Clinical Significance of Coagulate-Negative Staphylococci

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Although coagulate-negative staphylococci (C-NS) have been implicated in certain human infections, they are generally regarded as contaminants, and their clinical significance is questioned. To assess their role as pathogens, we studied 205 isolates of C-NS from wounds and body fluids (blood, urine, pleural and peritoneal fluids, etc.). Patient’s charts were reviewed, and, by using strict criteria, a determination was made regarding the clinical significance of these isolates. The organisms were then identified to determine whether certain species of C-NS were associated with specific infections. S. epidermidis sensu stricto accounted for 81% of the C-NS isolated. The frequencies of other species were: S. haemolyticus (6%), S. hominis (5%), S. capitis (4%), S. warneri (3%), and others (1%). Only two isolates were novobiocin resistant; neither was identified as S. saprophyticus. By using our criteria, 22% of the C-NS were considered to be clinically significant, and the majority of these (93%) was S. epidermidis. The most common source of the clinically relevant C-NS isolates was wounds. These data suggest that identification of C-NS species other than S. epidermidis may be of limited value in predicting clinical significance.

The coagulate-negative staphylococci (C-NS) historically have been regarded as saprophytes with little pathogenic potential; however, it is well known that under appropriate conditions, C-NS can produce serious human disease. C-NS are pathogenic when alterations in the integument allow these normal skin inhabitants to gain entry into the body, with resulting cerebrospinal fluid shunt infection (6, 20), prosthetic valve infection (1, 4), prosthetic joint infection (14, 23, 24), vascular graft infection (10), and occasionally natural valve endocarditis in intravenous drug abusers (16, 19). One species of C-NS (S. saprophyticus) has been reported to cause urinary infection in women (7, 11, 22). Other infections in which C-NS have been incriminated include peritonitis in patients receiving peritoneal dialysis (15, 18), infections associated with intravenous cannulae (17), skin abscesses (12), and eye and ear infections (3, 21).

In 1975 Kloos and Schleifer (8) described a simplified method to separate staphylococci into 11 taxonomic groups. We report an analysis of 205 consecutive isolates of C-NS, using this method of identification. Our purpose was to determine whether any particular species of C-NS was more likely to cause serious human infection and to assess the usefulness of this identification scheme in the clinical microbiology laboratory.

MATERIALS AND METHODS

Study design. A total of 205 consecutive isolates of C-NS obtained from specimens submitted from inpatients and outpatients at the Veterans Administration Medical Center during the calendar year 1981 were studied. The following criteria were required for inclusion: (i) C-NS were the only or predominant bacteria isolated; (ii) the culture was from body fluids (e.g., blood, cerebrospinal fluid, urine, pleural fluid, joint fluid, peritoneal fluid, etc.) or from a purulent, draining wound; (iii) clinical information was available to assess the significance of the culture. In all cases, the clinical records were reviewed, and the attending physician was queried for his opinion regarding the case; in selected cases, the patients were seen in consultation by a member of the Infectious Diseases Section.

Clinical relevance. Several factors were considered in determining the clinical significance of a C-NS isolate. These included the source of the culture, the relative numbers of organisms isolated, and the clinical findings in the patients. Organisms from a closed source such as blood, subarachnoid space, joint, or pleural space were considered more likely to be pathogenic than organisms isolated from an open source such as a wound. If organisms grew only in broth or if they grew only after 72 h of incubation, they were considered to be contaminants, although some may, in fact, have been pathogens.

The clinical findings which were considered to favor a significant infection included the presence of fever, local or generalized inflammation, and wounds which were fluctuant or drained purulent material. Associat-
ed laboratory findings included an elevated total leukocyte count with a granulocyte predominance and other indicators of infection such as the presence of leukocytes in cerebrospinal fluid or in the urine. In some instances, the patient’s response to specific therapy was used to judge clinical significance. The Centers for Disease Control criteria for determining the presence and classification of infections was used as a guide (5).

Microbiology. The identification of C-NS by the hospital microbiology laboratory was reconfirmed in our research laboratory. Once an organism was confirmed to be C-NS, it was further characterized by using the method of Kloos and Schleifer (8). This method uses 13 key characteristics, including: coagulase activity, hemolysis, nitrate reduction, and aerobic acid production from fructose, xylose, arabinose, ribose, maltose, lactose, sucrose, trehalose, mannitol, and xyitol. Lysostaphin susceptibility, phosphatase activity, and novobiocin susceptibility were also used to classify the isolates. The procedures for determining these characteristics were slightly modified from the original description and are summarized below.

