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ORIGINAL ARTICLE

Comparison between Vitek MS, Bruker Biotyper, Vitek2, and API20E for differentiation of species of the genus *Raoultella*

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Abstract

Rapid and reliable identification of microorganisms in the clinical laboratory is essential for an early and accurate diagnosis guiding timely therapy. However, conventional methods are sometimes unreliable and show controversial outcomes. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been reported as a rapid and reliable method for identification of bacteria and fungi isolated from clinical samples. Members of the genus *Raoultella* are increasingly recognized as clinically relevant. There are difficulties in their identification at the species level since sequencing the 16S rRNA or the *rpo*B genes does not show conclusive results. The aim of this study has been to compare two MALDI-TOF MS systems (Vitek MS and Bruker Biotyper) with Vitek2 and API20E systems for differentiation of *Raoultella* species. A collection of 97 clinical isolates of *Raoultella* species was identified with Vitek MS, in parallel with Vitek2 and API, and finally with Bruker Biotyper. Among the two most widely used MALDI-TOF MS platforms, results obtained with Vitek MS were slightly superior to those obtained with the Bruker Biotyper system, with sensitivities and specificities of 98.9/57.9% and 98.8/37.0%, respectively. The current commercial phenotypic identification systems are not optimized for the identification of *Raoultella* species. Our results indicate that MALDI-TOF-based identification is more accurate and sensitive than that provided by phenotypic methods.

Keywords MALDI-TOF MS · *Raoultella* · Vitek MS · Bruker Biotyper

Introduction

The genus *Raoultella*, belonging to the *Enterobacter-Escherichia* clade of the order "Enterobacteriales" [1], includes Gram-negative, oxidase-negative, capsulated, and non-motile facultatively anaerobic bacilli. *Raoultella* was recognized as a genus separated from *Klebsiella* in 2001 by Drancourt et al. [2] on the basis of the sequences of the 16S rRNA and *rpo*B genes. *Raoultella* spp. are emerging opportunistic pathogens which are being increasingly associated with infections of different locations in human patients, particularly in the gall bladder and pancreas [3, 4], the urinary tract [5–7], or infected wounds [8, 9].

The genus *Raoultella* includes four species: *Raoultella ornithinolytica*, *Raoultella planticola*, *Raoultella terrigena*, and *Raoultella electrica* [10]. *R. ornithinolytica* is considered to be more virulent than the other species, and it is associated with a higher attribute mortality rate [11, 12].

Identification of *Raoultella* isolates to species level is difficult because of the very close genetic relationship of the four species, and there is no biochemical or phenotypic profile that easily differentiates them from species of the genus *Klebsiella*, except for the use of L-sorbose and 3-*0*-methyl-D-glucose as carbon sources and ornithine decarboxylase (ODC) activity, which is always positive for *R. ornithinolytica* and variably positive for *R. terrigena* [2, 13, 14]. It has been reported that matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) shows high accuracy for *Raoultella* identification to the genus level, although there is

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no agreement on this regard [13, 15]. In the present study, we carried out a comparative evaluation of the performance of the two more extensively used MALDI-TOF MS platforms (Vitek MS and Bruker Biotyper) with Vitek2 and API20E systems, for identification at the species level of 97 clinical isolates originally phenotypically identified as *Raoultella* spp. by Vitek2, taking identification by molecular methods as the gold standard.

Materials and methods

Ninety-seven isolates, from clinical samples, of *Raoultella* spp. (63 *R. ornithinolytica*, 30 *R. planticola*, and 4 *R. terrigena*), were included in this study. First, isolates were identified with Vitek MS, in parallel with Vitek2 and API, and finally with Bruker Biotyper. By first using MALDI TOF, a more sensitive a priori system, we try to minimize a selection bias as much as possible.

Identification using phenotypic methods

The organisms were identified using two different methods: the Vitek2 automated system with Vitek2 GN cards (bioMérieux, Marcy-I'Étoile, France) and the API20E system (bioMérieux).

