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Characteristics of Coagulase-Negative Staphylococci That Help Differentiate These Species and Other Members of the Family *Micrococcaceae*

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One hundred reference strains and 1,240 clinical isolates representing 26 species of the family *Micrococcaceae* were used to evaluate the potential of tests for synergistic hemolysis, adherence to glass, pyroglutamyl-β-naphthylamide hydrolysis, and susceptibility to a set of five antimicrobial agents for differentiating these species and strains within the species. Sixty-eight percent of the clinical isolates exhibited synergistic hemolysis; 69% of the clinical staphylococci but none of the micrococci or stomatococci were adherence positive, and 92% of the strong positive adherence reactions were produced by strains of *Staphylococcus epidermidis*. Strains from 15 of the species were pyroglutamyl-β-naphthylamide positive, but this test separated *Staphylococcus xylosus* from other novobiocin-resistant staphylococci and *Staphylococcus intermedius* from other coagulase-positive species. A polymyxin B disk helped differentiate *S. epidermidis* from most other coagulase-negative staphylococci, and a bacitracin disk (10 U) helped differentiate *Staphylococcus haemolyticus* from most other novobiocin-susceptible staphylococci. All strains that were susceptible to furazolidone and resistant to Taxo A disks (bacitracin, 0.04 U; BBL Microbiology Systems, Cockeysville, Md.) were staphylococci. We observed a 91% correlation between species identification obtained with the Staph-Ident system (Analytab Products, Plainview, N.Y.) and conventional methods; but the micrococci and stomatococci were incorrectly identified as staphylococci with Staph-Ident, and several isolates of *S. epidermidis* were misidentified as *Staphylococcus hominis* because they were alkaline phosphatase negative. Both these problems can be prevented by adding the simple tests we describe to those already recommended when the Staph-Ident system is used to identify isolates of gram-positive, catalase-positive cocci.

Although the pathogenic role of coagulase-negative staphylococci is now well established, the clinical significance of the various species is still being defined. We should not disregard any of these organisms until their clinical significance is resolved, and since they are frequently opportunistic pathogens, we may never completely resolve the question of significance. Other members of the family *Micrococcaceae* are also being identified as opportunistic pathogens (7, 11).

Conventional methods used to identify species of staphylococci involve many tests that require the preparation of a variety of special media and reagents and 3 to 5 days of incubation to obtain results. A further complication is the range of reactivity within each species with many of the test systems. We used several techniques to study these organisms because we need a test or set of tests that (i) allow quicker species differentiation without the expense and expertise required by more sophisticated methods, and (ii) separate strains within a species as a measure of virulence.

Kloos and Wolfshohl (20) reported more than 90% agreement between the rapid Staph-Ident system (Analytab Products, Plainview, N.Y.) and conventional methods of identifying most species. Christensen et al. (6) described a test for slime production or adherence that might measure virulence and, therefore, clinical significance. In an earlier report (16), with strains representing 17 species of staphylococci, we suggested that synergistic hemolysis tests could discriminate among species, but more strains needed to be studied for many of the species. We have now examined reference and clinical strains representing 26 known species of the family *Micrococcaceae* for synergistic hemolysis, adherence, and other characteristics that could help differentiate them; the results of our studies are presented in this report.

MATERIALS AND METHODS

Cultures and growth conditions. A total of 1,340 strains representing 26 species of the family *Micrococcaceae* were examined during this study. The 100 reference strains (Table 1) were obtained from W. E. Kloos (Raleigh, N.C.) and from the American Type Culture Collection (Rockville, Md.). The set of 1,240 clinical isolates were from culture collections of laboratories at the Centers for Disease Control. 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 the strains were gram-positive cocci that produced catalase. They were tested for coagulase activity by both the slide and tube tests with rabbit plasma (Bacto Coagulase Plasma; Difco Laboratories, Detroit, Mich.). Coagulase-positive strains were tested for acid production from glucose and mannitol anaerobically and from maltose and mannitol aerobically, DNase and alkaline phosphatase activities, and pigment production (18). Coagulase-negative strains that produced lemon yellow colonies or rubbery white colonies were tested for acid production from glucose anaerobically and for resistance to lysostaphin (18). All of

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TABLE 1. Reference strains of members of the family *Micrococcaceae*[a]

Species	Strains received from:	
	W. E. Kloos	ATCC
Staphylococci		
S. auricularis	BO4	33750, 33751, 33752, 33753^T, 33754
S. capitis		27840^T, 27841, 27842, 27843, 35661
S. caprae		35538^T
S. carnosus	Hansen, MA	
S. caseolyticus		13548^T, 29750
S. chromogenes	6-IRRb, 93-IRRa, 95-8LRa, 1-HF-114RR, 2-LK5LF, 137-4RF, BB-124-LF	
S. cohnii	DM224, SH161A	29972, 29973, 29974^T, 35662
S. epidermidis	AW269, GH37, 1386-3	12228, 14990^T, 29887, 35547, 35983, 35984
S. gallinarum		35539^T
S. haemolyticus	GH59	29968, 29969, 29970^T
S. hominis	JL248	27844^T, 27845, 27846, 27847
S. hyicus	3813-D, Shy1, BOC38, 393-B, 451-B, 4827-A	11249^T
S. intermedius	RK12, 2092, 3681, 4272, 4975, 7021	29663^T
S. kloosii	SC210^T, DM3, DM93, GV243, PL427	
S. lentus	K-6	29070^T
S. saprophyticus	KL20, SM295, TW111	15305^T, 35552
S. sciuri		29059, 29060, 29061, 29062^T
S. simulans	6L31	27848^T, 27849, 27850, 27851
S. warneri		27836^T, 27837, 27838, 27839
S. xylosus	DM37	29966, 29967, 29971^T, 35033, 35663
Micrococci		
M. kristinae		27570^T
M. luteus		381, 4698^T
M. lylae		27566^T
M. roseus		186^T
M. varians		15306^T
Stomatococcus mucilaginosus		25296^T

[a] All of these strains were from either W. E. Kloos or the American Type Culture Collection (ATCC). A superscript T indicates type or neotype strains.

the reference strains, many of the coagulase-positive clinical isolates, and all of the coagulase-negative clinical isolates were tested with the Staph-Ident system (Analytab Products). This system contains tests for the production of alkaline phosphatase, β-glucosidase, β-glucuronidase, and β-galactosidase; for the utilization of urea and arginine; and for acid production from mannose, mannitol, trehalose, and salicin. The panels were inoculated and processed according to the procedures recommended by the manufacturer. The resulting biochemical profiles were expressed as four-digit numbers. The recommended additional tests for coagulase production, novobiocin susceptibility, and acid from xylose and arabinose were done as required to resolve some of the profiles into distinct species. The identifications of most of the coagulase-negative clinical isolates were confirmed by the methods of Kloos (W. E. Kloos, Clin. Microbiol. Newsl. **4**:75–79, 1982) and Kloos and colleagues (18, 19), with one modification. The carbohydrates in purple-agar base (Difco) were prepared and poured into the 16-mm wells of 24-well tissue-culture plates to ensure the isolation of individual reactions. These plates were prepared as follows: (i) 0.05 ml of a sterile 20% carbohydrate solution was placed in a sterile well, and (ii) 0.95 ml of sterile purple-agar base was added to the well and mixed gently. Each well of a plate contained a different carbohydrate solution, so that each plate of 21 carbohydrates was later exposed to only one inoculum. The plates were inoculated with overnight growth from a Trypticase soy broth (BBL) by dipping a sterile cotton or polyester fiber-tipped swab into the culture and touching each agar surface. All plates were then incubated and read as described in the reference method (19). The carbohydrates tested in these plates were glucose, maltose, trehalose, mannitol, xylose, xylitol, cellobiose, sucrose, turanose, mannose, ribose, raffinose, lactose, fructose, arabinose, galactose, melezitose, rhamnose, salicin, glycerol, and fucose.

