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Laboratory Identification and Epidemiology of Streptococcal Hospital Isolates

MARGARET A. HARDY, HARRY P. DALTON,* AND MARVIN J. ALLISON

Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia 23298

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A total of 343 streptococcal strains were identified to species on the basis of the progressive method of bacterial identification as advocated by *Cowan & Steel's Manual for the Identification of Medical Bacteria*. Comparative studies were also performed with these strains to determine the accuracy and feasibility of using various types of blood, differential media, and biochemical tests in conjunction with the progressive method of identification. The streptococcal species were then correlated with the type of specimen, sex, and age of hospitalized patients to obtain some insight into the epidemiology of hospital streptococcal isolates.

Over the years, interest in the study of the epidemiology of streptococcal infections has been sporadic (2). In 1942, Rantz and Kirby (29) published one of the first reports demonstrating the importance of non-group A streptococci in extrarespiratory infections. There followed a limited number of publications on this subject citing single or small collections of infections due to non-group A or D streptococci (21, 22, 26, 34). In the 1960s there was an increasing awareness of the emergence of group B infections (10, 17, 23), but it was not until Reinarz and Sanford in 1965 (30) and Feingold et al. in 1966 (14) again demonstrated the importance of various serological groups of streptococci as causative agents of extrarespiratory infections that sustained interest developed. These reports were soon followed by a comprehensive study by Duma et al. (9) of streptococcal bacteremia, emphasizing the spectrum of streptococcal groups that can be involved in infectious syndromes. Numerous recent publications demonstrate the increased interest in these organisms (1, 3, 7, 12, 25, 26, 33). In this report the laboratory identifications and origins of the streptococcal isolates from hospitalized patients are emphasized.

MATERIALS AND METHODS

Specimens. A total of 343 streptococcal isolates were obtained from 252 patients at the Medical College of Virginia from 1 June 1976 to November 1976. All specimens were plated on 5% sheep blood agar and incubated aerobically at 35°C (15).

Procedures for identification. Colonies determined to be streptococci (catalase-negative gram-positive cocci in chains) were first grouped and then identified to species by using the following spectrum of tests. (i) Hemolysis. Sheep, horse, and rabbit blood agar plates (Trypticase soy agar [Difco Laboratories] plus 5% blood [Granite Diagnostics]) were inoculated by streaking and stabbing a loopful of an 18-h Todd-Hewitt broth (THB; Difco) culture of an isolate into the agar. Hemolytic activity was noted as beta (complete hemolysis; clear, colorless zones surrounding colonies), alpha (incomplete hemolysis; green zones surrounding colonies), no reaction (red cells around colonies intact). Particular attention was paid to the stab area of the agar when doubt existed regarding the type of hemolysis around individual colonies. (ii) Bacitracin sensitivity. Commercial bacitracin disks (Difco) were utilized according to directions of the manufacturer. Any zone of inhibition extending beyond the edge of the disk was considered sensitive. (iii) Optochin sensitivity. Optochin disks (Difco) were utilized according to directions of the manufacturer. (iv) Bile-esculin reaction. Bile-esculin medium (Difco) was prepared and tested as described by Facklam and Moody (13). (v) Growth in salt broth. Salt broth consisted of brain heart infusion broth (Difco) plus 6% (wt/vol) NaCl. (vi) Arginine and esculin hydrolyses. Arginine (liquid medium) and esculin hydrolyses were determined as described by Cowan (6). (vii) Hippurate hydrolysis. Hippurate hydrolysis was determined by the rapid method of Hwang and Ederer (20), with the modification that the initial 2-h incubation period was increased to 4 h. (viii) CAMP test. The method for the CAMP test was that described by Darling (8). (ix) Pigment production in Columbia agar. Columbia agar medium (Baltimore Biological Laboratory) was prepared according to directions of the manufacturer and dispensed, inoculated, and incubated as recommended by Merritt et al. (24). (x) Acid production in carbohydrates. Purple broth base (Difco) was the basal medium to which 1% of each of the following carbohydrates was added and dissolved by heating: lactose, mannitol, salicin, sorbitol, trehalose, raffinose, sucrose, inulin, glycerol, arabinose, and sorbose. The medium was dispensed into tubes in 5-ml amounts before autoclaving. Inoculum for the tests was 2 drops of an 18-h THB culture of the isolate. All tests were incubated aerobically at 35°C for 5 days, and readings were taken

