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McMullen, Allison R.; Wallace, Meghan A.; Pincus, David H.; Wilkey, Kathy; and Burnham, Carey-Ann, ,"Evaluation of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for identification of clinically relevant filamentous fungi." Journal of Clinical Microbiology.54,8. 2068-2073. (2016).
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AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Evaluation of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of Clinically Relevant Filamentous Fungi

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Invasive fungal infections have a high rate of morbidity and mortality, and accurate identification is necessary to guide appropriate antifungal therapy. With the increasing incidence of invasive disease attributed to filamentous fungi, rapid and accurate species-level identification of these pathogens is necessary. Traditional methods for identification of filamentous fungi can be slow and may lack resolution. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid and accurate method for identification of bacteria and yeasts, but a paucity of data exists on the performance characteristics of this method for identification of filamentous fungi. The objective of our study was to evaluate the accuracy of the Vitek MS for mold identification. A total of 319 mold isolates representing 43 genera recovered from clinical specimens were evaluated. Of these isolates, 213 (66.8%) were correctly identified using the Vitek MS Knowledge Base, version 3.0 database. When a modified SARAMIS (Spectral Archive and Microbial Identification System) database was used to augment the version 3.0 Knowledge Base, 245 (76.8%) isolates were correctly identified. Unidentified isolates were subcultured for repeat testing; 71/319 (22.3%) remained unidentified. Of the unidentified isolates, 69 were not in the database. Only 3 (0.9%) isolates were misidentified by MALDI-TOF MS (including *Aspergillus amoenus* [*n* = 2] and *Aspergillus calidoustus* [*n* = 1]) although 10 (3.1%) of the original phenotypic identifications were not correct. In addition, this methodology was able to accurately identify 133/144 (93.6%) *Aspergillus* sp. isolates to the species level. MALDI-TOF MS has the potential to expedite mold identification, and misidentifications are rare.

Filamentous fungi are ubiquitous environmental microorganisms that have become increasingly important pathogens, especially in immunocompromised patients. Invasive fungal diseases have a high rate of morbidity and mortality, and accurate identification is necessary to guide appropriate antifungal therapy. Traditionally, the identification of filamentous fungi has required the use of different phenotypic methods in conjunction with macro- and microscopic assessment of the organism (1). This process requires highly trained mycologists, and, in some cases, turnaround time may be prolonged due to a requirement for extended incubation periods, potentially delaying appropriate therapy. Molecular methods, which can provide accurate identification to the species level, can be expensive, require specialized equipment or expertise, and are not commonly available in clinical laboratories.

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been utilized in the clinical microbiology laboratory for rapid and accurate identification of bacteria, mycobacteria, and yeasts (2–9). For filamentous fungi, databases have been limited, and unlike bacteria, filamentous fungi require additional processing steps to disrupt the cell wall, extract proteins, and inactivate the isolate (10). These additional processing steps and limited libraries have led laboratories to develop their own methodologies and databases to overcome these barriers to adoption (11–13). These limitations have hindered widespread implementation of this technology in the clinical laboratory for the identification of filamentous fungi.

Here, our objective was to evaluate the identification of filamentous fungi by the Vitek MS system with the Knowledge Base version 3.0 database and SpectraIdentifier R2.1.0 software that is currently in development. This updated library contains 82 unique species from 31 different genera of filamentous fungi.

(This work was presented in part as a poster at the 114th General Meeting of the American Society for Microbiology, Boston, MA, 17 to 20 May 2014 [14]).

MATERIALS AND METHODS

Clinical isolates. A total of 319 isolates of filamentous fungi representing 43 genera recovered from clinical samples submitted to the Barnes Jewish Hospital microbiology laboratory were analyzed; these isolates were recovered between 2008 and 2014. Isolates were identified using standard-of-care phenotypic characterization in the Barnes Jewish Hospital clinical microbiology laboratory. For MALDI-TOF MS analysis, all isolates were cultivated on Sabouraud dextrose agar (Remel, Lenexa, KS). Rapidly growing species were tested after 2 to 3 days of incubation, and slowly growing species were tested after 7 to 10 days of incubation at room temperature.

