Isolation and Characterization of Staphylococci from Human Skin

II. Descriptions of Four New Species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*¹

WESLEY E. KLOOS AND KARL H. SCHLEIFER

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607, and Lehrstuhl für
Mikrobiologie, Universität München, 8 Munich 19, Germany

Staphylococci were isolated from the skins of people living in North Carolina and New Jersey and were studied in an attempt to resolve their natural relationships. As a result of this study, four new species are proposed in this paper: *Staphylococcus warneri*, *S. capitis*, *S. hominis*, and *S. simulans*. The type strains of these species are ATCC 27836, ATCC 27840, ATCC 27844, and ATCC 27848, respectively. The new species were established on the basis of a variety of morphological, physiological, biochemical, and antibiotic characters. Cell wall composition was particularly useful in resolving species and correlated well with other characters. Characteristic pigment production was useful in distinguishing several of the different species. A summary of the character variation found in the species and a scheme for the classification of human cutaneous staphylococci are included in this paper. The predominant staphylococci found on human skin were *S. epidermidis* and *S. hominis*.

A brief account of the classification of staphylococci and their occurrence on human skin is presented in the accompanying paper (15). Most of the recent attempts at classifying cutaneous staphylococci have utilized the Baird-Parker scheme (2-4, 10, 11). This scheme involves the use of a small number of readily definable key characters to identify isolates at the species and subgroup levels. However, because of the small number of characters used and the general lack of information available on species character variation, the scheme is limited in accuracy and to the number of species and/or subspecies that it can resolve.

The specific purpose of our investigation was to identify the species of cutaneous staphylococci of humans. To attempt this, we reevaluated existing taxonomic criteria, explored the use of additional characters, and estimated species character variation in cutaneous populations.

The results of studies on cutaneous strains of *Staphylococcus epidermidis* (Winslow and Winslow) Evans 1916, *S. saprophyticus* Fairbrother 1940, and three new species, *S. cohnii*, *S. haemolyticus*, and *S. xylosus*, are presented in the accompanying paper (15). The present

paper is concerned with the classification of four additional new species of human cutaneous staphylococci; it also contains summary data on species character variation, occurrence of species on human skin, and a classification scheme.

MATERIALS AND METHODS

Bacterial strains. Staphylococci were isolated from the healthy skins of two groups of people. One group was composed of 20 people living in Raleigh, N.C., who were sampled once each month for 6 to 13 months. The second group was composed of 20 people living in New Jersey, who were sampled once during the winter. Samples were taken from two separate sites on the forehead and one site from one cheek, one anterior and external nare, chin, each axilla, each upper and lower arm, and each upper and lower leg.

Randomly selected representative strains of the new species proposed in this paper are listed in Tables 1, 2, 3, and 4, respectively. (Additional strains that were analyzed for only certain of the characters listed in the tables are too numerous to be cited here, but character information on these strains can be made available upon request to the authors.)

Procedures for isolating staphylococci. Sampling techniques and the composition of the isolation medium have been described previously (6).

Culture conditions. Culture conditions were similar to those previously described for the study of cutaneous micrococci (6).

Character determinations. Procedures for determining deoxyribonucleic acid (DNA) base composi-

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tion, colony morphology and pigment, cell morphology, motility, aerobic and anaerobic growth in thioglycolate, salt tolerance, growth temperature, catalase and benzidine activities, acetylmethylcarbinol production, nitrate reduction, carbohydrate reactions, and susceptibility to various antibiotics, lysozyme, and lysostaphin were similar to those described previously for micrococci (6). The minimal inhibitory concentrations (MIC) of antibiotics and lysostaphin are as denoted throughout the text.

Coagulase, hemolysis, deoxyribonuclease (DNase), phosphatase, and bacteriolytic activities were determined by procedures described in the companion paper (15).

Procedures for making cell wall hydrolysates and determining peptidoglycan type, teichoic acid, configuration of lactic acid, and the anaerobic fermentation of glucose have also been described previously (16).

RESULTS AND DISCUSSION

Characterization of *Staphylococcus* species. The characteristics of the *S. aureus* and other coagulase-positive strains isolated from human skin in this study were similar to those reported previously for various strains of these organisms (3, 14, 16). Descriptions of four new *Staphylococcus* species isolated from human skin are as follows.

(i) *S. warneri* sp. nov. (war. ner'i. M.L. gen. n. *warneri* of Warner; named for Arthur Warner, Jr., from whom this organism was originally isolated.) Several strains tentatively identified as members of this species were previously designated *S. epidermidis* (CCM 2445; reference 13) or *S. pyogenes albus* (ATCC 155; reference 16). Several strains, including SCH 5, SCH 7, and SCH 13, were isolated by one of us (K.H.S.) from dust and, based on their cell wall composition and the configuration of the lactic acid they produced, were placed together with the above-mentioned strains in *Staphylococcus* group II A3 (16). The following description of *S. warneri* is based on a total of 38 strains, unless noted otherwise.

Cells were gram-positive cocci, 0.5 to 1.2 μ m in diameter, nonmotile and nonsporeforming, occurring predominantly in pairs and singly, occasionally in tetrads. Only 12% of the strains studied had approximately equal numbers of pairs and tetrads.

Colonies on a P agar medium (6) were raised, usually had a slightly elevated center, and were circular, entire, smooth, glistening, and opaque. They were small and had a diameter of 3 to 5 mm. The consistency of colonies or culture streaks was usually sticky. Eighty percent of the strains were pigmented to some extent. Pigment occurred as a characteristic bright yellow-orange or yellow ring around the edge of

colonies or was a slight yellowish tint in the center of colonies. Twenty percent of the strains were unpigmented and had gray-white colonies.

Growth occurred in both the aerobic and anaerobic portions of the thioglycolate medium, indicating a facultatively anaerobic capability. Growth in the anaerobic portion was usually uniformly dense; however, 5% of the strains demonstrated less growth, and 8% had only individual colonies in the deeper, more anaerobic portion of this medium. Fifteen selected strains demonstrated fermentation of glucose by lowering the pH of a yeast extract-glucose broth from 6.8 to 4.7–5.0 after anaerobic incubation. These strains produced nearly equal amounts of D- and L-lactic acid from glucose.

All strains grew well at NaCl concentrations up to 10%. Sixty-two percent of the strains grew poorly or failed to grow at a NaCl concentration of 15%. The optimal growth temperature range was 25 to 40 C. Eighty-four percent of the strains grew poorly or failed to grow at 15 C, but 92% grew well at 45 C.

All strains had either weak or moderate catalase activity and were positive for the benzidine test. All failed to produce coagulases. Ninety-eight percent of the strains demonstrated weak or no hemolysin activity, 95% demonstrated weak or no DNase activity, 74% failed to reduce nitrates, and 78% failed to demonstrate phosphatase activity; there was variable bacteriolytic activity. All produced acetylmethylcarbinol.

All strains produced acid aerobically from glucose, fructose, sucrose, trehalose, and glycerol. Seventy-eight percent of the strains produced acid from maltose slowly, and 63% produced acid from mannitol. Fifty percent of the strains produced acid from ribose, and 24% produced acid from galactose. Only 2% of the strains produced acid from mannose, and 11% produced acid from lactose or turanose. All failed to produce acid from rhamnose, xylose, arabinose, gentiobiose, cellobiose, melezitose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

All strains were resistant to lysozyme and were slightly resistant to lysostaphin (MIC, 200 μ g/ml). All were susceptible to erythromycin (MIC, 0.4 to 1.6 μ g/ml), tetracycline (MIC, 0.4 to 0.8 μ g/ml), and novobiocin (MIC, 0.025 to 0.1 μ g/ml). Seventy-eight percent of the strains were susceptible to penicillin G (MIC, 0.012 to 0.025 μ g/ml), and 98% were susceptible or slightly resistant to streptomycin (MIC, 6.2 to 25 μ g/ml).

Fifteen selected strains studied contained

peptidoglycan of the type L-Lys-Gly_{3,3-4,5}, L-Ser_{0,6-1,4}. Their cell wall teichoic acid contained glycerol and glucose.

The guanine plus cytosine (G+C) content of the DNA, as determined in six strains, was 34.7 ± 0.2 mol%.

Some variable characters and the G+C content of the DNA of some representative strains of *S. warneri* are given in Table 1.

Strain ATCC 27836 (originally designated AW 25) is the type strain of *S. warneri*. A description of this strain follows.

Cells: Spheres, 0.8 to 1.0 µm in diameter, occurring in pairs, tetrads, and singly. Non-motile and nonsporeforming. Gram positive.

Agar colonies: Circular, entire, 3.0 to 3.5 mm in diameter, raised with slightly elevated center, smooth with glistening surface, sticky consistency. Originally opaque gray-white with yellow-orange edge; subcultures have lost pigment and are now gray-white.

