Characterization of Clinically Significant Strains of Coagulase-Negative Staphylococci

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On occasion, a patient may have two or more clinical cultures yielding a coagulase-negative staphylococcus. If these multiple isolates have the same phenotype, one might conclude that the same strain was reisolated from the patient, indicating its persistent and pathological presence. We examined the validity of this conclusion when we applied a number of characterizing systems to a collection of 143 isolates of coagulase-negative staphylococci collected during an outbreak of intravascular catheter-associated sepsis. The probability of classifying two random isolates as the same phenotype or species was as follows: $P = 0.356$ for phage typing, $P = 0.348$ for Baird-Parker biotyping, $P = 0.346$ for the API STAPH-IDENT (Analytab Products) system, $P = 0.327$ for Bentley et al. biotyping, and $P = 0.077$ for antimicrobial susceptibility patterns. Although antimicrobial susceptibility patterns had the lowest probability, a variability in test results of 7.7% and a tendency for strains to have similar antibiograms effectively raised the probability to $P = 0.897$. The combination of the API STAPH-IDENT with antibiograms resulted in a probability of $P = 0.037$ to $P = 0.147$. When all of the above methods were used together a probability of $P = 0.014$ was achieved. Five patients had isolates from two or more blood cultures spaced more than 1 day apart that were identical by all of the above criteria, thus confirming prolonged bacteremia. The collection was also examined for the incidence of slime production. Slime production was not associated with any of the above groups, but was associated with symptomatic infections ($P < 0.05$) and gentamicin resistance ($P < 0.01$). Slime production was strain stable and was of assistance in typing strains of coagulase-negative staphylococci.

On occasion, a patient may have two or more clinical cultures yielding a coagulase-negative staphylococcus. If these multiple isolates have the same phenotype, one might conclude that the same strain was reisolated from the patient, indicating its persistent and pathological presence. The strength of this conclusion is a function of both the number of phenotypes recognized by the characterizing system and the proportion of the total microbial population each phenotype represents.

In 1979 and 1980, the City of Memphis Hospital and the adjoining University of Tennessee Hospital experienced an outbreak of intravascular catheter-associated sepsis due to coagulase-negative staphylococci (15). In the course of our epidemiological investigations we prospectively collected a series of blood culture isolates of coagulase-negative staphylococci obtained from patients with symptoms of bacterial sepsis ("symptomatic patient strains," clinical groups 1 and 2 (15)). Some, but not all, of the organisms in this symptomatic patient strain collection were isolated from patients on more than one occasion. The task we assigned ourselves was thus to determine whether these multiple isolates were the same, indicating persistent bacteremia, or different, indicating contamination or transient bacteremia. To aid in this determination, we also collected a series of blood culture isolates from asymptomatic patients ("blood culture contaminants" (15) and isolates from the hands and nares of ward personnel ("cutaneous strains" (15)). These additional isolates represent the bacteriological setting of our study.

To decide whether the multiple isolates were the same or different, we first turned to the antimicrobial susceptibility reports ("antibiograms") that were routinely reported by the clinical microbiology laboratory. Because of ready availability and pattern variety, clinicians will frequently apply antibiograms toward the
identification of clinically significant strains of coagulase-negative staphylococci (10, 18, 20, 21, 34). Indeed, antibiograms have been suggested as the primary tool for the identification of coagulase-negative staphylococci (1). We repeated these determinations, and for reasons that are detailed below we decided to extend our studies to include other characterizing systems.

Phage susceptibility patterns ("phage typing") are widely used for strain identification, either alone (23, 45, 55) or in combination with other methods (12, 13, 36, 52). Although a standard phage typing set for the coagulase-negative staphylococci has not been established, we had one set available to us that has seen wide application (27, 42, 47, 50; D. G. Maki, M. Zilz, C. Alvarado, J. Robbins, and J. T. Parisi, Clin. Res. 30:373A, 1982). Unfortunately, many of our strains could not be typed.

We then turned to systems that type strains by their biochemical reaction patterns ("biotyping"). A number of such systems are available. We chose one, that of Bentley et al. (11), that was developed specifically for epidemiological research in hospital-acquired, coagulase-negative staphylococcal infections. Although the Bentley et al. system has seen limited application, it has some elements in common with the biotyping system of Baird-Parker (4-7). Until recently, the Baird-Parker system was the most widely used taxonomic system for the subclassification of the coagulase-negative staphylococci. It has often been used in clinical situations (2, 13, 33, 37, 39, 52), where most authors find that pathogens belong to biotype S (Staphylococci)-2 (26, 33). So that our results would be comparable to prior investigations, we also determined the biotype of our collection by the Baird-Parker system.