Received 15 April 2016 **Returned for modification** 5 May 2016
Accepted 19 May 2016
Accepted manuscript posted online 25 May 2016
Citation McMullen AR, Wallace MA, Pincus DH, Wilkey K, Burnham C-AD. 2016. Evaluation of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for identification of clinically relevant filamentous fungi. J Clin Microbiol 54:2068–2073. doi:10.1128/JCM.00825-16.
Editor: D. W. Warnock
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Mold inactivation and extraction. Isolates were processed according to the inactivation/extraction protocol for molds and Vitek MS identification (bioMérieux, Hazelwood, MO). In brief, hyphae and/or conidia were collected with a moistened sterile cotton swab (Puritan Medial Products, Guilford, ME) from a 1- to 2-cm diameter of growth and inoculated into 900 μl of 70% ethanol (Sigma-Aldrich, St. Louis, MO) in a microcentrifuge tube. The suspension was vortexed for 3 to 5 s and centrifuged at 10,000 × *g* for 2 min. The supernatant was discarded, and the pellet was resuspended with 40 μl of 70% formic acid (Sigma-Aldrich), followed by the addition of 40 μl of acetonitrile (Sigma-Aldrich). The suspension was vortexed for 3 to 5 s and centrifuged again at 10,000 × *g* for 2 min. One microliter of supernatant was transferred to the target slide. Once the sample was dry, 1 μl of matrix (α-cyano-4-hydroxy-cinnamic acid; bioMérieux) was applied and allowed to dry prior to analysis. For each isolate, a single extraction was performed and spotted in duplicate. An *Escherichia coli* reference strain (ATCC 8739) was directly spotted and overlaid with matrix on each acquisition group on each slide as a calibrant and quality control per the manufacturer's protocols.

MALDI-TOF MS analysis. Isolates were tested using the Vitek MS system. Spectra obtained from the Vitek MS were analyzed using the Knowledge Base, version 3.0 database and SpectraIdentifier R2.1.0 software. For data analysis, any Vitek MS identification and score reported were acceptable. If no identification was obtained, the isolate was repeated. Repeat testing consisted of resubbing the isolate, incubating it for the desired length of time, and repeating the inactivation and extraction procedure. If an isolate did not yield identification upon repeat testing, it was labeled as "no identification."

Evaluating spectra. Isolates that remained unidentified upon repeat of the MALDI-TOF MS analysis were further analyzed using the SARAMIS (Spectral Archive and Microbial Identification System) database. Isolates that were related to isolates previously identified or that had been sent for sequencing were compared to spectra in the database. Isolate spectra were grouped together in a taxonomy tree using the SARAMIS, version 4.12, software/analysis tool. Isolates that clustered with a relative taxonomy of approximately ≥65% were considered related at the species level. Outliers were removed from the taxonomy tree, and a superspectrum was created with the remaining isolate spectra. Superspectra added to the database were used for future isolate identification.

Sequencing. Isolates that had discrepant phenotypic identification compared to the MALDI-TOF MS identification and unidentified isolates were sent to the Fungus Testing Laboratory at the University of Texas Health Science Center (San Antonio, TX). Gene targets for sequencing were chosen based on the presumptive species identification by morphological characteristics. For the majority of the isolates, the internal transcribed spacer region 1/2 (ITS1/2) was sequenced; this was augmented with D1/D2 (domain of 26S), beta-tubulin (TUB), elongation factor (TEF), actin (ACT), or glyceraldehyde-3-phosphate dehydrogenase (GPD), as appropriate for the taxa being evaluated.

