

Evaluation of Accuracy and Repeatability of Identification of Food-Borne Pathogens by Automated Bacterial Identification Systems

JOSEPH A. ODUMERU,^{1*} MARINA STEELE,¹ LYNNE FRUHNER,¹
CAROLYN LARKIN,¹ JIANGDONG JIANG,¹ ELROY MANN,³
AND W. BRUCE MCNAB²

Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada N1H 8J7¹;
Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada,
N1G 4Y2²; and Health Canada, Health of Animals Laboratory, Guelph,
Ontario, Canada N1G 3W4³

Received 17 August 1998/Returned for modification 24 September 1998/Accepted 15 December 1998

The performances of five automated microbial identification systems, relative to that of a reference identification system, for their ability to accurately and repeatedly identify six common food-borne pathogens were assessed. The systems assessed were the MicroLog system (Biolog Inc., Hayward, Calif.), the Microbial Identification System (MIS; MIDI Inc., Newark, Del.), the VITEK system (bioMérieux Vitek, Hazelwood, Mo.), the MicroScan WalkAway 40 system (Dade-MicroScan International, West Sacramento, Calif.), and the Replianalyzer system (Oxoid Inc., Nepean, Ontario, Canada). The sensitivities and specificities of these systems for the identification of food-borne isolates of *Bacillus cereus*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., and verotoxigenic *Escherichia coli* were determined with 40 reference positive isolates and 40 reference negative isolates for each pathogen. The sensitivities of these systems for the identification of these pathogens ranged from 42.5 to 100%, and the specificities of these systems for the identification of these pathogens ranged from 32.5 to 100%. Some of the systems had difficulty correctly identifying the reference isolates when the results were compared to those from the reference identification tests. The sensitivity of MIS for the identification of *S. aureus*, *B. cereus*, *E. coli*, and *C. jejuni*, for example, ranged from 47.5 to 72.5%. The sensitivity of the Microlog system for the identification of *E. coli* was 72.5%, and the sensitivity of the VITEK system for the identification of *B. cereus* was 42.5%. The specificities of four of the five systems for the identification of all of the species tested with the available databases were greater than or equal to 97.5%; the exception was MIS for the identification of *C. jejuni*, which displayed a specificity of 32.5% when it was tested with reference negative isolates including *Campylobacter coli* and other *Campylobacter* species. All systems had >80% sensitivities for the identification of *Salmonella* species and *Listeria* species at the genus level. The repeatability of these systems for the identification of test isolates ranged from 30 to 100%. Not all systems included all six pathogens in their databases; thus, some species could not be tested with all systems. The choice of automated microbial identification system for the identification of a food-borne pathogen would depend on the availability of identification libraries within the systems and the performance of the systems for the identification of the pathogen.

Bacterial food-borne pathogens are an important food safety issue worldwide. Rapid and accurate identification of bacterial pathogens isolated from food samples is important both for food quality assurance and for the tracing of outbreaks of bacterial pathogens within the food supply. Automated microbial identification systems have become widely used in both clinical and food microbiology laboratories. These systems offer some important advantages over conventional methods, including reduced labor, reduced human error, increased sample throughput, and faster turnaround times for test results. Some examples of automated microbial identification systems currently on the market include the Microbial Identification System (MIS; MIDI Inc., Newark, Del.), the MicroScan WalkAway 40 system (Dade Diagnostics Corp., Mississauga, Ontario, Canada), the MicroLog system (Biolog Inc., Hayward, Calif.), the VITEK system (bioMérieux Vitek, Hazelwood,

Mo.), and the Replianalyzer system (Oxoid Inc., Nepean, Ontario, Canada).

While several studies have examined the performances of automated microbial identification systems for the examination of clinical isolates (11, 18, 21-27, 29), little work has been done to study the sensitivities and specificities of these systems for the testing of pathogens isolated from food samples. Many of the previous studies of clinical isolates were performed with versions of these automated microbial identification systems that contained now obsolete databases (11, 14, 18, 25, 28), and many of the studies did not include species that are often detected as pathogens in food samples. Therefore, it is essential to determine the sensitivities, specificities, and repeatabilities of these systems for the identification of important pathogens isolated from food samples.

The objectives of the study were (i) to determine the sensitivities and specificities of the MicroLog system, MIS, the VITEK system, the WalkAway 40 system, and the Replianalyzer system for the identification of food isolates of *Bacillus cereus*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., and verotoxigenic *Escherichia*

* Corresponding author. Mailing address: Laboratory Services Division, University of Guelph, 95 Stone Road West, Guelph, Ontario, Canada N1H 8J7. Phone: (519) 767-6243. Fax: (519) 767-6240. E-mail: jodumeru@lsd.uoguelph.ca.

