Numerical Taxonomy of Staphylococci Isolated from the Marine Environment

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A total of 220 strains of staphylococci isolated from marine and estuarine surface water samples were examined for 62 morphological, physiological, and biochemical characteristics. The taxonomic data were analyzed by numerical taxonomy; 86% of the cultures were classified as *Staphylococcus epidermidis* or *Staphylococcus hominis*, 5% were classified as *Staphylococcus aureus*, and 9% were classified as *Micrococcus* spp. An association was observed between species of *Staphylococcus* isolated from surface water samples and geographic source. *S. epidermidis*, the most common of the coagulase-negative staphylococci encountered in clinical laboratories, was the dominant species in samples of polluted water, whereas *S. hominis*, a less common species in clinical specimens, was dominant in water samples collected from unpolluted regions. The phenetic characteristics of the environmental staphylococcal isolates are presented.

The first major study (1) of the taxonomy of marine cocci was reported before publication of the first well-organized approach to the identification of these organisms (2). Consequently, species status was not assigned to the marine cocci because classification of these bacteria was in such confusion. In that study, Anderson (1) isolated 205 catalase-positive, gram-positive cocci from three water samples collected from the North Sea. A total of 90% of the 205 strains were fermentative, and 10% were either oxidative or asaccharolytic, when they were examined by using a modified glucose oxidation-fermentation medium. According to present classification schemes (3, 20, 23, 24, 26, 32, 40), the fermentative cocci should now be assigned to the genus *Staphylococcus*, and the oxidative and asaccharolytic strains should be assigned either to the genus *Micrococcus* or to *Planococcus*. It is possible that the asaccharolytic cocci examined were staphylococci which either did not grow well in oxidation-fermentation medium or did not produce enough acid from glucose to alter the pH indicator in the medium (15, 20, 24, 32). From this interpretation of the data, it may be concluded that staphylococci are present in marine waters at a greater frequency than has been believed previously.

McCallum (28) examined the bacterial flora of samples collected from the Bahama Bank and found very small numbers of staphylococci and micrococci. Those cocci that were isolated and

identified were associated primarily with sediment, not with water. However, from results of studies of beaches at Biscayne Bay, Fla., Buck (5) concluded that staphylococci were dominant in beach water, sediment, and sand compared with fecal coliform-organisms and fecal streptococci. A more recent study of high-salinity water of the East China Sea (35) and the Nansei area of the Pacific Ocean yielded samples containing up to 17% gram-positive cocci.

Thus, catalase-positive, gram-positive cocci indeed occur naturally in the marine environment, with the dominant genus of gram-positive cocci being *Staphylococcus*. In the study reported here, we characterized catalase-positive, gram-positive cocci isolated from seawater as part of a larger study of the bacterial communities of the surface waters of a deep sea site.

MATERIALS AND METHODS

Sampling. The following four locations were sampled during this study: (i) Narragansett Bay, R.I.; (ii) Chesapeake Bay; (iii) Atlantic Ocean coastal and open ocean waters east of Cape Henry, Va.; and (iv) a pharmaceutical waste dump site located in the Puerto Rico Trench area of the Atlantic Ocean, approximately 64 km north of Arecibo, Puerto Rico. The exact sampling locations, data, and methods have been reported elsewhere (14, 36).

Cultures. In addition to 220 fresh isolates from seawater samples collected at the sampling stations listed above, 19 clinical isolates, 24 reference strains of *Staphylococcus*, and 2 reference cultures of *Micrococcus* were included in the study. The reference cultures and their sources are listed in Table 1.

Characterization of cultures for taxonomic analyses. Cultures from seawater were isolated by using an

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

Species	Phenon	Strain(s) ^a
<i>S. sciuri</i> subsp. <i>sciuri</i>	E1	ATCC 29062 ^T , ATCC 29059
<i>S. sciuri</i> subsp. <i>lentus</i>	E1	ATCC 29070 ^T , K6
<i>S. hominis</i>	E7	ATCC 27846, ATCC 27844 ^T
<i>S. haemolyticus</i>	E7	DSM 20263 ^T (= ATCC 29970 ^T)
<i>S. haemolyticus</i>	E11b	DSM 20265 (= ATCC 29968)
<i>S. warneri</i>	E9	ATCC 27836 ^T , ATCC 27839
<i>S. epidermidis</i>	E10c	ATCC 14990 ^T , RM351
<i>S. capitis</i>	E11b	ATCC 27841, ATCC 27840 ^T
<i>S. aureus</i>	E12b	JL68, ATCC 12600 ^T
<i>S. simulans</i>	E12c	ATCC 27851, ATCC 27848 ^T
<i>S. cohnii</i>	E13	DSM 20260 ^T (= ATCC 29974 ^T), DSM 20261
<i>S. xyloso</i>	E13	DSM 20266 ^T (= ATCC 29971 ^T), DSM 20267
<i>S. saprophyticus</i>	E13	AW163, CCM 883 ^T (= ATCC 15305 ^T)