Coagulase. Clumping factor and coagulase activity were determined by using the conventional slide and tube tests, respectively, with rabbit plasma (Difco Laboratories) (9).

Hemolysis. Hemolytic activity was demonstrated with human blood agar (5% blood in P agar). P agar consists of 1% peptone, 0.5% yeast extract, 0.1% glucose, 0.5% NaCl, and 1.5% agar (8).

Nitrate reduction. Nitrate reduction was detected by using the conventional tube test with nitrate agar (Difco Laboratories) (9).

Aerobic acid production from carbohydrates. Acid production from various carbohydrates was detected as previously described (8) under aerobic conditions, using an agar plate method. Final carbohydrate concentration in the agar medium was 1%.

Novobiocin susceptibility. Novobiocin susceptibility was determined by using 5-μg novobiocin susceptibility disks (BBL Microbiology Systems) on inoculated P agar plates. An 18-h broth culture of the isolate was swabbed on a P agar plate, and a novobiocin disk was applied. The susceptibility plate was then incubated at 35°C for 18 to 24 h. Strains that had a zone of inhibition of 16 mm or greater were considered sensitive.

Lysostaphin susceptibility. Lysostaphin (Schwarz-Mann) susceptibility of strains was determined on P agar plates containing 50 μg of lysostaphin (220 U/mg) per ml of P agar. A loopful of a saline suspension was inoculated on the surface of a P agar plate containing lysostaphin and on a control P agar plate without lysostaphin. Up to 16 strains could be tested on a plate. Cultures were incubated for 24 h and then examined for growth.

RESULTS

Coagulase-positive staphylococci. Seven cultures identified as C-NS by the hospital microbiology laboratory on the basis of the tube coagulase test were found on retesting to be coagulase positive by the clumping factor (slide) test (9). All seven of these isolates were felt to be clinically significant. Most of these isolates were pigmented, produced hemolysis on sheep or human blood agar, and thus were reclassified as S. aureus (these are not included in the data). This 4% rate of isolation of tube coagulase-negative S. aureus emphasizes the need for accurate performance of both tests for differentiating staphylococci.

Sources of C-NS isolates. The sources of C-NS isolates are shown in Table 1. Drainage from wounds was the most frequent source of C-NS (43%), followed by blood (26%), urine (12%), peritoneal fluid (9%), cerebrospinal fluid (8%), joint fluids (1%), and pleural fluid (1%). In each instance, S. epidermidis was the most common C-NS species isolated. Other species were infrequently isolated from each of these sources.

Clinical significance. When the clinical findings were matched with the cultures of C-NS, it became clear that most were contaminants (Table 1). Only 41 of 198 (21%) cultures were considered by our criteria to be clinically significant. It was apparent that the source of the C-NS culture was important. A total of 24 of 84 (29%) wound cultures and 10 of 18 (55%) cultures from peritoneal fluids were felt to be clinically significant; all of the patients with peritonitis were undergoing peritoneal dialysis.

### Table 1. Distribution of 198 isolates of C-NS

<table>
<thead>
<tr>
<th>Species</th>
<th>Blood</th>
<th>Urine</th>
<th>Cerebrospinal fluid</th>
<th>Peritoneal fluid</th>
<th>Pleural fluid</th>
<th>Joint</th>
<th>Wound</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>40 (3)*</td>
<td>22 (3)</td>
<td>12 (0)</td>
<td>15 (10)</td>
<td>1 (0)</td>
<td>2 (1)</td>
<td>69 (21)</td>
<td>161 (38)</td>
</tr>
<tr>
<td>S. xylosus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cohnii</td>
<td>2 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. capitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. warneri</td>
<td>4 (0)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>4 (0)</td>
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<td></td>
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<tr>
<td>S. hominis</td>
<td>4 (0)</td>
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<td></td>
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<tr>
<td>S. simulans</td>
<td>1 (0)</td>
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</tr>
</tbody>
</table>

* Clinically significant isolates are shown within parentheses.
with Tenhoff catheters in place. In contrast, only 3 of 52 (5%) blood cultures and 3 of 24 (12%) urine cultures were judged to be clinically important. The three significant C-NS blood cultures represented bacteremia (two secondary to intravenous catheters, one secondary to a Swan-Ganz catheter), and all were due to S. epidermidis. The three significant C-NS urinary tract infections involved two patients with cystitis secondary to transurethral resection of the prostate, and one patient with community-acquired cystitis probably secondary to lower-tract obstructive syndrome. All of these were also caused by S. epidermidis.

