Efficacy of Microbial Identification System for Epidemiologic Typing of Coagulase-Negative Staphylococci

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The lack of an adequate typing system hampers our understanding of the epidemiology of infections caused by coagulase-negative staphylococci (CoNS). CoNS have become recognized as important nosocomial pathogens and the principal cause of infections associated with invasive devices. Sensitive, specific, and convenient methods are needed to evaluate whether implementing infection control guidelines reduces the risk of nosocomial infections from CoNS and other pathogens. The Microbial Identification System (MIDI) (Microbial ID Inc., Newark, Del.), a semiautomated system for fatty acid methyl ester analysis, shows considerable promise for clinical and epidemiologic applications. Its predictive accuracy and reliability were tested by using epidemiologically related and replicated CoNS isolates as well as CoNS from epidemiologically unrelated clinical infections, which were obtained from five established hospital culture collections in diverse geographic locations. Two hundred isolates were fully characterized in 5 days by one person using MIDI, and the results were similar to those produced by more expensive and time-consuming conventional typing methods. MIDI appears to be a useful screening tool that could be used before more expensive and labor-intensive molecular methods. It offers important advantages to hospital epidemiologists and clinical microbiologists who must identify and type CoNS isolates.

Staphylococcus epidermidis is the most frequent pathogen in intravascular (i.v.) device-associated sepsis, but the relative importance of different sources of infection is uncertain (17). If entry-site skin flora migrating along cannulae causes most i.v. device-associated infections caused by coagulase-negative staphylococci (CoNS), then infection control measures should focus on antisepsis, time limits for site use, and minimization of trauma at insertion sites. However, if exogenous flora contaminating i.v. hubs predominates, then emphasizing aseptic technique, including the use of gloves under either universal precautions or body substance isolation, would be appropriate to reduce the risk of cross-infection. Although most studies conclude that flora at insertion sites is the prime concern, evidence supporting that view might be biased by use of microbiologic rather than clinical definitions of infection. Evidence supporting an intra- rather than extraluminal route might also be biased by unstated blood culture practices. Drawing blood cultures through i.v. lines may contaminate lumens and lead to subsequent spread of biofilms. Further, variation in the quality of i.v. devices and i.v. care could periodically alter the proportion of infections caused by organisms colonizing i.v. sites versus those contaminating hubs. A convenient typing system would allow individual hospitals to monitor the origin of such infections and to periodically adjust the emphasis of their infection control program.

Epidemiologically useful typing systems employ methods that exploit documented species diversity, but this is often comparable to hitting a moving target with tools of uncertain precision, accuracy, or reliability. Existing typing systems are not adequate for tracing the origin of nosocomial CoNS infections (2). Convenient phenotypic methods often lack discriminatory power and give results that are not reproducible. Genotypic methods offer greater discriminatory power, but these molecular microbiology methods are less convenient and are not yet standardized for use in clinical laboratories. Identification of bacteria to the species level by fatty acid methyl ester analysis (FAME) through qualitative pattern recognition is well established. Eerola and Lehtonen recently demonstrated that FAME reliably distinguished species of different aerobic genera as well as some strains within species, but they noted that different univariate correlation methods for data analysis are not equivalent (4). Kotilainen et al. suggested that FAME might discriminate between epidemiologically related and unrelated strains (9).

The Microbial Identification System (MIDI) (Microbial ID Inc., Newark, Del.), a novel commercial system for clinical and environmental microbiology laboratories, automates quantitative analysis of over 200 fatty acid metabolic products and compares results with information in proprietary database libraries. New library entries can be created by individual laboratories when isolates with unrecognized patterns are encountered, and subsequent samples are automatically compared with updated libraries. MIDI identifies organisms to the species level through principal-component analysis of FAME peak area ratios. This quantitative multivariate statistical approach promises superior ability with respect to simple pattern recognition to differentiate between organisms.