^a The strains of staphylococci were obtained from W. E. Kloos, North Carolina State University, Raleigh. The strains of micrococci were obtained from J. F. Keiser, Walter Reed Army Medical Center, Washington, D.C.

incubation temperature of 25°C. However, the strains grew well at 35°C and were subsequently tested biochemically at this temperature by using methods described elsewhere (13, 15, 21, 24; Gunn, Ph.D. thesis, University of Maryland, College Park, 1981). Each strain was examined for a total of 62 unit characters. The salient, differentiating characters are shown in Tables 2 through 5.

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 265 strains and 62 characters.

Computer analysis. The data were analyzed by using the Jaccard coefficient (38), which excludes negative matches. Clustering was done by the unweighted average linkage method (39). The programs used, Taxan6 and IGPS3, were stored on a UNIVAC 1108 computer and are available on line at the University of Maryland Computer Science Center, College Park.

RESULTS

Results obtained by using the Jaccard coefficient revealed that 86% of the environmental gram-positive, coccus isolates were either *Staphylococcus epidermidis* or *Staphylococcus hominis*, 5% were *Staphylococcus aureus*, and less than 1% were *Staphylococcus cohnii*; 9% of the environmental isolates were *Micrococcus* spp. Gram-negative, aerobic, heterotrophic bacteria outnumbered the gram-positive cocci by 10:1 in open ocean waters and by approximately 2,000:1 in Chesapeake Bay top water samples. The criteria used to distinguish *Micrococcus* from *Staphylococcus* spp. included lysostaphin susceptibility, anaerobic fermentation of glucose in oxidation-fermentation medium, facultative growth in semisolid thioglycolate agar, and acid production from glycerol (15, 32, 33). The micrococci were included in this study to demonstrate the robustness of the test data set to distinguish between the two genera.

The 265 cultures examined comprised two major clusters of gram-positive, catalase-positive cocci at a level of similarity (*S* value) of $\geq 40\%$ (Fig. 1). Group IE included 246 strains of *Staphylococcus*; the second group, group IIE, included 19 strains of *Micrococcus*. Group IE was subdivided into *Staphylococcus sciuri* (group IEa) and other *Staphylococcus* spp. (group IEb) at an *S* value of $\geq 45\%$.

Characterization and identification of phenon. Phenon E1, with an intragroup *S* value of 66%, comprised four reference cultures of *S. sciuri*. This phenon was unique because it included strains of oxidase-positive staphylococci that produced acid from amygdalin, cellobiose, salicin, and esculin.

The next nine phenon (phenon E2 through E10) were related at an *S* value of $\geq 66\%$. Phenon E2 through E6 demonstrated an intragroup *S* value of 67% and comprised isolates that were identified as *S. hominis* according to the description of the species reported elsewhere (20, 32, 41). Two of the strains, one in phenon E2e and the other in phenon E6b, were coagulase positive, but otherwise the strains could be classified as *S. hominis*. None of the reference strains clustered with these phenon, whereas phenon E7, which had an intragroup *S* value of $\geq 75\%$, included reference cultures of *S. hominis* and *Staphylococcus haemolyticus*. Phenon E8 had an intragroup *S* value of 71% and comprised cultures that were identified as *S. hominis*; most of these organisms were isolated from water samples collected at the pharmaceutical dump site located north of Puerto Rico. Group E9 had an intragroup *S* value of 95% and included the two reference strains of *Staphylococcus warneri* (Table 1 and Fig. 1).

