemolytic group C *Streptococcus* that causes strangles in horses. To my knowledge, this species has not been isolated from humans. *S. equi* subsp. *equi* has a protein that induces opsonic antibodies in horses and is thought to be involved in the organism's virulence (125). Identification of this subspecies is based on the hemolytic reaction, demonstration of the group C antigen, differences in hydrolysis of esculin and starch, and fermentation of sorbitol and trehalose (Table 1).

Streptococcus equi subsp. zooepidemicus

Unlike *S. equi* subsp. *equi, S. equi* subsp. *zooepidemicus* is found in human infections. Most recently, this organism was associated with a large outbreak of nephritis in Brazil (5, 94). The publication by Nicholson et al. (94) provides references to previous nephritis outbreaks and other infections caused by this bacterium. Most human infections can be traced back to the consumption of contaminated dairy products. This organism is a frequent cause of bovine mastitis. *S. equi* subsp. *zooepidemicus* has a surface-exposed protein (Szp) that is antigenically variable, stimulates opsonic protective antibodies, and shares certain structural features with the *S. pyogenes* M protein (94). Although this protein does not have significant sequence homology to the M protein, it is tempting to speculate that Szp also plays an antiphagocytic role. *S. equi* subsp. *zooepidemicus* is identified by beta-hemolysis, group C antigen presence, hydrolysis of esculin (70% of strains) and starch, fermentation of sorbitol, and occasional fermentation of trehalose.

Streptococcus canis

S. canis is an organism that was described in 1986 as having the Lancefield group G antigen and was isolated from animals, most frequently dogs (hence the name "*canis*") (41). Extensive phenotypic testing of isolates from dogs was described in 1994 (135), and the isolation of *S. canis* from a human with sepsis was reported in 1997 (16). It is not possible to estimate how frequently *S. canis* is isolated from humans, because most clinical laboratories do not perform phenotypic tests on group G streptococci other than the hemolytic reaction and group determination. The phenotypic profile given in Table 1 for the identification of *S. canis* is that of nonhuman strains. It is not known whether human strains will have the same phenotypic characteristics.

Streptococcus anginosus Group

None of the beta-hemolytic streptococci has caused more confusion about terminology and classification than the group of organisms listed as *S. anginosus* (Table 1). There is no doubt that this group includes three distinct species and more subspecies. *S. anginosus, S. constellatus*, and *S. intermedius* were all collectively known as either *S. anginosus* or *S. milleri* at one time. The problem with the term "*S. milleri*" was that it was never accepted by the taxonomist as a confirmed taxonomic entity. DNA-DNA reassociation studies clearly show that the aforementioned species are distinct taxons (83, 142). There are beta-hemolytic strains of each of the three species, and the strains may possess one of four different Lancefield group antigens or no group antigen. Adding to the confusion is the fact that non-beta-hemolytic strains of the three species are more common than beta-hemolytic strains. Non-beta-hemolytic varieties of each of the three species are grouped into the general classification of viridans streptococci. Whiley et al. have proposed an identification scheme based on degradation of chromogenic substrates for the differentiation of the three species (139, 142). In addition, although these authors state that hemolysis and group antigens are of little value in differentiation of the species, more isolates of *S. constellatus* tend to be beta-hemolytic than of either *S. anginosus* or *S. intermedius.* Isolates of *S. intermedius* are rarely beta-hemolytic (139, 141, 142, 144). These authors also state that there tends to be an

association between the clinical sources and the three species. *S. anginosus* isolates are commonly isolated from urogenital and gastrointestinal sources, *S. constellatus* is often isolated from respiratory and many other sources, while *S. intermedius* strains are commonly identified from brain and liver abscesses. The distribution of the group antigens also shows some association with the species. Isolates of *S. intermedius* rarely have group antigens, while isolates of *S. anginosus* and *S. constellatus* often have group F, C, A, and G antigens (in order of frequency). Other investigators have also found similar distributions of the three species using Whiley's scheme and DNA reassociation as reference identification procedures (121). Very little is known about virulence factors produced by this group of bacteria. Two enzymes that may be considered virulence factors are α-N-acetylneuramidase (sialidase) and hyaluronidase; *S. intermedius* produces both of these enzymes, *S. constellatus* produces only hyaluronidase, and *S. anginosus* produces neither. The production of these enzymes is part of the identification scheme proposed by Whiley et al. (139, 141). Identification of the beta-hemolytic as well as non-beta-hemolytic species are given in Table 2. Whiley's scheme (139, 141) is considered a standard for phenotypic identification of the three species and potential subspecies. Table 2 has been constructed from the data presented in references 1, 14, 70, 71, 72, 121, 139, 141, 142, 144, and 145. The hemolytic reaction and Lancefield group antigens that are commonly associated with the three species are also included. Subspecies are also included. Column 2 under each of the species listed in Table 2 represents the original Whiley description of the species; column 1 under each of the species represents a beta-hemolytic variant of each species; and column 3 represents an official or unofficial subspecies designation of that particular species. This table should be used as a guide for identification of the species, and confirmation of subspecies may require additional molecular documentation. All the species and subspecies listed in this table should have the phenotypic characteristics of the *S. anginosus* group listed in Table 1. Column 1 under *S. anginosus* is the description of the group of strains identified but not proposed for subspecies status by Whiley et al. (145). Column 3 under *S. anginosus* is the description of the "motile *S. milleri*" strains (14, 70). Additional variants of *S. anginosus* have been described based on ribotypes, some of which correlate with the entities listed in Table 2 (71). Commercial identification systems that have three of the seven chromogenic substrates suggested by Whiley et al. are available. The Fluo-Card Milleri (Key Scientific, Round Rock, Tex.) includes β-D-fucosidase, β-glucosidase, and α-glucosidase (58). Compared to the Whiley scheme, this system identified 98% of *S. anginosus* strains, 97% of *S. constellatus* strains, and 88% of *S. intermedius* strains. The Rapid ID-32 Strep system (Bio-Mérieux, Marcy l'Etoile, France) (not available for clinical microbiology use in the United States) includes N-acetyl-β-glucosaminidase, β-glucosidase, and β-galactosidase (63). These authors did not use a reference method to compare their identification, and so all 70 strains in their study were placed into one of the three species. Limia et al. (90) evaluated the Rapid ID-32 Strep system as well as the Whiley system against a line blot hybridization assay. There was an 80% agreement between the genotypic method and Whiley's method. The agreement between the genotypic method and the Rapid

TABLE 2. Identification of the *S. anginosus* group[a]