Catalase and benzidine tests are positive.

Chemoorganotroph; metabolism is respiratory.

Facultatively anaerobic.

Temperature relationships: Good growth at 45 °C, poor growth at 15 °C.

Growth on NaCl agar: Good growth with 10% NaCl, no growth with 15%.

Coagulases not produced.

Phosphatase not produced.

Acetylmethylcarbinol produced.

Nitrates not reduced.

Hemolysins: Weak hemolysis of bovine, sheep, and human blood.

DNase activity is weak.

Acid produced aerobically from glucose, fructose, ribose, maltose, sucrose, trehalose, mannitol, and glycerol. No acid from galactose, mannose, rhamnose, xylose, arabinose, lactose, gentiobiose, cellobiose, melezitose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

Antibiotic susceptibilities: Slightly resistant to lysostaphin (MIC, 200 µg/ml) and streptomycin (MIC, 12.5 µg/ml). Susceptible to penicillin G (MIC, 0.025 µg/ml), erythromycin (MIC, 1.6 µg/ml), tetracycline (MIC, 0.8 µg/ml), and novobiocin (MIC, 0.025 µg/ml). Resistant to lysozyme.

Cell wall peptidoglycan: L-Lys-Gly_{4,2}, L-Ser_{0,5}.

Cell wall teichoic acid composition: Glycerol and glucose.

G+C content of the DNA: 34.2 mol%.

S. warneri can be distinguished from all other

staphylococci primarily on the basis of a combination of its colony morphology, carbohydrate reaction pattern, cell wall composition, lactic acid configuration, and the lack of nitrate reduction. There are also some significant differences between *S. warneri* and certain other species in the parameters of pigment pattern, cell arrangement, anaerobic growth pattern in thioglycolate, growth at different NaCl concentrations and temperature extremes, coagulase activity, hemolysis, acetylmethylcarbinol production, and phosphatase, DNase, and bacteriolytic activities. Several properties of *S. warneri* overlap with those of *S. haemolyticus*, *S. epidermidis*, and *S. hominis*, suggesting a relatively close relationship between these species.

(ii) *S. capitis* sp. nov. (ca'pi. tis. L. noun *capit* head; L. gen. noun *capitis* of the head; pertaining to that part of the human body where cutaneous populations of this species are usually the largest and most frequent.) Gordon (5) in 1905 reported an unusual *Staphylococcus* that he considered to be different from the commonly recognized *Staphylococcus epidermidis albus*. Strains of this unusual type were different from those of the above-mentioned species in that they failed to produce acid from maltose or lactose but produced acid from mannitol. They also were predominately found on the scalp as compared to the hands, face, or forearm. Although the few characters studied by Gordon will not conclusively identify his isolate as belonging to *S. capitis*, nevertheless, they are consistent with those of *S. capitis* and, hence, there is a reasonably good possibility that he was observing this species. A later study by Marples et al. (9) on the aerobic microflora of the scalp determined that the normal scalp cocci are predominately micrococci of type 3. However, according to Baird-Parker's scheme (3), the characteristics of type 3 micrococci are not those of *S. capitis*. Hence, there is some uncertainty concerning earlier reports of possible encounters with this species. The following description of *S. capitis* is based on a total of 47 strains, unless noted otherwise.

Cells were gram-positive cocci, 0.8 to 1.2 µm in diameter, nonmotile and nonsporeforming, occurring predominantly in pairs and tetrads.

Colonies on a P agar medium (6) were slightly convex, circular, entire, smooth, slightly dull to glistening, opaque and usually a distinctive chalk white (unpigmented). They were very small and had a diameter of 1 to 3 mm.

Growth occurred in both the aerobic and anaerobic portions of the thioglycolate medium, indicating a facultatively anaerobic capability.

TABLE 1. Variable characters of 17 strains of *Staphylococcus warneri*

Strain	Mol % G + C	Colony pig- ment ^a	Anaerobic thiogly- colate growth ^b	Anaerobic fermenta- tion of glucose (pH)	L-Lactic acid (%)	Growth on 15% NaCl agar	Growth at 15 C	He- moly- sis	Ni- trate reduc- tion	Phos- pha- tase	Deoxy- ribonu- clease	Bacterio- lytic activ- ity	Acid (aerobically) from					Penicillin G MIC (μg/ml)	Peptido- glycan (mol/mol of glu- tamic acid)
													Galac- tose	Ri- bose	Malt- ose	Lac- tose	Man- itol		
ATCC 27836	34.2	Ye	+	4.9	55	- ^c	±	±	-	-	±	+	-	±	+	0.025	0.8	1.2	
ATCC 27837	35.2	-	+	4.8	34	±	±	±	+	-	±	+	±	+	+	0.012	0.7	4.2	
ATCC 27838	35.0	Ye	+	5.0	64	-	±	-	-	-	±	+	-	-	+	0.025	1.3	3.5	
ATCC 27839	34.4	-	+	4.8	59	±	±	-	±	±	±	+	-	+	+	0.025	0.7	4.4	
WK 193	ND ^d	LY	+	4.7	57	±	±	-	-	±	-	-	-	+	+	0.025	0.8	4.0	
MK 222	ND	LY	±	5.0	44	-	±	-	+	-	±	-	-	+	-	0.025	0.6	4.2	
SM 219	ND	Y	+	4.9	45	-	±	±	±	-	±	+	-	+	-	0.025	1.4	3.3	
SM 251	ND	LY	+	4.7	40	-	±	-	-	±	-	+	-	+	-	0.025	0.6	4.2	
TW 98	ND	Y	+	4.7	60	-	±	±	±	-	ND	+	-	+	+	0.025	0.8	4.2	
JL 289	ND	-	+	4.8	52	±	±	-	-	-	ND	-	-	ND	±	0.012	0.6	4.3	
KH 127	ND	LY	+	4.7	59	±	±	-	-	-	ND	-	-	-	+	0.025	0.9	4.0	
MAW 314	34.5	Ye	+	4.7	55	±	±	-	-	-	±	+	-	+	+	0.025	0.7	4.2	
JM 25	ND	Ye	+	4.7	48	±	±	ND	-	-	±	+	-	+	+	0.025	1.3	3.8	
KL 105	34.9	Ye/-	+	4.8	60	-	±	-	-	-	±	±	+	+	+	3.1	0.6	4.4	
KL 195	ND	-/Ye	+	4.9	63	±	±	-	-	-	±	±	+	+	+	3.1	0.6	4.3	
MCS 4	ND	Ye	+	ND	ND	±	±	-	-	-	±	±	-	+	+	12.5	ND	ND	
ATCC 155 ^e	35.0	LYG	+	4.8	52	±	±	-	-	±	-	-	+	+	+	0.012	1.5	3.4	

^a Symbols for colony pigment: Ye, yellow edge; Y, yellowish center; LY, light yellowish center; LYG, light yellowish center in gray background; Y/-, predominantly yellow edge with gray-white sectors; -/Y, predominantly gray-white with yellow edge sectors; -, gray-white (unpigmented).

^b Symbols for anaerobic growth: +, uniform dense; ±, gradient of growth from dense to light down the tube.

^c Reactions: ++, strong positive (carbohydrates only); +, positive; ±, weak; -, negative.

^d ND, Not determined.

^e Strain ATCC 155 is being tentatively classified as *S. warneri*. Data on anaerobic glucose fermentation, lactic acid configuration, and cell wall composition were determined previously by Schleifer and Kocur (16), and the G+C content of the DNA was determined by Auletta and Kennedy (1).

Growth in the anaerobic portion was usually uniformly dense; however, 11% of the strains demonstrated less growth, and 21% had only individual colonies in the deeper, more anaerobic portion of this medium. Nineteen selected strains of this species demonstrated the fermentation of glucose by lowering the pH of a yeast extract-glucose broth from 6.8 to 4.2–4.7 after anaerobic incubation. These strains also produced predominately L-lactic acid and between 5 and 25% D-lactic acid from glucose.

Ninety percent of the strains grew well at NaCl concentrations up to 10%. All strains either grew poorly or failed to grow at a NaCl concentration of 15%. The optimal growth temperature range was 30 to 40 C. All strains failed to grow at 15 C but grew well at 45 C.

All strains had either weak or moderate catalase activity and were positive for the benzidine test. All failed to produce coagulases. All strains demonstrated either weak or no hemolysin activity on bovine or sheep blood but usually produced good hemolysin activity on human blood. Strains had variable acetylmethylcarbinol production and had weak or no phosphatase activity. Eighty-three percent of the strains reduced nitrates, and all strains demonstrated weak DNase and moderate to strong bacteriolytic activities.