daily. (xi) Growth on bile agar. Oxgall (10 or 40%, wt/vol; Difco) was incorporated into 3% sheep blood agar plates. The plates were inoculated by streaking one loopful of an 18-h THB culture onto the agar surface. Growth was determined as positive or negative after 3 days of incubation. (xii) Nitrofurazone sensitivity. Nitrofurazone (Sigma Chemical Co.) was incorporated into the media of 3% sheep blood agar plates at a concentration of 10 μg/ml. The plates were inoculated by streaking a loopful of an 18-h THB culture onto the agar surface and observed for growth after 24 and 48 h of incubation. (xiii) Citrate test. Two drops of an 18-h TBH culture was streaked over the surface of Simmons citrate (Difco) agar slants. All tests were examined for growth daily and for a color change (green to blue) for 7 days. (xiv) Serological grouping. Serological grouping was performed by the double enzyme method of Watson et al. (32). Grouping antisera were obtained from Burroughs, Wellcome and Co. for groups A, B, C, D, F, G, H, K, M, and O.

Identification scheme. The determination of a streptococcus species was divided into a two-part scheme, based on the progressive method of bacterial identification as advocated by Cowan (6). The physiological tests used in stage I served as a means for presumptive identification of all the streptococcal isolates. Depending upon the results obtained in stage I, the confirmatory identification of an organism was accomplished by a second group of tests, either stage IIa or stage IIb. The specific tests used in the identification scheme are shown in Tables 1 through 3.

RESULTS

The streptococci were separated into three divisions, based on reactions obtained in the two-phase scheme and hemolysis. Tables 1, 2, and 3 show the species represented within each division, as well as the number of isolates and the reactions obtained from them. There were five biochemical tests (bile-esculin, arabinose, growth in NaCl broth, arginine hydrolysis, and glycerol) which were either singly or in some combination pivotal for species identification within all three divisions.

The importance of bile-esculin medium was such that it readily allowed for the separation of group D streptococci (division III) from the non-group D streptococci (divisions I and II). In no instance in this study did this result in a false positive or a false negative, i.e. all group D species were positive and all non-group D species were negative.

Arginine hydrolysis was found to be most significant in differentiating among the species included in divisions II and III. Among the alpha-streptococci, arginine hydrolysis proved to be the main point of distinction between *S. sanguis* (arginine positive) and *S. salivarius* (arginine negative).

The *S. sanguis* and *S. mitis* strains listed as beta-hemolytic in division II were both alpha-hemolytic on surface streak cultures and beta-hemolytic on stab cultures. These strains could be placed in division I as nonidentified division I organisms.

If one applies the criteria of *Cowan & Steel's Manual for the Identification of Medical Bacteria* to *S. mitis*, it can be divided into *S. milleri* and *S. mitior*. The latter species does not hydrolyze either arginine or esculin (84 of 98 isolates), whereas the former species hydrolyzes either arginine or esculin (14 of 98 isolates).

Within group D, the enterococci (*S. faecalis, S. faecium*) were readily distinguished from the non-enterococci (*S. bovis, S. avium*), with the former proving to be arginine positive and the latter proving to be arginine negative. Of all the group D strains isolated, only 1 of 111 strains of *S. faecalis* gave an opposing result. All of the species included within division I proved to be arginine positive, with the exception of a single negative result occurring with a strain of *S. agalactiae* (1 of 34 isolates).

Growth in NaCl broth and the production of acid from arabinose were of importance in distinguishing among the species within division III. Among the group D isolations, growth in NaCl broth distinguished *S. faecalis, S. faecium,* and *S. avium* (all strains positive) from *S. bovis* (all strains negative). The production of acid from arabinose singled out *S. faecium* within the enterococci and *S. avium* within the non-enterococci. Of the six *S. faecium* strains isolated, all were arabinose positive. Also, four of five of the *S. avium* strains reacted with a positive result. The significance of these tests for the identification to species within divisions I and II were negligible because, with the exception of a few variants, these strains reacted negatively in both cases.

