Species Distribution of Coagulase-Negative Staphylococcal Isolates at a Community Hospital and Implications for Selection of Staphylococcal Identification Procedures

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A total of 499 coagulase-negative staphylococci (CoNS) were isolated from a variety of clinical specimens at a community hospital. Ten different species and many strains of CoNS were identified. Staphylococcus epidermidis was the most common isolate. The species distribution suggests that S. saprophyticus and, to a lesser extent, S. haemolyticus may be important in urinary tract infections. S. lugdunensis may be a significant isolate from wound infections. Frequently, mixed cultures were found with either multiple species or multiple strains of the same species of CoNS. These mixed cultures could not be detected by colony morphology upon initial overnight incubation of the cultures but could be distinguished following colony development for several days. In addition, sequential positive cultures from an individual patient often yielded different species or different strains of the same species which again could not be detected upon initial observations of colony morphology. Procedures for the identification of the CoNS need to be improved, and microbiology laboratories should consider the use of more definitive identification procedures for the CoNS.

The human skin represents a diverse environment, and a number of Staphylococcus species have evolved to inhabit the many varied microenvironments found on this complex organ (8). These microorganisms are frequently isolated in cultures from a variety of clinical specimens. In many cases, staphylococci are “picked up” from the skin during specimen collection and are not involved in any disease process. In some cases, they have been shown to cause serious infections (4–7, 11–17).

As a group, the staphylococci are among the most frequently isolated microorganisms in the hospital microbiology laboratory. Often, identification of the staphylococci is limited to a rapid screening test for Staphylococcus aureus, and non-S. aureus isolates are simply reported as coagulase-negative staphylococci (CoNS). In fact, these CoNS isolates include a variety of species and many different strains.

In this paper, we present data concerning the species and strain distribution of CoNS isolates from a variety of culture types performed at a nonteaching, 400-bed, community hospital in Raleigh, North Carolina.

A companion paper in this journal describes a comparison of the Vitek Systems (bioMerieux Vitek, Hazelwood, Mo.) Staphylococcus identification card with standard methods (1).

MATERIALS AND METHODS

Clinical specimens. Clinical specimens from a variety of sites were collected by routine methods and sent to the laboratory for culture. A total of 499 CoNS isolated from these specimens were included in the study.

Staphylococcus identification. Staphylococci isolated from routine specimens were first screened with a latex agglutination test (AccuStaph, Scot Laboratories). AccuStaph-negative isolates were then identified with the Vitek Gram-Positive Identification (GPI) card (bioMerieux Vitek). After initial identification by the Vitek System, the isolates were maintained on P agar for testing by conventional methods or were stored by desiccation on sterile fish spine insulator beads at 4°C for later use. Isolates were subcultured one to three times. This minimal amount of transfer prevented the significant accumulation of clonal variance. The final identification, including strain differentiation, was performed at North Carolina State University by previously described methods (10). Strain identification was based on the same multiple biochemical tests used for the species identification together with antibiotic resistance patterns, the morphology of aged (>5-day) colonies, and, in some cases, plasmid profiles done by previously described methods (2). Generally, members of the same strain demonstrated identical colony morphologies, biochemical test patterns, and antibiograms. However, in some cases, a quantitative biochemical difference or the loss of a specific plasmid and its associated antibiotic resistance between isolates characterized as a single strain were observed. This minor variation was believed to represent clonal variation within a strain.

Additional data. Additional laboratory data including the colony count, isolation in pure culture, leukocytes in the urine, and urine leukocyte esterase, etc., were collected for each patient included in the study as appropriate, on the basis of the source of the specimen.