Since the institution of these studies, a commercial system, API STAPH-IDENT (Analytab Products, Plainview, N.Y.), has become available for the identification of coagulase-negative staphylococcal species by the scheme of Kloos and Schleifer (31). The Kloos and Schleifer classification of the coagulase-negative staphylococci has replaced that of Baird-Parker. Because of its somewhat cumbersome methodology, however, it has seen limited application in clinical settings. The API STAPH-IDENT system remedies this problem by providing a reliable (3, 32), rapid micromethod for species identification. We have utilized this system, along with a newly recognized characteristic of coagulase-negative staphylococci, slime production (16), to further characterize our collection of isolates.

This extensive clinical, epidemiological, and microbiological characterization of our bacterial collection affords us the opportunity to compare the ability of each characterizing system and combination of systems to identify strains of clinically significant coagulase-negative staphylococci. It also affords us the opportunity to examine the relationships among these classification systems and to investigate relationships between virulence and phenotype.

(This paper was presented in part at the 83rd annual meeting of the American Society for Microbiology [G. D. Christensen and A. L. Bisno, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C298, p. 361].)

MATERIALS AND METHODS

Cultures. One hundred and forty-six isolates of Staphylococcus spp. were obtained from the clinical microbiology laboratories and serially coded by chronological receipt in our laboratory. Stock cultures were stored in outdated human blood at -70°C and later as lyophilized cultures. Working cultures were maintained on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood and transferred every 2 to 3 months. The procurement of these isolates has been previously described (15). The bacteriological identity of each isolate and its antimicrobial susceptibility were confirmed. All isolates were gram-positive, clustering cocci that produced catalase. All but one isolate were unable to ferment mannitol or produce coagulase. All but one isolate were susceptible to novobiocin, and all but one isolate fermented dextrose. These three exceptional strains were cutaneous isolates and were discarded. The remaining 143 isolates constitute the Memphis collection. Ninety-seven of these isolates were obtained from blood cultures from 58 patients. Sixteen of these 97 isolates were determined to be reisolations of the same strain from the same patient and were thus "duplicates." In this paper, "strain" refers to a distinct line of bacteria, isolated from an individual patient, which is represented by at least one isolate, but which may be represented by multiple identical isolates or duplicates. To avoid biasing some of the analyses, these duplicate isolates were excluded from the collection, leaving a final collection of 127 individual patient and personnel strains of coagulase-negative staphylococci. The remaining 81 blood culture strains included 35 strains from patients with suspected catheter-associated sepsis (symptomatic patient strains, clinical groups 1 and 2 [15]), and 46 strains from asymptomatic patients (blood culture contaminant strains [15]). In addition, the final collection included 46 strains isolated from the nares and hands of hospital personnel (cutaneous strains [15]).

Biotyping. Acid production from maltose and mannitol and the production of lipase, gluconidase, and gelatinase were detected as described by Bentley et al. (11). Acid production from lactose and production of acetoin were determined as described by Baird-Parker (6). Phosphatase activity was determined by the plate method of Bentley et al. (11) and by the tube method of Kloos and Schleifer (31), which is a modification of the method of Pennock and Huddy (44). Cultures were stored on P agar (53) at 4°C for 14 days and retested by the tube method. Control strains were obtained from the American Type Culture Collection (ATCC) and
included ATCC 155, 14990, 27836, 27840, 27844, and 27848.

Additional tests. Mannitol and dextrose fermentation tests were performed as described by Baird-Parker (6). Coagulase production was determined with rabbit plasma (Difco Laboratories, Detroit, Mich.). DNase production was determined with DNAse medium (Difco) as described by Bentley et al. (11). Mannose oxidation was performed as described by Bentley et al. (11). Phage typing was performed with 14 phages as described previously (15, 43).

API STAPH-IDENT strip system. API STAPH-IDENT strips were inoculated according to the manufacturer’s specifications with overnight cultures on Trypticase soy agar with 5% sheep blood. Swabs from the culture plate were agitated in 2 ml of 0.85% saline for an approximate turbidity of about 3 McFarland units. The strips were incubated at 37°C for 5 h and read. Cultures yielding unusual reaction patterns were repeated. In addition to the above control strains, ATCC strains 15305, 27626, 27845, 27846, 27847, 29972, 29968, and 29966 were also tested.

Slime production. Strains were examined for slime production as previously described (16) by inoculating 10 ml of Trypticase soy broth with a loopful of organisms from a blood plate culture and incubating overnight at 37°C. The test tubes were then emplaced of their contents and strained with trypan blue or safranin. Slime production was judged present if a visible film lined the walls of the tube. Ring formation at the liquid-air interface was not considered indicative of slime production.

