Clinical Importance of Identifying Coagulase-Negative Staphylococci Isolated from Blood Cultures: Evaluation of MicroScan Rapid and Dried Overnight Gram-Positive Panels versus a Conventional Reference Method

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We evaluated the clinical usefulness of species identification of blood isolates of coagulase-negative staphylococci as a predictor of the clinical significance of the isolates. In addition, we compared results of species identification obtained with MicroScan Rapid Gram-Positive Identification panels and Dried Overnight (Conventional) Gram-Positive Identification panels with those obtained by a tube reference method. Two hundred eighty-five blood isolates were tested, including 92 judged to represent true bacteremia and 193 judged to represent contamination. The most common species detected were Staphylococcus epidermidis, Staphylococcus hominis, and Staphylococcus haemolyticus. These three species accounted for nearly 98% of the clinically significant isolates and 89% of the contaminants. The isolation of other species almost always represented contamination. However, identification of the three most common species did not help distinguish pathogens from contaminants. Both the Rapid and the Dried Overnight Gram-Positive panels identified S. epidermidis strains accurately, but the panels performed less well for the other species. Analysis revealed that S. hominis was frequently misidentified due to the presence of a previously unknown subspecies. Based on the initial results, revised investigational Dried Overnight Gram-Positive Identification panels (CPID-2) were prepared and tested. The CPID-2 panels identified 85 to 95% of S. epidermidis strains, 76 to 86% of S. hominis strains, and 88 to 92% of S. haemolyticus strains with high probability (>85%) and, overall, represented a significant improvement over the other panels for identification of these staphylococcal species.

Coagulase-negative staphylococci (CoNS) are the most frequently isolated microorganisms in blood cultures, but 85% of isolates are contaminants, usually as a result of skin contamination at the time blood is obtained (15). Despite their frequency as contaminants, CoNS have become important nosocomial pathogens, in part because of the increased use of medical devices such as long-term indwelling intravenous catheters and other access devices, vascular grafts, and prosthetic heart valves and joints. Indeed, some hospitals now report CoNS to be among the most common etiologic agents of nosocomial bacteremia (1, 9, 15).

Many clinical microbiology laboratories do not identify CoNS to the species level even when these microorganisms are detected in blood or cerebrospinal fluid. However, as the pathogenic significance of CoNS increases, it may become more important to learn more about the epidemiology and pathogenic potential of individual species. This may be particularly important with regard to blood culture isolates, since it is often difficult to determine the clinical significance of an individual isolate. Numerous commercial systems and kits are now available for species identification of CoNS. In this evaluation, we sought to determine, based on species identification or possible biochemical profile, whether 92 CoNS blood isolates judged to be clinically important (15) could be differentiated from 193 strains determined to be contaminants. In addition, we examined the abilities of MicroScan Rapid Gram-Positive Identification panels and Dried Overnight (Conventional) Gram-Positive Identification panels to identify CoNS to the species level compared with a conventional reference method (8).

Blood cultures were obtained from patients with suspected bacteremia at Robert Wood Johnson University Hospital, New Brunswick, N.J., and Duke University Medical Center, Durham, N.C. Based on published criteria (14, 15), all CoNS isolated from positive cultures were judged to be pathogens or contaminants by an infectious disease physician. The variables assessed included each patient's history and physical exam results, hospital course, results of laboratory and imaging studies, and results of cultures from other sites. One isolate from each episode was stored at −70°C until testing. Ninety-two clinically significant isolates and 193 contaminants obtained from 1992 through 1995 were included for testing.

Prior to testing, isolates were subcultured twice on tryptic soy agar with 5% sheep blood to ensure purity and viability. The Rapid Gram-Positive Identification panels and the Conventional Gram-Positive Identification panels were tested concurrently according to the manufacturer's instructions. Bacterial suspensions for each panel were prepared from well-isolated colonies from a tryptic soy agar plate with 5% sheep blood incubated at 35°C for 18 to 24 h in a non-CO2 incubator.

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Instrumented incubation and identification was done with the WalkAway-40 system. Identification results were determined from the V.20.30 database. All instrumented testing was done in the laboratory of one of the investigators (M.P.W.). At the time isolates were set up for instrument identification, a coded subculture was sent to MicroScan for reference testing.

Reference testing utilized the scheme of Kloos and Schleifer (8) with modifications as noted. Conventional tests included tube coagulase; l-tryptophan; β-naphthylamide hydrolysis; nitrate reduction; acetoin production; urease production; arginine decarboxylation; and fermentation of arabinose, lactose, maltose, mannitol, sucrose, trehalose, xylose, and mannose. Additional biochemical tests were performed as needed. These included ornithine decarboxylase testing by the tube method and phosphatase, β-glucosidase, β-glucuronidase, and β-galactosidase testing by the STAPH-IDENT (bioMerieux Vitek, Inc., Hazelwood, Mo.) method. Isolates were also tested for susceptibility to bacitracin (0.04 and 10 U), furazolidone (100 μg), polymyxin B (100 U), and novobiocin (5 μg). Disk diffusion susceptibility testing was done on tryptic soy agar with 5% sheep blood. Subsequently, for some Staphylococcus hominis strains, testing was done on Mueller-Kprime-tionate agar (7).

