Numerical Taxonomy of Staphylococci Isolated from Clinical Sources

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A total of 264 staphylococcal and micrococcal isolates from clinical specimens were examined for 62 morphological, physiological, and biochemical characteristics. Numerical taxonomy was used to analyze the data. Forty percent of the strains were identified as *Staphylococcus epidermidis*, 25% were identified as *Staphylococcus saprophyticus* and related species, and 9% were identified as *Staphylococcus aureus*; the remaining strains were a mixture of other *Staphylococcus* spp. and *Micrococcus* spp. Our results were compared with the simplified scheme of Kloos and Schleifer. The phenetic characteristics of the species groups are presented. When applied to the identification of closely similar species, both the simplified scheme and numerical taxonomy have limitations, which should be recognized when a method for taxonomic analysis is selected.

Nine species of coagulase-negative staphylococci have been described (36, 59), some of which are associated with human disease. Among those found on human skin are *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, *Staphylococcus simulans*, *Staphylococcus xylosum*, *Staphylococcus saprophyticus*, *Staphylococcus auricularis*, and *Staphylococcus capitis* (33, 34). Staphylococci have been cultured from body cavities, including eyes (54), mouths (55), vaginas (3), and respiratory tracts (46). Typically, *Micrococcus* spp. comprise less than 20% of the gram-positive, catalase-positive cocci from human skin (34).

In hospital environments, specimen contamination and iatrogenic infections involve primarily staphylococci; it is well known that endogenous and nosocomial infections are often caused by staphylococci (42; S. Brake and F. J. Marsik, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C141, p. 298). The species of coagulase-negative cocci most commonly isolated from human infections (*S. epidermidis* and *S. saprophyticus*) constitute approximately 5% of the total isolates from urinary tract infections in humans (1, 15, 45, 47, 53, 70). Coagulase-negative cocci also are associated with endocarditis (64), colonization of ventriculoatrial shunts (28, 29), and eye infections (54). Indeed, coagulase-negative staphylococci possess a variety of virulence factors and are considered to be opportunistic pathogens (42). Because of the well-recognized resistance of these organisms to antibiotics, therapeutic problems are common (31, 42, 43). The coagu-

lase-negative staphylococci are ubiquitous (6), and the number of compromised hosts infected by multiply-antibiotic-resistant strains of these bacteria appears to be increasing (31, 43, 58).

Several studies reported before 1980 emphasized the lack of distinguishing characters useful in identifying newly described species of staphylococci (14, 21, 48, 68). However, more recently, new definitive tests have been used to strengthen the species definitions of certain *Staphylococcus* spp. previously held in question by some investigators (33, 41). Based on phenotypic and molecular genetic evidence, these *Staphylococcus* species can be assigned to species groups (37). The results of deoxyribonucleic acid (DNA) reassociation and immunological studies support both the establishment of the new species (32, 60) and the species group concept of Kloos et al. (37). The objective of the study reported here was to define efficient and economical methods for diagnostic laboratory identification of species groups of staphylococci.

MATERIALS AND METHODS

Organisms. *Staphylococcus* and *Micrococcus* spp. from clinical sources were obtained from the Walter Reed Army Medical Center, Washington, D.C. The fresh isolates were maintained on tryptic soy agar slants under oil at 5 to 10°C, and stock cultures were maintained in 5% glycerol-tryptic soy broth (Difco Laboratories, Detroit, Mich.) at -196°C.

Reference cultures. In addition to fresh clinical isolates, 24 reference strains of *Staphylococcus* and 1 reference strain of *Micrococcus* were included in this

study. These reference strains and their sources are listed in Table 1.

Characterization. Each strain was examined for a total of 64 unit characters. All test media were inoculated by using sheep blood agar cultures (England Laboratories, Beltsville, Md.) incubated at 35°C for 48 h. All media were used within 3 weeks of preparation. Media in tubes requiring a mineral oil seal were boiled before use to expel dissolved oxygen. Carbohydrate stock solutions were sterilized at a pressure of either 10 lbs/in² for 10 min (cellobiose, lactose, sucrose, arabinose, mannitol, galactose, α -methyl-*D*-mannoside) or 15 lbs/in² for 15 min (pyruvate) or were filter sterilized (amygdalin, glucose, maltose, sorbitol, salicin, raffinose, melibiose, trehalose, inulin, esculin, fructose, xylitol, melezitose, glycerol, sorbose). Appropriate volumes of each sterilized solution were added to sterile basal medium (33), and the media were mixed and dispensed into petri plates.

Media in plates and tubes that did not require the addition of reagents were discarded when a positive reaction occurred. Plates and tubes of media which remained negative after inoculation and incubation were observed for up to 7 days at 35°C, unless otherwise noted, and then discarded. Media in plates and tubes which required the addition of reagents were tested with the reagents at 3 or 7 days, depending on the test.

In most cases, eight cultures were inoculated per plate in 1-cm streaks across the agar surface. When a positive reaction occurred, the agar around the inoculation streak, including the growth, was removed with

a sterile loop to avoid interference with the growth of adjacent cultures.

Colonial morphology, micromorphology, and staining reactions. Production of red, grey, beige to white, or yellow pigment was determined from growth on heart infusion agar (Difco) incubated for 5 days. The Gram stain reaction was determined from heat-fixed smears prepared by the method of Paik and Suggs (51). Smears were prepared from tryptic soy broth cultures after incubation for 36 h. Each stained smear was examined for cell shape, Gram stain reaction, and aggregation pattern (packets and clumps, singles and pairs, and long chains). A culture was scored as positive for an aggregation pattern if more than 75% of the stained cells in a smear demonstrated the characteristic. Although motility was not included in the final data matrix, all strains were tested and found to be nonmotile in wet mount preparations.

Tolerance tests. Cultures were tested for growth at 15, 25, and 45°C on Mueller-Hinton medium (BBL Microbiology Systems, Cockeysville, Md.) after incubation for 7 days and for survival of exposure to 60°C for 30 min in tryptic soy broth (BBL). The ability to grow in the presence of 4 and 6.5% NaCl was determined by the method of Parker and Ball (52). Tolerance to 40% bile was tested on bile-esculin medium (Difco). Tolerance to 10% bile was determined by the method of Carlsson (7, 8).

Susceptibility tests. Standardized antibiotic susceptibility tests were performed by the method of Bauer et al. (4) and in accordance with the rules and regulations of the Federal Register (19, 20). The following antibiotic disk concentrations were used: gantrisin (BBL), 300 μ g; nitrofurantoin, 300 μ g; novobiocin, 5 μ g; kanamycin, 30 μ g; erythromycin, 15 μ g; streptomycin, 10 μ g; nalidixic acid, 30 μ g; tetracycline, 30 μ g; and, ampicillin, 10 μ g. Nonstandardized susceptibility tests were done as described by Gunn (25) for the following: bacitracin (Taxo A; BBL); optochin (Taxo P; BBL); methicillin (BBL), 5 μ g; clindamycin (BBL), 2 μ g; and SXT (BBL), 25 μ g. Any zone of inhibition around a bacitracin disk was recorded as a positive test for susceptibility. Susceptibility to optochin, methicillin, clindamycin, and SXT was recorded if the diameter of inhibition on sheep blood agar was ≥ 15 , 10, 16, and 11 mm, respectively.

