MICROBIOLOGICAL REVIEWS, June 1985, p. 126–139
0146-0749/85/020126-14$02.00/0

Vol. 49, No. 2

Coagulase-Negative Staphylococci and the Epidemiological Typing of *Staphylococcus epidermidis*

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INTRODUCTION

In the past two decades serious infections have been increasingly recognized in patients whose resistance to microbial disease has been compromised. Because of their prevalence on the skin and the frequent implantation of foreign devices into patients during hospitalization, coagulase-negative staphylococci (CNS) are ideally situated to cause serious infections in such individuals. Although numerous species of CNS have been recognized recently (96, 163), the majority are rarely pathogenic (93, 94). *Staphylococcus epidermidis* is the coagulase-negative species most frequently isolated from infections and is a significant nosocomial pathogen of prosthetic valves (117), cerebrospinal fluid shunts (169), joint prostheses (201), vascular prostheses (106), postoperative wounds (29), and the urinary tract (90). It is the most common organism recovered from the blood of patients undergoing bone marrow transplantation (204) or total parenteral nutrition with central venous catheters (158). *S. epidermidis* is also the most frequent cause of peritonitis in patients during continuous ambulatory peritoneal dialysis (193), and recent reports of its isolation from newborn nurseries (21) and the blood of children (58) and adults (198) with leukemia indicate that it is emerging as a major pathogen in these groups also.

The two classic species of CNS recognized previously have been redefined by Kloos and Schleifer (96, 163) so that 15 species are currently recognized (94). The commercial availability of two rapid identification kits has simplified the identification of this group of organisms to the species level (64). However, the usefulness of species identification in the clinical laboratory has not met with universal agreement (50, 65, 172) nor has there been complete acceptance of the reliability of some of the characters used by Kloos and Schleifer in their classification scheme (66, 171). On the other hand, with the ubiquitous presence of *S. epidermidis* in the environment, its habitation on the skin and in the nares of virtually everyone (93), and the documentation of this organism as a bona fide nosocomial pathogen, it is imperative that effective methods be used for the identification of specific strains in epidemiological studies. The length of carriage of *S. epidermidis* by patients and hospital personnel, whether patients are infected by strains from their own normal flora or by hospital-acquired strains, and the modes of transmission of *S. epidermidis* within the hospital are unknown. The identification of strains by antibiotic susceptibility patterns, biochemical characterization, bacteriophage typing, or serological typing has not been entirely satisfactory, and newer methods of strain identification by the molecular analysis of plasmids are now being used. The greater antimicrobial resistance of *S. epidermidis* compared with that of *Staphylococcus aureus* (14, 115), the conjugal transfer of its resistance with the possibility that *S. epidermidis* may serve as a reservoir of resistance plasmids for *S. aureus* (10, 39, 86), and the production by *S. epidermidis* of slime, enabling it to adhere to biomaterials (38, 82, 145, 174), have sparked new interest in this organism.

This review summarizes the history and classification of CNS and discusses the methods that are being used in the epidemiological typing of *S. epidermidis*. The characters used in the recent taxonomic scheme and the habitation of the CNS on humans and other mammals have been reviewed recently by Kloos (93). Infections produced by *S. epidermidis* and the current approaches to their management have been reviewed recently by Lowy and Hammer (109).

HISTORY

It has been over 100 years since Sir Alexander Ogston in 1883 introduced the name "staphylococcus" to describe the cluster-forming cocci which he observed as the cause of certain pyogenic abscesses in humans (136). Thus, staphylococcal taxonomy began what has since been fraught with controversy and confusion. The reasons for this are twofold: (i) the medical microbiologist was interested in identifying the pathogenic members of this group and any of their properties that enable him to trace the source of an infection; and (ii) the taxonomist was interested in how this organism fits into the general order of living things, not in disease or

the disease properties of the organism. As a result, the classification and nomenclature of staphylococci have had one of the most confusing histories of taxonomy in microbiology.

Although Ogston was the first to recognize the importance of staphylococci in disease and to name them, Rosenbach got the credit for the generic name *Staphylococcus* and the species name *aureus* because in 1884 he was the first to isolate the organism, grow it in pure culture, and study its laboratory characteristics (156). He demonstrated that two differently colored colonies were produced by this organism and he named the orange and white colony producers *Staphylococcus pyogenes aureus* and *Staphylococcus pyogenes albus*, respectively. Although pigmentation was used as the key character in this classification, the use of trinomials by Rosenbach suggests that he was uncertain about colony color being a key character. One year later, an organism producing a lemon-colored colony was named *Staphylococcus pyogenes citreus* (140). In 1891 the name *Staphylococcus epidermidis albus* was proposed for an attenuated form of *S. pyogenes albus* (199). Unfortunately, the name *Staphylococcus* was not readily accepted by others and most early classifications included these organisms in the genus *Micrococcus*, which had been used first in 1875 by Cohn (40). With 1883 as the starting point and 1923, the year of the first edition of *Bergey's Manual of Determinative Bacteriology* (27), as the end point, some 25 published classification systems did not recognize *Staphylococcus*; only 9 did (79). Most classifications in this period did not favor the separation of *Staphylococcus* from *Micrococcus*, although the Society of American Bacteriologists, which was influential in the publication of *Bergey's Manual*, did. Thus, *S. pyogenes aureus* became *Micrococcus aureus* and *S. pyogenes albus* became *Micrococcus pyogenes* (119). In 1900 *Staphylococcus* was divided into two subgenera: orange strains became *Aureococcus aureus* and white strains became *Albococcus epidermidis* (201). The two major factors affecting these early classifications were an undue emphasis on pigmentation and a preoccupation with distinguishing between pathogenic and nonpathogenic organisms, the latter concern being still with us today. Although the majority of the early classifications were based on cellular and colonial morphology, Andrewes and Gordon in 1905–1906 (8) proposed a classification of human staphylococci based on pigmentation and pathogenicity for guinea pigs; four species were recognized: *S. pyogenes* (orange, pale yellow, or white, highly pathogenic); *S. epidermidis albus* (white, feebly pathogenic); and two other species which were both white and nonpathogenic. The need to distinguish the pathogenic from the nonpathogenic species was evidenced by reports describing the use of a variety of serological or biochemical tests to achieve this (49, 80, 202, 203). The terms "pyococcus" and "saprococcus" were used to this end (61). Between 1923, when the first edition of *Bergey's Manual* was published, and 1948, when the sixth edition was published, the number of species listed under *Staphylococcus* varied from none to nine; the sixth edition deleted the genus *Staphylococcus* and relegated all staphylococci to the genus *Micrococcus*. The number of species listed under *Micrococcus* for this same period varied from 22 to 46.

In 1903 Loeb (107) demonstrated that *S. pyogenes aureus* was capable of coagulating goose plasma, and Much (128) repeated this test with rabbit and horse blood. Interestingly, this property was largely ignored as an important test until the mid-thirties. A period of time elapsed before the test was accepted universally and it did not become a key character until 1957, when the seventh edition of *Bergey's Manual* was published. In the meantime, the name *Staphylococcus saprophyticus* was proposed for coagulase-negative strains of staphylococci (53) and *Micrococcus* was regarded by some as an invalid generic name for staphylococci (173). In the mid-fifties there was renewed interest in the classification of staphylococci and several publications presented views either for or against separating *Staphylococcus* from *Micrococcus* (32, 51, 185, 192). One (51) of these studies suggested that these genera could be distinguished by their ability to grow anaerobically and to produce acid from glucose. This test formed the basis for the reintroduction of the genus *Staphylococcus* in the seventh edition of *Bergey's Manual* in 1957; in addition, two species, *S. aureus* and *S. epidermidis*, were recognized on the basis of anaerobic utilization of mannitol and the production of coagulase by the former. Thus, differentiation between micrococci and staphylococci appeared to be resolved. However, reliance on so few characters for generic and species differentiation, though appearing to be convenient and practical, was not taxonomically accurate. It failed to recognize the heterogeneity among strains of *S. epidermidis*. Today, this fact is quite evident and the storm created over this simplified classification for *S. epidermidis* does not show signs of abating.

CLASSIFICATION

With the publication of the seventh edition of *Bergey's Manual* in 1957 recognizing just two species of staphylococci, the separation of *S. aureus* from *S. epidermidis* was clearly defined. However, it placed all of the CNS into a single species which consisted of organisms with considerable heterogeneity. In addition, with the use of the test for the anaerobic production of acid from glucose, the definitive character separating *Staphylococcus* from *Micrococcus* was not standardized; some strains could be classified into either genus, depending upon the method used in this determination (60, 126). The use of numerical taxonomy in 1959 indicated that the coagulase-positive staphylococci were a homogeneous group but the CNS were not (78). In one study of catalase-positive cocci, it was suggested that all CNS and most mannitol-nonfermenting staphylococci be designated *S. saprophyticus* because no reliable criterion for their subdivision could be found (127). A detailed study of *S. epidermidis* in 1964 established the following characteristics for this organism: (i) ability to grow anaerobically in a standardized, complex medium containing glucose; (ii) inability to produce coagulase; (iii) inability to ferment mannitol; (iv) usually an anaerobic requirement for uracil; (v) reduction of nitrate to nitrite; (vi) requirement of biotin for growth. However, some isolates lacked one or more of these properties and their taxonomic status was uncertain (91). The first to recognize specific types within the heterogeneous group of CNS was Baird-Parker (17). He divided all staphylococci into six subgroups, of which subgroup I, containing *S. aureus*, and subgroups II through VI, containing only *S. epidermidis*, were differentiated on the basis of phosphatase and acetoin production and ability to form acid aerobically from lactose, maltose, and mannitol. In 1965 Baird-Parker (18) referred to subgroups II through VI as biotypes 1 through 5 and stated that most of the biotypes deserved species rank, but he did not wish to confuse the classification of staphylococci further by giving species names to these biotypes. In 1965 the International Commit-

tee on Systematic Bacteriology standardized the test for the anaerobic utilization of glucose, which separated *Staphylococcus* from *Micrococcus* (182).

The use of deoxyribonucleic acid (DNA) base composition, usually expressed as moles percent guanine plus cytosine (G+C) in the taxonomy of staphylococci, showed that strains of *S. aureus* or *S. epidermidis* had a low (31 to 40 mol%) G+C; strains of *Micrococcus* had a high (64 to 74 mol%) G+C (23). Subsequent work by others supported these results; based on DNA base composition, *Staphylococcus* and *Micrococcus* formed two distinct groups and, although some discrepancies did exist, this parameter showed a good correlation with anaerobic growth and the utilization of glucose by *Staphylococcus* but not *Micrococcus* (15, 30, 126, 157, 175). Because the determination of the G+C content is a highly specialized test that cannot be performed by most clinical laboratories, the anaerobic utilization of glucose remained the routine test for the separation of these genera. However, the observations (15, 101) that some staphylococci grow poorly or not at all under anaerobic conditions and some micrococci can produce small amounts of acid from glucose anaerobically led to the introduction of a new test to separate *Staphylococcus* from *Micrococcus* (52) based on anaerobic growth in a semisolid thioglycolate medium.