Phenon E10 was identified as *S. epidermidis* based on the description of Schleifer and Kloos

TABLE 2. Characteristics of phena^a

Test	Frequency in:						
	Phenon E1 (n = 4) ^b	Phenon E2 through E6 (n = 84) ^c	Phenon E7 (n = 5)	Phenon E8 (n = 46) ^d	Phenon E9 (n = 2)	Phenon E10 (n = 51) ^e	Phenon E11 (n = 13) ^f
Gram-stain: packets or clumps	75 ^g	1	100	98	100	31	100
Enzymes							
Nitrate reduction	100	77	100	75	0	63	85
Arginine dihydrolase	0	28	40	7	100	81	77
Lysozyme production	0	0	20	0	0	4	0
Gelatinase	50	13	20	24	0	78	0
DNase	0	2	0	0	0	30	0
Phosphatase	50	13	0	64	0	90	0
Tween 80 lipase	0	72	20	62	100	43	23
Coagulase	0	3	0	0	0	0	0
Growth at:							
15°C	100	9	20	13	100	0	8
45°C	0	61	0	42	0	69	70
Beta-hemolysis	0	13	0	7	0	8	85
Chocolate agar clearing	50	16	0	20	0	24	0
Voges-Proskauer	0	18	20	82	100	92	46
Yellow pigment	25	43	50	0	50	47	70
Lysostaphin susceptible	100	77	40	95	100	66	61
Anaerobic growth	25	93	50	91	100	100	100
Novobiocin susceptible	50	99	100	100	100	100	100
Acid from:							
Glucose (closed tube)	100	100	100	100	100	100	100
Fructose	100	90	80	96	100	98	100
Cellobiose	100	0	0	0	0	0	0
Galactose	25	12	20	7	0	93	0
Glycerol	25	100	100	82	0	98	46
Lactose	25	0	60	23	0	80	0
Maltose	75	100	100	96	100	100	85
Mannitol	50	26	40	15	0	21	30
Melezitose	50	20	100	6	0	33	0
Sorbitol	50	3	0	24	0	2	30
Sucrose	100	100	100	100	100	92	100
Trehalose	100	71	60	80	100	0	46

^a These phena (except phenon E1) comprised gram-positive, catalase-positive cocci that were nonmotile and oxidase negative and grew in the presence of 6.5 and 4.0% NaCl and on bile-esculin medium (Difco), but did not hydrolyze esculin in the presence of 40% bile. They produced acid aerobically from glucose, but not from arabinose, amygdalin, cellobiose, inulin, melibiose, pyruvate, raffinose, salicin, sucrose, α -methyl-*D*-mannoside, or xylitol. They did not produce coagulase or red pigment. Phenon E1 was oxidase and esculinase positive and produced acid from amygdalin, cellobiose, salicin, and sucrose.

^b Number of strains examined.

^c Composite frequency for phena E2a through E6b.

^d Composite frequency for phena E8a through E8c.

^e Composite frequency for phena E10a through E10d.

^f Composite frequency for phena E11a through E11d.

^g Percentage of positive reactions.

(32) and the relatedness of the organisms to the reference strains of *S. epidermidis*, strains RM351 and ATCC 14990^T (T = type strain). The four subphena of the phenon, subphena E10a through E10d, shared an intergroup *S* value of $\geq 66\%$. Of the 55 strains in phenon E10, 49 were identified by the KEY scheme of Kloos and Schleifer (21) as *S. epidermidis*. The other six strains shared characteristics of *S. epidermidis*

and *S. warneri*. Subphena E10a and E10b comprised isolates from Chesapeake Bay and Cape Henry water samples; in addition, three clinical strains clustered with subphenon E10b. Subphenon E10c comprised Narragansett Bay, reference, and clinical isolates. The single strain of subphenon E10d was an isolate from Chesapeake Bay water.

Phenon E11 has an intragroup *S* value of 66%

TABLE 3. Characteristics of *S. simulans* and clinical and environmental isolates of *S. aureus* (phenon E12)^a

Test	Frequency in <i>S. aureus</i>		Frequency in <i>S. simulans</i> reference and clinical isolates (phenon E12c; n = 3) ^c
	Environmental isolates (phenon E12a; n = 12) ^b	Reference and clinical isolates (phenon E12b; n = 7)	
Coagulase	100 ^d	100	0
Enzymes:			
DNase	100	100	0
Phosphatase	100	100	100
Lysozyme production	100	100	67
Nitrate reduction	100	100	100
Gelatinase	25 ^e	100	0
Tween 80 lipase	100	43	100
Chocolate agar clearing	93	86	0
Acid from:			
Maltose	93	100	0
Mannitol	100	100	100
Pyruvate	0	86	0
Galactose	0	100	100 (w) ^f
Glycerol	86	100	0
Lactose	0	100	100
Esculinase	72 (w)	14 (w)	0
Growth at:			
15°C	0	100	100
45°C	100	0	0
Yellow pigment	100	86	0
Voges-Proskauer	25	71	0