Substrate utilization. Heart infusion agar (Difco) was supplemented with one of the following ingredients: lysozyme substrate (Difco), soluble starch (Difco), gelatin (Difco), or Tween 80 (Sigma Chemical Co., St. Louis, Mo.). The test for lysozyme production (0.25% lysozyme substrate) was done by the method of Holt (30); the tests for gelatin (1.5%) and starch (0.2%) hydrolysis were done by the method of Oxborough and Faero (50); and the test for Tween 80 (1%) hydrolysis was done by the method of Crombach (9).

Carbohydrate fermentation tests. The purple agar (Difco) plate test of Kloos et al. (35) was used with the following substrates: arabinose, amygdalin, cellobiose, fructose, galactose, glycerol, inulin, lactose, maltose, mannitol, melibiose, melezitose, raffinose, salicin, sorbitol, sorbose, sucrose, α -methyl-*D*-mannoside, trehalose, and xylitol. Pyruvate fermentation was determined by the method of Gross et al. (24).

Biochemical tests. Phosphatase activity was determined by using the overnight broth method of Smith et al. (61). Esculin hydrolysis was detected by using

TABLE 1. List of reference strains of *Staphylococcus* and *Micrococcus*

Species	Phenon	Strain(s) ^a
<i>S. saprophyticus</i>	1a	AW 163, CCM 883 ^T (= ATCC 15305 ^T)
<i>S. xylosus</i>	2	DSM 20266 ^T (= ATCC 29971 ^T), DSM 20267
<i>S. cohnii</i>	3	DSM 20260 ^T (= ATCC 29974 ^T), DSM 20261
<i>S. capitis</i>	4	ATCC 27840 ^T , ATCC 27841
<i>S. haemolyticus</i>	4	DSM 20265 (= ATCC 29968)
<i>S. simulans</i>	5	ATCC 27851, ATCC 27848 ^T
<i>S. hominis</i>	7	ATCC 27846
<i>S. epidermidis</i>	8a	ATCC 14990 ^T , RM351
<i>S. haemolyticus</i>	9	DSM 20263 ^T (= ATCC 29970 ^T)
<i>S. hominis</i>	10	ATCC 27844 ^T
<i>S. aureus</i>	11a	JL68, ATCC 12600 ^T
<i>S. warneri</i>	12	ATCC 27836 ^T , ATCC 27839
" <i>S. lentus</i> "	13a	K6, ATCC 29070 ^T
<i>S. sciuri</i>	13b	ATCC 29059, ATCC 29062 ^T
<i>M. luteus</i>	14b	WRAMC 252

^a Reference strains of *Staphylococcus* species were obtained from W. E. Kloos, North Carolina State University, Raleigh.

ferric citrate reagent (44). Nitrate reduction was detected as described by Vera and Dumoff (69), using cultures incubated for 3 days in nitrate broth (Difco) containing Durham tubes. Reagents were prepared and used as described by Paik and Suggs (51). The oxidase test was performed by using the tetramethyl-*p*-phenylenediamine hydrochloride reagent (Sigma), as described by Paik and Suggs (51). Catalase was determined as described by McFaddin (44). The bile-esculin reaction was determined on bile-esculin medium (Difco) and was interpreted as described by Facklam (18) and McFaddin (44). The Voges-Proskauer test was done by the method of Paik and Suggs (51) after incubation of the organism in methyl red-Voges-Proskauer (MR-VP) broth for 3 days (BBL). Decarboxylase and dihydrolase were determined by using Moeller decarboxylase base (Difco) and L-lysine (Sigma), L-arginine (Sigma), or L-ornithine (Sigma), as described by Vera and Dumoff (69). After the first 160 cultures were tested, the lysine and ornithine tests were excluded from the study because all cultures examined were negative. Deoxyribonuclease (DNase) production was determined on DNase test agar (BBL), and the cultures were developed with 0.1 N HCl (69). The cultures were held for 3 days at 35°C before development with the HCl reagent. The CAMP test was done by the method of Darling (10) and was interpreted as described by Fuchs et al (22).

Hemolytic activity. Alpha-hemolytic, beta-hemolytic, and nonhemolytic reactions on sheep blood agar were determined by the method of Facklam (18).

Coagulase test. The slide test was used to screen cultures for coagulase production, and the tube test was used to confirm equivocal reactions (51). Sterile, diluted (1:4 with physiological saline), ethylenediaminetetraacetic acid-treated rabbit plasma (Difco) was used to perform these tests.

Peroxide production and chocolate agar clearing tests. Chocolate agar (England Laboratories) cultures were incubated for 7 days at 35°C. Peroxide production was detected by a yellowing of the agar medium around and beneath the bacterial growth (8). Clearing of chocolate agar was preceded by a darkening of the medium immediately surrounding the bacterial growth.

Oxidation-fermentation test. The modified method of Davis and Hoyle (11) and the Subcommittee on Taxonomy of Staphylococci and Micrococci (66) was used (26) for the oxidation-fermentation test.

Lysostaphin susceptibility. Lysostaphin susceptibility was determined by the method of Hajek (26, 27).

Anaerobic growth in thioglycolate semisolid agar. The method of Evans and Kloos (18), as modified by Davis and Hoyle (11), was used (26).

Coding of data. Characters were coded 1 if present or positive, 0 if absent or negative, and 9 if not applicable or not comparable. The final $n \times t$ matrix contained 264 strains and 64 characters. The physiological characters used in this study are listed in Tables 2 and 3.

Computer analyses. The data were analyzed by using the Jaccard coefficient (62), which excludes negative matches. Clustering was done by the unweighted average linkage method (63). The programs used, Taxan6 and IGPS3, are stored on the UNIVAC 1108 computer at the University of Maryland Computer Science Center.

RESULTS

The results obtained with the Jaccard coefficient showed that 94% of the 264 strains examined were included in 14 clusters (Fig. 1). At the 38% level of similarity (*S* value), all 264 strains fell into two main groups (*Staphylococcus* and *Micrococcus* spp.). The test characters associated with micrococci included resistance to lysostaphin, aerobic growth in semisolid thioglycolate agar, and inability to ferment glucose and glycerol. The staphylococci were further subdivided, at an *S* value of 52%, into groups identified as *Staphylococcus sciuri* and other *Staphylococcus* spp. The major group of staphylococci was subdivided into three clusters of novobiocin-resistant staphylococci (phena 1 through 3) and nine clusters of novobiocin-susceptible staphylococci (phena 4 through 12); 6% of the staphylococci could not be assigned to any of the described species (2, 36, 59) and may represent new species.

Characterization and identification of the phena. Phenon 1a, 2, 1b, and 3, which demonstrated an intergroup *S* value of 71%, comprised the novobiocin-resistant staphylococci (Tables 2 and 3 and Fig. 1). The distinguishing characteristics of these phena are summarized in Table 4. Phenon 1a, with an intragroup *S* value of 72%, was the second largest cluster in this study. All of the strains in this group were isolated from human urine, and the phenon was identified as *S. saprophyticus* by using the description of Schleifer and Kloos (59) and the relationships of this group of strains to reference cultures CCM 883^T (*T* = type strain) and AW163. In general, the characteristics of these strains matched the description of the species.

Phenon 2 demonstrated an intragroup *S* value of 86% and comprised two reference strains, *S. xylosus* DSM 20266^T and DSM 20267. These staphylococci were differentiated from the strains of phenon 1a by the nitrate and phosphatase tests (Table 2).

Phenon 1b, with an intragroup *S* value of 72%, was similar to phenon 1a, but differed in being unable to produce acid from lactose and xylitol.