Test	*S. anginosus*			*S. constellatus*				*S. intermedius*	
				subsp. constellatus			*subsp. pharyngis*		
Hemolysis	Beta-hemolytic	Non-beta-hemolytic	Non-beta-hemolytic[b]	Beta-hemolytic	Non-beta-hemolytic	Beta-hemolytic	Beta-hemolytic	Beta-hemolytic	Non-beta-hemolytic
Lancefield antigen[c]	C, F, G, N	A, C, F, G, N	F, G, N	F, N	F, N	F, N	C	N	N
β-D-Fucosidase	−	−	−	−	−	−	+	+	+
β-N-Acetylglucosaminidase	v	−	−	−	−	−	+	+	+
β-N-Acetylgalactosaminidase	−	−	−	−	−	−	−	+	+
α-N-Acetylneuramidase	−	−	−	−	−	−	−	+	+
β-Galactosidase	+	+	+	+	+	+	+	+	+
β-Glucosidase	−	v	−	+	+	+	+	>	>
α-Glucosidase	−	−	+	−	>	>	>	+	+
Hyaluronidase	−	−	−	+	>	+	+	+	+
Amygdalin (acidification)	+	+	+	>	>	+	+	>	>
Lactose (acidification)	+	−	+	>	>	+	+	+	+
Mannitol (acidification)	−	−	+	−	−	−	−	−	−
Raffinose (acidification)	−	−	+	−	−	−	−	−	−

[a] Data from references 14, 65–67, and 129–135. +, positive reactions ≥92% of strains; −, positive reaction ≤8% of strains; v, variable reactions positive in 8 to 91% of strains.
[b] Motile variant described in reference 14.
[c] N, no antigen.

ID-32 Strep system was 76%. The latter system was particularly inaccurate, with identification of only 57% of the *S. intermedius* strains. There have been reports that some of the problems with the Rapid ID-32 system is with preparation of the inoculum density and growth conditions (1). A third commercial system available for identification of the three species is the Becton Dickinson Microbiology Crystal Gram-Positive system (137). This system has β-*N*-fucosidase, β-glucosidase, and α-glucosidase. Very little information is available about the utility of this system. Molecular technology for specific identification of these species has been described. Pulsed-field gel electrophoresis (6), sequencing of specific genes (98) and 16S rRNA genes (12), and species-specific probes (70) have all been described. In summary, we know that beta-hemolytic strains of all species have been isolated from human infections (with and without various Lancefield antigens), but we know very little about the incidence and clinical significance of each of the redefined species and subspecies.

Streptococcus constellatus subsp. pharyngis

Whiley et al. have recently described a subspecies of *S. constellatus* that is beta-hemolytic and carries Lancefield's group C antigen (145). These beta-hemolytic group C streptococci have a predelection for the human throat and cause pharyngitis. Although still within the parameters of the criteria for inclusion in the species, the DNA-DNA reassociation percentages are different for the subspecies. Phenotypic differences in the chromogenic substrate degradation are also different for *S. constellatus* subspp. *pharyngis* and *constellatus*. A second group of beta-hemolytic group C and one strain of group G streptococci were also described in this report but not formally proposed as a subspecies (see column 1 of Table 2 under *S. anginosus*). This group was closely related to *S. anginosus* and was not necessarily associated with pharyngeal infections. The reactions listed in Table 2 in column 3 under *S. constellatus* subsp. *pharyngis* lists the tests to identify the subspecies.

Streptococcus porcinus

The beta-hemolytic streptococci that carry Lancefield group E, P, U, and V and four new antigens were included in a description of *S. porcinus* (30). *S. porcinus* has been isolated from the human genitourinary tracts of female patients of reproductive age (53). The incidence and the importance of this finding are inconclusive and are complicated by the fact that most human isolates of *S. porcinus* react with commercially prepared GBS grouping antisera (124). Slide agglutination group B reagent has also been reported to react with nonhuman strains (67). Lancefield extracts of these strains generally do not react with CDC group B antiserum (124). Most strains isolated from human sources react with antiserum prepared against one of the new group antigens. Careful observation of the hemolytic reaction can be a useful indicator of *S. porcinus*, and clinical microbiologists should be aware of the potential for misidentification of these isolates as *S. agalactiae*. Typical strains of *S. agalactiae* lyse red blood cells weakly, and colonies of GBS are surrounded by a small zone of lysis. On the other hand, typical strains of *S. porcinus* lyse red blood cells

in an agar plate in a much larger area around colony growth (124).

Streptococcus iniae

The original cultures of *S. iniae* were isolated from freshwater dolphins. *S. iniae* is also found in aquacultures of fish and can be transmitted to humans via the fish (138). An outbreak of bacteremic disease in humans who handle contaminated fish has been reported (138), but sporadic reports of this organism have been rare at the CDC. Beta-hemolytic streptococci without group antigen should be tested for the phenotypic characteristics listed in Table 1 for definitive identification.

Streptococcus phocae and Streptococcus didelphis

As noted above, *S. phocae* and *S. didelphis* have not been confirmed as being isolated from humans. *S. phocae* has been isolated from seals (119), and *S. didelphis* has been isolated from opossums (106). The data for identification of these two beta-hemolytic streptococci were obtained from these published reports and our own testing of the type strains for these two species (Table 1).

NON-BETA-HEMOLYTIC STREPTOCOCCI

As stated above, the determination of hemolysis is one of the most useful characteristics for the identification of streptococci. Nonhemolytic variants of *S. pyogenes, S. agalactiae*, and members of the *S. anginosus* group are well documented. Whether there are nonhemolytic variants of the other streptococcal species that are normally beta-hemolytic remains to be determined. It is not useful to try to distinguish between alpha-hemolysis and no hemolysis on blood agar plates. The composition of the medium including the type of blood and incubation atmospheres can influence whether "alpha" hemolysis occurs. There is no documented enzyme or toxin that affects red blood cells to produce alpha-hemolysis by streptococci. The greening or partial destruction of red blood cells is produced primarily by the production of hydrogen peroxide by the streptococci. The peroxide destroys some of the red blood cells and releases hemoglobin into the medium surrounding the streptococcal colony that appears green-like. If oxygen is removed from the growth atmosphere, peroxide is not formed, and cultures that appeared "alpha-hemolytic" will be nonhemolytic. The value of identification of Lancefield antigens on non-beta-hemolytic streptococci is also limited. While determination of the group D antigen for identification of *S. bovis* and determination of group R or other type antigens for *S. suis* are useful, determination of Lancefield antigens of all other non-beta-hemolytic streptococcal species, including the viridans streptococci, is of very little value for identification (50).

