

Occurrence of *Streptococcus milleri* among Beta-Hemolytic Streptococci Isolated from Clinical Specimens

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A total of 256 beta-hemolytic streptococcal isolates were subjected to serological and physiological tests to identify those which could be classified as *Streptococcus milleri*. *S. milleri* accounted for 75% of 70 group C isolates, 15% of 69 group G isolates, 75% of 16 nongroupable isolates, and 100% of 20 group F isolates examined. No *S. milleri* isolates were encountered among the 90 group A streptococci studied. Of the 95 beta-hemolytic *S. milleri* isolates examined, 81% were recovered from respiratory specimens.

Although *Streptococcus milleri* is not included in the approved lists of bacterial names (14), British authors have popularized this epithet as a species name for viridans streptococci which appear to be associated with abscesses and other purulent infections (10). Ball and Parker (1) have characterized *S. milleri* as a physiologically and serologically heterogeneous species consisting of a central group and two minor groups of organisms. They found the majority of *S. milleri* isolates examined to be nonhemolytic, nongroupable, and able to produce acetoin, hydrolyze esculin and arginine, and produce acid from trehalose, lactose, salicin, and sucrose. These organisms appear to be similar to those previously called *Streptococcus MG-intermedius* (6) and currently referred to, in an effort to adhere to approved bacterial nomenclature, as *Streptococcus intermedius* by Facklam et al. (8). Ball and Parker (1) also described a subgroup of isolates which were similar to this central group except for the ability to ferment additional carbohydrates. These organisms have been referred to as "mannitol-positive *S. intermedius*" by Facklam et al. (8). Finally, Ball and Parker noted a subgroup of isolates which lack one or more of the central group characteristics. This subgroup included isolates that were beta-hemolytic and that sometimes displayed Lancefield group A, C, F, or G antigens. Facklam originally recognized alpha-reacting and nonhemolytic members of this *S. milleri* subgroup as *Streptococcus anginosus-constellatus* (6) and more recently has referred to them as *Streptococcus constellatus* (8). Beta-hemolytic *S. milleri* isolates, with or without Lancefield group A, C, F, or G antigens, are referred to as *Streptococcus anginosus* by Facklam.

Beta-hemolytic *S. milleri* (*S. anginosus*) isolates appear to be identical with the "minute hemolytic streptococci" described by Long and Bliss (9) and shown by Bliss (2) to possess either group F or group G Lancefield antigens. Colman and Williams (4) proposed that these minute hemolytic organisms be included in the *S. milleri* species on the basis of physiological characteristics and cell wall composition. Subsequently, other authors (1, 10-12) advocated the inclusion of beta-hemolytic group A, group C, and nongroupable isolates in the *S. milleri* species on the basis of their physiological similarities to nonhemolytic *S. milleri* strains. Representatives of both groupable and nongroupable

beta-hemolytic *S. milleri* have been implicated as pathogens in a variety of infections (10, 11).

In view of the continued interest in the taxonomy and pathogenic potential of *S. milleri*, we investigated the occurrence of *S. milleri* among routine isolates of groupable and nongroupable beta-hemolytic streptococci. We examined beta-hemolytic isolates with group A, C, F, G, or no detectable Lancefield antigen with respect to physiological characteristics and comment on the frequency and distribution of *S. milleri* among the streptococci examined.

MATERIALS AND METHODS

Bacterial isolates. The streptococci studied were beta-hemolytic isolates present in moderate to abundant amounts in cultures of various specimen types. The representatives of each serological group were collected as consecutive isolates. Group C and G streptococci were gathered over a period of 3 months, group A and nongroupable isolates were gathered over a period of 2 months, and group F isolates were collected during a 3-week period. Throat specimens were planted on Selective Streptococcus agar containing sheep blood (GIBCO Diagnostics, Madison, Wis.), and streptococci from non-throat specimens other than blood were recovered from brucella agar plates containing 5% horse blood (GIBCO Diagnostics). All primary plates were incubated at 35°C in the presence of 3 to 5% CO₂. The streptococci were suspended in horse blood and stored frozen at -70°C. Thawed suspensions were propagated on brucella agar plates containing 5% horse blood, and this medium served as the source of inocula for tests performed in the study.

Serological characterization. Streptococci were tested for group A, C, F, and G antigens by one of two methods. These techniques were either microprecipitin testing (7) after extraction of antigens with lysozyme-albus enzyme mixture (15) or nitrous acid extraction of antigens followed by testing with Phadebact coagulation reagents according to the instructions of the manufacturer (Pharmacia Diagnostics, Piscataway, N.J.). Nongroupable isolates were examined with both Phadebact and lysozyme-albus methods and tested for group A, B, C, D, F, and G antigens.