TABLE 1. Reference isolates used to determine specificities of five automated microbial identification systems for common food-borne pathogens

Pathogen	Reference positive isolates	Reference negative isolates
<i>B. cereus</i>	35 laboratory isolates, ATCC 4342, ATCC 14579, ATCC 7004, ATCC 33018, ATCC 11778	16 <i>Bacillus</i> spp. (non- <i>B. cereus</i>), 2 <i>Bacillus amyloliquifaciens</i> , 1 <i>Bacillus circulans</i> , 1 <i>Bacillus megaterium</i> , 1 <i>Bacillus sphaericus</i> , 3 <i>Bacillus subtilis</i> , 3 <i>Bacillus licheniformis</i> , 2 <i>Bacillus pumilus</i> , 1 <i>Bacillus pasteurii</i> , 3 <i>Bacillus circulans</i> , 1 <i>Bacillus lentus</i> , 2 <i>E. coli</i> , 1 <i>S. aureus</i> , 1 <i>Staphylococcus haemolyticus</i> , 1 <i>L. monocytogenes</i> , 1 <i>Listeria seeligeri</i>
<i>C. jejuni</i>	35 laboratory isolates, ATCC 49349, ATCC 33560, ATCC 29428, ATCC 43432, ATCC 33291	29 <i>C. coli</i> , 3 <i>Campylobacter lari</i> , 1 <i>Campylobacter fetus</i> , 1 <i>Campylobacter hyointestinalis</i> , 5 <i>Ochrobactum anthropi</i> , 1 <i>Pseudomonas aeruginosa</i>
<i>L. monocytogenes</i>	35 laboratory isolates, ATCC 19111, ATCC 19112, ATCC 19117, ATCC 19114, ATCC 19116	15 <i>Listeria innocua</i> , 10 <i>Listeria welshimeri</i> , 7 <i>Listeria seeligeri</i> , 2 <i>Listeria murrayi</i> , 1 <i>Listeria ivanovii</i> , 1 <i>Listeria grayi</i> , 1 <i>S. haemolyticus</i> , 1 <i>Staphylococcus epidermidis</i> , 1 <i>Streptococcus agalactiae</i> , 1 <i>R. equi</i>
<i>S. aureus</i>	35 laboratory isolates, ATCC 25923, ATCC 29213, ATCC 9144, ATCC 12600, ATCC 27154	7 <i>Staphylococcus intermedius</i> , 6 <i>Staphylococcus xylosum</i> , 5 <i>Staphylococcus cohnii</i> subsp., 4 <i>Staphylococcus schleiferi</i> subsp., 4 <i>S. haemolyticus</i> , 2 <i>Staphylococcus lugdunensis</i> , 4 <i>Staphylococcus hyicus</i> , 1 <i>Staphylococcus sciuri</i> , 2 <i>Staphylococcus saprophyticus</i> , 3 <i>Staphylococcus epidermidis</i> , 1 <i>Staphylococcus chromogenes</i> , 1 <i>Micrococcus luteus</i>
Verotoxigenic <i>E. coli</i>	35 laboratory isolates, ATCC 43887, ATCC 43889, ATCC 43895, ATCC 43894, ATCC 25922	14 <i>Enterobacter</i> spp., 6 <i>Klebsiella</i> spp., 8 <i>Citrobacter</i> spp., 2 <i>Hafnia alvei</i> , 2 <i>Serratia</i> spp., 1 <i>Yersinia enterocolitica</i> , 1 <i>Pseudomonas alcaligenes</i> , 1 <i>Acinetobacter baumannii</i> , 1 <i>P. aeruginosa</i> , 1 <i>Kluyvera ascorbata</i> , 3 <i>Shigella</i> spp.
<i>Salmonella</i> spp.	35 laboratory isolates, ATCC 8326, ATCC 8391, ATCC 6962, ATCC 13076, ATCC 14028	14 <i>Enterobacter</i> spp., 6 <i>Klebsiella</i> spp., 2 <i>H. alvei</i> , 2 <i>Serratia</i> spp., 1 <i>Y. enterocolitica</i> , 10 <i>Citrobacter</i> spp., 3 <i>E. coli</i> , 1 <i>Edwardsiella tarda</i> , 1 <i>S. sonnei</i>

coli and (ii) to determine the repeatabilities of these systems for the identification of these food isolates.