^a These isolates were catalase-positive, gram-positive cocci that did not occur in chains and were negative for oxidase, amylase, hydrogen peroxide production, and production of acid from arabinose, amygdalin, cellobiose, inulin, melibiose, salicin, sorbitol, xylitol, and α -methyl-*D*-mannoside. They grew on bile-esculin agar, 4% NaCl, 6.5% NaCl, and 10% bile agar. These strains grew at 25°C, were susceptible to novobiocin and lysostaphin, grew anaerobically in semisolid thioglycolate broth, fermented dextrose both anaerobically and aerobically, and fermented sucrose.

^b Phenon E12a, but excluding two clinical strains.

^c This cluster included one clinical strain and two reference strains but no environmental isolates.

^d Percentage of positive reactions.

^e Nine strains isolated in February 1980 were negative. The three strains isolated in October 1979 were positive.

^f w, Weak positive reaction.

and included subphenon E11b, which was identified as *Staphylococcus capitis* based on the description of Kloos and Schleifer (20) and the similarity of the organisms to reference cultures of *S. capitis* strains ATCC 27840^T and ATCC 27841. *S. haemolyticus* DSM 20265 also clustered with this phenon. Subphenon E11b comprised a clinical isolate, three reference strains, and four environmental isolates from water samples collected at the Puerto Rico Trench dump site. The intragroup *S* value of the clinical isolate and the three reference strains was $\geq 79\%$, and the intergroup *S* value with the environmental isolates was 76%. Phenon E11a, E11c, and E11d, which clustered with *S. capitis*, possessed many of the characteristics of this species; i.e., the strains in these phenon were unable to ferment lactose and galactose and to produce the enzymes phosphatase, gelatinase, and deoxyribonuclease (DNase) (20).

Phenon E12, with an intragroup *S* value of $\geq 65\%$, comprised three subphenon; subphenon E12a and E12b were identified as *S. aureus* since the reference cultures of *S. aureus* strains JL68 and ATCC 12600^T were included in the phenon. Also, the strains fit the descriptions of the species given by Baird-Parker (3) and Kloos and Schleifer (20). Subphenon E12c was identified as *Staphylococcus simulans*, as described by Kloos and Schleifer (20). The animal pathogens *Staphylococcus intermedius* and *Staphylococcus hyicus* were not isolated from any of the water samples examined in this study. Criteria useful in the identification of these species have been reported elsewhere (7, 16, 27).

Phenon E13 had an intragroup *S* value of 64% and comprised the following strains of novobiocin-resistant staphylococci: *S. cohnii* DSM 20260^T and DSM 20261, *Staphylococcus saprophyticus* AW163 and CCM 883^T, and *Staphylo-*

TABLE 4. Bacteriophage types of *S. aureus* strains isolated from surface water samples collected at the Atlantic Ocean pharmaceutical dump site located 64 km north of Puerto Rico

Strain	Date of isolation	Bacteriophage type ^a
9B52	October 1979	80
15B803	October 1979	80
F1010	February 1980	80/77
12A2002	February 1980	80/77
F101	February 1980	80/77/53
F106	February 1980	80/77/53
F1013	February 1980	80/77/53
15B805	October 1979	96
F1011	February 1980	96/D11
F104	February 1980	53/D11
F107	February 1980	Nontypeable
F108	February 1980	Nontypeable

^a Data provided by C. Zierdt, National Institutes of Health, Bethesda, Md. (43).

coccus xylosus DSM 20266^T and DSM 20267. All members of this phenon were reference strains, except for a clinical strain of *S. saprophyticus* and an isolate of *S. cohnii* from a water sample collected in the Puerto Rico Trench.

Phenon E14 was classified *Micrococcus* sp. by using criteria contained in descriptions of the genus reported elsewhere (15, 32, 33).