Streptococcus pneumoniae

No changes in the classification have been made in the past few years for *S. pneumoniae*. This organism is still the leading cause of community-acquired pneumoniae, and the increasing prevalence of multidrug resistance is of great concern (147). Major developments in the formulation of vaccines for adults

TABLE 3. Identification of non-beta-hemolytic gram-positive cocci in chains[a]

Species or group	Antigen	Opt	BS	BE	Na	Pyr	Esc	Vp	Man	Mel	Sbl	Tre	St	Dx	Origin
S. pneumoniae	pn	+	+	−	−	−	v	−	−	+	−	v	−	−	Human
S. equinus (*S. bovis*)	D	−	−	+	−	−	+	+	−	−	−	v	−	−	Equine, bovine
S. gallolyticus (*S. bovis* I)	D	−	−	+	−	−	+	+	+	+	−	+	+	+	Human, koala bovine
S. pasteurianus (*S. bovis* II.2)	D	−	−	+	−	−	+	+	−	+	−	+	−	−	
S. infantarius (*S. bovis* II/1)	D(v)	−	−	−	−	−	v	+	−	+	−	−	+	−	Human, bovine
S. lutetiensis	D(v)	−	−	+	−	−	+	+	−	−	−	−	v	−	
S. suis[b]	Type 1–35 (R,S,T)	−	−	−	−	−	+	−	−	−	−	+	+	−	Swine, human
Viridans streptococci	A, C, G, F, none	−	−	−	−	−	v	v	v	v	v	v	v	v	Human
Other streptococci and genera	Unknown	−	−	v	v	v	v	v	v	v	v	v	v	v	Animal, human

[a] Abbreviations: pn, pneumococcal typing antiserum or Omni serum; letters, Lancefield group antigen; Opt, optochin; BS, bile solubility; BE, bile-esculin reaction; Na, growth in 6.5% NaCl broth; Pyr, pyrrolidonylarylamidase reaction; Esc, hyrolysis of esculin; Vp, Voges-Proskauer reaction; Man, Mel, Sbl, Tre, acidification of mannitol, melibiose, sorbitol, and trehalose broths; respectively; St, hydrolysis of starch; Dx, production of extracellular polysaccharide. See footnote in Table 2 for positive and negative reactions.

[b] Note that the only way to differentiate *S. suis* from viridans streptococci is by serologic typing.

(114) and children (18) are encouraging for control of pneumococcal infections. Isolation and identification of *S. pneumoniae* is still problematic since conventional culture techniques are far from perfect and identification of some strains is refractory. Phenotypic physiologic tests place *S. pneumoniae* in the viridans *Streptococcus, S. mitis* group. Ideally, *S. pneumoniae* is identified by a serologic technique such as omni serum or a slide agglutination test (95) or typing serum (120). Under most circumstances, if serologic techniques are not used, identification can be accomplished by determining susceptibility to optochin and/or bile solubility. Recent molecular techniques, such as PCR tests for targeted proteins, pneumolysin (59), and penicillin binding protein genes (9, 126), have been shown to improve the identification of culture-negative cases of pneumonia and identification of strains.

Streptococcus bovis Group: *S. bovis, S. equinus, S. gallolyticus, S. infantarius, S. pasteurianus, S. lutetiensis*

Table 3 lists the streptococcal species included in this section. DNA-DNA reassociation experiments have clarified the taxonomic classification of this group of streptococci, but the nomenclature of these species is confusing and subject to debate. The reasons for the changes listed in Table 3 for the *S. bovis* group begin with a report published in 1984 (56). These investigators examined a collection of strains, most of which were nonhuman isolates, and reported that the phenotypically described type strains of *S. bovis* and *S. equinus* were a single DNA group, i.e., the same species. Farrow et al. (56) identified seven different DNA groups in their study. These DNA groups are referred to throughout this section. Other investigators performed DNA reassociation studies on human isolates termed *S. bovis* biotype I and biotype II (the latter is sometimes referred to as *S. bovis* variant) and discovered that neither biotype I nor biotype II of human origin was sufficiently closely related to the reference type strains of the *S. bovis-S. equinus* DNA group to be included in the newly described species (38, 84). These investigators reported that the *S. bovis* biotype II isolates of human origin could be divided into two groups based on phenotypic characteristics determined in the

Rapid Strep system. These two groups were called *S. bovis* II/1 and *S. bovis* II/2. The investigators also reported that the human isolates of *S. bovis* II/2 were closely related to the *S. bovis* I strains by DNA reassociation studies.

Osawa et al. (96) demonstrated that strains isolated from koalas, dogs, cows, and other animals belonged to the Farrow et al. DNA group 2 (56) and proposed that these strains be called *S. gallolyticus* based on gallate-degrading capacity. These investigators also included isolates of *S. bovis* I and II from human infections in their studies. The human isolates of *S. bovis* I and II/2 joined the *S. gallolyticus* species based on DNA reassociation studies. Further studies (45) confirmed that no human strains called *S. bovis* by current identification procedures did in fact join the *S. bovis-S. equinus* group described by Farrow et al. (56). All the human isolates of *S. bovis* I and II/2 were suggested to be officially identified as *S. gallolyticus*. These experiments were done primarily by whole-cell protein analysis, which correlates with DNA reassociation studies. Nelms et al. reaffirmed these studies using 16S rRNA probes (93).

Recently, Schlegel et al. (107) described yet another species, *S. infantarius*, and further suggested that two subspecies *S. infantarius* subsp. *infantarius* and *S. infantarius* subsp. *coli* could be identified. These strains were closely related to the *S. bovis* group. DNA reassociation experiments showed that these human isolates were unique and corresponded to DNA group 4 described by Farrow et al. (56). The majority of strains in these studies were isolated from humans. The cultures described by Coykendall and Gustafson (38) and Knight and Shlaes (84) as *S. bovis* II/1 were a major part of the DNA group, *S. infantarius*. Strains of *S. bovis* II/1 are distributed in both subspecies. Even more recently Poyart et al. have suggested that the strains identified as *S. bovis* II.2 be renamed *S. pasteurianus* and the strains identified as *S. infantarius* subsp. *coli* be named *S. lutetiensis* (99). These suggestions were based on a combination of DNA homology, whole-cell protein, and sequencing of the *sodA* gene. The importance of this change in nomenclature can be translated back to the association of colonic cancer and the isolation of *S. bovis* from blood cultures of these patients. Ruoff et al. (104) showed that *S. bovis* I was more commonly

associated with colonic cancer patients than was *S. bovis* II/1. This translates into correct terminology that *S. gallolyticus* and *S. pasteurianus* are isolated from blood cultures of patients with colonic cancer more often than is *S. infantarius* or *S. lutetiensis.* Whether this terminology is accepted in the medical literature will depend on whether clinical microbiologists adopt procedures that accurately identify each of the new species with the published criteria.