The isolation of lysostaphin in 1964 provided another relatively simple and quick method for separating *Staphylococcus* from *Micrococcus*. *S. aureus* is very susceptible to lysis, *S. epidermidis* is less so, and *Micrococcus* is resistant (161, 170, 205). Lysostaphin susceptibility and the DNA base composition are closely correlated (92, 104). Lysostaphin susceptibility is also directly correlated to the chemical composition of the peptidoglycan of the cell wall. This enzyme is an endopeptidase which splits glycyl-glycine linkages in the penta- or hexapeptide crossbridge of the peptidoglycan of *Staphylococcus* (33, 63). Some staphylococci, especially some strains of *S. epidermidis*, are more resistant to lysis by lysostaphin because they contain variable amounts of serine in the interpeptide bridges (162, 187). However, the serine content of this interpeptide bridge can be lowered and sensitivity to lysostaphin increased by growing these strains in a medium containing sufficient amounts of glycine or by supplementing the medium with 0.1 to 0.3% glycine (162, 165). More recent data, however, suggest that, in addition to the growth conditions, other unknown factors also affect the lysis of CNS by lysostaphin (76).

Covalently linked to the peptidoglycan of staphylococci are the teichoic acids. These water-soluble polymers, comprising 30 to 50% of the dry weight of the cell wall, contain either glycerol or ribitol joined through phosphodiester bonds. Generally, *S. aureus* contains a ribitol teichoic acid with *N*-acetylglucosamine residues and *S. epidermidis* contains a glycerol teichoic acid with glucosyl residues (42). The *N*-acetylglucosamine ribitol teichoic acid of *S. aureus* is identical to polysaccharide A, a species-specific antigen, and the glucosylglycerol teichoic acid of *S. epidermidis* is identical to polysaccharide B, a common antigen (43, 44). The presence of ribitol or glycerol teichoic acids in *Staphylococcus*, either the absence of teichoic acids in *Micrococcus* or the presence of teichoic acids chemically and serologically distinct from those of *Staphylococcus* (16, 135), and the correlation of lysostaphin susceptibility with the G+C content in the DNA (92) indicate that, despite the difficulties in distinguishing *Staphylococcus* from *Micrococcus* by routine tests in the clinical laboratory, these genera are unrelated genetically. Other properties that point to the unrelatedness of these genera are the presence of aliphatic hydrocarbons in the neutral lipids of *Micrococcus* but not *Staphylococcus* (188), differences in their cell membrane menaquinone (88) and cytochrome (54) patterns, and differences in the degradation of DL-alanyl-β-naphthylamide and the decarboxylation of arginine (143). More recently, three selective media have been developed to distinguish *Staphylococcus* from *Micrococcus*: one contains erythromycin (0.4 μg/ml) and glycerol (only staphylococci can grow and ferment the glycerol) (164); another contains a nitrofuran (Furoxone; 50 μg/ml) (only micrococci can grow) (41); and the third contains sodium azide, potassium thiocyanate, lithium chloride, and glycine (only staphylococci can grow) (166).

The eighth and current edition of *Bergey's Manual* was published in 1974 and recognized a third species, *Staphylococcus saprophyticus*. This coagulase-negative organism had originally been classified as a *Micrococcus*. However, its ability to grow anaerobically in thioglycolate semisolid medium (52), its DNA base composition (126), its susceptibility to lysostaphin (163), its cell wall peptidoglycan and teichoic acid (44, 167), and its cell membrane menaquinones (89) all indicate that it is more closely related to the staphylococci. In addition to being coagulase negative, *S. epidermidis* and *S. saprophyticus* differ from *S. aureus* in being unable to ferment mannitol anaerobically (85, 163) but able to produce alpha-toxin (163), and neither species contains protein A in their cell walls (1). Also, in contrast to the heat-resistant nucleases of *S. aureus*, the nucleases of *S. epidermidis* and *S. saprophyticus* are heat sensitive (104). On the other hand, *S. saprophyticus* differs from *S. aureus* and *S. epidermidis* in being resistant (5.0 μg/ml) to novobiocin (163).

The eighth edition of *Bergey's Manual* identified four biotypes of *S. epidermidis* (27) by condensing Baird-Parker's biotypes 1 and 4 (18) into biotype 1. Although strains in biotype 4 were phosphatase negative by the plate method, with the more sensitive tube test (142), they are usually phosphatase positive. The basis for the identification of the four biotypes of *S. epidermidis* was the production of acetoin, phosphatase, and acid aerobically from lactose, maltose, and mannitol. Also identified for *S. saprophyticus* were four biotypes based on the production of acetoin and acid aerobically from arabinose, lactose, maltose, and mannitol (19). Again, Baird-Parker (19) indicated that the two coagulase-negative species identified should be considered tentative and should be divided into further species. This classification scheme provided the basis for many studies describing the sources of particular biotypes in nature (121, 132, 177) and laid the groundwork for the taxonomy which followed.

In 1975 Schleifer and Kloos published a series of papers redefining *S. epidermidis* and *S. saprophyticus* (96, 97, 163). Of the four biotypes of *S. epidermidis* and the four of *S. saprophyticus* identified earlier in the eighth edition of *Bergey's Manual* (27) and by Baird-Parker (19), only biotype 1 (formerly called subgroup II) was named *S. epidermidis* and only biotype 3 was named *S. saprophyticus*. Many new species of CNS (*Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus xylosus*, and *Staphylococcus simulans*) were recognized on the basis of extensive morphological, physiological, and biochemical characters and cell wall peptidoglycan and teichoic acid composition. These investigators subsequently confirmed the relationships between staphylococci in their classification scheme by DNA-DNA hybridization and comparative

immunological studies (99, 168). The following species groups were recognized. The *S. saprophyticus* group was composed of *S. saprophyticus*, *S. xylosus*, and *S. cohnii*. The *S. epidermidis* group consisted of *S. epidermidis*, *S. capitis*, and *S. warneri*. The *S. hominis* group, which exhibited a significant relationship to *S. epidermidis*, included *S. hominis* and *S. haemolyticus*. The *S. simulans* group was represented by this species only. In a study of the distribution of staphylococci on several major areas of human skin, *S. aureus* and *S. epidermidis* were the most predominant and persistent staphylococci isolated from the nares, whereas *S. epidermidis* and *S. hominis* were the most predominant and persistent staphylococci isolated from the axillae, head, legs, and arms. *S. capitis* was often isolated from the head and arms and *S. haemolyticus* was often isolated from the head, legs, and arms. *S. simulans*, *S. xylosus*, *S. cohnii*, *S. saprophyticus*, and *S. warneri* were only occasionally isolated from skin (95).

According to the classification scheme of Kloos, Schleifer, and co-workers (96, 98, 164), all strains of *S. epidermidis* sensu stricto produce acetoin and more than 80% of the strains reduce nitrate and produce phosphatase. All strains produce acid aerobically from glucose, fructose, maltose, sucrose, and glycerol, and 70 to 90% of the strains produce acid aerobically from galactose, mannose, and lactose. No acid is produced from mannitol, trehalose, rhamnose, xylose, or arabinose (Table 1). The primary characters of *S. epidermidis* can be summarized as follows: hemolysis on bovine, human, sheep, and rabbit blood agars, weak to none; novobiocin susceptible; maltose positive; mannitol negative; and trehalose negative. The secondary characters of *S. epidermidis* are the following: anaerobic growth in thioglycolate semisolid agar, phosphatase activity, moderate to strong or weak; nitrate reduction, moderate to strong or weak; mannose, moderate to strong acid or weak acid; acetoin production, positive; and 5-day colony size (diameter), 2 to 6 mm. The cell wall consists of peptidoglycan of type L-lysine-glycine$_{4-5}$, L-serine$_{0.7-1.5}$, and a teichoic acid composed of glycerol and glucose. The G+C content of its DNA is 33.5 ± 0.2 mol%. *S. epidermidis* is the most prevalent and persistent *Staphylococcus* on human skin and it makes up from 90 to 100% of the staphylococci isolated from the nares when *S. aureus* is absent. In the presence of a dominant strain of *S. aureus*, the number of *S. epidermidis* in the nares is greatly reduced. *S. epidermidis* is also present in large numbers in the axillae and perineum, and its natural host appears to be limited largely to humans. *S. epidermidis* is the coagulase-negative species most frequently isolated from human disease (50, 65, 114, 172).

TABLE 1. Key characters of *S. epidermidis*[a]

Character (% of strains)	Reaction or determination
Acetoin production (100)	Positive
Nitrate reduction (>80)	Positive
Phosphatase production (>80)	Positive
Acid (aerobically) from	
Glucose (100)	Positive
Fructose (100)	Positive
Maltose (100)	Positive
Sucrose (100)	Positive
Glycerol (100)	Positive
Galactose (70–90)	Positive
Mannose (70–90)	Positive
Lactose (70–90)	Positive
Mannitol (100)	Negative
Trehalose (100)	Negative
Rhamnose (100)	Negative
Xylose (100)	Negative
Arabinose (100)	Negative
Hemolysis on blood (bovine, human, sheep, or rabbit) agars	Weak to none
Novobiocin	Susceptible
Anaerobic growth in thioglycolate semisolid agar	Positive
Five-day colony size (diam)	2–6 mm
Type of peptidoglycan in cell wall	L-Lysine-glycine$_{4-5}$, L-serine$_{0.7-1.5}$
Composition of teichoic acid in cell wall	Glycerol and glucose
G+C content of DNA	33.5 ± 0.2 mol%

[a] Data from references 94 and 163.

TABLE 2. Species of CNS and their pathogenic significance[a]

Species	Pathogenic significance
S. epidermidis	Common pathogen
S. saprophyticus	Common pathogen
S. haemolyticus	Questionable or uncommon pathogen
S. hominis	Questionable or uncommon pathogen
S. warneri	Questionable or uncommon pathogen
S. saccharolyticus	Questionable or uncommon pathogen
S. cohnii	Questionable or uncommon pathogen
S. simulans	Questionable or uncommon pathogen
S. capitis	Undetermined or rare pathogen
S. auricularis	Undetermined or rare pathogen
S. xylosus	Undetermined or rare pathogen
S. carnosus	Undetermined or rare pathogen
S. sciuri	Undetermined or rare pathogen
S. lentus	Undetermined or rare pathogen
S. caseolyticus	Undetermined or rare pathogen

[a] Data from reference 94.

The simplified scheme proposed by Kloos and Schleifer in 1975 (97) to identify their nine species of CNS required the use of 13 key characters. Further simplification seemed necessary if it were to be used by clinical microbiology laboratories for routine use. In 1978 Brun et al. (34) modified a micromethod originally used by Peny and Buissiere (143) which used 63 different substrates to identify staphylococci. After preliminary tests on 77 substrates, Brun et al. (34) retained 19, 15 of which were used for the determination of species and 4 of which were used to identify biotypes. The substrates were placed in a rigid strip of inert plastic. There was excellent reproducibility and agreement with the conventional method of Kloos and Schleifer (97) for the identification of the nine species identified by Kloos and Schleifer. The test system developed by Peny and Buissiere (143) and modified by Brun et al. (34) was marketed under the name API 20E by API S.A. System, La Balme les Grottes, France, and consisted of 20 miniaturized biochemical tests in a plastic strip which are read after overnight incubation. The use of this kit (55, 62) does provide a rapid and simple means to identify the species proposed by Kloos and Schleifer, but this particular kit has not been extensively used in this country because in the United States, Analytab Products, Inc., Plainview, N.Y., introduced in 1982 the API STAPH-IDENT strip system that combined 10 minaturized biochemical tests that could be read after incubation for 5 h. Comparing the STAPH-IDENT system with the conventional methods of Kloos and Schleifer for the identification of CNS, there was a high degree (>90%) of congruence between the two methods (99). Even with the availability of these rapid identification kits for the identification of the CNS to the species level, there is no justification for their

routine use in the clinical laboratory because the number of potentially pathogenic species is small (Table 2). Their cost also precludes their routine use. Further use of the API 20E and the API STAPH-IDENT for the identification of CNS is reviewed in the section on epidemiological typing.