In addition to identifying genus and species, MIDI software provides two-dimensional principal-component plots and cluster analysis dendrograms to display the extent to which isolates are related. Highly related isolates cluster at relatively short
TABLE 1. Characterization of epidemiologically related isolates by laboratory of origin

<table>
<thead>
<tr>
<th>Group (reference)</th>
<th>No. of isolates</th>
<th>Source of isolates</th>
<th>Epidemiology shows time-space clustering</th>
<th>Biotype and susceptibility profile match</th>
<th>Plasmid profile</th>
<th>REA*</th>
<th>Hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (10)</td>
<td>10</td>
<td>Nursery blood cultures</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Blood and i.v. tip cultures</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>Blood and i.v. tip cultures</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Blood and i.v. tip cultures</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5 (3)</td>
<td>5</td>
<td>Surgical outbreak</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6 (6)</td>
<td>12</td>
<td>Repeated blood cultures</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*a*, clearly documented by contributing laboratory; ±, incompletely documented; −, not done.

*b* REA, restriction endonuclease analysis.

Distances from the origin on dendrograms, while less closely related isolates join at progressively greater distances. However, a critical distance, close enough to the origin to include all epidemiologically related isolates (sensitivity) but distant enough to exclude unrelated isolates (specificity), has not been validated.

We were unable to locate reports of studies that evaluated MIDI as an epidemiologic typing method for CoNS. Since MIDI may offer advantages to both clinical laboratories and hospital epidemiologists, we evaluated its discriminatory power (specificity), sensitivity, predictive value, and intralaboratory reliability.

**MATERIALS AND METHODS**

**Description of organisms.** One hundred eighty-three well-characterized CoNS isolates from clinical specimens were obtained from culture collections in five geographically distinct areas. The contributing laboratories used conventional methods to identify the CoNS and methods approved by the National Committee for Clinical Laboratory Standards to perform antimicrobial susceptibility testing. Identifications were confirmed subsequently by one of us (M.P.), using the scheme of Kloos and Schleifer. Some of these collections have been described elsewhere (3, 6, 10, 16).

One hundred fifty of the isolates were epidemiologically unrelated; the other 33 isolates comprised six different groups of epidemiologically related isolates: 10 S. haemolyticus blood culture isolates from neonates (Ontario), 3 pairs of matching S. epidermidis isolates from blood and intravascular cannula tip (British Columbia), 5 S. epidermidis isolates from an outbreak of surgical-site infections attributed to a surgeon's hand flora (Rhode Island), and 12 consecutive S. epidermidis isolates from one immunosuppressed patient (Iowa). The epidemiologic relatedness of the isolates was assessed by the following criteria, which are commonly accepted in infection surveillance programs (Table 1): (i) epidemiologic evidence of time-space clustering; (ii) matching biotype and susceptibility profile (no differences indicate that there is no evidence that isolates are different, one difference indicates that isolates might be different, and two or more differences indicate that isolates are different); and (iii) molecular methods (plasmid profile, restriction endonuclease analysis [plasmid or genome], and/or DNA-DNA hybridization [plasmid or genome]), if done, show no differences. A seventh group was created artificially by subculturing one isolate 18 times.

The 150 unrelated isolates were judged to be epidemiologically unrelated by methods that match or are superior to current infection surveillance practices. Forty-eight unrelated blood culture isolates collected over a 6-month period in one Ontario children's hospital exhibited unique combinations of biotype, susceptibility profile, and plasmid profile and were not clustered in time and space (10). Fifty-seven unrelated blood culture isolates from a university hospital in Iowa were unique in their combined biotype, susceptibility profile, slime production, synergistic hemolysis, plasmid profile, and restriction digest patterns of chromosomal DNA (6). Forty-two unrelated blood culture isolates from a British Columbia university hospital were selected from a collection spanning 3 years; they were considered to be unrelated episodes because they were not clustered in time or space. Three isolates from i.v. lines in a North Carolina university hospital also were considered to be unrelated on the basis of epidemiologic assessment, despite similar biotypes and susceptibility profiles (16).

