Species Identification and Antibiotic Susceptibilities of Coagulase-Negative Staphylococci Isolated from Clinical Specimens

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Identification of potentially significant coagulase-negative staphylococci isolated from clinical specimens was performed along with antibiotic susceptibility determinations. *S. epidermidis* accounted for 75% of these isolates, with *S. haemolyticus* and *S. hominis* being the second and third most frequently encountered species, respectively. Although there were many instances of single blood culture isolations of questionable significance, all three species were also found in multiple blood cultures from individual patients, indicating the ability to cause significant bacteremia. The most common source for most species was blood, except for *S. saprophyticus* and *S. simulans*, which were found more frequently in urine. Of urinary tract isolates, however, *S. epidermidis* was more common than *S. saprophyticus*. Antibiotic susceptibility profiles demonstrated that *S. haemolyticus* and *S. epidermidis* were frequently multiply antibiotic resistant. *S. haemolyticus* had a higher percentage of isolates that were oxacillin, cephalothin, aminoglycoside, erythromycin, and clindamycin resistant than did *S. epidermidis*. We found that species identification could be of benefit for both epidemiological as well as patient care purposes, and that this additional information is readily available, using convenient and rapid new methods.

Progress within the last 10 years in the taxonomy of staphylococci has led to a wider acceptance of and interest in the variety of species of staphylococci other than *S. aureus* and *S. epidermidis*. It is now possible to rapidly identify the different species of staphylococci by methods practical for diagnostic laboratories. Because of this, more laboratories can assess what potential clinical or epidemiological benefits this additional information might have.

The Microbiology Service of the Clinical Center, National Institutes of Health, has for the past 2 years been doing species identification of coagulase-negative staphylococci isolated from blood, normally sterile body fluids, and other potentially significant sources, such as high-colony-count urine samples or intravenous (i.v.) catheter tips. We present here a summary of the various species that were encountered and a comparison of their antibiotic susceptibility patterns. How this information is currently being reported to the physician and in what instances this additional information may be useful are discussed.

**MATERIALS AND METHODS**

**Staphylococcal isolates.** All tube coagulase (EDTA rabbit plasma; BBL Microbiology Systems, Cockeysville, Md.)-negative staphylococci which were submitted for antibiotic susceptibility testing were also submitted for species identification. This included all isolates from blood, other normally sterile body fluids and tissues, high-colony-count urine samples (>10⁵ organisms per ml), i.v. catheter with tips >15 colonies, and the predominant or only organism in moderate to heavy amounts from wounds, abscesses, or similar cultures. One known isolate of each species was obtained from P. B. Smith, Centers for Disease Control, Atlanta, Ga., and antibiotic susceptibility data on these isolates were included along with those of the patient isolates.

**Identification.** Species identification was done initially by using the criteria of Kloos and Schleifer (6, 11). All isolates that were species other than *S. epidermidis* were later reevaluated by using the StaphIdent system (Analytab Products, Plainview, N.Y.). Isolates that were unidentified by either scheme or in which both schemes were in conflict are classified in this report as unidentified.

**Antibiotic susceptibilities.** Antibiotic susceptibility tests were performed by a microdilution method (14). Disk diffusion check plates were used to check for organism purity and to detect resistance to semisynthetic penicillins (oxacillin disk), which are sometimes missed by dilution methods. The summaries of the susceptibility data include only a single determination per species per patient.

**Beta-lactamase detection.** Acidometric determination of beta-lactamase production was performed by
using growth from around the oxacillin disk (induced). Testing of certain isolates that were negative by the acidimetric test was done with a chromogenic cephalosporin, nitrocefin (Cefinase; BBL Microbiology Systems), which may be a more sensitive indicator of beta-lactamase production.

RESULTS

Table 1 summarizes the number of isolates identified and the sources from which they were recovered. For the various isolates submitted for susceptibility testing and identification on the basis of the criteria described above, the most common source for all species combined was blood (50%), followed by wounds and abscesses (17%) and i.v. catheter tips (11%). Blood was also the most frequent source for each of the individual staphylococcal species with the exception of *S. saprophyticus* and *S. simulans*, both of which were seen most frequently in urine cultures. Twenty-five percent (171/678) of isolates were species other than *S. epidermidis*, with *S. haemolyticus* and *S. hominis* being the second and third most frequently encountered species. These were also the three most common species isolated from blood cultures by Eng et al. (2) and from wounds and body fluids by Sewell et al. (12). Six percent (41) of the isolates were classified as unidentified because they either did not conform well with a particular species or because the conventional tube identification was at variance with the Staph-Ident determination. Of these 41, 16 were similar biochemically and are discussed later.

