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Hemolysins and Other Characteristics That Help Differentiate and Biotype *Staphylococcus lugdunensis* and *Staphylococcus schleiferi*

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Reference strains and clinical isolates representing the newly defined species *Staphylococcus lugdunensis* and *Staphylococcus schleiferi* were examined with the battery of tests previously recommended (G. A. Hébert, C. G. Crowder, G. A. Hancock, W. R. Jarvis, and C. Thornsberry, J. Clin. Microbiol. 26:1939–1949, 1988) for other species of coagulase-negative staphylococci (CNS). The Staph-Ident system (Analytab Products, Plainview, N.Y.) supplemented with tests for synergistic hemolysis, adherence to glass, pyroglutamyl-β-naphthylamide hydrolysis, and susceptibility to a set of five antimicrobial disks differentiated each of these species from other species of CNS and separated strains within each species into several biotypes. Most strains (95%) of *S. lugdunensis* produced a delta hemolysin like that seen with nine other species of CNS. Most strains (91%) of *S. schleiferi* produced a beta hemolysin, which is a unique characteristic among CNS. Most (95%) of the *S. schleiferi* but very few (12%) of the *S. lugdunensis* were adherence positive. Both hemolysins and adherins are potential virulence factors among CNS. Some (29%) of the *S. lugdunensis* were β-lactamase positive. The *S. lugdunensis* were resistant to polymyxin B and bacitracin (10 U), but the *S. schleiferi* were susceptible to both disks. Clinical isolates of *S. lugdunensis* were aligned in 18 biotypes because of eight biochemical profiles and eight physiologic subtypes; isolates of *S. schleiferi* were in 8 biotypes because of three biochemical profiles and subtypes. These tools for correctly identifying and then biotyping two more clinical species of CNS should enhance both epidemiologic and ecologic investigations.

Staphylococci are frequently isolated from human and animal specimens and environmental samples. There are currently 27 distinct species in the genus *Staphylococcus*, and 23 of those species are coagulase negative. The pathogenic role of several of the species of coagulase-negative staphylococci (CNS) is now well established, and CNS are recognized as one of the most frequent causes of nosocomial infections (8). Yet the question of clinical significance of the various species is still unresolved, because (i) many laboratories do not identify clinical isolates of CNS to the species level, (ii) the CNS are frequently opportunistic pathogens, and (iii) the natural habitats of many species of CNS include the skin and nares of humans (10).

The results from studies of phenotypic characteristics of 86 reference strains and 1,137 clinical isolates representing 19 different species of CNS were presented in earlier reports (5, 6); tests for synergistic hemolysis, adherence to glass, pyroglutamyl-β-naphthylamide (PYR) hydrolysis, and susceptibility to a set of five antimicrobial agents were used with the Staph-Ident system (Analytab Products, Plainview, N.Y.) to differentiate these species (6) and to define a biotyping scheme for epidemiologic studies (5).

Reference strains and clinical isolates representing two recently described species of CNS, *S. lugdunensis* and *S. schleiferi* (4), have now been examined for the characteristics described above and other phenotypic characteristics that could help differentiate and biotype them. The results of those studies are presented in this report, which includes comparisons of the 2 new species with the 19 previously discussed.

MATERIALS AND METHODS

Cultures and growth conditions. A total of 65 strains representing the species *S. lugdunensis* and *S. schleiferi* were examined. The 10 reference strains (Table 1) were obtained from the French National Reference Center for Staphylococci, Lyon, France. The set of 55 clinical isolates came from culture collections of several laboratories at the Centers for Disease Control; they were isolated between 1971 and 1990 in various states in the United States and from various sources including blood, abscess, urine, foot, toe, eye, nose, catheter, prosthesis, a perfusion machine, and an operating-room table. All the strains were subcultured on Trypticase soy agar containing 5% defibrinated sheep blood (TSA II; BBL Microbiology Systems, Cockeysville, Md.) and incubated aerobically for 18 to 24 h at 35°C. For long-term storage, 24-h growth was harvested in sterile rabbit blood and kept at −70°C. Strains removed from storage were subcultured on TSA II at least twice before testing.