Quality control was performed weekly during the evaluation with the appropriate quality control organisms for each panel type. Quality control testing was performed on the conventional test methods concurrently with the test isolates.

Species identification was based on the criteria of Kloos and Schleifer (8). Strains that could not be identified with confidence were sent to one of the investigators (W.K.) for expanded testing. The result obtained was considered the definitive reference result.

Instrument identification was categorized as high-probability agreement if the MicroScan result listed the reference identification as the first choice with a probability of ≥85%. Identification was categorized as low-probability agreement if the MicroScan result listed the reference identification at any probability of <85%. If the MicroScan panel did not list the reference identification at all or if the instrument listed at high probability a species other than the reference identification, the result was categorized as incorrect.

After initial testing, isolates were retested at MicroScan on a revised investigational Dried Overnight Gram-Positive Identification panel (CPID-2), which is similar in biochemical format to the Dried Overnight Gram-Positive Identification panel (CPID-1) currently available. The CPID-2 panel contains modifications to 15 tests, including all carbohydrate tests, to optimize reactions. The identification database was revised to improve accuracy and to provide the option of identifying the following to the subspecies level: Staphylococcus cohnii subsp. cohnii and Staphylococcus cohnii subsp. urealyticum, Staphylococcus capitis subsp. capitis and Staphylococcus capitis subsp. urealyticum, and Staphylococcus hominis subsp. hominis and a newly described subspecies, Staphylococcus hominis subsp. novobiocinicus (6). Seven species that are very rarely found in human clinical infections, including Staphylococcus equorum (3a), were deleted from the database.

Finally, a subset of 40 Staphylococcus epidermidis isolates, 26 Staphylococcus haemolyticus isolates, and 33 S. hominis isolates was tested in the laboratory of one investigator (M.P.W.) with the CPID-2 panels to validate the results obtained at MicroScan.

Where appropriate, statistical analyses with Fisher’s exact test were undertaken to assess differences in species identifications obtained with the different MicroScan panels.

Eleven different species of CoNS were isolated from the blood (Table 1). The most common overall were, in order of decreasing frequency, S. epidermidis, S. hominis, and S. haemolyticus. These three species accounted for 97.8% (76.1, 15.2, and 6.5%, respectively) of the isolates causing bacteremia. Of the contaminant CoNS, these three species accounted for 89% of the isolates (S. epidermidis, 60.6%; S. hominis, 18.1%; and S. haemolyticus, 10.4%). The range of species causing bacteremia was much narrower than that of species that were contaminants. Only one strain each of S. capitis and Staphylococcus lugdunensis caused bacteremia, whereas S. cohnii, Staphylococcus auricularis, Staphylococcus simulans, Staphylococcus caprae, Staphylococcus sciuri, and Staphylococcus warneri were isolated as blood contaminants. Eight of 14 clinically significant isolates of S. hominis (57.1%) belonged to the proposed new subspecies S. hominis subsp. novobiocinicus, whereas 5 of 35 S. hominis contaminants (14.3%) belonged to this proposed new subspecies.

All 285 CoNS strains were tested on the Rapid and the Conventional (CPID-1) MicroScan panels. Both panels performed well in identifying S. epidermidis but not as well in identifying less commonly isolated CoNS (Table 2). The Rapid panel identified 95.7% of the S. epidermidis isolates with high probability, significantly better than the CPID-1 panel, which identified 85.6% with high probability (P < 0.001). Both of these panel types failed to identify with high probability the majority of S. hominis isolates. This was due in part to a previously unknown subspecies, S. hominis subsp. novobiocinicus, which was novobiocin resistant and failed to ferment trehalose. Of the 49 S. hominis strains isolated, 13 (26.5%) were determined to be members of this subspecies.

The isolates were retested at MicroScan with the investigational CPID-2 panels and revised V.22 database. Two S. epidermidis isolates from the initial testing were unavailable for testing on the CPID-2 panels. The revised panel identified 95.1% of S. epidermidis isolates at high probability, which was significantly better than the CPID-1 panel (P < 0.001) but not significantly different from the Rapid panel results for this species. High-probability identification of S. hominis and S. haemolyticus with the CPID-2 panels was significantly improved in comparison with both the CPID-1 and Rapid panels (P < 0.001) (Table 2).
the isolates tested were identified at high probability, and an additional 21.2% were identified at low probability.

