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Comparative Evaluation of the New Titertek Enterobac Rapid Automated System (TTE-RAS) for Identification of Members of the Family *Enterobacteriaceae*

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The Titertek Enterobac Rapid Automated System (TTE-RAS; Flow Laboratories, SpA, Milan, Italy), a new semiautomated system for the identification of members of the family *Enterobacteriaceae*, was compared with the API 20E system (API System P.A., Montalieu Vercieu, France) by using 284 clinically isolated strains that were previously identified by conventional methods. Six strains from the American Type Culture Collection (Rockville, Md.) were included to evaluate the reproducibility of identification by both systems. Correct identifications at the species level were 93.7% with TTE-RAS and 96.1% with API 20E. Although some of the features of the TTE-RAS data base were not satisfactory, we consider this new miniaturized system to be a very valuable tool for the rapid identification of the most frequently isolated opportunistic bacteria.

In clinical bacteriology the rapid identification of members of the family *Enterobacteriaceae* is essential for diagnostic and therapeutic purposes and for epidemiological studies. A great improvement over the established methods of identification has been accomplished by use of miniaturized kits (3) and more recently by the introduction of semiautomated systems (2), which are faster to use and reduce the possibility of subjective errors in the identification of pathogens. These are important features for better management of the daily work load in clinical laboratories.

In this study we evaluated the overall features of the Titertek Enterobac Rapid Automated System (TTE-RAS; Flow Laboratories, SpA, Milan, Italy) (G. Marklein, H. Bakes, G. Barter, G. Hefner, R. Hasser, and D. Kurten, Fourth International Symposium on Rapid Methods and Automation in Microbiology and Immunology, Abstract P91, Berlin, 1984). Moreover, this system was compared with the API 20E system (API System P.A., Montalieu Vercieu, France) (1), a widely used miniaturized kit which uses classical tubed media as the reference method (5).

MATERIALS AND METHODS

Bacterial strains. A total of 284 bacterial strains isolated over a 4-month period from biological samples of different patients hospitalized at the Health Center of the II Medical School, University of Naples, Naples, Italy, were used for this study. They included the following (number of strains is given in parentheses): *Escherichia coli* (95), *Proteus mirabilis* (53), *Klebsiella oxytoca* (18), *Morganella morganii* (16), *Serratia marcescens* (14), *Hafnia alvei* (13), *Citrobacter freundii* (12), *Salmonella typhi* (11), *Enterobacter cloacae* (10), *Klebsiella pneumoniae* (8), *Enterobacter aerogenes* (7), *Proteus vulgaris* (6), *Enterobacter agglomerans* (5), *Providencia rettgeri* (4), *Serratia liquefaciens* (4), *Providencia stuartii* (3), *Klebsiella ozaenae* (3), *Citrobacter amalonaticus* (1), and *Citrobacter diversus* (1).

Six strains from the American Type Culture Collection, Rockville, Md. (*Citrobacter freundii* 8090, *Enterobacter aerogenes* 13048, *Escherichia coli* 25922, *Klebsiella pneumoniae* 13883, *Proteus vulgaris* 13315, *Serratia marcescens* 8100) were included to test the reproducibility of identification by both systems analyzed in this study.

Conventional identification methods. Each strain was isolated on MacConkey agar plates, and oxidase-negative bacteria were identified by using tubed media for the following tests: adonitol, sucrose, and sorbitol fermentation; arginine dihydrolase; citrate utilization; DNase; gas production from glucose; hydrogen sulfide; indole production; lysine and ornithine decarboxylase; motility; phenylalanine deaminase; urease; inositol; Voges-Proskauer; and *o*-nitrophenyl-β-D-galactopyranoside (ONPG). The use of Kliger iron agar, motility-indole-ornithine, and phenylalanine-urease broth allowed us to reduce to 13 the number of media used. All media and reagents were obtained from Difco Laboratories, Detroit, Mich.

TTE-RAS. TTE-RAS uses standard microdilution plates, each of which contains dehydrated substrates for 4 sets of 23 biochemical tests. The following were read at 620 nm: tetrathionate reductase, indole, Voges-Proskauer, urease, lysine decarboxylase, ornithine decarboxylase, citrate, malonate, glucose, rhamnose, sucrose, adonitol, inositol, xylose, sorbitol, and the control well, which was a blank used for sugar reactions. The second control well was for ONPG, β-xylosidase (ONPX), and growth; and the following tests were read at 414 nm: ONPX, H_2S, lactose, raffinose, maltose, ONPG, and trehalose.