Phenon 3 demonstrated an intragroup *S* value of 78% and comprised two reference strains, *S. cohnii* DSM 20260^T and DSM 20261, and one clinical isolate. Except for growth at 45°C and production of acid from sorbitol, these three strains were identical in phenotype to *S. cohnii* (59). Phenon 3 differed from phenon 1a in lacking sucrose fermentation.

Phenon 4, which was composed of 15 strains of staphylococci and demonstrated an intragroup *S* value of 74%, was identified as *S. capitis* based on the description of Schleifer and Kloos (59) and on the relationship of these strains to reference strains *S. capitis* ATCC

FIG. 1. Dendrogram showing relationships among gram-positive cocci.

27840^T and ATCC 27841. *S. haemolyticus* DSM 20265 also clustered with phenon 4.

Phenon 5 contained nine clinical isolates and two reference strains. This phenon was identified as *S. simulans* based on the relationships of the organisms to reference strains ATCC 27848^T and ATCC 27851 and the description of the species by Kloos and Schleifer (36). Although this phenon was more closely associated with coagulase-negative staphylococci, its characteristics, including beta-hemolysis, fermentation of mannitol, hydrolysis of arginine and Tween 80, and reduction of nitrate, were similar to the characteristics of phenon 11 (*S. aureus*). The intragroup *S* value for phenon 5 was 72%.

The phenon 6 intragroup *S* value was 78%, and this phenon comprised five clinical isolates; it contained no reference strains. Phenon 6, which was identified as *S. hominis* (36), included strains which grew poorly in semisolid thioglycolate agar but strongly fermented glucose. The apparent contradiction of poor growth under anaerobic conditions combined with strong anaerobic utilization of glucose has been reported previously for this species by Kloos and Schleifer (36).

The phenon 7 intragroup *S* value was 77%, and this phenon comprised one reference strain and four clinical strains. Phenon 7 was identified as atypical *S. hominis* based on the relationships of the organisms to reference strain *S. hominis* ATCC 27846 and the species description (36). This phenon differed from *S. hominis* type strain ATCC 27844, the sole member of phenon 10, by its inability to produce acid from trehalose and lactose and a positive arginine dihydrolase reaction; the first two of these characteristics are atypical for the species (36).

The phenon 8a intragroup *S* value was 83%, and this phenon comprised 70 clinical and 2 reference strains. The salient differential characteristics of *S. epidermidis* (phena 8a and 8b) and related phena are summarized in Table 5. A total of 14 unidentified strains were also included because of their phenotypic relationships with strains in these phena. Phenon 8a was identified as *S. epidermidis* based on relationships to reference strains RM351 and ATCC 14990^T and the description of Schleifer and Kloos (59). This phenon was relatively homogeneous and closely matched the description of the species. The phenon 8b intragroup *S* value was 79%, and this

TABLE 2. Physiological characteristics of clinical isolates and reference strains of staphylococci: phena 1 through 8^a

Characteristic or test ^b	Frequency in phenon:									
	1a (n = 56) ^c	2 (n = 2)	1b (n = 4)	3 (n = 3)	4 (n = 15)	5 (n = 12)	6 (n = 5)	7 (n = 5)	8a (n = 72)	8b (n = 34)
Cellular arrangement										
Packets or clumps	45 ^d	100	50	67	100	75	0	100	100	0
Singles or pairs	55	0	50	33	0	25	100	0	0	100
Enzymes										
NO ₃ reduction	2	100	25	0	100	100	100	100	99	77
Arginine dihydro- lase	0	0	25	0	100	100	100	80	81	38
Lysozyme produc- tion	100	100	50	100	0	58	0	20	1	0
Gelatinase produc- tion	21	100	0	0	0	27	0	0	57	32
Coagulase	0	0	0	0	0	0	0	20	0	0
DNase	2	0	0	0	20	33	0	80	10	3
Tween 80 lipase	4	0	0	67	7	100	20	0	67	71
Phosphatase	0	100	0	0	0	25	20	0	80	85
Growth on 6.5% NaCl	98	100	75	100	100	100	100	100	100	94
Growth at:										
15°C	96	100	100	100	0	42	20	20	0	3
45°C	2	100	25	0	27	58	100	80	39	88
β-Hemolysis	7	0	0	0	100	92	40	0	19	41
Bile-esculin medium: growth	100	100	100	100	100	100	100	100	97	85
Bile-esculin medium: black	7	0	0	0	13	0	20	0	4	6
Voges-Proskauer	52	0	75	67	73	17	20	60	99	74
Chocolate agar clear- ing	4	0	0	0	0	33	20	0	72	77
OF dextrose: fer- mentation ^e	100	100	100	100	93	91	100	100	99	100
Novobiocin suscepti- ble	5	0	0	0	93	100	100	100	99	100
Acid from:										
Arabinose	0	50	0	0	0	0	0	0	0	0
Cellobiose	2	0	0	0	0	0	0	0	0	0
Esculin hydrolysis	2	50	0	0	7	0	20	20	6	0
Fructose	100	100	100	100	100	83	100	80	99	100
Galactose	96	100	100	0	33	25	40	0	79	71
Glycerol	100	100	100	100	87	67	100	100	100	100
Inulin	2	0	0	0	0	0	0	0	0	0
Lactose	84	100	0	0	27	92	80	20	96	94
Maltose	100	100	100	100	87	17	100	100	100	100
Mannitol	96	100	100	100	87	92	100	100	0	0
Melezitose	23	0	75	0	0	0	0	25	58	47
Pyruvate	2	50	0	0	0	0	0	0	0	0
Sorbitol	2	50	0	67	13	0	0	0	0	0
Sorbose	0	0	0	0	0	0	0	0	0	3
Sucrose	98	100	100	0	100	100	100	100	99	100
Methyl-d-manno- side	4	0	0	0	0	0	0	0	0	0
Trehalose	75	100	75	100	47	75	0	0	0	6
Xylitol	79	100	0	0	0	0	0	0	0	3
Yellow pigment	43	100	100	0	8	0	0	75	0	0
White pigment	57	0	0	100	92	100	100	25	100	100
Lysostaphin suscepti- ble	82	100	50	100	58	100	60	75	96	82
Anaerobic growth	74	100	25	0	75	100	60	75	91	91

^a See Fig. 1.^b All strains were non-chain-forming, gram-positive cocci which grew at 25°C and on 4% NaCl, were amylase negative, did not produce hydrogen peroxide on chocolate agar, were oxidase negative, lacked red and gray colonial pigments, were not alpha-hemolytic, and did not produce acid from amygdalin, melibiose, raffinose, or salicin. All strains produced acid from glucose in oxidation-fermentation medium (open tubes) and were catalase positive.^c Number of strains examined.^d Percentage of strains positive.^e Fermentation in oxidation-fermentation medium containing dextrose.