Physiological characterization. Group A isolates were tested for susceptibility to bacitracin (7) and production of acetoin with the rapid VP test described by Bucher and von

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TABLE 1. Beta-hemolytic streptococci isolated from clinical specimens^a

Specimen	No. of each group of streptococcus isolated from various specimen types							
	Group A (<i>S. pyogenes</i>)	Group C		Group F (<i>S. milleri</i>)	Group G		Nongroupable	
		<i>S. milleri</i>	Large-colony		<i>S. milleri</i>	Large-colony	<i>S. milleri</i>	Unidentified
Throat	70	38	9	5	7	22	4	0
Sputum	1	7	1	8	3	3	5	4
Wound	18	3	5	5	0	11	3	0
Urine and genitourinary	1	4	1	2	0	7	0	0
Stool	0	1	1	0	0	3	0	0
Blood	0	0	0	0	0	2	0	0
Fluid (other than blood)	0	0	0	0	0	1	0	0

^a Streptococci were identified by use of the Rapid Strep system, presumptive tests, or conventional methods. See text for details.

Graevenitz (3). The first 50 isolates of group C and G streptococci and the group F and nongroupable isolates were identified with the Rapid Strep system according to the instructions of the manufacturer (API System S.A., Montalieu-Vercieu, France). The remaining group C and group G streptococcal isolates were screened for acetoin production with the rapid VP test. Beta-hemolytic behavior of all isolates was confirmed on horse blood agar plates with the streak and stab technique (7).

Certain isolates were subjected to physiological tests with conventional broth and agar media (5, 13). These streptococci were tested for the following characteristics: ability to ferment mannitol, lactose, raffinose, inulin, sorbitol, and melibiose; ability to hydrolyze arginine, esculin, and urea; and ability to produce polysaccharides from sucrose.

RESULTS

Identification of the isolates. All of the 90 beta-hemolytic group A isolates examined were susceptible to bacitracin and failed to produce acetoin. These organisms were presumptively identified as *Streptococcus pyogenes* and were not subjected to further testing. Beta-hemolytic group A *S. milleri* isolates are expected to be bacitracin resistant and Voges-Proskauer (VP) positive (1, 11).

The first 50 consecutive isolates of beta-hemolytic group C streptococci were identified with the Rapid Strep system. Eleven of these were *Streptococcus equisimilis* (large-colony-forming isolates) and thirty-eight were *S. milleri*. Of the *S. milleri* isolates, 17 were identified as *S. milleri* biotype 1, 19 were identified as *S. milleri* biotype 2, and 2 were identified as *S. milleri* without distinction between biotypes 1 or 2. One isolate produced a profile that could not be found in the code book, but with conventional testing it produced reactions typical of an *S. intermedius* strain negative for arginine hydrolysis; this isolate was therefore also classified as *S. milleri*. Of the 20 isolates subjected only to testing for acetoin production, 14 were positive and 6 were negative. The VP-positive organisms all formed small colonies, in contrast to the large-colony VP-negative isolates. Thus, by use of the Rapid Strep system and presumptive tests, 53 of the group C isolates were identified as *S. milleri* and 17 were identified as belonging to the large-colony group C streptococcal species.

In contrast to the group C isolates, the frequency of *S. milleri* among the 60 beta-hemolytic group G streptococci examined was low. Of the first 50 isolates tested with the Rapid Strep system, 7 *S. milleri* strains (1 of biotype 1, 4 of biotype 2, and 2 of biotype 3) were identified. Screening with the rapid VP test revealed three additional VP-positive

small-colony isolates that were presumptively identified as *S. milleri*, for a total of 10 *S. milleri* of the 60 isolates examined. Forty-two large-colony isolates were identified as group G by the Rapid Strep system, and seven large-colony isolates were VP negative in the screening test. One isolate could not be identified. It formed small colonies but was VP negative and β -glucuronidase positive (characteristics of large-colony group G streptococci) on the Rapid Strep strip. This isolate was negative in all tests on conventional media.

By use of the Rapid Strep system, 19 of 20 group F isolates and 12 of 16 nongroupable isolates were identified as *S. milleri* biotype 1. An additional group F isolate was identified as *S. milleri* biotype 2. Four of the nongroupable isolates produced profile numbers which could not be located in the Rapid Strep code book. When these isolates were tested on conventional media, all of them fermented lactose and inulin and hydrolyzed arginine but failed to ferment mannitol or sorbitol or to hydrolyze urea. Two isolates also fermented raffinose and melibiose and hydrolyzed esculin. Two isolates produced dextran, and one of these also fermented raffinose and melibiose.

