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Comparative Evaluation of the Bruker Biotyper and Vitek MS Matrix-Assisted Laser Desorption Ionization–Time Of Flight (MALDI-TOF) Mass Spectrometry Systems for Identification of Yeasts of Medical Importance

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We report the first comparative evaluation between the Bruker Biotyper MS (BMS) and the Vitek MS (VMS) for the identification of yeasts. The rate of correct identifications at the species level was comparable using the commercial databases (89.8% versus 84.3%; $P = 0.712$), but higher for BMS using an in-house-extended database (100% versus 84.3%; $P = 0.245$). Importantly, the rate of misidentification was significantly higher for VMS (1% versus 12.1%; $P < 0.0001$), including the rate of major errors (0% versus 4.5%; $P = 0.0036$).

The introduction of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) in the clinical microbiology laboratories is changing the approach to bacterial and fungal identification (1–4). In particular, several studies have already demonstrated the reliability of MALDI-TOF MS in the rapid identification of yeasts in different clinical settings (5–7), evidencing its cost-effectiveness in allowing the initiation of species-targeted antifungal therapy (7–9). To date, four MALDI-TOF MS systems are commercially available: the Microflex LT Biotyper (Bruker Daltonics, Bremen, Germany) (BMS), the Saramis system (bio-Mérieux, Marcy l'Etoile, France), the Vitek MS system (bioMérieux, Marcy l'Etoile, France) (VMS), and, very recently, the Andromas system (Andromas, Paris, France). Several comparative studies have already been performed using the most common systems (BMS and VMS), but, to the best of our knowledge, they have focused only on the identification of bacteria (10–13). Only very recently was a comparative study on yeasts performed using BMS and Saramis (bio-Mérieux, Marcy l'Etoile, France), the previously distributed version of VMS (14). In the present study, we evaluated the ability of BMS and VMS to identify a broad panel of yeasts of medical interest.

One hundred ninety-seven isolates from different human samples, previously identified by conventional biochemical techniques or by sequencing the internal transcribed spacer 1 (ITS1) and ITS2 regions, were blindly identified using the two systems. In order to minimize the risk of misidentification related to the use of incomplete and error-filled public databases (15), the sequences obtained were compared to reference data available in two databases: GenBank, searched by using the nucleotide BLAST tool (blast.ncbi.nlm.nih.gov), and the CBS (Centraalbureau voor Schimmelcultures) yeast database (www.cbs.knaw.nl). The panel included 157 (79.7%) isolates belonging to 30 *Candida* or *Candida*-related species (Table 1), and 40 (20.3%) isolates belonging to 15 non-*Candida* species (Table 2). Before processing for MS identification, each isolate was cultured on Sabouraud dextrose (Kima, Padua, Italy) agar and incubated for 24 h at 35°C. For BMS, proteins were extracted as recommended by the manufacturer. Briefly, a loopful of yeasts was suspended in one volume of water and three volumes of absolute ethanol, and after centrifugation, the pellets were processed with an equal amount of formic acid and acetonitrile and mixed by vortexing. One microliter of the mixture was spotted onto a polished steel target plate and covered with 1 μl of a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid when dried. Measurements were performed with a Microflex LT mass spectrometer (Bruker Daltonics), and spectra were recorded in the positive linear mode (laser frequency, 20 Hz; ion source 1 voltage, 20 kV; ion source 2 voltage, 16.7 kV; lens voltage, 8.5 kV). For VMS, fresh colonies were directly applied to two spots of the target plate and lysed with 0.5 μl of 25% formic acid, and when completely dried, they were covered with ready-to-use α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution, allowed to dry, and analyzed. The entire process took approximately 3 min for each isolate.

In the case of BMS, two databases were used for interpretation of the obtained spectra, the commercially available database (Bruker Daltonics library v. 3.0) and an in-house-developed extended database (BED). Identifications with confidence scores of ≥2.000 were considered reliable for optimal identification at the species level and were not further analyzed, whereas isolates identified with lower confidence scores (1.700 to 1.999) or not identified (<1.700) were further analyzed using the BED. For VMS, identifications were performed using the Vitek MS server v. 1.2.0 and were accepted when one spot gave a result with >90% confidence with no conflicting result with >85% confidence for the other spot or when concordant identifications were obtained from the two spots with at least one having a confidence level of >85%.