Antimicrobial susceptibility. Susceptibility to novobiocin (30-μg disk, Difco) and a panel of 10 antimicrobial agents (penicillin, ampicillin, oxacillin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, and tetracycline) was determined by the disk diffusion method (8) on commercially prepared Mueller-Hinton medium (BBL) after overnight incubation at 36°C. Beta-lactamase production was tested directly on the Mueller-Hinton agar plate by the chromogenic cephalosporin test (50).

Data processing. Data concerning clinical origin, biochemical reactions, species, phage type, antimicrobial susceptibility, and slime production were coded onto punched cards and key sorted. Some of the analyses were indexed to the isolate’s capacity for multiple antimicrobial resistance. “Resistant” refers to an isolate that was resistant to five or more antimicrobial agents, and “susceptible” refers to an isolate that was resistant to less than five antimicrobial agents. Isolates that produced beta-lactamase were considered resistant to ampicillin and penicillin and counted as resistant to two antimicrobial agents. Statistical analysis was by the chi-square test, with the Yates correction for continuity when appropriate.

The assignment probability for each characterizing system or combination of systems was calculated from the following formula: 

\[ P = (a/n)^2 + (b/n)^2 + (c/n)^2 + \ldots \]  

where \( a, b, c, \ldots \) equals the number of members in category \( A, B, C, \ldots \) and \( n \) equals the total number of categorized strains.

RESULTS

Phosphatase test. The test for phosphatase production is a critical discriminator in the Baird-Parker biotyping system, the Bentley et al. biotyping system, and the API STAPH-IDENT system. Each of these systems, however, employs different methods for phosphatase determination. The original Baird-Parker system (4, 5) and the Bentley et al. system (11) used the plate method to test for phosphatase production. Baird-Parker later modified his system (6, 7) by adopting a more sensitive tube test for phosphatase production (44). It was believed that with the more sensitive test the phosphatase-negative biotype S-5 would prove to be phosphatase positive. Thus, in the modified Baird-Parker system, biotype S-5 was deleted by combining it with the otherwise identical biotype S-2 (6, 7). Kloos and Schleifer also used the tube test in their studies, but did not include this test as a criterion for species identification (31). The developers of the API rapid identification systems, on the other hand, included a rapid micromethod test for phosphatase determination in the API STAPH-IDENT kit, which they believed was more accurate than the tube test (14). The test results separated isolates into either Staphylococcus epidermidis if positive or Staphylococcus hominis if negative.

We have examined all three methods. When compared with the plate method, the API STAPH-IDENT method was slightly less sensitive, but more specific, with a comparative accuracy of 91.3% (Table 1). In comparison with the plate method, the tube method was more sensitive (Table 1). In our hands, however, it failed to convert biotype S-5 to the phosphatase-positive biotype S-2; 55% of the strains that were phosphatase negative by the tube method remained in the S-5 category. We also stored the collection in the cold for 2 weeks, as Pal and Ray have reported that older cultures have an increased frequency of phosphatase production.

### TABLE 1. Comparison of different methods for phosphatase determination to the standard plate method

<table>
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<th>Methods compared</th>
<th>Sensitivity</th>
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<th>Comparative accuracy</th>
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<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<tr>
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<td>94.5</td>
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<tr>
<td>Tube method\d vs plate method</td>
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<td>69.1</td>
<td>80.4</td>
<td>95.0</td>
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<td>Tube method on 2-week-old plates\b vs plate method</td>
<td>100.0</td>
<td>49.1</td>
<td>72.0</td>
<td>100.0</td>
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</table>

\d ATCC 14990 and 27848 were positive; ATCC 155, 27840, 27844, and 27836 were negative.

\b ATCC 14990, 27848, 27844, and 27836 were positive; ATCC 155 and 27840 were negative.
(41). This procedure did increase the sensitivity of the tube test (Table 1), but 59% of the remaining 27 phosphatase-negative strains remained in the biotype S-5 category, whereas many other strains became unclassifiable. Previously reported positive strains (ATCC 14990 [28], ATCC 27848 [30]) and negative strains (ATCC 155 [48]; ATCC 27836, 27840, and 27844 [30]) behaved in a similar fashion. Since the API STAPH-IDENT method and the plate method were in closest agreement and since we could not successfully combine biotype S-5 with biotype S-2 by the tube method, we have chosen to retain for our analysis the original five Baird-Parker biotypes based on the plate method.