Identification. All tests were done with 24-h growth from a TSA II plate and included control strains from the collection of type strains of staphylococci (6). All of the 65 strains were gram-positive cocci that produced catalase. All 65 strains were tested for coagulase activity in both slide and tube tests with coagulase plasma (rabbit; Difco Laboratories, Detroit, Mich.) and in card tests with the Staphaurex latex suspension (Wellcome Diagnostics, Research Triangle Park, N.C.). The strains were tested for fibrinogen affinity factor with Staphyloslide (BBL Microbiology Systems) and production of ornithine decarboxylase in Moeller decarboxylase medium (Carr-Scarborough Microbiologicals, Stone Mountain, Ga.). All of the strains were tested with the Staph-Ident system of API (Analytab Products), which includes tests for (i) production of alkaline phosphatase, β-glucosidase, β-glucuronidase, and β-galactosidase; (ii) utilization of urea and arginine; and (iii) acid production from mannose, mannitol, trehalose, and salicin. The panels of the system were inoculated and processed according to the procedures rec-

TABLE 1. Characteristics of the 10 reference strains[a]

Species and strain	Results from test for:						Inhibition zone diam (mm)				
	FF	PYR	OC	APId	AH	SH	Nov	Poly	Bac	Fur	TXO
S. lugdunensis											
ATCC 43809[b]	+	+	+	6500	−	+	20	8	6	20	6
995	+	+	+	6400	−	+	18	8	6	20	6
1174	+	+	+	6000	−	+	17	8	16	20	6
1257	+	+	+	4500	+	+	18	9	6	23	6
N860165	+	+	+	6400	−	+	17	9	6	21	6
S. schleiferi											
ATCC 43808[b]	+	+	−	1141	+	−	20	12	15	20	6
N860173	+	+	−	1141	+	−	16	14	14	21	6
N860215	+	+	−	1041	+	−	17	13	15	21	6
N860265	+	+	−	1041	+	−	17	13	15	20	6
N860346	+	+	−	3041	−	−	16	12	13	19	6

[a] FF, Fibrinogen affinity factor; PYR, PYR hydrolysis; OC, ornithine decarboxylase; APId, Staph-Ident profile register number; AH, adherence to glass; SH, synergistic hemolysis; Nov, novobiocin (5 μg); Poly, polymyxin B (300 U); Bac, bacitracin (10 U); Fur, furazolidone (100 μg); TXO, Taxo A or bacitracin (0.04 U); +, positive; −, negative.

[b] Type strain of the species. The results of some of these tests with type strains are the same as those reported earlier (4) with European test systems, but the results for the other strains have not been reported individually and some of the tests used here were not used in the previous study.

ommended by the manufacturer. The resulting biochemical profiles were expressed as four-digit numbers.

Synergistic hemolysis. All of the isolates were tested for synergistic hemolysis, as previously described (6, 7). Briefly, a strain of *S. intermedius* (AB 148, ATCC 49052) was streaked down the center of a TSA II plate, and test strains were streaked perpendicular to but not touching the center inoculum. The plates were incubated aerobically at 35°C for 18 to 20 h and then held at room temperature for 4 to 6 h before the reactions were read. A zone of complete hemolysis (where the test strain was growing) within the zone of incomplete hemolysis caused by the beta lysin from the *S. intermedius* growth was a positive test.

Adherence. All of the isolates were tested for adherence to glass culture tubes, as described by Christensen et al. (1); the details of the qualitative procedure used were discussed previously (6). Briefly, after the cells had grown overnight in a glass tube of Trypticase soy broth, the turbid broth was poured out; the tubes were then rinsed once, filled with safranin to stain for 30 min, rinsed twice, and inverted to drain and dry. The dry tubes were examined for evidence of a stained film, and the reactions were recorded as negative, weakly positive, moderately positive, or strongly positive. Controls included the reference strains described by Christensen et al. (2); control strain ATCC 35983 (RP 12) was strongly positive, and strain ATCC 35982 (SP 2) was negative in the adherence tube test.

PYR hydrolysis. All strains were tested for PYR hydrolysis with the commercial kit of PYR broth and PYR reagent (Carr-Scarborough), as described previously (6). Briefly, growth from a TSA II plate was emulsified in the broth and incubated at 35°C for 2 h; PYR reagent was then added to each tube. The development of a dark color (purple red) within 2 min was recorded as a positive test; a pink, orange, or yellow color was recorded as negative. The controls included positive and negative staphylococci from the culture collection.

Disk diffusion susceptibility studies. All strains were tested for susceptibility to a set of five antimicrobial agents by a disk diffusion method with TSA II plates, as described previously (6); this is not the standard susceptibility test used in diagnostic testing. The five antimicrobial disks (BBL) used were novobiocin (5 μg), polymyxin B (300 U), bacitracin (10 U), furazolidone (100 μg), and Taxo A (bacitracin, 0.04 U). Briefly, the plates were streaked evenly in one direction with the rinsed, pressed swabs used to inoculate broth for the adherence tests. After placing the disks in position with forceps, the plates were put in plastic bags to conserve moisture and were then incubated at 35°C for 24 h. The diameters of the circular inhibition zones were carefully measured in reflected light with a sliding caliper. A zone size of 6 mm (the width of the paper disk) was recorded when no clear zone of growth inhibition was visible.