By comparison with other staphylococci, *S. capitis* produced acid aerobically from only a few different carbohydrates. All strains produced acid from glucose, fructose, mannose, and glycerol. Ninety-one percent of the strains produced acid slowly from sucrose, and 94% produced acid slowly from mannitol. Only 2% of the strains produced acid from trehalose or lactose. All failed to produce acid from galactose, rhamnose, xylose, arabinose, ribose, maltose, turanose, gentiobiose, cellobiose, melezitose, xylitol, sorbitol, arabitol, inositol, salicin, adonitol, dulcitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

All strains were resistant to lysozyme and were slightly resistant to lysostaphin (MIC, 200 µg/ml). All were susceptible to erythromycin (MIC, 0.4 to 0.8 µg/ml), streptomycin (MIC, 1.6 to 6.2 µg/ml), and novobiocin (MIC, 0.05 to 0.1 µg/ml). Ninety-four percent of the strains were susceptible to penicillin G (MIC, 0.025 to 0.05 µg/ml), and 85% were susceptible to tetracycline (MIC, 0.4 to 0.8 µg/ml).

Nineteen selected strains contained peptidoglycan of the type L-Lys-Gly_{3.5-4.3}, L-Ser_{0.8-1.2}. Their cell wall teichoic acid contained glycerol, and no neutral sugar or additional amino sugar was detected. Cell walls contained low amounts

of teichoic acid, and cell wall hydrolysates had to be concentrated for the detection of free glycerol. Cell walls also contained additional glutamic acid. In partial acid hydrolysates, diglutamic acid was found, indicating that a glutamic acid polymer is present. The additional glutamic acid can be extracted with hot trichloroacetic acid like the teichoic acid.

The G+C content of the DNA, as determined in five strains, was 33.6 ± 1.0 mol%.

Some variable characters and the G+C content of the DNA of some representative strains of *S. capitis* are given in Table 2.

Strain ATCC 27840 (originally designated LK 499) is the type strain of *S. capitis*. A description of this strain follows.

Cells: Spheres, 0.9 to 1.2 µm in diameter, occurring predominantly in tetrads and pairs. Nonmotile and nonsporeforming. Gram positive.

Agar colonies: Circular, entire, 2 to 2.5 mm in diameter, slightly convex, smooth with glistening surface. Opaque, chalk white.

Catalase and benzidine tests are positive.

Chemoorganotroph; metabolism is respiratory.

Facultatively anaerobic.

Growth at extreme temperatures: Good growth at 45 C, no growth at 15 C.

Growth on NaCl agar: Good growth with 10% NaCl, poor growth with 15%.

Coagulases not produced.

Phosphatase not produced.

Acetylmethylcarbinol not produced.

Nitrates are reduced.

Hemolysins: No hemolysis of bovine and sheep blood, but moderate hemolysis of human blood.

DNase activity is weak.

Acid produced aerobically from glucose, fructose, mannose, sucrose, mannitol, and glycerol. No acid from galactose, rhamnose, xylose, arabinose, ribose, maltose, lactose, trehalose, turanose, gentiobiose, cellobiose, melezitose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

Antibiotic susceptibilities: Slightly resistant to lysostaphin (MIC, 200 µg/ml); susceptible to penicillin G (MIC, 0.05 µg/ml), erythromycin (MIC, 0.4 µg/ml), streptomycin (MIC, 3.1 µg/ml), tetracycline (MIC, 0.4 µg/ml), and novobiocin (MIC, 0.05 µg/ml); resistant to lysozyme.

Cell wall peptidoglycan: L-Lys-Gly_{4.1}, L-Ser_{1.0}.

Cell wall teichoic acid: Glycerol.

G+C content of the DNA: 35.5 mol%.

S. capitis can be distinguished from all other

TABLE 2. Variable characters of 20 strains of *Staphylococcus capitis*

Strain	Mol % G + C	Anaerobic thiogly- colate growth ^a	Anaerobic fermenta- tion of glu- cose (pH)	L-Lactic acid (%)	Growth on 15% NaCl agar	Hemol- ysis	Acetyl- methyl- carbinol	Nitrate reduc- tion	Phos- phatase	Acid (aerobically) from			Penicillin G MIC (μ g/ml)	Tetra- cylene MIC (μ g/ml)	Cell wall glutamic acid (mol/mol lysine) ^b	Peptido- glycan (mol/mol of glu- tamic acid)	
										Su- crose	Man- nose	Man- nitrol				Ser- ine	Gly- cine
ATCC 27840	35.5	+	4.6	89	± ^c	-	-	+	-	+	+	0.050	0.4	1.6	1.0	4.1	
ATCC 27841	31.4	+	4.2	92	-	-	-	+	-	+	++	0.050	50.0	1.4	1.0	3.5	
ATCC 27842	31.0	+	4.6	90	±	-	±	+	-	+	+	50.0	50.0	1.5	1.0	4.0	
ATCC 27843	34.1	+	4.6	75	-	±	-	+	-	+	+	0.025	0.4	1.8	1.2	4.1	
RM 390	ND ^d	±	4.5	87	-	±	-	+	-	+	+	0.050	0.4	1.4	1.0	3.7	
GS 14	ND	+	4.6	89	-	-	-	+	-	+	+	0.050	0.8	1.6	1.0	3.9	
KES 14	ND	+	4.4	81	±	-	-	+	±	+	+	0.050	0.4	1.3	0.8	3.5	
SE 21	ND	+	4.7	90	ND	-	±	+	-	+	+	0.050	0.8	1.5	0.9	3.8	
CK 17	ND	±	4.4	91	±	-	±	+	-	+	+	0.050	0.4	1.6	1.0	4.0	
RK 23	ND	+	4.5	90	±	-	±	+	-	+	+	0.050	0.8	1.6	1.1	3.8	
KH 220	ND	±C	ND	ND	-	-	+	±	-	+	+	0.200	50.0	ND	ND	ND	
PM 378	35.8	+	4.4	90	-	-	±	+	-	+	++	0.025	0.4	1.7	1.0	3.8	
WK 11	ND	±C	4.3	92	-	±	-	+	-	+	++	0.050	0.4	1.6	1.1	3.9	
WK 445	ND	+	4.5	91	-	-	+	+	-	+	++	0.050	0.4	1.7	1.1	4.3	
DBM 213	ND	+	4.3	92	±	-	±	+	-	+	+	0.050	0.8	1.4	1.0	3.9	
EK 11	ND	+	4.7	82	±	-	-	-	±	+	+	0.050	0.4	1.8	1.1	3.8	
HM 11	ND	+	4.6	95	±	-	-	+	-	±	+	0.050	0.4	1.6	0.9	3.7	
BS 2	ND	±C	4.7	90	-	-	±	+	-	±	+	0.050	0.8	1.4	1.0	3.7	
CE 2	ND	+	4.7	87	±	-	±	+	-	+	+	0.050	0.4	1.5	1.0	3.7	
SK 13	ND	±C	4.3	88	±	-	-	+	-	+	+	50.0	50.0	1.6	1.1	3.9	

^a Symbols for anaerobic growth: +, uniform dense; ±, gradient of growth from dense to light down the tube; -C, gradient of growth plus individual colonies.^b Moles of glutamic acid per mole of lysine is 1.0 in all other staphylococci.^c Reaction: ++, strong positive (carbohydrates only); +, positive; ±, weak; -, negative.^d ND, Not determined.

staphylococci primarily on the basis of its small and slightly convex, chalk-white colony, carbohydrate reaction pattern, cell wall composition, and lactic acid configuration. There are also some significant differences between *S. capitis* and certain other species in the parameters of cell arrangement, anaerobic growth pattern in thioglycolate, growth at different NaCl concentrations and temperature extremes, coagulase activity, hemolysis, acetylmethylcarbinol production, nitrate reduction, and phosphatase, DNase, and bacteriolytic activities.

(iii) *S. hominis* sp. nov. (ho'mi.nis. L. noun *homo* man; L. gen. noun *hominis* of man; named for the host on whose skin this species is commonly found.) The following description of *S. hominis* is based on a total of 240 strains, unless noted otherwise.

Cells were gram-positive cocci, 1.0 to 1.5 μ m in diameter, nonmotile and nonsporeforming, occurring predominantly in tetrads with occasional pairs. Thirty-eight percent of the strains had approximately equal numbers of tetrads and pairs.

Colonies on a P agar medium (6) were raised to very slightly umbonate and, with age, usually developed a wide beveled edge and elevated center. The older colony profile appears to be species specific. Colonies were small, circular, and usually entire, smooth dull, and opaque. Their diameter was 3 to 4 mm. Colony pigment was variable; 64% of the strains had yellow-orange or yellowish pigment in the center of the colonies and 36% had gray-white (unpigmented) colonies. Older colonies (≥ 6 days) often showed concentric rings of lighter and darker colors.