**Biotyping.** Figure 1 cross-indexes the Baird-Parker system with the Bentley et al. system. Baird-Parker biotypes S-3 and Bentley et al. (B) biotypes B-7, B-8, and B-9 were not represented in this collection. The Baird-Parker biotype S-2 and the Bentley et al. biotype B-1 were nearly identical and constituted more than half of the collection under either typing system. The importance of the Baird-Parker biotype S-5 classification was evident, as S-5 was the second largest biotype with 26% of the strains. In the Bentley et al. scheme these same phosphatase-negative strains fell into two biotypes, B-2 and B-3, which included 29% of the strains and were the next largest biotypes after B-1. The major difference between the two systems was the gelatinase test, which subdivided the Baird-Parker biotypes S-2 and S-5 into the Bentley et al. biotypes B-1 and B-4 and biotypes B-2 and B-3, respectively. We noted a total of 11 separate phenotypes with the Baird-Parker biotyping system (Fig. 2A) and 10 phenotypes with the Bentley et al. biotyping system (Fig. 2B). Our distribution of biotypes for both systems was similar to previous reports with comparable collections (2, 11, 13, 33, 39, 52).

We also tested the collection for the oxidation of mannose and the production of DNase and beta-lactamase. The only noteworthy association between these tests and the preceding biotypes was between mannose oxidation and Baird-Parker biotypes S-6 and nongroupable strains (P < 0.001) and Bentley et al. nongroupable strains (P < 0.05). Mannose oxidation and DNase production were unusual (7.1 and 4.7%, respectively), whereas beta-lactamase production was common (90.6%). By using all 11 biochemical reactions, we identified a total of 30 phenotypes (Fig. 2D). Despite this combination of tests, 45.7% of the strains fell into a single phenotype.

**API STAPH-IDENT.** With the API STAPH-IDENT system, the largest and next largest species were *S. epidermidis* and *S. hominis* (Fig. 3), respectively, once again indicating the importance of the phosphatase determination in this system. Five strains could not be reliably identified. The histogram generated by identifying the species in our collection is shown in Fig. 2C. Our distribution was similar to that in one other report with the API STAPH-IDENT system and a similar clinical collection of bacterial strains (3). Other investigators using the standard methods of Kloos and Schleifer (3, 14, 19, 38) or the API STAPH miniaturized kit (22, 24) have noted fewer strains of *S. hominis* and more strains of *S. epidermidis*. DNase and beta-lactamase production and mannose oxidation were not associated with any of these species. Figure 3 is also a cross-index of the API STAPH-IDENT system and the Baird-Parker classification. Ninety percent of the S-2 biotype was classified as *S. epidermidis* with the API STAPH-IDENT, and 67% of the S-5 biotype fell into the *S. hominis* species. This similarity between Baird-Parker biotypes and Kloos and Schleifer species has been noted previously (36, 37).

**Phage typing.** By the rule of two strong differences (49), phage typing discriminated between 13 types (including nontypable) (Fig. 2E), but identified only four types with two or more members. Most (50.3%) organisms were untypeable. Nearly half (47.2%) of the strains were susceptible to bacteriophage 202, but only a small minority (16.5%) of these strains could be further typed. Phage 202 was primarily associated with *S. epidermidis*, but some members of *S. hominis, Staphylococcus warneri, Staphylococcus haemolyticus, Staphylococcus capitis, and Staphylococcus simulans* were also susceptible to this broadly reactive phage. Overall, 18.9% of the Memphis collection was typable by bacteriophages other than 202. Nine strains, eight cutaneous and one from a blood culture, belonged to the phage type 29/108/112/113/188/407-2/202 (type A); six strains, four cutaneous and two
blood cultures, were phage type 112/202 (type B). Except for one lactose negative variant, the nine type A strains were identical for the 11 biotyping reactions and fell in the S-2/B-1 subtype. Seven of these strains were S. epidermidis, one was S. warneri, whereas the lactose-negative variant was S. capitis. Three of the six type B strains were identical by their biotyping reactions and were identified as S. epidermidis. The combination of phage typing with biotyping subdivided the collection into 48 phenotypes (Fig. 2F).

Antimicrobial susceptibility patterns. Twelve percent of the beta-lactamase-producing strains were susceptible to ampicillin and penicillin by the disk diffusion method. There was a 7.7% disparity between the original antimicrobial susceptibility report and the redetermination; two antimicrobial agents, gentamicin and tetracycline, accounted for 49% of this variance. These discrepancies were equally divided between reports of resistance (3.6% found susceptible) and reports of susceptibility (4.2% found resistant).