TABLE 2. Characteristics of clinical isolates[a]

Organism (no. tested)	No. (%) of strains positive for:			
	Fibrinogen affinity	PYR hydrolysis	Synergistic hemolysis	Adherence tube test
S. lugdunensis (38)	30 (78.9)	38 (100.0)	36 (94.7)	4 (10.5)
S. schleiferi (17)	17 (100)	16 (94.1)	0 (0)[b]	17 (100)

[a] In ornithine decarboxylase tests, all strains of *S. lugdunensis* were positive and all strains of *S. schleiferi* were negative.

[b] A total of 15 isolates (88%) produced a distinct zone of incomplete hemolysis that appeared homologous with the beta hemolysin of *S. intermedius*.

β-Lactamase test. All strains were also examined for β-lactamase production by a rapid chromogenic cephalosporin test, as previously described (5). Briefly, a loopful of 18- to 24-h growth on TSA II was emulsified in 2 drops of nitrocefin solution in the well of a microdilution plate. The inoculated wells were sealed with strips of cellophane tape to prevent evaporation, incubated at room temperature, and observed for color change. Reactions were recorded after 1 h as negative (yellow), weakly positive (dark orange), or positive (red). When an isolate tested negative, it was retested after induction with oxacillin by repeating the test with the 18- to 24-h growth from around an oxacillin disk (5 μg). Known β-lactamase-positive and β-lactamase-negative strains of *S. aureus* were included as controls.

Antimicrobial profiles. All of the reference strains and most of the clinical isolates of both species were tested for susceptibility to 19 antimicrobial agents by either broth microdilution or agar disk diffusion or both, as previously described (11, 12). The drugs used during this study were penicillin, nafcillin, oxacillin, methicillin, amoxicillin-clavulanate, ampicillin-sulbactam, imipenem, cephalothin, tetracycline, minocycline, gentamicin, tobramycin, chloramphenicol, clindamycin, erythromycin, vancomycin, ciprofloxacin, rifampin, and sulfamethoxazole-trimethoprim.

RESULTS

All of the strains were negative in both of the coagulase tests with rabbit plasma and in the Staphaurex latex test for protein A and clumping factor. A fibrinogen affinity factor was detected in all of the reference strains of both species and in all of the clinical isolates of *S. schleiferi* but in only 79% of the clinical isolates of *S. lugdunensis* (Tables 1 and 2). All the *S. lugdunensis* and none of the *S. schleiferi* were positive for ornithine decarboxylase.

In the Staph-Ident panels, the *S. lugdunensis* produced β-glucosidase (95%), utilized urea (70%) and arginine (9%), and produced acid from trehalose (63%) and mannose (9%). All of the *S. schleiferi* produced alkaline phosphatase and

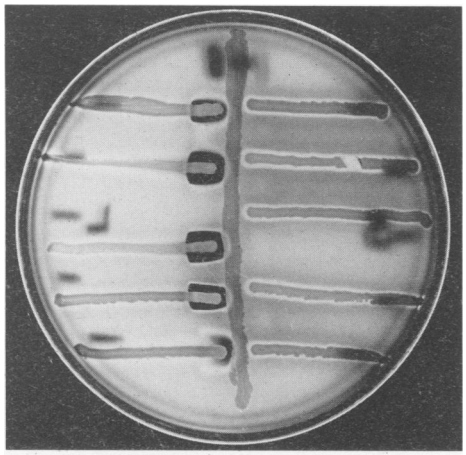

FIG. 1. Synergistic hemolysis between strain AB 148 of *S. intermedius* (vertical streak of growth) and the reference strains of *S. lugdunensis* (left) and *S. schleiferi* (right). Each of the *S. lugdunensis* produced a clear zone of synergistic, complete hemolysis within the zone of incomplete hemolysis produced by the beta lysin activity from the *S. intermedius*. All of the *S. schleiferi* were negative, and each produced a zone of incomplete hemolysis that appeared homologous with the beta lysin of the *S. intermedius*.

β-galactosidase, and all utilized arginine; 14% utilized urea, and 36% produced acid from mannose. The 43 strains of *S. lugdunensis* defined 10 distinct profile codes, and the 22 strains of *S. schleiferi* defined 3 distinct codes. Only two of the four-digit biochemical profile codes were found on the Staph-Ident profile register that accompanied each kit, and they were both generated by strains of *S. lugdunensis*; 11 strains had the 6400 profile of *S. warneri*, and 1 strain had the 2040 profile of *S. hominis*.