There were 16 C-NS cultures from cerebrospinal fluids, 12 of which were identified as S. epidermidis; none was judged clinically important and probably represented contamination due to inadequate preparation of the skin at the time of performing the lumbar puncture. Two pleural fluid samples contained C-NS; neither was felt to represent clinical empyema. One of two S. epidermidis joint fluid isolates represented community-acquired bursitis, whereas the other appeared to be a contaminant.

Identification of C-NS. Overall, of the 198 isolates, 161 (81%) were identified as S. epidermidis sensu stricto (Table 1); of these, 38 were considered to be of clinical importance. All other species accounted for only 37 isolates, with S. haemolyticus, S. hominis, and S. capitis being the most common; however, of these isolates, only 3 were felt to be of clinical significance. Two isolates were novobiocin resistant, but neither was identified as S. saprophyticus; both were identified as S. hominis.

DISCUSSION

The ability of C-NS to cause certain infections is well documented (1, 6, 7, 20, 22, 24); however, most laboratory cultures of C-NS are still considered to be contaminants of little clinical importance. Methods are now available to identify species of C-NS, but few studies have correlated specific C-NS with clinical infections (11, 13). Our data indicate that most C-NS are not clinically significant. Only 41 of 198 (21%) consecutive cultures which met our criteria were judged to signify relevant clinical infection. Moreover, significant infections due to C-NS were most often associated with material obtained from skin bacteria such as wounds or peritoneal fluid in patients with chronic indwelling peritoneal dialysis catheters. We have previously shown that peritonitis and catheter exit site infections are most commonly due to either S. aureus or C-NS which presumably originate from the patient’s nose or skin (C. M. Sewell, J. E. Claridge, C. Lacke, E. J. Weinman, and E. J. Young, Clin. Res. 29:818A, 1981). The next most frequent source of C-NS was blood culture; however, only 3 of 52 (5%) were judged to be clinically significant, and all were S. epidermidis. This compares favorably with the study by Marsik and Brake (11) in a different hospital population; they found that only 6 of 84 (7%) C-NS isolates from blood were clinically relevant, and all were due to S. epidermidis.

Of 24 urine cultures that grew C-NS, only 3 (12%) were associated with clinical infection. This contrasts with the study of Marsik and Brake (11), in which 15 of 64 (23%) C-NS in urines were significant. In that study, a colony count of 100,000 or more cells per ml of urine was considered significant but no mention was made of clinical symptoms, pyuria, etc. In our study, symptoms of urinary tract infection, leukocytes in the urine sediment, as well as a urine colony count of 100,000 or more cells per ml were necessary for clinical significance. By using these more stringent criteria, we may have underestimated the incidence of C-NS urinary tract infections, but there is less doubt regarding the relevance of C-NS in the urine of patients whose infections were deemed clinically significant. We did not isolate S. saprophyticus from the urine of any of our patients, whereas Marsik and Brake found S. saprophyticus to be the most common C-NS isolated from the urine (80%) (11). This is probably explained by the different patient populations studied. S. saprophyticus has been reported to cause urinary infection primarily in young women (7, 22); our patients were predominantly older men treated in a Veterans Administration Hospital.

A surprisingly large number of cerebrospinal fluid samples contained C-NS (8%) but none was associated with clinical or laboratory evidence of meningitis. This, too, attests to the frequency of contamination with these organisms when performing lumbar puncture. Moreover, we had no patients with cerebrospinal fluid shunts or other drainage devices which have been the most common sources of C-NS infections of the central nervous system (6, 20).

When C-NS isolates were further identified, it was found that S. epidermidis sensu stricto was the most common species isolated. This confirms the reports of Brun et al. (2) and Nord et al. (13) who also found S. epidermidis to be the most commonly identified clinical species of C-NS. It is also in accord with the study of Marsik and Brake (11), in which S. epidermidis caused all of the bacteremias and the majority of infections of bone, joints, and wounds. Our results differ from those of Marsik and Brake only in regard to urine, in which they found S. saprophyticus to be the most common C-NS isolated from urinary tract infections.
Although the numbers of cultures are small and the results must be considered in relation to the population studied (elderly men), the infrequent occurrence of C-NS other than *S. epidermidis* suggests that routine identification of species of C-NS may be of limited usefulness for predicting their clinical significance.

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LITERATURE CITED