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Advantages of Using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry as a Rapid Diagnostic Tool for Identification of Yeasts and Mycobacteria in the Clinical Microbiological Laboratory

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Yeast and mycobacteria can cause infections in immunocompromised patients and normal hosts. The rapid identification of these organisms can significantly improve patient care. There has been an increasing number of studies on using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for rapid yeast and mycobacterial identifications. However, studies on direct comparisons between the Bruker Biotyper and bioMérieux Vitek MS systems for the identification of yeast and mycobacteria have been limited. This study compared the performance of the two systems in their identification of 98 yeast and 102 mycobacteria isolates. Among the 98 yeast isolates, both systems generated species-level identifications in >70% of the specimens, of which *Candida albicans* was the most commonly cultured species. At a genus-level identification, the Biotyper system identified more isolates than the Vitek MS system for *Candida* (75/78 [96.2%]versus 68/78 [87.2%], respectively; $P = 0.0426$) and non-*Candida* yeasts (18/20 [90.0%]versus 7/20 [35.0%], respectively; $P = 0.0008$). For mycobacterial identification, the Biotyper system generated reliable identifications for 89 (87.3%) and 64 (62.8%) clinical isolates at the genus and species levels, respectively, from solid culture media, whereas the Vitek MS system did not generate any reliable identification. The MS method differentiated 12/21 clinical species, despite the fact that no differentiation between *Mycobacterium abscessus* and *Mycobacterium chelonae* was found by using 16S rRNA gene sequencing. In summary, the MALDI-TOF MS method provides short turnaround times and a standardized working protocol for the identification of yeast and mycobacteria. Our study demonstrates that MALDI-TOF MS is suitable as a first-line test for the identification of yeast and mycobacteria in clinical laboratories.

Yeast and nontuberculosis mycobacteria (NTM) are widely present in the environment. Some of them are potential pathogens causing infections not only in immunocompromised patients but also in normal hosts (1, 2). In general, a number of NTM are slowly growing organisms, and laboratory diagnosis usually takes >5 days. This slow diagnostic time limits patient care.

Yeast infections can cause significant mortality among critically ill and immunocompromised patients (3, 4). Although *C. albicans* has been found to be the most commonly identified pathogenic yeast species, other *Candida* species or yeast-like fungi, such as *Pichia*, *Cryptococcus*, *Saccharomyces*, and *Trichosporon* species, have also been reported to cause infection (5, 6). The rapid identification of pathogenic or intrinsically resistant species can be used to narrow therapy options, which might prevent treatment with potentially toxic antifungal agents, thus reducing negative clinical outcomes and costs.

Using the conventional phenotypic methods, some yeast species cannot be clearly differentiated. Molecular methods, such as the 18S rRNA gene or the internal transcribed spacer (ITS) region sequencing, can generate accurate species-level identification for many yeast isolates; however, these methods are time-consuming and technically demanding, and they are not readily available in diagnostic microbiological laboratories (7, 8).

Mycobacterium is another group of pathogens that can cause a wide spectrum of pulmonary and extrapulmonary infections. The antimicrobial resistance of some species influences the choice of treatment for these infections. Therefore, species-level identification for differentiating tuberculosis-causing mycobacteria and NTM is important for epidemiological, public health, and therapeutic reasons. Conventionally, the identification of mycobacteria has relied on well-established phenotypic methods, which assess morphological features, growth rates, preferred growth temperature, pigmentation, and biochemical profiles. However, as these tests are laborious and time-consuming, many clinical mycobacteriology laboratories still encounter the challenge of providing unequivocal identifications of *Mycobacterium* species (9). Recently, molecular methods, including PCR sequencing (10) and PCR hybridization (11), have become the new gold standards for mycobacterial identification. Although these methods are highly specific and might shorten the length of diagnostic procedures, a number of medically important *Mycobacterium* species, such as the *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex, *Mycobacterium abscessus*, and *Mycobacterium chelonae* cannot be easily differentiated by 16S rRNA gene sequencing due

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to the high similarity of their rRNA gene sequences (12). The sequencing of other genomic regions is necessary for complete genotyping; however, this requires specific technical expertise and operating costs that are unaffordable to clinical diagnostic laboratories.