Synergistic hemolysis. All of the reference strains and 95% of the clinical isolates of *S. lugdunensis* gave a distinct, clear zone of synergistic, complete hemolysis when tested against the beta hemolysin of *S. intermedius* (Tables 1 and 2). None

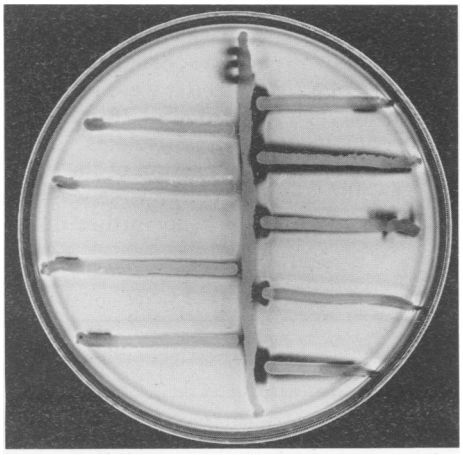

FIG. 2. Synergistic hemolysis between the type strain of *S. schleiferi* (ATCC 43808; vertical streak of growth) and the reference strains of *S. lugdunensis* (right) and *S. schleiferi* (left). The type strain of *S. schleiferi* produced a zone of incomplete hemolysis that was enhanced to complete hemolysis in the areas of hemolysin activity from each of the strains of *S. lugdunensis*. The other strains of *S. schleiferi* gave no reaction except the zone of incomplete hemolysis as produced by the type strain.

TABLE 3. Susceptibilities of clinical isolates to polymyxin B (300 U) disks on TSA II

Organism (no. of strains tested)	Mean (range) inhibition zone diam (mm)	No. of strains with inhibition zone diam[a] (mm) of:			
		6	7–9	10	>10
S. lugdunensis (38)	8.6 (6–12)	2	27	7	2
S. schleiferi (17)	14.4 (14–15)	0	0	0	17

[a] A 6-mm zone is the same as the diameter of the disk and implies no inhibition. Resistance to the polymyxin disk on TSA II is defined for CNS as an inhibition zone diameter of <10 mm (6). A total of 29 (76.3%) *S. lugdunensis* isolates and none of the *S. schleiferi* isolates had inhibition zone diameters of <10 mm.

of the *S. schleiferi* were positive in this test, but all reference strains and 88% of the clinical isolates produced a distinct zone of incomplete hemolysis that appeared homologous with beta hemolysin. The type strain of *S. schleiferi* was, therefore, used to test the other nine reference strains and many clinical isolates. Strains of *S. lugdunensis* that were positive in the standard test for synergistic hemolysis were also positive when *S. schleiferi* was used instead of *S. intermedius*. The reactions of all the reference strains are shown in Fig. 1 and 2.

Adherence to glass. One reference strain and only 4 of 38 (11%) clinical isolates of *S. lugdunensis* were positive in the adherence test (Tables 1 and 2); 2 of the clinical positives were only weakly positive, and only 1 was strongly positive. All clinical isolates and all but one reference strain of *S. schleiferi* were positive; most were moderately positive, but four were weakly positive and three were strongly positive.

PYR hydrolysis. All of the reference strains of both species were PYR positive. All of the clinical isolates of *S. lugdunensis* and all but one of the clinical isolates of *S. schleiferi* were also PYR positive (Tables 1 and 2).

Polymyxin B. The results of polymyxin disk susceptibility tests of reference strains on TSA II are shown in Table 1. Resistance to this disk is defined for CNS as an inhibition zone diameter of <10 mm (6). All the reference strains of *S. lugdunensis* were resistant to the disk, with recorded zone sizes of 8 to 9 mm. The results of polymyxin tests with clinical isolates are shown in Table 3. The mean inhibition zone diameter for *S. lugdunensis* was 8.6 mm; most (76%) of the clinical isolates were resistant, with inhibition zones of <10 mm, and two isolates were completely resistant to this drug, i.e., they gave no zone of inhibition with this disk. All of the reference strains and clinical isolates of *S. schleiferi* were susceptible to this disk, with measured zone sizes of 12 to 15 mm.