Baird-Parker (20) discussed the new changes in taxonomy since the publication of his classification scheme (17–19) and noted the probable relationship between the two classification schemes and observed that some of Kloos and Schleifer's species were so closely related that perhaps they should be regarded as subspecies. With DNA preparations from CNS, the polynucleotide divergence index, or the ratio of binding of DNA at both exacting and nonexacting temperatures, has been used to show that *S. capitis*, *S. hominis*, and *S. warneri* may be overclassified and may represent only one center of variation (67). Comparison of the biotype classification of Baird-Parker with the species identified by Kloos and Schleifer is difficult because substantially different characters were used to define the species. For example, *S. epidermidis* biotype 2 may consist of eight different Kloos and Schleifer species; similarily, *S. saprophyticus* biotype 1 may consist of seven different Kloos and Schleifer species. Five biotypes, including those of *S. saprophyticus*, give good correlations with the species of Kloos and Schleifer but the remaining four biotypes correlate poorly (112). With DNA-DNA homology data, genetically related CNS can give markedly different results with the biochemical tests commonly used in their classification, suggesting the unreliability of some of these tests (67). It is also possible that the differences observed between genetically related strains may be due to the presence of extrachromosomal genes that encode some of the characters studied (76). Some evidence suggests that certain of the genes controlling carbohydrate fermentations are linked to the genes controlling penicillinase production, which has been shown to be located on a plasmid (130, 160). However, except for the genetics of antimicrobial resistance, the genetics of other determinants in *S. epidermidis* has been largely unexplored.

A refinement of an earlier technique (194) of the bacteriolytic activity patterns on a bacterial substrate has been used as a tool for the taxonomy of *Micrococcaceae* (159). When heat-killed cells of *Micrococcus luteus* were used as a substrate in five different media, five patterns of lysis or lyogroups could be recognized. These lyogroups showed a correlation to the different species identified by Kloos and Schleifer. This system also separated staphylococci from micrococci because only the former produced a detectable bacteriolytic activity. The penicillin-binding proteins of staphylococci from different lyogroups (or species) contained penicillin-binding proteins which differed either in number or molecular weight from those of strains in other lyogroups (or species). This suggests that bacteria contain specific cell wall autolysin complexes which function in cell wall growth and cell division, and the lytic enzymes and penicillin-binding proteins should be considered important tests in bacterial taxonomy (56). The serological properties of the type strains of the species proposed by Schleifer and Kloos have been studied. Sixty-one of 275 strains identified biochemically did not react or agglutinated spontaneously; 12 of these identified as *S. warneri*, *S. haemolyticus*, or *S. hominis* were serologically indistinguishable, and of the remaining 202 strains, a correlation between species and serotype was observed with 77.2% of them. Among *S. epidermidis* species, several distinct serotypes were found (146). In a recent collaborative study of the biochemical and antibiotic resistance characters of 637 isolates from serious infections in patients from the United States, Canada, British Isles, England, West Germany, Denmark, Sweden, and Poland, *S. epidermidis* biotype 1 or *S. epidermidis* sensu stricto accounted for 473 of the 637 isolates. No other biotype was of importance and the identification of *S. xylosus* and *S. simulans* from blood or urine was noted infrequently. Resistance to antibiotics was common and *S. epidermidis* was more frequently resistant than other species (114). To determine the accuracy and reliability of the three methods, fermentation of dextrose, facultative growth in semisolid thioglycolate agar, and susceptibility to lysostaphin, used to distinguish staphylococci from micrococci, it was found that the most accurate, combining ease of use and speed in obtaining results, was the lysostaphin susceptibility test (71). In spite of these results, it is doubtful whether lysostaphin susceptibility will ever be used routinely in the clinical laboratory due to the many variables in the testing method, including the influence of the medium on susceptibility to lysis (164), and the cost of lysostaphin.

In 1982 (94) six new species of CNS were identified, *Staphylococcus auricularis*, *Staphylococcus lentus*, *Staphylococcus sciuri*, *Staphylococcus caseolyticus*, *Staphylococcus carnosus*, and *Staphylococcus saccharolyticus*, bringing to 15 the number of CNS proposed by Kloos and Schleifer (Table 2). However, the use of numerical taxonomy to study CNS from human and animal sources for 115-unit characters has still led some (66) to question the reliability of some of the tests recommended by Baird-Parker (18), Heczko et al. (73), Kloos and Schleifer (97), and Pelzer et al. (141) for the identification of CNS. The results of this study indicated that the subgeneric structure of *Staphylococcus* was more complex than was previously thought, and this was further substantiated by a study of the types of peptidoglycan and teichoic acid in the cell walls of representative staphylococcal strains from 18 clusters of the previous numerical taxonomic study (66). One strain with a new cell wall type, closely related strains in one cluster which could not be classified by the chemical analysis of their cell walls, and strains of three clearly defined species which belonged to different numerical taxonomic clusters were observed. Based on these results, it was concluded that the taxonomy of the CNS could not be regarded as a completely solved problem (171).

It is obvious that the classification scheme of Kloos and Schleifer has not met acceptance by everyone, and it is not reasonable to expect that any attempt to classify such a heterogeneous group of microorganisms ever will. Disagreements will always exist on just how many differences justify a new species. What is important is that Kloos, Schleifer, and co-workers have taken a highly heterogeneous group of microorganisms, classified them by the more traditional morphological and physiological characters, and then confirmed their classification scheme by using the modern taxonomic methods of cell wall composition, DNA-DNA hybridization, electrophoretic comparison of isofunctional enzymes, and the immunological relationships of proteins (93, 96, 163, 168). Classification schemes can no longer ignore the use of such powerful molecular and immunological methods. Of course, conflicts will always exist between the systematist and the medical microbiologist as long as one's primary interest in these organisms differs. Until the factors responsible for the disease potential of these organisms are identified and can be manipulated genetically, the preoccupation with distinguishing between pathogenic and nonpathogenic organisms, which occupied so much attention 100 years ago, will continue today.

EPIDEMIOLOGICAL TYPING OF *S. EPIDERMIDIS*

Microorganisms are typed to determine their genetic relatedness. A number of different methods have been used in epidemiological studies of infections by *S. epidermidis*. These include antimicrobial susceptibility patterns (antibiograms), biochemical characterization (biotyping), bacteriophage susceptibility patterns (phage typing), serological typing (serotyping), and molecular analysis of plasmids (plasmid profiles, restriction enzyme analysis, and DNA-DNA hybridization). To distinguish an individual strain, a typing system must be able to identify a sufficiently large number of phenotypes so that the probability of isolating any two phenotypes by chance alone would be less than $P = 0.05$. If each phenotype were represented equally, a typing system would have to distinguish at least 20 different phenotypes to accomplish this. Experience with most typing systems does not even approach this requirement (37). In spite of this shortcoming, a combination of different typing systems has been helpful in epidemiological studies on *S. epidermidis*. Unfortunately, some of the methods used are either highly specialized or expensive and exceed the capability of most clinical laboratories. However, because of the ubiquitous nature of *S. epidermidis* and its habitation on humans, the more methods available to type or distinguish strains of this organism, the more conclusive the evidence differentiating an infecting strain from a contaminant. Our knowledge concerning the origins, reservoirs, and modes of transmission of this important nosocomial pathogen is woefully lacking, and typing systems need to be used to obtain answers to these questions.

Antibiograms and Biotyping

Antibiograms are done routinely by the clinical microbiology laboratory according to standardized procedures. Because of their ready availability and the patterns obtained, clinicians frequently use the antibiogram to identify clinically significant strains of *S. epidermidis*. Antibiograms of *S. epidermidis* from different countries have been found to vary with the geographical location from which the isolates have been obtained (153), and it has been suggested recently that the antibiogram be used as a major epidemiological tool to identify *S. epidermidis* (2). The presence of a strain with a unique antibiogram could provide a marker for detecting similar strains. Because antibiograms are influenced by the antibiotics used within a hospital, this method of identification of a particular strain is more likely to be useful only within a localized environment. However, recent studies emphasize the problems inherent in the total reliance of the antibiogram as an epidemiological marker. In one study, despite the use of standardized Kirby-Bauer procedures by the laboratory personnel, there was a 7.7% disparity between the original antimicrobial susceptibility report and the redetermination (37). Another important consideration is the genetic stability of isolates. There is good evidence of the loss of resistance plasmids by *S. epidermidis* from patients during a period of hospitalization (137). Although methicillin resistance has been used as an epidemiological marker to study the frequency of resistant isolates of *S. epidermidis* from cardiac surgery patients (25), the widespread occurrence of methicillin-resistant strains of *S. epidermidis* today (12) makes the use of this resistance marker inadequate in epidemiological studies. It was observed as early as 1963 that CNS isolated from a hospital environment were more resistant to antibiotics than isolates from healthy carriers (103). This situation has not changed; in fact, it has been reported recently that *S. epidermidis* is the most resistant of the gram-positive cocci, showing increased resistance to almost all antimicrobial agents (14). It was reported in 1981 that in the last 5 years, resistance of *S. epidermidis* to erythromycin had increased from 32 to 50%; to chloramphenicol, from 13 to 21%; to gentamicin, from 2 to 24%; to kanamycin, from 29 to 51%; and to methicillin, from 22 to 33% (6). In spite of this increased resistance of *S. epidermidis* to antimicrobial agents and the ready availability of the antibiogram, it does not appear that the antibiogram can be relied upon as the sole determinant in strain identification.

In many instances antibiograms have been used in conjunction with biotyping to study infections produced by *S. epidermidis*. Many different schemes for biotyping CNS have been developed based upon numerous biochemical or physiological tests. There have been no attempts to standardize the methods or tests used or to compare the different schemes used. As a result, so many different schemes were used that a critical evaluation of them is difficult. One recent attempt has been made to do this (37). The comprehensive taxonomic studies of Baird-Parker (17–19) discussed above had a profound effect on the biotyping schemes used by many clinical studies even though Baird-Parker developed his scheme with CNS from a variety of nonclinical sources such as food, soil, water, air, and domestic animals. With isolates from the skin (132, 177), from the urine (87, 118, 120), or from various places in hospitals (102, 111), biotype 1 strains were the ones most commonly found, generally followed by biotypes 4, 3, and 2 in decreasing order of incidence. Although these studies established the predominance of the different biotypes in a hospital environment, they were ineffective in epidemiological studies because of the limited number of recognized phenotypes, the predominance of biotype 1 strains in the flora of the skin, and the widespread distribution of biotype 1 from clinical sources throughout the hospital (111).

A biotyping scheme specific for clinical isolates of CNS was developed by Bentley et al. (26). It utilized six (oxidation of maltose and mannitol and production of lipase, phosphatase, glucuronidase, and gelatinase) tests and was used to study the occurrence of different biotypes in disease and nondisease states. It was used successfully to study the transmission of chloramphenicol-resistant strains of *S. epidermidis* in a closed population (25) and in establishing that a patient with endocarditis complicating a prosthetic heart valve was infected with a strain with the same biotype for a period of almost 2 years despite intermittent therapy (199). However, this biotyping scheme also suffered from a relatively small number of recognized phenotypes and never received widespread use.