The nitrate reduction was performed in ONPG, ONPX, or ONPG-ONPX control wells. Each test was performed by rehydrating the substrates in the wells of microtiter plates with 100 μl of bacterial suspension in sterile saline solution to a turbidity equal to a 0.5 McFarland standard, followed by incubation for 5 h at 37°C. After the addition of specific reagent (indole II), the optical density variations were measured with a Multiscan multi-chromatic spectrophotometer. The data were analyzed with an Apple IIe personal computer which was equipped with a floppy disk containing the data base. All instruments were furnished by the manufacturers. The data base was established based on the biochemical profiles of 9,937 strains. It permits the identification of members of the family *Enterobacteriaceae* as well as *Acine-*

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TABLE 1. Identifications to the species level obtained by both systems analyzed in this study

Species	*n*	No. (%) identified by:	
		TTE-RAS	API 20E
Escherichia coli	95	93 (97.9)	94 (98.9)
Proteus mirabilis	53	47 (88.7)	53
Klebsiella oxytoca	18	18	17 (94.4)
Morganella morganii	16	16	15 (93.7)
Serratia marcescens	14	13 (92.8)	13 (92.8)
Hafnia alvei	13	6 (46.1)	12 (92.3)
Citrobacter freundii	12	12	12
Salmonella typhi	11	11	11
Enterobacter cloacae	10	9 (90.0)	10
Klebsiella pneumoniae	8	8	7 (87.5)
Enterobacter aerogenes	7	7	6 (85.7)
Proteus vulgaris	6	6	5 (83.3)
Enterobacter agglomerans	5	5	4 (80.0)
Serratia liquefaciens	4	3 (75.0)	4
Proteus rettgeri	4	4	3 (75.0)
Providencia stuarti	3	3	3
Klebsiella ozaenae	3	3	3
Citrobacter amalonaticus	1	1	0
Citrobacter diversus	1	1	1
Total	284	266 (93.7)	273 (96.1)

tobacter spp. (at the genus level only), *Pseudomonas maltophilia*, *Aeromonas hydrophila*, and *Plesiomonas shigelloides*. The test results are identified by the personal computer as + or − and include the percentage of relative probability, which is useful for the appraisal of the confidence level of identification. It also provides an indication of the need for further tests such as oxidase, nitrate reduction, or serology or a longer incubation period if the reading of the control growth well fails to exceed a predetermined threshold. If the probability of the first choice of identification is less than 80% or less than four times the second choice of identification, the program is such that the printout includes additional data for four biochemical tests (lactose, raffinose, maltose, trehalose).

API 20E. The well-known API 20E system was used following the instructions of the manufacturer. A suspension of each microorganism, at a turbidity of a 0.5 McFarland standard in sterile saline solution, was used to inoculate the galleries of biochemical tests. After incubation for 24 h at 37°C, the reagents for tryptophan deaminase, indole, and Voges-Proskauer tests were added. Strain identification is based on code numbers, generated from the combination of numerical values assigned to each positive reaction and matched with those reported in the API 20E Analytical Profile Index (1).

RESULTS

Each one of the six strains from the American Type Culture Collection was tested 10 times by both the TTE-RAS and API 20E systems. The results show that the reproducibility, although quite good (96.6%), was not absolute for either system. TTE-RAS showed two cases of identification only at the genus level (*Enterobacter gergoviae* instead of *Enterobacter aerogenes* and *Proteus mirabilis* instead of *Proteus vulgaris*), and API 20E showed two identifications at only the genus level (*Enterobacter agglomerans* instead of

TABLE 2. Results of TTE-RAS on 284 clinically isolated strains

Species	*n*	No. of species identified correctly	No. of species identified unacceptably	Probable error[a]	No. of species misidentified	Probable error[b]
Escherichia coli	95	93	1 (*P. mirabilis*, 74%; *E. coli*, 25%)	LDC− URE+	1 (*H. alvei*)	IND−
Proteus mirabilis	53	47	1 (*Acinetobacter* spp., 40%; *P. mirabilis*, 25%)	Data base	3 (*Acinetobacter* spp., 89%; *H. alvei* 11%) 1 (*K. ozaenae*) 1 (*M. morganii*)	LDC+ SOR+ H_2S−
Klebsiella oxytoca	18	18				
Morganella morganii	16	16				
Serratia marcescens	14	13			1 (*P. mirabilis*)	ODC+ URE+
Hafnia alvei	13	6	6 (*Acinetobacter* spp., 75%; *H. alvei*, 25%) 1 (*Acinetobacter* spp., 55%; *H. alvei*, 35%)	Data base ONPG		
Citrobacter freundii	12	12				
Salmonella typhi	11	11				
Enterobacter cloacae	10	9			1 (*Enterobacter gergoviae*)	SOR+
Klebsiella pneumoniae	8	8				
Enterobacter aerogenes	7	7				
Proteus vulgaris	6	6				
Enterobacter agglomerans	5	5				
Proteus rettgeri	4	4				
Serratia liquefaciens	4	3			1 (*Acinetobacter* spp.)	Data base
Providencia stuartii	3	3				
Klebsiella ozaenae	3	3				
Citrobacter amalonaticus	1	1				
Citrobacter diversus	1	1				

[a] Abbreviations: LDC, lysine decarboxylase; URE, urease.
[b] Abbreviations: IND, indole; LDC, lysine decarboxylase; SOR, sorbitol; ODC, ornithine decarboxylase; URE, urease.