DISCUSSION

An important part of any bacterial identification system is the ability to distinguish among species when recommended criteria are used (i.e., the robustness of the system). In the case of numerical taxonomy, successful classification and identification are affected by the test characters, the similarity coefficient, and the clustering method used for analyzing the test data (39). The methods used for testing cultures are very important. Some tests, in particular the micro or rapid test methods, do not always yield the same

result as conventional methods (4, 8, 11, 26, 27). A numerical analysis of 62 conventional nonmolecular tests easily identified most well-established species of *Staphylococcus*. The KEY scheme also served well for this purpose. However, species that reportedly differ by no more than 5 to 15% of their phenetic characteristics (11, 26) were difficult to identify. Since only 62 test characters were used in this study, additional phenotypic characterization, along with the results of deoxyribonucleic acid (DNA)-DNA hybridization studies, would be helpful before new species are designated. However, our analyses do provide support for the "species group" concept introduced by Kloos et al. in 1976 (22) for human strains of staphylococci, as well as differentiate micrococci from staphylococci (Fig. 1).

The *Micrococcus* spp. included in this study linked with *Staphylococcus* spp. at an *S* value of $\geq 40\%$. The characteristics associated with micrococci included resistance to lysostaphin, aerobic growth in semisolid thioglycolate agar, and inability to ferment dextrose and glycerol. In addition (15), FTO agar containing nitrofurantoin (6), modified oxidase and benzidine tests (10), and the production of acid from glycerol in the presence of erythromycin (33) have been reported by other investigators to be useful in distinguishing these genera. Even though some species of *Micrococcus* and *Staphylococcus* remain difficult to classify and identify, for the most part the results of the tests used in this study correlate well with data from DNA base composition analyses and other chemical systems used to separate the genera. A helpful review of the subject was published by Kloos in 1980 (18).

The four strains of *S. sciuri* included in this study linked with the other *Staphylococcus* spp. at an *S* value of only $\geq 45\%$, a strong indication of separate species status for this phenon. The results of DNA-DNA hybridization experiments support the notion that the two subspecies of *S.*

TABLE 5. Antibiotic susceptibility profiles for clinical strains of staphylococci and for staphylococcal isolates from a pharmaceutical dump site located north of Arecibo, Puerto Rico

Species	Group	No. of strains	% of strains susceptible to: ^a						
			Gantrisin	Nitrofurantoin	Methicillin	Clin-damycin	Erythromycin	Tetracycline	Ampicillin
<i>S. aureus</i>	Clinical	1,580	100 ^b	100 ^b	99	97	91	88	75
	Puerto Rico	12	100	75	100	100	100	67	44
<i>S. epidermidis</i> ^c	Clinical	579	73 ^d	100 ^d	88	70	66	60	78
	Puerto Rico	69	67	100	92	92	83	75	67

^a All clinical strains were tested at Walter Reed Army Medical Center, Washington, D.C., by using an agar dilution method (42).

^b Only 30 strains were tested for susceptibility to gantrisin and nitrofurantoin.

^c All coagulase-negative strains were placed in this species.

^d Only 14 strains were tested for susceptibility to gantrisin and nitrofurantoin.

FIG. 1. Dendrogram showing relationships between marine and estuarine isolates of gram-positive cocci.

sciuri deserve separate species status (18) (i.e., *S. sciuri* and "*Staphylococcus lentus*"). Although these two subspecies linked together at an *S* value of only $\geq 66\%$, they were similar in major biochemical differences (e.g., oxidase) and are combined in Fig. 1 and Table 2 as phenon E1. *S. sciuri* differed from "*S. lentus*" in being positive for gelatinase and phosphatase, clearing chocolate agar, being resistant to novobiocin, and being unable to ferment raffinose. Other characteristics of these species have been reported elsewhere (11, 19, 23). Although these organisms have been isolated from human and domestic and wild animal specimens (23), none was recovered in this study. The four strains examined grew well on agar containing 6.5% NaCl and strongly hydrolyzed esculin in the presence of 40% bile, characteristics not present in the other *Staphylococcus* spp. examined. Thus, colonial morphology and the results of the catalase test are characteristics that clinicians must use to separate *S. sciuri* and "*S. lentus*"

from group D enterococci, which demonstrate similar reactions in salt and esculin media (12). These two species are also negative for arginine dihydrolase, a characteristic which leads to misidentification as the group Q streptococcus "*Streptococcus avium*" which may be isolated from seawater, as well as from domestic animals (12; Gunn, Ph.D. thesis).

