The utility of the ENTERORapid 24 kit for the identification of *P. multocida* and *M. haemolytica*

G. Tefera, J. Smola

Institute of Microbiology and Immunology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: ENTERORapid 24 kit (PLIVA-Lachema, Czech Republic) was used for the identification of 321 strains isolated from the respiratory tract of different animal species in the Czech Republic and Ethiopia. A total of 207 (64.5%) strains were identified at the species level within 4 to 8 hours of incubation. In the same way, 39 (12.1%) strains were successfully classified at the genus level. The remaining strains (23.4%) were not identified nor classified to the family *Pasteurellaceae*. On the other hand, the accuracy of the ENTERORapid 24 kit for the identification of *P. multocida* and *M. haemoyltica* was observed using 9 reference strains and the identification results were compaired with the results of the RapiD 20E kit (bioMérieux, France), which required an overall examination time of 4 hours. According to our observation, the ENTERO Rapid 24 kit is the fastest system for the identification of *P. multocida* and *M. haemolytica* strains within 4 to 8 hours with a correct identification rate at the species level, with and without additional tests. For these reasons, we propose its modification for rapid identificaton of *P. multocida, M. haemolytica* and related bacterial species from the family *Pasteurellaceae* isolated from different animal species.

Keywords: *P. multocida; M. haemolytica; Pasteurellaceae*; Rapid identification; commercial kits

Bacterial species included within the genera *Pasteurella* and *Mannhiemia* have been classified and identified on the basis of their phylogenetic characteristics (Angen *et al.*, 1999). Furthermore, their phenotypic characteristics such as morphology, growth and biochemical activities are used for routine diagnosis of the strains. Most of the time, the rapid identification of the strains is determined by selected substrates of biochemical tests (Sperner *et al.*, 1999). Now a day, the classical tube tests are replaced by microwell bioche-mical tests, which are providing more accurate and rapid results (Bascomb *et al.*, 1997). However, a specific commercial biochemical test kit is not avialable for identification of bacterial species from the family *Pasteurellaceae*, especially for *P. multocida* and *M. haemolytica* until now. Practically, most of the commercial kits are designed for the identification *Enterobacteriaceae*. One of these kits is ENTERORapid 24, which is used for the identification of clinically important members of these bacteria. Becuaese of the similarity of the biochemical activities *Enterobacteriaceae* with *Pasteurellaceae*, the present study was carried out to evaluate the effectiveness of ENTERORapid 24 kit for the iden-tification of *P. multocida* and *M. haemolytica* strains, and other closely related bacterial species from the family *Pasteurellaceae.*

MATERIAL AND METHODS

Bacterial isolates

A total of 321 strains, isolated from clinical and section materials of animal's respiratory tract, were used in this study. They were cultured on blood agar and selective medium for *P. multocida* and *M. haemolytica.* These strains were taken from different animal species in the Czech Republic and Ethiopia (i.e. 247 bovine, 28 ovine, 22 caprine, 8 swine, 8 galline, 5 equine, 2 feline and 1 cunicle strains). Also, four reference strains of *P. multocida* (CCM 4370, CCM 4371, CCM 5419, CCM 5903) and five reference strians of *M. haemolytica* (CCM 5141,CCM 6169, CCM 6170, CCM 6174,CCM 6176) were used to evaluate the accuracy of the ENTERORapid 24 kit.

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Composition of biochemical kit

ENTERORapid 24 kit (PLIVA-Lachema, Czech Republic): It includes 24 tests (i.e. indole, lysine decarboxylase, ornithine decarboxylase, urease, saccharose, sorbitole, trehalose, glucose, pyrolidonylaminidase, esculin, cellobiose, melobiose, salicin, mannose, maltose, raffinose, acetoin, phenylalanine, malonate, β-galactosidase, β-glucuronodase, α-galactosidase, β-xylosidase, N-acetyl β-D-glucosaminidase).

RapiD 20 E (bioMérieux, France): The kit contains 21 tests. Fourteen of these tests are similar to the ENTERO Rapid 24 tests (i.e. lysine decarboxylase, ornithine decarboxylase, urease, phenylalanine deaminase, malonate, esculin, cellobiose, meliobiose, sucrose, trehalose, raffinose, glucose and indole) and the remaining

Table 1. Comparision results of fourteen biochemical tests of the ENTERORapid 24 and RapiD 20E kits for nine reference strains of *P. multocida* and *M. haemolytica*