The majority of these isolates were S. epidermidis strains that met Centers for Disease Control and Prevention criteria for clinical significance (5). Twenty-six of the 183 isolates were considered to be blood culture contaminants. They were included to study how closely they would relate to clinically significant isolates. One isolate was replicated 18 times (group 7) to test intralaboratory reliability. The entire set of 200 randomly numbered clones was analyzed blindly in a facility with an established MIDI system. Media were inoculated in class II laminar airflow hoods, and colony morphology was inspected for subcultures incubated at 37°C for 2 days (to check for contaminants) after isolates were identified from contributing laboratories and again just before MIDI analyses.

**MIDI technique.** Isolates were analyzed according to MIDI instructions (14). Briefly, a single colony was quadrant streaked onto Trypticase soy agar (TSA) plates (BBL) and harvested in log-phase growth after 24 (±2) h of incubation at 28°C in air. Approximately one 4-mm loopful of bacteria was harvested for each sample. Each sample was saponified (sodium hydroxide in methanol), methylated (hydrochloric acid in methanol), extracted (hexane in methyl tert-butyl ether), and cleaned (sodium hydroxide). Its organic layer was removed and injected into MIDI's gas chromatography system. Calibration standards and a negative control blank were run with each batch. One positive control specimen (Xanthomonas maltophilia, ATCC 13637) was run each day. Results were interpreted by using MIDI's aerobic bacterium library (version 3.30). Samples with total peak area of <80,000 or <85% of the peak area used by the naming algorithm were concentrated by evaporation, and the analysis of these flagged samples was repeated.

**Statistical analysis.** MIDI computes an index for each isolate through principal-component analysis (8) of cellular fatty acid content ratios. Cluster analysis of these indices was applied (8), with Euclidean distances calculated by the single-and Ward (18) linkage methods. Empirically, joining distances of replicated isolates (group 7) were examined to determine an
optimal strain-level joining distance. The MIDI system software and SYSTAT (version 4.1; Systat Inc., Evanston Ill.) were used for statistical analysis. The power to discriminate between unrelated isolates was calculated as Simpson's index, i.e., the probability that two isolates selected at random would be placed into different typing categories (7). A sensitivity index is proposed as the probability of placing two epidemiologically related isolates into the same typing category. It was evaluated by averaging the accuracy of MIDI's predictions (0, 1 scoring) for all possible combinations of two isolates within each group of related isolates (\( \alpha_{C_n} \), where \( n \) is the number of related isolates in each group). Positive predictive value was calculated by dividing the number of typing categories containing epidemiologically related isolates by the number containing two or more isolates (whether related or not). Sensitivity expresses the ability to type related isolates into the same group; positive predictive value expresses the accuracy of groupings among all isolates in the test set.

RESULTS

All 200 isolates were typeable, and their MIDI analysis was completed in 5 days by one of us (D.B.). Single-linkage and Ward's-method dendrograms produced similar conclusions; results obtained by Ward's method are reported here. A critical distance to distinguish between related and unrelated isolates was established empirically by examining the Euclidean distance at which isolates known to be related joined. Group 7 was taken as the gold standard for clonal origin. These replicated isolates all joined at 5.4 U or less; MIDI found three clusters joined at 0.7 to 2.1 U, and these were linked at 4.5 to 5.4 U. The former range is consistent with repeated analysis of the same isolate with previous versions of MIDI software (15). Thus, any isolates joining at less than 4.5 to 5.4 U were considered to be clustered into the same typing category, whereas isolates joining at more than 5.4 U were assigned to different categories. The colony morphology of the group 7 replicates was uniform on earlier subcultures but was not uniform on the TSA subculture immediately prior to MIDI analysis. The possibility of contamination during subculture was not further investigated. The single- and Ward linkage methods produced similar groupings. At least 46 typing categories were readily apparent, with a Simpson's index of \( \geq 0.93 \). Approximately half of the categories contained a single isolate. MIDI assigned epidemiologically unrelated isolates from different hospitals to clusters that also included related isolates and assigned 63% of the blood culture contaminant isolates to clusters containing clinically significant isolates. Overall, the positive predictive value of MIDI plus susceptibility profile results was approximately 30%. The sensitivity index for each group of epidemiologically related isolates is listed in Table 2. Joining distances are shown in Fig. 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of isolates (n)</th>
<th>No. of isolates in pairs</th>
<th>No. of pairs found/no. of pairs ( \left( \alpha_{C_n} \right) )</th>
<th>Sensitivity index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>4</td>
<td>6/45</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4</td>
<td>6/10</td>
<td>60</td>
</tr>
<tr>
<td>6a</td>
<td>12</td>
<td>10</td>
<td>29/66</td>
<td>44</td>
</tr>
</tbody>
</table>