The species of *Staphylococcus* that was most frequently isolated from seawater was *S. hominis*, an organism comprising less than 12% of the coagulase-negative staphylococci isolated in clinical laboratories (4, 17). Interestingly, *S. hominis* was not isolated from water samples of moderate salinity collected at polluted Narragansett Bay stations. Both *S. hominis* and *S. epidermidis* were isolated from seawater of moderate to high salinity collected at nonpolluted stations in the Chesapeake Bay and the Atlantic Ocean east of Cape Henry, Va., with *S. hominis* clearly the dominant species (14). Only *S. hominis* was isolated from high-salinity water sam-

ples collected at the deepwater dump site north of Puerto Rico. Therefore, it is obvious that seawater can harbor a predominance of either *S. hominis* or *S. epidermidis*, depending upon the salinity and distance from land. It is possible that coagulase-negative cocci phenotypically similar to human strains of *S. hominis* exist naturally in the ocean.

Using the numerical taxonomy programs available for this study, we found that the frequency of occurrence of features for *S. hominis* correlated well with the results obtained by Kloos and Schleifer (20) (Table 2, phenon E2 through E8). However, the results of the numerical analyses did not permit separation of *S. haemolyticus* type strain DSM 20263^T (= ATCC 29970^T) from the type and reference strains of *S. hominis*; this is not surprising since a close relationship between these two species, based on phenotypic characteristics (11, 19, 20, 22, 29), DNA relatedness (34), fatty acid composition (9), cell wall composition (20, 32), and other tests (41), has been recognized. Salient characters that are used to distinguish these two species include colony diameter, hemolysis, pigment production, anaerobic growth, production of acid from mannose, production of acid from mannitol, arginine utilization, and urease activity (19). In addition, a recent report evaluating a commercial *Staphylococcus* microtube identification kit cited the following two tests that are useful in distinguishing these species: β -glucosidase activity and β -glucuronidase activity (26). Thus, if in addition to the characters chosen for our analysis, colony diameter, production of acid from mannose, and urease, β -glucosidase, and β -glucuronidase activities had been included, a more clear separation may have been achieved. To test this hypothesis, several of the environmental strains of each phenon were tested by using the commercial kit, with the result that the staphylococci were assigned to the same species as they were by numerical taxonomy. Interestingly, *S. haemolyticus* reference strain DSM 20265 (= ATCC 29968) clustered with *S. capitis* (phenon E11b). Although strain DSM 20265 has been shown by other workers to be closely related to *S. capitis* (11), chemical analyses and DNA-DNA homology have been used to establish unequivocally the identity of this strain as *S. haemolyticus* (9, 20, 25, 32, 44).

Only two strains of *S. warneri* were analyzed; both were reference cultures. Phenon E9 linked closely with *S. hominis*, thereby revealing a close relationship. Kloos et al. (18, 22, 34) placed this species in the *S. epidermidis* species group along with *S. hominis*, *S. capitis*, *S. haemolyticus*, and *S. epidermidis*. Although *S. warneri* linked with *S. hominis*, phenon E9 was homogeneous, joining at an *S* value of 95%. The

frequencies of occurrence of features of this species correlated well with the frequencies determined by Kloos and Schleifer (20). The validity of separate species status is supported by the results of a numerical analysis and numerous reports of other workers. *S. warneri* appears to have diverged significantly from other *Staphylococcus* spp. in the *S. epidermidis* species group. It was not isolated from seawater.

Environmental isolates of *S. epidermidis* differed little from clinical strains described in the literature (19, 32, 41). The environmental strains were more frequently positive for DNase, gelatinase, and galactose fermentation than clinical strains. However, they were collected at sampling stations that were close to one another and possibly represented clones of the same strain. Their ability to ferment mannitol is interesting, since *S. epidermidis* reportedly does not ferment this substrate (32, 41). Although the KEY scheme (21) does not identify these strains as *S. epidermidis*, except for fermentation of mannitol these strains fit the description of this species; however, further phenotypic and DNA-DNA homology studies are needed to clarify their taxonomic position. Strains identified as *S. epidermidis* linked closely with *S. hominis* and *S. warneri* but were clearly a separate phenon. These results are supported by the results of other studies (19, 30, 32, 34, 41, 44) in which both phenetic and molecular or genetic analyses were used.

Since *S. epidermidis* strains were cultured primarily from seawater containing high fecal coliform counts (14), we suspected that these organisms were derived from human sources, especially because the collection point was near a sewage effluent discharge pipe at the sampling site in the Chesapeake Bay. Additional evidence supporting this hypothesis is provided by the absence of this species (the most common species in clinical specimens) in samples of high-salinity, nonpolluted water.