Thirty-one patterns of antimicrobial resistance were noted (Fig. 2G). When the strains were ranked according to the number of antimicrobial agents to which they were resistant (Fig. 4), the collection sorted itself into two populations. One population consisted of 52 strains resistant to four or fewer antimicrobial agents, with a peak of 22 strains that were only beta-lactamase producers. The second population consisted of 75 strains resistant to five or more antimicrobial agents, with a peak of 23 strains resistant to seven antimicrobial agents. Within these two populations, the antimicrobial susceptibility patterns were quite similar; 65.4% of the susceptible strains fell into two groups separated only by tetracycline susceptibility, and 45.3% of the resistant strains fell into four groups that differed only by the gentamicin and tetracycline susceptibility. The presence of these two populations reflects the earlier reported findings of a difference in the epidemiology between resistant strains, presumably of hospital origin, and susceptible strains, presumably of community origin (15). Of further note is the finding that almost all of the symptomatic patient strains were resistant (94.2%) as opposed to blood culture contaminants and cutaneous strains (45.6%, \( P < \)
STAPH-IDENT API

S. xylosus
H. hominis
S. capitis
S. haemolyticus
S. warneri
S. epidermidis
S. simulans
Baird-Parker Biotype
Non

groupable
S-4 S-2 S-5 S-6

Kloos & Schleifer
Species
Unknown

FIG. 3. Cross-index of species identified by the API STAPH-IDENT system and Baird-Parker biotypes.

0.001). This may represent the nosocomial origin of these infections or a greater virulence on the part of the resistant organisms.

Comparison of characterizing systems. Table 2 indicates the species and biotype make-up of selected subcollections of bacterial strains. Only the species and biotypes represented by nine or more members were included on this table. Generally, the make-up of the subcollections reflected the overall distribution of the species or biotype. The most notable exception to this, however, was in phage susceptibility. A greater proportion (72%) of strains susceptible to phage 202 were S. epidermidis than were nonsusceptible strains ($P < 0.001$). This difference was also reflected in the S. epidermidis similar biotypes S-2 and B-1 ($P < 0.001$ and $P < 0.001$, respectively). The opposite finding was noted for S. hominis and its similar biotypes S-5 and B-2 ($P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively), which were primarily but not exclusively phage nontypeable. A significantly greater proportion of cutaneous strains fell into the S-2 biotype than did blood culture strains ($P < 0.01$). S. epidermidis made up a significantly greater proportion of resistant strains than susceptible strains ($P < 0.05$); a similar but not significant trend was also noted for S-2 and B-1. Aside from multiple antimicrobial resistance, we did not note any association between phenotypes, phenotypic characters or species so far described, and the symptomatic patient strains (Table 2).

The ability of these various systems to describe the collection of strains can be visually compared by examining the histograms in Fig. 2.

Both biotyping systems, their combination, phage typing, and the API STAPH-IDENT system exhibited similar patterns: the majority of strains fell into a single large phenotype or species, whereas the minority of strains were scattered among a large number of phenotypes or species. This distribution would be expected for systems which were primarily taxonomic in nature, such as the API STAPH-IDENT system and the Baird-Parker biotyping. It is important to note that those systems intended for strain identification, such as Bentley et al. biotyping and phage typing, performed equally poorly. The combination of phage typing with biotyping, on the other hand, resulted in a more satisfactory classification; the single large phenotype was broken down into several moderately sized ones. Antibioms alone also subdivided the collection into a series of small and moderately sized phenotypes. When the antibioms were combined with biotyping and phage typing, a finer separation was achieved, yielding 95 phenotypes (Fig. 2). Biotyping and phage typing are primarily research tools for strain identification, whereas the API STAPH-IDENT microkit was designed for the clinical identification of species. It would be expected that in clinical use this system will also be combined with the antibioms for the identification of strains. Such a combination subdivided our collection into 56 phenotypes (Fig. 21).

These systems are compared in another manner in Table 3. By adding the squares of the percent frequency of each phenotype or species, we calculated the probability under each classification system of assigning by chance alone two randomly selected strains to a single phenotype or species (assignment probability, Table 3). Of all the systems, antibioms had the lowest

FIG. 4. Collection of 127 strains ranked by the number of antimicrobial agents to which each strain is resistant. Individual blocks indicate individual patterns of antibiotic susceptibility.
assignment probability of $P = 0.077$. On clinical application, however, this low probability was suspect. If the resistant and susceptible strains were considered separately, as they should be for community-acquired and hospital-acquired infections, and if the results of one or more susceptibility tests were discounted, such as the variable tetracycline and gentamicin tests, then the discriminating power of the antibiogram was greatly diminished. When we applied these considerations to our collection we found an assignment probability of $P = 0.897$ for susceptible strains and $P = 0.303$ for resistant strains (Table 3).

The assignment probability for Baird-Parker biotyping was high ($P = 0.348$). Again this would be expected for a primarily taxonomic system. The assignment probabilities for Bentley et al. biotyping, biotyping with 11 tests, and phage typing were also high ($P = 0.327, P = 0.240, and P = 0.356$, respectively). This was disappointing since these systems were intended for strain identification. By combining phage typing with biotyping a lower assignment probability of $P = 0.085$ was achieved, this was greatly improved by combining phage typing and biotyping with the antibiogram. With this combination we obtained an assignment probability of $P = 0.015$.