Growth occurred in the aerobic portion and 84% of strains produced slight growth in the anaerobic portion of the thioglycolate medium, indicating a limited facultatively anaerobic capability. Growth was much less intense in the deeper, more anaerobic, portion of the medium and was usually associated with the presence of a few very small individual colonies. Sixteen percent of the strains failed to produce any clear signs of visible anaerobic growth. Eighteen selected strains demonstrated the fermentation of glucose by lowering the pH of a yeast extract-glucose broth from 6.8 to 4.4–4.9 after anaerobic incubation. Considering the relatively poor anaerobic growth of this species, it seemed somewhat unusual to obtain acid from glucose under anaerobic conditions. However, it should be mentioned here that this species rapidly produces very strong acid from glucose aerobically, and even slight anaerobic growth or a slight

amount of oxygen present in the glucose medium might result in the production of significant quantities of acid such as were observed. The above-mentioned 18 strains could be subdivided into two groups on the basis of the type of lactic acid isomers produced from glucose. One group contained eight strains that produced only D-lactic acid, and another group contained 10 strains that produced both L- and D-lactic acid. The latter strains may contain, in addition to a D-lactate dehydrogenase, an L-lactate dehydrogenase. Such differences were also noted in *S. aureus* by Schleifer and Kocur (16). Most *S. aureus* strains produce L- and D-lactic acid, but a few strains produce only L-lactic acid. Ninety-five percent of the strains grew well at NaCl concentrations up to 7.5%, but 97% grew poorly at 10% and failed to grow at 15%. The optimal growth temperature range was 28 to 40 C. Seventy-two percent of the strains failed to grow at 15 C, and all strains grew well at 45 C.

All strains had either weak or moderate catalase activity and were positive for the benzidine test. All failed to produce coagulases and had weak or no hemolysin or DNase activities. Eighty-three percent of the strains had no phosphatase activity, weak or no bacteriolytic activity, and reduced nitrates. Strains had variable acetylmethylcarbinol production.

All strains produced acid aerobically from glucose, fructose, maltose, sucrose, and glycerol. Ninety percent of the strains produced acid from trehalose, 81% produced acid from turanose, 60% produced acid from lactose, 52% produced acid from galactose, and 51% produced acid from melezitose. Only 7% of the strains produced acid from mannitol, and 10% of the strains produced acid from mannose. All failed to produce acid from rhamnose, xylose, arabinose, ribose, gentiobiose, cellobiose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

All strains were resistant to lysozyme and were slightly resistant to lysostaphin (MIC, 100 to 200 μ g/ml). Seventy-seven percent of the strains were susceptible to penicillin G (MIC, 0.006 μ g/ml), 97% were susceptible to streptomycin (MIC, 1.6 to 6.2 μ g/ml), 93% were susceptible to erythromycin (MIC, 0.4 to 0.8 μ g/ml), 64% were susceptible to tetracycline (MIC, 0.4 μ g/ml), and 99% were susceptible to novobiocin (MIC, 0.1 to 0.2 μ g/ml). A relatively large proportion of the strains (36%) was resistant to tetracycline.

Eighteen selected strains contained peptidoglycan of the type L-Lys-Gly_{3,5-4,5}, L-Ser_{0,6-1,3}.

Their cell wall teichoic acid contained glycerol and, in addition, had 0.3 to 0.8 mol of glucosamine per mol of glycerol. No neutral sugar was found. This species produced only low levels of teichoic acids and, like *S. capitis*, required concentration of the cell wall hydrolysates for the detection of free glycerol.

The G+C content of the DNA, as determined in five strains, was 33.0 ± 1.0 mol%.

Some variable characters and the G+C content of the DNA of some representative strains of *S. hominis* are given in Table 3.

Strain ATCC 27844 (originally designated DM 122) is the type of strain of *S. hominis*. A description of this strain follows.

Cells: Spheres, 1.2 to 1.4 μm in diameter, occurring predominantly in tetrads and occasionally in pairs. Nonmotile and nonsporeforming. Gram positive.

Agar colonies: Circular, entire, 4 to 4.5 mm in diameter, slightly umbonate with wide, beveled edge and elevated center, smooth dull surface. Yellow-orange pigment is produced in the center of opaque colonies.

Catalase and benzidine tests are positive.

Chemoorganotroph; metabolism is respiratory.

Slightly facultatively anaerobic.

Growth at extreme temperatures: Good growth at 45 C, no growth at 15 C.

Growth on NaCl agar: Good growth with 7.5% NaCl, slight growth with 10%, no growth with 15%.

Coagulases not produced.

Phosphatase not produced.

Acetylmethylcarbinol produced weakly.

Nitrates are reduced.

Hemolysins: No hemolysis of bovine, sheep, or human blood.

DNase activity is weak.

Acid produced aerobically from glucose, fructose, galactose, maltose, lactose, sucrose, trehalose, turanose, and glycerol. No acid from mannose, rhamnose, xylose, arabinose, ribose, melezitose, mannitol, gentiobiose, cellobiose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

Antibiotic susceptibilities: Slightly resistant to lysostaphin (MIC, 100 $\mu\text{g}/\text{ml}$); susceptible to penicillin G (MIC, 0.006 $\mu\text{g}/\text{ml}$), streptomycin (MIC, 6.2 $\mu\text{g}/\text{ml}$), erythromycin (MIC, 0.4 $\mu\text{g}/\text{ml}$), and novobiocin (MIC, 0.1 $\mu\text{g}/\text{ml}$); resistant to tetracycline (MIC, 100 $\mu\text{g}/\text{ml}$); resistant to lysozyme.

Cell wall peptidoglycan: L-Lys-Gly_{4,1}, L-Ser_{0,8}.

Cell wall teichoic acid composition: Glycerol and glucosamine.

G+C content of the DNA: 33.7 mol%.

S. hominis can be distinguished from all other staphylococci primarily on the basis of a combination of its colony morphology and pigment pattern, predominant tetrad cell arrangement, poor or no detectable growth in thioglycolate, low NaCl tolerance, and carbohydrate reaction pattern. There are also some significant differences between *S. hominis* and certain other species in the parameters of cell wall composition, lactic acid configuration, temperature extremes of growth, coagulase activity, hemolysis, acetylmethylcarbinol production, nitrate reduction, and phosphatase, DNase, and bacteriolytic activities. Several of these properties overlap with those of *S. epidermidis*, *S. haemolyticus*, and *S. warneri*, suggesting a close relationship among these species.

(iv) *S. simulans* sp. nov. (sim'u.lans. L. part. adj. *simulans* imitating; named for having similarities to certain coagulase-positive staphylococci, including *S. aureus*.) The following description of *S. simulans* is based on a total of 13 strains.

Cells were gram-positive cocci, 0.8 to 1.5 μm in diameter, nonmotile and nonsporeforming, occurring predominantly in pairs and tetrads and occasionally singly.

Colonies on a P agar medium (6) were slightly raised, circular, entire, smooth, slightly glistening, translucent to slightly transparent, and usually gray-white (unpigmented). Twenty-three percent of the strains had a very slight tint of yellowish pigment. The colonies were relatively large and had a diameter of 5.0 to 7.5 mm.

Growth occurred in both the aerobic and anaerobic portions of the thioglycolate medium, indicating a facultatively anaerobic capability. Growth in the anaerobic portion of the medium was uniformly dense. This species demonstrated the fermentation of glucose by lowering the pH of a yeast extract-glucose broth from 6.8 to 4.7-5.0 after anaerobic incubation. The strains could be subdivided into two groups on the basis of the type of lactic acid isomers produced from glucose. One group contained six strains that produced only L-lactic acid, and another group contained seven strains that produced both L- and D-lactic acid. The occurrence of strains producing L- and D-lactic acid or only L-lactic acid was also found in *S. aureus* (16).

