Comparative evaluation of Enterosystem 18 R and API 20 E compared with the automatic system VITEK for the identification of the members of the *Enterobacteriaceae* Family.

A. Di Girolamo, G. Catamo, D. Paludi, A. Faraone, S. Brocco, M. Nicoletti, R. Piccolomini***

*Istituto di Medicina Sperimentale - * Cattedra di Microbiologia, Facoltà di Medicina e Chirurgia Università degli Studi di Chieti*
*** Liofilchem s.r.l., Roseto degli Abruzzi (Teramo)*

Summary. 126 bacterial strains belonging to the members of the Enterobacteriaceae Family (15 species) were used to test a new system for the identification of these germs, the Enterosystem 18 R (ENT 18 R), in confrontation with the system API 20 E comparatively with the system VITEK 30 jr. considered as reference element.
Both systems (ENT 18 R and API 20 E) correctly identified all the strains belonging to the genera Citrobacter, Hafnia, Morganella, Salmonella and Shigella. Discordance at the level of species was evidenced for the genera Enterobacter, Klebsiella and Serratia. The highest concordance at the level of species was shown by ENT 18 R for: *Citrobacter freudii*, *Hafnia alvei*, *Morganella morganii*, *Proteus mirabilis*, *Salmonella spp.* and *Shighella sonnei*, *E.coli*, *Proteus penneri* and *Serratia liquefaciens*.
The concordance percentages by the system API 20 E, in relation to the genus, were 89.7% (90.5% for ENT 18 R) and in relation to species were 79.3% (85.7% for ENT 18 R). Although the experimentation's limits, the Enterosystem 18 R shew, like API 20 E, characteristics of accuracy, sensitivity and reproducibility that set it as a valid identification support for *Enterobacteriaceae* in the clinical bacteriology laboratory.

Abstract. To evaluate the accuracy and utility of the ENTEROSYSTEM 18 R (ENT 18 R), we conducted a clinical preliminary comparison, with 126 *Enterobactericeae* isolates, of the ENT 18 R system with the API 20 E and an automated system, the VITEK 30 Jr., as reference identification. The ENT 18 R and the API 20 E yielded correct identification of 85.7% and 79.3%, respectively, at the species level. For ENT 18 R and API 20 E, 11 (8.7%) and 12 (9.6%) misidentifications, respectively, were observed. Although a further study is required, we think that the accuracy and the sensitivity of its identifications of both common and less-common isolates of *Enterobacteriaceae*, makes this new system fairly efficient in clinical bacteriology laboratories.

Introduction

The rising number of examinations to be carried out in clinical bacteriology laboratories is requiring the availability of systems able to simplify some of the manual phases, in order to obtain a rapid, accurate and reliable identification of microorganisms at both “genus” and “species” levels.
Among clinically important microorganisms, the Family of *Enterobacteriaceae* is undoubtedly important and every new system able to identify various strains inside it, if reliable, accurated and economic (1) as the systems already in use, has to be welcomed with interest by clinical microbiologists.
The purpose of this note is to illustrate the preliminary experimental results obtained by the comparison of a new identification system of clinical isolates belonging to the *Enterobacteriaceae* Family, the Enterosystem 18 R (Liofilchem s.r.l. - Roseto degli Abruzzi, Teramo) with the API 20 E system (Bio-Merieux Italia S.p.A. - Roma) now used in many bacteriology laboratories.
The automatic system VITEK 30 jr (Bio-Merieux Italia S.p.A. - Roma) was used as reference element, which uses specific cards (GNI) for the identification of *Enterobacteriaceae* through 32 biochemical characters.

Materials and methods

Through the three systems, in the laboratory of Clinical Bacteriology of the “Cattedra di Microbiologia dell’Università G. D'Annunzio di Chieti”, 126 bacterial strains of clinical isolation belonging to 15 species in *Enterobacteriaceae* Family were examined (Table 1).

Table 1. Bacterial strains subjected to comparative assay.

Genus	Species	No. strains
Citrobacter	freudii	8
Enterobacter	aerogenes	4
Enterobacter	cloacae	15
Enterobacter	sakazaki	2
Escherichia	coli	35
Hafnia	alvei	3
Klebsiella	oxytoca	6
Klebsiella	pneumoniae	13
Morganella	morganii	4
Proteus	mirabilis	24
Proteus	penneri	2
Salmonella	spp.	4
Serratia	liquefaciens	2
Serratia	marcescens	3
Shigella	sonnei	1
	TOTAL	126

Before carrying out the comparative assay, all bacterial strains were grown in Brain Heart Broth (Liofilchem) and then subcultivated in MacConkey Agar plates (Liofilchem) in order to ensure the purity and vitality of single colonies.

Enterosystem 18 R

The system was set to identify the members of oxidase-negative *Enterobacteriaceae* Family (4). It consists of a rectangular transparent plastic plate provided of lid in which 18 wells are positioned. Each well contains, respectively, a substratum for the biochemical reactions as follows. ONPG test, Lysine-, Ornithine-, Arginine-decarboxylase, Phenylalanine-deaminase, use of Citrate as sole Carbon source, Urease test, H2S production, use of Malonate as sole Carbon source, Voges-Proskauer reaction, Indole test, fermentation of the following sugars: Glucose, Mannitol, Inositol, Sorbitol, Sucrose, Arabinose, Raffinose.