Bacitracin (10 U). The results of the bacitracin disk susceptibility tests of reference strains on TSA II are shown in

TABLE 4. Susceptibilities of clinical isolates to bacitracin (10 U) disks on TSA II

Organism (no. of strains tested)	Mean (range) inhibition zone diam (mm)	No. of strains with inhibition zone diam[a] (mm) of:			
		6	7–9	10	>10
S. lugdunensis (38)	6.4 (6–14)	33	4	0	1
S. schleiferi (17)	16.4 (15–18)	0	0	0	17

[a] A 6-mm zone is the same as the diameter of the disk and implies no inhibition. Resistance to this bacitracin disk on TSA II is defined for CNS as an inhibition zone diameter of <11 mm (6). A total of 37 (97.4%) *S. lugdunensis* isolates and none of the *S. schleiferi* isolates had inhibition zone diameters of <11 mm.

TABLE 5. Disk susceptibilities of clinical isolates on TSA II

Organism (no. of strains tested)	Mean (range) inhibition zone diam (mm)		
	Novobiocin (5 μg)	Bacitracin (0.04 U)[a]	Furazolidone (100 μg)
S. lugdunensis (38)	18.2 (15–21)	6	21.1 (17–24)
S. schleiferi (17)	18.8 (18–20)	6	22.9 (22–25)

[a] A 6-mm zone is the same as the diameter of the disk and implies no inhibition. All isolates tested against this concentration of bacitracin had no visible zones of inhibition.

Table 1. Resistance to this disk is defined for CNS as an inhibition zone diameter of <11 mm (6). Most of the *S. lugdunensis* were completely resistant to this antimicrobial agent, with inhibition zones of 6 mm, but one strain had a 16-mm zone. The results of bacitracin tests with clinical isolates are shown in Table 4. The mean inhibition zone diameter for clinical isolates of *S. lugdunensis* was 6.4 mm, because most (87%) of them were completely resistant, and only one isolate had an inhibition zone diameter of >9 mm. All of the reference strains and clinical isolates of *S. schleiferi* were susceptible to this disk, with zone sizes of 13 to 18 mm.

Novobiocin, Taxo A, furazolidone. The results of novobiocin, Taxo A, and furazolidone disk susceptibility tests of reference strains and clinical isolates on TSA II are shown in Tables 1 and 5. All of these staphylococci were (i) susceptible to novobiocin, with inhibition zone sizes of 15 to 21 mm; (ii) susceptible to furazolidone, with inhibition zone sizes of 17 to 25 mm; and (iii) completely resistant to the lower concentration of bacitracin in the Taxo A disk, leaving only the 6-mm disk to measure.

β-Lactamase. All of the reference strains of both species were negative in the nitrocefin test for β-lactamase production. All of the clinical isolates of *S. schleiferi* were also negative, but 29% (11 of 38) of the clinical isolates of *S. lugdunensis* were positive.

Antimicrobial profiles. Most of these staphylococci were very susceptible to all of the antimicrobial agents tested by one or both methods; however, some resistance was detected in a few strains of each species. Clinical isolates of *S. lugdunensis* exhibited resistance to the penicillins (10 strains), tetracycline (2 strains), and gentamicin and tobramycin (1 strain); resistance to tetracycline was also detected in two of the isolates of *S. schleiferi*.

Characteristics. Since all of the strains of these new species were susceptible to novobiocin and all but one strain were also PYR positive, the other characteristics of the reference strains of these and the seven other species of CNS whose strains are both novobiocin susceptible and PYR positive are compared in Table 6.

TABLE 7. Definition of subtypes in the original subtyping scheme[a]

Subtype code	Results	
	Adherence tube test	Synergistic hemolysis
1a	+ strong	+
1b	+ moderate	+
1c	+ weak	+
2a	+ strong	−
2b	+ moderate	−
2c	+ weak	−
3	−	+
4	−	−

[a] For a review, see reference 5. +, Positive; −, negative

Biotyping. To separate strains of these two species into biotypes in the same way reported for the other CNS (5), the biochemical profiles obtained with the Staph-Ident system for each species were listed in the order of the frequency of occurrence and were assigned letter codes. Those original physiologic subtypes for biotyping were defined by the results obtained with the adherence and synergistic hemolysis tests; subtypes 1 and 2 were then further split into classes by degrees of adherence (Table 7).

The biotypes and subtypes of clinical isolates that resulted from these definitions are shown in Table 8. The 38 clinical isolates of *S. lugdunensis* were in eight biotypes and subdivided into only four more by the subtypes for a total of 12 profiles; 33 of 38 isolates were in subtype 3. The 17 clinical isolates of *S. schleiferi* were in only three biotypes but subdivided into five more by the subtypes for a total of eight profiles; 6 of 17 isolates were in biotype A2b.

Since the clinical isolates of *S. lugdunensis* gave variable but reproducible results with the tests for fibrinogen affinity factor and β-lactamase production, these tests results were combined with those for synergistic hemolysis and adherence to define the additional physiologic subtypes (Table 9).