All strains grew well at NaCl concentrations up to 10%. Seventy-seven percent of the strains grew poorly at a NaCl concentration of 15%, and

TABLE 3. Variable characters of 20 strains of *Staphylococcus hominis*

Strain	Mol % G+C	Colony pig- ment ^a	Anaerobic thiogly- colate growth ^b	Anaerobic fermenta- tion of glucose (pH)	L- Lactic acid (%)	He- moly- sis	Acetyl- methyl- carbinol	Ni- trate reduc- tion	Deoxy- ribonu- lease	Bac- terio- lytic activ- ity	Acid (aerobically) from						Peni- cillin G MIC (μ g/ml)	Erythro- mycin MIC (μ g/ml)	Tetra- cycline MIC (μ g/ml)	Peptido- glycan (mol/mol of glu- tamic acid)		Teichoic acid (mol of gluco- samine/ mol of glycerol)	
											Galactose	Mannose	Lactose	Trehalose	Turanose	Melzitose				Mannitol	Serine		Glycine
ATCC 27844	33.7	YO	±	4.5	0	- ^c	±	+	±	±	+	+	+	+	+	-	-	0.006	0.4	100.0	0.8	4.1	0.5
ATCC 27845	32.3	YO	±C	4.5	0	-	±	+	±	-	-	+	+	+	+	-	-	0.006	0.4	100.0	0.6	4.3	0.5
ATCC 27846	ND ^d	-	±C	4.6	0	-	-	+	-	+	-	±	+	+	+	-	-	0.006	0.4	0.4	1.0	3.9	0.3
ATCC 27847	29.9	-	±C	4.9	50	±	±	+	±	+	-	-	-	+	+	-	-	6.2	0.4	50.0	1.1	3.8	0.4
WK 52	ND	-	-C	4.5	0	±	±	+	±	±	+	+	+	+	+	-	-	0.006	0.4	0.4	0.9	3.9	0.8
PM 375	ND	-	-C	4.4	43	-	-	+	-	-	+	+	+	+	+	-	-	0.006	0.4	0.4	1.3	3.6	ND
SM 67	33.5	YO	-C	4.6	38	-	±	±	±	-	±	-	+	+	+	-	-	0.006	0.8	0.4	1.2	3.8	0.5
JL 248	ND	YO	-C	4.6	0	-	-	+	-	-	±	-	+	+	+	-	-	6.2	400.0	100.0	1.0	4.0	0.7
TW 52	ND	Y	±	4.5	49	-	-	+	-	-	+	+	+	+	+	-	-	0.006	0.4	200.0	1.3	3.6	0.5
TW 139	ND	Y	±	4.4	49	±	-	+	-	-	+	+	+	+	+	-	-	12.5	0.4	0.4	0.9	4.1	0.8
GH 149	ND	YO	-	4.5	0	-	-	+	-	-	+	-	+	+	+	-	-	0.006	0.4	0.4	1.2	3.8	0.5
KH 211	ND	Y	±C	4.5	0	-	±	+	±	±	+	+	+	+	+	-	-	25.0	0.8	0.4	0.7	4.4	0.3
AW 178	35.8	Y	-	4.4	42	-	-	+	-	±	+	+	+	+	+	-	-	6.2	0.4	100.0	0.8	4.1	0.4
RM 122	ND	Y	±C	4.5	51	-	-	+	-	±	+	+	+	+	+	-	-	0.006	0.8	0.4	0.9	4.3	0.4
MCS 13	ND	Y	-	4.5	0	-	±	+	-	±	-	±	+	+	+	-	-	0.050	200.0	100.0	1.1	3.3	0.5
GS 16	ND	Y	±	4.5	37	-	±	+	±	-	±	-	+	+	+	-	-	0.050	0.8	200.0	1.3	3.5	0.4
DGS 13	ND	-	±	4.5	47	±	±	+	-	-	±	+	+	+	+	-	-	0.006	400.0	0.4	1.1	3.6	0.8
BS 14	ND	-	±C	4.6	45	±	±	+	-	±	±	-	+	+	+	-	-	0.006	200.0	100.0	0.6	3.9	0.3
LK 47	ND	-	±	ND	ND	-	-	+	-	-	-	±	+	+	+	-	-	0.006	0.4	0.4	ND	ND	ND
MK 314	ND	Y	±	ND	ND	-	±	+	±	-	-	±	+	+	+	-	-	12.5	0.4	0.4	ND	ND	ND

^a Symbols for colony pigment: YO, yellow-orange center; Y, yellowish center; -, gray-white (unpigmented).^b Symbols for anaerobic growth: ±, gradient of growth from moderate to light down the tube; ±C, gradient of growth plus individual colonies; -C, individual colonies only (usually <50); -, no detectable growth.^c Reaction: ++, strong positive (carbohydrates only); +, positive; ±, weak; -, negative.^d ND, Not determined.

TABLE 4. Variable characters of 13 strains of *Staphylococcus simulans*

Strain	Mol % G+C	Anaerobic fermenta- tion of glucose (pH)	L-Lactic acid (%)	Growth on 15% NaCl agar	Growth at 15 C	Acetyl- methyl- carbinol	Nitrate reduc- tion	Phos- pha- tase	Bacteri- olytic activity	Acid (aerobically) from						Peni- cillin G MIC (μg/ml)	Erythro- mycin MIC (μg/ml)	Tetra- cycline MIC (μg/ml)	Peptido- glycan (mol/mol of glu- tamic acid)	
										Galac- tose	Man- nose	Ri- bose	Malt- ose	Tre- halose	Man- nitrol					
ATCC 27848	34.0	4.7	100	- ^a	+	-	+	±	+	-	±	+	±	±	±	3.1	0.8	200.0	0.2	4.9
ATCC 27849	36.9	4.7	63	±	+	±	+	±	+	+	+	-	+	+	+	0.012	0.4	0.4	0.1	5.7
ATCC 27850	36.7	4.8	60	±	+	-	+	±	+	+	-	+	+	+	+	0.025	400.0	200.0	0	6.0
ATCC 27851	34.2	4.8	100	-	±	-	+	±	+	+	±	+	+	+	+	1.6	0.8	400.0	0.1	4.7
AW 232	ND	4.8	100	●	+	+	+	-	±	+	-	-	+	+	+	0.012	0.4	100.0	0.2	5.6
SM 128	34.4	4.8	64	±	+	+	+	-	±	+	-	-	+	+	+	0.025	0.8	0.8	0	5.3
SM 81	ND	4.9	100	-	+	±	+	±	±	-	+	+	+	+	+	25.0	400.0	0.4	0	5.1
KL 299	ND	4.8	69	±	+	-	+	±	±	+	-	-	+	+	+	0.012	0.4	0.4	0	5.0
KL 104	ND	4.9	70	±	+	-	+	±	±	+	-	-	+	+	+	0.012	400.0	200.0	0	4.8
MAW 222	37.2	4.8	75	±	+	-	+	±	+	-	-	-	-	-	+	0.025	0.8	0.8	0.1	5.2
HK 12	ND	4.9	100	±	+	-	+	±	+	+	-	±	+	+	+	25.0	0.8	0.4	0.2	4.8
DW 196	ND	4.8	100	±	+	-	+	±	+	+	-	±	+	+	+	12.5	0.8	200.0	0.1	5.4
CE 13	ND	5.0	70	±	±	-	+	-	±	+	-	-	+	+	+	0.800	0.4	6.2	0	5.0

^a Reaction: + +, strong positive (carbohydrates only); +, positive; ±, weak; -, negative.

° ND, Not determined.

23% failed to grow at this concentration. The optimal growth temperature range was 25 to 40 C. Eighty-five percent of the strains grew well at 15 C, and all grew well at 45 C.

All strains had weak or moderate catalase activity and were positive for the benzidine test. All failed to produce coagulases. All strains demonstrated weak or no hemolysin activity on bovine or sheep blood but produced weak to good hemolysin activity on human blood. Seventy-seven percent of the strains failed to produce acetylmethylcarbinol or had weak phos-

phatase activity. Ninety-two percent of the strains reduced nitrates or demonstrated moderate to good bacteriolytic activity. All had weak to moderate DNase activity.

All strains produced acid aerobically from glucose, fructose, lactose, sucrose, and glycerol. Seventy-seven percent of the strains produced acid from mannose and trehalose, and 92% produced acid from mannitol. Fifty-four percent of the strains produced acid from ribose. Sixty-nine percent of the strains failed to produce acid from maltose; 31% produced only

TABLE 5. *Morphological characters of Staphylococcus species*

Species	Total no. of strains studied	Colony diameter ^a (mm)	Colony pigment					Colony profile			Colony edge		Cell arrangement			
			Yellow-orange	Yellowish	Gray or gray-white with yellowish tint	White with yellowish tint	Gray to gray-white	White	Raised (flat)	Low convex	Convex to slight umbonate	Entire	Undulate to crenate	Tetrads, <10% pairs and singles	Tetrads, 10-50% pairs and singles	Pairs and singles, 10- <50% tetrads
<i>S. aureus</i>	46	7.1 ● 0.1	32 ^b	2	44		22		100			100			18	82
<i>S. simulans</i>	13	6.5 ± 0.2			23		77		100			100		15	62	23
<i>S. xylosus</i>	102	7.0 ± 0.1	24	16	35	5	12	8	71	17	12	68	32		12	88
<i>S. cohnii</i>	42	5.9 ± 0.2			5	21	21	53	7	10	83	95	5		7	93
<i>S. saprophyticus</i> ..	83	6.5 ± 0.1	6	10	26	18	11	29	20	80		98	2		7	93
<i>S. haemolyticus</i> ...	117	5.4 ± 0.1		3	29	13	32	23	13	87		92	8	14	64	22
<i>S. warneri</i>	38	3.8 ● 0.1	5	38	37		20			22	78	98	2	12	60	28
<i>S. hominis</i>	240	3.7 ± 0.05	34	25	5		36			18	82	91	9	61	38	1
<i>S. epidermidis</i>	204	3.1 ± 0.04			4		96		100			99	1	2	17	66
<i>S. capitis</i>	47	2.4 ± 0.07						100		100		100		14	70	16

^a $\bar{x}$ and s._x.