RESULTS

Correct identifications by Vitek MS. A total of 319 isolates of filamentous fungi representing 43 genera were evaluated in this study. Out of those isolates, 213 (66.8%) were correctly identified using the Vitek MS Knowledge Base version 3.0 database and SpectraIdentifier R2.1.0 software (Table 1). An additional 32 isolates were identified after analyses with our modified SARAMIS database, for a total of 245 (76.8%) correct identifications. Eighty-six percent (125/144) of *Aspergillus* species isolates were initially identified, and eight more were correctly identified upon further analyses with the SARAMIS software; 98.9% of *Aspergillus flavus* and *Aspergillus fumigatus*, two common fungal pathogens within this genus, were correctly identified (93/94 isolates). MALDI-TOF MS was able to provide species-level identification for 13 *Aspergillus* species isolates which were identified only to the genus level using morphological characterization in the clinical laboratory (Table 2). Four isolates required additional analyses with the SARAMIS software. Only 17/26 (65%) *Fusarium* species isolates were identified. For the *Mucorales*, only one out of the three *Mucor* species isolates tested was identified and required the additional SARAMIS analysis. Two-thirds (6/9) of the *Rhizopus* species isolates were correctly identified using the Knowledge Base version 3.0 database. The database also performed well for the correct identification of the dimorphic fungi isolates, with 100% of the *Blastomyces dermatitidis* ($n = 4$) and *Histoplasma capsulatum* ($n = 2$) isolates identified. Of the dermatophytes tested, 100% of the *Trichophyton* sp. ($n = 12$) and *Microsporum* sp. ($n = 2$) isolates were correctly identified.

Misidentifications by Vitek MS. There were no misidentifications at genus level by the Vitek MS. At the species level, three isolates were misidentified. Two isolates of *Aspergillus amoenus* were identified as *Aspergillus versicolor*, and one isolate of *Aspergillus calidoustus* was identified as *Aspergillus ustus*.

Unidentified by Vitek MS. A total of 103 isolates were not identified by Vitek MS using the Knowledge Base version 3.0 database (Table 1). When these isolates were further analyzed using the modified SARAMIS database, the number of unidentified isolates was reduced to 71. These isolates were sequenced, and of the 71 unidentified isolates, only two isolates, *A. fumigatus* and *Rhizopus microsporus*, were included in the database.

Phenotypic identification errors. For isolates that were identified by phenotype and by Vitek MS, 10 discrepancies were observed (Table 3). This included isolates that were phenotypically identified as *A. fumigatus*, *Paecilomyces* spp., *Menispora* spp., and *Chrysosporium* spp. Sequencing results demonstrated that the Vitek MS system correctly identified all 10 isolates.

DISCUSSION

To our knowledge, this study represents the most comprehensive evaluation of the Vitek MS and Knowledge Base version 3.0 library for the identification of filamentous fungi. The majority of published studies examining large study sets of filamentous fungi utilize the Bruker Biotyper MALDI-TOF MS and the associated library and/or laboratory-developed libraries (10, 11, 15–18). Prior studies have evaluated the accuracy of MALDI-TOF MS for the identification of only a few genera or groups of organisms, such as *Aspergillus* spp. or the dermatophytes (12, 13, 19–26). In our study, which examined 319 isolates from 43 different genera, the Vitek MS version 3.0 library was able to correctly identify 66.8% of isolates and 76.8% of the isolates after additional SARAMIS analysis.

Seventy-one isolates (22.3%) were not identified using the Vitek MS or the SARAMIS database although only two of the unidentified isolates were included in the database and could have potentially been identified. These results are similar to those of other studies with augmented databases, and our study challenges the system with a larger number of genera in addition to an updated database and analysis software. Of note, in this investigation, there were no misidentifications at the genus level, and only three isolates were incorrectly identified at the species level. Two isolates, which were determined to be *A. versicolor* using sequencing, were identified as the closely related species *A. amoenus* by MALDI-TOF analyses. *A. amoenus* and *A. versicolor* are both members of the section *Versicolores*, and *A. versicolor* has been shown to cause onychomycosis and, rarely, pneumonia (27–29). A third isolate, *A. calidoustus*, was incorrectly identified as *A. ustus*

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TABLE 1 Performance of the Vitek MS version 3.0 library for the identification of filamentous fungi