The biotypes and additional subtypes of clinical isolates that resulted from this expanded scheme are shown in Table 10. The 8 biotypes of *S. lugdunensis* were subdivided into 10 more by these subtypes for a total of 18 profiles. Biotypes A6b and B6b contained the most isolates, but this wider distribution is a much-better reflection of the diversity seen among isolates of this species. The reverse was true with the clinical isolates of *S. schleiferi*; no further divisions were

TABLE 6. Characteristics of novobiocin-susceptible, PYR-positive reference strains of CNS[a]

Organism (no. tested)	No. positive for:			Inhibition zone sizes (mm)		Biochemical Staph-Ident profiles
	OC	AH	SH	Poly	Bac	
S. auricularis (6)	0	0	0	14–17	6–21	0040, 0440, 0441, 0541
S. caprae (1)	0	1	1	13	14	0040
S. carnosus (2)	0	0	0	13–14	12–13	1241
S. caseolyticus (2)	0	0	0	14–15	18–19	0000, 0040
S. chromogenes (7)	0	0	1	6	15–20	2000, 2040, 2400
S. haemolyticus (4)	0	1	4	12–14	9–13	0060, 0460, 0640, 4440
S. lugdunensis (5)	5	1	5	8–9	6–16	4500, 6000, 6400, 6500
S. schleiferi (5)	0	4	0	12–14	13–15	1041, 1141, 3041
S. simulans (2)	0	2	2	12–14	12–14	2061, 2461

[a] OC, Ornithine decarboxylase; AH, adherence to glass; SH, synergistic hemolysis; Poly, polymyxin B disk (300 U); Bac, bacitracin disk (10 U).

TABLE 8. Biotypes and original subtypes[a] of clinical isolates

Organism (no. tested) and biotype code	Staph-Ident profile code	No. of strains in subtype:						
		1b	1c	2a	2b	2c	3	4
S. lugdunensis (38)								
A	6000		2				11	
B	6400						9	
C	4400			1			6	1
D	6440						3	
E	4500	1					1	
F	4000						1	
G	2040						1	
H	0400						1	
S. schleiferi (17)								
A	1041			1	6	2		
B	1141			1	4	1		
C	3041				1	1		

[a] Subtypes were defined by degrees of adherence as shown in Table 7.

TABLE 10. Biotypes and additional subtypes[a] of clinical isolates

Organism (no. tested) and biotype code	Staph-Ident profile code	No. of strains in subtype:							
		5a	5b	6a	6b	6c	6d	7b	8c
S. lugdunensis (38)									
A	6000		2	3	8				
B	6400				7	1	1		
C	4400				3	2	1	1	1
D	6440			1		2			
E	4500	1			1				
F	4000				1				
G	2040				1				
H	0400				1				
S. schleiferi (17)									
A	1041							9	
B	1141							6	
C	3041							2	

[a] Subtypes were defined with the expanded scheme shown in Table 9.

made by these additional subtypes, so all 17 isolates were in the same subtype and differed only in Staph-Ident biotype code.

DISCUSSION

This report does not include detailed descriptions of many of the major phenotypic characteristics of these new species or the classical genetic data associated with such studies, because the initial report by Freney et al. (4) presented those basic observations. The purpose of this report is to add test data from the new species to the body of data on 19 other CNS (5, 6) to determine whether the previously recommended tests can still be used to correctly identify and then biotype isolates of CNS.

As stated in the initial report on synergistic hemolysis exhibited by staphylococci (7), a beta hemolysin produces a wide zone of incomplete hemolysis and a delta hemolysin produces a narrow zone of complete hemolysis on agar containing sheep erythrocytes; furthermore, a delta hemolysin potentiates the zone of a beta hemolysin to complete clearing on sheep cells. The activity of strains of *S. schleiferi* on TSA II is consistent with the definition for a beta hemolysin, and the activity of strains of *S. lugdunensis* on TSA II matches the definition of delta hemolysin. These distinct hemolytic activities are clearly demonstrated in Fig. 2 with the reference strains; delta hemolysin from each of the strains of *S. lugdunensis* produced a clear zone of complete hemolysis within the zone of incomplete hemolysis caused by the beta hemolysin from the strain of *S. schleiferi*. Most (91%) of the *S. schleiferi* produced a beta hemolysin, which is a unique characteristic among species of CNS. Beta hemolysin has been reported for only two other species of staphylococci, *S. aureus* and *S. intermedius*, and they are both coagulase positive. The delta hemolysin produced by most (95%) of the *S. lugdunensis* appeared no different from that reported earlier for nine other species of CNS (6, 7). The beta and delta hemolysins produced by these newly described species were not on the original list of phenotypic characteristics attributed to them (4) but have proved useful for differentiation. In addition, any hemolytic activity among species in in vitro synergistic relationships could relate to their virulence and, therefore, their clinical significance.