TABLE 3. Physiological characteristics of clinical isolates and reference strains of staphylococci: phena 9 through 13^a

Characteristic or test ^b	Frequency in phenon:						
	9 (n = 2) ^c	10 (n = 1)	11a (n = 20)	11b (n = 4)	12 (n = 2)	13a (n = 2)	13b (n = 2)
Cellular arrangement							
Packets or clumps	100 ^d	100	90	25	100	50	100
Singles or pairs	0	0	10	75	0	0	0
Enzymes							
Oxidase	0	0	0	0	0	100	100
NO ₃ reduction	50	100	100	100	0	100	100
Arginine dihydrolase	100	0	100	75	100	0	0
Lysozyme production	0	100	90	100	0	0	0
Gelatinase production	50	100	100	25	0	0	100
Coagulase	0	0	100	0	0	0	0
DNase	0	0	100	100	0	0	0
Tween 80 lipase	0	0	85	75	100	0	0
Phosphatase	50	0	95	100	0	0	100
Growth at:							
15°C	50	0	100	100	100	100	100
45°C	50	0	65	100	0	0	0
β-Hemolysis	50	0	95	0	0	0	0
Bile-esculin medium: black	0	0	80	50	0	50	100
Voges-Proskauer	100	0	85	50	100	0	0
Chocolate agar clearing	0	0	95	25	0	0	100
Novobiocin susceptible	100	100	100	100	100	100	0
Acid from:							
Arabinose	0	0	0	0	0	50	50
Amygdalin	0	0	0	0	0	100	100
Cellobiose	0	0	0	0	0	100	100
Esculin hydrolysis	0	0	80	25	0	100	100
Fructose	50	100	95	100	100	100	100
Galactose	0	0	30	25	0	50	0
Glycerol	100	100	90	100	0	50	0
Lactose	100	100	20	0	0	50	0
Maltose	100	100	100	100	100	50	100
Mannitol	0	0	100	100	0	50	50
Melibiose	0	0	5	0	0	0	0
Melezitose	100	100	0	0	0	0	100
Pyruvate	0	0	20	0	100	50	50
Raffinose	0	0	0	0	0	100	0
Salicin	0	0	0	0	0	100	100
Sorbitol	0	0	0	0	0	50	50
Sucrose	100	100	95	100	100	100	100
α-Methyl-d-mannoside	0	0	0	0	0	0	50
Trehalose	100	100	35	75	100	100	100
Yellow pigment	0	100	95	75	50	0	50
White pigment	100	0	5	25	50	100	50
Anaerobic growth	100	100	100	75	100	0	50

^a See Fig. 1.

^b All strains were non-chain-forming, gram-positive cocci which grew at 25°C and on 4% NaCl, were amylase negative, did not produce hydrogen peroxide on chocolate agar, did not produce gray or red colonial pigment, were not alpha-hemolytic, and did not produce acid from inulin, sorbose, or xylitol. All strains produced catalase, grew on 6.5% NaCl and bile-esculin medium, produced acid from glucose in oxidation-fermentation medium (sealed and open tubes), and were lysed by lysostaphin.

^c Number of strains examined.^d Percentage of strains positive.

phenon also was identified as *S. epidermidis*; however, it did not include *S. epidermidis* reference strains. The intergroup *S* value of phena 8a and 8b was 73%. These two clusters were similar in being predominantly nitrate and phosphatase

positive, nonhemolytic, and negative for fermentation of trehalose and mannitol; the two subphenon differed only in frequency of occurrence of selected characters were present. Interestingly, the characteristic most useful in distin-

TABLE 4. Characteristics useful in differentiating phenon 1 through 3

Test	Frequency in:			
	<i>S. saprophyticus</i> (phenon 1a; n = 56)	Atypical <i>S. saprophyticus</i> (phenon 1b; n = 4)	<i>S. xylosus</i> (phenon 2; n = 2)	<i>S. cohnii</i> (phenon 3; n = 3)
Nitrate reduction	2 ^a	25	100	0
Phosphatase	0	0	100	0
Gelatinase	21	0	100	0
Acid from:				
Arabinose	0	0	50	0
Lactose	85	0	100	0 ^b
Melezitose	23 ^b	75	0	0
Sucrose	98	100	100	0
Xylitol	79	0	100 ^b	0 ^b

^a Percentage of strains positive.^b Reactions that differed from the reactions given in the original species descriptions.

guishing these two subgroups (i.e., the uniformly different characteristic) was cellular arrangement in broth; phenon 8a cells occurred as packets or clumps, and phenon 8b cells occurred singly or in pairs.

The phenon 9 intragroup *S* value was 78%, and this phenon was composed of one clinical strain and one reference strain. Phenon 9 was identified as *S. haemolyticus* based on inclusion of reference strain DSM 20263^T and the description of the species (59). This phenon was closely related to phenon 10 (*S. hominis* ATCC 27844^T), with which the intergroup *S* value was 76%. On

the basis of the results of this study, phenon 9 and 10 should have been considered a single cluster, but were not because the two reference strains were type strains for two species (36, 59).

The phenon 11 intragroup *S* value was 72% and this phenon comprised two subclusters. The major difference between subphenon 11a and 11b was the absence of coagulase reactions in strains of subphenon 11b, which were identified in this study as coagulase-negative *S. aureus*. These four strains have not been subjected to cell wall analysis or phage susceptibility tests, tests which are used to identify coagulase-negative

TABLE 5. Characteristics useful in differentiating *S. epidermidis* from related species

Test	Frequency in:				
	<i>S. epidermidis</i> (phenon 8a and 8b; n = 106)	<i>S. capitis</i> (phenon 4; n = 15) ^a	<i>S. warneri</i> (phenon 12; n = 2)	<i>S. haemolyticus</i> - <i>S. hominis</i> (phenon 6, 7, 9, and 10; n = 13)	Uniden- tified species (n = 14) ^b
Phosphatase	82 ^c	0	0	15	7
Gelatinase	49	0	0	15	33
Chocolate agar clearing	74	0	0	8	0
Acid from melezitose	56	0	0	31	36
Growth at 15°C	1	0	100	23	29
Yellow pigment	0	8	50	31	57
Acid from mannitol	8	87	0 ^d	77	43
Acid from trehalose	2	47 ^d	100	62 ^d	64
DNase	8	20	0	31	21
Nitrate reduction	92	100	0	92	80
Acid from lactose	95	27	0	62	71
Acid from galactose	76	33 ^d	0	15	50
β-Hemolysis	26	100	0	23	64
Pairs of cells or single cells	32	0	0	39	50
Tween 80 hydrolysis	68	7	100	8	20
Voges-Proskauer	91	73	100	46	36
Anaerobic growth	91	58	100	76	50

^a Included two yellow-pigmented strains of "S. capitis-like" bacteria.^b Unidentified staphylococci not associated with named phenon in this study.^c Percentage of strains positive.^d Reactions that differed from the reactions given in the original species descriptions.

strains of *S. aureus*, but the biochemical and physiological characteristics of the strains fit the description of the species (2, 27). Four of the strains included in subphenon 11a only weakly coagulated rabbit plasma. The intragroup *S* value of subphenon 11a was 75%, whereas that of subphenon 11b was 76%. No reference strain fell into subphenon 11b; however, subphenon 11a did include reference strains JL68 and ATCC 12600^T and, based on the descriptions of the species provided by Baird-Parker (2) and Kloos and Schleifer (36), was identified as *S. aureus*. Other species of staphylococci which were not included in the present study but have been described by other investigators (12, 13, 27, 68) are listed in Table 6 for comparative purposes.

The phenon 12 intragroup *S* value was 95%, and this group included *Staphylococcus warneri* strains ATCC 27836^T and ATCC 27839. The characteristics of these strains were in accordance with the description of the species by Kloos and Schleifer (36), but these organisms differed from other staphylococci in being negative for nitrate reduction, phosphatase, and production of acid from lactose and mannitol.

