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Comparison of Vitek MS and MALDI Biotyper for Identification of *Actinomycetaceae* of Clinical Importance

Janina Ferrand,[a] Hélène Hochard,[b] Victoria Girard,[c] Nejla Aissa,[a] Baptiste Bogard,[a] Corentine Alauzet,[a] Alain Lozniewski[a]

Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Nancy, Nancy, France[a]; Laboratoire de Biologie Médicale, Centre Hospitalier Régional de Metz-Thionville, Metz, France[b]; bioMérieux, R&D Microbiologie, La Balme les Grottes, France[c]

The Vitek MS *in vitro* diagnostic (IVD) and MALDI Biotyper IVD systems were evaluated for the identification of 158 strains of *Actinomycetaceae*. Correct species-level identification rates of 60.7% and 58.2% were obtained with the Vitek MS system after direct deposit and with the MALDI Biotyper system after on-plate formic acid treatment, respectively.

The family *Actinomycetaceae* contains several genera, the members of which are increasingly recognized as human pathogens: *Actinomyces*, *Actinobaculum*, *Arcanobacterium*, *Mobiluncus*, *Trueperella*, and *Varibaculum* (1–3). For a proper identification of these Gram-positive asporogenous rods, the use of 16S rRNA gene sequencing is often required, since conventional bacteriological identification methods are not effective (4, 5).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has recently emerged as a fast and inexpensive approach for use in diagnostic microbiology. The purpose of this study was to compare the performances of two commercially available MALDI-TOF MS systems, the Vitek MS *in vitro* diagnostic (IVD) (bioMérieux, Marcy l'Etoile, France) and MALDI Biotyper IVD (Bruker Daltonics, Wissembourg, France) systems, for the identification of relevant isolates of *Actinomycetaceae*. It is known that due to the thick cell wall of Gram-positive bacteria, a preliminary extraction step may be warranted before the acquisition of mass spectra, as was already shown for the Biotyper system (6–9). However, to the best of our knowledge, the impact of such pretreatment has not systematically been evaluated for the Vitek MS system. This prompted us to investigate the effect of three sample preparation methods (i.e., direct deposit [DD], on-plate formic acid treatment [DD-FA], and ethanol-formic acid extraction [EXT]) on the identification rates.

A total of 158 strains, including 112 nonredundant clinical isolates collected from January 2005 to December 2013 in the Bacteriology Department of the University Hospital of Nancy, France, 28 strains from the collection of bioMérieux, and 18 type strains from the Culture Collection of the University of Gothenburg (CCUG) (Gothenburg, Sweden) and Collection of Institut Pasteur (CIP) (Paris, France) were tested (Table 1; see also Table S1 in the supplemental material). The strains were characterized by 16S rRNA gene sequencing using CLSI interpretive criteria (10, 11). Frozen isolates were subcultured twice on 5% sheep blood Columbia agar (bioMérieux) at 35°C in anaerobiosis for 24 to 48 h before analysis.

For both MALDI-TOF MS systems tested, bacterial samples were prepared using either DD, DD-FA, or EXT, as previously described (6, 12). The mass spectra acquired with the Vitek MS IVD system were analyzed using the Vitek MS IVD database version 2.0. With the MALDI Biotyper system, the mass spectra were obtained using the microflex LT MS (Bruker Daltonics). The results were analyzed using the MALDI Biotyper software (IVD version, with 5,627 database entries). Calibration, quality control, and interpretation of the results were performed according to the manufacturer's recommendations. All isolates were spotted in duplicate on a target plate, and the best result was chosen for use in the comparisons. The Cochran's Q test was used to identify overall differences between the three preparation methods for both systems tested. For significant differences ($P < 0.05$), *post hoc* McNemar's pairwise comparisons were performed. The P values correspond to McNemar's test results reported, when applicable.

