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Identification of Clinical Isolates of Non-*Enterobacteriaceae* Gram-Negative Rods by Computer-Assisted Gas-Liquid Chromatography

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Of 363 isolates of non-*Enterobacteriaceae* gram-negative rods analyzed, the Microbial Identification System (which chromatographically analyzes cellular fatty acids), using version 3.8 of the aerobic clinical library software, identified 328 (90.4%), of which 327 (99.7%) and 253 (77.1%) were correctly named to the genus and species levels, respectively.

By conventional methods, correct species identification of many oxidase-positive and -negative gram-negative rods, excluding members of the family *Enterobacteriaceae*, is frequently difficult and time-consuming (10, 11). Gas chromatographic analysis of bacterial fatty acid methyl esters has provided a reasonably accurate, rapid, and cost-effective alternative for identification of isolates of many aerobic gram-positive and gram-negative microorganisms (1, 2, 4–7, 10, 15, 16). The analysis has been automated and simplified with the introduction of the Microbial Identification System (MIS; Microbial ID, Inc., Newark, Del.), which includes a gas chromatograph with a flame ionization detector along with an autosampler, an integrator, and a computer. The system identifies and quantifies bacterial fatty acid methyl esters. The computer then searches a software library of fatty acid compositions, compares the fatty acid profile of the isolate with those of known species, and generates a report listing the most likely name of the isolate along with the extent of correlation of the isolate's profile with a species in the library (given as a "similarity index" [14]). The studies published to date on MIS identification of clinical isolates of aerobic gram-positive and gram-negative bacterial species have involved chromatographic analysis of isolates grown under conditions not common in clinical laboratories (Trypticase soy broth agar cultures incubated at 28°C [1, 10]).

The current study was undertaken to evaluate the accuracy of the relatively rapid MIS for identification of clinical isolates of a variety of oxidase-positive and -negative gram-negative bacterial species which had been cultured on the sheep blood agar (SBA) or chocolate agar media which are more commonly used than Trypticase soy broth agar in clinical laboratories. Species included in the study were all random fresh clinical isolates of oxidase-positive and -negative gram-negative rods (excluding the *Enterobacteriaceae*) which were recovered from 1 July 1994 until 20 October 1995 from patients in central Pennsylvania. Multiple isolates of the same species from the same patients were excluded from the study. Isolates of *Pseudomonas aeruginosa* which were studied were mucoid or nonpigmented. Single colonies of each isolate in the study were subcultured on 5% SBA or chocolate agar and incubated at 35°C (5 to 10% CO_2) for 1 to 4 days prior to analysis.

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Conventional identification. Colony characteristics on SBA, eosin methylene blue agar, or chocolate agar (all media were from BBL, Cockeysville, Md.), as well as the oxidase result and Gram stain morphology and reaction, were determined for each isolate. Depending on those results, a battery of additional tests was set up for each isolate from the following list: catalase, triple sugar iron agar, SIM's agar, tryptic soy broth cultures (to determine growth at 35 and 42°C and in the presence of 6.5% NaCl, as well as motility at 35°C), growth around X, V, and XV strips on brain heart infusion agar, relative growth on chocolate agar (aerobic and in 5 to 10% CO_2), lysine and ornithine decarboxylase (Moeller formulation), arginine dihydrolase (Moeller), urea, citrate, reduction of nitrate and nitrite, cetrimide agar (for growth, pigment, and fluorescence), acid production from O-F media (glucose, maltose, sucrose, lactose, xylose, and mannitol), DNase, egg yolk agar (for lecithinase and lipase), hydrolysis of casein, esculin, gel, and starch, methyl red–Voges-Proskauer, phenylalanine deaminase, and *o*-nitrophenyl-β-D-galactopyranoside (3, 8, 11, 12). Past antimicrobial agent responses for many of the species, as previously described by Gilardi (3), were also of assistance in determination of the identities of many isolates when compared with the responses determined in house for those isolates (9). The conventional identification was considered the correct identification.