The information in Table 3 was summarized from the publications just cited (38, 45, 56, 84, 93, 96, 97, 99, 107). Hopefully, additional studies will be undertaken to verify these changes in the nomenclature of this group, because cultures previously identified as *S. bovis* are frequently found in blood cultures of patients with bacteremia, sepsis, and endocarditis.

Streptococcus suis

S. suis is the name assigned to streptococci that were formally called Lancefield groups R, S, and T. Kilpper-Balz and Schleifer (81) determined that the strains representing group, R, S, and T and several other serotypes of strains with the same phenotypic characteristics were a single DNA group, which they called *S. suis.* Although some strains are beta-hemolytic and produce a hemolysin on agar plates containing horse blood agar, all strains of this species are alpha-hemolytic when grown on blood agar plates containing sheep blood (68). Early reports also included results that some strains of this species contained streptococcal group D antigen. However, later reports indicated that the group R and group D antigens were similar but not identical and the observed reactions were judged as cross-reactions in the group D antiserum. The discovery of additional serotypes of this species (64) led to a change in the nomenclature of how the capsular types were identified. There are now 35 different antigenic carbohydrate types of the species. Group R, the most common strain identified, is type 2, and group S is type 1. The important change to clinical microbiologists is that the only serotype identified from humans has been type 2 (group R).

Difficulties with the Lancefield extraction procedure, which is the reference procedure used for demonstrating group antigens in all other streptococci, have led to the recommendation that a capsular reaction be used (66) for identification of the various serotypes. This reaction is similar to the quellung reaction used to type *S. pneumoniae.* A modified Lancefield extraction may be used to identify type 2 by using group R-precipitating antiserum (64).

Lutticken et al. (92) reviewed the literature and summarized 44 cases of human infections caused by *S. suis.* There were 39 cases of meningitis and 5 cases of septicemia without meningitis. All cases were associated with the patient having worked with raw pork meat or having contact with pigs; none of the cases were from the United States. Trottier et al. reported the first case of infection cased by *S. suis* in North America; this was a case of human endocarditis from a Canadian patient (128). Type 2 *S. suis* cultures isolated from humans and pigs can be very similar. Chateillier et al. (25) performed random amplification polymorphic DNA (RAPD) analysis of 88 strains of type 2 *S. suis* including 8 isolates of human origin. Twenty-three different RAPD types were identified. Five of the eight human strains were placed in RAPD type 1, which contained

26 cultures isolated from pigs. By this procedure, the strains isolated from humans may be identical to those causing infections in pigs. *S. suis* is found in infections among pigs in the United States (25). Why no infections caused by this organism have been documented among U.S. patients is unknown. It is possible that laboratories may not have the microbiological capacity to identify the isolates. Phenotypically, *S. suis* resembles the viridans streptococcal species *S. gordonii, S. sanguinis,* and *S. parasanguinis* and therefore may be misidentified. Careful microbiological examination of alpha-hemolytic viridans streptococci isolated from human cerebrospinal fluid may result in identification of *S. suis* in the United States. Typing antisera and group R antiserum can be obtained from the Statens Serum Institute in Copenhagen, Denmark.

Viridans Streptococci

In 1997, Bruckner and Colonna listed 15 different streptococcal species that were included in the viridans streptococci (24). Table 4 includes 26 streptococcal species that have the phenotypic characteristics of typical viridans streptococci. In addition to being catalase-negative, gram-positive cocci arranged in chains, all the species listed in Table 4 have the phenotypic characteristics described for the viridans streptococci listed in Table 3. These characteristics are leucine aminopeptide positive, pyrrolidonylarylamidase negative, and no growth in 6.5% NaCl broth. Nearly all species, with the exception of those in the salivarius group, are bile-esculin negative. Species not yet identified from human sources are included because transmission of streptococcal species from nonhuman sources (*S. iniae, S. porcinus,* and *S. suis*) to humans has caused documented infections and there is no reason to believe that any of the species listed in Table 4 will not be isolated from human infections. The 26 species are arranged according to the six phenotypic characteristics listed in Table 4. Note that with this system, most individual species cannot not be identified but are placed in one of the six groupings. Not all investigators agree with this grouping of streptococcal species. Some have preferred to group *S. hyointestinalis* in the pyogenic group and *S. alactolyticus* in the *S. bovis-S. equinus* group based on sequence data of the 16S rDNA gene (76) and Fig. 1. Other investigators have included *S. acidominimus* and *S. uberis* in the viridans category (12). These two species are included in the category termed "other streptococci" because of their phenotypic characteristics (discussed below). The term "viridans streptococci" may not be the best to describe this group of organisms because many of the species do not express the hemolysis-like reaction (described as alpha-hemolysis, as discussed above) on blood agar plates. Many investigators refer to them as the oral streptococci; however, this designation does not truly represent the origin of all the species; some of them originate from gastrointestinal, vaginal, and dairy product sources. At this time, it is proposed that the term "viridans streptococci" be used to include streptococcal species with phenotypic characteristic described in Tables 3 and 4. There are two excellent reviews that summarize the molecular experiments that define the majority of viridans streptococcal species (37, 140). In addition, the corrected epithets of several species are given according to the rules of nomenclature (129). Interested investigators will need to devise a three-tier testing system for the definitive species iden-

TABLE 4. Identification of major groups of viridans *Streptococcus* species[a]

Group and species	Arginine	Esculin	Vogas-Proskauer	Mannitol	Sorbitol	Urea	Origin
Mutans group							
S. mutans	−	+	+	+	+	−	Human
S. sorbinus	−	+	+	+	+	−	Human, rat
S. cricetus	−	+	+	+	+	−	Rat, human
S. downei	−	−	+	+	+	−	Monkey
S. ferus	−	+	+	+	+	−	Rat
S. macaccae	−	+	+	+	+	−	Monkey
S. ratti	+	+	+	+	+	−	Rat, human
S. hyovaginalis	−	−	+	+	+	−	Swine
Salivarius group							
S. salivarius	−	+	+	−	−	v	Human
S. vestibularius	−	v	v	−	−	+	Human
S. infantarius	−	v	+	−	−	−	Human
S. alactolyticus	−	+	+	−	−	−	Swine, avian
S. hyointestinalis	−	−	+	−	−	−	Swine
S. thermophilus	−	−	+	−	−	−	Dairy product
Anginosus group							
S. anginosus	+	+	+	−	−	−	Human
S. constellatus	+	+	+	−	−	−	Human
S. intermedius	+	+	+	−	−	−	Human
Sanguinus group							
S. sanguinus	+	+	−	−	v	−	Human
S. parasangunis	+	v	−	−	−	−	Human
S. gordonii	+	+	−	−	v	−	Human
Mitis group							
S. mitis	−	−	−	−	v	−	Human
S. oralis	−	v	−	−	−	−	Human
S. cristatus	+	−	−	−	−	−	Human
S. infantis	−	−	−	−	−	−	Human
S. perois	−	−	−	−	−	−	Human
S. orisratti	−	+	−	−	−	−	Rat

