Evaluation of the VITEK Mass Spectrometry System for Rapid Identification of Medically Important Yeasts and Molds

1Shamma Shetye, 2Pratiksha Chheda, 3Shraddha Amberkar, 4Ganesh Madhu, 5Usha Mukundan

ABSTRACT

Background: Rapid identification of fungi and molds reduce turnaround time and cost for diagnosis of infections with these organisms in a clinical microbiology laboratory. We report here the clinical evaluation of the VITEK mass spectrometry system for rapid fungal identification in comparison to the internal transcribed spacer (ITS) DNA polymorphism method.

Methods: Total 136 archived isolates comprising 126 yeast and 10 molds were analyzed by mass spectrometry (VITEK system) and ITS sequencing for identification of fungi.

Results: Majority of the yeast isolates belonged to genus Candida (N = 123), followed by one isolate each of Trichosporon, Cryptococcus, and Rhodotorula. Amongst molds, Aspergillus (N = 4), Trichophyton (N = 3), Fusarium (N = 2) and Rhizopus (N = 1) were identified. Overall, correct species-level identification was obtained in 135/136 (99.26%) isolates with a single isolate of Candida auris misidentified as Candida haemulonii by VITEK MS.

Conclusion: The VITEK MS system, a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) system, is a reliable and rapid method for the identification of most of the fungi. Further expansion of the database of the VITEK MS for emerging pathogens is needed to enhance its performance.

Keywords: Candida auris, Candida haemulonii, Internal transcribed spacer, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry.


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Conflict of interest: None

INTRODUCTION

There has been an increase in the incidence of invasive fungal infections (IFIs), specifically C. albicans and invasive molds such as Aspergillus species.1 The high rate of morbidity and mortality due to fungal infections is seen in immunosuppressed patients with solid-organ and hematopoietic stem cell transplants.2

Rapid identification of fungal species helps in early successful therapy by choosing appropriate therapeutic options and avoiding the use of potentially toxic antifungal agents.3 This approach benefits the patient in terms of improved clinical outcome at reduced costs.3,4 There has been a significant development in laboratory diagnosis of fungal agents due to automated biochemical methods as well as molecular methods. Though these methods have enhanced the ability to identify different pathogenic species, there exist concerns related to cost, turnaround time and expertise.5 In rare situations the phenotypic method may not successfully identify less common fungal species. Molecular techniques such as deoxyribonucleic acid (DNA) sequencing are highly accurate but costlier and time consuming.6

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has recently been introduced for rapid and accurate identification of bacteria, mycobacteria, and yeasts and is now routinely used in the clinical microbiology laboratory.4,7-12 MALDI-TOF MS-based microbial identification relies on the generation of the unique organism-specific mass spectrum or “protein fingerprint” that is examined against a reference database to provide organism identification. The VITEK MS system (bioMérieux, Marcy l’Etoile, France) and Microflex LT Biotyper (Bruker Daltonics, Bremen, Germany) are commercially available systems that have been studied extensively.13,14

The protocol for MALDI-TOF MS-based identification is quite different for different groups of organisms such as gram-positive or gram-negative bacteria, mycobacteria, or fungi. The sample preparation methods vary between yeasts and, and certain mold genera are even more challenging. Though extensively used for bacterial identification, there are limited studies on MALDI-TOF MS-based identification of fungal isolates. The objective of this study was therefore to evaluate the performance of the VITEK MS MALDI-TOF mass spectrometer (bioMérieux, Marcy l’Etoile, France).
eux) in conjunction with the VITEK MS v 2.0 database in comparison with ITS DNA polymorphism for the identification of yeasts and molds isolated in the diagnostic clinical microbiology laboratory.

MATERIALS AND METHODS

A total of 136 archived isolates (126 yeasts and 10 molds) were subjected to MALDI-TOF MS analysis and ITS sequencing. These isolates were previously identified using standard phenotypic methods including VITEK 2 identification system (bioMérieux) and archived in sterile distilled water over a period of one year. The isolates were cultured on Sabouraud dextrose agar for 48–96 hours at 30°C before MALDI-TOF MS analysis. The study was carried out at Metropolis Healthcare Ltd. from Jan ’2017 to June ’2017.

Identification by VITEK MS

The yeast isolates were prepared for mass spectrometric analysis on target slide (bioMérieux) composed of a polypropylene carrier using the protocol described earlier. A small portion of a single fungal colony was picked up using a 1 µL loop and layered on to the slide, followed by application of 0.5 µL formic acid (bioMérieux). It was allowed to dry for about 2–3 minutes at room temperature. This was followed by application of 1 µL of cyano-4-hydroxycinnamic acid (CHCA) matrix solution (bioMérieux). Sixteen samples were prepared on different slots of the same slide. After drying, the slide was processed for mass spectrometric analysis on VITEK MS acquisition station using Myla v 2.4 middleware software.

