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Laboratory Evaluation of the AutoMicrobic System for Identification of *Enterobacteriaceae*

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The *Enterobacteriaceae* Biochemical Card used with the AutoMicrobic system (Vitek Systems, Inc., Hazelwood, Mo.) was compared with the API 20E system (Analytab Products, Plainview, N.Y.) for identifying members of the family *Enterobacteriaceae*. A total of 1,401 clinical isolates representing 18 species were identified by the AutoMicrobic and API 20E systems over a 5-month period. Discrepancies between these systems were resolved by conventional methods. Overall, 98.3% of the isolates were identified correctly by the AutoMicrobic system in 8 h, with 94.2% having an AutoMicrobic system confidence level ≥90%; 92.9% of the isolates were identified correctly by the 20E system after 24 h of incubation. Discrepancies between the two systems occurred in 3.1% of the isolates. Of these, 40.9 and 59.1% proved to be correct by the AutoMicrobic and API 20E systems, respectively.

Rapid and accurate identification of enteric bacteria is one of the most important roles of any clinical microbiology laboratory. Currently, most identifications are made with commercially produced systems, such as API 20E (Analytab Products, Plainview, N.Y.), Micro-ID (General Diagnostics, Morris Plains, N.J.), and Enterotube (Roche Diagnostics, Div. Hoffmann-La Roche, Inc., Nutley, N.J.) with conventional methods used as backup systems. The AutoMicrobic system (AMS) (Vitek Systems, Inc., Hazelwood, Mo.) was the first widely marketed automated system for rapid identification of *Enterobacteriaceae* (1, 3-5). The potential of the AMS has recently been expanded to the identification of nonfermenters and yeasts. The AMS also has the capacity to enumerate and selectively identify bacteria from urine specimens and to perform antimicrobic susceptibility testing. This study evaluates the clinical application of the *Enterobacteriaceae* Biochemical Card (EBC) of the AMS for the identification of members of the family *Enterobacteriaceae* under routine laboratory conditions.

MATERIALS AND METHODS

Organisms. All oxidase-negative organisms recovered on MacConkey agar from clinical specimens were tested by the API 20E system and by the AMS. All organisms were numerically coded. Identifications were compared only after testing with both systems. Any disagreement in identification was resolved with conventional biochemical tests (2).

AMS EBC. The EBC consists of a series of 26 biochemical tests contained in a plastic card which is sealed with mylar tape. Incubation for 8 h in the AMS is required before identification is considered complete. The AMS and EBC were used as previously reported (3).

API 20E system. The API system is a standardized, miniaturized version of conventional procedures for identification of members of the family *Enterobacteriaceae* (6). This system is used routinely in our laboratory. The API system is designed to identify gram-negative bacteria based on data from 23 standard biochemical tests.

An isolated colony was picked from MacConkey agar, emulsified in sterile saline, and used to innoculate the API 20E test strips. After overnight incubation at 35°C, biochemical results were recorded and interpreted according to the manufacturer's directions.

Conventional methods. Carbohydrate media used in the study were prepared with Taxo carbohydrate disks (BBL Microbiology Systems, Cockeysville, Md.) added to sterile phenol red base. All other media were made from dehydrated media (Difco Laboratories, Detroit, Mich.). *Shigella* antisera (Difco) were used for serological identification of *Shigella* spp. All conventional media were prepared and used according to the manufacturer's directions. Conventional testing was done for adonitol, arabinose, malonate, maltose, methyl red, motility, raffinose, and xylose.

Quality control organisms. As recommended by the manufacturer, six organisms were tested with the AMS at weekly intervals and with each new lot of reagents. The six control bacteria were *Shigella flexneri* (ATCC 12661), *Enterobacter cloacae* (ATCC 13883), *Proteus mirabilis* (ATCC 7002), *Citrobacter freundii* (ATCC 6750), and *Klebsiella pneumoniae* (ATCC 13883). In addition, an EBC was inoculated with saline as a sterility check.

TABLE 1. *Comparison of results for the AMS and the API system with 1,401 clinical isolates*

Organism	Total no. tested	No. (%) of isolates correctly identified by the AMS at 8 h	No. (%) of isolates correctly identified by the API system at 24 h
Citrobacter freundii	25	22 (88.00)	22 (88.00)
Enterobacter aerogenes	62	62 (100)	56 (90.32)
Enterobacter agglomerans	6	5 (88.33)	2 (33.33)
Enterobacter cloacae	58	57 (98.28)	55 (94.83)
Escherichia coli	636	631 (99.21)	635 (99.84)
Hafnia alvei	6	6 (100)	5 (83.33)
Klebsiella oxytoca	53	50 (94.34)	53 (100)
Klebsiella pneumoniae	260	254 (97.69)	237 (91.15)
Proteus mirabilis	166	163 (98.19)	166 (100)
Proteus vulgaris	6	6 (100)	6 (100)
Providencia rettgeri	5	5 (100)	3 (60.00)
Providencia stuartii	9	9 (100)	9 (100)
Morganella morganii	21	21 (100)	21 (100)
Serratia liquefaciens	1	1 (100)	0 (0.00)
Serratia marcescens	76	74 (97.37)	29 (38.16)
Shigella flexneri	1	1 (100)	0 (0.00)
Shigella sonnei	2	2 (100)	2 (100)
Total	1,401	1,377 (98.29)	1,302 (92.93)