[a] See Table 2 for definition of positive and negative reactions.

tification. The first determination is described in Table 3, the viridans streptococci group; the second determination is described in Table 4, the viridans streptococcal species group; and the third level needs to be similar to that described in Table 2 for the *S. anginosus* species group. A table similar to Table 2 using chromogenic substrates should be constructed for each viridans species group to identify definitive species (102, 105). All the phenotypic characteristics listed in Tables 1, 3, 4, and 5 in this review were obtained from conventional tests. The tables in the papers by Whiley and Beighton (140) and Ruoff et al. (102, 105) include most of the species listed in Table 4; however, a few additional species were described after the publication of their reports.

Streptococcus mutans Group

Whiley and Beighton (140) give an excellant review of the species origin included in the *S. mutans* group with the exception of *S. hyovaginalis.* This species is included in the mutans grouping because of similar phenotypic characteristics (Table 4). According to previous reports, the most common species isolated from human sources (primarily the oral cavity) are *S. mutans* and *S. sobrinus; S. cricetus* and *S. ratti* are rarely isolated (11). The identification scheme devised by these authors for differentiation of *S. mutans* and *S. sobrinus* included the fermentation of melibiose; *S. mutans* is positive and *S. sobrinus* is negative. In a previous study, we found that 88% of blood

TABLE 5. Identification of *Abiotrophia* and *Granulicatella* species[a]

Species	Pul	Suc	Tag	Tre	Hip	Arg	α-Gal	β-Glu	β-Gal	Origin
Abiotrophia defectiva	+	+	v	+	−	−	+	−	+	Human
Granulicatella adiacens	−	+	+	−	−	−	−	+	−	Human
Granulicatella para-adiacens	−	+	−	−	−	−	−	−	−	Human
Granulicatella balaenoptera	+	−	−	+	−	+	−	−	−	Whale
Granulicatella elegans	−	+	−	−	+	+	−	−	−	Human

[a] All strains are positive for pyrrolidonylarylamidase and leucine aminopeptidase and sensitive to vancomycin, and they show negative reactions for gas production in MRS broth, growth in 6.5% NaCl, or at 10 and 45°C. No reaction on bile-esculin medium. All strains require pyridoxal for growth and satillite around staphylococcus culture on blood agar plates. Abbreviations: Pul, Suc, Tag, Tre, acid production from pullulan, sucrose, tagatose, and trehalose broth, respectively; Hip, hydrolysis of hippurate; Arg, deamination of arginine; α-gal, production of α-galactosidase; β-glu, production of β-glucuronidase; β-gal, production of β-galactosidase; +, 85% or more of the strains positive; −, 15% or fewer of the strains positive; v, variable reactions (16 to 84% positive).

culture isolates (48 of 54) were melibiose positive, which indicates that the majority of isolates from human noncaries infections are *S. mutans* (49). *S. hyovaginalis* was described by Devriese et al. in 1997 (43). Several strains of this species were isolated from the genital tracts of female swine and appear to be part of the normal flora. Identification from human sources has not been documented.

Streptococcus salivarius Group

The *S. salivarius* group of bacteria is closely related to the *S. bovis-S. equinus-S. gallolyticus* group by both 16S rRNA gene analysis (140) and phenotypic characteristics (Tables 3 and 4). *S. alactolyticus* is included in the "*S. bovis*" group by some investigators (56, 130). Some strains of each of the species listed in the *S. salivarius* group react with streptococcal group D grouping antiserum, which would seem to indicate that they possess the group D antigen. This may not be the case. The problem with streptococcal group D antiserum is that this serum is rarely if ever tested for cross-reactions with non-beta-hemolytic streptococci. The *Streptococcus* grouping antisera are made for testing beta-hemolytic strains, and such sera are evaluated only with preparations of strains that are beta-hemolytic; therefore, group D antiserum may contain antibodies that cross-react with non-beta-hemolytic streptococci. Antigenic homology between strains of *Enterococcus faecalis* and members of the *S. Salivarius* group with streptococcal group D antiserum has not been published. Of the species listed in the *S. salivarius* group, only strains of *S. salivarius, S. vestibularius,* and *S. infantarius* have been isolated from humans. *S. salivarius* is commonly identified from a variety of human infections (38, 103). *S. vestibularius* was identified from the human oral cavity (146), and its association with human infections has not been confirmed. As discussed above, *S. infantarius* has been isolated from human infections (107). *S. infantarius* is listed in both the *S. bovis* group and the *S. salivarius* group because some *S. infantarius* strains are bile-esculin and esculin negative, which excludes them from the *S. bovis* group. These bile-esculin-negative strains were identified as *S. infantarius* by DNA-DNA reassociation studies. These strains are misidentified as *S. bovis* II/1 by the Rapid Strep system. Additional confusion will result in light of the latest findings that *S. bovis* II/1 can be either *S. infantarius* or *S. lutetiensis*. Accurate identification of these four species will establish their role in human infections. *S. hyointestinalis* isolated from the swine gut, was originally identified as *S. salivarius;* however, DNA-DNA reassociation studies demonstrated that this organism was unique and deserved species status (42). The taxonomic status of *S. thermophilus* has been in question for several years, and some investigators proposed that *S. thermophilus* should be a subspecies of *S. salivarius*. However, Schleifer et al. conducted DNA-DNA reassociation experiments under stringent conditions and determined that these strains deserved full species status (109). Data from the references noted in this section should be compiled to form a identification scheme for species identification.

Streptococcus anginosus Group

The species included in the *S. anginosus* group have been discussed in the section describing beta-hemolytic streptococcal species.