Hippurate hydrolysis, pigment production in Columbia agar, and the CAMP test were utilized in a comparison study to assess the merit of each in the presumptive identification of *S. agalactiae* (group B). Hippurate hydrolysis was used first as the screening method by which all of the hemolytic streptococcal isolates were tested. No false positives and no false negatives were obtained, i.e. all group B strains gave a positive reaction and all non-group B strains gave a negative reaction. Of the 34 group B strains, 10 (29%) failed to produce pigment on the Columbia agar. All group B strains provided a positive CAMP test.

Serological grouping was performed on all beta-hemolytic isolates and resulted in confirmation of the identification of each of the strains in division I. A random sampling of 25 of 142 of all alpha-hemolytic isolates was also performed. Of these, 17 of 25 (68%) proved to be nongroup-

TABLE 1. *Division I: 75 isolates of hemolytic streptococci from human sources showing reactions to a spectrum of tests*[a]

Test	No. of *S. pyogenes* isolates showing:				No. of *S. agalactiae* isolates showing:						No. of *S. equisimilis* isolates showing				No. of *S. anginosus* isolates showing:			No. of group G streptococcal isolates showing:			
	Complete hemolysis reaction	Positive reaction	Delayed positive reaction	Negative reaction	Incomplete hemolysis	Complete hemolysis	No hemolysis	Positive reaction	Delayed positive reaction	Negative reaction	Complete hemolysis	Positive reaction	Delayed positive reaction	Negative reaction	Complete hemolysis	Positive reaction	Negative Reaction	Complete hemolysis	Positive reaction	Delayed positive reaction	Negative reaction
Stage I																					
Hemolysis on:																					
Sheep blood agar	30				6	28					4				4			3			
Horse blood agar	30				6	27	1				4				4			3			
Rabbit blood agar	30				6	27	1				4				4			3			
Bacitracin sensitivity		30[b]						1		33				4			4		2		1
Optochin sensitivity				30						34				4			4				3
Arabinose (acid)				30					1	33				4			4				3
Bile-esculin medium				30						34				4			4				3
Growth in 6.5% NaCl		1	2	27																	
Arginine hydrolysis		30						33		1		4				4			3		
Glycerol (acid)			2	28				30	3			2	2				4			3	
Hippurate hydrolysis				30				34													
Columbia agar								24		10											
CAMP test								34													
Stage II																					
Growth on 10% bile			4	26				34				3		1		1	3			3	
Growth on 40% bile			1	29				34				1		3			4			1	2
Carbohydrates (acid)																					
Lactose		30						19	6	9		4				3	1		3		
Mannitol				30						34				4			4				3
Salicin		28		2				30	2	2		4				4			2		1

Sorbitol				30						34				4			4				3
Trehalose		29		1				28	4	2		4				4			2		1
Inulin		1		29				2		32				4			4				3
Esculin hydrolysis				30						34				4			4		3		
Serological grouping[c]			A(30)					B(34)				C(4)				F(4)				(G3)	

[a] The number of isolates and percentage of the total for each group were: *S. pyogenes*, 30 (40%); *S. agalactiae*, 34 (45.3%); *S. equisimilis*, 4 (5.3%); *S. anginosus*, 4 (5.3%); and group G streptococci, 3 (4%).

[b] Zone of inhibition varied from 9 to 16 mm.

[c] Numbers in parentheses indicate number of isolates in each serological group.

able with the antisera used. One of three of the *S. sanguis* isolates tested reacted with group H antisera, and 4 of 10 of the *S. mitis* strains tested reacted with group O antisera.

Within division III 31 of 126 isolates (24%) were grouped, including all *S. bovis*, *S. avium*, and *S. faecium* strains plus 15 *S. faecalis* strains.

The isolates are tabulated by clinical source in Table 4. All of the isolates listed for miscellaneous, urine, cerebrospinal fluid, and blood are from specimens in which they represent presumptive clinical infection. The majority of isolates (35%) came from the miscellaneous category, with infected wounds as the most common source. *S. faecalis* (34%) was the most frequent isolate in this category, followed by *S. mitis* (18%), *S. pyogenes* (15%), and *S. agalactiae* (14%). *S. milleri* was isolated from five urine, five respiratory, and four wound specimens. The respiratory isolates (28%) were studied, with no judgment being made as to their clinical significance.