Pyroglutamyl-β-naphthylamide hydrolysis. All strains were tested for pyroglutamyl-β-naphthylamide (PYR) hydrolysis by using the commercial kit of PYR broth (0.01% L-pyroglutamyl-β-naphthylamide) and PYR reagent (*N,N*-dimethylaminocinnamaldehyde) (both from Carr-Scarborough, Stone Mountain, Ga.) recommended for the rapid presumptive identification of group A streptococci and enterococci. A loopful of 24-h growth from a TSA II plate was emulsified in the PYR broth to a turbidity of a MacFarland no. 2 to 3 standard. The screw caps were tightened, and the suspensions were incubated at 35°C for 2 h. After incubation, 2 drops of PYR reagent were added to each tube without mixing. The development of a dark color (purple-red) within 2 min was recorded as a positive test result. A pink, orange, or yellow reaction was noted and recorded as a negative test result. The controls included positive and negative staphylococci from the previous day and uninoculated broth.

Synergistic hemolysis. All strains were tested for synergistic hemolysis as described in our earlier report (16). A strain of *Staphylococcus intermedius* (AB148) that produced betalysin was streaked down the center of a TSA II plate. 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 at room temperature for 4 to 6

TABLE 2. Characteristics of reference strains of *Micrococcaceae*

Organism (no.)	No. positive for:			Staph-Ident biochemical profile
	PYR hydrolysis	Synergistic hemolysis	Adherence	
S. auricularis (6)	6	0	0	0040, 0440, 0441, 0541
S. capitis (5)	0	5	0	0040, 0140, 0340
S. caprae (1)	1	1	1	0040
S. carnosus (2)	2	0	0	1241
S. caseolyticus (2)	2	0	0	0000, 0040
S. chromogenes (7)	7	1	0	2000, 2040, 2400
S. cohnii (6)	0	5	0	0000, 0200, 0400, 0240
S. epidermidis (9)	0	8	8	2040, 3000, 3040, 7040
S. gallinarum (1)	0	0	0	7351
S. haemolyticus (4)	4	4	1	0060, 0460, 0640, 4440
S. hominis (5)	0	0	1	2000, 2040, 2400
S. hyicus (7)	0	7	0	1540, 1560, 3440
S. intermedius (7)	7	0	7	1501, 3401, 3501, 3541, 7601
S. kloosii (5)	3	1	0	0600, 2201, 2601, 5600
S. lentus (2)	0	0	0	4710, 5600
S. saprophyticus (5)	0	0	3	2000, 2001, 2400
S. sciuri (4)	0	0	4	5610, 5710
S. simulans (5)	2	5	3	2061, 2461
S. warneri (4)	0	4	0	4640, 6600, 6640
S. xylosus (6)	6	4	5	6021, 6421, 6701, 7401, 7421
M. kristinae (1)	1	0	0	4510
M. luteus (2)	2	0	0	2040
M. lylae (1)	0	1	0	1040
M. roseus (1)	0	0	0	0040
M. varians (1)	0	0	0	2041
Stomatococcus mucilaginosus (1)	1	0	0	4510

h; the synergistic hemolysis reactions were read at approximately 24 h. A zone of complete hemolysis surrounding the test strain, but within the zone of incomplete hemolysis caused by the beta-lysin from the *S. intermedius* growth, was a positive test. Most of our reference strains of staphylococci and 260 clinical isolates of staphylococci representing 12 species were also tested for synergistic hemolysis with the alpha-toxin of *Clostridium perfringens*. Disks containing *C. perfringens* alpha-toxin (RIM CAMPtest disks; Austin Biological Labs, Austin, Tex.) were placed on TSA II plates near the overnight growth of test strains. Plates were reincubated aerobically at 35°C and read at 1, 2, 3, and 4 h. An arc-shaped zone between the disk and the test strain was a positive test result. The controls for both test systems included strains that tested positive and negative in previous analyses.

Adherence. All strains were tested for adherence to glass culture tubes as described by Christensen et al. (6). Several modifications of that original procedure are used to study staphylococci, and since this simple test may prove to be a tool for classifying these organisms, the qualitative procedure that we used was as follows. A sterile cotton or polyester fiber-tipped swab was used to transfer 24-h growth from a TSA II plate to a glass tube containing 5 ml of Trypticase soy broth. The swab was rinsed in the broth to achieve an inoculum turbidity of approximately a MacFarland no. 1 standard; the swab was then raised above the broth, pressed, and rotated against the inside wall of the tube to remove excess fluid and either discarded or used to inoculate a TSA II plate for the disk susceptibility tests. The broth tubes were recapped and incubated aerobically at 35°C for 18 to 20 h. The entire contents of the tubes were then carefully poured into a flask of concentrated disinfectant. The tubes were gently rinsed once with phosphate-buffered saline (pH 7.6) by slowly filling the tubes and then carefully pouring the rinse into the disinfectant. The tubes were then filled to the original broth level (half full) with a Gram-Safranin solution (Carr-Scarborough) and left at room temperature for 30 min. The safranin was then poured into the disinfectant, and the tubes were gently rinsed twice with phosphate-buffered saline before they were placed upside down to drain and dry overnight. The dry tubes were held up to a fluorescence bench lamp and observed by transmitted light for evidence of a film adhering to the walls of the tube; observations were recorded as negative, weakly positive, moderately positive, and strongly positive. All tubes were then recapped and stored in the dark. Controls included reference strains of *S. epidermidis* (ATCC 35983 [RP12] and ATCC 35984 [RP62A]) and uninoculated broth.

Disk-diffusion susceptibility studies. All strains were tested for susceptibility to a set of five antimicrobial agents by a disk-diffusion method using TSA II plates (15 by 100 mm); this is not the standard susceptibility test used in diagnostic testing. The 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). The TSA II plates were streaked with the rinsed, pressed swab that was used for the broth inoculum described above for the adherence test. The entire surface of each plate was streaked evenly in one direction for confluent growth. After allowing 10 min for the agar surface to dry, the five paper disks were placed on the plate with the Taxo A disk in the center and the others were equidistantly spaced around it. The plates were put in plastic bags to conserve moisture and incubated at 35°C for a full 24 h. The petri plate lids were then removed, and the diameters of the circular inhibition zones were carefully

TABLE 3. Comparison of the Staph-Ident biochemical system with our conventional identification results

Conventional identification[a]	No. of strains tested	Identification with the Staph-Ident system	
		No. (%) correct	Incorrect species of staphylococci (no. of strains/species)
S. epidermidis	373	335 (89.8)	*S. hominis* (34), *S. capitis* (3), *S. warneri* (1)
S. haemolyticus	104	102 (98.1)	*S. capitis* (1), *S. simulans* (1)
S. hominis	78	77 (98.7)	*S. capitis* (1)
S. simulans	42	42 (100.0)	
S. saprophyticus	28	25 (89.3)	*S. epidermidis* (1), *S. xylosus* (2)[b]
S. warneri	27	27 (100.0)	
S. capitis	12	12 (100.0)	
S. xylosus	12	12 (100.0)	
S. sciuri	3	3 (100.0)	
S. cohnii	2	1 (50.0)	*S. simulans* (1)
S. intermedius	4	4 (100.0)	
S. aureus	16	16 (100.0)	
Stomatococcus mucilaginosus	2	0 (0)	*S. sciuri* (1), unknown (1)
Micrococcus spp.	18	0 (0)	*S. capitis* (14), *S. hominis* (3), *S. saprophyticus* (1)

[a] By conventional methods we identified 65 of the clinical isolates as coagulase-negative staphylococci but could not place them in distinct species. The species identified by the Staph-Ident system for those strains were *S. saprophyticus* (18 strains), *S. warneri* (13 strains), *S. hominis* (7 strains), *S. haemolyticus* (3 strains), *S. hominis* or *S. haemolyticus* (5 strains), *S. capitis* (8 strains), *S. epidermidis* (6 strains), *S. cohnii* (2 strains), *S. simulans* (1 strain), and unknown (2 strains).

[b] These two strains were probably misidentified by conventional methods and correctly identified as *S. xylosus* with the Staph-Ident system.

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.

