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Routine Identification of Medical Fungi by the New Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight System with a New Time-Effective Strategy

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We report here a clinical evaluation of the Vitek MS system for rapid fungal identification. A strategy that uses a single deposit without prior protein extraction was utilized to save time and money. Clinical isolates from the Toulouse University hospital were used to evaluate the performance of the Vitek MS compared to that of both routine laboratory techniques and Vitek2. The Vitek MS performed well in the identification of yeasts and *Aspergillus* fungi (93.2% of correct identifications).

Fungal infections can lead to high morbidity and mortality, particularly in immunocompromised patients. An early treatment which considers the fungal species and any potential resistance is required to successfully treat these infections (5, 8, 14). Optimal therapy is also needed to reduce the overall cost of hospital care for patients with a fungal infection (15). Using conventional laboratory techniques, identification of the fungal species responsible for infection can take a long time (2 days to several weeks). It also requires specialized laboratory personnel and is costly. Given these issues, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based systems present an interesting alternative for the routine identification of fungal species. This technique allows accurate species identification (17), takes only a few minutes, and is cost-effective (7). Moreover, the spectrum of identifiable fungal species gradually spreads. While until recently, this technique involved only yeasts, new developments have allowed the use of MALDI-TOF MS for the identification of filamentous fungi, particularly *Aspergillus* species (1, 4).

We report here the clinical evaluation of the new Vitek MS system, a new and original algorithm called "advanced spectra classifier," and version 1 of an MS-identification (ID) database (bioMérieux, SA, Marcy l'Etoile, France) for the identification of yeasts and filamentous fungi.

The protocol typically used with this MALDI-TOF technology to identify fungal species requires an extraction procedure of fungal proteins before the deposit, as recommended with other mass spectrometry systems (2, 7, 18, 20). This procedure is time-consuming, because the colonies must be suspended in microtubes, mixed with various solvents, and centrifuged several times. Even if simplified procedures begin to appear, particularly for filamentous fungi (1, 4), these methods are not widely used for yeasts (19). The strategy of identification used by the Vitek MS system is simplified for both yeasts and filamentous fungi based on a single spot without prior extraction of fungal proteins, as indicated by the manufacturer. This strategy saves time and money and represents a major improvement over conventional and mass spectrometry techniques.

Yeasts isolated for 5 weeks from patients at the Parasitology-Mycology unit of the Toulouse University hospital were prospectively identified by routine laboratory techniques, the Vitek2 system for yeasts (bioMérieux, Saint Louis, MO), and the Vitek MS system. Fungi were isolated from patients from the respiratory, digestive, and genitourinary tracts, from the skin or superficial lesions, and from deep sites (blood, cerebrospinal fluid, and peritoneum).

For yeasts, when the three individual identification techniques were in concordance, the isolate was identified as the consensus species. When discordant results were obtained, definitive species identification was determined by sequencing of the rDNA region. Sequencing of the rDNA region was performed in an ABI Prism 3100 (Applied Biosystems) using fungus-specific universal primers to amplify the internal transcribed spacer 1 (ITS1) region. If the ITS1 region was not discriminating, the ITS2 or the large-subunit (28S) ribosomal gene region was sequenced (10, 13). The PCR sequences were analyzed by comparing them to sequences found in the GenBank database. For yeast and yeast-like identifications, we restricted the study to include only the first 60 isolates of *Candida albicans* to limit the proportion of this species and increase the diversity of other fungal species studied. Then, only the non-*albicans* isolates, selected as nongreen colonies on chromogenic CHROMagar Candida medium (Becton Dickinson, Heidelberg, Germany), were included in the study. The Vitek MS was used without prior protein extraction. A portion of one colony isolated from a Sabouraud agar plate (bioMérieux, Marcy l'Etoile, France) was applied directly onto the Vitek MS disposable target (single deposit) and was lysed with 0.5 μl of 25% formic acid. After drying completely at room temperature (1 to 2 min), 1 μl of ready-to-use α-cyano-4-hydroxycinnamic acid (CHCA) matrix (bioMérieux, Marcy l'Etoile) was applied to the spot, which was allowed to dry completely again (1 min). The Vitek2 system was used for yeast identification according to the manu-