A single colony of the strain under examination was emulsified in 5 mL of sterile physiological solution and corrected to reach an opacity of 1.0 MacFarland.

Four drops (0.2 mL) of the single bacterial suspension were then added into every well of the system. Where specified, two drops of sterile vaseline oil were added to create anaerobiosis conditions in the indicated wells.

The system was then placed in an incubator regulated at 37 °C for a time period of 8 hours to a maximum of 24 hours. The identification of the germ under examination was carried out highlighting the positive reactions related to the various tests and entering them in a data collection form. Through the obtained numeric code, the type of germ was detected by the use of the Bacterial Code Table (1990 edition).

API 20 E

The system was inoculated and incubated following manufacturer's instructions. After 24 hours incubation the germs were identified through the numeric code found on the Profit Index of the system and obtained according to the biochemical reactions developed by the germ.

VITEK 30 jr.

The computerized automatic system which uses specific cards for the identification of Gram negative germs containing 32 specific biochemical reactions was used according to manufacturer's instructions.

Conventional methods
Where necessary, single bacterial species were identified through conventional biochemical tests according to Edwards and Ewing's methods.

Quality Control
Reference strains for quality control were added to the identification tests when the two systems were compared and to the system used as reference. The strains were the following: *Escherichia coli* ATCC 25933, *Klebsiella pneumoniae* ATCC 13882 and *Proteus vulgaris* ATCC 13315. These strains were tested in three separate situations so as to ascertain the reproducibility of the results obtained with the Enterosystem 18 R.

Results

Of the 126 bacterial strains subjected to the comparative test between the two non automatic systems and the automatic VITEK system, 114 were correctly identified, at the level of genus, from the Enterosystem 18 R; 113 were correctly identified by by the API 20 E (Table 2).

Table 2. Agreeing identifications at the "genus" level of the Enterosystem 18 R (ENT 18 R) and API 20 E compared to the VITEK system.

Genus	No.	No. agreements	
		ENT 18 R	API 20 E
Citrobacter	8	8	8
Enterobacter	21	16	14
Escherichia	35	35	33
Hafnia	3	3	3
Klebsiella	19	15	17
Morganella	4	4	4
Proteus	26	26	26
Salmonella	4	4	4
Serratia	5	2	3
Shigella	1	1	1
TOTAL	126	114 (90.5%)*	113 (89,7%)*

* = % of agreement (ACCURACY) with VITEK at the level of genus

Both systems correctly identified all the strains belonging to to the genera Citrobacter, Hafnia, Morganella, Salmonella and Shigella.

The concordance was also complete, at the level of "genus", of the Enterosystem 18 R for for the Escherichia genus.

The Enterosystem 18 R provided 5 disagreeing interpretations in for the 21 *Enterobacter* spp. (Table 3), in 3 cases of which the strains were recognized as *E.coli* and in 2 cases, respectively, as *Klebsiella pneumoniae* and *Klebsiella ozaenae*; in 4 strains of *Klebsiella* spp. two were identified, respectively, one as *E.coli* and the other as *Hafnia alvei*, while the system did not provide twice a code number included in the Bacterial Code List, even though the results of the biochemical tests included in the result system were agreeing with those obtained with the API 20 E and with the VITEK system; finally for the 5 *Serratia* spp. the Enterosystem 18 R provided two different

identifications (*Enterobacter cloacae* and *Klebsiella ozaenae*, respectively). Furthermore, the disagreeing identifications are reported in Table 3, at the level of genus, shown by the API 20 E.

Table 3. Disagreeing identifications for genus shown by ENTEROSYSTEM 18 R (ENT 18 R) and by API 20 E in comparison with the automatic system VITEK.

Genus	No.	IDENTIFICATION			
		ENT 18 R	No.	API 20 E	No.
Enterobacter	21	E.coli	3	E.coli	2
		K.pmeumoniae	1	K.pmeumoniae	3
		K.ozaenae	1	K.ozaenae	1
				K.oxytoca	1
Escherichia	35			Ent. cloacae	1
				K.oxytoca	1
Klebsiella	19	E.coli	1	E.coli	2
		Hafnia alvei	1		
		Not identified	2		
Serratia	5	Ent.cloacae	1		
		K.ozaenae	1 (*)	K.ozanae	1 (*)

(*) = Both Enterosystem 18 R and API 20 E identified the same strain as *K.ozaenae*, but VITEK recognized it as *Serratia marcescens*.

Table 4. Disagreeing identifications in species shown by Enterosystem 18 R (ENT 18 R) and by API 20 E in comparison with the automatic system VITEK.