Table 5 records the age and sex of 252 patients who accounted for an equal number of streptococcal isolates; of the 252 patients, there were 119 males and 133 females.

Patients in the 0- to 10-year age group had the highest number of isolates (21%). *S. faecalis* (19%), *S. mitis* (21%), and *S. pyogenes* (19%) were the species most frequently encountered in this age group. As might be anticipated, 17 of 18 (94%) of the *S. faecalis* isolates were obtained from the urinary tract. A total of 40% of the *S. pyogenes* isolations were obtained from the respiratory tract, with 60% being obtained from wound cultures. Although the majority of *S. mitis* strains would be expected to have been cultured from the respiratory tract, only 2 of 11 (18%) were from this source in the 0- to 10-year age group. A total of 54% were from wounds, and 28% were from urine infections.

There was an increase in incidence of group D isolates in the sixth and seventh decades of life. There were 41 group D isolates found in this age span; 36 of 41 (88%) were *S. faecalis*, and 23 of 36 (64%) were obtained from the urinary tract.

DISCUSSION

The use of the scheme of *Cowan & Steel's Manual for the Identification of Medical Bacteria* for the identification of streptococci proved to be both convenient and reproducible. It should be pointed out that many of the tests included in the present study were for comparison purposes only and would not be done on a routine basis. Certainly there is no need to simultaneously inoculate blood from three different species or use both hippurate and the CAMP tests.

TABLE 2. *Division II: 142 isolates of alpha-streptococci from human sources showing reactions to a spectrum of tests*[a]

Test	No. of *S. sanguis* isolates showing:				No. of *S. pneumoniae* isolates showing:			No. of *S. salivarius* isolates showing:				No. of *S. mitis* isolates showing:					No. of *S. mutans* isolates showing:			
	Incomplete hemolysis	Complete hemolysis	Positive reaction	Negative reaction	Incomplete hemolysis	Positive reaction	Negative reaction	Incomplete hemolysis	Positive reaction	Delayed positive reaction	Negative reaction	Incomplete hemolysis	Complete hemolysis	Positive reaction	Delayed positive reaction	Negative reaction	Incomplete hemolysis	Positive reaction	Delayed positive reaction	Negative reaction
Stage I																				
Hemolysis on:																				
Sheep blood agar	13	1			9			4			11	97	1				6			
Horse blood agar	13	1			9			4			11	97	1				6			
Rabbit blood agar	13	1			9			4			11	92	1			5	6			
Bacitracin sensitivity				14			9		1		14			5		93				6
Optochin sensitivity				14		9					15					98				6
Arabinose (acid)			1	13			9				15					98			2	4
Bile-esculin medium				14			9				15					98				6
Growth in 6.5% NaCl			1	13			9		5		10			15		83		4		2
Arginine hydrolysis			14				9				15			5		93				6
Glycerol (acid)				14			9				15					98			1	5
Stage IIa																				
Growth on 10% bile			7	7			9		12		3			22		76		6		
Growth on 40% bile			7	7			9		11		4			11		87		6	4	1
Carbohydrates (acid):																				
Lactose			14			9			14		1			89	2	7		6		
Mannitol				14			9				15					98		6		
Salicin			10	4			9		14		1			26		72		5		1
Sorbitol				14			9				15					98		6		
Trehalose			14			6	3		14		1			33		65		6		
Inulin			12	2		1	8		13	1	1			2		96		2		4

Esculin hydrolysis		11	3	1	8	13	2		10	88	4	2
Nitrofurazone sensitivity[b]		7	7			7	8		36	62	3	3
Serological grouping[c]		H(1), NG(2)		NG(3)		NG(3)			NG(10), O(4)		NG(2)	

[a] The number of isolates and percentage of the total for each group were: *S. sanguis*, 14 (9.9%); *S. pneumoniae*, 9 (6.4%); *S. salivarius*, 15 (10.6%); *S. mitis*, 98 (69.0%); and *S. mutans*, 6 (4.2%).