MALDI-TOF MS identification

Identification was performed with both Vitek MS V2.0 Knowledge Base-Clinical Use (bioMérieux, Marcy-I'Étoile, France) and MALDI Biotyper (Bruker Daltonics GmbH, Bremen, Germany). Both libraries are for in vitro diagnostic (IVD) and not for research use only (RUO). The identifications are gold standard (molecular methods) based. To be called correctly to species, it should achieve a score > 2 in the case of Bruker and a score of 99.9% in the case of Vitek MS.

Identification using molecular methods

All the strains were subjected to PCR amplification of the *Klebsiella oxytoca peh*X gene to distinguish between this species and *Raoultella* [16]. In addition, the *bla* gene was amplified and digested with the *Not*I enzyme for identification at the species level of *R. ornithinolytica* and *R. planticola*, as described elsewhere [17]. Lastly, in those strains that did not amplify the *bla* gene, the housekeeping genes *rpoB*, *gyrA*, and *parC* were sequenced for the identification of *R. terrigena* [18].

Data were analyzed with SPSS 20.0 software (IBM Corp, Armonk, NY, USA). Two-by-two concordances were estimated by Cohen's *kappa* coefficient of agreement (*k*) and concordance index (CI).

Results

The enzyme *Nof*I digested the amplification of the *bla* gene in 63 isolates, identifying them as *R. ornithinolytica* while the 30 undigested ones were identified as *R. planticola*. In the four isolates in which the gene *bla* was not amplified, the *rpoB*, *gyrA*, and *parC* genes were sequenced and those four isolates

Table 1 Sensitivity, specificity, and predictive values of four methods for the identification of *Raoultella* at the species and genus level

	Raoultella ornithinolytica, *n* = 63	*Raoultella planticola*, *n* = 30	*Raoultella terrigena*, *n* = 4	Total no. (%) of strains, *n* = 97
Vitek MS				
Correct identification at species level	62 (98.41)	22 (73.34)	4 (100)	88 (90.72)
Not identified	1 (1.59)	8 (26.66)	0 (0)	9 (9.28)
Identification at genus level				96 (98.26)
Bruker Biotyper				
Correct identification at species level	63 (100)	17 (56.67)	0 (0)	80 (82.47)
Not identified	0 (0)	13 (43.33)	4 (100)	17 (17.53)
Identification at genus level				97 (100)
Vitek®2				
Correct identification at species level	10 (15.87)	29 (96.66)	0 (0)	39 (40.21)
Not identified	53 (84.13)	1 (3.33)	4 (100)	58 (59.79)
Identification at genus level				96 (98.96)
API20E				
Correct identification at species level	10 (15.87)	0 (0)	2 (50)	12 (12.37)
Not identified	53 (84.13)	30 (100)	2 (50)	85 (87.63)
Identification at genus level				18 (18.55)

Table 2 Identification results for *R. ornithinolytica* ($n = 63$), *R. planticola* ($n = 30$), and *R. terrigena* ($n = 4$) by Vitek MS, Bruker Biotyper, Vitek®2, and API20E

	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
	Value (%)	95%CI	Value (%)	95%CI	Value (%)	95%CI	Value (%)	95%CI
Vitek MS	98.88	93.90–99.97	57.89	33.50–79.75	91.67	73.90–89.06	91.67	86.65–94.91
Bruker	98.77	93.31–99.97	37.04	19.40–57.63	82.47	77.88–86.28	90.91	57.29–98.68
Vitek®2	95.12	83.47–99.40	14.93	7.40–25.74	40.62	37.72–43.60	83.33	53.76–55.24
API20E	13.04	6.93–21.68	62.50	35.43–84.80	66.67	46.67–82.01	11.11	7.82–15.50