Streptococcus sanguinis Group (Formerly Known as *S. sanguis*)

As stated above, some investigators have preferred to arrange the viridans *Streptococcus* species differently from the classification listed here. For example, some have included the *S. sanguinis* and *S. mitis* groups together based on 16S rRNA sequence analysis (76), Fig. 1, and phenotypic characteristics (140). According to the phenotypic characteristics listed in Table 4, these two groups can be separated for the most part by their reactions in the arginine and esculin tests. There are exceptions. *S. gordonii* was split from the *S. sanguinis* species (79). Although there are many similarities between the two species, they can be differentiated by immunoglobulin A1 IgA1 protease, amygdalin, and alkaline phosphatase reactions (79). *S. parasanguinis* was split from the *S. mitis* grouping (143) and hence has some characteristics of both the *S. sanguinis* and *S. mitis* groups. The authors described characteristics that helped to differentiate *S. parasanguinis* from *S. sanguinis*, but the differentiation was based on percent positive reactions in 14 different tests; inulin fermentation was the only test that was positive for all strains of *S. sanguinis* and negative for all strains of *S. parasanguinis*. Formation of the extracellular polysaccharide dextran on 5% sucrose agar by both *S. sanguinis* and *S. gordonii* but not by *S. parasanguinis* also helps to differentiate these species. All three of these species are commonly found in viridans streptococcal type infections. *S. sanguinis* is probably the most frequently identified viridans *Streptococcus* species from patients with subacute bacterial endocarditis. It is difficult to assess the association of any of the newly described and redefined viridans streptococcal species with specific human infections.

Streptococcus mitis Group

The *S. mitis* group of viridans streptococci has caused considerable confusion for both clinical microbiologists and taxonomists. One of the problems has been the use of invalid species names such as *Streptococcus viridans* and *S. mitior*. Although these names may convey a meaning to physicians managing patients, they are troublesome to taxonomists in that there are no official reference strains which to study. Another problem has been the use of the terminology "biotypes." For example, "*S. sanguis* biotype II" is a common designation (50). The biotype describes strains with certain phenotypic characteristics but does not have official taxonomic status, and therefore no "type" strains are usually available for study. In addition, the type strain for *S. mitis* was determined to be inappropriate based on molecular experiments. The addition of *S. oralis* (21, 82) and *S. cristatus* (65), formally called *S. crista*, added to the complexity of the *S. mitis* group. Beighton et al. presented a reasonable scheme based on 23 tests (including 10 chromogenic tests) to differentiate the *S. sanguinis* and *S. mitis* groups recognized in 1991 (10). Since then, two more species, *S. peroris* and *S. infantis*, have been added to the *S. mitis* group (77). *S. mitis, S. oralis, S. cristatus, S. peroris*, and *S. infantis* have all been isolated from the human oral cavity. Even more recently, *Streptococcus orisratti* (151) has been added to the *S. mitis* group. *S. orisratti* possesses Lancefield's group A antigen, and this phenotypic characteristic can be used to iden-

tify this species once it is determined that the strain in question has phenotypic characteristics consistent with the *S. mitis* group. Devising a scheme for identification of all six species in the *S. mitis* group will take additional work. It is not yet possible to correlate the new and revised species in this group with human infections. Whether any of the newly described species are associated with human infections is unknown.

Options for Identification of Viridans Streptococcal Species

What are the options for identifying the viridans streptococcal species? It is apparent that the use of conventional tests such as those used at CDC does not differentiate most species. Nearly 40 conventional tests have been used to test all the type strains as well as other reference strains of each species, and successful differentiation of species has not been accomplished. Ruoff et al. in the last two editions of the *Manual of Clinical Microbiology* included identifications tables that could be used to identify the viridans streptococcal species known at that time (102, 105). These tables included conventional tests as well as a series of fluorogenic substrates that had to be prepared in house because they were not available from commercial medium sources. Commercially available systems do not have all the species in their databases, so that the identifications generated by those systems are compromised, at least to some extent. Probably the best one could hope for was reported by Kikuchi et al. (78). When DNA homology studies were used to confirm the identifications, 87% of 156 strains of 13 different species in the database were correctly identified and 56% of 15 strains of 6 species not in the database were incorrectly identified using the Rapid ID 32 Strep system (bioMérieux). In the latter case, a result of "unidentified" would be correct. Unfortunately, DNA-DNA reassociation procedures at this time are not applicable for most clinical laboratories. 16S rRNA gene-sequencing procedures have been used to show relationships between many of the streptococcal species; however, strict criteria for determining species have not been established. For example, the sequence identities of the 16S rRNA genes for the types strains of *S. mitis, S. oralis*, and *S. pneumoniae* are greater than 99% similar (76). This does not leave much room for sequence variability that occurs in many strains. In addition, *S. macedonicus, S. wais*, and *S. gallolyticus* appear to be separate species based on 16S rRNA sequences (Fig. 1); however, DNA-DNA reassociation and *sodA* sequencing studies indicate that the type strains representing the species are a single species, *S. gallolyticus* (99). Rudney and Larson (101) used restriction fragment polymorphism of rRNA ribotyping for six species and found that 91% of 53 isolates were reported as one of three species. Whole-cell protein analysis is thought to correlate very well with DNA-DNA reassociation, but Vandamme et al. (132) did not get correlation between their whole-cell protein profiles and species identification. This is in contrast to the results obtained by most other authors using this technique but would seem to point to a need for a standardized procedure. Garnier et al. (60) targeted the D-alanine–D-Alanine ligases of six viridans species, applied PCR reaction technology with specific primers, and successfully identified the six species. However, they were unable to differentiate the *S. anginosus* group of species. Two investigations have been reported examining the tDNA intergenic spacer length polymorphism (tDNA-PCR), with different results (4, 40). These investigators used different detection systems, which may explain the differences in the results; however, neither report indicated a completely successful result in differentiating all species tested. The most successful molecular technique reported to identify viridans streptococcal species to date is that reported for examining the sequence of the gene encoding the manganese-dependent superoxide dismutase ($sodA_{int}$) (98, 99). Poyart et al. (98, 99) reported differentiation of 29 streptococcal species including 16 viridans species. *S. mitis, S. oralis*, and *S. pneumoniae* were clearly differentiated by *sodA* sequencing, as opposed to the situation for 16S rRNA gene sequencing. Although other technologies that may be used to study strain-to-strain relationships for epidemiologic studies include electrophoretic isoenzyme typing (62), pulsed-field gel electrophoresis (149), and various other PCR-based methods (2), these procedures are not useful for the identification of specific species.

