JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1994, p. 1908–1910
0095-1137/94/$04.00+0

Vol. 32, No. 8

Gas-Liquid Chromatography of Cellular Fatty Acids for Identification of Staphylococci

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Received 7 March 1994/Returned for modification 21 April 1994/Accepted 17 May 1994

A commercially available, computer-assisted microbial identification system (MIS) employs gas-liquid chromatographic analyses of bacterial fatty acids. The MIS was used to identify 470 isolates of *Staphylococcus* species. The accuracy of the MIS was compared with the accuracies of conventional methods. There was a complete agreement between the MIS and conventional methods in the identification of 413 (87.8%) strains. For 36 of 45 misidentified strains, the correct identification was listed by the MIS as a choice but not as the first choice. Twelve strains could not be matched. All strains of *Staphylococcus cohnii*, *S. epidermidis*, *S. intermedius*, *S. lugdunensis*, *S. schleiferi*, *S. sciuri*, *S. simulans*, and *S. xylosus* were correctly identified. Two species, *S. hominis* and *S. saprophyticus*, accounted for 52.6% (30 of 57) of the misidentifications. Seventy-eight organisms were retested. Identification of 73 organisms remained unchanged, and for five organisms, the second choice became first and vice versa. The overall performance of the MIS is acceptable, and the system can be used as an alternate identification method for staphylococci.

Coagulase-negative staphylococci are some of the most important agents of nosocomial infections (6, 13, 15). They can be primary pathogens and are a major cause of morbidity in a variety of situations, including those involving patients who have undergone cardiovascular surgery (1), patients with prosthetic devices (7, 18), and immunocompromised patients (24). Several manual and automated methods for the identification of staphylococci are commercially available (16, 21).

We undertook this study to determine the ability of the Microbial Identification System (MIS) (Microbial ID Inc., Newark, Del.) to identify staphylococci. The results of the MIS analyses were compared with those of conventional methods (9). The MIS employs cellular fatty acid analysis for the identification of bacteria. The system identifies fatty acid methyl esters, dimethyl acetyls, aldehydes, and unknown compounds unique to a species by high-resolution gas-liquid chromatography. The database of the MIS consists of libraries with analyses of cellular fatty acid profiles. The principal components of an analysis of the cellular fatty acids of an unknown organism are compared with those of the analyses stored in the database by a covariance matrix and pattern recognition software. The results produced by MIS include the total area under the curve, the names of the fatty acid derivatives, their retention times and amounts, and a list of probable identifications with similarity index values. The similarity index expresses the closeness of the profile of an unknown organism to representative profiles contained in the library entry of each known class. Pattern recognition techniques are used to compare profiles statistically.

MATERIALS AND METHODS

Four hundred seventy strains of *Staphylococcus* species were tested. The names of the species and the number of strains included in the study are shown in Table 1. Almost all the strains were isolated from clinical specimens submitted to our laboratory at Victoria Hospital, London, Canada, and a few were from animal sources. Strains isolated from significant sources such as blood, cerebrospinal fluid, peritoneal fluid, and catheter tips were saved and included in the study. Duplicate isolates from the same patients were excluded. A concerted effort to include all staphylococcal species seen in our clinical laboratory and at least 10 strains of each species was made. Strains were either freshly isolated or previously isolated and stored frozen. Frozen organisms were subcultured at least three times before identification. Isolates were grown on Columbia agar with 5% horse blood. The plates were incubated for 5 days to ensure a pure growth (9), and a single colony was picked. Identification of the strain by conventional methods and with the MIS system was carried out with a subculture of this colony. The identification of isolates as *Staphylococcus* species was confirmed by Gram stain and acid production from glycerol in the presence of erythromycin (0.4 μg/ml) (19). The following American Type Culture Collection (ATCC) strains were used for quality control: *Staphylococcus aureus* ATCC 25923, *S. cohnii* ATCC 29974, *S. epidermidis* ATCC 14990, *S. haemolyticus* ATCC 29970, *S. hominis* ATCC 27845, *S. intermedius* ATCC 29663, *S. saprophyticus* ATCC 15305, *S. sciuri* ATCC 29062, *S. warneri* ATCC 27836, and *S. xylosus* ATCC 29971.