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TABLE 1 Performance of Vitek MS for the identification of clinical fungal isolates present in the database

Species present in the Vitek MS database	No. of isolates (% of total isolates)	No. (%) of isolates with each outcome		
		Correct identification	Not contributive[b]	Error
Yeasts[a]	188 (83.9)	184 (97.9)	4 (2.1)	0 (0)
Aspergillus	36 (16.1)	36 (100)	0 (0)	0 (0)
Total	224 (100)	220 (98.2)	4 (1.8)	0 (0)

[a] Yeasts and yeast-like fungi.
[b] Includes the "no identification" and "low discrimination" results.

facturer's instructions. Routine identification techniques were performed as follows: *C. albicans* was identified using CHROMagar chromogenic medium associated with the formation of chlamydospores on potato/carrot/bile agar (Bio-Rad, Marnes-La-Coquette, France), *Candida dubliniensis* and *Candida krusei* were identified by the rapid latex agglutination test (Fumouze Diagnostics, Levallois-Perret, France), and all other yeasts were identified by ID32 C (bioMérieux, la Balme, France).

Aspergillus spp. were isolated for 10 weeks from patients at the Parasitology-Mycology unit. *Aspergillus* isolates were identified by routine laboratory tests using macroscopic and microscopic morphological criteria and by the Vitek MS system. The Vitek MS analysis was also performed for these filamentous fungi on a single spot without prior protein extraction according to a more simplified protocol that excluded the formic acid lysis. The ready-to-use CHCA matrix was applied directly after the deposit under laminar flow to avoid contamination. For *Aspergillus* species, the reference identification was the sequencing, which was performed in an ABI Prism 3100 (Applied Biosystems) using fungus-specific universal primers to amplify the β-tubulin region (Bt2a-Bt2b) according to Glass et al. (9). If the β-tubulin region was not discriminating, the ITS1, the ITS2, or the large-subunit (28S) ribosomal gene region was sequenced (10, 13). The PCR sequences were analyzed by comparing them to sequences found in the GenBank and CBS databases.

For all the fungi, analysis was performed on the Vitek MS instrument in a positive linear mode. Species identification was conducted using a new spectrum classifier algorithm and spectral database (MS-ID version 1). The results obtained using the Vitek MS were compared to those of conventional identification methods: routine laboratory fungal identification for all the fungi and Vitek2 for yeasts and yeast-like fungi.

Two hundred thirty-six isolates, representing 27 species of fungi, were analyzed.

With all species included, the Vitek MS performed as well as conventional methods (no significant differences) in the identification of fungal isolates. A total of 93.2% of the isolates were correctly identified by Vitek MS, compared to 94.1% and 88.0% by routine laboratory identification methods and by Vitek2, respectively.

Considering only the species present in the Vitek MS database, 98.2% of the total yeasts had a correct identification, including 97.9% of yeasts and 100% of *Aspergillus* species (Table 1).

Out of the 192 (81.4%) yeast and yeast-like isolates, 184 (95.8%) had correct species identification by Vitek MS, compared to 183 (95.3%) correct species identifications by the routine laboratory identification tests and 169 (88.0%) by Vitek2 (Table 2).

Out of the 44 sequenced *Aspergillus* isolates, 36 (81.8%) had correct species identification by Vitek MS and 39 (88.6%) had correct identification by routine laboratory morphological identification.

These performances are comparable to those reported in previous studies (2, 7, 18, 20) despite the use of a single deposit without prior protein extraction. This study is the first to present such excellent identification performance with a method of extraction which saves time and money. Indeed, in a recent publication using the Microflex system and Biotyper database (Bruker), the authors tried to simplify the extraction procedure for yeast but the identification performance (log scores) was lower than that with the standard procedure. Indeed, 17.2% of the isolates showed a log score of below 1.7, the reliability cutoff recommended by Bruker Daltonics (19).