Independent testing and data analysis. The standard tests for identification of strains by conventional methods and some of the Staph-Ident tests were done by two of us separately from any other analyses. The tests for PYR hydrolysis, synergistic hemolysis, tube adherence, disk-diffusion susceptibilities on TSA II, and some of the Staph-Ident panels were done independently by another one of us, who had no prior knowledge of the identity of the strains. When the results were combined and compared, all aberrant values were confirmed by repeated testing of coded samples. Since the identity of some strains was not confirmed by the conventional methods, the number of strains used in the analysis of the Staph-Ident system is less than that reported in the other analyses (see Table 3 versus Tables 4, 5, and 7 to 11). All data were stored and analyzed with computer software (dBASE III PLUS; Ashton-Tate, Torrance, Calif.) and a portable computer (COMPAQ) upgraded to 640 kilobytes.

RESULTS

The four-digit biochemical profiles obtained with Staph-Ident tests of the 26 species of reference strains are given in Table 2. *Stomatococcus mucilaginosus*, the 5 species of micrococci, and 8 of the 20 species of staphylococci shown in Table 2 are not on the Staph-Ident Profile Register that accompanies each test kit. These strains, however, generated some of the same numeric profiles as those species currently on that register and could, therefore, be misidentified without additional testing. For the other 12 species of staphylococci, all but 4 of the reference strains were correctly identified by this system; two strains of *S. epidermidis* had the 2040 profile of *S. hominis*, one strain of *S. cohnii* had the 0240 profile of *S. capitis*, and one strain of *S. intermedius* had the 7601 profile of *S. xylosus*. A comparison of the Staph-Ident test results with our conventional methods for identifying clinical isolates is shown in Table 3. Of the 766 staphylococcal isolates tested by both methods, 701 (91.5%) were identified to the species level by conventional methods and 93.6% (656 of 701) of the isolates identified to the species level were correctly identified with the Staph-Ident system. The 45 incorrect identifications among the staphylococci were 1 isolate each of *S. cohnii* and *S. hominis*, 2 isolates of *S. haemolyticus*, 3 isolates of *S. saprophyticus*, and 38 isolates of *S. epidermidis*. Two of the three incorrect *S. saprophyticus* isolates were probably misidentified by conventional methods and correctly identified as *S. xylosus* with Staph-Ident; they had the 7061 profile of *S. xylosus*. Most (34 isolates) of the incorrect *S. epidermidis* were identified as *S. hominis*; 21 isolates had the 2040 profile, and 13 had the 2000 profile of *S. hominis*.

All the micrococci were incorrectly identified with Staph-Ident as staphylococci because they had the same biochemical profiles as staphylococci: 14 had *S. capitis* profile 0040, 3 had *S. hominis* profiles 2040 and 2000, and 1 had *S. saprophyticus* profile 2040. One of the stomatococci was identified as *Staphylococcus sciuri* profile 4710, and the other had an unknown profile, 0510. So, of the 721 isolates identified by conventional methods, 90.98% (656 of 721) were correctly identified with Staph-Ident. All but 2 of the 65 isolates that were identified as coagulase-negative staphylococci by conventional methods but were not differentiated further, were identified to the species level with the Staph-Ident system. We could not confirm these Staph-Ident identifications by conventional methods.

The distribution of species among the clinical isolates is shown in Table 4. Of the 1,240 isolates examined, 1,175 (94.8%) were identified as a species of *Staphylococcus* (1,155 strains), *Micrococcus* (18 strains), or *Stomatococcus* (2

TABLE 4. Characteristics of clinical isolates

Organism	No. of strains tested	No. (%) of strains positive for:		
		PYR hydrolysis	Synergistic hemolysis	Adherence tube test
S. epidermidis	672	0 (0)	487 (72.5)	556 (82.7)
S. haemolyticus	133	133 (100.0)	132 (99.2)	56 (42.1)
S. hominis	103	1 (1.0)	54 (52.4)	58 (56.3)
S. simulans	48	46 (95.8)	46 (95.8)	34 (70.8)
S. saprophyticus	40	2 (5.0)[a]	1 (2.5)[a]	29 (72.5)
S. warneri	40	2 (5.0)	25 (62.5)	2 (5.0)
S. capitis	15	0 (0)	14 (93.3)	1 (6.7)
S. xylosus	13	13 (100.0)	7 (53.8)	11 (84.6)
S. sciuri	6	0 (0)	0 (0)	2 (33.3)
S. cohnii	2	0 (0)	2 (100.0)	1 (50.0)
S. intermedius	4	4 (100.0)	0 (0)	4 (100.0)
S. aureus	79	2 (2.5)	26 (32.9)	58 (73.4)
Staphylococcus spp.[b]	65	13 (NR[c])	43 (NR)	32 (NR)
Micrococcus spp.	18	15 (83.3)	0 (0)	0 (0)
Stomatococcus mucilaginosus	2	2 (100.0)	1 (50.0)	0 (0)

[a] These strains were probably misidentified by conventional methods; they were identified as *S. xylosus* with the Staph-Ident system.

[b] Coagulase-negative staphylococci.

[c] NR, Not relevant.

TABLE 5. Adherence-positive staphylococci

Organism (no.)	No. (%) of strains positive	No. (%) of strains at each level		
		Strongly positive	Moderately positive	Weakly positive
Clinical staphylococci (1,220)	844 (69)	218 (18)	456 (37)	170 (14)
S. epidermidis (672)	556 (83)	200 (30)	276 (41)	80 (12)
S. haemolyticus (133)	56 (42)	4 (3)	36 (27)	16 (12)
S. hominis (103)	58 (56)	6 (6)	38 (37)	14 (14)
S. simulans (48)	34 (71)	1 (2)	24 (50)	9 (19)
S. saprophyticus (40)	29 (73)	2 (5)	25 (63)	2 (5)
S. aureus (79)	58 (73)	1 (1)	20 (25)	37 (47)
S. xylosus (13)	11 (85)	0	10 (77)	1 (8)
S. intermedius (4)	4 (100)	0	4 (100)	0
S. sciuri (6)	2 (33)	0	2 (33)	0
S. capitis (15)	1 (7)	1 (7)	0	0
S. cohnii (2)	1 (50)	0	1 (50)	0
S. warneri (40)	2 (5)	0	0	2 (5)
Staphylococcus spp.[a] (65)	32 (NR[b])	3 (NR)	20 (NR)	9 (NR)

[a] Coagulase-negative staphylococci.
[b] NR, Not relevant.

strains). The identified staphylococci included 672 *S. epidermidis*, 133 *S. haemolyticus*, 103 *S. hominis*, 48 *S. simulans*, 40 *S. saprophyticus*, 40 *S. warneri*, 15 *S. capitis*, 13 *S. xylosus*, 6 *S. sciuri*, 2 *S. cohnii*, 4 *S. intermedius*, and 79 *S. aureus*. The remaining 65 (5.2%) isolates were identified as coagulase-negative staphylococci but could not be further differentiated into species by conventional methods. We did not identify the micrococci to the species level for this study.

PYR hydrolysis. Our early pilot tests of staphylococci for PYR hydrolysis involved a few reference strains of several different species. These strains were tested after 1, 2, 3, and 4 h of incubation in PYR broth before the final reagent was added. In the 1-h tests, there were many very distinct purple positive reactions, as well as several light rose or dark pink reactions that were difficult to evaluate. These were much less frequent in the 2-h tests, but occurred again after the longer incubations of 3 and 4 h. In the 2-h pilot tests, all the reference strains examined for a given species were positive, or all were negative, when a dark purple-red reaction was considered the only positive result. For this reason, all our strains were examined after 2 h of incubation. The data on PYR hydrolysis by reference strains (Table 2) show that, except for two species, the strains within a species gave identical results: either all were positive or all were negative. All the reference strains of *S. auricularis*, *S. caprae*, *S. carnosus*, *S. caseolyticus*, *S. chromogenes*, *S. haemolyticus*, *S. intermedius*, *S. xylosus*, *Micrococcus kristinae*, *M. luteus*, and *Stomatococcus mucilaginosus* were positive. However, only three of the five strains of *S. kloosii* and two of the five strains of *S. simulans* were positive. All reference strains in the remaining species were negative. The PYR data from clinical isolates (Table 4) reflect the same reactions and the same consistency among strains of a species. All the clinical isolates of *S. haemolyticus*, *S. xylosus*, *S. intermedius*, and *Stomatococcus mucilaginosus* were positive. All but two (96%) of the *S. simulans* and most (83%) of the micrococci were positive. All the clinical isolates of *S. epidermidis*, *S. capitis*, *S. sciuri*, and *S. cohnii* were negative; and only one isolate of *S. hominis* and two isolates each of *S. saprophyticus*, *S. warneri*, and *S. aureus* were positive. The two PYR-positive isolates of *S. saprophyticus* were the same two isolates that were probably misidentified and should be listed as *S. xylosus*. Most of the isolates of *S. aureus* and a rare isolate each of *S. xylosus* and *S. saprophyticus* gave a pink (negative) reaction in this test; many isolates of *S. saprophyticus* and *S. warneri* gave an orange (negative) reaction.