^b Percentages of strains possessing the character indicated.

TABLE 6. *Physiological characters of Staphylococcus species*

Species	Total no. of strains studied	Anaerobic growth in thioglycolate semisolid medium					Growth on:					Growth at:				
							10% NaCl agar			15% NaCl agar		15 C			45 C	
		Uniformly dense	Gradient: dense to light	Scattered colonies >50	Scattered colonies <50	None	+	●	-	+	±	+	●	-	+	±
<i>S. aureus</i>	46	98 ^b	2				96	4			60	36	4	100		
<i>S. simulans</i>	13	100					100				77	23	85	100		
<i>S. xylosus</i>	102	68	11	14	4	3	100			38	48	14	100		19	62
<i>S. cohnii</i>	42	24	47	17	7	5	100			21	64	15	100		76	7
<i>S. saprophyticus</i>	83	59	30	9	1	1	100			30	70		100		38	26
<i>S. haemolyticus</i>	117	20	8	66	4	2	100			15	45	40		43	57	100
<i>S. warneri</i>	38	87	5	8			100			8	54	38	8	76	16	92
<i>S. hominis</i>	240		10	44	30	16		97	3		3	97		28	72	100
<i>S. epidermidis</i>	204	98	1	1			16	79	5		4	96		21	79	100
<i>S. capitis</i>	47	68	11	21			90	10			51	49		100	100	

^a Growth: +, good; ±, weak; -, none.

^b Percentages of strains possessing the character indicated.

weak acid from this carbohydrate. Eight percent of the strains produced weak acid from galactose. All failed to produce acid from rhamnose, xylose, arabinose, turanose, gentiobiose, cellobiose, melezitose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, fucose, tagatose, lyxose, or sorbose.

All strains were resistant to lysozyme and were susceptible to lysostaphin (MIC, 12.5 to 25 $\mu\text{g/ml}$). All were susceptible to streptomycin (MIC, 1.6 to 3.1 $\mu\text{g/ml}$) and novobiocin (MIC, 0.1 to 0.2 $\mu\text{g/ml}$). Fifty-four percent of the strains were susceptible to penicillin G (MIC, 0.012 $\mu\text{g/ml}$), and 77% were susceptible to erythromycin (MIC, 0.4 to 0.8 $\mu\text{g/ml}$). Forty-six percent of the strains were susceptible to tetracycline (MIC, 0.4 $\mu\text{g/ml}$). This species contained peptidoglycan of the type L-Lys-Gly₅₋₆. The cell wall teichoic acid contained glycerol and galactosamine.

The G+C content of the DNA, as determined in six strains, was 35.6 ± 0.6 mol%.

Some variable characters and the G+C content of the DNA of some representative strains of *S. simulans* are given in Table 4.

Strain ATCC 27848 (originally designated MK 148) is the type strain of *S. simulans*. A description of this strain follows.

Cells: Spheres, 0.8 to 1.0 μm in diameter, occurring predominantly in pairs and tetrads and occasionally singly. Nonmotile and non-sporeforming. Gram positive.

Agar colonies: Circular, entire, 6.5 to 7.0 mm in diameter, slightly raised, smooth with slightly glistening surface. Translucent to slightly transparent and gray-white (unpigmented).

Catalase and benzidine tests are positive.

Chemoorganotroph; metabolism is respiratory.

Facultatively anaerobic.

TABLE 7. Biochemical characters of *Staphylococcus* species

Species	Total no. of strains studied	Coagulase		Hemolysis				Acetyl-methyl-carbinol production				Nitrate reduction			Phosphatase			Deoxyribonuclease			Bacteriolytic activity		
		+	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-		
<i>S. aureus</i>	46	100 ^a		84	14	2	98	2		98	2		96	4		100			88	12			
<i>S. simulans</i>	13		100		54	46	8	15	77	92		8		77	23		100			54	38		8
<i>S. xylosus</i>	102		100	5	10	85	21	43	36	83		17	70	10	20		21	79	14	20		66	
<i>S. cohnii</i>	42		100	14	31	55	41	45	14	3	3	94		17	83		21	79	14	24		62	
<i>S. saprophyticus</i>	83		100		3	97	77	23		5	3	92		2	14	84			100			100	
<i>S. haemolyticus</i>	117		100	70	28	2	66	15	19	83	7	10		2	20	78		34	66	12	36		52
<i>S. warneri</i>	38		100	2	42	56	95	5		18	8	74	10	12	78		5	63	32	45	25		30
<i>S. hominis</i>	240		100		30	70	38	26	36	61	23	16	<1	16	83			42	58	17	22		61
<i>S. epidermidis</i>	204		100	31	69	99	<1			60	28	12		89	6	5		83	17	34	32		34
<i>S. capitis</i>	47		100		23	77	25	33	42	79	4	17			28	72		100			92		8

^a Reactions: +, positive; ±, weak; -, negative.

^b Percentages of strains giving the reaction indicated.

TABLE 8. Carbohydrate reactions of *Staphylococcus* species: monosaccharides under aerobic conditions

Species	Total no. of strains studied	D(+) Glucose			β-D(-) Fructose			D(+) Galactose			D(+) Mannose			D(+) Xylose			L(+) Arabinose			D(-) Ribose		
		++ ^a	+	•	++	+	-	++	•	-	++	±	-	+	-	±	+	-	±	++	±	-
<i>S. aureus</i>	46	100 ^b				100		100			89	11		100			100			100		
<i>S. simulans</i>	13	100				100			8	92	77		23	100			100			23	31	46
<i>S. xylosus</i>	102	56	38	6	95	5		62	29	9	95	5		92	8	76			24	15	24	61
<i>S. cohnii</i>	42	86	12	2	100				10	90	51	25	24	100			100					100
<i>S. saprophyticus</i>	83	85	14	1	92	5	3		8	92			100	100			100				5	95
<i>S. haemolyticus</i>	117	98	2		24	36	40	29	24	47	3		97	100			100			6	11	83
<i>S. warneri</i>	38	100			79	21		5	19	76	2		98	100			100			45	5	50
<i>S. hominis</i>	240	100			100			33	19	48	9	1	90	100			100					100
<i>S. epidermidis</i>	204	100			100			80	5	15	85	8	7	100			100			2	2	96
<i>S. capitis</i>	47	100			79	21				100	89	11		100			100					100

^a Reactions: ++, strong acid; +, moderate acid; ±, weak acid; -, negative.

^b Percentages of strains giving reaction indicated.

TABLE 9. Carbohydrate reactions of *Staphylococcus* species: di- and trisaccharides and sugar alcohols under aerobic conditions

Species	Total no. of strains studied	Disaccharides										Trisaccharide		Sugar alcohols					
		Maltose		α -Lactose			Sucrose			D(+) Trehalose		D(+) Turanose		D(+) Melezitose		D-Mannitol		Xylitol	
		++, + ±	-	++, + ±	-	++	+	-	++	+	+	±	+	+	±	+	±		
<i>S. aureus</i>	46	100 ^b	31	88	4	8	100			100		74	26		100	96	2	2	100
<i>S. simulans</i>	13			100			100			77			100		100	77	15	8	100
<i>S. xylosus</i>	102	82	11	63	11	26	77	19	4	96	4	35	53	12	100	100	5	5	82
<i>S. cohnii</i>	42	28	53	31		69	10	2	88	100		5	95		100	62	26	12	62
<i>S. saprophyticus</i>	83	98	2	85		15	88	12		94	6	92	8		100	100	1	4	21
<i>S. haemolyticus</i>	117	100		33	3	64	60	40		95	5	22	25	53	100	95	4	60	20
<i>S. warneri</i>	38	68	27	3	8	89	100			100	11	89			100	53	47	4	100
<i>S. hominis</i>	240	100		44	16 ^c	40	19	81		90	10	81	19	51	100	63	37	93	100
<i>S. epidermidis</i>	204			76	2	24	100			2	98	35	31	19	81	7	100	100	100
<i>S. capitis</i>	47				2	98		91	9	2	98		100		100	92	2	6	100

^a Reactions: ++, strong acid; +, moderate acid; ±, weak acid; -, negative.
^b Percentages of strains giving reaction indicated.
^c Slow development of distinctive (+) (yellow—acid) colonies in (-) (gray) culture streak. Small percentage of the culture population appears to be capable of producing acid from lactose.

Growth at extreme temperature: Good growth at 15 and 45 C.

Growth on NaCl agar: Good growth with 10% NaCl, no growth with 15%.

Coagulases not produced.

Phosphatase activity weak.

Acetylmethylcarbinol not produced.

Nitrates are reduced.

Hemolysins: No hemolysis of sheep blood,

weak hemolysis of bovine blood, good hemolysis of human blood.