Antimicrobial Susceptibilities of Viridans Streptococcal Species

Viridans streptococci are isolated from a variety of infections but most significantly from patients with subacute bacterial endocarditis and from neutropenic patients with cancer. In these two instances, knowledge of the antimicrobial susceptibility of the infecting organisms is useful for good patient management. The question is whether knowledge of the taxonomic identity of infecting bacteria is useful for predicting the antimicrobial susceptibility of the organism. In view of the multiple changes in taxonomy and nomenclature of this group of gram-positive cocci, it is difficult to say exactly if it is possible to predict potential susceptibility problems related to specific species. However, some generalities can be made regarding susceptibilities and species. First of all, antimicrobial resistance is substantial in the viridans streptococci as a group. Penicillin resistance in the viridans streptococci is as high as 48% in strains from the United States, 45% in strains from Canada, and 33% in strains from Latin America (97). Specific species in this study were not adequately identified, but *S. mitis* was the most common species group identified and the most likely to be resistant. Other studies with better identification procedures have indicated that *S. mitis* and *S. oralis* strains are the most common found in blood cultures of cancer patients and are commonly resistant to β-lactam antimicrobials (3, 46, 73, 123). Although not all these investigators used identification procedures that would allow identification to the species level according to today's standards, the identification certainly would be in the *S. mitis* grouping. The species distribution is somewhat different in endocarditis patients; *S. sanguinis, S. oralis*, and *S. gordonii*, in descending order, are the most common strains isolated from cultures of blood of endocarditis patients (46). Although the incidence is not as high in the *S. sanguinis* group as in the *S. mitis* group, antimicrobial resistance is present. One other generality can be made with the *S. anginosus* group. Resistance to β-lactam antimicrobial was only at the intermediate level, but resistance to macrolides was found in 17% of the strains (91). More recently, Tracy et al. (127) used 16S rRNA sequencing to identify the species of the *S. anginosus* group and found no difference in antimicrobial

TABLE 6. Identification of unusual *Streptococcus* species and other genera of gram-positive cocci in chains[a]

Species	LAP	PYR	NaCl	BE	Esc	Arg	VP	Hip	Man	Sbl	Origin
S. acidominimis	+	+	−	−	+	−	−	−	+	−	Food
S. pluranimalium	+	−	+	+	+	−	−	−	−	−	Bovine, other animals
S. thoraltensis	+	−	+	+	+	+	+	v	+	+	Swine
S. uberis/parauberis	+	+	+	−	+	v	v	+	+	+	Bovine
S. urinalis	+	+	+	+	+	+	+	−	−	−	Human
Dolosicocccus paucivorans	−	+	−	−	−	−	−	−	−	−	Human
Facklamia species	+	+	+	−	v	v	−	v	−	v	Human
Ignavigranuum	+	+	+	−	−	−	−	−	−	−	Human
Globicatella sanguinus	−	v	+	+	+	−	−	+	+	v	Human

[a] Abbreviations: LAP, leucine aminopeptidase; PYR, pyrrolidonylarylamidase; NaCl, growth in 6.5% NaCl broth; BE, bile-esculin reaction; Esc, esculin hydrolysis; Arg, deamination of arginine; VP, Voges-Proskauer reaction; Hip, hydrolysis of hippurate; Man and Sbl, acid production in mannitol and sorbitol broths. See Table 2 for definition of positive and negative reactions.

susceptibilities of the three species (*S. anginosus, S. constellatus*, and *S. intermedius*). These authors expressed the opinion that it was unnecessary to identify the infecting organism to the species level and that identification to the "milleri group" was sufficient for patient management. Whether the same can be said for the other viridans groups remains to be established. There appears to be antimicrobial susceptibility differences in some of the other species within these groups.

NUTRITIONALLY VARIANT STREPTOCOCCI, *ABIOTROPHIA* AND *GRANULICATELLA* SPECIES

The nutritional variant group of gram-positive bacteria has been described as satelliting, pyridoxal-dependent, vitamin B$_6$-dependent, cell wall-deficient (L-form), and finally nutritionally variant streptococci (NVS) before being reclassified as *Streptococcus adjacens* and *Streptococcus defectivus* (19). Kawamura et al. demonstrated that the two NVS species were phylogenetically distant from the streptococci and proposed that they be given new genus status, *Abiotrophia adjacens* and *Abiotrophia defectivus* (75). Recently, three new species have been added to the *Abiotrophia* genus, *A. elegans* (98), *A. balaenopterae* (89), and *A. para-adiacens* (74). Collins and Lawson further proposed that some of the *Abiotrophia* species were phylogenetically distinct from each other and proposed the establishment of the genus *Granulicatella* to include *G. adiacens, G. balaenopterae*, and *G. elegans* in this new genus while *A. defectiva* remains in the *Abiotrophia* genus (35). Note the corrected epithets. Table 5 includes all the species of "NVS" and an identification scheme based on the published reports (35, 74, 75, 89, 100) and our own results examining 100 strains of NVS taken from the CDC stock culture collection (26). Our results indicate that of 101 isolates from 97 patients (58 with endocarditis), 55 were *G. adiacens*, 43 were *A. defectiva*, and 3 were *G. elegans*. Other authors using slightly different species identification criteria found 15 *G. adiacens*, 13 *G. para-adiacens*, 9 *A. defectiva*, and 8 *G. elegans* strains among 45 endocarditis patients (74). NVS overall is reported to cause approximately 5% of all cases of endocarditis (22). These reports indicate that all the species except *G. balaenopterae* have been isolated from human infections. Patients with endocarditis due to NVS are more difficult to treat than those infected with viridans streptococci. As many as 41% of patients may fail to

respond to antimicrobial therapy, and combination therapy is often recommended (22).

UNUSUAL *STREPTOCOCCUS* SPECIES AND OTHER GRAM-POSITIVE COCCI IN CHAINS

The last group of *Streptococcus* species and related genera consists of bacterial strains that do not fit into any of the species or species groups discussed above. None of these strains are beta-hemolytic, which eliminates them from the species listed in Table 1. Note that in Table 3, the last species-group listed is "other streptococci and genera." The species listed in Table 6 are not viridans streptococci, because all viridans streptococci are leucine aminopeptidase positive and pyrrolidonylarylamylase-negative and do not grow in 6.5% NaCl broth. The species in Table 6 have at least one reaction different from this pattern. These species are bile insoluble and optochin resistant (eliminating the pneumococci) and do not have defined group antigens (eliminating the *S. bovis* and *S. suis* groups). The identifications based on the reactions listed in Table 6 are a guide for potential identification. The majority of reactions listed for the species *S. acidominimus, S. pluranimalium, S. thoraltensis, S. uberis, S. parauberis*, and *S. urinalis* were taken from published materials (13, 39, 42, 43, 44). In most cases, only the type strain for each species has been tested in our laboratory. In addition, none of these six species has been isolated from human infections.