MATERIALS AND METHODS

Bacterial isolates. The bacterial isolates used in this study included 40 reference positive isolates and 40 reference negative isolates each of *B. cereus*, *C. jejuni*, *L. monocytogenes*, *S. aureus*, *Salmonella* species, and verotoxigenic *E. coli* (Table 1). All of the reference positive isolates were obtained from food samples and included five American Type Culture Collection (ATCC) strains and 35 laboratory isolates from a wide variety of food sources. The reference negative isolates were cultured from food, clinical, or environmental samples and included five ATCC strains and 35 laboratory isolates which were related to but not identical to the pathogen of interest. All isolates were confirmed as either reference positive isolates or reference negative isolates by using reference identification tests, and only those isolates which were correctly identified by the reference identification tests at a good confidence level were included in the study.

Preparation of study isolates. Stock cultures of reference positive isolates and reference negative isolates were subcultured from a frozen state onto Trypticase soy agar (TSA) with 5% sheep blood (BBL, Cockeysville, Md.) and were incubated aerobically at 35°C for 24 h; however, *C. jejuni* reference positive isolates and reference negative isolates were incubated microaerophilically for 48 to 72 h. Second and third subcultures were performed with the media and incubation conditions recommended by the manufacturer of each identification system. Isolates from the third subculture was used to test each system.

Reference identification tests. The identities of the reference positive isolates and the reference negative isolates of verotoxigenic *E. coli* and *Salmonella* spp. were confirmed with the API 20E identification kit (bioMérieux Vitek) according to directions that accompanied the product. The identities of the reference positive isolates and the reference negative isolates of *B. cereus* were confirmed by a biochemical testing scheme consisting of Gram staining, catalase reaction, hemolysin production on TSA plus 5% sheep blood (BBL), and lecithinase production on *B. cereus* selective agar (Oxoid) supplemented with polymyxin B (Oxoid) and egg yolk emulsion (Oxoid). The identities of the *B. cereus* isolates were further confirmed by using the API 50 CHB (bioMérieux Vitek) test kit. The identities of the *C. jejuni* reference positive isolates and reference negative

isolates were confirmed by using a biochemical identification scheme consisting of Gram staining, catalase reaction, oxidase reaction, the presence of corkscrew-like motility as seen under a dark-field microscope, hippurate hydrolysis, and sensitivity to nalidixic acid and resistance to cephalothin (20). The reference method used in this study to confirm the identities of the *L. monocytogenes* reference positive isolates and reference negative isolates was a biochemical scheme, which included Gram staining, catalase reaction, esculin hydrolysis on Oxford agar, hemolysis on horse blood agar, CAMP reaction, fermentation of rhamnose, xylose, and mannitol, and the presence of tumbling motility. A modified version of the simplified scheme of Kloos and Schleifer (13) was used to confirm the identities of the *S. aureus* reference positive isolates and reference negative isolates. This scheme consisted of Gram staining, hemolysin production on TSA plus 5% sheep blood (BBL), colony pigment production, catalase reaction, tube coagulase test, urea and nitrate reactions, and fermentation of arabinose, lactose, maltose, mannitol, sucrose, trehalose, and xylose. These reference identification tests were designed to identify the reference positive isolates to the species level and to confirm that the reference negative isolates were species other than that of the reference positive isolate to which they were being compared.

Bacterial identification systems. The automated microbial identification systems included in this study are listed in Table 2. The WalkAway 40 and the VITEK systems both entail inoculation of a microbial suspension into prepared microwell plates for the WalkAway 40 system or test cards for the VITEK system. These microwell plates and test cards contain a variety of conventional and proprietary biochemical substrates and antibiotics. Growth of bacteria within the microwells (WalkAway 40 system) or test card wells (VITEK system) results in biochemical substrate changes which can be interpreted by a specialized plate reader (WalkAway 40 system) or automated test card reader (VITEK system) to produce a biochemical profile. This profile can be compared to the profiles of known microorganisms to generate an identification. Operation of the MicroLog system also involves inoculation of a microbial suspension into specialized microwell plates. The wells of these plates contain buffered media with different carbon sources and an indicator dye, tetrazolium violet. The dye is reduced when different carbon sources are utilized, resulting in a biochemical profile which can be compared to the profiles of known microorganisms to generate an identification. The Replianalyzer is similar to the WalkAway 40 and VITEK systems in that a profile of biochemical reactions is generated and compared to those of

TABLE 2. Summary of automated bacterial identification systems included in the study and the food-borne bacterial pathogens used to test these systems^a