Received 29 March 2013 Returned for modification 25 April 2013
Accepted 10 May 2013
Published ahead of print 15 May 2013
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doi:10.1128/JCM.00841-13

TABLE 1 *Candida* and *Candida*-related genera

Species	No. (%) of isolates										
		With Vitek MS result				With Bruker MS result					
						Standard database				BED[a]	
	Tested	Correct identification at the species level	Correct identification at the genus but not at the species level	No identification	Error	Correct identification at the species level	Correct identification at the genus but not at the species level	No identification	Error	Suboptimal confidence score	Unreliable confidence score
Candida aaseri	1				1			1			1
Candida albicans	35	35				35					
Candida blankii	1				1			1			1
Candida dubliniensis	2	1		1		2				1	
Candida glabrata	29	28		1		29					
Candida guilliermondii	3	3				3					
Candida inconspicua	2	2					2			2	
Candida intermedia	1	1				1				1	
Candida kefyr	3	3				3				1	
Candida krusei	14	14				14					
Candida lambica	1	1				1				1	
Candida lipolytica	1	1				1				1	
Candida lusitaniae	7	7				7				4	
Candida metapsilosis	3		1	1	1	3				2	
Candida nivariensis	2		1		1	1		1			1
Candida norvegensis	3	3				3					
Candida orthopsilosis	5		5			3		2		1	2
Candida parapsilosis	16	16				16				1	
Candida pararugosa	1			1		1				1	
Candida pelliculosa	2	2				2				1	
Candida slooffiae	1		1					1			1
Candida sphaerica	1	1				1				1	
Candida tropicalis	16	16				16				2	
Candida utilis	1	1						1			1
Candida valida	1	1				1					
Debaryomyces hansenii	1	1				1				1	
Pichia caribbica	1		1					1			1
Pichia fabiani	1			1				1			1
Pichia manshurica	1		1			1					
Pichia onychis	1			1				1			1
Total	157 (100)	137 (87.3)	10 (6.4)	6 (3.8)	4 (2.5)	145 (92.3)	2 (1.3)	10 (6.4)	0	21 (13.4)	10 (6.4)

[a] Number of isolates with suboptimal confidence scores (>1.7 but <2.0) or giving unreliable (<1.7) identification with the BMS standard database but optimally (>2.0) identified using the Bruker extended database (BED).

TABLE 2 Non-*Candida* yeasts

Species	No. (%) of isolates										
		Vitek MS				Bruker MS					
						Standard database				BED[a]	
	Tested	Correct identification at the species level	Correct identification at the genus but not at the species level	No identification	Error	Correct identification at the species level	Correct identification at the genus but not at the species level	No identification	Error	Suboptimal confidence score	Unreliable confidence score
Arxiozyma telluris	1				1	1					
Cryptococcus gattii	5		4		1	5				5	
Cryptococcus neoformans	10	10				10					
Geotrichum candidum	4	4				4				3	
Lodderomyces elongisporus	1			1		1					
Magnusiomyces capitatus	3	3				3				1	
Malassezia pachydermatis	1	1				1				1	
Rhodotorula glutinis	1				1			1			1
Rhodotorula mucilaginosa	2	1			1	1		1			1
Rhodotorula slooffiae	1		1					1			1
Saccharomyces cerevisiae	5	5				5				3	
Trichosporon asahii	1	1						1			1
Trichosporon inkin	3	3						3			3
Trichosporon mucoides	1	1				1				1	
Williopsis spp.	1				1			1			1
Total	40 (100)	29 (72.5)	5 (12.5)	1 (2.5)	5 (12.5)	32 (80)	0	8 (20)	0	14 (35)	8 (20)

[a] Number of isolates with suboptimal confidence scores (>1.7 but < 2.0) or giving unreliable (<1.7) identification with the BMS standard database but optimally (>2.0) identified using the Bruker extended database (BED).

Regarding *Candida* and *Candida*-related species (Table 1), BMS correctly identified 145 (92.3%) isolates at the species level, two (1.3%) at the genus but not at the species level (two isolates of *Candida inconspicua* identified as *Pichia cactophila*, a species already reported to be genetically closely related to *C. inconspicua* [14, 16]), while 10 (6.4%) isolates were not identified with the commercially available database. No major errors (i.e., genus misidentification) were observed. Interestingly, all isolates with unreliable or suboptimal identification were correctly identified by the BED (Table 3). In the case of VMS, 137 (87.3%) isolates were identified at the species level and 10 (6.4%) were correctly identified as belonging to the genus *Candida* but misidentified at the species level (Table 1 and Table 3). In six (3.8%) cases, it was not possible to achieve any reliable identification, whereas, more importantly, four (2.5%) isolates were completely misidentified (Table 1 and Table 3).

With respect to non-*Candida* species (Table 2), BMS identified 32 (80%) isolates at the species level, whereas in eight cases (20%) it was not possible to achieve a reliable identification. When the spectra of the unidentified isolates were matched against the extended database, a reliable identification at the species level was obtained in all cases (Table 3). VMS correctly identified 29 (72.5%) isolates, whereas five (12.5%) isolates (four isolates of *Cryptococcus gattii* misidentified as *Cryptococcus neoformans* and one isolate of *Rhodotorula slooffiae* misidentified as *Rhodotorula minuta*) were correctly identified at the genus but not at the species level (Table 3). One (2.4%) isolate did not yield a reliable identification, whereas, importantly, five (12.5%) isolates were completely misidentified.