Microorganisms	No.	IDENTIFICATION			
identified by VITEK		ENT 18 R	No.	API 20 E	No.
Enterobacter aerogenes	4	Enterobacter cloacae	1	Enterobacter cloacae	2
Enterobacter sakazaki	2			Enterobacter cloacae	2
Klebsiella oxytoca	6	Klebsiella pneumoniae	1	Klebsiella pneumoniae	2
		Klebsiella ozaenae	1		
Klebsiella pneumoniae	13	Klebsiella pneumoniae	1		
		K.ozaenae	2	Klebsiella ozaenae	4
Protteus penneri	2			Proteus vulgaris	2
Serratia liquefaciens	2			Serratia marcescens	1
Serratia marcescens	3	Serratia liquefaciens	1	Serratia liquefaciens	1

Table 5. Agreement and disagreement of identification, at the levels of “genus” and “species” shown by ENTEROSYSTEM 18 R (ENT 18 R) and API 20 E systems compared to the VITEK system.

		ENT 18 R			API 20 E		
Microorganisms identified by VITEK	No.	Agreement	Disagreement in “genus”	Disagreement in “species”	Agreement	Disagreement in “genus”	Disagreement in “species”
Citrobacter freundii	8	8	-	-	8	-	-
Enterobacter aerogenes	8	3	-	1	2	-	2
Enterobacter cloacae	4	10	5	-	8	7	-
Enterobacter sakazaki	15	2	-	-	0	-	2
Escherichia coli	2	35	-	-	33	2	-
Hafnia alvei	35	3	-	-	3	-	-
Klebsiella oxytoca	6	3	1	2	3	1	2
Klebsiella pneumoniae	13	7	3	3	8	1	4
Morganella morganii	4	4	-	-	4	-	-
Proteus mirabilis	24	24	-	-	24	-	-
Proteus penneri	42	2	-	-	0	-	2
Salmonella spp.	244	4	-	-	4	-	-
Serratia liquefaciens	22	2	-	-	1	-	1
Serratia marcescens	43	0	2	1	1	1	1
Shigella sonnei	11	1	-	-	1	-	-
TOTAL	126	108	11	7	100	12	14
% of agreement		85,70%			79,30%		

In Table 4 the disagreeing identifications at the level of species are reported, shown by the two compared systems.

The Enterosystem 18 R, specially, identified as *Enterobacter cloacae* 1 strain of the 4 *E.aerogenes*. Two strains of 6 *Klebsiella oxytoca* were wrongly identified as *Klebsiella pneumoniae* and *Klebsiella ozaenae*. In 13 *Klebsiella pneumoniae,* one was identified as *Klebsiella oxytoca* and two as *Klebsiella ozaenae*. In three strains of *Serratia marcescens*, the system identified 1 strain as *Serratia liquefaciens*.

Discussion and Conclusion

While indicating as ACCURATE the percentage of agreement of the tested method with the reference one (VITEK), it is claimable that according to the tests carried out, the Enterosystem 18 R proved to be accurate with 90,5% identification agreement of the examined strains for the genus, and 85,7% for the species (Table 5).

With the API 20 E system the percentages of agreement were 89,7% for the genus and 79,3% for the species (Table 5).The highest number of agreements at the level of species was obtained for *Citrobacter freundii, Hafnia alvei, Morganella morganii, Proteus mirabilis, Salmonella* spp. and *Shigella sonnei* (100% for both systems); *E.coli, Proteus penneri* and *Serratia liquefaciens* (100% with the Enterosystem 18 R).

In both cases the reproducibility was excellent (100%) that permits to claim Enterosystem is as precise as API 20 E.

The sensitivity and specificity of Enterosystem 18 R also seemed to be lightly superior to those shown by API 20 E in this experimentation.

In conclusion, and considering the inherent limitations of this study, from the comparative evaluation of the two systems, it is evident that the Enterosystem 18 R has characteristics which allow its use in a bacteriology clinical laboratory routine just like the API 20 E, for a correct identification of the Members belonging to the Enterobacteriaceae Family.

Furthermore, it is to be suggested that thank to the excellent reading and reproducibility of the results shown in wells, and to the rationality which the biochemical tests were carried out with, the system Enterosystem 18 R could be studied to be automatized, though with the due considerations.

Thanks

We thank Liofilchem s.r.l. - Roseto degli Abruzzi (TE) - for the technical support.

References

1. Bartlett RC. Establishing clinical relevance. p.9-43. Medical Microbiology, John Wiley & Sons, Inc., New York, 1974.
2. Edwards PR, Ewing WH (ed.). Identification of *Enterobacteriaceae* 3rd ed. Burgess Publishing Co., Minneapolis 1972.
3. Ewing WH. Differentiation of *Enterobacteriaceae* by biochemical reactions, revised. U.S. Department of Health, Education and Welfare publication no. (CDC) 74-8270. Center for Disease Control, Atlanta 1973.
4. Piccolomini R, Di Girolamo A, Catamo G, Cellini L, Allocati N, Ravagnan G. Enterosystem 18 R: description and comparative evaluation with conventional methods for identification of members of the family of *Enterobacteriaceae*. J. Clinical Microbiol., 29, (10), 2300-2304, 1991.