Species	Reference test	System used				
		WalkAway 40	MIS	VITEK	Replianalyzer	MicroLog
<i>L. monocytogenes</i>	Biochemical scheme	Yes	Yes	Yes	No	Yes
<i>S. aureus</i> ^b	Biochemical scheme	Yes	Yes	Yes	No	Yes
<i>B. cereus</i> ^b	Biochemical scheme, API 50 CHB	No	Yes	Yes	No	Yes
<i>C. jejuni</i>	Biochemical scheme	No	Yes	No	No	No
<i>Salmonella</i> spp.	API 20E	Yes	Yes	Yes	Yes	Yes
<i>E. coli</i>	API 20E	Yes	Yes	Yes	Yes	Yes

^a The following database versions of the automated microbial identification systems were used in this study: WalkAway 40 system, version 20.37; MIS, version 3.8; VITEK system, version DSAMSO-R10.3; Replianalyzer system, version 2.11; MicroLog system, version 3.5.

^b *S. aureus* and *B. cereus* cultures were not tested with the Biolog databases because these databases were in the process of being revised at the time of the study.

known microorganisms, but this system uses agar plates rather than microwell plates. MIS is significantly different from the other systems because it is based on a comparison of the fatty acid methyl ester profiles for unknown microorganisms to those for known microorganisms to generate an identification.

Isolates to be identified with MIS were grown and processed as described in the MIS operating manual (17) for the CLIN library and were tested with the Sherlock, version 1.06, CLIN library database, version 3.8. Isolates to be identified with the Microscan WalkAway 40 system were grown and processed as described in the WalkAway 40 system operating manual (5), inoculated into Dried Overnight Gram Positive ID panels (*L. monocytogenes* and *S. aureus*) or Dried Overnight Gram Negative ID panels (*Salmonella* spp. and verotoxigenic *E. coli*), and evaluated with the Microscan Data Management System, version 20.57, database. Isolates to be identified with the Replianalyzer system were grown and processed as described in the Replianalyzer operating manuals (3) and were evaluated with the Replianalyzer, version 2.11, database. Isolates to be identified with the MicroLog system were grown and processed as described in the Biolog system manual (6), inoculated into GP MicroPlates (*L. monocytogenes*) or GN MicroPlates (*Salmonella* spp. and verotoxigenic *E. coli*), and analyzed with the version DE, release 3.5, database. Isolates to be identified with the VITEK system were grown and processed as described in the VITEK operator's manual (7), inoculated into the GPI card (*L. monocytogenes* and *S. aureus*), the GNI card (*Salmonella* spp. and verotoxigenic *E. coli*), or the BAC card (*B. cereus*), and evaluated with the VITEK, version DSAMSO-R10.3, database. Reference negative isolates and reference positive isolates of each pathogen were examined with the same systems under the same conditions.

Analysis of results. The different systems generated identification results in different formats. Most of the automated systems had a specific minimum level of probability that was required for the identification of an unknown organism to be interpreted with good confidence. The MicroLog system required a minimum similarity index of 0.500. MIS required a minimum similarity index of 0.300 with a minimum separation of 0.100 between the first identification and any secondary identifications. The WalkAway 40 system required a probability of greater than or equal to 85%. The Replianalyzer system required a probability of 95%. The VITEK system did not have a specific probability cutoff level for acceptable identifications. A probability of greater than or equal to 95% was used so that the results obtained with the VITEK system could be directly compared with those obtained with the other systems.

The sensitivities of the test systems were determined by testing each system with 40 known reference positive isolates of each of the six pathogens. Sensitivity in this study was defined as the proportion of the reference positive isolates which were correctly identified with the automated microbial identification systems with an acceptable identification confidence level, as specified by the system's manufacturer. The proportion of reference positive isolates which were correctly identified with the automated microbial identification systems but with unacceptably low confidence levels was also examined.

The specificities of the test systems were determined by testing each system with 40 isolates of bacteria which were not the pathogen of interest but which showed similarities in terms of their biochemical reactions and Gram staining results to those of the pathogen of interest. Specificity in this study was defined as the proportion of reference negative isolates which were not identified as the pathogen of interest.

The repeatabilities of the test systems were determined by performing repeat analyses for 20 randomly selected ATCC strains and laboratory isolates from among both the reference positive isolates and the reference negative isolates of each pathogen. The second subculture of the first analysis was used as a starting point for the replicate analysis of each isolate, and the replicate analysis was performed once for each isolate on days different from the days of performance of the initial analysis. The identification results and the confidence levels of the identifications were examined. Repeatability of identification was defined as the proportion of repeat analyses which generated the same identification with similar confidence levels, i.e., having either acceptable or unacceptable confi-

dence levels in both analyses. The proportion of repeat analyses which generated the same identification but at different confidence levels was also examined.