Conventional identification. Conventional identification of *Staphylococcus* species was based on colony morphology; coagulase and catalase production; growth under anaerobic conditions; resistance to novobiocin, polymyxin B, furazolidine, and bacitracin; production of oxidase, urease, pyrrolidonyl-arylamidase, β-glucosidase, β-glucuronidase, β-galactosidase, and ornithine decarboxylase; arginine utilization; esculin hydrolysis; acetoin production; and acid production from trehalose, mannitol, turanose, xylose, cellobiose, arabinose, maltose, lactose, sucrose, raffinose, and *N*-acetylglucosamine, as outlined by Kloos and Lambe (9). β-Glucosidase, β-galactosidase, and β-glucuronidase discs were obtained from Rosco Diagnostica, Taatstrup, Denmark.

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TABLE 1. Results of identification of *Staphylococcus* species with MIS

Organism	No. of strains (%)	No. of strains (%)		
		Correctly identified	Incorrectly identified	Not identified
S. aureus	76	69		7
S. epidermidis group				
S. capitis	25	18	7	
S. epidermidis	77	77		
S. haemolyticus	63	56	7	
S. hominis	36	17	16	3
S. warneri	39	33	4	2
S. intermedius	12	12		
S. lugdunensis	26	26		
S. saprophyticus group				
S. cohnii	14	14		
S. saprophyticus	37	26	11	
S. xylosus	15	15		
S. schleiferi	11	11		
S. sciuri	18	18		
S. simulans	21	21		
Total	470 (100)	413 (87.8)	45 (9.6)	12 (2.6)

MIS method. Strains were fractionally streaked in four quadrants of a plate containing Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood and were incubated at 35°C for 24 h in ambient air. The growth from the third quadrant was harvested and placed in a virgin tube (13 by 100 mm) with a Teflon screw cap. Lysis of cells, saponification, methylation of fatty acids, and extraction of methyl esters into the organic phase were carried out as described previously (12). All reagents were obtained from vendors recommended in the MIS manual. Following preparation, the washed organic extract was placed in an autosampler vial with an aluminum crimp cap. Specimens were processed on a Hewlett-Packard 5890A gas chromatograph with a Hewlett-Packard 7673A automatic sampler and integrator. The parameters of chromatography were those recommended in the operational manual of the MIS (12). For identification, version 3.7 of the clinical aerobic library was used. If the area under the curve was inadequate, the identification was considered questionable. Identifications of all such strains were repeated.

Reproducibility test. After the completion of our study, 78 organisms were recultured, harvested, and retested with the MIS. The strains for reproducibility testing were selected so that all the species were represented and the number of strains of each species was proportional to the number tested. Results of repeat tests were used only for the evaluation of the reproducibility of the MIS results.

RESULTS

A total of 470 strains was evaluated. For 31 strains, the area under the curve was inadequate because not enough cells were harvested for the cellular fatty acid extraction. The strains were retested with the MIS and were evenly distributed among the various staphylococcal species. Identifications obtained with the MIS were compared with those obtained by the conventional methods. There was complete agreement between the MIS and the conventional methods in the identification of 413 (87.8%) strains. Forty-five strains (9.6%) were incorrectly identified in that the correct identification was not the first choice. For 36 of the incorrectly identified strains, the correct identification was given as a choice. For the other nine strains, the correct designation was not listed among the choices. The MIS was unable to identify 12 (2.6%) strains and produced a "no-match" result for these. Detailed results are shown in Table 1.

TABLE 2. MIS results for strains that were incorrectly identified

Organism	No. of strains	Species (no. of identifications) as identified with MIS
S. epidermidis group		
S. capitis	7	*S. epidermidis* (3), *S. haemolyticus* (3), *S. warneri* (1)
S. haemolyticus	7	*S. warneri* (5), *S. lugdunensis* (2)
S. hominis	16	*S. epidermidis* (16)
S. warneri	4	*S. epidermidis* (1), *S. aureus* (3)
S. saprophyticus group, *S. saprophyticus*	11	*S. cohnii* (8), *S. xylosus* (3)

There were no discordant results between the MIS and the conventional methods for strains of *S. cohnii*, *S. epidermidis*, *S. intermedius*, *S. lugdunensis*, *S. schleiferi*, *S. sciuri*, *S. simulans*, and *S. xylosus*. Two species, *S. hominis* and *S. saprophyticus*, accounted for 52.6% (30 of 57) of the incorrectly identified results. On the basis of DNA hybridization, biochemical reactions, immunological characteristics, and cell wall composition, staphylococcal species have been divided into related groups (20). Interestingly, 40 of the 45 incorrectly identified strains were designated as a species within the same group (Table 2). This was especially true for *S. hominis* and *S. saprophyticus*. In the case of *S. hominis*, 16 of 36 strains were misidentified as *S. epidermidis*, while 11 of 37 strains of *S. saprophyticus* were identified as *S. cohnii* or *S. xylosus* (Table 2). Other incorrect identifications were evenly distributed among several species and did not show any trend.