DNase activity weak.

Acid produced aerobically from glucose, fructose, ribose, maltose (very weak), lactose, sucrose, trehalose, mannitol, and glycerol. No acid from galactose, mannose, rhamnose, xylose, arabinose, turanose, gentiobiose, cellobiose, melezitose, xylitol, sorbitol, inositol, salicin, adonitol, dulcitol, arabitol, erythritol, erythrose, raffinose, melibiose, fucose, tagatose, lyxose, or sorbose.

Antibiotic susceptibilities: Susceptible to lysostaphin (MIC, 25 μ g/ml), streptomycin (MIC, 3.1 μ g/ml), erythromycin (MIC, 0.8 μ g/ml), and novobiocin (MIC, 0.1 μ g/ml); slightly resistant to penicillin G (MIC, 3.1 μ g/ml); resistant to tetracycline (MIC, 200 μ g/ml); resistant to lysozyme.

Cell wall peptidoglycan: L-Lys-Gly_{4,6}, L-Ser_{0,2}.

Cell wall teichoic acid composition: Glycerol and galactosamine.

G+C content of the DNA: 34.0 mol%.

S. simulans can be distinguished from all other staphylococci primarily on the basis of a combination of its colony morphology, lack of acetylmethylcarbinol production, carbohydrate reaction pattern, and cell wall composition. There are also some significant differences be-

TABLE 10. *Lysostaphin susceptibility of Staphylococcus species*

Species	Total no. of strains studied	Level of susceptibility ^a				
		12.5 (+)	25 (+)	50 (+)	100 (+)	200 ($\pm$)
<i>S. aureus</i>	51	10	82	8		
<i>S. simulans</i>	13	23	77			
<i>S. xylosus</i>	101		27	67	6	
<i>S. cohnii</i>	42			100		
<i>S. saprophyticus</i>	83			28	72	
<i>S. haemolyticus</i>	116				34	66
<i>S. warneri</i>	38				8	92
<i>S. hominis</i>	178				41	59
<i>S. epidermidis</i>	152				68	32
<i>S. capitis</i>	48				8	92

^a Expressed as MIC (numbers) and spot inhibition (symbols in parentheses). MICs are expressed as micrograms per milliliter. Spot inhibition is expressed as: +, complete inhibition; $\pm$, partial inhibition. Data are given as percentages of strains.

TABLE 11. *Antibiotic susceptibilities of Staphylococcus species*

Species	Total no. of strains studied ^a	Penicillin G					Novobiocin			Erythromycin			Tetracycline				
		Level of susceptibility ^b					Level of susceptibility			Level of sus- ceptibility			Level of sus- ceptibility				
		0.003-0.006 (18-15)	0.012-0.025 (14-12)	0.050-0.1 (11-9)	0.2-0.4 (8-6)	0.8-6.2 (5-3)	12.5-100 (2-0)	0.025-0.2 (14-8)	0.4-0.8 (7-6)	1.6-3.1 (5-3)	6.2-12.5 (2-1)	0.4-1.6 (8-3)	3.2-25 (2-1)	100-400 (0)	0.4-0.8 (12-8)	1.6-6.2 (7-5)	50-200 (2-0)
<i>S. aureus</i>	51		45 ^c	20		8	27	96		2	2	96		4	100		
<i>S. simulans</i>	13		46	8		23	23	100				77		23	46	8	46
<i>S. xylosus</i>	101		2	26	47	25				32	68	62	26	12	80	2	18
<i>S. cohnii</i>	42			2	91	7				12	88	62	17	21	84	14	2
<i>S. saprophyticus</i> ...	83				95	5		1		8	91	82	7	11	93	4	3
<i>S. haemolyticus</i> ...	116		49	4	<1	25	21	98			2	95		5	90	<1	9
<i>S. warneri</i>	38		79	5		13	3	100				100			100		
<i>S. hominis</i>	178	65	12			21	2	99		<1	<1	93		7	64		36
<i>S. epidermidis</i>	152	49	4			36	11	99	<1		<1	96		4	83		17
<i>S. capitis</i>	48		29	65			6	100				100			85		15

^a Strains were isolated from persons who were not exposed to antibiotics for at least 3 months prior to collecting. It would be expected that the above base-level frequencies might shift toward higher frequencies of resistant strains during or shortly after antibiotic exposure (8).

^b Expressed as MIC (numbers) and disk zone inhibition (numbers in parentheses). MICs are expressed as micrograms per milliliter. Disk zone inhibition represents the width (in millimeters) of the zone of antibiotic inhibition, measured from the edge of a 2-U penicillin G, 5- μ g novobiocin, 2- μ g erythromycin, and 30- μ g tetracycline sensitivity disk (Difco), respectively.

^c Data are given as percentages of strains.

TABLE 12. Scheme for the classification of human cutaneous staphylococci^a

Species	Colony diameter (mm)	Colony pigment	Colony profile and light transmission ^b	Cell arrangement (pre-dominant) ^c	Anaerobic growth in thioglycolate ^d	Terminal pH in extract-glucose medium	Lactic acid isomer(s)	Growth on 10% NaCl agar	Growth at 15°C	Growth at 45°C	Coagulase (pref. rabbit)	Hemolysis (pref. bovine)	Acetyl-methyl-carbinol	Nitrate reduction	Phosphatase	Deoxy-ribonuclease
<i>S. aureus</i>	6-8	(+)	RT	(PS)	+	4.3-4.6	DL(L)	+	++	+	+	(+)	+	+	+	+
<i>S. simulans</i>	5-7	(-)	RT	V	+	4.7-5.0	DL.L	+	(+)	+	-	±,-	(-)	(+)	(+)	±
<i>S. xylosus</i>	5-9	(-)	(RST)	PS	V	4.9-5.6	L>D	+	+	(-)	-	(-)	±,-	(+)	(+)	(-)
<i>S. cohnii</i>	5-7	(-)	(CO)	PS	V	5.3-6.0	L ₂ DL	+	+	(+)	-	(-±)	+	-	(-)	(-)
<i>S. saprophyticus</i>	5-8	V	(LCO)	PS	(+,±)	5.0-5.4	DL	+	+	V	-	-	(+)	-	(-)	-
<i>S. haemolyticus</i>	4-8	(-)	(LCO)	V	(±C)	4.9-5.5	D	+	±,-	+	-	(+)	+	(+)	(-)	±,-
<i>S. warneri</i>	3-5	(+)	(CO)	V	(+)	4.7-5.0	DL	+	(+)	+	-	±,-	+	(-)	(-)	±,-
<i>S. hominis</i>	3-4	V	(CO)	T	c	4.4-4.9	DL,D	±	(-)	+	-	(-)	V	(-)	(-)	±,-
<i>S. epidermidis</i>	2-4	-	RT	V	+	4.3-4.6	L	(±)	(-)	+	-	±,-	+	(+)	(+)	(±)
<i>S. capitis</i>	1-3	-	CO	(T)	V	4.2-4.7	L>D	+	-	+	-	(-)	V	(+)	(-)	±

Species	Acid (aerobically) from										Resistance to		Cell-wall composition										
	β-D(-)-Fructose	D(+)-Galactose	D(+)-Mannose	D(+)-Xylose and/or L(+)-Arabinose	D(-)-Ribose	Maltose	α-Lactose	Sucrose	D(+)-Trehalose	D(+)-Turannose	D(+)-Melirose	D-Mannitol	Xylitol	Novobiocin ^e (MIC, ≥3.1 μg/ml)	Lysozastaphin (MIC, ≥100 μg/ml)	Peptidoglycan (mol/mol of glutamic acid)							
																Serine	Glycine	Ribitol	Glycerol	Glucose	Glucosamine	Galactosamine	Glutamic acid polymer (glutamic acid ≥1.3 mol/mol lysine)
<i>S. aureus</i>	+	+	+	-	Δ	+	(+)	+	(+)	+	-	+	-	-	-	0-0.3	5.0-6.0	+	+	-	+	-	-
<i>S. simulans</i>	+	+	+	-	Δ	+	(+)	+	(+)	+	-	+	-	-	-	0-0.2	4.7-6.0	+	+	-	+	-	-
<i>S. xylosus</i>	+	+	+	+	Δ	+	Δ	+	+	+	-	+	(-)	-	-	0-0.1	4.9-5.7	+	+	ND ^g	+	-	-
<i>S. cohnii</i>	+	+	+	+	Δ	+	(+)	+	(+)	+	-	+	(+)	+	+	0-0.2	4.9-6.0	+	+	+	(+)	-	-
<i>S. saprophyticus</i>	+	+	+	+	Δ	+	(+)	+	+	+	-	+	(+)	+	(+)	0.6-0.8	4.1-5.1	+	+	ND	+	-	-
<i>S. haemolyticus</i>	+	+	+	+	Δ	+	+	+	Δ	+	-	+	-	-	+	0.9-1.4	3.3-4.0	+	+	+	+	-	-
<i>S. warneri</i>	+	+	+	+	Δ	+	+	+	(-)	+	-	+	-	-	+	0.6-1.4	3.3-4.4	+	+	+	+	-	-
<i>S. hominis</i>	+	+	+	+	Δ	+	+	+	(+)	+	-	+	-	-	+	0.6-1.3	3.3-4.3	+	+	+	+	-	-
<i>S. epidermidis</i>	+	+	+	+	Δ	+	+	+	(+)	+	-	+	-	-	+	0.7-1.5	4.0-5.0	+	+	+	+	-	-
<i>S. capitis</i>	+	+	+	+	Δ	+	+	+	(+)	+	-	+	-	-	+	0.8-1.2	3.5-4.3	+	+	+	+	-	+