The relatively poor performance of the API STAPH-IDENT system was not surprising ($P = 0.346$), as again this system was not intended for strain identification. When used in combination with the antibiogram, however, the discriminating power of both systems was greatly enhanced, achieving an assignment probability of $P = 0.037$. Even with the above-mentioned reservations regarding antibiograms, when both systems were used together the probability for susceptible strains was $P = 0.119$ and that for resistant strains was $P = 0.147$.

The combination of all available methods resulted in an assignment probability of $P = 0.014$. Despite this low probability, the entire collection of 143 isolates included eight patients with 25 isolates representing nine phenotypes. Five patients, all symptomatic, had isolates recovered on more than 1 day with identical phenotypes. Four of these sepsis cases were due to *S. epidermidis*, and one was due to *S. hominis*.

**Slime production.** In the course of these investigations, we noted that some strains of coagulase-negative staphylococci produced slime, as evidenced by their growth as a viscid film in Trypticase soy broth (16). The incidence of slime production in the Memphis collection was 44% (Table 4). Previous reports of similar phenomena suggested that slime production may be confined to biotype S-2 (4, 9). In our collection, slime production was found in all the major Baird-Parker biotypes and staphylococcal species except *S. warneri* (Table 2); it was not associated with any particular biochemical reaction. Slime production was also unrelated to phage susceptibility (Table 4). A higher, although not significant, proportion of resistant strains produced slime. Slime production was

<table>
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<tr>
<th>Subcollection (n)</th>
<th>% of species (n)</th>
<th>% Baird-Parker biotype (n)</th>
<th>% Bentley et al. biotype (n)</th>
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<tr>
<td>Total</td>
<td>53</td>
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<td>8</td>
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*a Data are expressed as row percent.

$P < 0.01$.

$P < 0.05$.

$P < 0.001$. 

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<thead>
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<th>Subcollection (n)</th>
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<th>% Bentley et al. biotype (n)</th>
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<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Phage-nontypable strains (64)</td>
<td>38d</td>
<td>36b</td>
<td>11</td>
</tr>
<tr>
<td>Phage 202-typable strains (60)</td>
<td>72</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Slime producers (56)</td>
<td>57</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td>Total (127)</td>
<td>53</td>
<td>24</td>
<td>8</td>
</tr>
</tbody>
</table>
TABLE 3. Comparison of the probability of assigning two random coagulase-negative staphylococcal cultures to the same type under various typing systems and their combinations

<table>
<thead>
<tr>
<th>Typing system</th>
<th>Assignment probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phage typing</td>
<td>0.356</td>
</tr>
<tr>
<td>Baird-Parker</td>
<td>0.348</td>
</tr>
<tr>
<td>API STAPH-IDENT</td>
<td>0.346</td>
</tr>
<tr>
<td>Bentley et al.</td>
<td>0.327</td>
</tr>
<tr>
<td>All biotyping (11 tests)</td>
<td>0.240</td>
</tr>
<tr>
<td>Phage typing and biotyping (11 tests)</td>
<td>0.085</td>
</tr>
</tbody>
</table>

Antibiogram

- Considering only sensitive strains, dividing tetracycline: 0.897
- With API STAPH-IDENT: 0.119
- With slime production: 0.282
- With API STAPH-IDENT and slime production: 0.076

- Considering only resistant strains, dividing tetracycline and gentamicin: 0.303
- With API STAPH-IDENT: 0.147
- With slime production: 0.163
- With API STAPH-IDENT and slime production: 0.086

- Considering all strains, all 10 antimicrobial agents: 0.077
- With API STAPH-IDENT: 0.037
- With slime production: 0.049
- With API STAPH-IDENT and slime production: 0.026
- With phase typing and biotyping (11 tests): 0.015
- With phase typing, biotyping (11 tests), and API STAPH-IDENT: 0.014

associated with gentamicin resistance ($P < 0.01$) and symptomatic infections ($P < 0.05$) (Table 4).

Slime production appeared strain stable. The presence or absence of this characteristic was constant for strains isolated from multiple blood cultures obtained on the same day (8 strains, 17 isolates) or different days (5 strains, 13 isolates). Of further note, the three identical members of phage type A were all slime producers. These three strains were cutaneous isolates and probably represented multiple isolations of a common environmental strain.

Slime production was an efficient discriminator between strains. Of the 11 biochemical reaction tests, only the plate method for phosphatase determination evenly divided the collection (57% positive, 43% negative). Slime production also evenly divided the collection and was independent of other biochemical reactions and phage type.