Streptococcus acidominimis

The type strains of *S. acidominimis* have been poorly described, and most reference strains have not had the phenotypic characteristics of the original description of the species in 1922. Recently, Devriese et al. examined the reference strains, firmly established the correct type strain for the species, and described better phenotypic criteria for identification (44). Although it had been reported that *S. acidominimus* was identified from humans in 1977 (50), we have recently revised that finding. The human isolates previously identified as *S. acidominimus* are now identified as *Facklamia sourekii* (86). The reactions listed in Table 6 are those obtained from Devriese et al. (42–44) and our testing of the current type strain for the species. At this time, we have not confirmed any human iso-

lates of *S. acidominimus* when using the revised identification criteria.

Streptococcus pluranimalium

The new species *S. pluranimalium* was described by Devriese et al. (44). The strains resembled *S. acidominimus*, and in fact some of the reference strains of *S. acidominimus* in culture collections were reidentified as *S. pluranimalium*. *S. pluranimalium* has been isolated from bovine mastitis; bovine vagina, cervix, and tonsils; and canary lung and lesions (44). No human isolates have been confirmed. Presumptive identification can be made on the basis of the reactions listed in Table 6.

Streptococcus thoraltensis

The new species *S. thoraltensis* was also described by Devriese et al. (43). Cultures of this species were recovered from the intestinal tracts of swine. Identification of this species from humans has not been documented. Presumptive identification can be made by matching reactions with those listed in Table 6.

Streptococcus uberis and Streptococcus parauberis

The two species *S. uberis* and *S. parauberis* are placed in Table 6 because differentiation of the two species by conventional phenotypic tests is not possible. Species-specific probes have been developed for identification of both species (13). *S. uberis* can be found in up to 20% of cases of bovine mastitis (13). Confirmation of isolation *S. uberis* or *S. parauberis* from human infections has not been documented. All the isolates of *S. uberis* previously reported from humans (50) have been reidentified as *Globicatella sanguinis* (see below).

Streptococcus urinalis

The description of *S. urinalis* was made on the basis of a single strain isolated from a case of human cystitis (31). Since the publication of that report, a second isolate of *S. urinalis* has been identified, a blood culture isolate with no additional information. Phenotypically, *S. urinalis* is similar to *S. uberis, G. sanguinis*, and enterococci. In addition to the tests described in Table 6, isolates should be confirmed as not being enterococci by use of the GenProbe *Enterococcus* probe because *S. urinalis* has overlapping phenotypic characteristics with the enterococci.

Dolosicoccus paucivorans

D. paucivorans was initially described based on one organism isolated from a culture of blood from a patient with pneumonia (34). Two additional cultures have been isolated from blood samples. One patient had a diagnosis of pneumonia, and the other report did not include clinical information. The appearance of dolosicocci on blood agar plates is similar to that of the viridans streptococci, but the positive pyrrolidonylarylamidase and negative leucine aminopeptidase reactions clearly differentiate this bacterium from the viridans streptococci (Table 6).

Facklamia species and Ignavigranum ruoffiae

Five species of *Facklamia* have been described, *F. hominis, F. ignova, F. languida, F. sourekii*, and *F. tabacinasalis. F. languida* is described as not forming chains; however, in our hands some chains are apparent in Gram stains prepared from thioglyolate broth (86). All but *F. tabacinasalis* have been isolated from human infections. Most isolates have been from blood cultures, but very little clinical information was provided with the isolates (85). Table 6 lists the tests necessary for presumptive identification of the *Facklamia* and *Ignavigranum* genera. Identification of the species has been recently reviewed (51). Some strains exhibit decreased susceptibilies to the β-lactam and macrolide antimicrobials (85).

Globicatella sanguinis

G. sanguinis (sanguis) was described in 1992 from a collection of cultures from human sources identified as *Streptococcus uberis*-like (28). They were called *S. uberis*-like because of phenotypic similarities. Isolates of this species are also similar to *Aerococcus viridans* in that both species are leucine aminopeptidase negative and pyrrolidonylarylamidase positive and hydrolyze hippurate. However, the cellular arrangement of *A. viridans* in the Gram stain is in pairs and clusters, as opposed to chains (*G. sanguinis*). *G. sanguinis* is differentiated from *S. uberis* by the leucine aminopeptidase test (*S. uberis* is positive) and the bile-esculin test (*S. uberis* is negative, but *G. sanguinis* is positive). In a recent study of 28 strains of *G. sanguinis*, 20 were isolated from cultures of blood from patients with diagnoses including bacteremia, sepsis, and endocarditis. Urinary tract infections were associated with three urine culture isolates (117). These data seem to indicate that this species may be an important pathogen in humans. *G. sanguinis* can be identified using the phenotypic tests listed in Table 6.

New Species Not Yet Tested

Five new *Streptococcus* species, all of which appear to fall into the viridans *Streptococcus* grouping, and one *Facklamia* species have been described that have not yet been tested in our conventional test system. *S. australis* (148) appears to fit into the *S. mitis* grouping, and because it deaminates arginine, it most closely resembes *S. cristatus. S. australis* was isolated from the human oral cavity. *S. ovis* (33), isolated from vaginal swabs of sheep, perhaps fits into the *S. mutans* group. *S. ovis* is reported to form acid in mannitol and sorbitol broths like the other members of this group. *S. sinensis* (150) appears to fit into the *S. anginosus* group by both phenotypic and genetic tests. *S. sinensis* was isolated from a human with endocarditis. *S. entericus* (134), isolated from cattle intestines, phenotypically fits roughly into the *S. mitis* group. Yet another recently described species, *S. gallinaceus* (31), isolated from chickens phenotypically resembles species in the *S. anginosus* group, because the cultures are mannitol positive, a trait rarely observed in the *S. anginosus* group, they may be identified fairly easily. *F. miroungae* (69) was isolated from an elephant seal. Phenotypic tests indicate that this species is closely related to *F. hominis*. An exact position of any of these new species cannot be determined until they are tested with conventional

tests, and this has not been reported by the authors of these reports.

CONCLUSIONS

The changes in the nomenclature and taxonomy of the *Streptococcus* genus are numerous and varied. Eleven species and four subspecies of streptococci are beta-hemolytic and can be identified by Lancefield grouping and a few phenotypic tests. Nearly all these species and subspecies are isolated from human infections. However, among the non-beta-hemolytic species, 26 different species of viridans streptococci, 5 different species of what was termed *S. bovis*, 5 different species of nutritionally deficient streptococci, 9 different species of other streptococci, and 3 new genera of gram-positive cocci in chains have been described. The majority of changes in the non-beta-hemolytic streptococci have been the addition of species. These additions for the most part have complicated the recognition of each specific species. It is apparent that conventional tests and commercially available devices and systems have not incorporated all the taxonomic changes into the identification procedures. In my opinion, the development of molecular genetic procedures is required before the non-beta-hemolytic streptococci can be accurately identified.

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