In our study, failure of identification (low discrimination or no identification) occurred in 7.8% and 6.4% of cases for Vitek2 and Vitek MS, respectively. Failure of identification with the Vitek MS was principally due to the restricted number of *Aspergillus* species present in the current database (*A. fumigatus*, *A. flavus*, *A. niger*, *A. versicolor*). Nevertheless, none of the species absent from the database were misidentified, showing the good specificity of the method. Wrong species identification (a major error) occurred in 1 isolate (0.4%) for the Vitek MS (*Candida palmioleophila* misidentified as *Candida haemulonii*), compared to more than 4% with conventional identification methods. However, this error can be explained by the fact that *Candida palmioleophila* was not in the database. Generally, the Vitek MS has the advantage of giving a "no identification" response rather than making an erroneous identification. Comparing the performance of each technique by species, *C. albicans*, *C. dubliniensis*, *Candida glabrata*, *C. krusei*, *Candida parapsilosis*, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* were correctly identified by all 3 techniques. All *Candida tropicalis*, *Candida inconspicua*, *Candida guilliermondii*, *Candida kefyr*, and *Candida lusitaniae* isolates were correctly identified by Vitek MS but not by ID32 C (62.5% and 60% correct identification for *C. inconspicua* and *C. lusitaniae*, respectively) or Vitek2 (0% to 75% correct identification for *C. tropicalis*, *C. inconspicua*, *C. guilliermondii*, and *C. kefyr*). Thus, all common yeast isolates were perfectly identified by Vitek MS with far fewer misidentifications than conventional identification methods (Table 2). In particular, *C. inconspicua*/*Candida norvegensis*, which are difficult to distinguish by conventional methods, were correctly identified by Vitek MS. Rare yeast isolates (*Candida nivariensis*, *C. palmioleophila*, *Candida orthopsilosis*) were not identified by Vitek MS or by the conventional techniques, but it is worth noting that these species are not present in the MS-ID database. *Candida palmioleophila* is often misidentified in clinical diagnosis (6, 11), and *Candida nivariensis* is a clinically emerging pathogenic fungus. *C. orthopsilosis*, newly recognized as a member of the *C. parapsilosis* complex, is frequently incorrectly identified by conventional methods as *C. parapsilosis* sensu stricto. Two studies show that *C. orthopsilosis*/*Candida metapsilosis* and *C. nivariensis* can be identified by MALDI-TOF (16, 18). Nevertheless, in the two cases, the original manufacturer's spectral database library was secondarily modified to include several rare yeast isolates. For the clinical routine diagnosis, it is of interest to further develop the Vitek MS database to include these new species, considering that these fungi can possess dif-

TABLE 2 Identification of clinical fungal isolates using the Vitek MS, the Vitek2, and routine laboratory fungal identification