Distribution of *S. milleri*. Table 1 shows the distribution of the isolates examined among different specimen types. Respiratory (throat and sputum) isolates accounted for 79% of the *S. pyogenes* isolates, 59% of the group C large-colony streptococci, and 51% of the group G large-colony streptococci. The following *S. milleri* isolates were also found in respiratory sources: 85% of group C, 65% of group F, 100% of group G, and 75% of nongroupable isolates. Thus, for beta-hemolytic *S. milleri* as for *S. pyogenes* and large-colony group C and G beta-hemolytic streptococci, respiratory specimens were the most common source of isolates in this study. Beta-hemolytic *S. milleri* was also isolated from wounds, urine, and genitourinary specimens, and one isolate was recovered from stool.

DISCUSSION

All of the beta-hemolytic *S. milleri* isolates examined in this study shared the common trait of small-colony formation, although morphology of the colonies varied. Some isolates formed surface colonies that appeared slightly alpha-hemolytic, but all isolates were judged as beta-hemolytic when the area around subsurface growth was examined microscopically. A positive VP reaction (assayed with either the Rapid Strep system or the rapid VP test) coincided with small-colony formation, except in the case of one group G isolate.

Physiological characteristics determined by the Rapid Strep system were variable among the beta-hemolytic iso-

lates identified as *S. milleri*. The *S. milleri* biotype 1 distinguished by the Rapid Strep system predominated among group F (19 of 20 isolates) and nongroupable (12 of 12 isolates) *S. milleri*. Only 17 of the 37 group C and 1 of the 7 group G *S. milleri* isolates examined were *S. milleri* biotype 1. *S. milleri* biotype 2 accounted for all the remaining *S. milleri* identifications, with the exception of two group G isolates identified as *S. milleri* biotype 3 and two group C isolates for which a distinction between biotypes 1 and 2 could not be made. Facklam and co-workers (8) recently found that three of four group C isolates, eight group F isolates, four group A isolates, and seven nongroupable *S. milleri* isolates were categorized as *S. milleri* biotype 1 with the Rapid Strep system, while the remaining group C isolate was identified as *S. milleri* biotype 2.

Of the 95 beta-hemolytic *S. milleri* isolates examined in the present study, 81% were recovered from respiratory specimens. Poole and Wilson (11) previously noted a lower incidence (37%) of respiratory isolates in a study of 131 beta-hemolytic *S. milleri* strains. They recovered 14% of their isolates from patients with appendicitis; abdominal surgery and vaginal isolates accounted, respectively, for 13 and 24% of the beta-hemolytic *S. milleri* in their study. While Poole and Wilson noted that their isolates were recovered during "the course of routine bacteriological investigations," they did not state that the organisms were consecutive isolates, as were the streptococci in the study reported here. The possibility that Poole and Wilson's isolates were selectively collected might account for the differences in distribution among specimen types, but their study demonstrates that beta-hemolytic *S. milleri* may be isolated from many body sites.

We made no attempt to establish whether the isolates examined in the present study were clinically significant. Bucher and von Graevenitz (3) have observed that while large-colony group C and G streptococci have been implicated as etiological agents of pharyngitis, no convincing evidence exists for the pathogenic role of beta-hemolytic *S. milleri* in throat infections. They recommended performing rapid VP tests on beta-hemolytic group C and G throat isolates to separate large-colony strains from *S. milleri*. We found that 81% of 47 group C throat isolates and 24% of 29 group G throat isolates were *S. milleri*. The common occurrence of these organisms in throat cultures and the time and economic resources required to identify them point to a need for further study of their clinical significance.

In the study reported here, *S. milleri* was not found among the beta-hemolytic group A isolates examined, but it accounted for approximately 75% of the group C isolates, 15% of the group G isolates, 75% of the nongroupable isolates, and, as expected, 100% of the group F beta-hemolytic isolates. These findings are in agreement with those of Lawrence et al., who observed that in a collection of 167 beta-hemolytic streptococci with group A, C, F, G, or no

detectable antigen, *S. milleri* accounted for the majority of the group C, F, and nongroupable isolates but was rare among the group A and G streptococci studied (J. Lawrence, D. M. Yajko, and W. K. Hadley, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C52, p. 308).

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