Another biotyping scheme which utilized the production of acid aerobically from galactose, fructose, and mannose and the production of gelatinase and urease was developed by Holt to study CNS from the shunts, ventricles, and bloodstreams of children with colonized cerebrospinal fluid shunts (83). This scheme was successful in showing whether staphylococci in the same or different subgroups colonized the blood and shunt, or recolonized replaced shunts. Interestingly, Holt discussed the colonization of cerebrospinal fluid shunts by CNS in 1971 and stated, "The greatly increasing use of many kinds of such prostheses makes it safe to predict that colonization by relatively benign bacteria and fungi will soon become a major laboratory and clinical problem" (84). It is obvious that this prediction has come

TABLE 3. Studies using phage typing for the identification of strains of CNS

Type of infection, specimen, or study	Cultures typed with the phage set of:			
	Verhoef et al.	Pulverer et al.	Dean et al.	Parisi et al.
Endocarditis or prosthetic valve implantation	28[a], 195	NR[b]	113	9
Cerebrospinal fluid shunt	190, 195	NR	NR	9
Surgical wounds, including foreign body and bypass infections	29, 105, 149, 181	134, 181	149	36, 181
Clinical and nonclinical specimens	4, 59, 189, 196	31, 59, 148, 153	45, 47, 70	4, 137, 176, 184
Ecological	190, 197	74	45	NR
Other	NR	74	NR	183

[a] Reference number.
[b] NR, None reported.

true; the CNS, and especially *S. epidermidis*, are important nosocomial pathogens today.

Other biotyping schemes were developed but their use never became widespread (3, 4, 73, 124, 141). Some of these schemes utilized relatively few tests (3, 73, 141); others used too many tests (124) or contained too many steps to be practical (4).

With the recognition of the new species of Kloos and Schleifer (96, 163), the emphasis changed from biotyping to the identification of different species of CNS in clinical isolates. The most common species of CNS in urine was *S. epidermidis* (90, 115), also the most common species from all other clinical specimens (50, 65, 172). The availability of the API STAPH-IDENT heightened interest in the CNS because it provided a rapid and reasonable method to identify CNS. However, additional tests are needed to increase the accuracy of this system (7, 68), and the lack of sensitivity of the phosphatase reaction has led to the misidentification of *S. epidermidis* with *S. hominis* (5, 7, 48, 68). When compared with the DMS Staph-Trac system (DMS Laboratories, Inc., Flemington, N.J.) (now distributed by Analytab Products, Inc., under the name API Systems Staph-Trac), which is based on the prototype miniaturized biochemical test system developed by Peny and Buissiere (143), described by Brun et al. (34) and developed and marketed in Europe by API S.A. System under the name of API 20E, the API STAPH-IDENT proved equivalent (64). The fundamental problem with either of these commercial kit systems in epidemiological studies is that neither was intended for the identification of specific strains. With either kit the number of biochemical profiles identifying an isolate as *S. epidermidis* is limited and the majority of isolates are usually contained within one or a few of these profile numbers (48, 64, 68). A combination of the antibiogram with either the API STAPH-IDENT or the DMS Staph-Tract can be helpful in the identification of a specific strain in an epidemiological study (37), and a preliminary study has shown how the antibiogram can be utilized to characterize isolates within a biochemical profile (J. T. Parisi and B. H. Hamory, Diagn. Microbiol. Infect. Dis., in press).

Phage Typing

With the recognition in the early 1970s that the CNS were capable of causing serious infections, the need for additional epidemiological tools with which to study these organisms was soon realized. The success of phage typing *S. aureus* naturally led to the use of the same international set of typing phages for *S. epidermidis*. However, the results were discouraging. CNS were rarely lysed by phages isolated from *S. aureus* (144, 151). So, the need for the development of a phage-typing system for CNS was obvious. The first sets of typing phages in the early 1970s were developed by Verhoef, van Boven, and Winkler in The Netherlands (190, 191, 197); Pulverer, Pillich, and Krivankova in West Germany and Czechoslovokia (151, 154); and Dean, Williams, Hall, and Corse in England (45). The phages were obtained by either induction of lysogenic bacteria with mitomycin C or ultraviolet irradiation or the cross-culture technique of spotting supernatant fluids of cultures on plates spread with another culture. The results obtained with these early typing sets were not totally successful. The highest percentage of typability was 75.5% (196), but the lytic patterns were generally quite long (45) and strains were differentiated into too many phage types to be of any real epidemiological value (154). An interesting characteristic of all of these typing sets, and the ones which followed, was the use of typing phages at concentrations of 100 times or greater than the routine test dilution of the phage. With concentrated phage suspensions, it is sometimes difficult to distinguish between true cell lysis and inhibition reactions resulting from cell lysis without phage multiplication ("lysis from without"). The use of such concentrated suspensions of typing phages may be an indication of the overall greater resistance of *S. epidermidis* to phages than *S. aureus*. Pulverer and co-workers (152, 153) continued to isolate new phages and modify their typing set. In the United States, because Parisi and co-workers found that the use of Verhoef et al.'s phages were unsatisfactory with their cultures of *S. epidermidis*, they isolated their own set of typing phages in the mid-1970s (176, 184). They reported what appeared to be a geographic variation in phage sensitivity: phages were more active on strains isolated in the same locality as the bacterial strains from which the phages were obtained (138). They also found that their phages were considerably more active against isolates of CNS identified as *S. epidermidis* sensu stricto than against the other Kloos and Schleifer species of CNS (88).

The four phage typing sets just described have been used in the majority of the studies where phage typing has been used (Table 3). There are three characteristics that a phage-typing system should possess if it is going to be useful in epidemiological studies. (i) It should be able to type or lyse a large proportion of the strains. In general, the typing sets in current use have not demonstrated a high degree of typability, even with the concentrated suspensions of phages which are commonly used. To increase the typability of untypable strains, a "reverse" typing procedure has been used (47). In this method, the patterns of lysis produced by supernatants of the untypable strains upon the propagating strains of the phages in the typing set are compared. One is, in essence, comparing the host ranges of the phage or phages carried by the untypable strain, with the propagating strains of the phages in the typing set serving as the indicator strains. This method does not appear to be a practical solution to the enhancement of typability. (ii) A phage-typing set should demonstrate reproducibility in that different isolates derived from the same bacterial clone should give essentially identi-

cal reactions or at least fall within the guidelines of the "two strong differences" rule; i.e., two cultures are different when one is lysed strongly by two or more phages which produce no lysis on the other culture. Obviously some subjectivity is involved in determining what constitutes a "strong" reaction and a "weak" reaction. Also, subculturing and retyping such related isolates should give identical or nearly identical lytic reactions. (iii) A typing set should be able to discriminate epidemiologically related strains from unrelated strains. A large number of different lytic patterns should be identified with the typing set and the distinction between epidemiologically related and unrelated strains should be obvious.

At the International Conference on Phage Typing of CNS in 1977, it was decided that the different typing sets used in laboratories throughout the world should be evaluated with a carefully selected set of cultures from incidents of serious human disease. *S. epidermidis* comprised the majority (75.5%) of the 400 cultures assembled from laboratories in the United States, Canada, British Isles, England, West Germany, Denmark, Poland, and Sweden. These cultures were sent to the Central Public Health Laboratory in London. There they were examined biochemically and characterized with regard to their antibiotic resistance characters. These were then sent blinded to the 12 laboratories that participated in the study. These laboratories were requested to examine the cultures under their normal typing conditions, to record the phage reactions they obtained, and to return these results, together with a completed questionnaire with details of the phages and methods used, to London. The typing system was then analyzed in regard to typability, reproducibility, and discrimination. These results were published recently (46) and can be summarized as follows: none of the typing systems was satisfactory for all parameters tested. The phage sets with the highest percentage of typability were not satisfactory in their ability to discriminate between epidemiologically related and unrelated strains. Reproducibility was unsatisfactory in that no system gave identical results for significantly more than 50% of the typable pairs of strains. The occurrence of geographical variation could not be substantiated but there was some indication that typability might be increased by the adaptation of existing phages to untypable strains. However, it was not possible to make valid comparisons between individual phages in different typing sets because the typing methods used were not the same and agreement on a standard phage typing method for the CNS is essential in any subsequent study.

Although the results of this study were not encouraging, phage typing has been used successfully in many of the studies cited in Table 3. Combined with the antibiogram and either the API STAPH-IDENT or the DMS Staph-Trac (API Systems Staph-Trac), phage-typing credibility can be increased, and it has been suggested that perhaps phage typing should be reserved for epidemic rather than endemic situations (37). Where phage typing has been used in ecological studies (Table 3), it has shown a large number of distinguishable strains of *S. epidermidis* from swab cultures of a single site and how the flora varies over a period of several weeks. These studies show the difficulty in conducting ecological or epidemiological studies with this organism. The presence of staphylococci and micrococci as normal flora complicates the identification and isolation of *S. epidermidis*. How many colonies can reasonably be screened to obtain a representative sample of the *S. epidermidis* population at that site? There has been one recent attempt to resolve some of these problems (Parisi and Hamory, in press).

Serotyping

The serotyping of *S. epidermidis* as an epidemiological tool has not developed to the extent that phage typing has. This is due to the difficulties of preparing specific antisera and the standardization of typing methods. The cross-agglutination between *S. epidermidis* and *S. aureus* was recognized early (122, 146). However, cross-absorption tests indicated that *S. epidermidis* had its own set of type agglutinogens (1). Of the group precipitinogens present in *S. aureus*, antigen D, but not protein A, is shared with *S. epidermidis* (81). Whereas ribitol teichoic acid with *N*-acetylglucosaminyl residues is characteristic of the cell walls of *S. aureus*, a glycerol teichoic acid with glucosyl residues is present in the cell walls of *S. epidermidis* (42, 108, 123). These are referred to as polysaccharide A and polysaccharide B, respectively, in the early literature.

Serotyping, usually in combination with biotyping, has been used successfully in identifying a strain of *S. epidermidis* possessing a particular agglutinogen (144) or polysaccharide B (1, 108) or thermostable and thermolabile antigens (186). It would seem that the availability of other methods of typing *S. epidermidis* precludes the further development of methods of serotyping *S. epidermidis* in epidemiological studies.

Molecular Analysis of Plasmids

The rapid development of techniques for studying bacterial plasmids has given the epidemiologist new tools with which to study nosocomial infections. The use by Falkow and co-workers (129) of agarose gel electrophoresis to detect plasmids and to estimate their molecular weights has brought this technique within the capability of some clinical microbiology laboratories. When combined with restriction enzyme digestion of plasmid DNA, it is possible to "type" plasmids. Restriction endonucleases catalyze double-stranded cleavages at specific recognition sites in DNA. Upon exposure of plasmid DNA to these enzymes, the production of different-sized fragments depend upon the number and location of the specific recognition sites within the plasmid. The DNA fragments can then be separated by electrophoresis and the patterns obtained with different plasmids can be compared to determine their degree of relatedness. Thus, two plasmids of the same size may be judged to be identical or entirely different in their base sequences based on their fragment patterns. It is also possible for two different-sized plasmids to have some identical-sized fragments, indicating possible homology. However, restriction enzyme analysis provides only preliminary information on plasmid homologies because DNA fragments of similar sizes do not necessarily have identical base sequences. More definitive evidence of the extent of homology between two plasmid molecules must be obtained by DNA-DNA hybridization. To accomplish this, the DNA of one plasmid is radiolabeled by in vitro nick translation (155) to determine DNA hybridization by the Southern blot technique (178). After electrophoresis of the unlabeled plasmids, the electrophoresed plasmids are transferred to nitrocellulose and then exposed to the labeled probe. The plasmids containing sequences homologous to those present in the probe can be detected as bands by autoradiography of the nitrocellulose. Although this latter technique of DNA-DNA hybridization is primarily a research tool, there may be instances when it could be used to advantage in an epidemiological study. Since many research laboratories perform this technique routinely, it may be possible for a clinical laboratory to get this work done with

a minimum of effort and expense in the event of a particular outbreak.