Organism	No. (%) of isolates				
	Total	Identified by Knowledge Base version 3.0	Identified by Knowledge Base version 3.0 plus modified SARAMIS database	Unidentified	Misidentified
Acrodontium salmoneum	1	0 (0.0)	0 (0.0)	1	0
Alternaria spp.	11	10 (90.9)	11 (100)	0	0
Aspergillus spp.	144	125 (86.8)	133 (93.6)	8	3
A. amoenus	2	0 (0.0)	0 (0.0)	0	2
A. calidoustus	1	0 (0.0)	0 (0.0)	0	1
A. carneus	1	0 (0.0)	0 (0.0)	1	0
A. chevalieri	1	0 (0.0)	0 (0.0)	1	0
A. clavatus	2	0 (0.0)	1 (50.0)	1	0
A. flavus	31	28 (90.3)	31 (100.0)	0	0
A. fumigatus	63	61 (96.8)	62 (98.9)	1	0
A. lentulus	2	2 (100.0)	2 (100.0)	0	0
A. nidulans	1	1 (100.0)	1 (100.0)	0	0
A. niger	22	19 (86.3)	22 (100)	0	0
A. sydowii	5	5 (100.0)	5 (100.0)	0	0
A. tennesseensis	1	0 (0.0)	0 (0.0)	1	0
A. terreus	8	8 (100.0)	8 (100.0)	0	0
A. unguis	1	1 (100.0)	1 (100.0)	0	0
A. westerdijkiae	3	0 (0.0)	0 (0.0)	3	0
Beauveria spp.	1	0 (0.0)	0 (0.0)	1	0
Bipolaris spp.	2	2 (100.0)	2 (100.0)	0	0
Blastomyces dermatitidis	4	4 (100.0)	4 (100.0)	0	0
Chaetomium spp.	1	0 (0.0)	0 (0.0)	1	0
Chrysosporium spp.	2	0 (0.0)	1 (50.0)	1	0
Cladosporium spp.	12	2 (16.7)	10 (83.3)	2	0
Coniochaeta velutina	1	0 (0.0)	0 (0.0)	1	0
Coprinellus radians	1	0 (0.0)	0 (0.0)	1	0
Coprinopsis cinerea (*Hormographiella*)	1	0 (0.0)	0 (0.0)	1	0
Curvularia spp.	5	0 (0.0)	3 (60.0)	2	0
Engyodontium album	1	0 (0.0)	0 (0.0)	1	0
Eutypella scoparia	3	3 (100.0)	3 (100.0)	0	0
Exophiala spp.	2	0 (0.0)	0 (0.0)	2	0
Fusarium spp.	26	17 (65.4)	17 (65.4)	9	0
Ganoderma resinaceum	1	0 (0.0)	0 (0.0)	1	0
Histoplasma capsulatum	2	2 (100.0)	2 (100.0)	0	0
Irpex lacteus	1	0 (0.0)	0 (0.0)	1	0
Libertella spp.	4	0 (0.0)	2 (50.0)	2	0
Malbranchea filamentosa	1	0 (0.0)	0 (0.0)	1	0
Microsporum spp.	2	2 (100.0)	2 (100.0)	0	0
Mucor spp.	3	0 (0.0)	1 (33.3)	2	0
Myrmecridium schulzeri	2	0 (0.0)	0 (0.0)	2	0
Neurospora sitophila	1	0 (0.0)	0 (0.0)	1	0
Nodulisporium spp.	1	0 (0.0)	0 (0.0)	1	0
Oxyporus corticola	1	0 (0.0)	0 (0.0)	1	0
Paecilomyces spp.	4	4 (100.0)	4 (100.0)	0	0
Paraconiothyrium brasiliense	1	0 (0.0)	0 (0.0)	1	0
Penicillium spp.	20	12 (60.0)	12 (60.0)	8	0
Phialemonium obovatum	1	0 (0.0)	0 (0.0)	1	0
Phialophora americana	1	0 (0.0)	0 (0.0)	1	0
Pithomyces chartarum	3	0 (0.0)	0 (0.0)	3	0
Pseudallescheria boydii	10	9 (90.0)	10 (100.0)	0	0
Rasamsonia argillacea complex	4	3 (75.0)	4 (100.0)	0	0
Rhizopus spp.	9	6 (66.7)	6 (66.7)	3	0
Scopulariopsis spp.	6	0 (0.0)	4 (66.7)	2	0
Talaromyces spp.	2	0 (0.0)	0 (0.0)	2	0
Trametes spp.	5	0 (0.0)	2 (40.0)	3	0
Trichoderma spp.	3	0 (0.0)	0 (0.0)	3	0
Trichophyton spp.	12	12 (100.0)	12 (100.0)	0	0
T. interdigitale	5	5 (100.0)	5 (100.0)	0	0
T. rubrum	2	2 (100.0)	2 (100.0)	0	0
T. tonsurans	5	5 (100.0)	5 (100.0)	0	0
Xylomelasma sordida	1	0 (0.0)	0 (0.0)	1	0
Total	319	213 (66.8)	245 (76.8)	71 (22.3)	3 (0.9)