RESULTS

A total of 499 CoNS were identified. The species distribution can be seen in Table 1. Although this type of comprehensive study of CoNS isolates, including the new species and subspecies included in this study, has not been reported, others have found the same three species, S. epidermidis, S. haemolyticus, and S. hominis, to be the most common clinical isolates in that order (16). For each species, there were almost as many identifiable strains as there were isolates. The species distribution by culture type can be seen in Table 2. It should be noted that, subsequent to this study,
TABLE 1. Species distribution of CoNS isolated from a variety of clinical sources

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates*</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. auricularis</td>
<td>1 (1)</td>
<td>0.2</td>
</tr>
<tr>
<td>S. capitis</td>
<td>2 (2)</td>
<td>0.4</td>
</tr>
<tr>
<td>S. capitis subsp. capitis</td>
<td>10 (10)</td>
<td>2.0</td>
</tr>
<tr>
<td>S. capitis subsp. ureolyticus</td>
<td>8 (7)</td>
<td>1.6</td>
</tr>
<tr>
<td>S. cohnii</td>
<td>1 (1)</td>
<td>0.2</td>
</tr>
<tr>
<td>S. cohnii subsp. cohnii</td>
<td>1 (1)</td>
<td>0.2</td>
</tr>
<tr>
<td>S. cohnii subsp. urealyticum</td>
<td>2 (2)</td>
<td>0.4</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>37 (37)</td>
<td>7.4</td>
</tr>
<tr>
<td>S. hominis</td>
<td>14 (14)</td>
<td>2.8</td>
</tr>
<tr>
<td>S. lugdunensis</td>
<td>5 (5)</td>
<td>1.0</td>
</tr>
<tr>
<td>S. simulans</td>
<td>12 (12)</td>
<td>2.4</td>
</tr>
<tr>
<td>S. warner</td>
<td>20 (20)</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the numbers of different strains identified.

we have shown that some strains of S. lugdunensis may give a false-positive result when the AccuStaph latex screen is used. This means that some S. lugdunensis isolates may have been screened as coagulase positive and not included in this study population.

**Blood and urine isolates.** The largest number of isolates were from blood and urine cultures. Of 85 patients from whom CoNS were isolated from the blood, 6 patients had CoNS isolated from multiple blood cultures (Table 3). For two of these patients, the isolates were not the same species. For the remaining four patients, the same species was isolated, but strain identification revealed different strains from two of these patients. Only two patients had the same species and strain isolated from two separate blood cultures.

CoNS were isolated from the urine of 102 patients. Nine of these patients had more than one urine sample cultured. Seven of these patients had the same species grown from multiple urine samples. Of these seven, only one patient had the same strain grown from multiple urine samples. This was a strain of S. haemolyticus from a 70-year-old female. Both of her cultures had colony counts of >10,000 but <100,000. The relative percentage of S. haemolyticus isolates from urine, 24.2% (38 of 157), was significantly higher than the overall 13.4% occurrence (67 of 499) found in this study.

Of 158 isolates of CoNS from urine cultures, 29 gave colony counts of >100,000. A total of 94 isolates gave colony counts of >10,000 but <100,000. High colony counts did not consistently correspond to factors generally associated with infection.

Several other factors related to urine cultures, including isolation in pure culture, urine leukocyte esterase, and leukocytes in the urine, were also monitored. The factor that correlated most closely with S. saprophyticus isolates from urine cultures was a positive result from a urine leukocyte esterase test, found for four of five patients from whom this species was isolated. S. saprophyticus was found only in urine cultures. Others have shown S. saprophyticus to be associated with urinary tract infections (11, 12). In addition to S. saprophyticus, patients tended to have positive urine leukocyte esterase tests more often when S. lugdunensis, S. simulans, and S. haemolyticus species were isolated from their urine.

**Isolates from other sites.** The remainder of the CoNS were isolated from discharge, drainage, catheter, tissue, wound, ear, eye, spinal fluid, gastric, genital, lymph node, nose, and sputum cultures. From 170 patients, 27 cultures yielded mixed CoNS species and 26 additional cultures yielded mixed strains of the same species. In a few cases, more than one culture was done on a patient, and in one case, two separate CVP tip cultures yielded the same S. haemolyticus strain. S. lugdunensis was found more frequently in wound cultures and cultures of tissue, as has been reported in the literature (6).