When DD was used, the rates of correct identifications were higher with the Vitek MS system than with the Biotyper system at the species level (60.7% [96 of 158 strains] versus 27.2% [43 of 158 strains], respectively; $P < 0.000001$) but not at the genus level (67.7% [107 of 158 strains] versus 63.3% [100 of 158 strains], respectively) (Table 1; see also Table S1 in the supplemental material). At the genus level, 10 isolates belonging to unknown species were misidentified with the Vitek MS system, while no errors were observed with the Biotyper system (Table 1). When considering solely the species included in the database, the Vitek MS system correctly identified to the genus and species levels 92.5% (98/106) and 90.6% (96/106) of the strains tested, respectively (see Table S1 in the supplemental material). Using the same criteria, 66.2% (100/151) and 28.5% (43/151) of the strains were correctly identified by the Biotyper system to the genus and species levels, respectively.

In comparison to DD, neither DD-FA nor EXT significantly modified the rates of correct identification obtained with the Vitek MS system, both at the genus level (DD versus DD-FA, 67.7% versus 70.3%, respectively, and DD versus EXT, 67.7% versus 66.4%, respectively) and species level (DD versus DD-FA, 60.7%

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Address correspondence to Janina Ferrand, j.ferrand@chu-nancy.fr.

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TABLE 1 Identification of 158 *Actinomycetaceae* isolates by the Vitek MS IVD and MALDI Biotyper

Genus (no. of isolates/ no. of species[a])	Preparation method[b]	No. (%) of isolates by system									
		Vitek MS IVD					MALDI Biotyper IVD				
		Correctly reported		Misidentified			Correctly reported		Misidentified		
		Species level	Genus level[c]	Species level	Genus level	Not identified	Species level	Genus level[c]	Species level	Genus level	Not identified
Actinobaculum (21/2)	DD				3 (14.3)	18 (85.7)	3 (14.3)	11 (52.4)			10 (47.6)
	DD-FA				7 (33.3)	14 (66.7)	16 (76.2)	20 (95.2)			1 (4.8)
	EXT				4 (19)	17 (81)	17 (81)	20 (95.2)			1 (4.8)
Actinomyces (113/24)	DD	79 (69.9)	90 (79.6)	11 (9.7)	5 (4.4)	18 (15.9)	26 (23)	73 (64.6)	3 (2.7)		40 (35.4)
	DD-FA	82 (72.6)	94 (83.2)	12 (10.6)	2 (1.8)	17 (15)	60 (53.1)	98 (86.7)	4 (3.5)		15 (13.3)
	EXT	78 (69)	87 (77)	9 (8)	2 (1.8)	24 (21.2)	81 (71.7)	101 (89.4)	5 (4.4)	2 (1.8)	10 (8.8)
Arcanobacterium (6/1)	DD	4 (66.7)	4 (66.7)			2 (33.3)	4 (66.7)	5 (83.3)			1 (16.7)
	DD-FA	4 (66.7)	4 (66.7)		1 (16.7)	1 (16.7)	5 (83.3)	5 (83.3)			1 (16.7)
	EXT	5 (83.3)	5 (83.3)			1 (16.7)	5 (83.3)	5 (83.3)			1 (16.7)
Mobiluncus (2/2)	DD	2 (100)	2 (100)				1 (50)	1 (50)			1 (50)
	DD-FA	2 (100)	2 (100)				1 (50)	1 (50)			1 (50)
	EXT	2 (100)	2 (100)				1 (50)	1 (50)			1 (50)
Trueperella (12/2)	DD	11 (91.7)	11 (91.7)			1 (8.3)	9 (75)	10 (83.3)			2 (16.7)
	DD-FA	11 (91.7)	11 (91.7)			1 (8.3)	10 (83.3)	11 (91.7)		1 (8.3)	
	EXT	11 (91.7)	11 (91.7)			1 (8.3)	10 (83.3)	11 (91.7)			1 (8.3)
Varibaculum (4/1)	DD				2 (50)	2 (50)					4 (100)
	DD-FA					4 (100)					4 (100)
	EXT					4 (100)					4 (100)
Total (158/32)	DD	96 (60.7)	107 (67.7)	11 (7)	10 (6.3)	41 (25.9)	43 (27.2)	100 (63.3)	3 (1.9)		58 (36.7)
	DD-FA	99 (62.7)	111 (70.3)	12 (7.6)	10 (6.3)	37 (23.4)	92 (58.2)	135 (85.4)	4 (2.5)	1 (0.6)	22 (13.9)
	EXT	96 (60.7)	105 (66.4)	9 (5.7)	6 (3.8)	47 (29.7)	114 (72.2)	138 (87.4)	5 (3.2)	2 (1.3)	18 (11.4)