The fatty acid composition of *S. aureus* was sufficiently distinct from that of the coagulase-negative staphylococci that all strains either were correctly identified or were found to have a no-match result. *S. aureus* strains accounted for the most (7 of 12) no-match results. The seven *S. aureus* isolates with no-match results were quantitatively but not qualitatively different in fatty acid composition from those that were correctly identified. In these isolates, three to six fatty acids were outside the minimum or maximum peak area value for corresponding fatty acids as given in the summary sheets of the MIS. These summary sheets show the average cellular fatty acid composition, standard deviation, coefficient of variation, and maximum and minimum peak area values for fatty acids within the species for the strains included in the database. The peak area values are presented as a percentage of the total fatty acid peak areas.

Results of the reproducibility test. The first and second choices for 73 organisms remained unchanged. Five organisms, i.e., two strains of *S. haemolyticus*, two strains of *S. hominis*, and one strain of *S. saprophyticus*, underwent a change of designation. In all cases, the first choice was replaced with the previous second choice and vice versa.

DISCUSSION

It has been shown previously that the fatty acid profiles of members of the genus *Staphylococcus* are distinct from those of

other bacteria. Coagulase-negative staphylococci show quantitative but not qualitative differences in their fatty acid compositions (3, 11, 14, 20). There is, however, considerable overlap in average fatty acid compositions, their standard deviations, and their minimum and maximum values (11, 14). Our data indicate that the fatty acid profile of *S. aureus* obtained with the MIS is distinct and can be used to separate these strains from other species. Durham and Kloos, in their study of the fatty acid compositions of various staphylococcal species, also found *S. aureus* to be a fairly homogeneous group (3).

Our results indicate that intraspecies similarity indices in the MIS database are high for strains of *S. cohnii*, *S. epidermidis*, *S. intermedius*, *S. lugdunensis*, *S. schleiferi*, *S. sciuri*, *S. simulans*, and *S. xylosus*, whereas similarity indices between these and other species appear to be low. Therefore, it is not surprising that the first designations for 16 of the 36 strains of *S. hominis* were *S. epidermidis*. Similarity in the fatty acid profiles of *S. hominis* and *S. epidermidis* has been demonstrated previously (3). Kotilainen et al. (11) also found that strains of these two species form one related cluster. In their study, they found that strains of *S. capitis*, *S. haemolyticus*, *S. lugdunensis*, and *S. warneri* formed another cluster. In our study, for 11 of the 14 incorrectly identified strains of *S. capitis* and *S. haemolyticus*, the first choice was one of the species within this cluster. In contrast to the observations of Durham and Kloos (3), the MIS identified all the strains of *S. cohnii* and *S. xylosus* correctly but had difficulty separating strains of *S. saprophyticus* from these two species.

Several commercial kits for the identification of staphylococci are available (16), and some, like the MIS, are automated (21). No one system can identify all staphylococcal species with complete reliability (16). Many of these kits have been evaluated, and results of those evaluations have been published. The performance of the kits and their identification rates depend on the strains tested, the species mix of test organisms, and the supplementary tests performed, making it difficult to compare the results of different studies directly. However, overall identification rates of ≥85% have been reported for several commercially available kits such as the API Staph-Ident (10), the DMS Staph-Trac (5), the Minitek (2), and the Pos ID panel (8) and the Rapid Pos combo panel (23) (MicroScan), as well as the Vitek GPI (17). The MIS in the present study correctly identified 87.8% of 470 staphylococci. This performance is similar to that of other commercially available systems, although the MIS has not been directly compared with any commercially available identification kit.

The MIS is completely automated after the initial step of cellular fatty acid extraction. In addition, the database of the MIS contains identification libraries for various gram-negative, gram-positive, aerobic, and anaerobic bacteria (4, 22). It is possible to load cellular fatty acid extracts of various kinds of bacteria on the autosampler carousel at the same time. The MIS program is capable of sorting the organisms and making the appropriate identification. The MIS also allows the user to develop his or her own identification libraries. We have found that the creation of such libraries and their use in the identification of isolates may further enhance the performance of the system. Our study suggests that the MIS is an acceptable alternative for the identification of staphylococci.

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