[b] In nitrofurazone tests, positive reaction = sensitive and negative reaction = resistant.

[c] Numbers in parentheses indicate number of isolates in each serological group.

The tests utilized in stage II were of no value in differentiating the species in division I or in differentiating division I from divisions II and III. The most useful characteristics were hemolysis, which provided the initial criterion for placement in division I, and group antigens, which provided the final identification.

It was quite unusual to find 17% of the division II streptococci tolerant to 6.5% NaCl, as Facklam has reported that only 2 to 4% of the common viridans streptococci are tolerant to 6.5% NaCl (12). To preclude the possibility that these organisms were members of the genus *Aerococcus*, the morphology of these organisms on gram stain was confirmed to be gram-positive cocci in chains. Also, the catalase activity was always negative, and all of the strains were glycerol negative.

As would be expected from previous studies, the majority of group A infections occurred in wounds and the upper respiratory tract. One-third of all group A infections occurred in children under 10 years of age, the majority a result of skin infections. When all ages are considered, 60% of *S. pyogenes* infections were associated with skin or wound infections. Duma et al. found in their study of streptococcal bacteremia that almost half of the patients with group A streptococcal bacteremia had an associated skin or wound infection, and frequently the skin was considered the source and/or portal of entry (9).

S. agalactiae (group B), the third most frequent isolation overall, was found in 55% of all non-respiratory infections. It was the only streptococcal species recovered from every type of clinical specimen, from males and females alike. In a recent pediatric study by Cuniff and Bump (7), group B was found to be responsible for 50% of all extrarespiratory infections.

S. equisimilis (group C) has in the past been most commonly associated with skin and puerperal infections (1, 4, 27). The group C isolates of this study were found to be equally distributed between wounds and the respiratory tract and to occasionally cause disease in humans (4, 9).

S. anginosus (group F) has been reported to be the agent responsible for infections in the sinuses, wounds, and meninges of humans. Rantz (28) and Duma et al. (9) both reported cases of bacteremia after tooth extraction.

Lancefield group G is a streptococcus without a name. The designation *S. canis* has been suggested due to its frequent association with infections in dogs. In humans, the most common infections with which it has been associated have been puerperal, skin, wound, cerebrospinal fluid, and, occasionally, septicemia (5, 9, 14, 18), and Armstrong et al. found skin, gastrointestinal

TABLE 3. *Division III: 126 isolates of group D streptococci from human sources showing reactions to a spectrum of tests*[a]

Test	Enterococci												Non-enterococci							
	No. of *S. faecalis* isolates showing:						No. of *S. faecium* isolates showing:						No. of *S. bovis* isolates showing:			No. of *S. avium* isolates showing:				
	Incomplete hemolysis	Complete hemolysis	No hemolysis	Positive reaction	Delayed positive reaction	Negative reaction	Incomplete hemolysis	Complete hemolysis	No hemolysis	Positive reaction	Delayed positive reaction	Negative reaction	Incomplete hemolysis	Positive reaction	Negative reaction	Incomplete hemolysis	Complete hemolysis	Positive reaction	Delayed positive reaction	Negative reaction
Stage I																				
Hemolysis																				
Sheep blood agar	106	5					6						4			4	1			
Horse blood agar	77	33	1				6						4			4	1			
Rabbit blood agar	73	33	5				4	1	1				4			4	1			
Bacitracin sensitivity				1		110						6			4					5
Optochin sensitivity						111						6			4					5
Arabinose (acid)				2		109				6					4				11	1
Bile-esculin medium				111						6				4				5		
Growth in 6.5% NaCl				111						6					4			5		
Arginine hydrolysis				110		1				6					4					5
Glycerol (acid)				108	3						5	1			4			4	1	
Stage IIb																				
Citrate				10	97	4						6			4					5
Carbohydrates (acid):																				
Lactose				103	7	1				6				4				5		
Mannitol				111						5		1		1	1			5		
Raffinose				2	1	108				1	1	4		3	1					5
Salicin				111						6				4				4	1	
Sorbitol				109	1	1						6			4			5		
Sucrose				105	3	3				6				4				5		
Trehalose				111						6				4				5		
Sorbose						111						6			4			5		
Serological grouping[b]				D(5)						D(6)				D(4)				D(5)		

[a] The number of isolates and percentage of the total for each group were: *S. faecalis*, 111 (88.1%); *S. faecium*, 6 (4.8%); *S. bovis*, 4 (32.%); and *S. avium*, 5 (4.0%).