Overall, the BMS correctly identified 177 (89.8%) isolates at the species level in comparison with 166 (84.3%) isolates correctly identified by VMS (two-tailed Fisher's exact test; $P = 0.712$). BMS yielded an unreliable identification more frequently than VMS, 18 (9.2%) and seven (3.6%), respectively ($P = 0.039$). In the case of BMS, all unidentified spectra were retested by the Bruker extended database (BED), thus allowing reliable identification. Finally, the rate of overall misidentification was significantly ($P < 0.0001$) higher for VMS (24 isolates, 12.1%) than for BMS (two isolates, 1%), with a higher rate ($P = 0.0036$) of major errors (e.g., technically reliable but incorrect identification at the genus level) (nine isolates [4.5%] versus 0 isolates). A similar trend was also observed when the *Candida* and the non-*Candida* species were considered separately.

From a technical point of view, the paired-spots approach for VMS was useful for only 17 (14 *Candida* and 3 non-*Candida*) of the 197 isolates (8.6%). In particular, 13 isolates (11 *Candida* and 2 non-*Candida*) were reliably identified (>90%) in one spot but did not give any identification in the other one, whereas 4 isolates (3 *Candida* and 1 non-*Candida*) gave concordant identifications, with one spot one giving a result with >85% confidence. Although not directly investigated in this study, the real cost-effectiveness of the two-spot approach, compared to possible retesting in case of a preliminary unreliable identification, may deserve further investigation.

Previous studies showed MALDI-TOF MS to be reliable and cost-effective in identifying different fungal species of medical interest (5–7), but to date none had comparatively investigated the performances of the two most common commercially available MALDI instruments. In this study, a broad panel of medically important *Candida* and non-*Candida*-related yeasts was tested, thus justifying the observed lower rate of correct identifications in comparison with other studies (1, 2, 6, 9, 17, 18). Indeed, our overall rate of correct identifications was very similar to that reported in a recent study on a comparable panel of yeasts (14). Several points emerging from our study deserve further discussion. First, BMS and VMS share almost 100% accuracy of identi-

TABLE 3 Cases of misidentification[a]