* For group 6, the 10 isolates in pairs consisted of one cluster of 8 and a separate cluster of 2. There are 66 distinct ways to draw combinations of 2 from 12 items. Thus, although 10 of 12 isolates were found in pairs, the probability of drawing any 2 of the 12 and finding them paired by MIDI was 44% (29/66), not 83% (10/12). If group 6 consisted of only the 10 isolates with identical plasmid profiles, then the sensitivity index would become 64% (29/45).

FIG. 1. Joining distances of groups 1 to 6. Dotted lines indicate a 4.5- to 5.4-U joining distance limit. Group numbers are indicated at left. Group 5 is shown as patients' (p) and surgeon's (s) isolates; group 6 is shown as two clusters, 6a and 6b (see text).
FIG. 2. Section of MIDI dendrogram containing group 5 isolates. Dotted lines indicate a 4.5- to 5.4-U joining distance limit. A to H, distinct antimicrobial drug susceptibility profiles; 0, epidemiologically unrelated isolates, 5p and 5s, four patients’ isolates and one surgeon’s isolate, respectively, of group 5. All 5p and 5s isolates exhibited susceptibility pattern D. MIDI separated the patients’ isolates from the surgeon’s isolate.

Group 1 consisted of 10 *S. haemolyticus* blood culture isolates from a neonatal nursery. The isolates had identical susceptibility profiles, restriction endonuclease patterns, and patterns of hybridization to three drug resistance probes. This resistant strain was endemic in several adjacent hospitals, and the affected infants had overlapping hospitalization dates during a 5-month period, but there was no conclusive evidence to establish or to disprove common-source infection or cross-infection. MIDI identified two isolates (obtained within 3 days of each other) as related to each other and to a third (joined at 1.9 Euclidean distance units) and to a fourth (at 5.4 U). MIDI typed the other six isolates as unrelated.

Group 2 consisted of matching blood culture and intravascular cannula tip isolates from a single patient. MIDI placed the blood and tip isolates (and other epidemiologically unrelated isolates) in a single category. This patient appeared to
have three different CoNS isolates on the intravascular line tip; only one matched the blood isolate's susceptibility profile. MIDI correspondingly typed the isolates with matching susceptibility profiles as related and placed the other two into separate, distinct categories. The correctly paired isolates joined at 2.6 Euclidean distance units and subsequently were confirmed to be *S. epidermidis*, whereas the other two cannula tip isolates were *Staphylococcus intermedius* and *Staphylococcus auricularis*. Susceptibility profile differences distinguished the two related isolates from most of the epidemiologically unrelated isolates in this MIDI typing category.

Group 3 consisted of paired blood and cannula isolates from another patient. MIDI placed these two and other epidemiologically unrelated isolates in a single category. The correctly paired isolates joined at 1.2 Euclidean distance units. Susceptibility profile differences correctly retained the blood and cannula isolates in one subcategory and put mutually unrelated isolates in multiple distinct subcategories.

Group 4 also consisted of paired blood and cannula isolates from one patient. These were correctly matched at a distance of 3.8 U. Unrelated isolates also were included in this group, but susceptibility profile differences correctly separated related from unrelated isolates.