For molds, a wet swab was used to take a small piece (1 cm²) from Sabouraud dextrose agar plate, suspended in a 1.5 mL microcentrifuge tube containing 500 µL 70% ethanol and vortexed. The tube was centrifuged at 3000 g for 2 minutes. The supernatant was discarded and 40 µL of 70% formic acid was added to the pellet and vortexed. This was followed by addition of 40 µL of 100% acetonitrile. The tube was vortexed and centrifuged at 3000 g for 2 minutes. One µL of the supernatant was added to the target slide and was allowed to dry at room temperature. This was followed by application of 1 µL CHCA matrix on the sample spot and dried at room temperature. The slide was analyzed using Myla v2.4 middleware software on VITEK MS platform.

Molecular Identification by Inferior Temporal Sulcus Sequencing

The molecular identification of all isolates in the test collection was carried out by sequencing of ITS. Briefly, DNA was extracted and purified directly from single fungal colonies with the DNA Min Kit (Qiagen). PCR was set up using primers ITS1 (5′-TCC GGA GAA CCT TGC GG-3′) and ITS4 (5′-TCC TCG TAT GA TAT GC-3′) as described earlier. Amplification of the ITS region was carried out using the following conditions: denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s, and elongation at 72°C for 60 s, with a final extension step of 10 min at 72°C. The resultant PCR product (300 bp to 880 bp) (Fig.1) was sequenced and the obtained sequences were compared to ITS sequences that are found in public database: NCBI (https://blast.ncbi.nlm.nih.gov/). The identity of each isolate was determined by sequence similarity of the ITS regions, specifically using those results with 97% similarity and 99% coverage and the species showing maximum score was considered.

Ethics

This study was conducted as part of clinical laboratory test validation on archived fungal isolates and not on patients or patient material. Hence, ethical committee approval was not required.

RESULTS

We analyzed 136 fungal isolates comprising of 126 yeast and 10 molds by VITEK MS and DNA sequencing of ITS region. 8 genera and 21 different species were identified (Table 1). Majority of the yeast isolates belonged to genus Candida (N = 123), followed by one isolate each of Trichosporon, Cryptococcus, and Rhodotorula. Out of 126 yeast isolates, ITS region sequencing revealed identical results in 125 (99.20%). One isolate identified as C. haemulonii, turned out to be C. auris on molecular typing. This isolate was further evaluated on VITEK 2 system for confirmation and it was identified as C. auris confirming the findings by DNA sequencing. Thus VITEK MS failed to correctly identify this medically important species.

Fig. 1: Gel image for ITS PCR (300–800 bp), M-100 bp ladder, 1: C. albicans, 2: C. krusei, 3: C. tropicalis, 4: C. parapsilosis, 5: C. guilliermondii, 6: R. mucilaginosa, 7: A. niger, 8: C. neoformans, 9: C. glabrata, 10: C. keyfr, 11: A. flavus
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**Table 1: Correlation between MALDI-TOF MS (VITEK MS) and Molecular (ITS Sequencing) identification of yeasts and molds**

<table>
<thead>
<tr>
<th>Identification by VITEK MS</th>
<th>Identification by ITS sequencing</th>
<th>Correct Identification No. (%)</th>
<th>Misidentification Isolate No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>Candida albicans</td>
<td>73 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>Candida tropicalis</td>
<td>14 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>Candida glabrata</td>
<td>12 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida kefyr</td>
<td>Candida kefyr</td>
<td>7 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>Candida krusei</td>
<td>7 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida guilliermondii</td>
<td>Candida guilliermondii</td>
<td>4 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>Candida parapsilosis</td>
<td>2 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida zeylanoides</td>
<td>Candida zeylanoides</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida inconspicua</td>
<td>Candida inconspicua</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>Candida lusitaniae</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Candida haemulonii</td>
<td>Candida auris</td>
<td>0 (0%)</td>
<td>1 (100%)*</td>
</tr>
<tr>
<td>Trichosporon asahii</td>
<td>Trichosporon asahii</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Rhodotorula mucilaginosa</td>
<td>Rhodotorula mucilaginosa</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>Cryptococcus neoformans</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Aspergillus niger</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus sydowii</td>
<td>Aspergillus sydowii</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>Aspergillus flavus</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>Aspergillus versicolor</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>Trichophyton mentagrophytes</td>
<td>3 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>Fusarium solani</td>
<td>2 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>Rhizopus homothallicus</td>
<td>Rhizopus homothallicus</td>
<td>1 (100%)</td>
<td>-</td>
</tr>
</tbody>
</table>

*Isolate misidentified as Candida haemulonii

C. albicans (N = 73) was the most common pathogen detected, followed by C. tropicalis (N = 14) and C. glabrata (N = 12).

Successful mass spectra were generated for all the 10 mold isolates and there was a 100% correlation with DNA sequencing data. Aspergillus (N = 4), Trichophyton (N = 3), Fusarium (N = 2) and Rhizopus (N = 1) were different molds that were identified. Overall, VITEK MS could correctly identify, up to species level, 135 out of 136 (99.26%) fungal isolates that were archived in the laboratory.