**Application of typing systems.** Seven patients had positive blood cultures on multiple days with isolates that had the same antibiogram, or an antibiogram that differed only by the gentamicin susceptibility. Subsequent analysis ruled out persistent bacteremia in these patients since the isolates were found to be of a different biotype or phage type. The application of the API STAPH-IDENT system or a test for slime production would have indicated that four of these seven sets were composed of different strains. If slime production was combined with the API STAPH-IDENT system, each of these sets would have been demonstrated to be composed of different members. Slime production in combination with the antibiogram had an assignment probability of $P = 0.049$ (with a predicted maximum probability for susceptible strains of $P = 0.282$ and for resistant strains of $P = 0.163$) (Table 3). The combination of slime production with the API STAPH-IDENT and antibiograms resulted in an assignment probability of $P = 0.026$, which approached the low probability achieved by combining the antibiogram with biotype and phage type.

**DISCUSSION**

Investigations into the bacteriology of the coagulase-negative staphylococci have taken two opposite approaches. Taxonomists seek to group these diverse organisms into classes or species with common properties. On the other hand, in the smaller arena of opportunistic human infections where commensal coagulase-negative staphylococci abound, medical microbiologists need to detect minor differences between related organisms to recognize the continued presence of an infecting strain or the environmental distribution of an epidemic strain.

The taxonomic approach is exemplified by the work of Kloos and Schleifer, who have systematically reorganized the taxonomy of the coagulase-negative staphylococci on the basis of phenotypic characterization and DNA homology studies (30, 46). At this point, the clinical significance of these species designations is unknown. All clinical studies using the newly designated

**TABLE 4. Comparison of the frequency of slime production in the subcollections**

<table>
<thead>
<tr>
<th>Collection</th>
<th>Slime producers</th>
<th>Nonslime producers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic patient strains</td>
<td>22 (63)*</td>
<td>13 (37)*</td>
</tr>
<tr>
<td>Blood culture contaminant strains</td>
<td>16 (35)*</td>
<td>30 (65)*</td>
</tr>
<tr>
<td>Cutaneous strains</td>
<td>18 (39)*</td>
<td>28 (61)*</td>
</tr>
<tr>
<td>Resistant strains</td>
<td>38 (51)</td>
<td>37 (49)</td>
</tr>
<tr>
<td>Gentamicin-resistant strains</td>
<td>21 (70)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Susceptible strains</td>
<td>18 (35)</td>
<td>34 (65)</td>
</tr>
<tr>
<td>Phage-nonpylicable strains</td>
<td>28 (49)</td>
<td>36 (56)</td>
</tr>
<tr>
<td>Phage 202-typable strains</td>
<td>25 (42)</td>
<td>35 (58)</td>
</tr>
<tr>
<td>Complete collection, 127 strains</td>
<td>56 (44)*</td>
<td>71 (56)*</td>
</tr>
</tbody>
</table>

* Data from reference 16.
staphylococcal species, including this one, indicate that *S. epidermidis* is the major human pathogen for all coagulase-negative staphylococcal infections exclusive of the urinary tract (22, 24, 38). If one accepts the data of Marples and Richardson (35, 37) and our observation that *S. epidermidis* is generally equivalent to Baird-Parker biotype S-2, then this pathological association probably also reflects the earlier observation of a relationship between biotype S-2 and disease (26, 52). The problem in making this association is that both the current and obsolete taxonomies recognize that *S. epidermidis* (and biotype S-2) is also the major class of staphylococci on human skin (7, 29). Thus, the association of species with illness may only reflect opportunity rather than virulence.

In addition to *S. epidermidis*, other species have also been reported to be human pathogens such as *S. haemolyticus* (21, 38), *S. hominis* (21), and *S. warneri* (21). In this study, one patient (patient no. 9 [15]) sustained *S. hominis* sepsis, and another (patient no. 24 [15]) died with *S. hominis* pneumonia. The species designation of *S. hominis* could be criticized in this situation since the API STAPH-IDENT system appears to identify many more strains as *S. hominis* than either the methods of Kloos and Schleifer or the API STAPH system (3). Since this identification depends upon a single test, phosphatase production, which is not as sensitive as the tube method, these *S. hominis* strains could be considered "phosphatase-negative" *S. epidermidis* (3). We believe however, that there are several arguments in favor of the *S. hominis* designation. The API STAPH-IDENT system has been examined by Kloos and Wolfshohl and found to be very accurate, particularly in resolving the identification of strains of *S. epidermidis* group into *S. epidermidis*, *S. hominis*, and other species (32). Many authors have expressed dissatisfaction with the tube method for phosphatase determination (14, 35, 37, 40, 54, 56). In our own collection the *S. hominis* strains were not susceptible to phage 202, whereas the *S. epidermidis* strains were susceptible to phage 202. Finally, in continuing studies on the surface characteristics of these organisms we have found distinct differences between *S. hominis* and *S. epidermidis* (Christensen and Bisno, Abstr. Annu. Meet. Am. Soc. Microbiol., 1983, C298, p. 361).