by MALDI-TOF MS. These two species are closely related, and previous studies have shown that most, if not all, clinical isolates of *A. calidoustus* were previously misidentified as *A. ustus*. The ability to correctly identify *A. calidoustus* in the clinical lab is important due to its resistance to multiple antifungals, including azoles (30, 31). However, the misidentifications encountered in this investigation are unlikely to be clinically significant as a result of the similarity of these species.

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TABLE 2 Species-level identification of *Aspergillus* sp. isolates by MALDI-TOF MS

Aspergillus spp. identified by MALDI-TOF MS	Identified only to genus level by clinical lab (no. of isolates)
A. flavus/A. oryzae	2
A. fumigatus	2
A. nidulans	1
A. sydowii	4
A. terreus	3
A. unguis	1

TABLE 3 Discrepant identification between phenotype and MALDI-TOF MS

Phenotypic identification (n)[a]	MALDI-TOF MS identification	Sequencing identification
Aspergillus fumigatus (2)	*Aspergillus lentulus*	*Aspergillus lentulus*
Menispora spp. (2)	*Eutypella scoparia*	*Eutypella scoparia*
Paecilomyces spp. (1)	*Aspergillus sydowii*	*Aspergillus sydowii*
Paecilomyces spp. (4)	*Rasamsonia argillacea* complex	*Rasamsonia argillacea* complex
Chrysosporium spp. (1)	*Pseudallescheria boydii*	*Pseudallescheria boydii*

[a] n, number of isolates.

Identification using MALDI-TOF MS may overcome the difficulties with traditional identification methods, especially with sterile molds and species that are morphologically similar but may differ genetically. Using MALDI-TOF MS, we were able to identify 13 different *Aspergillus* spp. that were identified only to the genus level using routine phenotypic identification in the clinical laboratory. Additionally, our study demonstrated that there were 10 isolates incorrectly identified using traditional phenotypic methods. This represents just over 3% of all isolates examined in this study. Two isolates identified as *A. fumigatus* were confirmed to be *Aspergillus lentulus* by both MALDI-TOF MS analyses and sequencing. *A. lentulus* was previously considered to be a variant of *A. fumigatus*, but additional studies suggest that this is a unique species (26, 32). *A. lentulus* has been shown to cause invasive aspergillosis and has increased azole, amphotericin B, and caspofungin MICs, and this differentiation is clinically important (33–35). Another study examining the ability of the Vitek MS system to differentiate species of *Aspergillus* showed that the research-use-only (RUO) system is able to accurately identify the closely related species of *A. fumigatus* and *A. lentulus* (26).