Phenon 13 comprised two subclusters with an intragroup *S* value of 61%. The phenon 13a intragroup *S* value was 62%, and this phenon was composed of two reference strains of "*S. sciuri* subsp. *lentus*", strains ATCC 29070^T and K6. The phenon 13b intragroup *S* value was

83%, and this group was identified as *S. sciuri* subsp. *sciuri* based on characteristics reported by Kloos et al. (38). Phenon 13b included two reference cultures of *S. sciuri* subsp. *sciuri*, strains ATCC 29059 and ATCC 29062^T. The characteristics useful in differentiating subphenon 13a from subphenon 13b were positive oxidase test and production of acid from amygdalin, cellobiose, esculin, and salicin.

Subphenon 14a and 14b comprised six strains of micrococci and were included in this study to establish the robustness of the character data set for delineating groups of catalase-positive cocci encountered in clinical laboratories. The subphenon 14a intragroup *S* value was 92%, and this phenon consisted of two strains of *Micrococcus* spp. which could not be further identified by using the descriptions of species of micrococci provided by Kloos et al. (39). The phenon 14b intragroup *S* value was 65%, and this phenon consisted of three clinical strains and one reference strain fitting the description of *Micrococcus luteus* (39).

All of the strains examined in this study were negative for bile solubility, susceptibility to optochin and bacitracin, indole and H₂S production (SIM medium), motility, ornithine and lysine decarboxylases, growth on MacConkey agar, xanthinase, tyrosinase, ribonuclease, hydrolysis of elastin, reactivity to streptococcus antisera A, B, C, D, F, and G (Difco), and

TABLE 6. Characteristics useful in differentiating *S. aureus* and related staphylococci

Test ^a	Frequency in:			
	<i>S. aureus</i> (phenon 11a and 11b; n = 24)	<i>S. simulans</i> (phenon 5; n = 12)	<i>S. intermedius</i> ^b	<i>S. hyicus</i> ^c
Enzymes				
Oxidase	0 ^d	0	100 (w) ^e	0
Coagulase	83	0	100	19
Phosphatase	96	25	100	100
Gelatinase	88	27	94	100
Acid from:				
Mannitol	100	92	86	13
Maltose	100	17	0	11
Voges-Proskauer	79 ^f	17	0	0
β-Hemolysis	79	92 ^f	22	0
Yellow pigment	92	0	0	21
Heat-stable nuclease	100 ^g	77 ^g	100	>74
Anaerobic fermentation of mannitol	96 ^g	97 ^g	0	0 ^h

^a All four species were positive for lysozyme production and the DNase, Tween 80 lipase, and arginine dihydrolase reactions.

^b Data from reference 27. This species is associated with warm-blooded animals.

^c Data from reference 13. This species is associated with warm-blooded animals.

^d Percentage of strains positive.

^e w, Weak positive reaction.

^f Reactions different from those given in the original species descriptions.

^g Results from reference 68.

^h Results from reference 12.

production of phenylalanine deaminase. These characters were eliminated from the numerical analysis, as were the following characters which were found to be positive for all strains: survival at 60°C for 30 min, growth in medium supplemented with 2% NaCl, and growth on eosin-methylene blue agar.

Tests which were difficult to reproduce or interpret included the CAMP test, growth on mitis-salivarius agar, citrate utilization, and extracellular polysaccharide production. Thus, these tests were eliminated from the numerical analysis.

After 100 cultures had been tested, the following antibiotic susceptibility tests were omitted from the analysis because results were identical for all species examined: clindamycin, methicillin, SXT, gantrisin, nitrofurantoin, kanamycin, erythromycin, streptomycin, nalidixic acid, tetracycline, and ampicillin.

DISCUSSION

From the results of this study, it is clear that phenotypic heterogeneity exists within some *Staphylococcus* species and that other species are phenotypically homogeneous. Although some species are not as easily distinguished from each other as other species are, the major groups of staphylococci can be recognized with reasonable accuracy and efficiency in the laboratory. The species group concept, as delineated by Kloos et al. (37), provides a useful system for clustering closely related species of staphylococci into major groups. Schleifer et al. (60) have suggested dividing the original *S. epidermidis* group into the following two subgroups: the *S. epidermidis* group and the *S. hominis* group. The first subgroup includes *S. epidermidis*, *S. capitis*, and *S. warneri*; and the second subgroup includes *S. hominis* and *S. haemolyticus*. This system is a conservative approach to the classification of staphylococci, but is reasonable, since for years clinical laboratories have been content with lumping coagulase-negative, catalase-positive cocci under the name *S. epidermidis*. Although the species group concept is supported by molecular genetic data, it offers little advantage over the classification scheme provided by Baird-Parker (2) in *Bergey's Manual of Determinative Bacteriology*. However, this approach is important in that it gives clinical laboratories a method for improved resolution of the species of *Staphylococcus*. Thus, data gathered for not-so-well-described species can be statistically analyzed and handled by using groups of closely related strains (i.e., species groups).

Four major species groups of staphylococci were observed in this study and could be distinguished by a numerical analysis of conventional

tests. These groups are identical in species composition to the species groups described by Schleifer et al. (60), who used molecular genetic methods of analyses. The results of the present study are summarized in Table 7.

The first species group, *S. sciuri*, is associated with animals and has not been reported to be associated with clinical material. This group is characteristically oxidase positive and produces acid from amygdalin, cellobiose, and salicin. Feltham (21) suggested a new genus name for these bacteria, "*Cellobiosococcus*." The uniqueness of this species is certainly evident and is supported by the results of our study. However, the results of DNA composition, DNA-DNA homology, and comparative immunological studies do not support the assignment of these bacteria to a new genus (32, 60); rather, the data suggest that the two subspecies of *S. sciuri* should be given separate species status within the genus *Staphylococcus* (i.e., "*Staphylococcus lentus*" and *S. sciuri*) (32). The results of numerical analyses clearly support this suggestion, since the two subspecies were linked at an *S* value of 62%. A number of tests can be used to distinguish the two subspecies (Table 3). Since these bacteria are usually not cultured from clinical material, their taxonomy is not discussed further here. Additional information on *S. sciuri* is contained in the report of Kloos et al. (38).

The second species group, *S. saprophyticus*, comprises the following three novobiocin-resistant species of staphylococci: *S. saprophyticus*, *S. cohnii*, and *S. xylosus*. It is important to distinguish these species since the first is a common pathogen of urinary tracts and *S. cohnii* and *S. xylosus* are typically nonpathogenic (33). This group corresponds to lyogroup IV based on bacteriolytic activity patterns, as described by Valardo and Satta (68). The three species show only minor differences when they are compared by the assay system used by Valardo and Satta (68). However, the reassociation results of Pulverer et al. (56) and Schleifer et al. (60) emphasize the validity of the three species. Also, immunological studies have revealed that *S. saprophyticus* and *S. xylosus* are more closely related to each other than to *S. cohnii* (60), although the three are similar enough in other characters to be assigned to the same species group. The phenotypic analyses in our study support the close association that reportedly exists among the three species and the distinctness of their phenetic species group.