RESULTS

The sensitivities and specificities of the automated microbial identification systems for each of the pathogens of interest are summarized in Table 3. The data indicate the sensitivity and specificity of each system for the pathogens of interest when identification results with acceptable confidence levels were analyzed. The repeatabilities of the different automated micro-

TABLE 3. Summary of sensitivities and specificities of several automated bacterial identification systems tested with food-borne pathogens

Pathogen and system	% Sensitivity ^a	% Specificity ^b
<i>Listeria</i> species		
WalkAway 40	100	100
MIS	90	100
VITEK	97.5	100
MicroLog	97.5	100
<i>S. aureus</i>		
WalkAway 40	97.5	100
MIS	47.5	100
VITEK	95	95
<i>B. cereus</i>		
MIS	55	97.5
VITEK	42.5	97.5
<i>C. jejuni</i> , MIS		
	72.5	32.5
<i>Salmonella</i> species		
WalkAway 40	97.5	100
MIS	85	100
VITEK	100	100
Replianalyzer	95	100
MicroLog	95	100
<i>E. coli</i>		
WalkAway 40	100	100
MIS	52.5	97.5
VITEK	100	97.5
Replianalyzer	90	100
MicroLog	72.5	100

^a Proportion of reference positive strains which were correctly identified with an acceptable confidence rating.

^b Proportion of reference negative strains which were not identified as the pathogen of concern with an acceptable confidence rating.

TABLE 4. Repeatabilities of identifications generated by automated bacterial identification systems against food-borne pathogens and closely related species

Pathogen and system	Repeatability (%) in replicate analyses ^a	
	Reference positive isolates	Reference negative isolates
<i>L. monocytogenes</i>		
Biochemicals	100	100
WalkAway 40	100	90
MIS	30	55
VITEK	100	85
MicroLog	100	85
<i>S. aureus</i>		
Biochemicals	100	85
WalkAway 40	100	75
MIS	60	90
VITEK	90	65
<i>B. cereus</i>		
Biochemicals	100	100
MIS	90	80
VITEK	60	80
<i>C. jejuni</i>		
Biochemicals	100	100
MIS	90	75
<i>Salmonella</i> spp.		
API 20E	100	90
WalkAway 40	95	90
MIS	90	65
VITEK	100	100
Replianalyzer	100	85
MicroLog	85	65
<i>E. coli</i>		
API 20E	100	90
WalkAway 40	100	90
MIS	70	65
VITEK	95	100
Replianalyzer	100	85
MicroLog	70	65

^a Proportion of replicate analyses which generated the same identification with similar confidence rating.

bial identification systems for reference positive isolates and reference negative isolates for the pathogens of interest are presented in Table 4. The values represent the proportions of repeat analyses which generated the same identification with similar confidence levels.

DISCUSSION

There are a number of factors to be considered when determining which automated microbial identification system is most appropriate for the detection of food-borne pathogens within a particular laboratory. These factors include the initial investment that is required, operating costs, technician time, range of organisms within the system's database, and the ability of the system to correctly identify food pathogens of interest. The last two variables were examined in this study. The automated microbial identification systems evaluated included MIS, the WalkAway 40 system, the MicroLog system, the VITEK system, and the Replianalyzer system.

The WalkAway 40 system, the VITEK system, and the MicroLog system all contained databases for the identification of

Listeria spp. MIS contained a database for the identification of *L. monocytogenes* specifically. To compare the systems fairly, only identifications to the genus level are reported in Table 3. All four of the systems performed well in identifying *L. monocytogenes* to the genus level, with sensitivities of 90 to 100% and specificities of 100%. A much lower specificity was observed when MIS was used to identify *L. monocytogenes* to the species level because *Listeria innocua* isolates were frequently identified as *L. monocytogenes*. The repeatabilities observed for the different systems were also good, with the exception of the MIS, which showed a lower repeatability than the other systems. The repeatability of the MIS was increased to 70% when species identification, but not confidence rating, was considered. While all of these systems could be used reliably as a screening method for the detection of *Listeria* spp. to the genus level, further biochemical tests would be required to determine whether the isolate was a potentially pathogenic *L. monocytogenes* isolate or a nonpathogenic *Listeria* species. Presumptive identification of *Listeria* species from colonies on selective media, such as Oxford agar, requires only a few simple tests, so there is at present little advantage in using these automated identification systems for the identification of *L. monocytogenes*.