This study was designed to address several issues of importance to clinicians and microbiologists with regard to CoNS. First, we wished to determine whether the species of CoNS found to cause true bacteremia differed substantively from those of CoNS isolated as blood contaminants. Although S. epidermidis, S. hominis (both subspecies), and S. haemolyticus accounted for nearly 98% of CoNS isolates found to be clinically significant in blood cultures in the recent study, the same three species accounted for 89% of contaminants. Therefore, the presence of one of these three species in blood cultures cannot be used to distinguish clinically important bacteremia from contamination. On the other hand, since species other than S. epidermidis, S. hominis, and S. haemolyticus were extremely rare causes of bacteremia, the argument can be made that the isolation of these other species from blood likely represents contamination. While this may be true in most instances, there is documentation in the literature from blood cultures, especially in laboratories that do not have molecular typing capabilities on site or immediately available.

A second major issue we wished to address in this study was the accuracy of MicroScan Rapid Gram-Positive Identification panels and Dried Overnight (Conventional) Gram-Positive Identification panels for the identification of clinical isolates of CoNS from blood cultures. Although two reports (3, 12) have shown that the systems accurately identify strains of S. epidermidis but are less accurate in identifying other CoNS species (2, 5, 10), the results of the current study support the latter reports. Whereas the Rapid and the Dried Overnight (CPID-1) panels as currently marketed identified strains of S. epidermidis with high probability in the great majority of instances (95.7 and 85.6%, respectively), they identified S. hominis strains and S. haemolyticus strains with high probability only half the time or less. In the case of S. hominis, this was due in part to the presence of the previously unknown subspecies, S. hominis subsp. novobiosepticus, which was consistently identified as S. equorum by the currently marketed MicroScan database.

Based on the initial results of this study, the CPID-2 panels were developed and tested both at MicroScan and independently in the laboratory of one of the investigators (M.P.W.). As shown in Table 2, the changes resulted in substantial improvements in the ability of this panel to identify strains of S. hominis and S. haemolyticus. The revisions to the conventional overnight panels have been incorporated into the commercially available MicroScan panels and revised V.22 software for MicroScan instruments. The isolation of CoNS from blood cultures remains a clinical dilemma in many cases (11, 13, 15), and physicians and microbiologists often cannot determine with certainty the clinical significance of these isolates. When only a single blood culture grows a CoNS strain, the isolate virtually always represents contamination (15). However, when two or more blood cultures grow CoNS, full identification and susceptibility testing may be worthwhile. If the isolated strains have the same biochemical profile and an identical expanded antibiogram, it is likely that the strains are identical, although only molecular methods can provide proof. This finding increases the probability that the microorganisms represent clinically significant bacteremia. By contrast, if the biochemical profiles and antibiograms differ, the strains are far more likely to represent contamination. Given the clinical uncertainties associated with the isolation of CoNS from blood, the additional information provided by full identification when more than one blood culture grows these strains may help clinicians in patient management.

In conclusion, the results of this study at two university medical centers showed that the overwhelming majority of CoNS isolated from blood cultures (98% of clinically significant isolates and 89% of contaminants) represented three spe-

### Table 2: Species identification of CoNS with three types of MicroScan panels

<table>
<thead>
<tr>
<th>Species</th>
<th>Rapid Gram-Positive Identification panels</th>
<th>Dried Overnight Gram-Positive Identification panels (Conventional)</th>
<th>Revised Conventional Gram-Positive Identification panels (CPID-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>No. (% of total) of isolates identified*</td>
<td>No. of isolates</td>
</tr>
<tr>
<td>S. epidermidis/</td>
<td>187</td>
<td>179 (95.7)</td>
<td>5 (2.7)</td>
</tr>
<tr>
<td>S. hominis/</td>
<td>49</td>
<td>16 (32.7)</td>
<td>14 (28.6)</td>
</tr>
<tr>
<td>S. haemolyticus/</td>
<td>26</td>
<td>13 (50.0)</td>
<td>5 (19.2)</td>
</tr>
<tr>
<td>S. capitis/</td>
<td>8</td>
<td>6 (75.0)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>S. lugdunensis/</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>S. cohnii/</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>S. auricularis/</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. simulans/</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S. caprae/</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. scuiri/</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. warneri/</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*HP, correct at high probability (≥85%); LP, correct at low probability (<85%); I, incorrect.
cies: \(S. \text{ epidermidis} \), \(S. \text{ hominis} \), and \(S. \text{ haemolyticus} \). Thus, routine species identification is not likely to assist in determining the clinical significance of these strains. In addition, this study demonstrated problems with MicroScan Rapid and Dried Overnight panels in identifying \(S. \text{ hominis} \) and \(S. \text{ haemolyticus} \). The revised conventional panel (CPID-2) developed as a result of this study represents a substantial improvement of the MicroScan system.

REFERENCES
3a. Kloos, W. Personal communication.