Species	No. of isolates	Vitek MS identification (confidence level [%])	Bruker MS identification (confidence score)	
			Standard database	BED
Candida aaseri	1	*Williopsis* spp. (99.0)	*Candida lambica* (1.425)	***Candida aaseri* (2.054)**
Candida blankii	1	*Malassezia furfur* (99.6)	No reliable identification	***Candida blankii* (2.035)**
Candida dubliniensis	1	*Candida dubliniensis/Candida albicans* (99.9/99.9)	***Candida dubliniensis* (1.954)**	***Candida dubliniensis* (2.056)**
Candida glabrata	1	*Geotrichum fermentans/Malassezia pachydermatis* (99.9/98.2)	***Candida glabrata* (2.493)**	
Candida inconspicua	2	***Candida inconspicua* (99.9)**	*Pichia cactophila* (1.998)[b]	***Candida inconspicua* (2.432)**
		***Candida inconspicua* (99.9)**	*Pichia cactophila* (1.764)[b]	***Candida inconspicua* (2.286)**
Candida metapsilosis	3	*Candida parapsilosis* (99.9)	***Candida metapsilosis* (2.144)**	
		Malassezia pachydermatis (88.6)	***Candida metapsilosis* (1.993)**	***Candida metapsilosis* (2.300)**
		Candida parapsilosis (64.5)	***Candida metapsilosis* (1.957)**	***Candida metapsilosis* (2.438)**
Candida nivariensis	2	*Candida glabrata* (89.7)	***Candida nivariensis* (2.180)**	
		Geotrichum capitatum (95.1)	*Candida nivariensis* (1.609)	***Candida nivariensis* (2.267)**
Candida orthopsilosis	5	*Candida parapsilosis* (98.4)	Not reliable identification	***Candida orthopsilosis* (2.015)**
		Candida parapsilosis (99.9)	***Candida orthopsilosis* (2.104)**	
		Candida parapsilosis (99.9)	***Candida orthopsilosis* (1.925)**	***Candida orthopsilosis* (2.031)**
		Candida parapsilosis (99.9)	No reliable identification	***Candida orthopsilosis* (2.384)**
		Candida parapsilosis (99.9)	***Candida orthopsilosis* (2.157)**	
Candida pararugosa	1	*Candida freischussii/Candida parapsilosis* (99.9)	***Candida pararugosa* (1.712)**	***Candida pararugosa* (2.156)**
Candida slooffiae	1	*Candida utilis* (98.3)	*Candida slooffiae* (1.644)	***Candida slooffiae* (2.003)**
Candida utilis	1	***Candida utilis* (99.9)**	Not reliable identification	***Candida utilis* (2.197)**
Debaryomyces hansenii	1	***Candida famata* (99.9)**	***Debaryomyces hansenii* (1.868)**[c]	***Debaryomyces hansenii* (2.032)**
Pichia caribbica	1	*Candida guilliermondii* (99.9)	No reliable identification	***Pichia caribbica* (2.070)**
Pichia fabiani	1	Not reliable identification	No reliable identification	***Pichia fabiani* (2.099)**
Pichia manshurica	1	*Candida guilliermondii* (94.2)	***Pichia manshurica* (2.219)**	
Pichia onychis	1	*Candida valida* (68.1)	Not reliable identification	***Pichia onychis* (2.205)**
Arxiozyma telluris	1	*Kloeckera apiculata* (97)	***Arxiozyma telluris* (2.094)**	
Cryptococcus gattii	5	*Cryptococcus neoformans* (99.9)	***Cryptococcus gattii* (1.913)**	***Cryptococcus gattii* (2.050)**
		Cryptococcus neoformans (99.9)	***Cryptococcus gattii* (1.830)**	***Cryptococcus gattii* (2.060)**
		Cryptococcus neoformans (99.9)	***Cryptococcus gattii* (1.810)**	***Cryptococcus gattii* (2.083)**
		Cryptococcus neoformans (98.4)	***Cryptococcus gattii* (1.953)**	***Cryptococcus gattii* (2.011)**
		Kodamaea ohmeri (99.6)	***Cryptococcus gattii* (1.725)**	***Cryptococcus gattii* (2.135)**
Lodderomyces elongisporus	1	*Candida pelliculosa/Cryptococcus laurentii* (99.9/96.9)	***L. elongisporus* (2.011)**	
Rhodotorula glutinis	1	*Geotrichum candidum* (96.1)	No reliable identification	***Rhodotorula glutinis* (2.007)**
Rhodotorula mucilaginosa	1	*Candida dubliniensis* (99.3)	*R. mucilaginosa* (1.514)	***R. mucilaginosa* (2.025)**
Rhodotorula slooffiae	1	*Rhodotorula minuta* (99.9)	Not reliable identification	***Rhodotorula slooffiae* (2.126)**
Trichosporon asahii	1	***Trichosporon asahii* (99.9)**	*Trichosporon asahii* (1.681)	***Trichosporon asahii* (2.102)**
Trichosporon inkin	3	***Trichosporon inkin* (99.9)**	No reliable identification	***Trichosporon inkin* (2.013)**
		***Trichosporon inkin* (99.9)**	No reliable identification	***Trichosporon inkin* (2.062)**
		***Trichosporon inkin* (99.9)**	No reliable identification	***Trichosporon inkin* (2.045)**
Williopsis spp.	1	*Candida utilis* (99.9)	No reliable identification	***Williopsis* spp. (2.010)**

[a] All isolates misidentified or not reliably identified by one or both systems are reported. The high scores obtained with the BED are probably biased by the fact that the spectra in the extended database had been acquired with the mass spectrometer used in the present study; however, they had been generated using, for each species, at least one isolate different from those investigated in this paper. Correct identifications are in bold.
[b] *Pichia cactophila* is genetically closely related to *Candida inconspicua* (14, 16).
[c] *Debaryomyces hansenii* is the teleomorph of *Candida famata*.

fication for the most common yeasts isolated in clinical settings. As a matter of fact, the 78 isolates (50.3% of *Candida*-related isolates) belonging to the most common *Candida* species with potentially different susceptibilities to fluconazole (*C. albicans*, *C. glabrata*, and *C. krusei*) were correctly identified by both systems, except for one *C. glabrata* isolate not reliably identified by VMS. Second, VMS is more prone to misidentification than BMS. As an example, its database was not able to distinguish the recently identified *C. metapsilosis* and *C. orthopsilosis* within the so-called *C. parapsilosis* complex, species with different propensities for biofilm production (19, 20). A similar behavior was also observed in relation to *Cryptococcus gattii* misidentified by VMS as *C. neoformans*, two species whose prompt differentiation may allow more accurate treatment choices (21, 22). Importantly, all misidentifications, including those regarding rarer species, were reported with a high confidence score, indicating the actual major drawback of VMS. On the other hand, BMS did not give any misidentification but yielded a higher rate of "no reliable identification" results. Finally, this last observation highlights the most important actual advantage of BMS, that is, the possibility of supplementing

its existing database by implementation with spectra of well-characterized isolates.

In conclusion, the more recently introduced VMS system proved to be as accurate as BMS in the identification of the most common medically important yeasts, but on the other hand, it yielded a lower rate of correct identification and a higher rate of major errors for rarer *Candida* and non-*Candida* species due to its nonexpandable database.

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