TABLE 9. Definition of additional subtypes in the expanded scheme

Subtype code	Results[a] from tests for:			
	Synergistic hemolysis	Adherence to glass	Fibrinogen affinity	β-Lactamase production
5a	+	+	+	+
5b	+	+	+	−
5c	+	+	−	+
5d	+	+	−	−
6a	+	−	+	+
6b	+	−	+	−
6c	+	−	−	+
6d	+	−	−	−
7a	−	+	+	+
7b	−	+	+	−
7c	−	+	−	+
7d	−	+	−	−
8a	−	−	+	+
8b	−	−	+	−
8c	−	−	−	+
8d	−	−	−	−

[a] +, Positive; −, negative.

Adherence also helped differentiate these species because most (95%) of the *S. schleiferi* were positive and most (88%) of the *S. lugdunensis* were negative. Adherence is presumed to be evidence of slime production (1), which has been associated with the ability of an organism to cause disease in patients with prosthetic devices (3). This characteristic has been reported among strains of all major species of staphylococci associated with human infections (6) and has also been useful to clinicians in evaluating clinical significance among cases of nosocomial septicemia (9).

Susceptibility to the set of five antimicrobial disks on TSA II again proved useful for differentiating species. *S. lugdunensis* was resistant to polymyxin B and the higher concentration of bacitracin, but *S. schleiferi* was susceptible to both of the disks. Both species were susceptible to novobiocin, a characteristic shared with 11 other species of CNS; *S. lugdunensis* is currently, however, the only species of CNS that is susceptible to novobiocin but resistant to both polymyxin B and bacitracin (10 U). Like all species of staphylococci, both of these were susceptible to furazolidone and resistant to Taxo A disks.

All of the *S. lugdunensis* and all but one of the *S. schleiferi* were PYR positive, as were nine of the other species of CNS reported earlier (6). Only three of those species (*S. hae-*

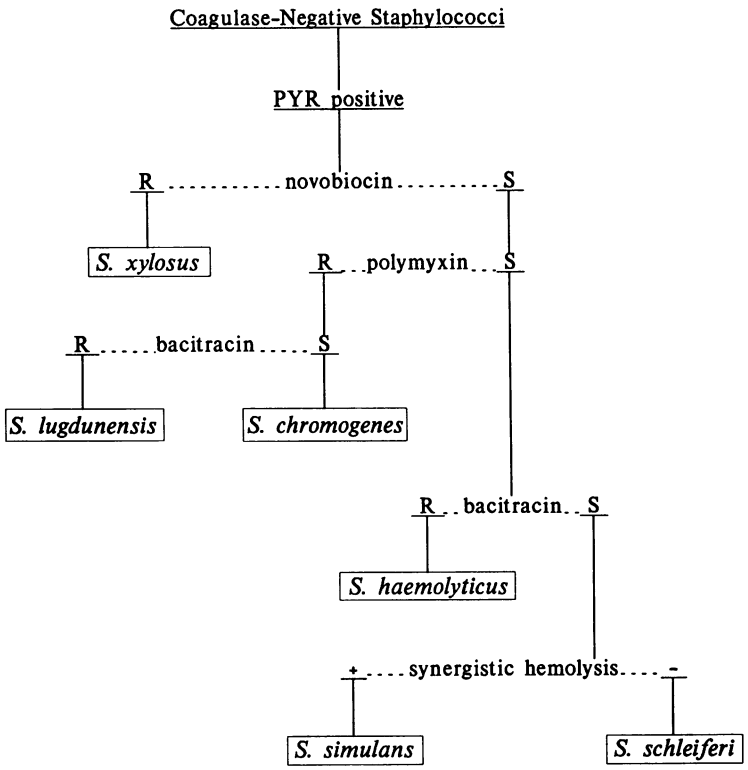

FIG. 3. Flow chart of characteristics that help differentiate some of the PYR-positive species of CNS. The R and S used here indicate resistant and susceptible, respectively, as defined in this report and a previous report (6) but do not refer to resistant and susceptible as defined by a standardized susceptibility test.

molyticus, *S. simulans*, and *S. xylosus*) are usually associated with human infections, however, and they can easily be distinguished from each other and from these new species with other tests (Fig. 3). The novobiocin disk can be used to separate the resistant *S. xylosus* from the other PYR-positive CNS. The polymyxin B disk can be used to separate the resistant *S. lugdunensis* from *S. haemolyticus*, *S. simulans*, and *S. schleiferi*. The bacitracin disk can be used to separate the resistant *S. haemolyticus* from *S. simulans* and *S. schleiferi*, and the synergistic hemolysis test can be used to separate *S. schleiferi* from the positive *S. simulans*.