[b] Numbers in parentheses indicate number of isolates in each serological group.

TABLE 4. *Distribution of streptococci in clinical specimens*

Streptococcal species	Source of specimen: Respiratory No.	%	Miscellaneous[a] No.	%	Urine No.	%	Cerebrospinal fluid No.	%	Blood No.	%	Total	% within division
Division I												
S. pyogenes	12	12.8	18	15.1	0	0	0	0	0	0	30	40.0
S. agalactiae	5	5.3	16	13.5	11	9.7	1	100	1	6.7	34	45.3
S. equisimilis	2	2.1	2	1.7	0	0	0	0	0	0	4	5.3
S. anginosus	3	3.2	1	0.8	0	0	0	0	0	0	4	5.3
Group G	1	1.1	2	1.7	0	0	0	0	0	0	3	4.0
Total	23	24.5	39	32.8	11	9.7	1	100	1	6.7	75	
Division II												
S. sanguis	8	8.5	6	5.0	0	0	0	0	0	0	14	9.9
S. pneumoniae	6	6.4	3	2.5	0	0	0	0	0	0	9	6.3
S. salivarius	7	7.5	3	2.5	3	2.6	0	0	2	13.3	15	10.6
S. mitis	48	51.1	21	17.7	23	20.2	0	0	6	40.0	98	69.0
S. mutans	1	1.1	0	0	4	3.5	0	0	1	6.7	6	4.2
Total	70	74.5	33	27.7	30	26.3	0	0	9	60.0	142	
Division III												
S. faecalis	0	0	40	33.6	69	60.5	0	0	2	13.3	111	88.1
S. faecium	0	0	4	3.4	1	0.9	0	0	1	6.7	6	4.8
S. faecium subsp. *durans*	0	0	0	0	0	0	0	0	0	0	0	0
S. bovis	0	0	1	0.8	1	0.9	0	0	2	13.3	4	3.2
S. equinus	0	0	0	0	0	0	0	0	0	0	0	0
S. avium	1	1.1	2	1.7	2	1.8	0	0	0	0	5	4.0
Total	1	1.1	47	39.5	73	64.1	0	0	5	33.3	126	

[a] Abscesses and wounds from various anatomical origins, genitourinary tract, umbilicus, placenta, and postmortem specimens.

tract, and respiratory tract infections due to group G streptococci second in frequency only to group A (1). In this study two of the three group G isolates were recovered from wound infections, with the remaining isolate from the respiratory tract. Two of the three isolates would have been identified as group A if bacitracin sensitivity had been the sole criterion.

S. sanguis, S. salivarius, S. mitior, S. milleri, and *S. mutans* have been collectively known in the past as *S. viridans* and were thought to be just an undefinable cluster of organisms. Their identification to species has been overlooked and regarded as unnecessary. *S. mutans* has been overlooked and regarded as unnecessary. *S. mutans* has been associated with dental caries, and the remaining species have been associated with endocarditis. Parker and Ball have shown the advantages of identification to species in their excellent study on systemic streptococcal infections (25). They showed that *S. milleri* was most often isolated from purulent lesions, whereas this study suggests that the respiratory or urinary tract may be the source of these organisms. In this study all of these streptococci were found in a variety of extrarespiratory infectious situations in substantial numbers. Respiratory isolates aside, it could be argued that these organisms were, in fact, bystanders or contaminants, but such is not likely to be the case based on the manner in which they were chosen for inclusion in this study.

S. faecalis accounted for 61% of all urine streptococcal isolates. Approximately 65% of these occurred in patients over the age of 50 years; almost 60% were females, in contrast to previous findings in which they occurred almost four times more often in elderly males (9). *S. faecalis* was also commonly isolated from wounds (40%), either alone or with other organisms. Horvitz and Von Graevenitz have questioned the significance of enterococcus isolates from wounds (19). Although our study does not determine the true clinical significance of enterococcus isolates from wounds, it must be remembered that these wound cultures were not taken prophylactically, which means that the physician submitted the culture because there was clinical evidence of an infectious process. Also, the isolation of enterococci in large numbers as the sole isolate recorded could indicate significance and should not be totally ignored.