Synergistic hemolysis. Most or all the reference strains (Table 2) of *S. capitis*, *S. caprae*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hyicus*, *S. simulans*, *S. warneri*, *S. xylosus*, and *M. lylae* were positive in the synergistic hemolysis test. As we reported earlier (16), the positive *S. hyicus* reactions were unique; the clear area of complete hemolysis was shaped like an arc. Only one strain each of *S. chromogenes* and *S. kloosii* was positive. The same general pattern of results was seen with the clinical isolates of staphylococci (Table 4) except for *S. hominis*; 52.4% (54 of 103) of the clinical isolates of *S. hominis* were positive, but all 5 of the reference strains of *S. hominis* were negative. The single positive isolate of *S. saprophyticus* was one of the two isolates that was probably misidentified and should be listed as *S. xylosus*. The data obtained with the disks containing *C. perfringens* alpha-toxin are not shown, but the reactions of the reference and clinical strains tested with the *C. perfringens* alpha-toxin were identical to those seen with the beta-lysin of *S. intermedius* except for two species; all strains of *S. hyicus* and *S. chromogenes* were negative by this method. Many of the positive strains produced clear zones after only 2 h, and all reactions were complete within 4 h.

Adherence to glass. Most or all the reference strains of *S. caprae*, *S. epidermidis*, *S. intermedius*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, and *S. xylosus* were positive in the adherence test (Table 2). One reference strain each of *S. haemolyticus* and *S. hominis* was also positive. All the reference and clinical strains of *Micrococcus* and *Stomatococcus* were negative. From 71 to 100% of the clinical isolates of *S. aureus*, *S. epidermidis*, *S. intermedius*, *S. saprophyticus*, *S. simulans*, and *S. xylosus* were positive for adherence (Table 4). Fewer of the clinical isolates of *S. haemolyticus* (42%), *S. hominis* (56%), and *S. sciuri* (33%); only two strains of *S. warneri*; and one strain each of *S. capitis* and *S. cohnii* were positive. Sixty-nine percent (844 of 1,220) of the clinical staphylococci were adherence positive; 18% were strongly positive, 37% were moderately positive, and 14% were weakly positive (Table 5). One-fourth (218 of 844) of the adherence-positive strains were strongly positive, and 92% (200 of 218) of these were *S. epidermidis* isolates. The few other strongly positive strains were scattered among only six species: *S. hominis*, *S simulans*, *S. haemolyticus*, *S. capitis*, *S. saprophyticus*, and *S. aureus*. The two positive strains of *S. warneri* were weakly positive.

Novobiocin. The results of novobiocin disk susceptibility tests of reference strains on TSA II are shown in Table 6. The diameters of the inhibition zones were 15 to 23 mm for strains in those staphylococcal species that have been historically defined as novobiocin susceptible and 6 mm (the diameter of the paper disk, i.e., no inhibition) to 11 mm for those defined as novobiocin resistant (*S. cohnii*, *S. gallinarum*, *S. kloosii*, *S. saprophyticus*, *S. sciuri*, and *S. xylosus*). The two strains of *S. lentus*, a species defined as slightly resistant to novobiocin, had zone diameters of 12 and 13 mm. The results of novobiocin tests with clinical isolates are shown in Table 7. The mean inhibition zone diameters of the resistant species were 6.0 to 10.0 mm. All strains of *S. saprophyticus*, *S. xylosus*, *S. sciuri*, and *S. cohnii*; four strains of *S. epidermidis*; two strains of *S. haemolyticus*; and

TABLE 6. Disk susceptibilities of reference strains on TSA II

Organism (no.)	Range of inhibition zone diam (mm) to:				
	Novobiocin (5 μg)	Polymyxin B (300 U)	Bacitracin		Furazolidone (100 μg)
			10 U	0.04 U	
S. auricularis (6)	20–22	14–17	6–21	6[a]	27–35
S. capitis (5)	19–22	12–14	13–16	6	20–24
S. caprae (1)	19	13	14	6	19
S. carnosus (2)	16–17	13–14	12–13	6	21–24
S. caseolyticus (2)	15–16	14–15	18–19	6	19–22
S. chromogenes (7)	20–22	6	15–20	6	19–23
S. cohnii (6)	6–11	12–16	13–26	6	17–25
S. epidermidis (9)	16–22	6	14–18	6	16–25
S. gallinarum (1)	11	12	17	6	18
S. haemolyticus (4)	16–21	12–14	9–13	6	20–22
S. hominis (5)	18–22	12–14	13–18	6	21–25
S. hyicus (7)	20–22	6	15–20	6	19–22
S. intermedius (7)	20–23	11–13	13–16	6	23–25
S. kloosii (5)	6–10	12–14	13–15	6	22–23
S. lentus (2)	12–13	11–13	14–15	6	21–27
S. saprophyticus (5)	6	12–14	12–16	6	20–23
S. sciuri (4)	10–11	11–12	10–12	6	16–18
S. simulans (5)	17–22	12–14	12–14	6	20–23
S. warneri (4)	17–21	11–14	18–20	6	18–20
S. xylosus (6)	6	6–13	13–15	6	16–19
M. kristinae (1)	18	10	21	10	6
M. luteus (2)	26–27	15–16	33–35	6–18	6
M. lylae (1)	21	14	27	12	6
M. roseus (1)	30	19	47	25	6
M. varians (1)	24	14	30	12	6
Stomatococcus mucilaginosus (1)	17	6	22	10	6

[a] A 6-mm zone is the diameter of the paper disk and implies no inhibition.

one strain each of *S. hominis* and *Micrococcus* spp. had inhibition zone diameters of <12 mm. Among the 122 strains with inhibition zone diameters of 12 to 16 mm were 25 *S. epidermidis*, 54 *S. haemolyticus*, 16 *S. hominis*, 7 *S. simulans*, 5 *S. warneri*, 1 *S. capitis*, and 14 *S. aureus*. The remaining staphylococci, the other 17 micrococci, and both stomatococci had inhibition zone diameters of >16 mm. When the TSA II plates of strains in some species were viewed by transmitted light, a circular band of hemolysis (width, 2 to 4 mm) was associated with the novobiocin disks. Starting at the disk, we saw a clear zone of no growth, then frequently a narrow zone of light-to-moderate growth, and then a ring of hemolysis and heavier growth at the edge of the regular growth area of no inhibition. Among the reference strains, this hemolytic ring was seen with all the *S. haemolyticus* and *S. simulans*, four of the five *S. capitis*, and

TABLE 7. Susceptibilities of clinical isolates to novobiocin (5 μg) disks on TSA II

Organism	No. of strains tested	Mean (range) inhibition zone diam (mm)[a]	No. of strains with the following inhibition zone diam (mm):						
			6	7–9	10–11	12–15	16	>16	<12 (% of total)
S. epidermidis	672	20.0 (8–28)		1	3	6	19	643	4 (0.6)
S. haemolyticus	133	16.8 (13–23)	2			24	30	77	2 (1.5)
S. hominis	103	18.5 (10–27)			1	11	5	86	1 (1)
S. simulans	48	18.4 (15–23)				2	5	41	0 (0)
S. warneri	40	18.3 (15–22)				2	3	35	0 (0)
S. capitis	15	19.3 (15–24)				1		14	0 (0)
S. intermedius	4	20.7 (20–22)						4	0 (0)
S. aureus	79	18.6 (14–22)				7	7	65	0 (0)
Micrococcus spp.	18	23.2 (11–30)			1			17	1 (5.6)
Stomatococcus mucilaginosus	2	19.0 (18–20)						2	0 (0)
S. saprophyticus	40	6.2 (7–11)	37	2	1				40 (100)
S. xylosus	13	6.6 (10–11)	11		2				13 (100)
S. sciuri	6	10.0 (9–11)		1	5				6 (100)
S. cohnii	2	6.0 (NA[b])	2						2 (100)

[a] The mean is the average of all values, but the range does not include the 6-mm zones that imply no inhibition.
[b] NA, Not applicable.