The environmental strains of *S. capitis* that clustered in phenon E11b differed from clinical strains primarily in pigment production. *S. capitis* reportedly grows as tiny, chalk white colonies (20, 41); however, in this study, the environmental strains were yellow, and the clinical strains were non-pigmented. Similar yellow-pigmented "*S. capitis*-like" strains have been encountered in clinical specimens at the Walter Reed Army Medical Center (Gunn, unpublished data). These strains have been examined at a reference center (W. Kloos, personal communication) by using DNA-DNA hybridization, and they are not *S. capitis*. Many factors influence pigmentation, including length of the incubation period, temperature of incubation, growth medium, etc. Some investigators recommend that

colony pigmentation should not be used in taxonomic studies of bacteria because of its inconsistent nature (29) and the considerable amount of subjective evaluation that is required for determining colony pigmentation. Clearly, however, the strains that were identified as *S. capitis* in this study and clustered with reference cultures of the species were yellow pigmented, and pigmentation was used as a clue to their mistaken identity as *S. capitis*. Additional characterization of these strains is needed to further clarify the species status of the yellow-pigmented strains. All evidence considered, however, we conclude that *S. capitis* is a separate species based on the results of our studies and those of other workers (19, 20, 34, 41, 44).

Strains identified as *S. aureus* (phena E12a and E12b) and *S. simulans* (phenon E12c) linked at an *S* value of $\geq 65\%$. Phenon E12a comprised 2 clinical and 12 environmental strains of *S. aureus* isolated from seawater collected at several stations in the Atlantic Ocean north of Puerto Rico. Phage typing revealed that at least two types were present (Table 4). Phenon E12b consisted of two reference strains and five clinical strains of *S. aureus*. The salient characteristics of the phena are given in Table 3. Multiple antibiotic resistance was not less prevalent in the environmental strains of *S. aureus* than in clinical strains isolated at the Walter Reed Army Medical Center (Table 5).

S. simulans (phenon E12c) differed from *S. aureus* (phena E12a and E12b) in coagulase production, DNase production, clearing of chocolate agar, yellow pigmentation, and production of acid from maltose and glycerol. Although all three clinical strains of *S. simulans* were DNase negative, Varaldo and Satta (41) reported that *S. simulans* strains were weakly positive for production of this enzyme. Weakly positive DNase tests were encountered in many strains of all species examined in this study. The subjectivity in interpreting weak DNase reactions was alleviated by using DNase test agar in plates poured to a depth of more than 6 mm. Thus, only strongly DNase-positive species, such as *S. aureus*, yielded positive reactions. Kloos and Schleifer (20) reported that many strains gave weakly positive DNase tests in their studies. Although Kloos and Schleifer (20) and Varaldo and Satta (41) found a close relationship between *S. simulans* and *S. aureus*, as observed here, Schleifer et al. (34) demonstrated only very low DNA-DNA homology between these species. Nevertheless, the species are phenotypically similar. In fact, on occasion, a coagulase-negative strain of *S. aureus* might be confused with *S. simulans* if appropriate tests are not done.

The only isolate from seawater that was identified as a member of the *S. saprophyticus*

species group (19, 22) was a single strain of *S. cohnii*, which is isolated infrequently in clinical laboratories (17). The characteristics of this strain fit the description of the species given by Schleifer and Kloos (32).

Although the KEY identification scheme of Kloos and Schleifer (21) was originally designed to identify human *Staphylococcus* spp., it was useful in that it compared well with the numerical taxonomy data. The KEY scheme was introduced in 1975, and since then proposed and new species of *Staphylococcus* from human and animal sources have been described (e.g., *Staphylococcus auricularis*, *S. intermedius*, *S. hyicus*, *S. sciuri*, "*S. lentus*," and *Staphylococcus carnosus*) (16, 19, 23, 27, 31). Anyone using the KEY scheme must know that the new species exist and must modify their use of this scheme accordingly to obtain accurate results.

In conclusion, staphylococci were isolated from seawater samples collected in near-shore, offshore, and deepwater sites. These isolates possess a wide range of degradative activities and may contribute to the detoxification of wastes and recycling of nutrients in the sea. The species most likely to be present in open ocean water appears to be *S. hominis*, but both *S. hominis* and *S. epidermidis* can be present in coastal and estuarine waters. From results of this study, both the ecology and the systematics of staphylococci have been broadened to include estuarine and marine isolates.

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