Chromatographic identification. Isolated colonies of clinical isolates of bacteria were quadrant streaked onto 5% SBA (for most genera) or chocolate agar (for *Haemophilus* spp.) and incubated for 24 ± 2 h at 35°C in an aerobic atmosphere supplemented with 5 to 10% CO_2. Fatty acid methyl ester extracts were prepared and then analyzed on a 5890 series II gas-liquid chromatograph (Hewlett-Packard, Avondale, Pa.) in accordance with the manufacturer's specifications (13). An extract of a control strain (*Xanthomonas maltophilia* ATCC 13637) and an external calibration mixture (Microbial ID, Inc.) were chromatographically analyzed each day of testing. The software library in the MIS computer used to identify the isolates was Clinical Aerobes, version 3.8. The computer printout for each isolate listed one or more possible species choices, with a similarity index ranging from 0 to 1.000 accompanying each choice. For this study, the MIS result was considered correct if the correct species name of an isolate was listed by the MIS computer as the first or highest choice, regardless of the similarity index, as suggested in a previous study (10).

When the MIS result was either a misidentification to the

TABLE 1. Comparison of MIS with conventional tests for identification of oxidase-positive and -negative gram-negative rods and diplococci

Species identified with conventional tests	No. of isolates tested	No. (%) of isolates tested by MIS			Predictive value (%) of species identification by MIS[b]
		Correctly identified to species level	Incorrectly identified to species level[a]	Unidentified (no match)	
Acinetobacter spp.					
A. baumannii	86	52 (60)	29 (34)	5 (6)	52/55 (95)
A. calcoaceticus	3		3 (100)		0/6 (0)
A. haemolyticus	11	7 (64)	3 (27)	1 (9)	7/28 (25)
A. johnsonii	1	1 (100)			1/8 (13)
A. junii	1		1 (100)		
A. lwoffii	0				0/1 (0)
A. radioresistens	2		2 (100)		
Aeromonas spp.					
A. caviae	4	3 (75)	1 (25)		3/3 (100)
A. hydrophila	3	3 (100)			3/4 (75)
A. sobria	1			1 (100)	
Agrobacterium tumefaciens	4	3 (75)		1 (25)	3/3 (100)
Alcaligenes spp.					
A. faecalis	6	6 (100)			6/6 (100)
A. xylosoxidans subsp. *xylosoxidans*	8	8 (100)			8/8 (100)
Bordetella bronchiseptica	3	3 (100)			3/4 (75)
Burkholderia cepacia	6	5 (83)		1 (17)	5/5 (100)
Capnocytophaga sputigena	1			1 (100)	
Comamonas spp.					
C. acidovorans	12	12 (100)			12/12 (100)
C. testosteroni	26	26 (100)			26/26 (100)
CDC[c] groups					
EF-4	2	2 (100)			2/2 (100)
EO-2	1			1 (100)	
Flavimonas oryzihabitans	3	3 (100)			3/3 (100)
Flavobacterium spp.					
F. breve	1			1 (100)	
F. gleum	8	7 (88)		1 (13)	7/15 (47)
F. indologenes	7		5 (71)	2 (29)	
F. meningosepticum	10	5 (50)	3 (30)	2 (20)	5/5 (100)
F. odoratum	3	3 (100)			3/3 (100)
Haemophilus spp.					
H. aphrophilus	2		1 (50)	1 (50)	
H. influenzae	0				0/2 (0)
H. parahaemolyticus	1		1 (100)		
H. parainfluenzae	7	5 (71)	2 (29)		5/7 (71)
Moraxella spp.					
M. lacunata	2	2 (100)			2/2 (100)
M. nonliquefaciens	1			1 (100)	
M. osloensis	1	1 (100)			1/1 (100)
Neisseria elongata	4	4 (100)			4/4 (100)
Ochrobactrum xanthropi	6	6 (100)			6/6 (100)
Oligella urethralis	11	11 (100)			11/11 (100)
Pasteurella multocida	8	7 (88)		1 (13)	7/7 (100)
Plesiomonas shigelloides	2	2 (100)			2/2 (100)