The small number of strains of *S. cohnii* and *S. xylosus* identified in this study correlates with the frequency of occurrence of these organisms on human skin (34). *S. saprophyticus*, the most common species of the group and the species

TABLE 7. Identification of species groups of *Staphylococcus*

Test	Frequency in: ^a			
	<i>S. sciuri</i> - " <i>S. lentus</i> " group	<i>S. saprophyticus</i> - <i>S. cohnii</i> - <i>S. xylosum</i> group	<i>S. epidermidis</i> - <i>S. capitis</i> - <i>S. warneri</i> - <i>S. haemolyticus</i> - <i>S. hominis</i> group	<i>S. aureus</i> - <i>S. simulans</i> group
Coagulase	0 ^b	0	<1	56
Oxidase	100	0	0	0
Acid from:				
Amygdalin	100	0	0	0
Cellobiose	100	0	0	0
Salicin	100	0	0	0
Mannitol	50	97	19	97
Novobiocin susceptible	50	4	94	100
Growth at 15°C	100	97	6	81
Lysozyme production	0	97	6	81
Nitrate reduction	100	6	90	100
Phosphatase	50	3	60	72
DNase	0	2	12	78
Tween 80 hydrolysis	0	7	53	89
Growth at 45°C	0	6	55	67
Arginine dihydrolase	0	2	68	97
β-Hemolysis	0	6	36	83
Voges-Proskauer	0	53	80	58

^a The following numbers of strains were examined: *S. sciuri*, 2; "*S. lentus*," 2; *S. saprophyticus*, 60; *S. cohnii*, 3; *S. xylosum*, 2; *S. epidermidis*, 106; *S. capitis*, 15; *S. warneri*, 2; *S. haemolyticus*-*S. hominis*, 20; *S. aureus*, 24; *S. simulans*, 12.

^b Percentage of strains positive.

implicated in human urinary tract infections, for the most part fits the description of the species (59). Interestingly, however, Schleifer and Kloos (59) observed that none of 83 strains examined by them was positive for melezitose fermentation. In our study, 23% of the strains examined were positive for this test. Devriese and Van De Kerckhove (14) partially explained this discrepancy, which they also noted. These authors demonstrated that variability in melezitose fermentation by staphylococci was a result of the medium used. When the type strain of *S. saprophyticus* was examined by Devriese and Van De Kerckhove (14), it occasionally fermented melezitose if a medium other than the medium of Schleifer and Kloos (59) was used for testing. Our results support the significance of the medium used for detecting fermentation of carbohydrates and bring into question the reliability of identification schemes which rely heavily on carbohydrate fermentation reactions to identify staphylococci isolated from clinical sources. A new *Staphylococcus* identification kit (STAPH-IDENT; Analytab Products, Plainview, N.Y.) which uses six enzymatic and four carbohydrate tests has been reported to distinguish species previously difficult to separate by the simplified scheme of Kloos and Schleifer (35). Preliminary studies in our laboratory with the test kit to identify 209 strains isolated from clinical sources (Gunn, Keiser, and Colwell, unpublished data) confirm this report. The re-

sults of this study indicate that oxidase activity, lysozyme production, and Tween 80 hydrolysis also are useful for identifying the species of *Staphylococcus*.

The third species group, *S. epidermidis*, consists of lyogroups III, V, and VI of Valardo and Satta (68), which represent the species *S. capitis* (lyogroup III), *S. epidermidis* (lyogroup V), and *S. warneri*, *S. hominis*, and *S. haemolyticus* (lyogroup VI).

The results of our study and other studies (21, 59, 68) show that *S. epidermidis* differs from other species in the *S. epidermidis* group in phosphatase activity, gelatinase production, clearing of chocolate agar, growth at 15°C, Tween 80 hydrolysis, DNase, and production of acid from mannitol and trehalose (Table 5). Two biotypes of *S. epidermidis* have been isolated from clinical specimens, but more information is needed to establish the importance of reliability of the methods used for distinguishing these biotypes.

S. capitis (lyogroup III) was readily distinguished from *S. hominis*, *S. haemolyticus*, and *S. warneri* by the bacteriolytic system of Valardo and Satta (68). The phenotypic differences among these species include responses in carbohydrate fermentation tests and colony characteristics (33, 36, 41, 59). Durham and Kloos (16) reported that *S. capitis* differs from the other three species in cellular fatty acid composition. Although the fatty acid composition of *S. war-*

neri was different, it was more closely related to the fatty acid composition of *S. capitis* than to the fatty acid compositions of *S. hominis* and *S. haemolyticus*. The fatty acid compositions of the latter two species were virtually indistinguishable. Using numerical taxonomy, Feltham (21) reported that *S. capitis* and *S. warneri* share a high degree of intergroup similarity. This author also reported that *S. haemolyticus* DSM 20265 clustered with strains of *S. capitis*, corroborating our findings reported here. However, the results of further character and chemical analyses, including DNA-DNA homology, have unequivocally established that this strain is *S. haemolyticus* (16, 36, 40, 59, 71). Thus, we concluded that strain DSM 20265 belongs to the species *S. haemolyticus*, but the character set chosen for the numerical analysis was not sufficient to distinguish this phenotypically aberrant strain from the *S. capitis* phenon.

Based on overall phenotypic similarity, there seems to be little support for separating *S. hominis* and *S. haemolyticus* into two distinct species. The results of other workers (21, 36, 48, 56, 60, 68) also show few differences between these two taxa. However, the two species show obvious differences when DNA-DNA hybridization and other chemical techniques are used to examine them (16, 40, 71). The search for new test methods to distinguish *S. hominis* from *S. haemolyticus* has led to the development of a new commercial test kit that was tested and described by Kloos and Wolfshohl (41). In this system, tests for urease, β -glucosidase, β -glucuronidase, and arginine dihydrolase were used to distinguish the two species. Of the four tests, the first three were not examined in our numerical analysis reported here, and this certainly hampered proper identification of the strains included in these closely related species.

Although *S. warneri* could not be distinguished from *S. hominis* and *S. haemolyticus* by Valardo and Satta (68), the results of Feltham (21) and our results indicate that *S. warneri* is indeed a separate species. Studies of fatty acid composition (16) and the immunological relationships of catalase (60) and DNA-DNA reassociation assays (60) stress the uniqueness of *S. warneri* and its close relationship to the *S. epidermidis* group of Schleifer et al. (60). Simple phenotypic differences determined with a commercial test kit also differentiate this species from other staphylococci (40).

Feltham (21) reported that *S. hominis* ATCC 27846 clustered with staphylococci identified as *S. epidermidis* biotype 3 and suggested that this strain was misidentified by Kloos and Schleifer (36). More recently, Kloos (32) reaffirmed the opinion that *S. hominis* ATCC 27846 is correctly named, based on DNA-DNA hybridization data

and phenotypic characterization. These differences of opinion led us to classify the strains representing phenon 7 and including *S. hominis* ATCC 27846 as atypical *S. hominis*. Since *S. haemolyticus* and *S. hominis* are similar in character analysis and since both species are questionable or uncommon human pathogens (33), they are combined into one entry in Table 5.

A total of 14 strains that could not be identified are included in Table 5, but because of their similarity are grouped with 13 *S. haemolyticus*-*S. hominis* strains in Table 7.

A coagulase-positive culture was identified by numerical taxonomy as *S. hominis*. The occurrence of coagulase-positive strains of staphylococci other than *S. aureus* has been reported previously (23, 65). It is acknowledged that on occasion, the coagulase test may lead to incorrect identification of staphylococci as *S. aureus* (5, 23, 49, 65, 67). Indeed, the strain included in this study would have been incorrectly identified if a complete array of tests (more than the minimum used for identification) had not been done. The coagulase-positive, non-*S. aureus* strain examined in this study is being tested further to verify its identity.

