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Description and Evaluation of the Semiautomated 4-Hour ATB 32E Method for Identification of Members of the Family *Enterobacteriaceae*

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A study was performed to compare the rapid identification system ATB 32E (API-bioMérieux SA, La Balme-les-Grottes, France) with conventional biochemical methods for identifying 414 isolates of the family *Enterobacteriaceae* and the genus *Aeromonas*, mainly of clinical origin. Overall, 395 strains (95.4%) were correctly identified, with 48 (11.6%) requiring extra tests for complete identification. Ten strains (2.4%) were not identified, and nine (2.9%) were misidentified. The ATB 32E is a suitable alternative for rapid identification of members of the family *Enterobacteriaceae*.

Members of the family *Enterobacteriaceae* are the causative agents of more than 50% of nosocomial infections. They are responsible for 50% of septicemias, 60 to 70% of enteric infections, and 70% of urinary infections (10). In addition to well-represented species, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Hafnia alvei*, *Serratia marcescens*, *Citrobacter freundii*, *Proteus mirabilis*, and *Morganella morganii*, a large number of new species of *Enterobacteriaceae* have been described in the last few years (5): 37 species were recognized in 1957, 54 were recognized in 1984, and more than 140 were recognized in 1989. Consequently, it seemed essential to propose systems that contained a sufficient number of tests well adapted to the identification of these species. An automated system was the only answer to the complex problem posed by the large number of species and tests and to the need for rapid diagnosis.

The present report evaluates the new ATB 32E 4-h system for the identification of members of the family *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacteria. The study involved 395 gram-negative bacteria isolated mainly from clinical specimens. They were members of the *Enterobacteriaceae* family and included 39 reference strains (Table 1). Nineteen *Aeromonas* strains were also tested. Most of the clinical strains commonly encountered in the laboratory had been previously identified by the API 20E gallery. For strains identified at the *Serratia* and *Aeromonas* genus levels, carbon substrate assimilation tests were also used (6). Recently described or rare members of the family *Enterobacteriaceae*, especially those sampled in the environment (Table 1) had been previously identified by conventional methods (1, 2, 4, 11, 12).

ATB 32E system. The ATB 32E system (API-bioMérieux, La Balme-les-Grottes, France) includes 32 wells containing a dehydrated reagent that corresponds to various biochemical reactions: 4 enzymatic reactions with chromogenic substrates (α-galactosidase, β-galactosidase, β-D-glucosaminidase, and alkaline phosphatase); 8 conventional tests (lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, tetrathionate reduction, indole production, malonate utilization, and urea and esculin hydrolysis); 16 carbon substrate fermentation tests (arabinose, mannitol, cellobiose, glucuronate, mannose, maltose, trehalose, 5-ketogluconate, palatinose, galacturonate, raffinose, sucrose, sorbitol, rhamnose, melibiose, and adonitol) as well as a fermentation control; and 2 inhibition tests (coumarate and colistin). The oxidase test was also carried out.

After growth on a bromocresol purple agar medium (bioMérieux), a bacterial suspension was prepared in 2 ml of sterile saline equivalent to an 0.5 McFarland standard turbidity. Fifty-five microliters of the suspension was deposited into each well. After a 4-h incubation at 37°C, a reading was taken with an ATB 1520 reader (API-bioMérieux) linked to an ATB 1545 computer. Interpretation was obtained with ATB Plus software. Visual reading and interpretation with a code book was also possible. When a discrepancy between API 20E and ATB 32E was observed, definitive identification of the isolate was determined by conventional biochemical tests, as previously mentioned.

RESULTS

Of the 414 strains tested, 347 (83.8%) were correctly identified without additional tests, and 48 (11.6%) were correctly identified after further testing. A total of 395 (95.4%) were correctly identified by the ATB 32E system; 10 (2.4%) were not identified, and 9 (2.2%) were misidentified (Table 1).

Most species were correctly identified by the system, in particular those often found in clinical laboratories, such as *E. coli* (30 of 31 strains), *C. freundii* (16 of 17), *Citrobacter amalonaticus* (5 of 5), *Citrobacter diversus* (15 of 15), *Enterobacter aerogenes* (6 of 6), *Enterobacter agglomerans* (10 of 10), *H. alvei* (7 of 7), *P. mirabilis* (17 of 17), *Proteus vulgaris* (10 of 10), *M. morganii* (10 of 11). *Providencia rettgeri* (10 of 11), and *Providencia stuartii* (6 of 7). However, other bacteria frequently encountered in clinical labo-

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TABLE 1. Origin and accuracy of ATB 32E identification of 414 strains