Unlike the other species of CNS, both *S. lugdunensis* and *S. schleiferi* could produce a fibrinogen affinity factor; this feature and the production of ornithine decarboxylase by *S. lugdunensis* were reported earlier (4). All tests for the fibrinogen affinity factor with strains of *S. schleiferi* were positive, although a few strains were slow or weak. A fibrinogen affinity factor was never detected in some of the strains of *S. lugdunensis* and was seen with other strains of the species only after several more transfers on TSA II. This characteristic is, therefore, useful for identifying *S. schleiferi* but not reliable for identifying *S. lugdunensis*. Some strains of *S. epidermidis* and rare strains of *S. haemolyticus* and *S. warneri* have been reported to decarboxylate ornithine, and since *S. haemolyticus* is also PYR positive, the ornithine reaction is not completely reliable for identifying isolates of *S. lugdunensis*.

Strains of both *S. lugdunensis* and *S. schleiferi* were active in the Staph-Ident panels and generated codes that could help identify the species. Although some strains of *S. lugdunensis* gave codes for *S. warneri* and *S. hominis*, neither of those species is PYR positive. The Staph-Ident profile combined with the results from a few additional tests like those described in this and an earlier report (6) made this system even more useful by identifying more species of staphylococci. The biochemical profiles from this system also served as a convenient basis for biotyping strains of each species.

The original battery of tests (6) can still be used to correctly identify and then biotype isolates of staphylococci. New tests can be added, however, to incorporate these two new species into the scheme. Adding the test for ornithine decarboxylase can help confirm the identification of *S. lugdunensis*, and adding the test for fibrinogen affinity factor can help confirm the identification of *S. schleiferi* as well as biotype isolates of *S. lugdunensis*.

The original subtyping scheme (5) was especially helpful for differentiating strains of *S. schleiferi* but was less helpful with strains of *S. lugdunensis*. Since the purpose of biotyping is to help the user, biotyping should be flexible and adaptable to the needs of the user. *S. lugdunensis* is an important, newly defined clinical species of CNS, and to understand its ecological niche and clinical relevance, we need tools to differentiate the isolates. Both of the tests added for biotyping with the expanded subtyping scheme, fibrinogen affinity and β-lactamase, measure secreted substances which may relate to their pathogenicities. Hemolysins and adherins may also be virulence factors, but their roles in the virulence of the various clinical species of CNS are still not clearly defined, because the species have not been identified in many of the published reports. This expanded subtyping scheme, which incorporates synergistic hemolysis, adherence, fibrinogen affinity, and β-lactamase production, may serve as a very useful tool for measuring

the clinical significance of *S. lugdunensis* in epidemiologic studies.

Some of the clinical isolates examined during this study were collected recently, but most were from the frozen collections of several laboratories. Some of the frozen strains had been identified as *Staphylococcus* species, and a few were identified as *S. warneri* and *S. haemolyticus*, but most were simply identified as a CNS. The oldest of these isolates was collected in 1971 from the foot of a patient at the Veterans Administration hospital in Atlanta, Ga.; it is an *S. lugdunensis*, biotype B6c, which means that it produces a delta hemolysin and β-lactamase but does not produce an adherin or a fibrinogen affinity factor.

Some of the *S. lugdunensis* in this collection were resistant to the penicillins; the low level of antimicrobial resistance among these isolates was otherwise comparable with that originally reported for these species (4). Some (29%) of these *S. lugdunensis* produced a β-lactamase; other clinical isolates of this species collected in the United States have also been reported to be β-lactamase positive (T. E. Herchline, J. Barnishan, L. Ayers, and R. J. Fass, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 701, 1989), but none of the isolates reported from France (4) were β-lactamase positive.

All of the tests used in this study are easy to set up and to interpret, are read at or before 24 h, and use routine or readily available commercial products. They are also inexpensive, reproducible tests that can be done in any clinical laboratory. If more clinical laboratories would identify their isolates of CNS to the species level, we could soon resolve the question of clinical significance for the various species of CNS. Furthermore, if the battery of tests discussed in this report were used to identify those isolates, the test results could be used to biotype selected groups of organisms for further evaluation in epidemiologic investigations.

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