Compaired tests	Bacterial species							
	1		2		3		4	
	results[a]	similarity[b] (%)	results	similarity (%)	results	similarity (%)	results	similarity (%)
Indole[1]	+	100	+	100	+	100	–	100
Indole[2]	+		+		+		–	
Lysine[1]	(–)	90	(–)	91.4	(–)	87.5	–	100
Lysine[2]	–		–		–		–	
Ornithine[1]	d	84	d	86.2	–	100	–	100
Ornithine[2]	–		–		–		–	
Urease[1]	–	100	–	100	–	100	–	100
Urease[2]	–		–		–		–	
Sucrose[1]	+	100	+	100	+	100	+	100
Sucrose[2]	+		+		+		+	
Trehalose[1]	+	100	+	100	+	100	–	100
Trehalose[2]	+		+		+		–	
Glucose[1]	+	100	+	100	+	100	+	100
Glucose[2]	+		+		+		+	
Esculin[1]	–	100	–	100	–	100	–	100
Esculin[2]	–		–		–		–	
Cellobiose[1]	–	100	–	100	–	100	–	100
Cellobiose[2]	–		–		–		–	
Meliobiose[1]	–	100	–	100	–	100	–	100
Meliobiose[2]	–		–		–		–	
Phenylalanine[1]	–	100	–	100	–	100	–	100
Phenylalanine[2]	–		–		–		–	
Raffinose[1]	–	100	–	100	–	100	–	100
Raffinose[2]	–		–		–		–	
Malonate[1]	–	100	–	100	–	100	–	100
Malonate[2]	–		–		–		–	
Sum in %	98		98.3		99		100	

[1] = tests of the ENTERORapid 24 kit, [2] = tests of the RapiD 20E kit

1 = *P. multocida* ssp. *multocida* (two strains), 2 = *P. multocida* ssp. *septica* (one strain),

3 = *P. multocida* ssp. *gallicida* (one strain), 4 = *M. haemolytica* (five strains)

[a] = results of both kits, [b] = similarity of both kits in %

+ = 80–100% positive test results, (+) = 70–79% positive test results, d = 31–69% positive test results

(–) = 16–30% positive test results, – = 0–15% positive test results

tests are distinct from them (i.e. *o*-nitro-phen-yl-β-D-galactopyranoside, sodium citrate, arabinose, xylose, anoditol, rhamnose and cytochrome oxidase).

Result reading

The testing process follows a specific protocol described easly in the user instructions. The results are read after 4 hours of inubation according to the manufacturer, and after 8 hours of incubation because of the slow metabolic activities of *P. multocida* and *M. haemolytica*. The result reading was made visually based on the eventual color change of the tests. The commer-cial tests were checked using proposed bacterial strains.

RESULTS

The reliability and accuracy of the ENTERORapid 24 kit for the identification of *P. multocida* and *M. haemolytica* strains was determined using 9 reference strains of animal origin. The biochemical profile of fourteen tests of the ENTERORapid and RapiD 20E kits were compaired. As it is illustrated on Table 1, both kits are similar within 98% for *P. multocida* subsp. *multocida*, 98.3% for *P. multocida* subsp. *septica*, 99% for *P. multicida* subsp. *gallicida* and 100% for *M. haemolytica*. Based on these, the ENTERORapid 24 kit was used for the identification of 321 field strains, from which 207 were identified at the species level after 4 and 8 hours of incubation (Table 2). The identified species were *P. multocida* (118 strains – 36.8%), *M. haemolytica* (64 strains – 20.0%), *P. trehalosi* (20 strains – 6.2 %), *P. canis* (3 strains – 0.9%) and *H. somnus* (2 strains – 0.6%). On the other hand, 39 strains were successfully classified at the level of genus that *Pasteurella* (31 strains – 9.6%), *Actinobacillus* (6 strains – 1.9%) and *Mannheimia* (2 strains – 0.6%). The remaining 75 strains (23.4%) were not identified nor classified to the familiy *Paseturellaceae*. Based on these results, the basic tests for the identification of *P. multocida*, *M. haemolytica* and other species from the family *Pasteurellaceae* are summarized on Table 3.

DISCUSSION

The reliability of ENTERORapid 24 commercial kit was analyzed using reference and field strains. First of all, the results of the ENTERORapid 24 kit were compared with the RapiD 20E kit to evaluate their similarity using the reference strains. According to our observation, the similarity of both kits was between 98–100% for *P. multocida* and 100% for *M. haemolytica*. Nekhorosheva *et al.* (2000) registered a 84.6–100% corellation of both kits for *Enterobacteriaceae*. Based on these results, 207 (64.5%) field strains were identified at the species level and 39 field strains (12.1%) at the genus level by the ENTERO Rapid 24 kit. The remaining field strains (23.4%) were not icluded to the family *Pasteurellaceae*. Later on, they were identified to the family *Pseudomonadaceae*. The composition of the ENTERORapid 24 kit is very satisfactory that it contains the basic tests for the identification of *P. multocida and M. haemolytica*. Particularly, indole, urease,

Table 2. Identification results of 321 field strains reached by the ENTERORapid 24 Kit