The WalkAway 40 system, the VITEK system, and MIS all contained databases for the identification of *S. aureus*. The MicroLog and the Replianalyzer system databases for the identification of *Staphylococcus* and related species were being developed or upgraded, and they were not included in this study. The WalkAway 40 and the VITEK systems both showed good sensitivities and specificities for the identification of *S. aureus*. MIS showed 100% specificity for the identification of *S. aureus* but a sensitivity of only 47.5%. This sensitivity of MIS for the identification of *S. aureus* increased to 87.5% if correct identifications with low confidence levels were included, suggesting that the fatty acid compositions of the food isolates examined were slightly different from those of the isolates within the database. The specificity of the system was reduced to 90% when results with low confidence levels were included in the analysis. Repeatability levels for all three systems were slightly higher for reference positive isolates than for reference negative isolates, ranging from 60 to 100% when both identification and confidence level were considered. Presumptive identification of *S. aureus* requires only a few biochemical tests which are available in most nonautomated methods; hence, there is little advantage in using these automated identification systems for the identification of this organism.

Both MIS and the VITEK system contained databases for the identification of *B. cereus* isolates. At the time of this study, the MicroLog system database for *B. cereus* was being upgraded; therefore, this system was not evaluated for *B. cereus*. Neither MIS nor the VITEK system was able to identify *B. cereus* with a high sensitivity, even if correct identifications with low confidence levels were included. Some investigators have proposed that *B. cereus*, *Bacillus mycoides*, and *Bacillus thuringiensis* are very closely related and should be merged into a single species (2, 8). When the results were reanalyzed with the definition of a correct identification expanded to include *B. mycoides* and *B. thuringiensis* species, the sensitivities of MIS and the VITEK system increased to 82.5 and 67.5%, respectively. The two systems showed a high specificity (97.5%) for *B. cereus*. The repeatability of the VITEK system for this organism was somewhat low at 60%, while MIS showed a repeatability of 90%. The results obtained by the API 50 CHB test kit corresponded well with those obtained by the biochemical reference scheme when tests were performed with *B. cereus* isolates.

MIS was the only system with a database for the identification of *Campylobacter* species. The sensitivity and specificity of this system for the identification of *C. jejuni* were 72.5 and 32.5%, respectively. The sensitivity increased to 92.5% if correct identifications with low confidence levels were included. However, the specificity of the system declined to 30% when other *Campylobacter* species misidentified as *C. jejuni* with low confidence levels were included in the estimation of specificity. The low specificity of MIS for *C. jejuni* may be due to the fact that most of the reference negative isolates used in the study were *Campylobacter coli* and the system appears to have difficulty distinguishing *C. coli* from the closely related species *C. jejuni*. Both *C. jejuni* and *C. coli* are common food-borne pathogens; thus, MIS may be useful as a screening method for the identification of pathogenic *Campylobacter* isolates. The repeatability of MIS was found to be 90% for *C. jejuni* and 75% for related species when the same identification and confidence levels were considered in the analysis. The identification of *C. jejuni* is similar to that of *L. monocytogenes* in that presumptive identification from selective media requires only a few simple laboratory tests; hence, there is little advantage in the use of automated systems for the identification of *Campylobacter* to the genus level unless these systems are able to differentiate pathogenic and nonpathogenic *Campylobacter* species with high specificities and sensitivities.

All of the systems included in this study had developed databases for the identification of *Salmonella* spp., and all of the systems showed good sensitivities for *Salmonella* identification, with values ranging from 85 to 100%. Specificities of 100% were observed for all of the systems tested, suggesting that false-positive identifications would not be a problem for *Salmonella* isolates. The repeatabilities of the different systems when they were tested with *Salmonella* spp. and related isolates were highest with the VITEK, the WalkAway 40, and the Replianalyzer systems and were somewhat higher for reference positive isolates than for reference negative isolates.

While all of the systems included in this study had databases for the identification of *E. coli*, none of these databases distinguished between verotoxigenic *E. coli* and other *E. coli* strains. One study, however, reported that a limited number of biochemical profiles were generated when *E. coli* O157:H7 strains were screened with the MicroScan Overnight Panel System (1). The Replianalyzer database may also detect *E. coli* O157:H7 on the basis of a sorbitol-negative reaction. The WalkAway 40, the VITEK, and the Replianalyzer systems appeared to be well adapted for the identification of *E. coli* isolates, with sensitivities of 90 to 100% and specificities of 97.5 to 100%. Both the MicroLog system and MIS reported somewhat lower sensitivities; however, these sensitivities increased to acceptable levels if correct identifications with low confidence levels were included in the analysis. Inclusion of results with lower confidence levels in the analysis resulted in a slightly lower specificity of MIS for this organism. The WalkAway 40, VITEK, and Replianalyzer systems appeared to be the most repeatable for verotoxigenic *E. coli* and related isolates, while MIS and the MicroLog system had somewhat lower repeatabilities.