**DISCUSSION**

The main objective of a diagnostic laboratory is to deliver accurate and clinically useful results in the shortest possible time. For fungal identification, though microscopy offers quick presumptive diagnosis, growth on culture media, followed by biochemical or morphological identification is necessary for confirmatory diagnosis. Molecular methods, like DNA sequencing, though gold standard for the identification of fungi at the species level, are expensive and require specialized equipment and expertise and are not commonly available in clinical laboratories. When species belonging to the same genus have different antifungal susceptibility profiles, a reliable species-level identification of the isolate is crucial for therapeutic decision-making. MALDI-TOF MS offers a balance between speed, cost, and accuracy for fungal identification in clinical settings. The procedures for preanalytic processing of organisms and analysis by MALDI-TOF MS are technically simple and reproducible and commercial databases, and interpretive algorithms are available for the identification of a wide spectrum of clinically significant organisms. The platform has been successfully employed in clinical microbiology laboratories to identify bacterial pathogens and yeasts, but not for identification of molds. Recent progress in extraction protocols and composition of comparative libraries, support potential application of MALDI-TOF MS for mold identification in clinical microbiology laboratories.

MALDI-TOF technology patented by VITEK® examines the patterns of proteins detected directly from intact bacteria or lysed yeast and mold preparations. The sample to be analyzed is mixed with another compound, called a matrix, applied to a metal plate and irradiated with a laser. The matrix absorbs the laser light and vaporizes, along with the sample, in the process gaining an electrical charge (ionization). Electric fields then guide the ions into the time of flight mass spectrometer, which separates them according to their mass to charge (m/z) ratio, and ultimately the quantity of each ion is measured. Detection is achieved at the end of the flight tube. VITEK® MS contains a comprehensive IVD-CE marked database for bacteria and fungi, including mycobacteria, Nocardia and molds.

For molecular identification of fungal species, we chose to sequence Internal Transcribed Spacers 1 and 2 (ITS 1 and ITS 2 regions) since it has been successfully used for identification of medically important yeast and Aspergillus species in previous studies. The ITS region
has been more reliable in comparison to the large-subunit RNA gene (D1-D2 region) for the identification of closely related *Aspergillus* species.\(^8\)

In this study on 136 fungal isolates (126 yeast and 10 molds) showed 99.26% concordance of results between mass spectrometry and molecular-based identification. All the medically important *Candida* species, such as *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. guilliermondii*, *C. krusei*, *C. glabrata*, and *C. kofyr* were all successfully and accurately identified by VITEK MS. However, *C. auris*, a multidrug-resistant yeast was mistyped as *C. haemulonii* on MALDI-TOF MS (IVD) analysis. The same isolate was identified as *C. auris* on ITS sequencing as well as on VITEK 2 v.8.01 system. Several studies published recently report that *C. auris* in routine microbiology laboratories remains under-reported, as 90% of the isolates characterized by commercial biochemical identification systems are misidentified primarily because of a lack of the yeast in their databases.\(^{19-25}\) A comprehensive study from India investigated 102 clinical isolates previously identified as *C. haemulonii* or *C. famata* by VITEK 2 system and found that 88.2% of the isolates were *C. auris* on ITS sequencing.\(^22\) There is another study from India where a total of 125 clinical fungal culture isolates (yeasts and filamentous fungi) were studied and all 88 yeast isolates were correctly identified by MALDI-TOF/MS. *C. auris* was however, not amongst the study isolates.\(^26\) Previous database of VITEK 2 lacked *C. auris* strain; hence all the strains were misidentified as *C. haemulonii*, but *C. auris* was identified by Bruker’s MALDI Biotyper Microbial Identification system.\(^22\)

In a multicentric study, out of 852 yeast isolates, 24 (2.8%) were not identified and 5 (0.6%) were misidentified in comparison to molecular typing of D2 region of the 26S rRNA gene.\(^4\) Since the majority of the yeast isolates were identified up to species level (96.1%), authors conclude that MS is superior to the phenotypic identification systems.\(^4\) A study by Iriart et al. reported that none of the *Aspergillus* species absent from the database were misidentified, showing the good specificity of the method.\(^2\) Incorrect species identification on VITEK MS was observed for an isolate of *Candida palmioleophila* which was misidentified as *Candida haemulonii*.\(^2\) It is to be noted that it is possible to use an updated research-use-only (RUO) library or database, which can also be updated in-house. Also, the profile for identification of *C. auris* is built into the upcoming IVD database of VITEK MS, as confirmed by the manufacturer (bioMérieux, Marcy l’Etoile, France).

Regarding molecular analysis, the phylogenetically closely-related species sometimes cannot be identified correctly by sequence analysis ITS.\(^18\) However, we did not come across any such problem, probably due to limited sample size.

**CONCLUSION**

This study highlights the usefulness of MALDI-TOF MS (VITEK system) as a good alternative to conventional methods for rapid identification of yeasts and molds in clinical settings so that appropriate therapy can be instituted at the earliest. It is also cost-effective. However, a larger number of general encompassing several species of fungi by MALDI TOF-MS and molecular typing should be added to their database.

**REFERENCES**


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