The fourth species group, *S. aureus*, was originally included in our study of coagulase-negative staphylococci because of the possibility of encountering strains of *S. aureus* that fail to produce coagulase. The characteristics of the *S. aureus* group are summarized in Table 6. Schleifer et al. (60) assigned *S. simulans* to a species group separate from *S. aureus*, as did Valardo and Satta (68) with their lyogroup system. However, Valardo and Satta reported that the bacteriolytic and phenotypic patterns of *S. simulans* (lyogroup II) did not differ greatly from those of *S. aureus* (lyogroup I). Two species, *Staphylococcus hyicus* and *Staphylococcus intermedius*, have not been reported as human pathogens, although they are associated with warm-blooded animals. The significance of these species lies in the fact that they often produce coagulase and can be associated with food derived from animals.

The characteristics of *S. aureus* recorded in the present study followed the species description closely (2, 36), except for hydrolysis of esculin. The observation that 80% of the strains of *S. aureus* (subphenon 11a) hydrolyzed esculin is unusual; Quadi et al. (57) studied 364 staphylococci and found that only 4 strains hydrolyzed this glycoside. These four strains were identified as *S. aureus* and comprised only 3% of the coagulase-positive cultures examined. In the present study, more than 70% of the strains in phenon 11 hydrolyzed esculin. The difference between the results of these two studies can be attributed to the methods used to detect esculin

hydrolysis. Quadri et al. (57) used a rapid test and recorded results after incubation of the strains for 4 and 24 h. In the present study, all *S. aureus* strains were negative after incubation for 24 h, both in esculin broth and on bile-esculin agar. At 72 h, the tests were only weakly positive. The *S. sciuri* strains were strongly positive within 3 days. The ability of *S. sciuri* to degrade esculin has been reported elsewhere (21), without mention of the ability of coagulase-positive strains to degrade esculin. Useful differential characteristics of *S. aureus* and related phena are summarized in Table 6.

We concluded that major species groups of staphylococci can be identified in clinical laboratories. Characteristics heretofore not reported were found in this study to be useful in identifying species of *Staphylococcus*. Some species, viz., *S. haemolyticus* and *S. hominis*, were difficult to identify, and because of these difficulties it may prove best for clinical laboratories to identify new isolates as *Staphylococcus* species groups rather than as individual species per se.

LITERATURE CITED

1. Bailey, R. R. 1973. Significance of coagulase-negative *Staphylococcus* in urine. *J. Infect. Dis.* 127:179-182.
2. Baird-Parker, A. C. 1974. Family I. *Micrococcaceae* Pribam 1929, 385, p. 478-490. in R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
3. Bartlett, J. G., A. B. Onderdonk, E. Drude, C. Goldstein, M. Anderka, S. Alpert, and W. M. McCormack. 1977. Quantitative bacteriology of the vaginal flora. *J. Infect. Dis.* 136:271-277.
4. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45:493-496.
5. Bayliss, B. G., and E. R. Hall. 1965. Plasma coagulation by organisms other than *Staphylococcus aureus*. *J. Bacteriol.* 89:101-105.
6. Buck, J. D. 1976. Pollution microbiology of Biscayne Bay beaches. *Fla. Sci.* 39:111-120.
7. Carlsson, J. 1965. Zoogaea-forming streptococci, resembling *Streptococcus sanguis*, isolated from dental plaque in man. *Odontol. Revy.* 16:348-358.
8. Carlsson, J. 1968. A numerical taxonomic study of human oral streptococci. *Odontol. Revy.* 19:137-160.
9. Crombach, W. H. J. 1978. *Caseobacter polymorphus* gen. nov., sp. nov., a coryneform bacterium from cheese. *Int. J. Syst. Bacteriol.* 28:354-366.
10. Darling, D. L. 1975. Standardization and evaluation of the CAMP reaction for the prompt, presumptive identification of *Streptococcus agalactiae* (Lancefield group B) in clinical material. *J. Clin. Microbiol.* 1:171-174.
11. Davis, G. H. G., and B. Hoyling. 1974. Observations on anaerobic glucose utilization tests in *Staphylococcus-Micrococcus* identification. *Int. J. Syst. Bacteriol.* 24:1-5.
12. Devriese, L. A., and V. Hajek. 1980. A review. Identification of pathogenic staphylococci isolated from animals and foods derived from animals. *J. Appl. Bacteriol.* 49:1-11.
13. Devriese, L. A., V. Hajek, P. Oeding, S. A. Meyer, and K. H. Schleifer. 1978. *Staphylococcus hyicus* (Sompolsky 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogenes* subsp. nov. *Int. J. Syst. Bacteriol.* 28:482-490.
14. Devriese, L. A., and A. Van DeKerckhove. 1980. Carbohydrate dissimilation tests in the identification of staphylococci. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 45:65-72.
15. Digranes, A., and P. Oeding. 1975. Characterization of *Micrococcaceae* from the urinary tract. *Acta. Pathol. Microbiol. Scand. Sect. B* 83:373-381.
16. Durham, D. R., and W. E. Kloos. 1978. Comparative study of the total cellular fatty acid of *Staphylococcus* species of human origin. *Int. J. Syst. Bacteriol.* 28:223-228.
17. Evans, J. B., and W. E. Kloos. 1972. Use of shake cultures in a semisolid thioglycollate medium for differentiating staphylococci from micrococci. *Appl. Microbiol.* 23:326-331.
18. Facklam, R. R. 1973. Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. *Appl. Microbiol.* 26:138-145.
19. Federal Register. 1972. Rules and regulations. Antibiotic susceptibility discs. *Fed. Regist.* 37:20525-20529.
20. Federal Register. 1973. Rules and regulations. Antibiotic susceptibility disc correction. *Fed. Regist.* 38:2576.
21. Feltham, R. K. A. 1979. A taxonomic study of the *Micrococcaceae*. *J. Appl. Bacteriol.* 47:243-254.
22. Fuchs, P. C., C. Christy, and R. N. Jones. 1978. Multiple-inocula (replicator) CAMP test for presumptive identification of group B streptococci. *J. Clin. Microbiol.* 7:232-233.
23. Garcia, M. L., B. Moreno, and M. S. Bergdoll. 1980. Characterization of staphylococci isolated from mastitic cows in Spain. *Appl. Environ. Microbiol.* 39:548-553.
24. Gross, K. C., M. P. Houghton, and L. B. Senterfit. 1975. Presumptive speciation of *Streptococcus bovis* and other group D streptococci from human sources by using arginine and pyruvate tests. *J. Clin. Microbiol.* 1:54-60.
25. Gunn, B. A. 1976. SXT and Taxo A disks for presumptive identification of group A and B streptococci in throat cultures. *J. Clin. Microbiol.* 4:192-193.
26. Gunn, B. A., F. L. Singleton, E. R. Peele, R. R. Colwell, J. F. Keiser, and C. O. Kapfer. 1981. Comparison of methods for identifying *Staphylococcus* and *Micrococcus* spp. *J. Clin. Microbiol.* 14:195-200.
27. Hajek, V. 1976. *Staphylococcus intermedius*, a new species isolated from animals. *Int. J. Syst. Bacteriol.* 26:401-408.
28. Holt, R. J. 1969. The classification of staphylococci from colonized ventriculo-atrial shunts. *J. Clin. Pathol.* 22:475-482.
29. Holt, R. J. 1971. The opportunist pathogenicity of coagulase-negative staphylococci. *J. Clin. Pathol.* 24:770-772.
30. Holt, R. J. 1971. Lysozyme production by staphylococci and micrococci. *J. Med. Microbiol.* 4:375-379.
31. John, J. F., Jr., P. K. Gramling, and N. M. O'Dell. 1978. Species identification of coagulase-negative staphylococci from urinary tract isolates. *J. Clin. Microbiol.* 8:435-437.
32. Kloos, W. E. 1980. Natural populations of the genus *Staphylococcus*. *Annu. Rev. Microbiol.* 34:559-592.
33. Kloos, W. E. 1982. Coagulase-negative staphylococci. *Clin. Microbiol. Newslett.* 4:75-79.
34. Kloos, W. E., and M. S. Musselwhite. 1975. Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl. Microbiol.* 30:381-395.
35. Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol.* 1:82-88.
36. Kloos, W. E., and K. H. Schleifer. 1975. Isolation and characterization of staphylococci from human skin. II. Descriptions of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. *Int. J. Syst. Bacteriol.* 25:62-79.