^a Characters were selected based on their ease of distinction and wide differences (≥70%) in their type frequencies between two or more species. A single listed symbol denotes a type frequency of 90 to 100%; parentheses around symbols denote a type frequency of 70 to 89%; two symbols are listed for a character when either type is in frequency below 70%, but together equal 80 to 100%. Symbols for characters (unless noted otherwise): +, positive; ±, weak; -, negative; V, variable (+, ±, and -).
^b Symbols for colony profile and light transmission: RT, raised (flat) and slightly translucent; CO, convex and opaque; LCO, low convex and opaque.

^c Symbols for cell arrangement: PS, pairs and single cells; T, tetrads; V, variable (pairs, singles, and tetrads common).

^d Symbols for anaerobic growth: +, dense uniform; ±, gradient of growth from dense to light down tube; ±C, large individual colonies; c, small individual colonies to absence of visible growth; V, variable (dense uniform, gradient growth, and individual colonies).

^e Without exposure.

^f ND, Not determined. (All of the strains were not tested. Some of the strains of *S. xylosus* and *S. saprophyticus* tested contained glucose, and most of the strains of *S. saprophyticus* tested contained glucosamine.)

tween *S. simulans* and certain other species in the parameters of anaerobic growth in thioglycolate, lactic acid configuration, growth at different NaCl concentrations and temperature extremes, coagulase activity, hemolysis, acetylmethylcarbinol production, nitrate reduction, and phosphatase, DNase, and bacteriolytic activities. Several of the properties of *S. simulans* overlap with those of five unusual coagulase-positive strains isolated in this study that appear to be similar to strains previously described by Reeder and Ekstedt (14) and Oeding (12). There is also an overlap of several properties with those of human strains of *S. aureus*. It would appear then that *S. simulans* is closely related to the coagulase-positive staphylococci.

Character variation in *Staphylococcus* species. Summary data of the morphological, physiological, biochemical, and antibiotic characters found in the various *Staphylococcus* species isolated from human skin are shown as follows: colony morphology and pigment and cell arrangements, Table 5; anaerobic growth in thioglycolate, growth on 10% and 15% NaCl agar, and growth at 15 and 45 C, Table 6; coagulase, hemolysis, acetylmethylcarbinol production, nitrate reduction, phosphatase, DNase, and bacteriolytic activities, Table 7; carbohydrate reactions, Tables 8 and 9; lysostaphin susceptibility, Table 10; and antibiotic susceptibilities, Table 11. A summary scheme showing the key characters used for the classification of cutaneous *Staphylococcus* species is presented in Table 12. This information is based on species described in the companion paper (15) and this one.

Species colony morphology and pigment patterns are illustrated in Fig. 1. These characters were very useful in making the preliminary species identification of strains on the original isolation plates. Species having very distinctive colony characteristics include *S. hominis*, *S. warneri*, *S. epidermidis*, and *S. capitis*. It was somewhat difficult to differentiate a certain few strains of *S. xylosus*, *S. saprophyticus*, *S. cohnii*, and *S. haemolyticus* from one another using these characters alone.

It should be pointed out that the species character variation observed in this study, through very similar for the strains isolated from cities in North Carolina and New Jersey, may be somewhat different with strains obtained from certain other geographical areas. For example, in this study we did not recover any of the violet-pigmented strains of *S. epidermidis* found by Marples (7) on people living in Philadelphia, Pa., and recently found by one of us (W.E.K.) and W. C. Noble (unpublished data) on people living in London, England. Strains originating from animals other than man may also show somewhat different character variation from the human strains.

Occurrence of *Staphylococcus* species on human skin. A summary of the occurrence and distribution of *Staphylococcus* species on humans is shown in Table 13. As can be seen from the data, some species show preferences for certain regions of the cutaneous habitat. *S. epidermidis* was a predominant species in all regions tested, including the head, nares, axillae, arms, and legs. *S. hominis* was a predominant species on the head, axillae, arms, and

TABLE 13. Occurrence of *Staphylococcus* species on human skin

Species	% of 40 individuals with species	Mean % of skin sites occupied by species/individual					Mean % of species of total staphylococci (CFU) recovered/site per individual ^a				
		Head	Nares	Axillae	Arms	Legs	Head ^c	Nares	Axillae	Arms	Legs
		4 ^b	2	2	4	4	1.6 × 10 ⁴ ^c	7.8 × 10 ⁵	1.0 × 10 ⁶	81	108
<i>S. aureus</i>	52	12	43	4	11	9	5	30	0.02	2	4
<i>S. simulans</i>	12	3	2	1	7	2	1	0.1	0.05	2	3
<i>S. xylosus</i>	42	15	5	5	11	8	4	0.06	0.2	3	3
<i>S. cohnii</i>	35	17	15	6	18	19	3	0.3	0.2	5	6
<i>S. saprophyticus</i> ..	70	31	12	17	28	32	3	0.1	0.3	7	6
<i>S. haemolyticus</i>	78	21	5	8	28	26	4	0.3	0.9	6	6
<i>S. warneri</i>	52	15	12	0	9	9	1	0.3	0	0.8	1
<i>S. epidermidis</i>	100	87	78	92	82	62	46	63	64	32	26
<i>S. hominis</i>	100	62	22	48	69	77	22	5	34	34	41
<i>S. capitis</i>	65	44	12	3	27	17	11	0.8	0.3	8	4

^a The number of colonies isolated from each site to determine the percentage of species present represents an estimated 22 ± 1% of the total staphylococcus colony-forming units (CFU) recoverable.

^b Number of sites per individual.

^c Average number of staphylococci per site per individual.

legs, and to a lesser extent in the nares. *S. capitis* was a predominant species on the head and to a lesser extent on the arms. *S. aureus*, as expected from a considerable number of earlier reports, was often a predominant species in the nares.

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REPRINT REQUESTS

Address reprint requests to: Dr. Wesley E. Kloos, Department of Genetics, North Carolina State University, Raleigh, N.C. 27607.

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FIG. 1. Colonies of *Staphylococcus* species isolated from human skin. The "seed" inoculum was incubated at 34 C for 5 days (unless otherwise noted). (All rows read from left to right.) (A) *S. aureus*: top, GH 38; middle row, KL 207, JL 68, KES 1; bottom row, GH 39, MAW 306, HK 91 (*Staphylococcus* sp. related to/or a variety of *S. aureus*). (B) *S. simulans*: ATCC 27848 (MK 148), ATCC 27849 (AW 215), ATCC 27850 (KL 240). (C) *S. xylosus*: top row, AW 124, DM 37, AW 244, GH 30; middle row, DSM 20266 (KL 162), KH 168, TM 76, RM 349; bottom row, DBM 194, GH 9 (*Staphylococcus* sp. related to/or variety of *S. xylosus*). (D) *S. cohnii*: DM 224, DSM 20260 (GH 137), MK 247. (E) *S. saprophyticus*: top row, TW 111, KL 20, DM 100; bottom row, SE 11, GH 196, SM 118. (F) *S. haemolyticus*: top row, DM 70, TW 268, GS 1, MK 67; bottom row, KH 99, DSM 20263 (SM 131), RM 419. (G) *S. warneri*: top row, MAW 314, ATCC 27838 (MK 155), ATCC 27836 (AW 25); bottom row, ATCC 27837 (RM 130), ATCC 27839 (DW 138), DM 229. (H1) *S. hominis* (incubated 5 days): top row, ATCC 27844 (DM 122), GH 180, LK 89, JM 16; bottom row, ATCC 27845 (KL 243), BS 14, ATCC 27847 (KES 13), ATCC 27846 (MK 129). (H2) *S. hominis* (incubated 7 days): colony identification same as above as in (H1). (I) *S. epidermidis*: top row, KL 275, MK 214, AW 269; bottom row, RK 13, JRM 1, KH 141. (J) *S. capitis*: ATCC 27840 (LK 499), ATCC 27842 (MCS 11), ATCC 27841 (DM 267).