Table 2 compares the susceptibility patterns of the different coagulase-negative species. As shown, 72 to 88% of *S. epidermidis*, *S. haemolyticus*, and *S. hominis* isolates were resistant to penicillin at minimum inhibitory concentrations (MICs) of greater than 0.05 μg/ml. Staphylococci with MICs of greater than 0.05 μg/ml are generally assumed to be penicillinase producers (3); isolates of these three species were tested acridometrically for beta-lactamase production and were positive. In contrast to these results, all five isolates of *S. saprophyticus* had MICs of greater than 0.05 μg/ml but equal to or less than 1.0 μg/ml, although they were beta-lactamase negative by both acidimetric and chromogenic cephalosporin tests. Both isolates of *S. cohnii* were also beta-lactamase negative despite MICs greater than 0.05 μg/ml. Four isolates of *S. simulans* had MICs of greater than 0.05 μg/ml but equal to or less than 3 μg/ml; one was positive and three were negative for beta-lactamase by both methods. All isolates with MICs of equal to or less than 0.05 μg/ml (for all species) were checked for beta-lactamase production, and two isolates (one *S. epidermidis* and one *S. capitis*) were found to be beta-lactamase producers.

Resistance to oxacillin was highest among isolates of *S. haemolyticus* (76%), followed by *S. epidermidis* (38%). All of the oxacillin-resistant isolates of *S. haemolyticus* showed distinct oxacillin resistance by microdilution methods (MICs of greater than 6 μg/ml), whereas 18 isolates of *S. epidermidis* and 1 isolate each of *S. hominis* and *S. capitis* were susceptible by MIC determinations but were observed to be resistant.

<table>
<thead>
<tr>
<th>Species</th>
<th>All sources</th>
<th>Blood</th>
<th>i.v. catheter tips</th>
<th>Wounds, abscesses, lesions, etc.</th>
<th>Urine</th>
<th>Sterile fluids</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em></td>
<td>507 (75)</td>
<td>253 (76)</td>
<td>61 (80)</td>
<td>94 (80)</td>
<td>29 (63)</td>
<td>39 (70)</td>
<td>31 (67)</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>49 (7)</td>
<td>20 (6)</td>
<td>7 (9)</td>
<td>12 (10)</td>
<td>4 (9)</td>
<td>5 (9)</td>
<td>1 (2)</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>36 (5)</td>
<td>22 (6)</td>
<td>3 (4)</td>
<td>1 (1)</td>
<td>0</td>
<td>8 (14)</td>
<td>2 (4)</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>19 (3)</td>
<td>13 (4)</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>4 (9)</td>
</tr>
<tr>
<td><em>S. capitis</em></td>
<td>13 (2)</td>
<td>5 (1)</td>
<td>2 (3)</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>4 (9)</td>
</tr>
<tr>
<td><em>S. simulans</em></td>
<td>7 (1)</td>
<td>1 (&lt;1)</td>
<td>0</td>
<td>2 (2)</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>5 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. cohnii</em></td>
<td>3 (&lt;1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Unidentified (Staph-Ident no. 4500 or 6500)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16 (2)</td>
<td>4 (1)</td>
<td>1 (1)</td>
<td>4 (3)</td>
<td>4 (9)</td>
<td>0</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Other unidentified</td>
<td>25 (4)</td>
<td>18 (5)</td>
<td>1 (1)</td>
<td>4 (3)</td>
<td>0</td>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>678</td>
<td>336</td>
<td>76</td>
<td>118</td>
<td>46</td>
<td>56</td>
<td>46</td>
</tr>
</tbody>
</table>

<sup>a</sup> Greater than 15 colonies.  
<sup>b</sup> Sole organism or in moderate-to-heavy amounts.  
<sup>c</sup> Greater than 10<sup>3</sup> organism per ml.  
<sup>d</sup> Includes peritoneal, pleural, synovial, and cerebrospinal fluids.  
<sup>e</sup> Includes a variety of specimen types such as bone marrow, tissue biopsies, bile, etc.  
<sup>f</sup> See the text for description.
to oxacillin by the lack of an inhibitory zone around the oxacillin disk. Oxacillin resistance in all other species was low.