Species	No. of isolates	% of total isolates	No. (%) of isolates with each outcome							
			Routine identification tests		Vitek2[i]			Vitek MS		
			Correct identification	Error	Correct identification	Not contributive[c]	Error	Correct identification	Not contributive[c]	Error
Candida albicans	60	25.4	60 (100)		60 (100)			59 (98.3)	1 (1.7)[d]	
Candida parapsilosis	46	19.5	46 (100)		44 (95.7)	1 (2.2)[d]	1 (2.2)[d]	45 (97.8)	1 (2.2)[d]	
Candida glabrata	31	13.1	31 (100)		31 (100)			31 (100)		
Candida krusei	10	4.2	10 (100)		10 (100)			10 (100)		
Candida dubliniensis	1	0.4	1 (100)		1 (100)			1 (100)		
Candida tropicalis	8	3.4	8 (100)		3 (37.5)	5 (62.5)[d]		8 (100)		
Candida kefyr	4	1.7	4 (100)		3 (75)	1 (25)[d]		4 (100)		
Candida lusitaniae	8	3.4	5 (62.5)	3 (37.5)[d]	8 (100)			8 (100)		
Candida guilliermondii	4	1.7	4 (100)			3 (75)[d]	1 (25)[d]	4 (100)		
Candida inconspicua	5	2.1	3 (60)	2 (40)[d]	2 (40)	2 (40)[d]	1 (20)[d]	5 (100)		
Candida nivariensis	1	0.4		1 (100)[b]			1 (100)[b]		1 (100)[b]	
Candida norvegensis	1	0.4	1 (100)				1 (100)[b]	1 (100)		
Candida palmioleophila	1	0.4		1 (100)[b]			1 (100)[b]			1 (100)[b]
Candida orthopsilosis	2	0.8		2 (100)[b]			2 (100)[b]		2 (100)[b]	
Geotrichum candidum	2	0.8	2 (100)[a]			2 (100)[d]			2 (100)[d]	
Geotrichum capitatum	3	1.3	3 (100)		2 (66.7)	1 (33.3)[d]		3 (100)		
Cryptococcus neoformans	1	0.4	1 (100)		1 (100)			1 (100)		
Saccharomyces cerevisiae	4	1.7	4 (100)		4 (100)			4 (100)		
All yeasts[e]	192	81.4	183 (95.3)	9 (4.7)	169 (88.0)	15 (7.8)	8 (4.2)	184 (95.8)	7 (3.7)	1 (0.5)
Aspergillus flavus	2	0.8	2 (100)		NA	NA	NA	2 (100)		
Aspergillus fumigatus	33	14.0	33 (100)		NA	NA	NA	33 (100)		
Aspergillus niger	1	0.4	1 (100)		NA	NA	NA	1 (100)		
Aspergillus sydowi	2	0.8		2 (100)[f]	NA	NA	NA		2 (100)[b]	
Aspergillus terreus	1	0.4	1 (100)		NA	NA	NA		1 (100)[b]	
Aspergillus tubingensis	1	0.4		1 (100)[g]	NA	NA	NA		1 (100)[b]	
Aspergillus calidoustus	1	0.4		1 (100)[h]	NA	NA	NA		1 (100)[b]	
Aspergillus nidulans	2	0.8	2 (100)		NA	NA	NA		2 (100)[b]	
Aspergillus puniceus	1	0.4		1 (100)[h]	NA	NA	NA		1 (100)[b]	
All *Aspergillus* spp.	44	18.6	39 (88.6)	5 (11.4)	NA	NA	NA	36 (81.8)	8 (18.2)	0 (0)
Total	236	100.0	222 (94.1)	14 (5.9)	169 (88.0)	15 (7.8)	8 (4.2)	220 (93.2)	15 (6.4)	1 (0.4)

[a] Genus level only (ID 32C).
[b] Not in database.
[c] Includes the "no identification" and "low discrimination" results.
[d] Present in the database.
[e] Yeasts and yeast-like fungi.
[f] Morphologically confused with *Aspergillus versicolor*.
[g] Morphologically confused with *Aspergillus niger*.
[h] Morphologically confused with *Aspergillus ustus*.
[i] NA, not applicable.

ferences in antifungal susceptibilities, particularly in their resistance to azoles (3, 11, 12).

Although present in the MS-ID database, *Geotrichum candidum* was not identified by the Vitek MS system, possibly because of the difficulties of spotting due to the compact mycelium of *Geotrichum* spp.

Few studies to evaluate the routine identification by Vitek MS have included filamentous fungi. All the *Aspergillus* isolates included in the database were correctly identified by Vitek MS when compared to DNA sequencing results. The direct-spotting method without extraction or lysis was easy to use. No cross-contamination in the spot was observed, perhaps because of the use of the laminar flow and the deposit of CHCA matrix directly after the fungus on the spot, allowing the trapping of the spore. Nevertheless, to protect the operator and as recommended for the routine morphological identification, dimorphic fungi (*Coccidioides* spp., *Histoplasma* spp., etc.) will not be treated by this method if these species are suspected in the sample. If the database is expanded, the Vitek MS appears promising for the identification of molds in clinical routine diagnosis and may represent an important advance in the diagnosis of mycosis due to filamentous fungi.

In conclusion, the new Vitek MS system, utilizing a strategy of a single deposit without prior protein extraction, has an excellent performance profile for the identification of a large panel of yeasts and for *Aspergillus* fungi. It represents a major improvement in terms of time and money saving. Nevertheless, continuously updating the database is necessary to extend the identification of all filamentous fungi and of emerging pathogenic fungal species.

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