TABLE 5. *Relationship of streptococcal species to patient sex and age for 252 isolates*

Streptococcal species	Age and sex distribution[a]																										
	0-10 years			11-20 years			21-30 years			31-40 years			41-50 years			51-60 years			61-70 years			71-80 years			81-90 years		
	No.	M	F	No.	M	F	No.	M	F	No.	M	F	No.	M	F	No.	M	F	No.	M	F	No.	M	F	No.	M	F
Division I: hemolytic streptococci																											
S. pyogenes	10	7	3	2	1	1	3	0	3	3	2	1	2	1	1	5	1	4	1	1	0	2	0	2	1	1	0
S. agalactiae	4	0	4	1	1	0	3	0	3	5	1	4	6	3	3	2	0	2	0			1	0	1	0		
S. equisimilis	1	1	0	0			0			1	0	1	0			0			0			0			0		
S. anginosus	1	0	1	0			0			1	0	1	0			0			0			0			0		
Group G	0			0			0			1	0	1	0			0			0			0			0		
Total	16	8	8	3	2	1	6	0	6	11	3	8	8	4	4	7	1	6	1	1	0	3	0	3	1	1	0
Division II: alpha-streptococci																											
S. sanguis	1	1	0	1	0	1	0			1	0	1	0			2	1	1	1	1	0	1	0	1	0		
S. pneumoniae	3	1	2	1	1	0	0			0			0			0			2	2	0	0			0		
S. salivarius	1	0	1	1	0	1	0			3	0	3	0			0			1	1	0	0			0		
S. mitis	11	6	5	11	3	8	3	2	1	4	3	1	9	7	2	7	3	4	13	11	2	6	1	5	1	0	1
S. mutans	0			0			0			0			0			0			11	0	1	2	2	0	0		
Total	16	8	8	14	4	10	3	2	1	8	3	5	9	7	2	9	4	5	18	15	3	9	3	6	1	0	1
Division III: group D streptococci																											
S. faecalis	18	7	11	3	2	1	10	3	7	9	7	2	7	4	3	13	5	8	23	11	12	15	7	8	2	0	2
S. faecium	0			1	1	0	1	1	0	0			0			1	0	1	0			0			0		
S. bovis	1	0	1	0			0			0			0			1	1	0	0			0			0		
S. avium	1	1	0	0			0			0			0			1	1	0	2	2	0	0			0		
Total	20	8	12	4	3	1	11	4	7	9	7	2	7	4	3	16	7	9	25	13	12	15	7	8	2	0	2

[a] M, Male; F, female.

Although *S. faecalis* and *S. faecium* can be cultured from similar sources, the incidence of *S. faecium* is much less (31). Of 117 enterococcal strains encountered during this work, only 5% were *S. faecium*. This is similar to the findings of Gross et al. that *S. faecium* constituted 7% of 2,503 group D clinical isolates (16).

An interesting finding among the group D isolation was the occurrence of *S. avium* (4%). This incidence was comparable to *S. bovis* (3%) and *S. faecium* (5%). In 1970 Facklam and Moody reported that *S. avium* should rarely be of consequence to a clinical laboratory concerned with the diagnosis of human infection (13). A 1972 study by Facklam supported this (11). In 1975 Gross et al. reported that *S. avium* constituted 2% of all group D isolations from a variety of clinical sources (16). Because of these previous reports we found a 4% recovery rate to be of note. As this species provides a positive bile-esculin reaction and grows in 6.5% NaCl, most laboratories would be likely to automatically identify it as an enterococcus. The use of sorbose allows for *S. avium* to be separated, because it is the only group D species which can produce acid with the carbohydrate. The need for such separation may become apparent when more is learned about the penicillin susceptibility of this organism.

It is not our recommendation that every streptococcal isolate routinely be identified to species by every clinical microbiology laboratory. However, it is apparent from this study and from the literature that more epidemiological studies, complete with identification to species, need to be done to determine the true pathogenesis of many streptococcal species.

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