**DISCUSSION**

The overall species distribution of CoNS isolated and identified in this study was similar to the normal distribution on the skin as previously reported (9). Within species, almost all isolates could be shown to be different strains. For example, for 322 isolates of S. epidermidis, it was possible to distinguish 320 different strains. For this study, a strain was...
considered to be "composed of cell populations of common or clonal origin" (8). The question of whether phenotypic variants should be considered separate strains or clonal variants from the same parent has been raised by Deighton et al. (3). It is our opinion that the majority of isolates with different colony morphologies found in our study represent different strains. Isolates showing different colony morphologies also demonstrated different biochemical patterns, different antibiotic resistance patterns, and/or different plasmid profiles. These strain differences could be of significance. When a single culture yields mixed strains or when multiple cultures yield different strains, it may be more likely that the mixed strains suggest contamination. However, true mixed infections with multiple strains cannot be ruled out. This important question needs further investigation.

The sites from which the organisms were isolated show some propensity of certain species for specific culture types. Further studies are needed to clearly demonstrate the clinical significance of the isolation of individual species from specific sites. In this study, identical strains of S. haemolyticus were isolated from successive urine cultures from a 70-year-old woman, suggesting that this species may cause urinary tract infections.

The repeated isolation of a microbial agent from multiple blood cultures from an individual patient has often been touted as an indication of infection. In evaluating CoNS isolated from blood cultures, it may be important not only to establish the repeated isolation of CoNS but to demonstrate the repeated isolation of the same species and strain. With these more stringent criteria, only 2 of the 85 patients in our patient population from whom CoNS were isolated from the blood would be considered to be infected. When critically evaluated, true septicemia caused by the CoNS and confirmed by the repeated isolation of the same species and strain may be less common than is often reported.

Sequential urine cultures are seldom performed. Isolation in pure culture together with high colony counts is generally accepted as an indicator of infection. In this study, many of the urine cultures with high counts grew multiple species or multiple strains. Because the colony morphologies of these mixed cultures were indistinguishable at 24 to 48 h, all colonies would erroneously be included in the count as if the colonies were a pure culture. It seems clear that the colony count alone, without determining whether the colonies represent multiple species or strains, is probably of limited value in assessing the clinical significance of CoNS isolated from urine cultures.

From the findings in this study, it is clear that one might expect to isolate a variety of species and many different strains of CoNS in the hospital laboratory. The frequent reporting of CoNS from a variety of clinical sources can lead to confusion for the clinician and often may result in unnecessary antibiotic treatment. A variety of clinical criteria, such as fever, elevated leukocytes, and presence of an invasive device, etc., has been suggested for assessing the need for treatment when CoNS are isolated. These criteria are often subjective and nonspecific and only suggest that the patient has an infection, without directly demonstrating the infection to be caused by the isolated organism.

To fully assess an individual staphylococcal isolate, the microbiology laboratory must be able to determine the species and strain of clinical isolates. Upon initial isolation, after overnight incubation, the colony morphologies of most of the species and strains of CoNS are indistinguishable. It is generally not possible to determine whether multiple colo-

nyes found on a primary isolation plate represent a single species or mixed species.

Species differentiation and strain differentiation currently require specialized testing including the observation of colony morphology after several days of incubation at 34 to 35°C followed by 2 days of growth at room temperature (2, 10). Since CoNS are seldom identified in clinical microbiology laboratories, the significance of mixed cultures with multiple species or strains is not known. Primary isolation media capable of demonstrating different species and more rapid procedures for strain identification may be needed to fully evaluate clinical isolates of CoNS. Procedures for the identification of the CoNS need to be improved, and microbiology laboratory workers should consider the use of more definitive identification procedures for the CoNS.

Until better procedures are available, laboratory workers may want to consider incorporating the procedure of incubating plates growing multiple colonies of staphylococci for 3 days and then keeping the plates for an additional 2 days at room temperature and making observations as to whether the cultures were pure or mixed on the basis of a close examination of colony morphology. Although this lengthy process does not provide clinically timely information, it may be of value in those cases in which empiric treatment fails and will help to enhance our recognition of the diversity of species and strains isolated from clinical materials.

ACKNOWLEDGMENTS

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REFERENCES