TABLE 8. Susceptibilities of clinical isolates to polymyxin B (300 U) disks on TSA II

Organism	No. of strains tested	Mean (range) inhibition zone diam (mm)[a]	No. of strains with the following inhibition zone diam (mm):				
			6	7–9	10	>10	<10 (% of total)
S. epidermidis	672	6.4 (7–16)	535	118	12	7	653 (97.2)
S. haemolyticus	133	12.0 (10–15)			7	126	0 (0)
S. hominis	103	13.3 (7–19)	7	2	5	89	9 (8.7)
S. simulans	48	13.0 (9–15)		1		47	1 (2.1)
S. saprophyticus	40	14.4 (12–19)				40	0 (0)
S. warneri	40	12.9 (7–16)		1		39	1 (2.5)
S. capitis	15	13.4 (11–15)				15	0 (0)
S. xylosus	13	12.4 (11–16)				13	0 (0)
S. sciuri	6	12.7 (12–13)				6	0 (0)
S. cohnii	2	16.0 (15–17)				2	0 (0)
S. intermedius	4	11.8 (10–14)			1	3	0 (0)
S. aureus	79	6.1 (7–8)	69	10			79 (100)
Micrococcus spp.	18	13.4 (10–16)	1		1	16	1 (5.6)
Stomatococcus mucilaginosus	2	6.0 (NA[b])	2				2 (100)

[a] The mean is the average of all values, but the range does not include the 6-mm zones that imply no inhibition.
[b] NA, Not applicable.

five of the seven *S. intermedius*. With the clinical isolates, the ring was seen with most *S. haemolyticus*, *S. simulans*, and *S. capitis*; with a few *S. aureus*; and rarely with *S. hominis*, *S. warneri*, and *S. xylosus*. The narrow inner band of light-to-moderate growth measured 1 to 5 mm and was most often seen with *S. haemolyticus*. The total inhibition zone diameter for one of these strains included one-half the inner band.

Polymyxin B. The results of polymyxin B disk susceptibility tests of reference strains on TSA II are shown in Table 6. All the reference strains of *S. epidermidis*, *S. chromogenes*, and *S. hyicus*; the strain of *Stomatococcus mucilaginosus*; and one strain of *S. xylosus* were completely resistant to the 300-U disk, with a recorded zone size of 6 mm (i.e., no inhibition). The single strain of *M. kristinae* had a zone of 10 mm, and all the other reference strains had zones of 11 to 19 mm. The results of polymyxin B tests with clinical isolates are shown in Table 8. The mean inhibition zone diameters for these species were 6.0 to 16.0 mm. Most of the strains of *S. epidermidis* (80%) and *S. aureus* (87%), seven strains of *S. hominis* (7%), both strains of *Stomatococcus mucilaginosus*, and a single strain of *Micrococcus* (6%) were completely resistant to this antimicrobial agent, i.e., gave no zone of inhibition with this disk. The strains with inhibition zone diameters of <10 mm included 97% of the *S. epidermidis*, 100% of the *S. aureus* and *Stomatococcus mucilaginosus*, 8.7% of the *S. hominis*, one strain each of *S. simulans* and *S. warneri*, and a *Micrococcus* sp. A few of the strains in other species had zones of only 10 mm, but most had larger zones, of 11 to 19 mm.

Bacitracin (10 U). The results of bacitracin (10 U) disk susceptibility tests of reference strains on TSA II are shown in Table 6. Most of the staphylococci, all of the micrococci, and *Stomatococcus mucilaginosus* were susceptible to this antimicrobial agent, with inhibition zone diameters of 11 to 26 mm for staphylococci and much larger inhibition zone diameters for members of the other genera; however, two strains each of *S. haemolyticus* and *S. auricularis* and one strain of *S. sciuri* had smaller inhibition zone diameters (<11 mm). The results of bacitracin tests with clinical isolates are shown in Table 9. The mean inhibition zone diameters for these species were 7.7 to 27.0 mm. Most of the clinical isolates showed some susceptibility to this antimicrobial agent; however, 90.2% of the *S. haemolyticus* strains; the six *S. sciuri* strains; a few strains each of *S. saprophyticus* and *S. xylosus*; two strains each of *S. epidermidis* and *S. simulans*; and one strain each of *S. hominis*, *S. warneri*, and *S. cohnii* had inhibition zone diameters of <11 mm.

Taxo A (bacitracin, 0.04 U). The results of Taxo A disk susceptibility tests of reference strains on TSA II are shown in Table 6. None of the staphylococci had inhibition zones at this concentration of bacitracin; all strains grew up to the paper disk, leaving nothing but the disk to measure. All the micrococci and *Stomatococcus mucilaginosus* were susceptible, with inhibition zone diameters of 10 to 25 mm. The results of Taxo A tests with clinical isolates are shown in Table 10. The mean inhibition zone diameters for these species were 6.0 to 13.6 mm. None of the staphylococci were susceptible, but the two stomatococci and all the micrococci were susceptible, with inhibition zones of 8 to 19 mm.

Furazolidone. The results of furazolidone disk susceptibility tests with reference strains are shown in Table 6. All staphylococci were susceptible, with inhibition zones of 16 to 35 mm. All micrococci and *Stomatococcus mucilaginosus* were completely resistant. The results of furazolidone tests with clinical isolates are shown in Table 10. The mean inhibition zone diameters for these species were 6.0 to 24.2 mm. All the staphylococci and the two stomatococci were susceptible, with inhibition zones of 12 to 34 mm. All the micrococci were completely resistant.

Summary. A summary of the results obtained with the 1,175 clinical isolates in all of these tests is shown in Table 11. The species were grouped on the basis of our test results to show their relationships.

DISCUSSION

Although a large collection of reference strains and clinical isolates was examined in this study, very few strains of several of the species less frequently isolated in clinical settings were available; therefore, further testing should be done with additional strains in those species to confirm our results. The more common species were, however, well represented in this data set.

When Kloos and Wolfshohl (20) evaluated the Staph-Ident system, they observed a 95% correlation between species

TABLE 9. Susceptibilities of clinical isolates to bacitracin (10 U) disks on TSA II

Organism	No. of strains tested	Mean (range) inhibition zone diam (mm)[a]	No. of strains with the following inhibition zone diam (mm):				
			6	7–9	10	>10	<11 (% of total)
S. epidermidis	672	16.6 (10–30)	1		1	670	2 (0.3)
S. haemolyticus	133	7.7 (7–16)	60	47	13	13	120 (90.2)
S. hominis	103	17.0 (12–26)	1			102	1 (1.0)
S. simulans	48	13.8 (10–24)			2	46	2 (4.2)
S. saprophyticus	40	15.1 (7–28)	1	2	2	35	5 (12.5)
S. warneri	40	19.3 (16–25)	1			39	1 (2.5)
S. capitis	15	15.1 (11–21)				15	0 (0)
S. xylosus	13	14.0 (11–19)	3			10	3 (23.1)
S. sciuri	6	7.7 (7–9)	1	5			6 (100.0)
S. cohnii	2	14.5 (6–23)	1			1	1 (50.0)
S. intermedius	4	15.2 (12–20)				4	0 (0)
S. aureus	79	16.1 (12–19)				79	0 (0)
Micrococcus spp.	18	25.8 (23–29)				18	0 (0)
Stomatococcus mucilaginosus	2	27.0 (26–28)				2	0 (0)

