Comparison of the MicroScan System with the API Staph-Ident System for Species Identification of Coagulase-Negative Staphylococci

ZAFAR HUSSAIN,1,* LUBA STOAKES,1 DONALD L. STEVENS,2 BEREND C. SCHIEVEN,1 ROBERT LANNIGAN,1 AND COLINA JONES2

Department of Clinical Microbiology, Victoria Hospital Corporation, London, Ontario N6A 4G5; and Microbiology Laboratory, Department of Laboratory Medicine, St. Joseph's Hospital, Hamilton, Ontario N8Z 3Z5. Canada

Received 12 July 1985/Accepted 19 September 1985

To evaluate the accuracy of the MicroScan System (American Hospital Supply Corp., Sacramento, Calif.) for identification of coagulase-negative staphylococci, we tested 175 clinical isolates of coagulase-negative staphylococci. The results obtained by the MicroScan system were compared with those of the API Staph-Ident system (Analytab Products, Plainview, N.Y.). Forty-three discrepancies between the two systems were resolved by the conventional method of Kloos and Schleifer (W. E. Kloos and K. H. Schleifer, J. Clin. Microbiol. 1:82–88, 1975). The MicroScan and the Staph-Ident systems correctly identified 146 (86.4%) and 154 (88%) of 175 strains, respectively. The API system failed to identify phosphatase-negative Staphylococcus epidermidis. The MicroScan system demonstrated the greatest accuracy in the identification of S. epidermidis and S. saprophyticus, whereas lesser accuracy was achieved with S. hominis, S. warneri, and S. sciuri.

Coagulase-negative staphylococci (C-NS) were formerly considered to be nonpathogenic; however, recently they have been implicated in a variety of infections in both immunocompromised and otherwise healthy individuals (4). This has led to increased interest in the identification of C-NS, particularly since some species of staphylococci encountered in clinical material are highly resistant to various antibiotics (3). MicroScan (American Hospital Supply Corp., Mahwah, N.J.) has marketed new panels for the identification and MIC determination of aerobic gram-positive cocci and Listeria spp. (positive combination). The system now uses autoSCAN-4, which is an improved version of autoSCAN-3, to read and interpret MicroScan panels. This study was undertaken to evaluate the accuracy of the MicroScan system of C-NS identification. The results obtained with the MicroScan system were compared with those of the API Staph-Ident System (Analytab Products, Plainview, N.Y.).

MATERIALS AND METHODS

In total, 175 C-NS isolates were tested and included the following species: Staphylococcus epidermidis, 42; S. saprophyticus, 35; S. warneri, 23; S. haemolyticus, 17; S. hominis, 17; S. simulans, 13; S. capitis, 12; S. cohnii, 8; S. sciuri, 5; and S. xylosus, 3. For quality control the following American Type Culture Collection (ATCC) strains were tested: S. aureus ATCC 25923; S. epidermidis ATCC 14990; S. cohnii ATCC 29974; S. haemolyticus ATCC 29970; S. intermedius ATCC 29663; S. sciuri ATCC 29062; S. warneri ATCC 27836; S. xylosus ATCC 29971; S. saprophyticus ATCC 15305, and S. hominis ATCC 27844.

All strains were isolated from human clinical sources. All isolates were grown on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% horse blood (BA) at 35°C for 18 to 24 h. Stock cultures were stored in buffered glycerol broth at −70°C. Working cultures were maintained on BA slants and were transferred every 6 to 8 weeks. All isolates were confirmed as CNS by Gram stain, catalase, slide and tube coagulase with citrated rabbit plasma (BBL Microbiology Systems), and acid production from glycerol in the presence of erythromycin (0.4 μg/ml), as described by Schleifer and Kloos (7).

MicroScan system. MicroScan panels for the identification and susceptibility testing of gram-positive organisms (positive combination) are supplied and stored frozen. The panels use 27 tests for identification of the Micrococcaceae and the Streptococcaceae. Of this, the following 18 tests are utilized for the identification of members of the Micrococcaceae: susceptibility to crystal violet, 0.05 μg of bacitracin (Micrococcus screen) per ml and 1.6 μg of novobiocin and optochin per ml; fermentation of raffinose, lactose, trehalose, and mannose; production of beta-D-glucuronidase, beta-D-galactopyranosidase, urease, indoxyl phosphate, and alkaline phosphatase; hydrolysis of pyrrolidonyl-beta-naphthylamide, and 40% bile esculin; Voges-Proskauser; dehydrogenization of arginine; and reduction of nitrate.