*S. haemolyticus* was the most resistant species to both erythromycin (80%) and clindamycin (64%). *S. epidermidis* and *S. hominis* also showed a significant percentage of isolates resistant to both of these agents. The other species were more susceptible to both antibiotics, although more isolates were susceptible to clindamycin than to erythromycin as was also observed with *S. haemolyticus* and *S. epidermidis.*
Significant resistance to cephalothin was only seen with *S. haemolyticus* (76%), and this was paralleled by resistance to cefoxitin. Limited experience with cefotaxime also showed resistance as might have been expected. With the exception of one isolate of *S. cohnii*, all other species were susceptible to cephalothin and cefoxitin.

Resistance to gentamicin (76%) and tobramycin (76%) was greatest for *S. haemolyticus*, followed by *S. epidermidis* and *S. hominis*. For all other species, most isolates were susceptible. Although 36, 49, and 14% of *S. epidermidis*, *S. haemolyticus*, and *S. hominis* isolates, respectively, had MICs of greater than 16 to amikacin, all of these isolates were susceptible at 32 μg/ml and were therefore in an intermediate MIC range, which our laboratory classifies as moderately resistant (greater than 4 but less than 32 μg/ml).

Chloramphenicol resistance was low for most species, with *S. haemolyticus* having the highest percentage of resistance (42%). Tetracycline susceptibilities were variable among the different species, with over one-half of the isolates of *S. hominis* showing resistance.

All isolates were tested against vancomycin and showed 100% susceptibility at an MIC of equal to or less than 5 μg/ml.

**DISCUSSION**

A total of 678 coagulase-negative staphylococci were identified from patient specimens. These were selected because they had a potential of being clinically significant on the basis of source, amount of organism, or a combination of both parameters. Even with these guidelines it is difficult in many instances to assess the true relevance of these organisms. Twenty-five percent of these isolates were identified as species other than *S. epidermidis*. It was sometimes possible to use the species identification to help assess the significance of two or more blood cultures on a patient or a blood culture and an i.v. catheter tip. Species identification also verified the suspicion of two different colony types with different antibiograms from repetitive blood cultures on several patients. We observed one patient with multiple positive blood cultures containing a mixture of *S. epidermidis* and *S. hominis* and two other patients with repetitive blood cultures containing a mixture of *S. epidermidis* and *S. haemolyticus*. Two different species were also seen in several high-colony-count catheter tip cultures. *S. epidermidis*, *S. haemolyticus*, and *S. hominis* yielded blood culture isolates that were evaluated by the physicians as being clinically relevant, requiring antibiotic therapy or removal of i.v. lines or both. These judgments were usually based on the finding of more than one blood culture positive or clinical evidence of infection or both. Although 13 of the 19 isolates of *S. warneri* were from blood, none of these was readily apparent as being significant based on the finding of multiple blood cultures containing the same species. It is likely, however, that some of these were treated on the basis of clinical assessment alone.

Three of the seven *S. simulans* and all of the *S. saprophyticus* isolates were from urine. The association of *S. saprophyticus* with urinary tract infection is well documented (4, 8). In our hospital, however, 29 of 46 or 63% of coagulase-negative staphylococci isolated from urine with significant colony counts were identified as *S. epidermidis*, whereas only 11% of the isolates were *S. saprophyticus*. The *S. saprophyticus* isolates were all from women; information available on four of them showed that they were outpatients at the time of culture. Our findings are similar to those of Nicolle et al. (9), who found 70% *S. epidermidis* and 17% *S. saprophyticus*.

Of the 41 isolates that fell into the unidentified category, 16 of these were biochemically similar; in addition to being coagulase negative and susceptible to novobiocin, significant biochemical reactions were acid production from sucrose, maltose, mannose, and trehalose, variable reactions from mannitol and lactose, and the ability to reduce nitrate. These isolates had Staph-Ident profile numbers of either 4500 or 6500, suggestive of either *S. warneri* or *S. aureus*. Acid production from mannose and nitrate reduction are not typical of *S. warneri*. The Staph-Ident system does not include nitrate reduction in its battery of tests, which makes it more likely to identify these isolates as *S. warneri*. The distinction between this group of organisms and *S. warneri* is probably of less concern to clinical laboratories than to taxonomists, however, as long as the identification system (such as Staph-Ident) is able to identify these consistently and reproducibly.