According to the limitations encountered with the currently available methods for identifying yeast and mycobacteria, an alternative strategy may become necessary for clinical laboratories to consider in order to overcome this problem. The use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) for the rapid identification of clinical yeast and mycobacterial isolates has been evaluated in some studies (13–25). However, there has been a limited number of direct comparison studies focusing on the performance of the two MALDI-TOF systems to rapidly identify yeast and mycobacterial clinical isolates (26–28).

In this study, we evaluated the performance of two MALDI-TOF MS systems, the Bruker Microflex LT Biotyper system (Bruker Daltonics, Bremen, Germany) and the Vitek MS system (bioMérieux, Marcy l'Etoile, France), for the rapid identification of clinical yeast and mycobacteria to the species level compared to the phenotypic and molecular methods currently used.

MATERIALS AND METHODS

Sample collection. A total of 98 yeast and 102 mycobacterial isolates were collected for this study. Among the 98 yeast isolates, 48 of them were reference strains from different quality assurance programs or old clinical cases. We collected an additional 50 clinical yeast isolates encountered randomly in the routine laboratory workflow over 2 weeks. Different types of specimens, including bronchial aspirate lavage fluid, blood culture, cerebrospinal fluid, ear swabs, endocervical swabs, high vaginal swabs, nails and skin, sputum, and wound swabs, were collected. All the strains were cultured and isolated from selective fungal isolation Sabouraud's dextrose agar for 48 to 96 h at 30°C to ensure purity prior to further testing. For the 102 mycobacterial isolates, 10 isolates were collected from the College of American Pathologists quality assurance panels, and the other 92 isolates were collected from clinical specimens, including sputum, blood culture, bronchial aspirate, body fluid, urine, and wound swabs. Auramine O and Ziehl-Neelsen carbol fuchsin acid-fast stains were used for acid-fast bacilli screening. All mycobacterial strains were cultured on Lowenstein-Jensen (LJ), Stonebrink, and Middlebrook 7H10 solid media with incubations at 31°C, 35°C, and 42°C for 5 to 60 days to ensure purity prior to further testing.

Identification by biochemical and molecular methods. The identification of clinical yeast isolates was first generated by the Vitek II identification system (bioMérieux, Marcy l'Etoile, France). For samples with ambiguous results, one or more tests, including assessment of morphology on Sabouraud's dextrose agar, the germ tube test for *Candida* species, urease assimilation for *Cryptococcus* species, and sequencing of the ITS1-5.8S-ITS2 region, were performed to confirm the Vitek II identification (29).

For clinical identification of mycobacteria, sequencing of the 16S rRNA gene (10) or the *hsp65* gene (30) was performed on positive cultures. Photoreactivity and biochemical tests, including nitrate reduction, arylsulphatase production, pigment production, and 5% NaCl tolerance results were used to further identify isolates that could not be differentiated by the molecular method mentioned above (12).

Identification by MALDI-TOF MS method. In this study, we used the ethanol-formic acid sample preparation method for the Bruker Biotyper and Vitek MS systems. For protein extraction from yeast isolates, 1 to 3 yeast colonies were suspended into 100 μl high-performance liquid chromatography (HPLC)-grade H_2O (Fluka, USA) and 300 μl absolute ethanol (Sigma-Aldrich, USA). After 5 min of room temperature incubation, the mixture was centrifuged at 13,000 rpm for 2 min. Subsequently, the supernatant was carefully removed and the pellet was air dried for 30 min. The pellet was then resuspended into 30 to 50 μl formic acid (Fluka, USA) with 5 min incubation for the general yeast strains. For yeast colonies that were isolated from cerebrospinal fluid specimens or were macroscopically mucoid, the formic acid incubation was prolonged to 15 min in order to completely destroy the suspected *Cryptococcus* organisms. An equal volume of acetonitrile (Fluka, USA) was then added, followed by centrifugation at 13,000 rpm for 2 min. The supernatant was then spotted on target slides in duplicate for MALDI-TOF analysis.