TABLE 2. *Organisms correctly identified by the AMS, with a percent confidence >0.9000, and in agreement with the API system or conventional methods*

Organism	No. (%) of isolates identified by the AMS
Escherichia coli	617 (97)
Klebsiella pneumoniae	242 (93)
Klebsiella oxytoca	49 (92)
Proteus mirabilis	146 (88)
Enterobacter aerogenes	61 (98)
Enterobacter cloacae	52 (90)
Enterobacter agglomerans	5 (83)
Proteus vulgaris	6 (100)
Providencia stuartii	7 (78)
Morganella morganii	20 (95)
Citrobacter freundii	19 (76)
Citrobacter diversus	8 (100)
Providencia rettgeri	5 (100)
Serratia marcescens	73 (96)
Serratia liquefaciens	1 (100)
Hafnia alvei	6 (100)
Shigella flexnei	1 (100)
Shigella sonnei	2 (100)
Total	1,320 (94.2)

TABLE 3. *Organisms identified by the AMS and in agreement with the API system or conventional methods but having an AMS percent confidence level <0.9000*

Organism	No. of isolates identified by the AMS	Mean AMS confidence level
Proteus mirabilis	17	0.7765
Escherichia coli	14	0.7035
Klebsiella pneumoniae	12	0.7689
Enterobacter cloacae	5	0.6473
Citrobacter freundii	3	0.7500
Providencia stuartii	2	0.7976
Klebsiella oxytoca	1	0.8859
Enterobacter aerogenes	1	0.8830
Morganella morganii	1	0.7695
Serratia marcescens	1	0.7447
Total	57	0.7726

In our laboratory the API system is routinely quality controlled daily with the following organisms: *K. pneumoniae* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 10145), and *E. cloacae* (ATCC 13047).

RESULTS AND DISCUSSION

Table 1 summarizes the comparison between the AMS and the API system using 1,401 clinical isolates. The AMS correctly identified 98.3% of the isolates at 8 h, and 92.9% were correctly identified by the API system at 24 h.

The AMS correctly identified 94.2% of the 1,401 isolates, with a confidence level >0.9000 (Table 2). An additional 57 isolates (Table 3) were identified correctly by the AMS, with a confidence level <0.9000. Of the 57 isolates, 17 were *P. mirabilis*, 14 were *Escherichia coli*, and 12 were *K. pneumoniae*. Of the 17 *P. mirabilis* isolates, 13 had the same AMS biochemical profile, being citrate and H_2S negative. This accounted for the low confidence level. No similarity in the biochemical profiles of the 14 *E. coli* isolates could be found, accounting for their low

TABLE 4. *Discrepancies between the AMS and the API systems*[a]

API identification	AMS identification	AMS confidence level[b]
Citrobacter freundii	*Enterobacter agglomerans*	0.6436
Citrobacter freundii	*Enterobacter cloacae*	0.9676
Citrobacter freundii	*Klebsiella ozaenae*	0.6905
Enterobacter agglomerans	*Klebsiella ozaenae*	0.6600
Enterobacter cloacae	*Enterobacter agglomerans*	0.9514
Escherichia coli	*Salmonella typhi*	0.4855
Escherichia coli	*Salmonella typhi*	0.4855
Escherichia coli	*Citrobacter freundii*	0.6185
Escherichia coli	*Salmonella typhi*	0.8328
Escherichia coli	*Providencia stuartii*	0.8283
Klebsiella pneumoniae	*Enterobacter aerogenes*	0.7774
Klebsiella pneumoniae	*Enterobacter cloacae*	0.7424
Klebsiella pneumoniae	*Enterobacter aerogenes*	0.6676
Klebsiella pneumoniae	*Enterobacter agglomerans*	0.9999
Klebsiella pneumoniae	*Enterobacter aerogenes*	0.8830
Klebsiella pneumoniae	*Klebsiella rhinoscleromatis*	0.8562
Klebsiella oxytoca	*Enterobacter aerogenes*	0.7274
Klebsiella oxytoca	*Enterobacter aerogenes*	0.9855
Klebsiella oxytoca	*Klebsiella ozaenae*	0.4069
Proteus mirabilis	*Morganella morganii*	0.6921
Proteus mirabilis	*Morganella morganii*	0.9066
Serratia marcescens	*Morganella morganii*	0.6921
Serratia marcescens	*Serratia liquefaciens*	0.7754
	Yersinia enterocolitica	0.8697

[a] Organisms confirmed by conventional methods to be correctly identified by the API system and incorrectly identified by the AMS.
[b] Mean, 0.7560.