Bacterial species or other taxa	Origin and number of tested strains								Total	
	bovine	ovine	caprine	swine	galline	equine	feline	cunicle	number	%
P. multocida	91	9	7	7	3	1	–	–	118	36.8
M. haemolytica	57	4	2	1	–	–	–	–	64	20
P. trehalosi	11	5	3	–	–	–	–	1	20	6.2
P. canis	2	–	–	–	–	–	1	–	3	0.9
H. somnus	2	–	–	–	–	–	–	–	2	0.6
Pasteurella sp.	25	1	3	–	–	1	1	–	31	9.6
Actinobacillus sp.	–	5	1	–	–	–	–	–	6	1.9
MLO	1	–	–	–	1	–	–	–	2	0.6
Unclassified*	58	4	6	–	4	3	–	–	75	23.4
Sum	247	28	22	8	8	5	2	1	321	100

* = they were not classified nor identified to the family *Pasteurellaceae*

Table 3. Summary results of biochemical activities of selected bacterial species from the family *Pasteurellaceae* reached by ENTERORapid 24 kit

Tests	Selected bacterial species										
	1	2	3	4	5	6	7	8	9	10	11
Indole	+	(+)	–	–	–	–	–	–	–	–	(–)
Lysine	(–)	(–)	(–)	–	–	–	–	–	–	–	(–)
Otnithine	d	(+)	(–)	+	+	d	–	–	d	d	+
Urease	–	–	–	–	–	–	–	–	–	d	+
Sucrose	+	+	+	+	–	+	+	+	+	+	+
Sorbitol	d	+	d	–	–	d	+	+	d	+	–
Trehalose	(+)	–	–	–	–	–	–	–	–	–	–
Glucose	+	+	+	+	+	+	+	+	+	+	d
Pyrrolidonylamidase	–	–	–	–	–	–	+	+	–	–	–
Esculin	(–)	–	(+)	d	d	(–)	+	–	–	–	+
Cellobiose	(–)	–	–	d	–	(–)	–	–	–	–	(–)
Meliobiose	(–)	(–)	–	d	(–)	d	–	–	–	–	(–)
Salicin	–	–	–	(+)	–	–	+	–	d	–	d
Mannose	+	+	–	(–)	d	d	d	d	d	+	d
Maltose	–	(–)	(–)	+	(–)	+	+	+	d	–	d
Raffinose	–	–	–	d	–	–	–	d	–	–	–
Aceton	–	–	–	–	–	–	–	–	–	–	–
Phenylalanine	–	–	–	–	–	–	–	–	–	–	–
Malonate	(–)	–	–	d	(–)	–	–	–	–	–	d
β-Galactosida	d	(–)	–	+	(+)	+	+	+	+	+	+
β-Glucuronidase	–	–	–	–	–	–	–	–	–	–	–
α-Galactosidase	d	(–)	–	d	(+)	–	–	–	–	–	+
β-Xylosidase	d	(–)	–	d	d	(–)	+	–	–	–	+
N-acetyl-β-D-gluco-saminidase	(+)	(–)	(–)	+	(+)	(–)	–	d	d	–	+

1 = *P. multocida*, 2 = *P. canis biovar* 2, 3 = *P. avium biovar* 2, 4 = *P. trehalosi*, 5 = *Pasteurella* spp., 6 = *M. haemolytica*, 7 = *M. glucosida,* 8 = *M. ruminalis*, 9 =*Mannheimia-like organisms*, 10 = *Actinobacillus* spp., 11 = *Haemophilus* spp.
+ = 80–100% positive, (+) = 70–79% positive, d = 31–69% positive
– = 0–15% positive, (–) = 16–30% positive

trehalose, manose and maltose are the critical tests for the identification of both species. Especifically, indole positive and trehalose negative results are typical for *P. multocida*, whereas indole negative and trehalose positive results are typical for *M. haemolytica*. Both *P. multocida* and *M. haemolytica* are urease negative; in this regard, they differ from other species in the family *Pasteurellaceae* (Holt *et al.*, 1994). The ENTERO Rapid 24 kit basically adapt to existing laboratory workflow in the identification *P. multocida* and *M. haemolyica* strains because of its accurate and rapid result.

CONCLUSION

Based on the results of this study, we propose the modification of the ENTERORapid 24 kit for rapid identification of the family *Pasteurellaceae* after 4 to 8 hours of incubation in veterinary microbiology.

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Corresponding Author

MVDr. G. Tefera, Institute of Microbiology and Immunology, University of Veterinary and Pharmaceutical Sciences, Palackého 1–3, 612 42 Brno, Czech Republic
Tel. +420 5 41 56 22 73, 41 56 22 82, fax +420 5 41 21 26 46, e-mail: teferag@vfu.cz