For protein extraction from mycobacterial isolates, a previously published protocol was used, with a few modifications (22). Briefly, one loopful of colony from Lowenstein-Jensen or Middlebrook 7H10 medium was suspended into 300 μl HPLC-grade H_2O (Fluka, USA) inside a biosafety class 2 cabinet. Next, 900 μl absolute ethanol was added with vortexing. After incubation at room temperature for 10 min, the suspension was centrifuged at 13,000 rpm for 2 min and the supernatant was removed. The pellet was washed and suspended into 500 μl H_2O followed by centrifugation at 13,000 rpm for another 2 min. The supernatant was then removed and the pellet was resuspended into 50 μl H_2O. Heat inactivation was followed by incubation of the tube at 95°C for 30 min. After incubation, the samples were cooled to room temperature and 1.2 ml precooled absolute ethanol (Sigma-Aldrich, USA) was added. The mixture was centrifuged at 13,000 rpm for 2 min and the supernatant was removed. The residual ethanol was further removed by an extra centrifugation step at 13,000 rpm for 2 min and by pipetting. The pellet was air dried for 10 min. A small spatula amount of 0.5-mm silica beads (BioSpec Products, USA) and about 10 to 50 μl of acetonitrile (Fluka, USA) were added to the tube. The tube was vortexed vigorously at maximum speed for 2 to 5 min. About 10 to 50 μl 70% formic acid was added and vortexed for 5 s. Finally, the tube was centrifuged at 13,000 rpm for 2 min. The supernatant was then spotted on target slides in duplicate for MALDI-TOF analysis.

Bruker Microflex LT with Biotyper 3.0 system. One microliter of each purified extract was transferred to an individual spot on the Bruker 96-spot reusable stainless steel target plate. Each spot was covered with 1 μl alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics, Germany). The target plate was then read and analyzed by the Bruker Microflex LT system. The protein profile of each specimen with an *m/z* of 3,000 to 15,000 was generated based on a minimum 240 laser shot measurements. The profiles were further analyzed by using the Biotyper version 3.0 software (Bruker Daltonics, Germany), which queried a CE-IVD database of >2,000 species and the Mycobacteria and Filamentous Fungus Library (version 1.0), which includes 90 different species of mycobacteria and >100 different species of fungi. The software returned the top 10 identification matches along with confidence scores ranging from 0.0 to 3.0. We reached a conclusion with the results by using the top-scoring identification. Scores of ≥2.0 were considered high-confidence identifications at the species level, while scores of 1.7 to 1.99 were considered intermediate confidence at a genus-level identification only. Scores of <1.7 were considered to be unreliable identifications, according to the manufacturer's recommendation.

bioMérieux Vitek MS IVD system. One microliter of each extracted supernatant was transferred to an individual spot on the 48-well Vitek MS-DS disposable target slide. Each spot was covered with 1 μl ready-to-use Vitek MS HCCA matrix (bioMérieux, Marcy l'Etoile, France). The target plate was then read and analyzed by the Vitek MS *in vitro* diagnostic (IVD) system. The protein profile of each specimen with an *m/z* of 3,000 to 15,000 was generated based on 100 measurements. The profiles were further matched with the Vitek MS reference CE-IVD certified database (version 2.0), which includes >1,400 species (10 *Mycobacterium* species and >90 species of fungi) and returned the best identification match, along with a confidence percentage from 0% to 99.9%. The spots with results of 90 to 98% confidence were considered to be high-confidence identifications at the genus level, while confidence results of >98% were

TABLE 1 Identification results of yeast isolates by Biotyper and Vitek MS IVD ($n = 98$)