TABLE 5. *Discrepancies between the AMS and the API system*[a]

AMS identification	API identification	AMS confidence level[b]
Citrobacter freundii	*Escherichia coli*	0.9727
Enterobacter aerogenes	*Serratia liquefaciens*	0.9928
Enterobacter agglomerans	*Enterobacter cloacae*	0.9880
Enterobacter agglomerans	*Enterobacter cloacae*	0.6896
Enterobacter agglomerans	*Enterobacter cloacae*	0.6619
Enterobacter agglomerans	*API group II*	0.9820
Enterobacter cloacae	*Citrobacter freundii*	0.9922
Hafnia alvei	*Proteus mirabilis*	0.9999
Klebsiella pneumoniae	*Enterobacter aerogenes*	0.9921
Klebsiella pneumoniae	*Enterobacter aerogenes*	0.9101
Klebsiella pneumoniae	*Klebsiella ozaenae*	0.9960
Klebsiella pneumoniae	*Klebsiella oxytoca*	0.6802
Klebsiella pneumoniae	*Enterobacter aerogenes*	0.9959
Serratia marcescens	*Serratia liquefaciens*	0.9996
Serratia marcescens	*Serratia liquefaciens*	0.9996
Serratia marcescens	*Serratia liquefaciens*	0.9998
Serratia marcescens	*Serratia liquefaciens*	0.9998
Serratia marcescens	*Serratia liquefaciens*	0.9996

[a] Organisms confirmed by conventional methods to be correctly identified by the AMS and incorrectly identified by the API system.
[b] Mean, 0.9365.

confidence levels. Of the 12 *K. pneumoniae* isolates, 10 were urea negative by the AMS and the API system. The fact that the isolates were urea negative accounted for the low confidence levels.

Discrepancies occurred in 3.1% of the isolates. Of these, 40.9 and 59.1% were correctly identified by the AMS (Table 4) and the API system (Table 5), respectively.

There were notable common factors among some of the species incorrectly identified by the AMS. Seven of the nine *Klebisella* spp. were misidentified as *E. aerogenes*. Of the five isolates

of *E. coli* misidentified by the AMS, four identified as *Salmonella typhi*, even though they were arabinose positive on the EBC. This illustrates the lack of significance that the AMS gives to any one test in an identification (i.e., all tests on the EBC are weighted equally). *S. typhi* is considered arabinose negative (100%) (2). The low confidence factor of these misidentifications (average, 0.65) and the fermentation of arabinose alerted the technologist to check the indole reaction. This is easily done by puncturing the mylar tape over the third well (growth control) and adding Kovács reagent. The reading is the same as with the API 20E system or conventional methods. These isolates were all indole positive. Three isolates of *P. mirabilis* were misidentified as *Morganella morganii*. This was a result of the xylose being negative on the EBC. By conventional methods these isolates were xylose positive. This misidentification was probably due to an underinoculation or to a slow reaction. These isolates were known swarmers, and the entry of this information into the identification scheme would have alerted trained personnel to the misidentification. There were few misidentified species exhibiting a reoccurring error in their AMS biochemical profiles. The number of *Klebsiella* sp. misidentified represented 2.9% of the total number of *Klebsiella* isolates, that of *E. coli*, 0.79%, and that of *P. mirabilis*, 1.8%.

All quality control organisms run weekly during this study were identified correctly by the AMS, with consistently high confidence levels. In addition to the five members of the family *Enterobacteriaceae*, *P. aeruginosa* was used as a non-glucose-fermenting control. *P. aeruginosa* was always glucose negative and was identified by the AMS as a non-*Enterobacteriaceae* organism. EBCs with AMS saline inoculum were run simultaneously as sterility controls and were always negative.

The quality control organisms used in the API system were always correctly identified. The use of these organisms provided positive and negative results for each biochemical reaction.

This study provides evidence for the reliability of the AMS in delivering fast and accurate identification of members of the family *Enterobacteriaceae*. The bacteria tested represent a wide range of *Enterobacteriaceae* commonly recovered in clinical laboratories. The low numbers of some species isolated are indicative of the unequal distribution of organisms in clinical settings. The analysis of the organisms isolated in this 5-month period provide a practical guideline for comparing, in clinical laboratories, the identification of members of the family *Enterobacteriaceae* by the AMS with a widely accepted method such as the API system.

This report does not closely scrutinize the biochemical profiles of the EBC or the mechanisms of the AMS. A detailed description of the AMS and EBC have been previously reported (3).

The capacity of the AMS to identify members of the family *Enterobacteriaceae* and the high correlation of this identification with the API system has been established in these analyses. In addition, color changes are interpreted spectrophotometrically with the AMS, eliminating errors in interpreting color changes inherent in the API system. These facts, along with the short incubation period, make the AMS a useful instrument for clinical laboratories.

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