Species	No. or identity of strains								
	Tested	Clinical isolates	Environmental isolates	Of unknown origin	Reference[a]	Correctly identified	Correctly identified with extra tests	Incorrectly identified	Not identified
Eschericia coli	31	30			ATCC 11775	30	1		
Escherichia vulneris	11		10		ATCC 33821	11			
Escherichia fergusonii	4	2	2			3	1		
Escherichia hermannii	5		4		ATCC 33560	5			
Leclercia adecarboxylata	5		5			5			
Enterobacter cloacae	15	14			ATCC 13047	15			
Enterobacter aerogenes	6	5			ATCC 15038	6			
Enterobacter agglomerans	10			9	CDC 164571	10			
Enterobacter sakazakii	5	1		3	ATCC 29544	4	1		
Enterobacter amnigenus	3	1	1		ATCC 33072	1	1		1
Enterobacter gergoviae	5	3	1		ATCC 33028	5			
Enterobacter intermedium	5	1	4			5			
Enterobacter taylorae	9	1	7		CDC 464184	9			
Citrobacter diversus	15	14			ATCC 27156	15			
Citrobacter freundii	17	16			ATCC10787	16			1
Citrobacter amalonaticus	5	1		3	ATCC 24405	5			
Hafnia alvei	7	6			ATCC 25927	7			
Serratia marcescens	17	15		1	ATCC 264	12	5		
Serratia liquefaciens	3			3			3		
Serratia rubidaea	3		1	1	ATCC 27593	3			
Serratia ficaria	5		4		ATCC 33105	4			1
Serratia fonticola	5		4		ATCC29847	5			
Serratia grimesii	2	2				2			
Serratia odorifera	3			2	NCTC 11214	3			
Serratia plymuthica	3		3			3			
Buttiauxella agrestis	4		2	1	ATCC 33320	3	1		
Budvicia aquatica	5		5			5			
Kluyvera cryocrescens	4		1	2	ATCC 149239	4			
Kluyvera ascorbata	4		1	2	ATCC 33433	3	1		
Klebsiella ornithinolytica	5	3	1		CDC 463684	4	1		
Klebsiella oxytoca	18	15		3		13	3	1	1
Klebsiella pneumoniae subsp. *pneumoniae*	17	15		1	ATCC 23357	15	1		1
Klebsiella pneumoniae subsp. *ozaenae*	5	2		2	ATCC 11297	2	2		1
Klebsiella terrigena	5		4		CDC 9015-82	5			
Salmonella arizonae	4	3			ATCC 12323	4			
Salmonella typhi	1	1				1			
Salmonella spp.	14	14				9	4	1	
Shigella flexneri	6	6				3	3		
Shigella sonnei	7	7				6	1		
Shigella boydii	3	3					3		
Proteus mirabilis	17	16			ATCC 4675	17			
Proteus penneri	7	1		5	CDC 412483	2	5		
Proteus vulgaris	10	8	1		ATCC 13315	7	2	1	
Providencia rettgeri	11	10			ATCC 14505	10	1		
Providencia alcalifaciens	4	2		1	ATCC 9886	4			
Providencia stuartii	7	6			ATCC 25825	6	1		
Morganella morganii	11	10			ATCC 25829	10			1
Yersinia enterocolitica	8	6	1		ATCC 23715	5	1	1	1
Yersinia fredericksenii	2		1		CIP 80-29	1	1		
Yersinia intermedia	4	1	2		ATCC 29909	4			
Yersinia pseudotuberculosis	7			6	ATCC 23207	7			
Yersinia ruckeri	6		5		ATCC 29473	6			
Aeromonas hydrophila	6	1	5			3	3		
Aeromonas sobria	8		8				1	5	2
Aeromonas caviae	5		5			4	1		

[a] ATCC, American Type Culture Collection, Rockville, Md.; CDC, Centers for Diseases Control, Atlanta, Ga.; NCTC, National Collection of Type Cultures, London, England; CIP, Collection de l'Institut Pasteur, Paris, France.

ratories required further tests: *S. marcescens* (5 of 17 strains), *K. oxytoca* (3 of 18), and *K. pneumoniae* subsp. *ozaenae* (2 of 5). The correct identification of most *Salmonella* and *Shigella* species tested was possible at the genus level only, apart from *Salmonella arizonae* (4 of 4 strains), *Salmonella typhi* (1 of 1), and *Shigella sonnei* (6 of 7).

Most of the uncommon or newly described organisms were correctly identified by the system: *E. fergusonii* (3 of 4

TABLE 2. Analysis of incorrect identifications with the ATB 32E system

Correct identification	Initial test result	Discrepant biochemical test(s)[a]
Salmonella typhimurium	*Salmonella choleraesuis*	ARA, αGAL
Citrobacter freundii	No identification	CEL
Klebsiella oxytoca	No identification	CEL, IND, GAT
Klebsiella oxytoca	*Klebsiella pneumoniae* subsp. *pneumoniae*	IND, MNT
Klebsiella pneumoniae subsp. *pneumoniae*	No identification	TTR
Klebsiella pneumoniae subsp. *ozaenae*	No identification	URE, RHA, MNT, COL, αGAL
Enterobacter amnigenus	No identification	ESC, CMT
Serratia ficaria	No identification	ESC, ARA, CMT, PNPG
Proteus vulgaris	*Proteus penneri*	IND, PLE
Morganella morganii	No identification	IND, COL, CMT, GRT
Yersinia enterocolitica	*Enterobacter agglomerans*	URE, CMT
Yersinia enterocolitica	No identification	ADO, CEL
Aeromonas sobria (*n* = 5)	*Aeromonas caviae/Vibrio alginolyticus* (*n* = 5)	IDP (n = 5), ONAG (*n*= 1)
Aeromonas sobria	No identification	MNE, IDP
Aeromonas sobria	No identification	ESC, PPA, IDP