Yeast/fungal species	No. of isolates	MALDI-TOF MS results (no. [%]) using[a]: Biotyper plus fungus RUO database: Unreliable ID[b] (score, <1.6)	Biotyper plus fungus RUO database: Genus-level ID only (score, 1.6–1.99)	Biotyper plus fungus RUO database: Species-level ID (≥2.0)	Vitek MS IVD: Unreliable ID[b] (<90.0%)	Vitek MS IVD: Correct ID (90.0–97.9%)	Vitek MS IVD: Correct ID (≥98.0%)
Candida spp.							
C. albicans	24	0 (0)	2 (8.3)	22 (91.7)	1 (4.2)	0 (0)	23 (95.8)
C. boidinii	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
C. dubliniensis	3	0 (0)	1 (33.3)	2 (66.7)	3 (100)	0 (0)	0 (0)
C. glabrata	5	0 (0)	0 (0)	5 (100)	0 (0)	1 (20.0)	4 (80.0)
C. guilliermondii	2	0 (0)	1 (50.0)	1 (50.0)	0 (0)	0 (0)	2 (100)
C. kefyr (*Kluyveromyces marxianus*)	3	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)
C. krusei (*Issatchenkia orientalis*)	3	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)
C. lipolytica	2	0 (0)	1 (50.0)	1 (50.0)	0 (0)	0 (0)	2 (100)
C. magnoliae	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
C. norvegensis	4	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)
C. parapsilosis	18	0 (0)	5 (27.8)	13 (72.2)	4 (22.2)	1 (5.6)	13 (72.2)
C. pararugosa	1	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
C. rugosa	2	1 (50)	1 (50)	0 (0)	1 (50)	0 (0)	1 (50)
C. tropicalis	7	0 (0)	0 (0)	7 (100)	0 (0)	0 (0)	7 (100)
C. (*Clavispora*) *lusitaniae*	2	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	2 (100)
Subtotal	78	3 (3.8)	12 (15.4)	63 (80.8)	10 (12.8)	2 (2.6)	66 (84.6)
Other yeast and yeast-like fungi							
Blastoschizomyces capitatus	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Cryptococcus humicola (*Trichosporon mucoides*)	2	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)
Cryptococcus neoformans	7	1 (14.3)	1 (14.3)	5 (71.4)	6 (85.7)	0 (0)	1 (14.3)
Galactomyces candidum	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Pichia anomala (*Candida pelliculosa*)	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Pichia ohmeri	2	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
Pseudozyma parantarctica	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Saccharomyces cerevisiae	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Trichosporon asahii	2	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)
Trichosporon dermatis	2	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
Subtotal	20	2 (10.0)	8 (40.0)	10 (50.0)	13 (65.0)	0 (0)	7 (35.0)
Total	98	5 (5.1)	20 (20.4)	73 (74.5)	23 (23.5)	2 (2.0)	73 (74.5)

[a] RUO, research use only; ID, identification.
[b] Includes the no identification and low discrimination results.

considered to be high-confidence identifications at the species level. For spots with confidence results of <90.0%, these were considered to be unacceptable identifications. For specimens showing multiple identification results that were from the same genus, we concluded that to be a genus-level match only. For specimens showing multiple identification results that included some results with mismatched genus or family identifications, we concluded that these were unreliable identification.

Statistical methods. Pearson's chi-square test and Fisher's exact test were used to compare the results obtained by the two systems with the same specimens.

RESULTS

In total, all 98 yeast and 102 mycobacterial isolates in this study were cultured and identified. The phenotypic and molecular methods required an average of 4 working days for the identification of the yeast isolates, whereas the identification of the mycobacterial isolates required 14 days to complete (starting from the time the culture was available for testing). In comparison, the MALDI-TOF MS method took an average of 3 h to complete a species-level identification for most of the yeast and mycobacterial isolates.