Plasmid profiles. Plasmid profiles were first used to distinguish different strains of *S. epidermidis* by Parisi and Hecht (137). With crude cell lysates of cultures from sick neonates, they showed that isolates with the same phage type, biotype, and antibiogram had distinctly different plasmid profiles in agarose gel after electrophoresis. In other instances, almost identical plasmid profiles were observed in cultures with the same phage type, biotype, and markedly different antibiograms. The presence in the nursery of two unique strains, as determined by their plasmid profiles, could be documented for a period of over 3 months. Others have combined the use of plasmid profiles with phage typing and antibiograms to establish the epidemiological relationship of isolates from patients with prosthetic valve endocarditis (13) or the identity of multiple isolates in a neutropenic patient with *S. epidermidis* arthritis following catheter-induced bacteremia (85a) or the identity of multiple isolates from a surgeon and his patients who have undergone coronary artery bypass surgery (D. G. Maki, M. A. Zilz, R. McCormick, C. Alvarado, C. Hassemer, and J. T. Parisi, submitted for publication). This technique has also been used to differentiate skin contaminants from cultures responsible for prosthetic valve endocarditis, cerebrospinal fluid or ventriculostomy infections, intravenous catheter sepsis, urinary tract infections, and osteomyelitis (11).

Although the number of instances where this relatively new technique has been useful in epidemiological studies is few, it shows promise of becoming an important one. The techniques available for the rapid isolation from *S. epidermidis* of crude extracts of DNA without the necessity of removing the chromosomal DNA takes this technique out of the research laboratory and places it within the capability of some clinical laboratories. *S. epidermidis* appears to be ideally suited for this type of analysis because it harbors an abundance of plasmids, many of which are cryptic. One factor that reduces the sensitivity of this typing method is the tendency of strains to lose plasmids, resulting in the possibility of chromosomally identical strains producing different plasmid profiles. This has been documented in *S. epidermidis* with antibiotic resistance plasmids (137; P. A. Mickelsen, J. J. Plorde, J. McClure, F. D. Schoenknecht, F. C. Tenover, K. Gordon, and L. S. Tompkins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, L22, p. 311) and cryptic plasmids (D. G. Maki, M. A. Ziliz, C. Alvarado, C. Hassemer, J. Robbins, and J. T. Parisi, submitted for publication). This makes the interpretation of such profiles difficult because two isolates may have similar but not identical plasmid profiles, and it may not be practical to formulate rules regarding the establishment of identity of strains with similar but not identical profiles, unless additional molecular analysis is done (see below).

Restriction enzyme analysis and nucleic acid hybridization. The technique of restriction enzyme analysis and nucleic acid hybridization provides additional information on the nucleotide sequences within the plasmid and makes it possible to "type" plasmids to determine the identity between them. One instance where this technique has been used to advantage has been documented (Maki et al., submitted for publication). In this case, it was possible to show that two cultures of *S. epidermidis* isolated from two different coronary artery bypass patients were identical to that carried by the surgeon who performed the graft, even though each of the patients contained cultures which lacked a single different plasmid from those of the surgeon. Restriction enzyme digestions of the plasmids shared by the surgeon and his two patients yielded the same fragments. To confirm their homology, when the plasmids were transferred to nitrocellulose and exposed to a labeled probe of the plasmids in the surgeon's strain, DNA-DNA hybridization occurred in plasmids that comigrated, a test of their identity. Although this is a highly specialized technique, its sensitivity can establish unequivocally the identity of strains with similar but not identical plasmid profiles.

Slime production

The ability of some CNS to produce a mucoid substance which enabled them to adhere to smooth glass surfaces or to colonize Holter shunts was observed in 1972 (22). However, it has been only recently that this property of slime production was found to be an efficient discriminator between strains of *S. epidermidis* (38). Slime production appears to be a stable property of strains with this capability, the test for its production can be performed easily and can be quantitated, and this property is associated with bacterial adherence to intravascular catheters (38, 145). When used in conjunction with the antibiogram and the API STAPH-IDENT, slime production had a high discriminatory power (37). Besides aiding in the identification of strains, this property may provide some insight into the ability of *S. epidermidis* to produce infections in patients with indwelling foreign devices. There is good evidence that the attachment of *S. epidermidis* to the synthetic polymers of biomaterials is not dependent solely upon the production of slime but is a multifactorial event involving surface tension, surface free energy, surface charge, and hydrophobicity (110). Nevertheless, the great interest shown in slime production by *S. epidermidis* recently (57, 69, 82) indicates that this property is important not only in our understanding of the pathogenic potential of *S. epidermidis* but also in epidemiological studies.

CONCLUSIONS

Historically, the classification and taxonomy of the CNS have undergone numerous changes and revisions. The ability of some members of this heterogeneous group of organisms to produce disease in individuals whose resistance to microbial disease has been compromised has, no doubt, contributed to the renewed interest in them. The use of modern taxonomic methods based on molecular parameters has led to the current recognition of 15 species of CNS. However, questions have been raised as to the validity of some of these species. Although the commercial availability of two rapid identification kits has simplified the identification of this group of organisms to the species level, there is no evidence that these identification kits should be used routinely in the clinical laboratory. Obviously, those species that most frequently produce disease need to be identified, but how this can be done quickly, simply, and cheaply awaits further work.

S. epidermidis sensu stricto is the CNS most frequently isolated from disease. It is also the most prevalent and persistent staphylococcus on the skin. A number of methods has been used in epidemiological studies of infections by *S. epidermidis*. Some of these (antibiograms, biotyping, plasmid profiles, and slime production) are within the capability of most clinical laboratories; others (phage typing, serotyping, restriction enzyme analysis, and DNA-DNA hybridization) are primarily research tools. Some of these methods provide more conclusive epidemiological data than others, and the use of a combination of methods is ideal. As the significance

of *S. epidermidis* in disease becomes more recognized and accepted, the more effort clinical laboratories will have to make to help answer the questions we have regarding the origins, reservoirs, and modes of transmission of this nosocomial pathogen.

ACKNOWLEDGMENT

I thank Karen Ehlert for her skilled secretarial assistance.

LITERATURE CITED

1. **Aasen, J., and P. Oeding.** 1971. Antigenic studies on *Staphylococcus epidermidis*. Acta Pathol. Microbiol. Scand. Sect. B **79**:827–834.
2. **Aber, R. C., and D. C. Mackel.** 1981. Epidemiologic typing of nosocomial microorganisms. Am. J. Med. **70**:899–905.
3. **Akatov, A. K., and M. L. Khatenever.** 1976. Identification and biological typing of *Staphylococcus epidermidis* strains isolated in hospitals, p. 119–124. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
4. **Akatov, A. K., M. L. Khatenever, and L. A. Devriese.** 1981. Identification of coagulase-negative staphylococci isolated from clinical sources, p. 153–161. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
5. **Aldridge, K. E., C. W. Stratton, L. S. Pattersen, M. E. Evans, and R. L. Hodges.** 1983. Comparison of the Staph-Ident system with a conventional method for species identification of urine and blood isolates of coagulase-negative staphylococci. J. Clin. Microbiol. **17**:516–520.
6. **Allen, J. R., A. W. Hightower, S. M. Martin, and R. E. Dixon.** 1981. Secular trends in nosocomial infections: 1970–1979, p. 14–17. *In* A. E. Dixon (ed.), Nosocomial infections. Yorke Medical Books, New York.
7. **Almeida, R. J., and J. H. Jorgensen.** 1983. Identification of coagulase-negative staphylococci with API STAPH-IDENT system. J. Clin. Microbiol. **18**:254–257.
8. **Andrewes, F. W., and M. H. Gordon.** 1905–1906. Report on the biological characters of the staphylococci pathogenic for man. Rep. Med. Off. Loc. Govt. Bd. App. B **7**:543–560.
9. **Archer, G. L.** 1978. Antimicrobial susceptibility and selection of resistance among *Staphylococcus epidermidis* isolates recovered from patients with infections of indwelling foreign devices. Antimicrob. Agents Chemother. **14**:353–359.
10. **Archer, G. L., and J. L. Johnston.** 1983. Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. Antimicrob. Agents Chemother. **24**:70–77.
11. **Archer, G. L., A. W. Karchmer, N. Vishniavsky, and J. L. Johnston.** 1984. Plasmid-pattern analysis for the differentiation of infecting from noninfecting *Staphylococcus epidermidis*. J. Infect. Dis. **149**:913–920.
12. **Archer, G. L., and M. J. Tenebaum.** 1980. Antibiotic-resistant *Staphylococcus epidermidis* in patients undergoing cardiac surgery. Antimicrob. Agents Chemother. **17**:269–272.
13. **Archer, G. L., N. Vishniavsky, and H. G. Stiver.** 1982. Plasmid pattern analysis of *Staphylococcus epidermidis* isolates from patients with prosthetic valve endocarditis. Infect. Immun. **35**:627–632.
14. **Atkinson, B. A., and V. Lorian.** 1984. Antimicrobial agent susceptibility patterns of bacteria in hospitals from 1971 to 1982. J. Clin. Microbiol. **20**:791–796.
15. **Auletta, A. E., and E. R. Kennedy.** 1966. Deoxyribonucleic acid base composition of more members of the *Micrococcaceae*. J. Bacteriol. **92**:28–34.
16. **Baddiley, J., J. H. Brock, A. L. Davison, and M. D. Partidge.** 1968. The wall composition of micrococci. J. Gen. Microbiol. **54**:393–396.
17. **Baird-Parker, A. C.** 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. **30**:409–427.
18. **Baird-Parker, A. C.** 1965. Staphylococci and their classification. Ann. N.Y. Acad. Sci. **128**:4–25.
19. **Baird-Parker, A. C.** 1974. The basis for the present classification of staphylococci and micrococci. Ann. N.Y. Acad. Sci. **236**:7–14.
20. **Baird-Parker, A. C.** 1979. Methods for identifying staphylococci and micrococci, p. 201–210. *In* F. A. Skinner and D. W. Lovelock (ed.), Identification methods for microbiologists. Academic Press, London.
21. **Baumgart, S., S. E. Hall, J. M. Campos, and R. A. Polin.** 1983. Sepsis with coagulase-negative staphylococci in critically ill newborns. Am. J. Dis. Child. **137**:461–463.
22. **Bayston, R., and S. R. Penny.** 1972. Excessive production of mucoid substance in staphylococcus SIIA: a possible factor in colonization of Holter shunts. Dev. Med. Child. Neurol. **14**(Suppl. 27):25–28.
23. **Belozersky, A. N., and A. S. Spirin.** 1960. Chemistry of the nucleic acid of microorganisms, p. 147–185. *In* E. Chargaff and J. N. Davidson (ed.), The nucleic acids. Academic Press, London.
24. **Bentley, D. W.** 1973. Methicillin resistance in *Staphylococcus epidermidis* epidemiologic studies, p. 650–658. *In* J. Jeljaszewiscz (ed.), Staphylococci and staphylococcal infections. S. Karger, Basel.
25. **Bentley, D. W., J. J. Hahn, and M. H. Lepper.** 1970. Transmission of chloramphenicol-resistant *Staphylococcus epidermidis*: epidemiologic and laboratory studies. J. Infect. Dis. **122**:365–375.
26. **Bentley, D. W., R. Haque, R. A. Murphy, and M. H. Lepper.** 1968. Biotyping, an epidemiological tool for coagulase-negative staphylococci, p. 54–59. Antimicrob. Agents Chemother. 1967.
27. **Bergey's Manual of Determinative Bacteriology.** First ed., 1923; 2nd ed., 1925; 3rd ed., 1930; 4th ed., 1934; 5th ed., 1939; 6th ed., 1948; 7th ed., 1957; 8th ed., 1974. The Williams & Wilkins Co., Baltimore.
28. **Blouse, L. E., L. N. Kolonel, C. A. Watkins, and J. M. Atherton.** 1975. Efficacy of phage typing epidemiologically related *Staphylococcus epidermidis* strains. J. Clin. Microbiol. **2**:318–321.
29. **Blouse, L. E., G. D. Lathrop, L. N. Kolonel, and R. M. Brockett.** 1978. Epidemiologic features and phage types associated with nosocomial infections caused by *Staphylococcus epidermidis*. Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:119–135.
30. **Bohacek, J., M. Kocur, and T. Martinec.** 1967. DNA base composition and taxonomy of some micrococci. J. Gen. Microbiol. **46**:369–379.
31. **Brandis, H., C. Schick, and W. Lenz.** 1978. Preliminary results of phage typing of coagulase-negative staphylococci with the set of typing phages of Pulverer and co-workers. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:72–77.
32. **Breed, R. S.** 1956. *Staphylococcus pyogenes* Rosenbach. Int. Bull. Bacteriol. Nomencl. Taxon. **6**:35–42.
33. **Browder, H. P., W. A. Zygmunt, J. R. Young, and P. A. Tavormina.** 1965. Lysostaphin: enzymatic mode of action. Biochem. Biophys. Res. Commun. **19**:383–389.
34. **Brun, Y., J. Fleurette, and F. Forey.** 1978. Micromethod for biochemical identification of coagulase-negative staphylococci. J. Clin. Microbiol. **8**:503–508.
35. **Brun, Y., J. Fleurette, F. Forey, and B. Gonthier.** 1981. Antibiotic susceptibility of coagulase-negative staphylococci, p. 137–145. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
36. **Christensen, G. D., A. L. Bisno, J. T. Parisi, B. McLaughlin, M. G. Hester, and R. W. Luther.** 1982. Nosocomial septicemia due to multiply antibiotic-resistant *Staphylococcus epidermidis*. Ann. Intern. Med. **96**:1–10.
37. **Christensen, G. D., J. T. Parisi, A. L. Bisno, W. A. Simpson, and E. H. Beachey.** 1983. Characterization of clinically significant strains of coagulase-negative staphylococci. J. Clin. Microbiol. **18**:258–269.
38. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1983. Experimental foreign body infections in mice