Panels were removed from freezer storage and were allowed to thaw at room temperature. The panels were inoculated by stationary-phase technique according to the manufacturer's instructions. With a sterile loop, 4 to 5 large or 5 to 10 small colonies were picked from BA plates. The colonies were emulsified in 0.5 ml of inoculum broth (Todd-Hewitt broth with 0.1% TWEEN 80) and were incubated for 4 to 6 h at 35°C. Of the bacterial suspension, 50 μl was mixed into 25 ml of inoculum water (sterile distilled water with 0.02% TWEEN 80). If not turbid the inoculum broth was further incubated for 12 to 18 h before processing. The inoculum water was poured into a sterile disposable plastic trough. The 95-pronged lid of the trough was used to inoculate a previously thawed panel. According to the manufacturer this system transfers 5 μl of inoculum to each microwell of the panel, providing a final suspension of 105 CFU/ml. Arginine and urease wells were overlaid with mineral oil. The panels were then incubated in stacks three to five high overnight at 35°C in air. The panels were read on
the MicroScan instrument (autoSCAN-4). Correctness of automated reading was checked by visual reading and appropriate changes were made if necessary. The reagents for pyrrolidonyl-beta-naphthylamide, Voges-Proskauer, and nitrate were added in accordance with the product insert. Reagents were added only to the panels which showed three carbohydrate or the beta-D-galactopyranosidase reaction as positive; otherwise the panels were reincubated for an additional 18 to 24 h. Whether the reagents were added after 24 or 48 h, MICs, indoxyl phosphatase, and alkaline phosphatase results were recorded only after 18 to 23 h of incubation. The biochemical profile produces a six-digit code number that autoSCAN compares with its data base and identifies the organism. Profile numbers resulting in a "rare biotype" response were telephoned to an autoSCAN central computer for identification. Identiﬁcations were considered correct only if the probability value was ≥85 as speciﬁed by the manufacturer.

API Staph-Ident System. The API Staph-Ident system consists of 10 microcupules with dehydrated substrates. The system was used according to the instructions of the manufacturer. Growth from a BA plate was removed and suspended in 5 ml of 0.85% saline solution (API) to obtain turbidity equivalent to no. 3 McFarland standard. Each microcupule was inoculated with 2 drops of the organism suspension and was incubated for 5 h at 35°C in ambient air. After incubation the results of the first nine tests were recorded on the API report sheet. Two drops of Staph-Ident reagent were added to the β-galactosidase microcupule and the reaction was recorded after 30 s. A four-digit code number was obtained from the biochemical proﬁle of each isolate. The profle number was looked up in the Staph-Ident profle register for the isolate identification. If the profle number was not listed, the manufacturer’s computer data base was consulted by telephone.

Conventional identiﬁcation. All isolates showing discrepancies in the classiﬁcation of MicroScan and API Staph-Ident systems were identiﬁed by Kloos and Schleifer’s simpliﬁed scheme (5). For the purpose of this study, the results of the conventional system were considered to be correct. A number of strains required supplemental testing for novobiocin susceptibility, acid production from xylose and arabinose, and production of coagulase for complete identiﬁcation by the API system. Of these strains the required tests were performed, using the technique of Kloos and Schleifer. Novobiocin susceptibility was performed with P-agar with 1.6 μg of novobiocin per ml as previously described (5).

RESULTS

A total of 175 staphylococci were used in this study. The species identiﬁcations obtained by the API Staph-Ident and the MicroScan systems were compared. In the classiﬁcation of 132 (75.4%) strains, there was agreement between the two systems. A 43 discordant results were resolved by the conventional scheme of Kloos and Schleifer. The API and MicroScan correctly identiﬁed 22 and 14 of these strains, respectively. Both systems misidentiﬁed the remaining seven C-NS. Table 1 demonstrates the numbers and percentages of correct identiﬁcations by the API and MicroScan systems for each species.

The species most frequently misidentiﬁed by Staph-Ident was S. epidermidis. Ten of 11 misidentiﬁed S. epidermidis strains were classiﬁed as S. hominis and eight of these were phosphatase negative by both the API and the conventional systems. The Staph-Ident strip alone could not identify

<table>
<thead>
<tr>
<th>C-NS species</th>
<th>No. of isolates tested</th>
<th>By Staph-Ident</th>
<th>By MicroScan after 24 h of incubation</th>
<th>By MicroScan after 48 h of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>42</td>
<td>31 (73.8)</td>
<td>18 (42.0)</td>
<td>41 (97.6)</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>35</td>
<td>34 (97.1)</td>
<td>24 (68.5)</td>
<td>34 (97.1)</td>
</tr>
<tr>
<td>S. warneri</td>
<td>23</td>
<td>18 (78.3)</td>
<td>3 (13.0)</td>
<td>16 (69.5)</td>
</tr>
<tr>
<td>S. hominis</td>
<td>17</td>
<td>15 (88.2)</td>
<td>3 (17.0)</td>
<td>6 (35.2)*</td>
</tr>
<tr>
<td>S. haemolyticus</td>
<td>17</td>
<td>16 (94.0)</td>
<td>3 (17.0)</td>
<td>14 (82.3)*</td>
</tr>
<tr>
<td>S. simulans</td>
<td>13</td>
<td>12 (92.3)</td>
<td>7 (53.0)</td>
<td>12 (92.3)</td>
</tr>
<tr>
<td>S. capitis</td>
<td>12</td>
<td>12 (100)</td>
<td>0</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td>S. cohnii</td>
<td>8</td>
<td>8 (100)</td>
<td>0</td>
<td>7 (87.5)</td>
</tr>
<tr>
<td>S. sciuri</td>
<td>5</td>
<td>5 (100)</td>
<td>2 (40)</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>3</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
</tr>
</tbody>
</table>

* Two further strains were correctly identified but had low probability values (<85).
* One further strain was correctly identified but had a low probability value (<85).