Among the 98 yeast isolates, 78 of them (78.0%) were found to be *Candida* species (15 different species) and 22 of them (22.0%) were found to belong to other yeast-like fungi (11 different species) (Table 1). Through the use of MALDI-TOF MS for identification, 73 out of 100 (73.0%) samples overall were correctly identified at the species level by the Biotyper and Vitek MS systems in concordance with the phenotypic and molecular method results. *Candida* was found to be the most commonly found genus in the collection. At the genus-level identification, the Biotyper system was observed to have a significantly better identification rate among *Candida* strains than the Vitek MS system (Biotyper, 75/78 [96.2%]; Vitek MS, 68/78 [87.2%]; $P = 0.0426$). The same significant observation was also found with other yeast-like fungi (Biotyper, 18/22 [81.8%]; Vitek MS, 7/22 [31.8%]; $P = 0.0008$). At the species-level identification, the two systems demonstrated a similar identification rate (Biotyper, 64/78 [82.1%]; Vitek MS, 67/78 [85.9%]). *C. albicans* (24/78 [30.8%]) was found to be the most commonly cultured *Candida* species, while the Biotyper and Vitek MS systems generated species-level identifications for 22/24 (91.7%) and 23/24 (95.8%) of the *C. albicans* isolates. Among the 15 different *Candida* species included in this cohort, Biotyper and

TABLE 2 Identification results of mycobacterial isolates by Bruker Biotyper system ($n = 102$)

Mycobacterium sp.	No. of isolates	MALDI-TOF MS Biotyper results (no. [%])[a]			
		Unreliable ID		Genus-level ID only	Species-level ID
		No ID generated	Incorrect ID (ID generated)		
M. avium complex[b]	10	0	0	3 (30)	7 (70)
M. arupense	2	0	0	0	2 (100)
M. abscessus	18	0	0	3 (16.7)	15 (83.3)
M. chelonae	1	0	0	0 (0)	1 (100)
M. fortuitum	6	0	0	0	6 (100)
M. gordonae	9	0	0	0	9 (100)
M. gastri/kansasii	20	0	0	9 (45)	11 (55)
M. lentiflavum	4	0	0	0	4 (100)
M. marinum	4	0	0	1 (25)	3 (75)
M. mucogenicum	1	0	0	1 (100)	0
M. neoaurum	3	0	0	1 (33.3)	2 (66.7)
M. nebraskense[c]	1	0	1 (100) (*M. avium*)	0	0
M. novocastrense	1	0	1 (100) (*M. austroafricanum*)	0	0
M. paraffinicum[c]	4	1 (25)	3 (75) (*M. bovis BCG*, *M. avium*, *M. intracellulare*)	0	0
M. parascrofulaceum	8	1 (12.5)	3 (37.5) (*M. avium*, *M. scrofulaceum*)	4 (50)	0
M. scrofulaceum	1	0	0	1 (100)	0
M. simiae	2	2 (100)	0	0	0
M. szulgai	1	0	0	1 (100)	0
M. tuberculosis complex[d]	4	0	0	0	4 (100)
M. tusciae[c]	1	0	1 (100) (*M. vaccae*)	0	0
M. xenopi	1	0	0	0	1 (100)
Total	102	4 (3.9)	9 (8.8)	25 (24.5)	64 (62.8)

[a] ID, identification.
[b] *Mycobacterium avium* complex includes *M. avium*, *M. intracellulare*, *M. chimaera*, *M. colombiense*, *M. vulneris*, *M. marseillense*, *M. bouchedurhonense*, and *M. timonense*.
[c] Species not included in the database.
[d] *M. tuberculosis complex* includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. canettii*, and *M. pinnipedii*.