challenged with slime-producing *Staphylococcus epidermidis*. Infect. Immun. **40**:407–410.
39. **Cohen, M., E. S. Wong, and S. Falkow.** 1982. Common R-plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis* during a nosocomial *Staphylococcus aureus* outbreak. Antimicrob. Agents Chemother. **21**:210–215.
40. **Cohn, F.** 1875. Untersuchungen uber Bacterien II. Beitr. Biol. Pflanz. **1**:127–224.
41. **Curry, J. C., and G. E. Borovian.** 1976. Selective medium for distinguishing micrococci from staphylococci in the clinical laboratory. J. Clin. Microbiol. **4**:455–457.
42. **Davison, A. L., and J. Baddiley.** 1963. The distribution of teichoic acids in staphylococci. J. Gen. Microbiol. **32**:271–276.
43. **Davison, A. L., and J. Baddiley.** 1964. Glycerol teichoic acids in the walls of *Staphylococcus epidermidis*. Nature (London) **202**:874.
44. **Davison, A. L., J. Baddiley, T. Hofstad, N. Losnegard, and P. Oeding.** 1964. Teichoic acids in the walls of staphylococci. Serological investigations on teichoic acids from the walls of staphylococci. Nature (London) **202**:872–874.
45. **Dean, B. A., R. E. O. Williams, F. Hall, and J. Corse.** 1973. Phage typing of coagulase-negative staphylococci and micrococci. J. Hyg. **71**:261–270.
46. **de Saxe, M. J., J. A. Crees-Morris, R. R. Marples, and J. F. Richardson.** 1981. Evaluation of current phage-typing systems for coagulase-negative staphylococci, p. 197–204. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
47. **de Saxe, M. J., and C. M. Notley.** 1978. Experiences with typing of coagulase-negative staphylococci and micrococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:46–59.
48. **Doern, G. V., J. E. Earls, P. A. Jeznach, and D. S. Parker.** 1983. Species identification and biotyping of staphylococci by the API Staph-Ident system. J. Clin. Microbiol. **17**:260–263.
49. **Dudgeon, L. S.** 1908. The differentiation of the staphylococci. J. Pathol. Bacteriol. **12**:242–247.
50. **Eng, R. H., C. Wang, A. Person, T. E. Kiehn, and D. Armstrong.** 1982. Species identification of coagulase-negative staphylococcal isolates from blood cultures. J. Clin. Microbiol. **15**:439–442.
51. **Evans, J. B., W. L. Bradford, and C. F. Niven, Jr.** 1955. Comments concerning the taxonomy of the genera *Micrococcus* and *Staphylococcus*. Int. Bull. Bacteriol. Nomencl. Taxon. **5**:61–66.
52. **Evans, J. B., and W. E. Kloos.** 1972. Use of shake cultures in semisolid thioglycolate medium for differentiating staphylococci from micrococci. J. Bacteriol. **23**:326–331.
53. **Fairbrother, R. W.** 1940. Coagulase production as a criterion for the classification of the staphylococci. J. Pathol. Bacteriol. **50**:83–88.
54. **Faller, A. H., and K. H. Schleifer.** 1981. Comparative studies on the cytochrome pattern of staphylococci and micrococci, p. 49–55. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
55. **Feltham, R. K. A.** 1979. A taxonomic study of the Micrococcaceae. J. Appl. Bacteriol. **47**:243–254.
56. **Fontana, R., P. E. Veraldo, P. Canepari, and G. Salta.** 1981. Analysis of the penicillin binding proteins in the membranes of staphylococci of different lyogroups (or species), p. 67–70. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
57. **Franson, T. R., N. K. Sheth, H. D. Rose, and P. G. Sohnle.** 1984. Quantitative adherence in vitro of coagulase-negative staphylococci to intravascular catheters: inhibition with D-mannosamine. J. Infect. Dis. **149**:116.
58. **Friedman, L. E., A. E. Brown, D. R. Miller, and D. Armstrong.** 1984. *Staphylococcus epidermidis* septicemia in children with leukemia and lymphoma. Am. J. Dis. Child. **138**:715–719.
59. **Frihandler, I., P. Gill, L. Martinez, and S. I. Vas.** 1978. Comparison of "invasive" and "non-invasive" strains of *Staphylococcus epidermidis* by phage typing. Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:136–139.
60. **Garvie, E. I., T. M. Higgs, and F. K. Neave.** 1961. Unusual varieties of *Staphylococcus aureus*. J. Pathol. Bacteriol. **81**: 255–259.
61. **Geisse, A.** 1914. Die differenzierung pathogenes und saprophytischer staphylokokken. Z. Hyg. **76**:282–306.
62. **Gemmell, C. G., and J. E. Dawson.** 1982. Identification of coagulase-negative staphylococci with the API Staph system. J. Clin. Microbiol. **16**:874–877.
63. **Ghuysen, J. M., D. J. Tipper, C. H. Birge, and J. L. Strominger.** 1965. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. IV. The soluble glycopeptide and its sequential degradation by peptidases. Biochemistry **4**: 2245–2254.
64. **Giger, O., C. C. Charilaou, and K. R. Cundy.** 1984. Comparison of the API Staph-Ident and DMS Staph-Trac systems with conventional methods used for the identification of coagulase-negative staphylococci. J. Clin. Microbiol. **19**:68–72.
65. **Gill, V. J., S. T. Selepak, and E. C. Williams.** 1983. Species identification and antibiotic susceptibilities of coagulase-negative staphylococci isolated from clinical specimens. J. Clin. Microbiol. **18**:1314–1319.
66. **Goodfellow, M., G. A. Alderson, M. R. Nahaie, G. Peters, F. Schumacher-Perdreau, G. Pulverer, P. B. Heczko, and M. Modarski.** 1983. Numerical taxonomy of staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **256**:7–24.
67. **Goodfellow, M., M. Modarski, A. Tkacz, K. Szyba, and G. Pulverer.** 1980. Polynucleotide sequence divergence among some coagulase-negative staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **246**:10–22.
68. **Grasmick, A. E., N. Naito, and D. A. Bruckner.** 1983. Clinical comparison of the AutoMicrobic system Gram-Positive Identification card, API Staph-Ident, and conventional methods in the identification of coagulase-negative staphylococci. J. Clin. Microbiol. **18**:1323–1328.
69. **Gray, E. D., G. Peters, M. Verstegen, and W. E. Regelman.** 1984. Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. Lancet **i**:365–367.
70. **Grigorova, M., and D. Bailjosov.** 1976. Enzyme activity and phage sensitivity of *Staphylococcus epidermidis*, p. 175–177. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
71. **Gunn, B. A., F. L. Singleton, E. R. Peele, R. R. Colwell, J. K. Keiser, and C. O. Kapper.** 1981. Comparison of methods for identifying *Staphylococcus* and *Micrococcus*. J. Clin. Microbiol. **14**:195–200.
72. **Heczko, P. B.** 1973. The taxonomy of *Staphylococcus epidermidis*, p. 41–46. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. S. Karger, Basel.
73. **Heczko, P. B., J. Jeljaszewicz, and G. Pulverer.** 1974. Classification of Micrococcaceae isolated from clinical sources. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **229**:171–177.
74. **Heczko, P. B., A. Klein, A. Kasprowicz, and G. Pulverer.** 1978. Use of a phage set for the ecological typing of coagulase-negative staphylococci. Zentralbl. Bakteriol. Parasitenkol. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:157–164.
75. **Heczko, P. B., G. Pulverer, A. Kasprowicz, and A. Klein.** 1977. Evaluation of a new bacteriophage set for typing of *Staphylococcus epidermidis* strains. J. Clin. Microbiol. **5**:573–577.
76. **Heddaeus, H., B. Heczko, M. Modarski, and G. Pulverer.** 1981. Relationships between some characters of coagulase-negative staphylococci and DNA:DNA sequence homology, p. 23–26. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
77. **Heddaeus, H., P. B. Heczko, and G. Pulverer.** 1979. Evaluation of the lysostaphin-susceptibility test for the classification of staphylococci. J. Med. Microbiol. **12**:9–15.
78. **Hill, L. R.** 1959. The Adansonian classification of the staphylococci. J. Gen. Microbiol. **20**:277–283.
79. **Hill, L. R.** 1981. Taxonomy of the staphylococci, p. 33–62. *In*