33.7% of the isolates (59 of 175) and supplementary tests were required. The most frequently required supplementary test was susceptibility to novobiocin (43 isolates), followed by acid production by arabinose and xylose (16 isolates) and coagulase production (1 isolates only).

All 175 MicroScan panels were read both visually and with the autoSCAN-4. Occasionally the automated readings were corrected; however, all reading errors by the autoSCAN were observed in the susceptibility test to novobiocin, bacitracin, or crystal violet and were invariably due to the presence of air bubbles or scratches on the plastic well. Barring these errors there was a complete agreement between automated and visual evaluation of the panels. Only in 63 (36%) instances was the identification complete after overnight incubation; 112 isolates required additional incubation of 24 h because of insufficient positive reactions. The number of isolates of each species that could be identiﬁed after 24 h is also shown in Table 1. The MicroScan correctly identiﬁed 83.4% of C-NS. The identiﬁcation rate was excellent (97%) for the two most common C-NS pathogens, S. epidermidis and S. saprophyticus, and acceptable (>80%) for some of the less commonly isolated species, such as S. haemolyticus, S. simulans, S. capitis, S. cohnii, and S. xylosus. The MicroScan demonstrated lesser accuracy in the identiﬁcation of S. hominis (35.2%), S. warneri (69.5%), and S. sciuri (60%). The strains of S. hominis that were not correctly classiﬁed either had low probability value or were designated as S. warneri or S. haemolyticus. These errors arose mainly because pyrrolidonyl-beta-naphthylamide, beta-D-galactopyranosidase, or lactose failed to give a positive reaction or because arginine or mannose was positive. The misidentiﬁcations of S. warneri strains occurred mainly due to false-positive lactose and novobiocin and false-negative urease reactions. The misidentiﬁed strains of S. warneri were classiﬁed as S. hominis or S. haemolyticus. The sample of S. sciuri was too small to allow any comment.

DISCUSSION

Overall performance of the MicroScan and the API Staph-Ident systems were comparable. Both correctly identiﬁed 83.4 and 88% of 175 C-NS tested, respectively. Most of the misidentiﬁcation with the MicroScan occurred in association with S. hominis (11 of 17) and S. warneri (7 of 23) strains. Most of the strains of S. hominis and S. warneri that were
misidentified by the MicroScan system were classified as S. warneri or S. haemolyticus in the first instance and as S. haemolyticus or S. hominis in the latter. S. hominis, S. warneri, and S. haemolyticus are biochemically closely related and difficulties in the identification of these species also occur when the conventional scheme of Kloos and Schleifer is used, as has been reported previously (3, 6).

The rate of identification by API was equal to or better than the MicroScan system for all the species tested except S. epidermidis. Eight strains of phosphatase-negative S. epidermidis were classified as S. hominis by the Staph-Ident. The difficulty in the identification of phosphatase-negative S. epidermidis by the API system has also been noted by others (1).

One major disadvantage of the MicroScan system was that for 112 (64%) strains the identification was not complete until after 48 h, although the system required no supplementary tests. This delay may be attributable to the small inoculum (10^5 CFU/ml), which cannot be changed for the determination of MIC. However, the size of inoculum usually allows picking of colonies from the direct plate without the need of a purity plate. Although the Staph-Ident is a 5-h system, it requires heavy inoculum, and in many instances, 33.7% of the time in the present study, supplementary tests are required. Both of these may delay the availability of the final result in a diagnostic laboratory.

The MicroScan panels can be evaluated both visually and automatically with the use of autoSCAN-4 reader. We consider this a definite advantage of the system. The MicroScan panels also test for MICs of 12 antibiotics. Christensen et al. (2) have demonstrated that antibiograms in combination with biograms are superior to biogram or antibiogram alone in identifying the “same” strains of C-NS. This is helpful and important if the patient has multiple C-NS isolates and in the case of cross-infections in the hospital setting.

ACKNOWLEDGMENTS

We thank the staff of the Microbiology Laboratory of Victoria Hospital, London, Ont., for their technical assistance and Krista Harlow for clerical assistance.

This study was supported in part by American MicroScan, Sacramento, Calif.

LITERATURE CITED