The antibiotic susceptibility data have been interesting to us in several ways. The most obvious finding was observed in smaller numbers by others (10), that is, that all of the species, *S. haemolyticus* has the most antibiotic-resistant profile. Approximately 64 to 76% of our isolates of *S. haemolyticus* showed resistance to oxacillin, cephalothin (and cefoxitin), erythromycin, clindamycin, gentamicin, and tobramycin. Although *S. epidermidis* can also show significant multiple resistance patterns, in our hospital only 33 to 51% showed resistance to oxacillin, erythromycin, clindamycin, gentamicin, and tobramycin. An important distinction from *S. haemolyticus* is the lack of in vitro
coagulase-negative staphylococci the care of many cancer (formerly S. aureus that are resistant to the penicillinase-resistant penicillins such as oxacillin and methicillin should also automatically be regarded as resistant to the cephalosporins, this cross-resistance has not been a consistent finding for coagulase-negative staphylococci even when the usual techniques for enhancing cephalosporin resistance are used (7). Our in vitro data obtained by using conventional microdilution methods suggests that although oxacillin-resistant S. haemolyticus shows this cross-resistance, the other species do not. Studies by Karchmer et al. (5), however, with methicillin-resistant S. epidermidis isolates, showed their strains to have resistant subpopulations when tested with high inocula. Whether the in vitro cephalothin susceptible or resistant determination for oxacillin (or methicillin)-resistant coagulase-negative staphylococci is meaningful is still subject to question (5).

S. saprophyticus and S. cohnii isolates show relative resistance to penicillin at MICs which are traditionally associated with beta-lactamase production. However, all of our isolates of S. saprophyticus and S. cohnii were beta-lactama nonproducers by both acidometric and nitrocefin tests.

All isolates of all species tested showed susceptibility to vancomycin. Because of the relatively high percentage of resistance to oxacillin and also the question of cephalothin susceptibility, vancomycin has been used frequently in our hospital as the starting antibiotic for staphylococcal coverage until susceptibility determinations are available.

Our laboratory has been reporting the various species of staphylococci for the last 6 months. For most specimens, the isolation of coagulase-negative staphylococci represents only normal flora, and these isolates are not identified any further. We report these as Staphylococcus species, coagulase negative. For potentially significant isolates (e.g., from blood, other normally sterile fluids, etc.) where antibiotic susceptibilities are performed, we now identify these isolates by the Staph-Ident determination, along with the necessary additional tests, with the notation that these isolates were formerly called S. epidermidis. S. haemolyticus would thus appear in the physician's report as S. haemolyticus (formerly S. epidermidis).

For many hospitals, species identification of coagulase-negative staphylococci may not merit the additional time and resources needed. For hospitals such as ours which are involved in long-term care of many cancer patients, other seriously compromised patients, and cardiac surgery patients who may acquire significant infections with coagulase-negative staphylococci (1, 13), we are hopeful that species identification can be of use in furthering our understanding of the microbiology of the organisms as well as their role in disease. More specifically, an increase in antibiotic resistance among coagulase-negative staphylococci should now be looked at more critically as to whether there is an increase in the frequency of S. haemolyticus as opposed to a generalized increase in resistance among all species. Identification can sometimes also facilitate or at least add another parameter to help evaluate the significance (or lack) of sporadically positive blood cultures containing coagulase-negative staphylococci. We have also been able to correlate positive blood cultures with positive i.v. catheter tips containing S. haemolyticus and S. hominis, and, as mentioned above, we have documented mixed bacteremia with two species of coagulase-negative staphylococci. We may also begin to recognize particular potential infectious roles of the different species; for example, 12 of 49 (24%) S. haemolyticus isolates were obtained from wounds, abscesses, and lesions, whereas only 1 of 36 (3%) S. hominis isolates occurred from these sources. More information on the invasive potential of the different species may lead to a better understanding of how to interpret the significance of coagulase-negative staphylococci.

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LITERATURE CITED

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