[a] The mean is the average of all values, but the range does not include the 6-mm zones that imply no inhibition except for the single strain of *S. cohnii*.

identification obtained by Staph-Ident and their conventional method, when the tube coagulase and novobiocin susceptibility tests were added to the profile, as recommended by Analytab Products. They also reported that 8 to 14% of the Staph-Ident profiles overlapped two species. Their data showed two strains of *S. epidermidis* with the 2040 profile and one with the 2000 profile of *S. hominis*; all three strains were verified as *S. epidermidis* by DNA-DNA hybridization. Phosphatase activity is considered a secondary characteristic in identification schemes, and 10 to 20% of the clinical isolates of *S. epidermidis* are phosphatase negative (13, 22); yet, the profile register for Staph-Ident excludes all phosphatase-negative staphylococci from identification as *S. epidermidis*. Despite this problem, which others have also noted (1), we found a good correlation (94%) between our conventional methods and this simple, rapid system for identifying those species of staphylococci most frequently associated with human illness. In addition, two of the isolates reported as *S. saprophyticus* in this study were probably correctly identified with Staph-Ident as *S. xylosus* and were misidentified by conventional methods; they had the 7061 profile of *S. xylosus*, which includes positive reactions in the alkaline phosphatase and β-glucuronidase tests. Most isolates of *S. xylosus* produce strong β-glucuronidase activity, and many produce alkaline phosphatase, whereas *S. saprophyticus* does not produce β-glucuronidase and usually does not produce alkaline phosphatase (20). The other species of staphylococci, the micrococci, and the stomatococci that were included in this study are rarely isolated from human clinical specimens, and many are primarily of veterinary interest; they would, however, be incorrectly identified with this system as one of the species of coagulase-negative staphylococci. Perhaps, after more strains of these species are studied, several of them could be added to the profile register. The Staph-Ident profile and the origin of the isolate, combined with the results from a few additional tests like those described in this report, could make this system even more useful by identifying several more species.

TABLE 10. Susceptibilities of clinical isolates to bacitracin (Taxo A) and furazolidone disks on TSA II

Organism	No. of strains tested	Mean (range) inhibition zone diam (mm)	
		Bacitracin (0.04 U)	Furazolidone (100 μg)
S. epidermidis	672	6[a] (all 6)	23.1 (12–34)
S. haemolyticus	133	6 (all 6)	21.3 (15–31)
S. hominis	103	6 (all 6)	24.2 (18–31)
S. simulans	48	6 (all 6)	22.3 (18–30)
S. saprophyticus	40	6 (all 6)	22.2 (17–29)
S. warneri	40	6 (all 6)	20.6 (17–26)
S. capitis	15	6 (all 6)	23.2 (20–30)
S. xylosus	13	6 (all 6)	20.3 (16–24)
S. sciuri	6	6 (all 6)	20.5 (20–22)
S. cohnii	2	6 (all 6)	22.5 (22–23)
S. intermedius	4	6 (all 6)	22.7 (20–27)
S. aureus	79	6 (all 6)	18.4 (14–22)
Staphylococcus spp.[b]	65	6 (all 6)	22.8 (16–29)
Stomatococcus mucilaginosus	2	10.0 (8–12)	17.5 (14–21)
Micrococcus spp.	18	13.6 (9–19)	6 (all 6)

[a] A 6-mm zone is the diameter of the disk and implies no inhibition.
[b] Coagulase-negative staphylococci.

A 4-h test for PYR hydrolysis has been reported to differentiate non-beta-hemolytic streptococcal species (4); we used the same commercial reagents in our study of staphylococci. A recent report by Oberhofer (21) supported the use of a 4-h PYR test for routine presumptive identification of group D enterococci, group A streptococci, and *S. haemolyticus*. During that study, Oberhofer (21) found that all 26 strains of *S. haemolyticus*, 2 of 7 strains of *S. warneri*, and 1 strain of *S. intermedius* were PYR positive; other strains, including *S. aureus*, *S. epidermidis*, *S. hominis*, *S. saprophyticus*, and *S. capitis*, were PYR negative. In our study, we examined more isolates from more species and found that more species were PYR positive. We have added *S. auricularis*, *S. caprae*, *S. carnosus*, *S. caseolyticus*, *S. chromogenes*, *S. kloosii*, *S. simulans*, *S. xylosus*, *M. kristinae*, *M. luteus*, and *Stomatococcus mucilaginosus* to the list of PYR-positive species reported earlier (21). However, this test easily separates *S. saprophyticus* from *S. xylosus*, *S. aureus* and *S. hyicus* from *S. intermedius*, and *S. hyicus* from *S. chromogenes*.

Except for *S. hominis*, the pattern of results described in our earlier report on synergistic hemolysis (16) was confirmed in this expanded study. The 14 clinical isolates of *S.*

TABLE 11. Summary of characteristics observed with 1,175 clinical isolates[a]

Organism (no.)	% of strains positive for:			% of strains with specific inhibition zone diam for[b]:				
	PYR hydro	Syner hemol	Adherence	Novob (<12)	PolyB (<10)	Bacit (<11)	Fur (>6)	TXO (>7)
S. epidermidis (672)	0	73	83	0.6	97	0.3	100	0
S. hominis (103)	1	52	56	1	9	1	100	0
S. warneri (40)	5	63	5	0	3	3	100	0
S. capitis (15)	0	93	7	0	0	0	100	0
S. sciuri (6)	0	0	33	100	0	100	100	0
S. cohnii (2)	0	100	50	100	0	50	100	0
S. saprophyticus (40)	5	3	73	100	0	13	100	0
S. xylosus (13)	100	54	85	100	0	23	100	0
S. haemolyticus (133)	100	99	42	2	0	90	100	0
S. simulans (48)	96	96	71	0	2	4	100	0
S. intermedius (4)	100	0	100	0	0	0	100	0
S. aureus (79)	3	33	73	0	100	0	100	0
Stomatococcus mucilaginosus (2)	100	50	0	0	100	0	100	100
Micrococcus spp. (18)	83	0	0	6	6	0	0	100

[a] Abbreviations: PYR hydro, PYR hydrolysis; Syner hemol, synergistic hemolysis; Adherence, adherence to glass; Novob, novobiocin, 5 μg; PolyB, polymyxin B, 300 U; Bacit, bacitracin, 10 U; Fur, furazolidone, 100 μg; TXO, Taxo A or bacitracin, 0.04 U.
[b] Values in parentheses are the specific inhibition zone diameters.

hominis tested earlier (16) and all 5 of the *S. hominis* reference strains were negative for synergistic hemolysis, and when retested during this study, they were still negative; but 54 of the 89 clinical isolates of *S. hominis* obtained since that study are positive for synergistic hemolysis. The *S. hominis* and *S. epidermidis* reactions are no longer sufficiently different to clearly separate these two species, but this activity was seen less often with *S. hominis* (52%) than with *S. epidermidis* (73%) strains. Most of the micrococci were negative, but the reference strain of *M. lylae* gave a very strong positive reaction, which may prove helpful if tests of clinical strains agree with the reference data. The reference method for the synergistic hemolysis test uses a living strain of *S. intermedius* on a TSA II plate. Two other methods were examined as part of our initial study (16), and another method was examined in this study. Both the beta-lysin disk from that study (16) and the alpha-toxin disk in this study are reasonable alternatives to a viable strain of *S. intermedius* or *S. aureus* (16) for this test on TSA II plates. Since we have not yet used other types of blood agar plates for any of these synergistic hemolysis tests, we cannot suggest any alternate media formulas.

Adherence to smooth surfaces has been reported for strains of all major species of staphylococci associated with human infections except *S. warneri* (5). We found two weakly positive strains of *S. warneri* in adherence tests with glass tubes, but some of our weakly positive strains might be discarded by some grading standards. None of the micrococci or stomatococci were positive, but 69% of the clinical staphylococci and 83% of the *S. epidermidis* isolates were positive. The strong positive reactions were very easy to see and were good markers for species identity, because 92% of them proved to be *S. epidermidis*. Adherence is presumed to be evidence of slime production, and slime production has been postulated to be both a colonization (6) and a virulence (9, 17) factor. The ability of an organism to produce slime has been reported to be significantly associated with its ability to cause disease in patients with prosthetic devices (9) and to be useful to the clinician in evaluating clinical significance in cases of nosocomial septicemia (17).