[a] One strain of *Actinomyces meyeri*, 2 strains of *Actinomyces odontolyticus*, 3 strains of *Actinomyces turicensis*, 1 strain of *Arcanobacterium haemolyticum*, and 3 strains of *Trueperella pyogenes* were included in the strain collection used to create Vitek MS database.
[b] DD, direct deposit; DD-FA, direct deposit-formic acid treatment; EXT, ethanol-formic acid extraction.
[c] Total number of isolates correctly reported to the species level and to the genus level only (including strains misidentified to the species level but correctly identified to the genus level).

versus 62.7%, respectively, and DD and EXT, 60.7%, respectively) (Table 1). With the Biotyper system, the use of DD-FA permitted the achievement of higher identification rates at the genus level (DD versus DD-FA, 63.3% versus 85.4%, respectively; $P < 0.000001$) and species level (DD versus DD-FA, 27.2% versus 58.2%, respectively; $P < 0.000001$). The use of EXT permitted the achievement of higher identification rates at the species level, compared to DD-FA (DD-FA versus EXT, 58.2% versus 72.2%, respectively; $P < 0.000001$) but not at the genus level (EXT, 87.4%). Genus-level misidentifications were not significantly modified using DD-FA or EXT with the Vitek MS system (DD and DD-FA, 6.3%, and DD versus EXT, 6.3% versus 3.8%, respectively). With the Biotyper system, one repeatable genus misidentification occurred using DD-FA, whereas two repeatable genus misidentifications were observed using EXT (Table 1; see also Table S2 in the supplemental material).

To the best of our knowledge, this is the first study to compare the performances of the Vitek MS IVD and MALDI Biotyper IVD systems with a large panel of strains of *Actinomycetaceae*. When DD was used, the rate of species-level identification was significantly lower with the Biotyper system, despite its higher species coverage, than that obtained with the Vitek MS system. This difference may at least partially be explained by the fact that the Biotyper database was built with spectra obtained after ethanol-formic acid extraction (13), in contrast to the Vitek MS database, which was built using spectra obtained without any pretreatment step. This may also explain why the performance of the Biotyper system was increased when samples were pretreated. When DD-FA, which represents, in contrast to EXT, an acceptable alternative to DD in a routine workflow (6), was used, we found that the Biotyper identified, compared to the Vitek MS system after DD, more isolates to the genus level ($P < 0.000001$) but not to the species level (Table 1). Genus-level misidentifications occurred more frequently with the Vitek MS system than with the Biotyper system. It is noteworthy that only few of these errors might have resulted in erroneous or delayed diagnosis of actinomycosis (see Table S2 in the supplemental material) (14). Isolates that were incorrectly identified at the genus level by the Vitek MS system produced spectra that had a few number of peaks, some of which are common with other genera claimed in the Vitek MS version 2.0 database. This suggested that this problem might be solved, at least partially, by expanding the database. This was confirmed by the fact that when spectra obtained after DD were analyzed with an expanded database (Vitek MS version 3.0, which contains ad-

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ditional reference spectra of *Actinobaculum schaalii*, *Actinomyces israelii* and *Actinomyces naeslundii*), only two isolates (belonging to species not included in the version 3.0 database: *Actinomyces cardiffensis* and *Actinomyces hongkongensis*) were misidentified at the genus level without any potential clinical impact. It is noteworthy that in this case, identification rates were increased at the genus level (72.2%) and species level (69%).

In conclusion, the two commercially available MALDI-TOF MS systems tested were globally effective in identifying *Actinomycetaceae*. The performance of the Vitek MS IVD system was not improved by adding an extraction step, while using an on-plate formic acid extraction prior to analysis with the MALDI Biotyper permitted the optimization of the system performance. The accuracy of both systems needs to be further increased by expanding the spectral database.

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Alain Lozniewski is a member of the Scientific Board of bioMérieux, France.

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