Other phenotypic misidentifications include five isolates originally identified as *Paecilomyces* spp. but identified *as Aspergillus sydowii* (one isolate) and *Rasamsonia argillacea* complex (four isolates) by MALDI-TOF MS. Members of the *R. argillacea* complex, previously known as *Geosmithia* spp., are commonly misidentified as *Paecilomyces* or *Penicillium* spp. and are a known, yet rare, cause of human infection in immunocompromised hosts. *R. argillacea* has been identified as a colonizing organism in cystic fibrosis patients and as a cause of pulmonary infections. It is resistant to voriconazole, with variable resistance to itraconazole and amphotericin B (36). Two isolates identified as *Menispora* spp. were determined to be *Eutypella scoparia* and one isolate identified phenotypically as *Chrysosporium* spp. was identified as *Pseudallescheria boydii* by both MALDI-TOF MS and sequencing. All four of these organisms are environmental molds and are typically considered contaminants except for *P. boydii*, which has been implicated in a variety of illnesses in immunocompromised patients and in near-drowning victims (37). Accurate identification of this organism is important due to its decreased susceptibility to many antifungals, including some azoles, amphotericin B, and micafungin (38).

MALDI-TOF MS for the identification of molds presents unique challenges not encountered with other organism types, such as bacteria and yeasts. As a result of the unique properties of the filamentous fungi, such as robust cell walls and lower growth rates, there have been many barriers to the development of robust methods for MALDI-TOF MS identification. To date, a major limitation of MALDI-TOF MS for mold identification has been the breadth of commercially available databases. Despite this, a meta-analysis examining the use of MALDI-TOF MS for the identification of yeasts and filamentous fungi showed that while the analytical performance characteristics are not as desirable as for the yeasts, the method is able to provide accurate and rapid mold identification (17). Of note, investigations using expanded laboratory-developed databases have returned a much higher rate of mold identification (11, 15). Studies examining MALDI-TOF MS for the identification of filamentous fungi have used different MALDI-TOF MS systems, databases (both manufacturer developed and laboratory developed), and extraction protocols. This makes it difficult to directly compare the results from different studies. That said, our study demonstrated a challenge with *Fusarium* sp. identification, even with the additional SARAMIS analyses. *Fusarium* spp. are important pathogens, especially in immunocompromised hosts, and accurate and rapid diagnosis is important. This challenge has not been reported in prior publications although earlier studies have evaluated different systems and/or hardware (10, 11, 15, 39).

For the *Mucorales*, another group of clinically important pathogens, our study identified only 70% of the isolates; this is consistent with previous literature, where the rates of correct identifications for *Mucor* spp. and *Rhizopus* spp. range from 66.7% to 100% (10, 11, 15, 39). Previous studies have shown that MALDI-TOF MS is a rapid and accurate method for the identification of dermatophytes. There have been many studies examining this group of molds, with identification rates of >90% for these studies (12, 13, 20–22). Our study correctly identified 100% (14/14) of the dermatophytes tested. Finally, there have been relatively few studies examining dimorphic fungi. For our study, we correctly identified the two *H. capsulatum* and four *B. dermatitidis* isolates tested.

The strength of this study is the evaluation of a large number of unique isolates using an updated Vitek MS database. However, this study is not without its limitations, including the facts that the isolates evaluated are from a single medical center and that some important species were underrepresented due to lack of available isolates, especially the thermally dimorphic fungi. In addition, 32 of the isolates were identified using the SARAMIS database; the ability of a laboratory to fully utilize an augmented database with the SARAMIS database and superspectra will vary, depending on the access of the laboratory to a bank of well-curated fungal isolates for comparison.

In summary, the Vitek MS and Knowledge Base version 3.0 library are able to accurately identify filamentous fungi isolated

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from clinical specimens. While nearly one-fourth of the isolates tested were not found in the database, the addition of the SARAMIS software allows the user to easily analyze and add new spectra to the database. This feature will be a tremendous benefit for laboratories with large collections of clinical isolates. Additional studies examining the effect of expedited mold identification on patient outcomes are needed.

ACKNOWLEDGMENTS

We thank the Barnes Jewish Hospital Clinical Mycology laboratory for assistance with isolate storage.

Supplies for this study were provided by bioMérieux. C.-A.D.B. received research support from bioMérieux. D.H.P. and K.W. are employees of bioMérieux.

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