The clinical application of taxonomic systems may or may not predict an organism's virulence. On the other hand, there have been many attempts to use these systems for strain identification (2, 19, 52). These attempts are a misapplication of these systems, as their taxonomic purpose is to group strains rather than differentiate between them. A review of those occasions when taxonomic classifications were applied to clinical culture collections indicates that the calculated assignment probabilities are high and similar to the findings in this report (Table 5). The high assignment probability in each of these cases derives from the bacterial homogeneity of human commensal and pathogenic coagulase-negative staphylococci.

To identify individual strains of coagulase-negative staphylococci, a characterizing system must describe 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. Assuming that each phenotype was equally represented, a strain identification system would have to recognize at least 20 phenotypes to achieve this goal. Generally, the type distribution is unequal; consequently the number of required types greatly increases. For instance, if one type accounted for 22% of the population, the system would have to recognize at least 380 other types, each of which must account for not more than 0.2% of the total population to keep the assignment probability below *P* = 0.05.

Experience with most strain identification systems does not even approach these requirements (Table 5). Published reports of phage typing have assignment probabilities that vary between *P* = 0.133 and *P* = 0.425. The major problem with phage typing has been the inability of the current typing to type the majority of the clinical isolates. Despite this limitation, phage typing has proven valuable in identifying epidemic strains in several circumstances (12, 36; Maki et al., Clin. Res. 30:37A, 1982). Since we could not identify an epidemic strain, and the epidemiology of our outbreak was more characteristic of a hyperendemic process, it may be that phage typing coagulase-negative staphylococci is better reserved for epidemic rather than endemic situations.

Biotyping systems for strain identification are limited by the relatively small number of recognized phenotypes and the bacterial homogeneity of human strains. These limitations are reflected in the high assignment probabilities calculated from prior studies, which range from *P* = 0.202 to *P* = 0.369.

Despite the widespread application of antibiograms to the problem of identifying pathogenic strains of coagulase-negative staphylococci, there has been surprisingly little attention paid to the practical or theoretical utility of this approach. In our experience, antibiograms had the greatest discriminatory power, although the assignment probability was still above the 0.05 level. This discriminatory power was misleading, however, since the strain origin and the variability in antimicrobial susceptibility test reports dimin-
In the situation of a community-acquired illness where the tetracycline susceptibility was discounted, the assignment probability was higher than that of any other system that we tested or reviewed. It does not appear that antibiograms can be relied upon as the sole method for strain identification.

With current technology, the combination of various typing systems is the most advantageous method for strain identification. Such a combination of antibiograms, phage typing, and biotyping allowed us to identify five patients with sepsis. This required a prodigious effort that was far beyond the capacity of most clinical laboratories and was impractical for routine clinical use.

Now that the coagulase-negative staphylococci are well recognized nosocomial pathogens, efforts to improve their clinical laboratory diagnosis should be made. The combination of the antibiogram with the API STAPH-IDENT system goes a long way toward accomplishing this.
goal. These two systems complement each other and would allow a microbiology laboratory to identify clinically significant strains with some confidence. In our experience such a combination achieved an assignment probability of less than $P = 0.05$.

Despite the advantage of this combination of systems, we recognize that in certain circumstances the assignment probability may still remain greater than $P = 0.1$. In such situations, a clinical laboratory might consider employing additional typing procedures. One such criterion might be the test for slime production, since it is easily performed, has a high discriminatory power, and appears strain stable. In our study, when the test for slime production was used in conjunction with antibiograms and the API STAPH-IDENT system, the assignment probability approached that of all our methods used together.

Besides aiding in the identification of strains, slime production may provide some insight into the pathogenesis of these infections. Since slime production is associated with bacterial adherence to smooth surfaces in vitro (16), it would seem reasonable that this factor is important in the colonization of a foreign body. Indeed, slime production was a common finding in the group of strains from patients with symptomatic infections. We also have preliminary evidence that slime production is associated with cerebrospinal fluid shunt infections (G. D. Christensen, W. A. Simpson, E. H. Beachey, A. L. Bisno, and F. F. Barrett, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, 1982, abstr. no. 649). In addition, Needham and Stempskey, who use methods similar to ours, have just reported an association between illness and slime production (C. Needham and W. Stempskey, Abstr. Annu. Meet. Am. Soc. Microbiol., 1983, B193, p. 55). Whether slime production is a virulence factor per se is not as clear. In our study, four of the five patients with documented prolonged bacteremia had strains that were not slime producers. On the other hand, we have evidence that slime production is associated with virulence in a mouse model for foreign body infections (17) and a rat model of bacterial endocarditis (L. M. Baddour, G. D. Christensen, M. G. Hester, and A. L. Bisno, submitted for publication).

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LITERATURE CITED