Group 5 consisted of *S. epidermidis* isolates from a surgeon's hands, two infected surgical wounds, and two blood cultures. Temporal clustering, biotyping, antibiograms, plasmid analysis, *EcoRI* restriction endonuclease digests of plasmid DNA, and ribotyping documented that this was a common-source outbreak. MIDI typed the four isolates from patients as related (three joined at 1.4 U and the fourth joined at 2.8 U), but the surgeon's isolate was in a different category (joined at 10.6 U). Susceptibility profile differences created subcategories correctly distinguishing the patient outbreak isolates from unrelated isolates. This is illustrated in Fig. 2 and 3: isolates joining to the right of the distance marked by dotted lines in Fig. 2 did not cluster into the typing category containing the patient outbreak isolates.

Group 6, a series of 12 *S. epidermidis* isolates obtained from an immunosuppressed patient over a 5-month period, was identified by MIDI as consisting of two clusters and two unrelated isolates. One cluster consisted of two isolates from blood cultures drawn on the same day (joined at 2.1 U). The second cluster contained eight isolates obtained over several months (joined at 4.5 U). Plasmid patterns of the 2 isolates identified as unrelated were different from those of the other 10 isolates, which were identical. Susceptibility profiles characteristic of the two clusters differed. Therefore, at least two distinct clusters may be correctly identified by MIDI for group 6.

**DISCUSSION**

A microbiologic approach to evaluating typing systems typically assesses the discriminatory power for detecting clonal origin without considering epidemiologic accuracy. Simpson's index, which is a microbiologic analog to the epidemiologic statistic specificity, measures the degree to which typing systems recognize unrelated isolates as being unrelated (7). There is no microbiologic analog to sensitivity, an epidemiologic measure of how well tests recognize true cases (i.e., the proportion of related isolates recognized as being related). Assessing typing systems also involves an added complication in that related isolates may not all type into a single cluster (Table 2, group 6); thus, we proposed a novel sensitivity index based on pairwise linkages.

From a microbiologic perspective, reproducible typing methods provide stronger evidence when they detect differences between two or more isolates because that appear to be identical might be discriminated by more powerful typing methods or with a different substrate(s) or enzyme(s). This is consistent with an epidemiologic perspective: changing the specificity has a relatively greater impact when positive test results are used for confirmation (strain differences as evidence disproving an outbreak), and changing the sensitivity has a relatively greater impact when negative test

### FIG. 3. Number of differences between antimicrobial drug susceptibility profiles of isolates shown in Fig. 2. Subtyping within an MIDI cluster was based upon profiles differing by two or more drugs. For example, group 5 isolates (from a surgical-site infection outbreak) all had a profile arbitrarily designated D, which could be distinguished from profiles of epidemiologically unrelated isolates (differing from profile A by three drugs and from B by four drugs, etc.).

### FIG. 4. Joining distances of repeated assays. C, 25 concentrated samples versus samples with insufficient peak area; M, 9 samples exhibiting colony variants (size, texture, or color) on the TSA plates; *, median values; ( ), a 95% confidence interval for the median; boxes, 25th to 75th percentiles; +, extreme values beyond the data range indicated by the line. Assays of three concentrates and five morphology variants joined the original assay at more than 5.4 Euclidean distance units, and thus the isolates were typed as dissimilar.
results are used to reach a diagnosis (lack of strain differences failing to disprove an outbreak). Selecting a cutoff threshold closer to the MIDI dendrogram origin improves specificity but decreases sensitivity; moving the threshold further from the origin improves sensitivity but decreases specificity.