Enterobacter aerogenes and *Serratia liquefaciens* instead of *Serratia marcescens*). There was no generic misidentification by either system.

The following criteria for the identification of all clinically isolated strains were applied: species identification if the system gave a percentage of probability higher than 90%; misidentification if the results suggested with the same level of probability were discordant with the reference method; unacceptable identification when the percentage of probability was lower than 90%.

Identification at the species level was obtained by TTE-RAS in 266 cases (93.7%) and by the API 20E system in 273 cases (96.1%) (Table 1).

Table 2 shows the overall results of TTE-RAS on 284 clinical isolates, with tentative explanations for the discrepancies with regard to the reference method. Unacceptable identifications and misidentifications occurred in nine cases (3.1%). Seven cases of unacceptable identification involved *Hafnia alvei* and five cases of misidentification involved *Proteus mirabilis*. The biochemical discrepancies between TTE-RAS and the conventional methods, which may explain the failure of identification at the species level, involved lysine decarboxylase (four cases); urea hydrolysis (two cases); sorbitol (two cases); and ONPG, indole, ornithine decarboxylase, and H_2S in one case each.

Moreover, identification to the species level was missed in eight particular cases without discordances in possible discriminative biochemical reactions.

DISCUSSION

The results obtained by testing the strains from the American Type Culture Collection allowed us to rate as acceptable the identification reproducibility of the new rapid test system, TTE-RAS. The high degree of identification at the species level in comparison with the conventional methods makes TTE-RAS a reliable method for the identification of members of the family *Enterobacteriaceae*.

The failure to obtain the correct identification in eight cases was not because of discrepancies of biochemical reactions, as compared with the reference method, but was due to the faulty data base of TTE-RAS. In fact, if lysine decarboxylase or ornithine decarboxylase or both reactions were reported as positive, the data base selects as a more probable identification the genus *Acinetobacter*. However, this genus, according to current information (4, 6), is considered negative for both lysine and ornithine.

Taken together, the data support the conclusion that TTE-RAS basically is capable of performing an accurate identification of all the most frequently isolated strains in clinical microbiology.

Our experience suggests that the new system (TTE-RAS), if carefully used and if one keeps in mind the problems with its data base, is a very valuable system for routine use, particularly in emergencies, because in addition to a simplified technical procedure, it requires a very short time (5 h ± 20 min) of incubation to obtain a satisfactory identification.

The information provided by a system such as TTE-RAS can be of great advantage for microbiologists, because it eliminates the preliminary report and the need for further investigations. This results in a sizable reduction of the work load for the microbiology laboratory. The advantages of a miniaturized system appear to be greatest to both the physician and the microbiologist when, as is possible today, in the same period of time rapid identification and antimicrobial susceptibility tests are accomplished together.

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LITERATURE CITED

1. **Aldridge, K. E., B. B. Gardner, S. J. Clark, and J. M. Matsen.** 1978. Comparison of Micro-ID, API 20E, and conventional media system in identification of *Enterobacteriaceae*. J. Clin. Microbiol. 7:507–513.
2. **Freney, J., J. Fleurette, P. Laban, J. P. Gayral, and M. Desmonceaux.** 1983. Système automatique d'identification des bacilles à gram nègatif, p. 455–466. *In* H. Leclerc (ed.), Les bacilles à gram negatif d'intèrêt médical en santé publique. taxonomie. Identification. Applications, vol. 114. Les Editions Institut National de la Santé et de la Recherche Medicale, Paris.
3. **Freney, J., P. Laban, M. Desmonceaux, H. Alexandre, B. Poggi, J. P. Gayral, and J. Fleurette.** 1985. An automatic micromethod for the identification of gram negative bacilli by carbon substrate assimilation tests, p. 377–389. *In* K.-O. Habermehl (ed.), Rapid method and automation in microbiology and immunology. Springer-Verlag, Berlin.
4. **Gilardi, G. L.** 1978. Identification of miscellaneous glucose non fermenting gram negative bacteria, p. 45–65. *In* G. L. Gilardi (ed.), Glucose non fermenting gram negative bacteria in clinical microbiology. CRC Press, Inc., Boca Raton, Fla.
5. **Koneman, E. W., J. D. Allen, V. R. Dowell, Jr., and H. M. Sommers.** 1983. Diagnostic microbiology, 2nd ed. J. B. Lippincott Co., Philadelphia.
6. **Rubin, S. J., P. A. Granato, and B. L. Wasilauskas.** 1980. Glucose nonfermenting gram-negative bacteria, p. 263–287. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.