In summary, none of the systems appeared to offer any advantage over biochemical identification schemes for *L. monocytogenes* identification; however, very good results (>90% sensitivity) were obtained for the identification of *S. aureus*, *Salmonella* spp., and verotoxigenic *E. coli* by the WalkAway 40 and the VITEK systems. The Replianalyzer system had a $\geq 90\%$ sensitivity for the identification of *Salmonella* and verotoxigenic *E. coli*. The MicroLog system had >90% sensitivity for the identification of *Salmonella* spp. and >90% sensitivity for the identification of verotoxigenic *E. coli* when

correct identification results with or without acceptable confidence ratings were included in the results. Similarly, MIS had >90% sensitivity for the identification of the *B. cereus* group, *C. jejuni*, and *Salmonella* spp. when correct identification results with or without acceptable confidence ratings were included in the analysis. The lower sensitivities observed when some of these pathogens were tested with MIS may be influenced by the fact that the reference systems for these species were based on biochemical reactions, while the MIS identifications are based on the analysis of the fatty acid composition of the unknown microorganism. MIS showed promising results for the identification of *Campylobacter* species, and both the VITEK system and MIS showed a fairly good ability to identify *B. cereus* group isolates.

ACKNOWLEDGMENTS

We acknowledge the financial support received from the Enhanced Food Quality and Safety Program administered by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the contributions of consumable supplies by Dade Diagnostics Corp., MicroLog Inc., bioMérieux VITEK, and Oxoid Canada Inc. We also thank the following people for contributing bacterial isolates for this study: David Woodward, National Laboratory for Enteric Pathogens, Health Canada; Peter Boleszczuk and Mike Brodsky, Ontario Ministry of Health, Laboratory Services Branch, Etobicoke, Ontario, Canada; Luba Stokes, Victoria Hospital, London, Ontario, Canada; Roger Shuttleworth, University Hospital, London, Ontario, Canada; Noni Smart, Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada; and C. E. Park, Health Canada, Ottawa, Ontario, Canada.

REFERENCES

- Abbott, S. L., D. F. Hanson, T. D. Felland, S. Connell, A. H. Shum, and J. M. Janda. 1994. *Escherichia coli* O157:H7 generates a unique biochemical profile on MicroScan conventional gram-negative identification panels. *J. Clin. Microbiol.* **32**:823–824.
- Ash, C., J. A. E. Farrow, M. Dorsh, E. Stackebrandt, and M. D. Collins. 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int. J. Syst. Bacteriol.* **41**:343–346.
- AutoMed Cathra Systems. 1994. Cathra microbiology manual, Cathra Replidex manual, Cathra Replianalyzer II manual. AutoMed Cathra Systems, Arden Mills, Minn.
- Bannerman, T. L., K. T. Kleeman, and W. E. Kloos. 1993. Evaluation of the VITEK systems Gram-Positive Identification card for species identification of coagulase-negative staphylococci. *J. Clin. Microbiol.* **31**:1322–1325.
- Baxter Diagnostics Inc. 1992. MicroScan operators manual for Walkaway systems. Baxter Diagnostics Inc., West Sacramento, Calif.
- Biolog Inc. 1992. MicroLog operating manual. Biolog Inc., Hayward, Calif.
- bioMérieux Vitek. 1996. VITEK System manual. bioMérieux Vitek, Hazelwood, Mo.
- Carlson, C. R., D. A. Caugant, and A. B. Kolstø. 1994. Genotypic diversity among *Bacillus cereus* and *Bacillus thuringiensis* strains. *Appl. Environ. Microbiol.* **60**:1719–1725.
- Champagne, C. P., R. R. Laing, D. Roy, A. A. Mafu, and M. W. Griffiths. 1994. Psychrotrophs in dairy products: their effects and their control. *Crit. Rev. Food Sci. Nutr.* **34**:1–30.
- Holmes, B., M. Costas, M. Ganner, S. L. W. On, and M. Stevens. 1994. Evaluation of Biolog system for identification of some gram-negative bacteria of clinical importance. *J. Clin. Microbiol.* **32**:1970–1975.
- Hussain, Z., L. Stokes, D. L. Stevens, B. C. Schieven, R. Lannigan, and C. Jones. 1986. Comparison of the MicroScan system with the API Staph-Ident system for species identification of coagulase-negative staphylococci. *J. Clin. Microbiol.* **23**:126–128.
- Klingler, J. M., R. P. Stowe, D. C. Obenhuber, T. O. Groves, S. K. Mishra, and D. L. Pierson. 1992. Evaluation of the Biolog automated microbial identification system. *Appl. Environ. Microbiol.* **58**:2089–2092.
- Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol.* **1**:82–88.
- Kloos, W. E., and C. G. George. 1991. Identification of *Staphylococcus* species and subspecies with the MicroScan Pos ID and Rapid Pos ID panel systems. *J. Clin. Microbiol.* **29**:738–744.
- Knight, M. T., D. W. Wood, J. F. Black, G. Gosney, R. O. Rigney, and J. R. Agin. 1990. Gram-Negative Identification card for identification of *Salmonella*, *Escherichia coli*, and other *Enterobacteriaceae* isolated from foods:

- collaborative study. *J. Assoc. Off. Chem.* **73**:729-733.
16. **Kramer, J. M., and R. J. Gilbert.** 1989. *Bacillus cereus* and other *Bacillus* species, p. 21-70. In M. P. Doyle (ed.), *Foodborne bacterial pathogens*. Marcel Dekker, Inc., New York, N.Y.
 17. **Microbial ID Inc.** 1993. *Microbial Identification System operating manual*, version 4. Microbial ID Inc., Newark, Del.
 18. **Miller, J. M., J. W. Biddle, V. K. Quenzer, and J. C. McLaughlin.** 1993. Evaluation of Biolog for identification of members of the family *Micrococaceae*. *J. Clin. Microbiol.* **31**:3170-3173.
 19. **Miller, M., and D. L. Rhoden.** 1991. Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. *J. Clin. Microbiol.* **29**:1143-1147.
 20. **Nachamkin, I.** 1995. *Campylobacter* and *Arcobacter*, p. 483-491. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
 21. **O'Hara, C. M., and J. M. Miller.** 1992. Evaluation of the autoSCAN-W/A system for rapid (2 hour) identification of members of the family *Enterobacteriaceae*. *J. Clin. Microbiol.* **30**:1541-1543.
 22. **O'Hara, C. M., F. C. Tenover, and J. M. Miller.** 1993. Parallel comparison of accuracy of API 20E, Vitek GNI, Microscan Walk/Away Rapid ID, and Becton Dickinson Cobas Micro ID-E/NF for identification of members of the family *Enterobacteriaceae* and common gram-negative, non-glucose-fermenting bacilli. *J. Clin. Microbiol.* **31**:3165-3169.
 23. **Osterhout, G. J., V. H. Shull, and J. D. Dick.** 1991. Identification of clinical isolates of gram-negative nonfermentative bacteria by an automated cellular fatty acid identification system. *J. Clin. Microbiol.* **29**:1822-1830.
 24. **Pfaller, M. A., D. Sahn, C. O'Hara, C. Ciaglia, M. Yu, N. Yamane, G. Scharnweber, and D. Rhoden.** 1991. Comparison of the AutoSCAN-W/A Rapid Bacterial Identification system and the Vitek AutoMicrobic system for identification of gram-negative bacilli. *J. Clin. Microbiol.* **29**:1422-1428.
 25. **Rhoads, S., L. Marinelli, C. A. Imperatrice, and I. Nachamkin.** 1995. Comparison of MicroScan WalkAway system and Vitek system for identification of gram-negative bacteria. *J. Clin. Microbiol.* **33**:3044-3046.
 26. **Robinson, A., Y. S. McCarter, and J. Tetreault.** 1995. Comparison of Crystal Enteric/Nonfermenter System, API 20E System, and the Vitek Automicrobic System for identification of gram-negative bacilli. *J. Clin. Microbiol.* **33**:364-370.
 27. **Stager, C. E., and J. R. Davis.** 1992. Automated systems for identification of microorganisms. *J. Clin. Rev.* **5**:302-327.
 28. **Stokes, L., B. C. Schieven, E. Ofori, P. Ewan, R. Lannigan, and Z. Hussain.** 1992. Evaluation of Microscan Rapid Pos combo panels for identification of staphylococci. *J. Clin. Microbiol.* **30**:93-95.
 29. **Stokes, L., M. A. John, R. Lannigan, B. C. Schieven, M. Ramos, D. Harley, and Z. Hussain.** 1994. Gas-liquid chromatography of cellular fatty acids for identification of staphylococci. *J. Clin. Microbiol.* **32**:1908-1910.