For this study, we established a criterion reference, or gold standard, from epidemiologic and microbiologic information available for clinical isolates. At three gold-established empirically, MIDI demonstrated high discriminatory power (Simpson's index, \( r = 0.93 \)) and sensitivity that was highest among those isolates with the strongest evidence of an epidemiologic relationship. Specificity was further improved without loss of sensitivity by combining MIDI with antimicrobial susceptibility results. MIDI grouped together some isolates that were unrelated epidemiologically; this is probably a consequence of MIDI measuring phenotypic characteristics of taxonomically similar (but epidemiologically unrelated) strains. MIDI also separated isolates that were grouped together by other methods; the separation might be erroneous or might indicate that MIDI is a more powerful epidemiologic typing method. Results were specific to the software version used, and an earlier MIDI library produced significantly inferior results; this may also vary with the extent of diversity among organisms analyzed together, since dendrogram structure is a function of both the mathematical algorithms and the data set covariance structure. Fine distinctions may be lost if relatively diverse organisms are analyzed together.

Although MIDI results supplemented by susceptibility results closely paralleled the gold standard results, MIDI did not place all isolates of group 5 into one category. This is of concern because the isolate from the surgeon's hand was identical to the patient isolates by whole plasmid analysis, plasmid restriction endonuclease analysis, and several analyses of chromosomal DNA, which are definitive.

Growth of CoNS was often relatively light after 24 h on TSA plates. Although better growth may be achieved on blood agar at 37°C and improved reproducibility has been reported for other aerobic bacteria under these conditions (13), MIDI's 37°C blood agar library was not as well established as its aerobic 28°C TSA library. The problem of light growth was easily overcome by using a heavier inoculum. Streaking two TSA plates might provide an adequate inoculum for strains that grow poorly. This solution has the additional advantage of allowing the cells to be reharvested immediately if a critical sample is ruined by an error in technique. Concentrating extracts for a second chromatography run, the standard procedure when MIDI's printout warns of unacceptably low peak areas, was also simple and effective: 84% of inadequate samples attained acceptable peak areas after they were concentrated. Three of 25 such samples were reassigned to a different category as new peaks emerged above the detection limits (Fig. 4).

Compared with current alternatives, FAME by gas-liquid chromatography may be particularly cost-effective for epidemiologic typing of clinical or environmental isolates. FAME has been used to identify staphylococci to the subspecies level (9, 12). However, discerning epidemiologic relationships is more difficult than simply establishing taxonomic relationships. MIDI was used in a small study of Pseudomonas cepacia in cystic fibrosis centers; a low but promising ability to discriminate among 42 isolates from five centers was determined (Simpson's index, 0.775) (11). The present study of CoNS involves a large number of isolates selected specifically to minimize bias from erroneous inclusion of strains related to each other through unrecognized common-source infection or cross-infection. In comparison with available molecular typing methods, MIDI promises to be a relatively powerful, rapid, and inexpensive screening tool but does not replace definitive genotypic methods.

MIDI conveniently accommodates large numbers of isolates and can type every isolate, and the costs for materials for complete identification are low (about $1.30 per isolate). One-tube sample preparation (saponification, methylation, extraction, and sample cleanup) and automated analysis accommodate the identification of up to 45 samples per operator-day per machine. Depending upon experience, one person can process as many as 30 to 50 isolates in about 4 h. Performance of gas chromatography requires an additional 30 min per sample. MIDI's waste can be recycled or biodegraded. Compared with other typing systems, a relatively small amount of plastic is discarded. Glass, water, and sodium chloride are the major waste products.

This study suggests that MIDI results plus susceptibility profiles provide sufficient discriminatory and predictive power to support epidemiologic screening for cross-infection or outbreaks. The overall 30% positive predictive accuracy found is commensurate with other measures used in hospital epidemiology to interpret infection surveillance data (1). Higher accuracy may be expected when analysis is restricted to isolates from a suspected outbreak. If MIDI is a cost-effective alternative to conventional biochemical systems for identification of microbes, then its additional value to infection surveillance programs is an attractive benefit. MIDI offers important advantages in speed, reliability, and convenience. These results are promising and warrant further prospective studies with CoNS and other nosocomial pathogens to confirm MIDI's predictive accuracy and reliability.

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