[a] ARA, Arabinose; αGAL, α-galactosidase; CEL, cellobiose; IND, indole; GAT, galacturonate; MNT, malonate; TTR, tetrathionate reductase; URE, urease; RHA, rhamnose; COL, colistin; ESC, esculin; CMT, coumarate; PNPG, p-nitrophenyl-β-D-galactopyranoside; PLE, palatinose; GRT, glucuronate; ADO, adonitol; IDP, indoxyl phosphate; ONAG, *O*-nitrophenyl-*N*-acetyl-β-D-glucosaminide; MNE, mannose; PPA, phenylalanine desaminase.

strains), *Escherichia hermannii* (5 of 5), *Escherichia vulneris* (11 of 11), *Klebsiella terrigena* (5 of 5), *Klebsiella ornithinolytica* (4 of 5), *Enterobacter gergoviae* (5 of 5), *Enterobacter taylorae* (9 of 9), *Serratia fonticola* (5 of 5), *Serratia grimesii* (2 of 2), *Serratia plymuthica* (3 of 3), *Budvicia aquatica* (5 of 5), *Kluyvera ascorbata* (3 of 4), *Kluyvera cryocrescens* (4 of 4), and *Yersinia ruckeri* (6 of 6).

The majority of errors occurred mainly in identifying *Aeromonas* species; in particular, five of eight *Aeromonas sobria* were misidentified. The eight nonidentified enterobacteria were randomly distributed. The discrepant biochemical tests are listed in Table 2.

DISCUSSION

The large number of tests available in the system allows an accurate identification of most commonly encountered clinical enterobacteria as well as most recently described species. Theoretically, the system is able to identify other species, such as *Edwardsiella hoshinae*, *Edwardsiella tarda*, *K. pneumoniae* subsp. *rhinoscleromatis*, *Rahnella aquatilis*, *Tatumella ptyseos*, *Moellerella wisconsensis*, *Cedecea lapagei*, *Cedecea neteri*, *Ewingella americana*, *Serratia proteamaculans*, *Plesiomonas shigelloides*, *Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio metschnikovii*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*, contained in its data base. Identification can be carried out directly from the primary plate, since the required inoculum is generally obtained with only one or two colonies.

Most of the strains of *Salmonella arizonae*, *Salmonella typhi*, and *Shigella sonnei* were correctly identified by the system. Strains belonging to the other species or serotypes of the genera *Salmonella* and *Shigella* were identified only at the genus level, except for one strain of *Salmonella typhimurium* misidentified as *Salmonella choleraesuis*. In any case, identification of *Salmonella* spp. and *Shigella* spp. must include serological confirmation.

The other misidentified members of the family *Enterobacteriaceae* were as follows: a strain of *K. oxytoca* came out as *K. pneumoniae* subsp. *pneumoniae*, one strain of *P. vulgaris* was identified as *Proteus penneri*, and one strain of *Yersinia enterocolitica* was identified as *Enterobacter agglomerans*. For the first two strains, the discrepancy was due to an indole reaction that was too low to be detected by the reader. The third strain, *Y. enterocolitica*, was strongly atypical, since it exhibited negative ornithine decarboxylase, urease, and sorbitol fermentation reactions (9). The only *E. coli* that required additional testing was not directly identified, partly because the system failed to detect an indole reaction. The strain demonstrated other unusual features, such as a positive reaction to adonitol, sucrose, and raffinose (3). Additional testing was required to discriminate five *S. marcescens* strains from *Serratia liquefaciens*, because of the biochemical proximity of the two species in the data base. They can be easily differentiated by using a simple test such as D-xylose fermentation (7). On the other hand, all of the three *S. liquefaciens* strains required additional testing before being identified.

Only one of the eight *A. sobria* strains tested was correctly identified after further testing. Two strains were not identified, and the remaining five strains were incorrectly attributed to groups that systematically included the following choices: *Aeromonas hydrophila/Aeromonas caviae* or *V. alginolyticus*. On the other hand, the system failed to discriminate the *A. hydrophila* and *A. caviae* species, which is not surprising, since studying this group of bacteria is not easy with the conventional phenotype tests (8), and the system is not specifically adapted to the identification of oxidase-positive bacteria. In fact, *Aeromonas* species are identifiable at the genus level only.

In conclusion, ATB 32E is a rapid, semiautomated system which is suitable for the identification of members of the family *Enterobacteriaceae*.

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