The conventional methods we used to identify coagulase-negative staphylococci to the species level included a novobiocin disk-diffusion susceptibility test on P agar (19). Any strain that produced an inhibition zone of 5 mm or less from the edge of the 5-μg disk (total inhibition zone of <17 mm) was reported to be resistant, and any with a zone of >16 mm was reported to be susceptible (19). The mean inhibition zone diameters produced by our strains in susceptible species ranged from 26.2 to 33.1 mm on P agar; the range of zone sizes for strains in resistant species was 6 to 13 mm. The mean inhibition zone diameters produced by the susceptible strains tested on TSA II ranged from 16.8 to 20.7 mm; the zone sizes for resistant species ranged from 6 to 11 mm on TSA II. If we defined susceptibility to novobiocin on TSA II as any inhibition zone size of >16 mm and resistance as <17 mm, then 42% of the *S. haemolyticus*, 17% of the *S. hominis*, 15% of the *S. simulans*, and 13% of the *S. warneri* isolates were resistant to novobiocin. Based on our data, however, we defined a zone diameter of >11 mm as susceptible and a zone diameter of <12 mm as resistant to novobiocin on TSA II (Table 7). This breakpoint does not agree with that recommended in an earlier study (12), in which 11 species of staphylococci were examined for novobiocin susceptibility by disk diffusion on three different media incubated for 18 h: P agar, TSA II, and Mueller-Hinton agar. Goldstein et al. (12) found both TSA II and Mueller-Hinton agar to be satisfactory for novobiocin tests, as long as a zone size of >16 mm was used to define susceptibility. Our tests on P agar were incubated for 18 h, but our tests on TSA II were incubated for a full 24 h. The longer incubation time is recommended for disk diffusion studies of staphylococci (C. Thornsberry, J. M. Swenson, C. N. Baker, L. K. McDougal, S. A. Stocker, and B. C. Hill, Antimicrob. Newsl. **4**: 47–55, 1987).

When resistance to a polymyxin B disk was defined as an inhibition zone diameter of <10 mm, 97% of the *S. epidermidis* and all the *S. aureus*, *S. chromogenes*, *S. hyicus*, and *Stomatococcus mucilaginosus* strains tested were resistant. Only 12 of the other strains were resistant. Resistance to polymyxin B is, therefore, a test that helps differentiate *S. epidermidis* from other clinical species of coagulase-negative staphylococci. When resistance to a bacitracin disk (10 U) was defined as an inhibition zone diameter of <11 mm, 90% of the *S. haemolyticus*, all the *S. sciuri*, and one of the two *S. cohnii* strains tested were resistant. Only 14 other strains were resistant. Resistance to bacitracin is, therefore, a test

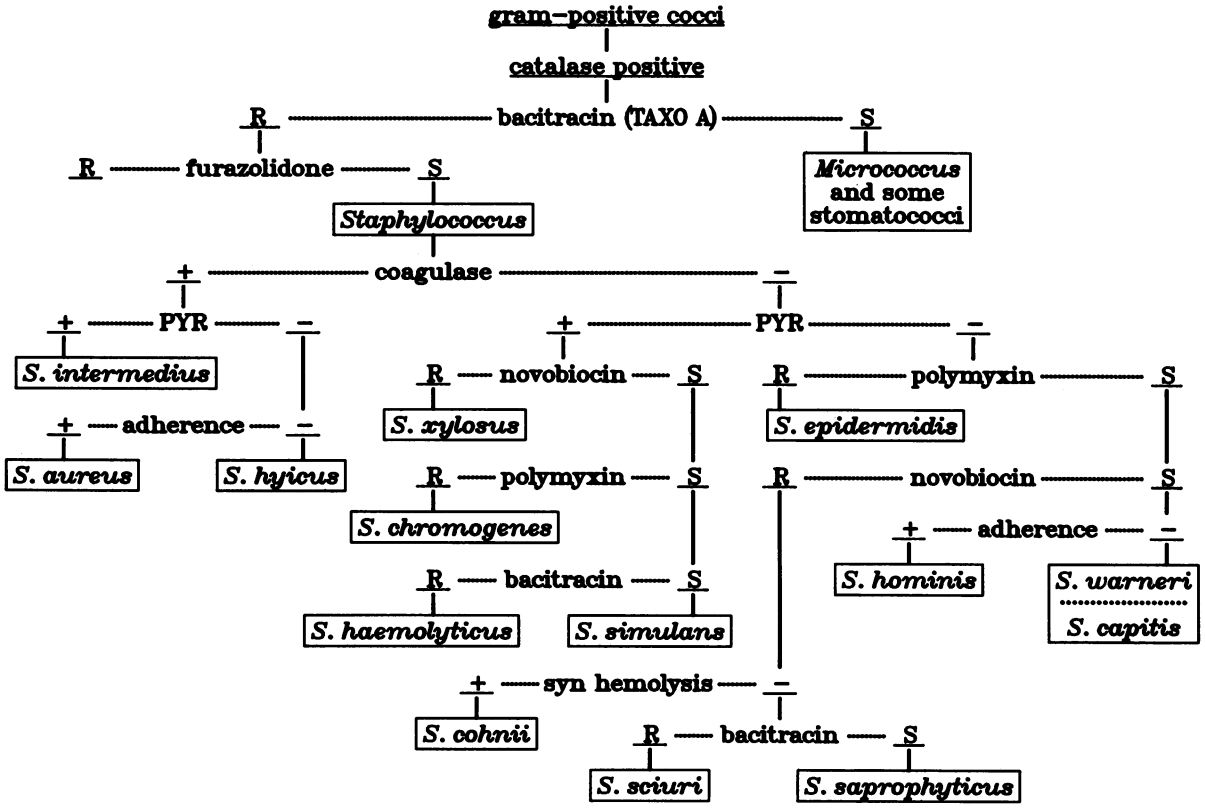

FIG. 1. Characteristics that help to differentiate species of staphylococci and some other members of the family *Micrococcaceae*. The R and S used here indicate resistant and susceptible, respectively, as defined in this report but do not refer to resistant and susceptible as defined by a standardized susceptibility test.

that helps differentiate *S. haemolyticus* from other clinical species of novobiocin-susceptible staphylococci.

Isolates of *Micrococcus* spp. are seldom reported in clinical studies; this could be because they are rarely pathogens, but it could also be because they are rarely recognized. Several tests are currently recommended to separate staphylococci from micrococci, including resistance to lysostaphin, glucose fermentation, and anaerobic growth in semisolid thioglycolate agar. Most staphylococci are reported to be susceptible to lysostaphin, to ferment glucose, and to grow in both the aerobic and anaerobic portions of the thioglycolate agar. Most micrococci are reported to be resistant to lysostaphin, to not ferment glucose, and to grow only in the aerobic portion of the thioglycolate agar. All these tests require special reagents or media. In addition, Gunn et al. (14) reported that 83% of the staphylococci and 4% of the micrococci examined were susceptible to lysostaphin, that 3 of 444 strains of staphylococci were negative for glucose fermentation, and that 86% of the staphylococci and 2% of the micrococci grew in the anaerobic portion of the thioglycolate agar. They further stated that, when identification problems arise, all three tests and several others may be needed to distinguish between these two genera.

Susceptibility to the Taxo A disk (bacitracin, 0.04 U) is routinely used in many clinical laboratories to presumptively identify group A *Streptococcus pyogenes*. This disk test has also been reported as a method for separating staphylococci from micrococci (10); the staphylococci were completely resistant and the micrococci gave inhibition zones of >10 mm in diameter. In a later study, Baker et al. (3) found 94.6% of staphylococci resistant to the Taxo A disk and all micrococci susceptible when an inhibition zone diameter of >10 mm was considered susceptible. In both studies, the medium was Mueller-Hinton agar and incubation was for only 18 h. We used TSA II incubated for a full 24 h and found that all 1,313 strains of staphylococci were resistant with no zones to measure, but all 24 micrococci and all three stomatococci gave inhibition zones of >7 mm in diameter. The Taxo A disk effectively separated our staphylococci from the micrococci and stomatococci.