were definitely identified as *R.* terrigena. Vitek MS, Bruker Biotyper, and Vitek2 assigned a correct genus identification to 96 (98.9%), 97 (100%), and 96 (98.9%) isolates, respectively. On the other hand, API galleries allowed genus identification for only 18 (18.5%) isolates. Vitek MS, Bruker Biotyper, Vitek2, and API globally assigned a correct species identification in 88 (90.7%), 80 (82.4%), 39 (40.2%), and 12 (12.3%) cases. Vitek MS correctly identified 62 (98.4%) *R. ornithinolytica*, 22 (73.3%) *R. planticola*, and 4 (100%) *R. terrigena*. Bruker Biotyper correctly identified 63 (100%) *R. ornithinolytica*, 17 (56.6%) *R. planticola*, and none of (0%) *R. terrigena*. Vitek2 correctly identified 10 (10.3%) *R. ornithinolytica*, 29 (96.6%) *R. planticola*, and none of (0%) *R. terrigena*, and it did not assign any identification in one isolate. Finally, API galleries correctly identified 10 (10.3%) *R. ornithinolytica*, 0 (0%) *R. planticola*, and 2 (50%) *R. terrigena* and identified 2 additional isolates as *R. ornithinolytica* when the strips were incubated for 48 h; despite these results, the API20E developer system indicated in many cases "possibility of *Raoultella*" (Table 1).

In comparison with molecular methods, the sensitivities and specificities of Vitek MS, Bruker Biotyper, Vitek2, and API20E results were 98.8/57.8% (95%CI 93.9–99.9%/33.5–79.7%), 98.7/37.0% (95%CI 93.3–99.9%/19.4–57.6%), 95.1/14.9% (95%CI 83.4–99.4%), and 13.0/62.5% (95%CI 6.9–21.6%/35.4–84.8%), respectively. We have also calculated kappa coefficient (κ) and concordance index (CI) between Vitek MS with molecular techniques and Bruker Biotyper with molecular techniques showing a result of κ 0.664 (95%CI 48.2–84.6%) with an appropriate degree of agreement for the first system and κ 0.446 (95%CI 28.2–61.0%) with a moderate degree of agreement for the second system (Table 2).

Of note the 10 (15.8%) *R. ornithinolytica* correctly identified by Vitek2 were the same isolates as those correctly identified by API20E in the first 24 h, and they were the only isolates showing ODC activity. Only in two additional strains the ODC was positivized by incubating the API20E gallery for 48 h. Malonate became positive by all *R. ornithinolytica* and *R. planticola* isolates. The 2-nitrophenyl-β-D-galactopyranoside (ONPG) test was positive in all *R. ornithinolytica*, *R. planticola* and *R. terrigena* isolates. The Voges-Proskauer reaction was positive in all strains of *R. planticola* and *R. terrigena*, and in 60 (95.2%) of *R. ornithinolytica* [19].

Discussion

Both Vitek2 and API20E only rely on the ODC to differentiate *K. oxytoca* (ODC negative) from *R. ornithinolytica* (ODC positive). Moreover, the Vitek2 system does not include *R. terrigena* in its database.

In our study, the two MALDI-TOF MS systems that have been evaluated are more efficient than the Vitek2 system or API20E galleries for a reliable identification of *Raoultella* at a genus level, so this proteomics-based identification is an appropriate alternative to the phenotypic tests. Although Vitek2 is almost as reliable as MALDI-TOF for identification of the genus *Raoultella*, with a sensitivity of 95.1% (but with the limited specificity of 14.9), it needs 24 h until results are available. Within the two MALDI-TOF systems studied, Vitek MS is slightly better than the Bruker Biotyper one, with a sensitivity and specificity of 98.8/57.8% from the first to 98.7/37.0% of the second. Finally, API galleries did not assign correct identification at species level of most clinical isolates of *R. ornithinolytica* and *R. planticola* and identified about 50% of the uncommon species *R. terrigena*, being aware of the limited number of strains studied.

The finding of low correlation between *R. ornithinolytica* isolates identified by both molecular methods and MALDI-TOF with the positivity of ODC is justified by the existence of ODC-negative *R. ornithinolytica* isolates, as has been outlined by Walckenaer et al. [17].

Vitek2 and API require additional biochemical tests to identify the ODC-negative *R. ornithinolytica* isolates. The current commercial phenotypic identification systems are not optimized for the identification of *Raoultella* species.

In summary, although more information is required about the reliability of MALDI-TOF MS for the identification of *Raoultella* species, it would be expected that more cases of infections by these microorganisms could be diagnosed with these identification approaches [20].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This work has gone through the ethics committee of our hospital without receiving any kind of objection.

Informed consent This work has not needed any informed consent.

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