37. Kloos, W. E., K. H. Schleifer, and W. C. Noble. 1976. Estimation of character parameters in coagulase-negative *Staphylococcus* species, p. 23–41. In J. J. Eljaszewicz (ed.), *Staphylococci and Staphylococcal diseases*. G. Fisher, Stuttgart.
38. Kloos, W. E., K. H. Schleifer, and R. F. Smith. 1976. Characterization of *Staphylococcus sciuri* sp. nov. and its subspecies. *Int. J. Syst. Bacteriol.* 26:22–37.
39. Kloos, W. E., T. G. Tornabene, and K. H. Schleifer. 1974. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. *Int. J. Syst. Bacteriol.* 24:79–101.
40. Kloos, W. E., and J. F. Wolfshohl. 1979. Evidence for deoxyribonucleotide sequence divergence between staphylococci living on human and other primate skin. *Curr. Microbiol.* 3:167–172.
41. Kloos, W. E., and J. F. Wolfshohl. 1982. Identification of *Staphylococcus* species with the API STAPH-IDENT system. *J. Clin. Microbiol.* 16:509–516.
42. Males, B. M., W. A. Rogers, Jr., and J. T. Parisi. 1975. Virulence factors of biotypes of *Staphylococcus epidermidis* from clinical sources. *J. Clin. Microbiol.* 1:265–261.
43. Marsik, F. J., and J. T. Parisi. 1973. Significance of *Staphylococcus epidermidis* in the clinical laboratory. *Appl. Microbiol.* 25:11–14.
44. McFaddin, J. F. 1976. Biochemical tests for identification of medical bacteria. The Williams & Wilkins Co., Baltimore.
45. Møller, J. K., C. Christiansen, and N. Mortensen. 1973. DNA base composition of coagulase-negative staphylococci associated with urinary tract infection. *Acta. Pathol. Microbiol. Scand. Sect. B* 81:559–562.
46. Murray, P. R., and J. A. Washington II. 1975. Microscopic and bacteriologic analysis of expectorated sputum. *Mayo Clin. Proc.* 50:339–344.
47. Namavar, F., J. de Graaf, and D. M. MacLaren. 1977. Micrococci and urinary tract infection. *Lancet* ii:1223–1224.
48. Namavar, F., J. de Graaf, and D. M. MacLaren. 1978. Taxonomy of coagulase-negative staphylococci: a comparison of two widely used classification schemes. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 44:425–434.
49. Orth, D. C., L. R. Chugg, and A. W. Anderson. 1971. Comparison of animal sera for suitability in coagulase testing. *Appl. Microbiol.* 21:420–425.
50. Oxborrow, G. S., and M. S. Favero. 1967. A combination medium for demonstrating starch and gelatin hydrolysis. *Am. J. Clin. Pathol.* 33:334–335.
51. Paik, G., and M. T. Suggs. 1974. Reagents, stains, and miscellaneous test procedures, p. 930–950. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
52. Parker, M. T., and L. C. Ball. 1976. Streptococci and aerococci associated with systemic infection in man. *J. Med. Microbiol.* 9:275–302.
53. Pereira, A. T. 1962. Coagulase-negative strains of *Staphylococcus* possessing antigen 51 as agents of urinary infection. *J. Clin. Pathol.* 15:252–260.
54. Perkins, R. E., R. B. Kindsin, M. V. Pratt, I. Abrahamson, and H. M. Leibowitz. 1975. Bacteriology of normal and infected conjunctiva. *J. Clin. Microbiol.* 1:147–149.
55. Pike, E. B. 1962. The classification of staphylococci and micrococci from the human mouth. *J. Appl. Bacteriol.* 25:448–455.
56. Pulverer, G., M. Mordarski, A. Tkacz, K. Szyba, P. Heczko, and M. Goodfellow. 1978. Relationships among some coagulase-negative staphylococci based upon deoxyribonucleic acid reassociation. *FEMS Microbiol. Lett.* 3:51–56.
57. Quadri, S. M. H., M. I. DeSilva, and S. Zubairi. 1980. Rapid test for determination of esculin hydrolysis. *J. Clin. Microbiol.* 12:472–474.
58. Sabath, L. D., F. F. Barrett, C. Wilcox, D. A. Gerstein, and M. Finland. 1969. Methicillin resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*, p. 302–306. *Antimicrob. Agents Chemother.* 1968.
59. Schleifer, K. H., and W. E. Kloos. 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. *Int. J. Syst. Bacteriol.* 25:50–61.
60. Schleifer, K. H., S. A. Meyer, and M. Rupprecht. 1979. Relatedness among coagulase-negative staphylococci: deoxyribonucleic acid reassociation and comparative immunological studies. *Arch. Mikrobiol.* 122:93–101.
61. Smith, R. F., D. Blasi, and S. L. Dayton. 1973. Phosphatase activity among *Candida* species and other yeasts isolated from clinical material. *Appl. Microbiol.* 26:364–367.
62. Sneath, P. H. A. 1957. Some thoughts on bacterial classification. *J. Gen. Microbiol.* 17:184–200.
63. Sneath, P. H. A., and R. R. Sokal. 1974. Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman, San Francisco.
64. Speller, D. C., and R. G. Mitchell. 1973. Coagulase-negative staphylococci causing endocarditis after cardiac surgery. *J. Clin. Pathol.* 26:517–522.
65. Sperber, W. H., and S. R. Tatin. 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. *Appl. Microbiol.* 29:502–505.
66. Subcommittee on Taxonomy of Staphylococci and Micrococci. 1965. Minutes of first meeting. *Int. Bull. Bacteriol. Nomencl. Taxon.* 15:107–108.
67. Turner, F. J., and B. S. Schwartz. 1958. The use of a lyophilized human plasma standardized for blood coagulation factors in the coagulase and fibrinolytic tests. *J. Lab. Clin. Med.* 52:888–894.
68. Valardo, P. E., and G. Satta. 1978. Grouping of staphylococci on the basis of their bacteriolytic-activity patterns: a new approach to the taxonomy of the *Micrococcaceae*. II. Main characters of 1,054 strains subdivided into "lyogroups." *Int. J. Syst. Bacteriol.* 28:148–153.
69. Vera, H. D., and M. Dumoff. 1974. Culture media, p. 881–929. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
70. Williams, D. N., M. E. Lund, and D. J. Blazejic. 1976. Significance of urinary isolates of coagulase-negative *Micrococcaceae*. *J. Clin. Microbiol.* 3:556–559.
71. Zimmerman, R. J., and W. E. Kloos. 1976. Comparative zone electrophoresis of esterases of *Staphylococcus* species isolated from mammalian skin. *Can. J. Microbiol.* 22:771–779.