A selective medium (FTO agar), composed of Trypticase soy agar with yeast extract, Tween 80, oil red O, and furazolidone, was designed to inhibit staphylococci in a study of corynebacteria from skin specimens (23). In a later study, Curry and Borovian (8) found that FTO agar prevented the growth of staphylococci and permitted the growth of micrococci, and they suggested that this characteristic might be used clinically by adding a 50-μg disk to the normal battery of susceptibility tests. In a more recent study of furazolidone (100-μg) disks on Mueller-Hinton agar, Baker (2) found that 99% of staphylococci were susceptible and all micrococci were resistant, and Baker suggested that this test be used with the Staph-Ident strip to prevent the incorrect identification of micrococci as staphylococci. All of our staphylococci were susceptible to the furazolidone (100-μg) disk and all of our micrococci were resistant. We cannot say how useful this test might be for stomatococci, because the three strains that we tested gave divergent results; the reference strain was resistant with no inhibition zone, whereas the two clinical isolates gave inhibition zones of 14

and 21 mm. Further work with many more strains of this genus needs to be done to resolve this conflict.

Analysis of the characteristics observed with the 1,175 identified clinical isolates (Table 11) suggests the following. (i) The furazolidone and Taxo A (bacitracin, 0.04 U) disks should be used together for better separation of staphylococci and micrococci. All the isolates that were susceptible to furazolidone and resistant to Taxo A were staphylococci. (ii) The polymyxin B disk can be used to separate *S. epidermidis* from most other clinical isolates of coagulase-negative staphylococci. (iii) The bacitracin disk (10 U) can be used to separate *S. haemolyticus* from most other novobiocin-susceptible staphylococci. (iv) The PYR test can be used to separate *S. xylosus* from other novobiocin-resistant staphylococci and *S. intermedius* from other coagulase-positive species. (v) The synergistic hemolysis and adherence tests could be useful in separating strains within most of the species into biotypes (15).

Further analysis of our data allowed us to prepare a flow chart of characteristics that help differentiate these organisms (Fig. 1). All of the tests shown in this chart are easy to do, are read at or before 24 h, and use readily available commercial products. For this chart to be valid, however, all of the tests must be done as we have described them in this report, until it is demonstrated that another medium or method is equivalent. These tests require (i) TSA II plates for the synergistic hemolysis and antimicrobial susceptibility tests, (ii) Trypticase soy broth and the Gram stain-safranin reagent for adherence tests, and (iii) a PYR test kit containing tubes of PYR broth and the developing reagent.

The Staph-Ident strip can be used with confidence to identify most clinical isolates of staphylococci, provided that the few simple tests already recommended by Analytab Products are also done. The simple additional tests we described in this report further increase the accuracy of this system. The susceptibility tests with furazolidone and Taxo A disks on TSA II prevent the misidentification of micrococci and stomatococci as staphylococci, and the test for resistance to polymyxin B prevents the misidentification of *S. epidermidis* as *S. hominis*. The tests for resistance to bacitracin and for PYR hydrolysis help to identify some of the species and support the identification of several others. Only commercial media and reagents already available in many clinical laboratories are needed for these extra tests. Further testing of additional strains of micrococci, stomatococci, and the other less frequently encountered species of staphylococci may show that many of them could also be identified with the combination of simple tests reported in this study.

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LITERATURE CITED

1. **Almeida, R. J., and J. H. Jorgensen.** 1983. Identification of coagulase-negative staphylococci with the API STAPH-IDENT system. J. Clin. Microbiol. **18**:254–257.
2. **Baker, J. S.** 1984. Comparison of various methods for differentiation of staphylococci and micrococci. J. Clin. Microbiol. **19**: 875–879.
3. **Baker, J. S., M. F. Hackett, and D. J. Simard.** 1986. Variations in bacitracin susceptibility observed in *Staphylococcus* and *Micrococcus* species. J. Clin. Microbiol. **23**:963–964.
4. **Bosley, G. S., R. R. Facklam, and D. Grossman.** 1983. Rapid identification of enterococci. J. Clin. Microbiol. **18**:1275–1277.
5. **Christensen, G. D., J. T. Parisi, A. L. Bisno, W. A. Simpson, and E. H. Beachey.** 1983. Characterization of clinically significant strains of coagulase-negative staphylococci. J. Clin. Microbiol. **18**:258–269.
6. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. **37**: 318–326.
7. **Coudron, P. E., S. M. Markowitz, L. B. Mohanty, P. F. Schatzki, and J. M. Payne.** 1987. Isolation of *Stomatococcus mucilaginosus* from drug user with endocarditis. J. Clin. Microbiol. **25**:1359–1363.
8. **Curry, J. C., and G. E. Borovian.** 1976. Selective medium for distinguishing micrococci from staphylococci in the clinical laboratory. J. Clin. Microbiol. **4**:455–457.
9. **Davenport, D. S., R. M. Massanari, M. A. Pfaller, M. J. Bale, S. A. Streed, and W. J. Hierholzer, Jr.** 1986. Usefulness of a test for slime production as a marker for clinically significant infections with coagulase-negative staphylococci. J. Infect. Dis. **153**: 332–339.
10. **Falk, D., and S. J. Guering.** 1983. Differentiation of *Staphylococcus* and *Micrococcus* spp. with the Taxo A bacitracin disk. J. Clin. Microbiol. **18**:719–721.
11. **Fosse, T., Y. Peloux, C. Granthil, B. Toga, J. Bertrando, and M. Sethian.** 1986. Meningitis due to *Micrococcus luteus*. Eur. J. Clin. Study Treat. Infect. **13**:280–281.
12. **Goldstein, J., R. Schulman, E. Kelley, G. McKinley, and J. Fung.** 1983. Effect of different media on determination of novobiocin resistance for differentiation of coagulase-negative staphylococci. J. Clin. Microbiol. **18**:592–595.
13. **Gunn, B. A., J. F. Keiser, and R. R. Colwell.** 1983. Numerical taxonomy of staphylococci isolated from clinical sources. Int. J. Syst. Bacteriol. **33**:738–750.
14. **Gunn, B. A., F. L. Singleton, E. R. Peele, R. R. Colwell, J. K. Keiser, and C. O. Kapfer.** 1981. Comparison of methods for identifying *Staphylococcus* and *Micrococcus* spp. J. Clin. Microbiol. **14**:195–200.
15. **Hébert, G. A., R. C. Cooksey, N. C. Clark, B. C. Hill, W. R. Jarvis, and C. Thornsberry.** 1988. Biotyping coagulase-negative staphylococci. J. Clin. Microbiol. **26**:1950–1956.
16. **Hébert, G. A., and G. A. Hancock.** 1985. Synergistic hemolysis exhibited by species of staphylococci. J. Clin. Microbiol. **22**: 409–415.
17. **Ishak, M. A., D. H. M. Groschel, G. L. Mandell, and R. P. Wenzel.** 1985. Association of slime with pathogenicity of coagulase-negative staphylococci causing nosocomial septicemia. J. Clin. Microbiol. **22**:1025–1029.
18. **Kloos, W. E., and J. H. Jorgensen.** 1985. Staphylococci, p. 143–153. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
19. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–88.
20. **Kloos, W. E., and J. F. Wolfshohl.** 1982. Identification of *Staphylococcus* species with API STAPH-IDENT System. J. Clin. Microbiol. **16**:509–516. (Erratum **18**:1020, 1983.)
21. **Oberhofer, T. R.** 1986. Value of the L-pyrrolidonyl-β-naphthylamide hydrolysis test for identification of select gram-positive cocci. Diagn. Microbiol. Infect. Dis. **4**:43–47.
22. **Parisi, J. T.** 1985. Coagulase-negative staphylococci and the epidemiological typing of *Staphylococcus epidermidis*. Microbiol. Rev. **49**:126–139.
23. **Smith, R. F.** 1969. A medium for the study of the ecology of human cutaneous diphtheroids. J. Gen. Microbiol. **57**:411–417.