Continued on following page

TABLE 1—*Continued*

Species identified with conventional tests	No. of isolates tested	No. (%) of isolates tested by MIS			Predictive value (%) of species identification by MIS[b]
		Correctly identified to species level	Incorrectly identified to species level[a]	Unidentified (no match)	
Pseudomonas spp.					
P. aeruginosa	37	35 (95)	2 (5)		35/36 (97)
P. alcaligenes	1		1 (100)		
P. diminuta	5	5 (100)			5/6 (83)
P. fluorescens	2	1 (50)	1 (50)		1/12 (8)
P. mendocina	1		1 (100)		
P. pseudoalcaligenes	0				0/1 (0)
P. putida	12	2 (17)	10 (83)		2/9 (22)
P. stutzeri	10	1 (10)	6 (60)	3 (30)	1/1 (100)
P. vesicularis	2	1 (50)	1 (50)		1/1 (100)
Roseomonas spp.	4			4 (100)	
Sphingobacterium spp.					
S. multivorum	3	1 (33)	1 (33)	1 (33)	1/1 (100)
S. spiritivorum	0				0/1 (0)
Sphingomonas paucimobilis	13	9 (69)		4 (31)	9/9 (100)
Weeksella spp.					
W. virosa	5	2 (40)		3 (60)	2/3 (67)
W. zoohelcum	1		1 (100)		
Xanthomonas maltophilia	9	9 (100)			9/9 (100)
Total	363	253 (70)	75 (21)	35 (10)	253/328 (77)

[a] All isolates but one (*P. alcaligenes*) incorrectly identified to the species level were correctly identified to the genus level.
[b] Number of correct MIS identifications for a species/total number of times the MIS identified isolates by that species name (correctly and incorrectly).
[c] CDC, Centers for Disease Control and Prevention.

species level or "no match" (not identifiable by the MIS), appropriate conventional tests were repeated to confirm the species identification. Also, a fresh extract from a subculture of the isolate was chromatographed again. In addition to calculating the percentage of isolates of each species that were correctly identified by the MIS, the predictive values for the accuracy of MIS identifications for different species were also determined by dividing the number of correct MIS identifications for a species by the total number of times the MIS identified isolates by that species name.

Of the 363 isolates studied, the MIS identified 328 (90.4%), of which 327 (99.7%) were correct to the genus level and 253 (77.1%) were correct to the species level (Table 1). Only nine isolates (one of *Acinetobacter junii*, two of *A. radioresistens*, one of *Capnocytophaga sputigena*, one of Centers for Disease Control and Prevention group EO-2, and four of *Roseomonas* spp.) were encountered from species or groups for which the MIS software library had no data. Of these, six (67%) were correctly called "no match" by the system and three (all *Acinetobacter* spp.) were called other species within the correct genus. The predictive value of an MIS species identification (the probability that the MIS species identification was accurate) was 77% overall but was ≥95% for 25 (62.5%) of the 40 species or groups which were named by the MIS during the study (Table 1).

Although only 60% of the isolates of *A. baumannii* that were chromatographed were correctly assigned by the system to that species, an MIS identification of an unknown isolate as *A. baumannii*, the most frequently isolated species in the *Acinetobacter* genus, had a 95% predictive value for accuracy (52 of 55 MIS identifications of that species were correct). All 10 of the isolates of *P. putida* that were misidentified by the chromatographic system were named as the closely related species *P. fluorescens*. The isolate that was misidentified by the MIS to the genus level was a urease-negative, motile isolate of *P. alcaligenes* which was called *Bordetella bronchiseptica*/Kingella by the gas-liquid chromatography system.

The results of this study, obtained with conventional SBA or chocolate agar cultures incubated aerobically (CO_2) at 35°C and the MIS Clinical Aerobes software library, agree with those of an earlier study in which isolates were cultured on Trypticase soy broth agar incubated at 28°C prior to extraction for chromatographic analyses (10). Both studies indicate that the MIS provides a sophisticated, rapid alternative for accurate identification of many of the clinical isolates of oxidase-positive and -negative, non-*Enterobacteriaceae* gram-negative rods. Just correct identification of the genera of some of these isolates with the MIS may be of considerable assistance in accurate identification of the species by conventional methods. The high predictive values of MIS for accuracy of identification to the genus level, and, to a lesser extent, to the species level, indicate that the chromatographic system can effectively supplement or replace time-consuming, traditional biochemical studies for many of the isolates encountered in clinical laboratories.

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