A. MacDonald and G. Smith (ed.), The staphylococci. Aberdeen University Press, Aberdeen, U.K.
80. **Hine, T. G. M.** 1922. Serological classification of the staphylococci. Lancet **ii**:1380–1382.
81. **Hofstad, T.** 1965. Studies on the antigenic structure of the 80/81 complex of *Staphylococcus aureus*. 6. Antigenic properties of a polysaccharide-polypeptide compound. Acta Pathol. Microbiol. Scand. **65**:143–150.
82. **Hogt, A. H., J. Dankert, J. A. deVries, and J. Feijen.** 1983. Adhesion of coagulase-negative staphylococci to biomaterials. J. Gen. Microbiol. **129**:2959–2968.
83. **Holt, R.** 1969. The classification of staphylococci from colonized ventriculo-atrial shunts. J. Clin. Pathol. **22**:475–482.
84. **Holt, R.** 1971. The colonization of ventriculo-atrial shunts by coagulase-negative staphylococci, p. 81–87. *In* M. Finland, W. Marget, and K. Bartmann (ed.), Bacterial infections. Springer-Verlag, New York.
85. **Hugh, R., and M. A. Ellis.** 1968. The neotype strain of *Staphylococcus epidermidis* (Winslow and Winslow, 1908) Evans 1916. Int. J. Syst. Bacteriol. **18**:231–239.
85a. **Hutton, J. P., B. H. Hamory, J. T. Parisi, and L. J. Strausbaugh.** 1985. *Staphylococcus epidermidis* arthritis following catheter-induced bacteremia in a neutropenic patient. Diagn. Microbiol. Infect. Dis. **3**:119–124.
86. **Jaffe, H. W., H. M. Sweeney, R. A. Weinstein, S. A. Kabins, C. Nathan, and S. Cohen.** 1982. Structural and phenotypic varieties of gentamicin resistance plasmids in hospital strains of *Staphylococcus aureus* and coagulase-negative staphylococci. Antimicrob. Agents Chemother. **21**:773–779.
87. **Jakubicz, P., and J. Borowski.** 1976. *Staphylococcus epidermidis* and micrococci as an etiologic agent in urinary tract infection, p. 141–143. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
88. **Jefferson, S., and J. T. Parisi.** 1979. Bacteriophage typing of coagulase-negative staphylococci. J. Clin. Microbiol. **10**: 396–397.
89. **Jeffries, L., M. A. Cawthorne, M. Harris, B. Cook, and A. T. Diplock.** 1968. Menaquinone determination on the taxonomy of Micrococcaceae. J. Gen. Microbiol. **54**:365–380.
90. **John, J. F., P. K. Gramling, and N. M. O'Dell.** 1978. Species identification of coagulase-negative staphylococci from urinary tract isolates. J. Clin. Microbiol. **8**:435–437.
91. **Jones, D., R. H. Deibel, and C. F. Niven, Jr.** 1963. Identity of *Staphylococcus epidermidis*. J. Bacteriol. **85**:62–67.
92. **Klesius, P. H., and V. T. Schuhardt.** 1968. Use of lysostaphin in the isolation of highly polymerized deoxyribonucleic acid and in the taxonomy of aerobic *Micrococcaceae*. J. Bacteriol. **95**:739–743.
93. **Kloos, W. E.** 1980. Natural populations of the genus *Staphylococcus*. Annu. Rev. Microbiol. **34**:559–592.
94. **Kloos, W. E.** 1982. Coagulase-negative staphylococci. Clin. Microbiol. Newsl. **4**:75–79.
95. **Kloos, W. E., and M. S. Musselwhite.** 1975. Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. Appl. Microbiol. **30**: 381–395.
96. **Kloos, W. E., and K. H. Schleifer.** 1975. Isolation and characterization of staphylococci from human skin. II. Description of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. Int. J. Syst. Bacteriol. **25**:62–79.
97. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–88.
98. **Kloos, W. E., K. H. Schleifer, and W. C. Noble.** 1976. Estimation of character parameters in coagulase-negative *Staphylococcus* species, p. 23–41. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
99. **Kloos, W. E., and J. Wolfshohl.** 1979. Evidence for deoxyribonucleic sequence divergence between staphylococci living on human and other primate skin. Curr. Microbiol. **3**:162–172.
100. **Kloos, W. E., and J. Wolfshohl.** 1982. Identification of *Staphylococcus* species with the API STAPH-IDENT system. J. Clin. Microbiol. **16**:509–516.
101. **Kocur, M., and N. Mortensen.** 1967. Comparison of methods for estimation of anaerobic production of acid from glucose and mannitol in staphylococci and micrococci. Acta Pathol. Microbiol. Scand. **71**:141–146.
102. **Krynski, S., E. Becla, P. Goluszko, and A. Kraususki.** 1973. Sensitivity of *Staphylococcus epidermidis* to antibiotics and mercury salts in dependence on orgin and attachment to Baird-Parker's subgroups, p. 646–649. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. S. Karger, Basel.
103. **Krynski, S., J. Borowski, E. Becla, J. Galinski, A. Niemiro, R. Szmanska-Malottke, and M. Wroczynski.** 1963. On the correlation between the nature and antibiotic sensitivity of the bacterial flora of the air and contamination of hospital rooms. Am. J. Hyg. **78**:111–122.
104. **Lachica, R. V. F., P. D. Hoeprich, and C. Genigeorgis.** 1971. Nuclease production and lysostaphin susceptibility of *Staphylococcus aureus* and other catalase-positive cocci. Appl. Microbiol. **21**:823–826.
105. **Lathrop, G. D., R. M. Brockett, and L. E. Blouse.** 1978. Epidemiologic surveillance for *Staphylococcus epidermidis* infections related to cardiopulmonary bypass. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:108–118.
106. **Liekweg, W. G., and L. J. Greenfield.** 1977. Vascular prosthetic infections: collected experience and results of treatment. Surgery **81**:335–342.
107. **Loeb, L.** 1903. The influence of certain bacteria on the coagulation of the blood. J. Med. Res. **10**:407–419.
108. **Losnegard, N., and P. Oeding.** 1963. Immunochemical studies on polysaccharides from *Staphylococcus epidermidis*. I. Isolation and chemical characterization. Acta Pathol. Microbiol. Scand. **58**:482–492.
109. **Lowy, F. D., and S. M. Hammer.** 1983. *Staphylococcus epidermidis* infections. Ann. Intern. Med. **99**:834–839.
110. **Ludwicka, A., B. Jansen, T. Wadstrom, and G. Pulverer.** 1984. Attachment of staphylococci to various synthetic polymers. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **256**:479–489.
111. **Males, B. M., W. A. Rogers, Jr., and J. T. Parisi.** 1975. Virulence factors of biotypes of *Staphylococcus epidermidis* from clinical sources. J. Clin. Microbiol. **1**:256–261.
112. **Marples, R. R.** 1981. Coagulase-negative staphylococci: classification and problems, p. 13–18. *In* H. I. Maibach and R. Aly (ed.), Skin microbiology. Springer-Verlag, New York.
113. **Marples, R. R., R. Hone, C. M. Notley, J. F. Richardson, and J. A. Crees-Morris.** 1978. Investigation of coagulase-negative staphylococci from infections in surgical patients. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:140–156.
114. **Marples, R. R., and J. F. Richardson.** 1981. Characters of coagulase-negative staphylococci collected for a collaborative phage-typing study, p. 175–180. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
115. **Marsik, F. J., and S. Brake.** 1982. Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. J. Clin. Microbiol. **15**:640–645.
116. **Marsik, F. J., and J. T. Parisi.** 1973. Significance of *Staphylococcus epidermidis* in the clinical laboratory. Appl. Microbiol. **25**:11–14.
117. **Masur, H., and W. D. Johnson.** 1980. Prosthetic valve endocarditis. J. Thorac. Cardiovasc. Surg. **80**:31–37.
118. **Meers, P. D., W. Whyte, and G. Sandys.** 1975. Coagulase-negative staphylococci and micrococci in urinary tract infections. J. Clin. Pathol. **28**:270–273.
119. **Migula, W.** 1990. System der Bakterien. 2. Specielle Systematik der Bakterien, p. 135–137. G. Fischer, Jena, Germany.
120. **Mitchell, R. G.** 1968. Classification of *Staphylococcus albus* strains isolated from the urinary tract. J. Clin. Pathol. **21**:93–96.
121. **Mitchell, R. G., V. G. Alder, and K. Rosendal.** 1973. The

classification of coagulase-negative Micrococcaceae from human and animal sources. J. Med. Microbiol. **7**:131–135.
122. **Morse, S. I.** 1962. Studies on the chemistry and immunochemistry of cell walls of *Staphylococcus aureus*. J. Exp. Med. **116**:229–245.
123. **Morse, S. I.** 1963. Isolation and properties of a group antigen of *Staphylococcus albus*. J. Exp. Med. **117**:19–26.
124. **Mortensen, N.** 1973. Characterization of staphylococci and micrococci by carbohydrate breakdown tests, p. 38–40. *In* J. Jeljasewicz (ed.), Staphylococci and staphylococcal infections. S. Karger, Basel.
125. **Mortensen, N., and M. W. Bentzon.** 1976. Classification of staphylococci and micrococci by numerical taxonomy, p. 63–70. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
126. **Mortenson, N., and M. Kocur.** 1967. Correlation of DNA base composition and acid formation from glucose of staphylococci and micrococci. Acta Pathol. Microbiol. Scand. **69**:445–457.
127. **Mossel, D. A. A.** 1962. Attempt in classification of catalase-positive staphylococci and micrococci. J. Bacteriol. **84**:1140–1147.
128. **Much, H.** 1908. Uber eine Vorstufe des Fibrinfermentes in Kulturen von *Staphylokokkus aureus*. Biochem. J. **14**:143–155.
129. **Myers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoresis method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.
130. **Nazar, K., P. B. Heczko, and G. Pulverer.** 1977. Transduction of penicillin resistance together with ability to ferment mannitol and ribose in *Staphylococcus epidermidis*. J. Gen. Microbiol. **99**:449–452.
131. **Noble, W. C.** 1969. Distribution of the Micrococcaceae. Br. J. Dermatol. Suppl. **1**:27–32.
132. **Noble, W. C.** 1969. Skin carriage of the Micrococcaceae. J. Clin. Pathol. **22**:249–253.
133. **Nord, C. E., S. Holta-Oie, A. Ljungh, and T. Wadstrom.** 1976. Classification of coagulase-negative staphylococcal species from human infections, p. 106–111. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
134. **Nord, C. E., and L. Sjoberg.** 1978. Phage typing and biotyping of coagulase-negative staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:78–82.
135. **Oeding, P., B. Myklestad, and A. L. Davison.** 1967. Serologic investigation on teichoic acids from the walls of *Staphylococcus epidermidis* and *Micrococcus*. Acta Pathol. Microbiol. Scand. **69**:458–464.
136. **Ogston, A.** 1883. Micrococcus poisoning. J. Anat. Physiol. **17**:24–58.
137. **Parisi, J. T., and D. W. Hecht.** 1980. Plasmid profiles in epidemiologic studies of infections by *Staphylococcus epidermidis*. J. Infect. Dis. **141**:637–643.
138. **Parisi, J. T., H. W. Talbot, Jr., and J. M. Skahan.** 1978. Development of a phage typing set for *Staphylococcus epidermidis* in the United States. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:60–67.
139. **Parker, M. T.** 1981. Infection and colonization by the "other" staphylococci, p. 156–174. *In* A. MacDonald and G. Smith (ed.), The staphylococci. Aberdeen University Press, Aberdeen, U.K.
140. **Passet, J.** 1885. Uber Mikroorganismen der eiterigen Zellgewebsentzundung des Menschen. Fortschr. Med. **3**:33–43.
141. **Pelzer, K., G. Pulverer, J. Jeljaszewicz, and J. Pillich.** 1973. Modification of Baird-Parker's classification system of *Staphylococcus albus*. Med. Microbiol. Immunol. **158**:249–257.
142. **Pennock, C. A., and R. B. Huddy.** 1967. Phosphatase reaction of coagulase-negative staphylococci and micrococci. J. Pathol. Bacteriol. **93**:685–688.
143. **Peny, J., and J. Buissiere.** 1970. Micromethode d' identification des bacteries. Ann. Inst. Pasteur Paris **118**:10–18.
144. **Pereira, A. T.** 1962. Coagulase-negative strains of *Staphylococcus* possessing antigen 51 as agents of urinary tract infection. J. Clin. Pathol. **15**:252–253.
145. **Peters, G., R. Locci, and G. Pulverer.** 1982. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J. Infect. Dis. **146**:479–483.
146. **Pillet, J., and B. Orta.** 1954. Recherches sur la composition antigenique des staphylocoques non pathogenes. I. Mise en evidence d' agglutinogenes specifiques et d' un agglutinogene common aux souches pathogenes et non pathogenes. Ann. Inst. Pasteur Paris **86**:752–758.
147. **Pillet, J., and B. Orta.** 1981. Species and serotypes in coagulase-negative staphylococci, p. 147–152. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
148. **Pillich, J., G. Pulverer, A. Klein, and M. Vojtiskova.** 1978. A proposal for further modification of the phage-typing system for coagulase-negative staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **241**:83–94.
149. **Polaczek-Kornecka, B., Z. Dziadur-Goldsztajn, and A. Komorowski.** 1976. Coagulase-negative staphylococci in clinical surgery, p. 145–147. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
150. **Pulverer, G., G. Damen, and M. Neugebauer.** 1973. Antibiotic resistance of *Staphylococcus albus*. Med. Microbiol. Immunol. **158**:32–43.
151. **Pulverer, G., and J. Pillich.** 1971. Pathogenic significance of coagulase-negative staphylococci, p. 91–96. *In* M. Finland, W. Marget, and K. Bartmann (ed.), Bacterial infections. Springer-Verlag, New York.
152. **Pulverer, G., J. Pillich, and M. Haklova.** 1976. Phage typing set for the species *Staphylococcus albus*, p. 153–157. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer, Stuttgart.
153. **Pulverer, G., J. Pillich, and A. Klein.** 1975. New bacteriophages of *Staphylococcus epidermidis*. J. Infect. Dis. **132**: 524–531.
154. **Pulverer, G., J. Pillich, and M. Krivankova.** 1973. Differentiation of coagulase-negative staphylococci by bacteriophage typing, p. 503–508. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. S. Karger, Basel.
155. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
156. **Rosenbach, F. J.** 1884. Mikro-Organismen bei den Wund-Infections-Krankheiten des Menschen, p. 19–21. J. F. Bergman, Wiesbaden.
157. **Rosypal, S., A. Rosypalova, and J. Horejs.** 1966. The classification of micrococci and staphylococci based on their DNA base composition and Adansonian analysis. J. Gen. Microbiol. **44**:281–292.
158. **Sanders, R. A., and G. F. Sheldon.** 1976. Septic complications of total parenteral nutrition. Am. J. Surg. **132**:214–219.
159. **Satta, G., P.E. Veraldo, G. Grazi, O. Soro, and C. A. Romanzi.** 1981. Significance of bacteriolytic activity in the taxonomy and routine identification of the Micrococcaceae, p. 57–60. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
160. **Schaefler, S.** 1972. Polyfunctional penicillinase plasmid in *Staphylococcus epidermidis:* bacteriophage restriction and modification mutants. J. Bacteriol. **112**:697–706.
161. **Schindler, C. A., and V. T. Schuhardt.** 1964. Lysostaphin: a new bacteriolytic agent for the *Staphylococcus*. Proc. Natl. Acad. Sci. U.S.A. **51**:414–421.
162. **Schleifer, K. H., L. Huss, and O. Kandler.** 1969. Die Beeinflussung der Aminosauresequenz des Serinhaltigen des Mureins von *Staphylococcus epidermidis* Stamm 24 durch die Nahrbodenzusammensetzung. Arch. Mikrobiol. **68**:387–404.
163. **Schleifer, K. H., and W. E. Kloos.** 1975. Isolation and characterization of staphylococci from human skin. I. Amended description of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii, Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. Int. J. Syst. Bacteriol. **25**:50–61.
164. **Schleifer, K. H., and W. E. Kloos.** 1975. A simple test system

for the separation of staphylococci from micrococci. J. Clin. Microbiol. 1:337–338.
165. **Schleifer, K. H., and W. Kloos.** 1976. Separation of staphylococci from micrococci, p. 3–9. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
166. **Schleifer, K. H., and E. Kramer.** 1980. Selective medium for isolating staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe C 1:270–280.
167. **Schleifer, H. H., and M. Kocur.** 1973. Classification of staphylococci based on chemical and biochemical properties. Arch. Mikrobiol. 93:65–85.
168. **Schleifer, K. H., S. A. Meyer, and M. Rupprecht.** 1979. Relatedness among coagulase-negative staphylococci: deoxyribonucleic acid reassociation and comparative studies. Arch. Microbiol. 122:93–101.
169. **Schoenbaum, S. C., P. Gardner, and J. Shillito.** 1975. Infections of cerebrospinal fluid shunts, epidemiology, clinical manifestations, and therapy. J. Infect. Dis. 131:543–552.
170. **Schuhardt, V. T.** 1965. Discussion of the paper: staphylococci and their classification. Ann. N.Y. Acad. Sci. 128:19–25.
171. **Schumacher-Perdreau, F., H. Rotering, and G. Pulverer.** 1983. Cell wall analysis and taxonomy of staphylococci. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 256:25–36.
172. **Sewell, C. M., J. E. Clarridge, E. J. Young, and R. Guthrie.** 1982. Clinical significance of coagulase-negative staphylococci. J. Clin. Microbiol. 16:236–239.
173. **Shaw, C., J. M. Stitt, and S. T. Cowan.** 1951. Staphylococci and their classification. J. Gen. Microbiol. 5:1010–1023.
174. **Sheth, N. K., T. R. Franson, H. D. Rose, F. L. A. Buckmire, J. A. Cooper, and P. Sohnle.** 1983. Colonization of bacteria on polyvinyl chloride and Teflon intravascular catheters in hospitalized patients. J. Clin. Microbiol. 18:1061–1063.
175. **Silvestri, L. G., and L. R. Hill.** 1965. Agreement between deoxyribonucleic acid base composition and taxometric classification of gram-positive cocci. J. Bacteriol. 90:136–140.
176. **Skahan, J. M., and J. T. Parisi.** 1977. Development of a bacteriophage-typing set for *Staphylococcus epidermidis*. J. Clin. Microbiol. 6:16–18.
177. **Smith, R. F., and C. L. Bettge.** 1972. Comparative characteristics of human and porcine staphylococci and their differentiation in burn xenografting procedures. Appl. Microbiol. 24:929–932.
178. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–527.
179. **Sovadina, M.** 1975. Classification of coagulase-negative staphylococci of human origin. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 230:313–319.
180. **Speller, D. C. E., and R. G. Mitchell.** 1973. Coagulase-negative staphylococci causing endocarditis after cardiac surgery. J. Clin. Pathol. 26:517–522.
181. **Staal, M. J., E. E. Stobberingh, M. J. Haverkorn, and C. P. A. van Boven.** 1981. Application of phage-typing and biotyping to the epidemiology of coagulase-negative staphylococci in cardiovascular surgery, p. 189–195. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal infections. Gustav Fischer Verlag, Stuttgart.
182. **Subcommittee on Taxonomy of Staphylococci and Micrococci.** 1965. Int. Bull. Bacteriol. Nomencl. Taxon. 15:109–110.
183. **Sugarman, B., and E. Pesanti.** 1980. Treatment failures secondary to *in vivo* development of drug resistance by microorganisms. Rev. Infect. Dis. 2:153–168.
184. **Talbot, H. W., Jr., and J. T. Parisi.** 1976. Phage typing of *Staphylococcus epidermidis*. J. Clin. Microbiol. 3:519–523.
185. **Thatcher, F. S., and W. Simon.** 1957. Some physiological and toxigenic properties of members of the genus *Micrococcus* in relation to taxonomy. Int. Bull. Bacteriol. Nomencl. Taxon. 7:21–36.
186. **Tierno, P. M., Jr., and G. Stotzky.** 1978. Serologic typing of *Staphylococcus epidermidis* biotype 4. J. Infect. Dis. 137:514–523.
187. **Tipper, D. J.** 1969. Structures of the cell wall peptidoglycans of *Staphylococcus epidermidis* Texas 26 and *Staphylococcus aureus* Copenhagen. II. Structure of neutral and basic peptides from hydrolysis with Myxobacter AL-1 peptidase. Biochemistry 8:2192–2202.
188. **Tornabene, T. G., S. J. Morrison, and W. E. Kloos.** 1970. Aliphatic hydrocarbon contents of various members of the family Micrococcaceae. Lipids 5:929–937.
189. **Tselenis-Kotsowilis, A., G. Dimitracopoulos, and J. Papavassiliou.** 1978. A comparative study of *Staphylococcus epidermidis* isolates from clinical material and healthy carriers. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 241:101–107.
190. **van Boven, C. P. A.** 1976. Phage typing of *Staphylococcus epidermidis*, p. 149–152. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
191. **van Boven, C. P. A., J. Verhoef, and K. C. Winkler.** 1969. Bacteriophage typing of coagulase-negative staphylococci. Antonie van Leeuwenhoek J. Microbiol. Serol. 35:232.
192. **van Eseltine, W. P.** 1955. On the inadvisability of separating the genera *Micrococcus* and *Staphylococcus*. Int. Bull. Bacteriol. Nomencl. Taxon. 5:53–60.
193. **Vas, S. I., D. E. Low, and D. G. Oreopoulos.** 1981. Peritonitis, p. 344–365. *In* K. D. Nolph (ed.), Peritoneal dialysis. Martinus Nijhoff Publishers, The Hague.
194. **Veraldo, P. E., G. Satta, and G. Grazi.** 1976. Grouping of staphylococci on the basis of the bacteriolytic activity patterns, p. 95–99. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag, Stuttgart.
195. **Verhoef, J., P. K. Peterson, D. N. Williams, M. Laverdiere, and L. D. Sabath.** 1978. Atrio-ventricular shunt infections and endocarditis due to *Staphylococcus epidermidis*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A 241:95–100.
196. **Verhoef, J., C. P. A. van Boven, and K. C. Winkler.** 1971. Characters of phages from coagulase-negative staphylococci. J. Med. Microbiol. 4:413–424.
197. **Verhoef, J., C. P. A. van Boven, and K. C. Winkler.** 1972. Phage-typing of coagulase-negative staphylococci. J. Med. Microbiol. 5:9–19.
198. **Wade, J., S. C. Schimpff, K. A. Newman, and P. H. Wiernick.** 1983. *Staphylococcus epidermidis*: an increasing cause of infections in patients with granulocytopenia. Ann. Intern. Med. 97:503–508.
199. **Watanakunakorn, C., and C. M. Hamburger.** 1970. *Staphylococcus epidermidis* endocarditis complicating a Starr-Edwards prothesis. Arch. Intern. Med. 126:1014–1020.
200. **Welch, W. H.** 1891. Conditions underlying the infection of wounds. Am. J. Med. Sci. 102:439–465.
201. **Wilson, P. D.** 1977. Joint replacement. South. Med. J. 70:55S–60S.
202. **Winslow, C. E. A., and A. R. Rodgers.** 1906. A statistical study of the generic characters in the Coccaceae. J. Infect. Dis. 3:485–546.
203. **Winslow, C. E. A., W. Rothberg, and E. I. Parsons.** 1920. Notes on the classification of the white and orange staphylococci. J. Bacteriol. 5:145–167.
204. **Young, L. S.** 1981. Infection in the compromised host. Hosp. Pract. 16:73–84.
205. **Zygmunt, W. A., H. P. Browder, and P. A. Tavormina.** 1968. Susceptibility of coagulase-negative staphylococci to lysostaphin and other antibiotics. Appl. Microbiol. 16:1168–1173.