Vitek MS generated species-level identifications for 12 and 13 different species, respectively. For non-*albicans Candida* species, *Candida dubliniensis* and *Candida pararugosa* were differentiated by the Biotyper system but not the Vitek MS system. On the other hand, *Candida boidinii*, *Candida magnoliae*, and *Candida rugosa* were differentiated by the Vitek MS only. For the other 22 yeast-like fungi, 11 different species were identified. Six out of the 11 species, including *Blastoschizomyces capitatus*, *Cryptococcus humicola*, *Galactomyces candidum*, *Pichia anomala*, *Pseudozyma parantarctica*, and *Trichosporon dermatis*, required the use of a molecular method to confirm their identities because of ambiguous Vitek II results. The Biotyper and Vitek MS systems generated species-level identifications in 5 and 6 species, respectively. *Cryptococcus neoformans*, *P. anomala*, *Saccharomyces cerevisiae*, and *Trichosporon asahii* were identified by the two systems. *C. humicola* was identified by the Biotyper system only, while *B. capitatus* and *G. candidum* were identified by the Vitek MS system only. We found that other rarely identified species, such as *Pichia ohmeri*, *Prototheca wickerhamii*, *P. parantarctica*, and *T. dermatis*, were not identified by either system.

For the 102 mycobacterial isolates, 20 different species were differentiated by the biochemical and molecular methods (Table 2). The MALDI-TOF MS method only identified 11 different species. The Biotyper system correctly identified 89/102 (87.3%) isolates at the genus level, whereas only 64 of them (62.8%) were identified to the species level. Instead, the Vitek MS system failed to generate any identification for all 102 isolates growing on the solid-phase Lowenstein-Jensen (LJ) and Middlebrook 7H10 media. Individual mass peaks were observed in most of the spectra generated from the Vitek MS system. The identification results of the colonies extracted from the LJ and Middlebrook 7H10 media were compared. However, no differences were observed for the identification results between the isolates extracted from the two media.

Among the 11 different *Mycobacterium* species that were identified by the Biotyper system, *M. tuberculosis* complex was successfully differentiated from other NTM species, including 8 slowly growing mycobacteria (*M. avium* complex, *Mycobacterium arupense*, *Mycobacterium gordonae*, *Mycobacterium gastri*, *Mycobacterium kansasii*, *Mycobacterium lentiflavum*, *Mycobacterium marinum*, and *Mycobacterium xenopi*) and 4 rapidly growing mycobacteria (*M. abscessus*, *M. chelonae*, *Mycobacterium fortuitum*, and *Mycobacterium neoaurum*), at the species level. However, this method did not differentiate the species among the *M. tuberculosis* and *M. avium* complexes. For *Mycobacterium* species, such as *M. abscessus* and *M. chelonae*, which were not easily distinguished by 16S rRNA gene sequencing, the MALDI-TOF MS method differentiated them clearly. For other NTM, including *Mycobacterium nebraskense*, *Mycobacterium novocastrense*, *Mycobacterium paraffinicum*, *Mycobacterium simiae*, and *Mycobacterium tusciae*, the Biotyper system did not generate reliable identifications. In this study, the *M. nebraskense*, *M. novocastrense*, and *M. tusciae* species had been misidentified as *M. avium*, *Mycobacterium austroafricanum*, and *Mycobacterium vaccae*, respectively. For *M. paraffinicum*, 1 out of 4 strains were not identified and the other 3 strains were misidentified as *Mycobacterium bovis* BCG, *M.*

avium, or *M. intracellulare*. Among the 8 *Mycobacterium parascrofulaceum* strains, 4 were not identified at the genus level and 1 strain was not identified. The other 3 strains were misidentified as *M. avium* or *Mycobacterium scrofulaceum*.

DISCUSSION

MALDI-TOF MS has been proven to be a promising tool for the rapid identification of clinical bacterial isolates. With the introduction of more protein spectra of organisms into the identification database and the development of a refined methodology, this technology can now be used for the rapid identification of other slowly growing organisms, such as mycobacteria and fungi.

Phenotypic and molecular methods versus MALDI-TOF MS. Our study demonstrated that use of phenotypic and molecular tests (including a series of biochemical tests or ITS/16S rRNA gene sequencing) generated more yeast (phenotypic and molecular, 25 species, versus MS, 22 species) and mycobacterium (phenotypic and molecular, 22 species, versus MS, 11 species) species-level identifications than the use of the MALDI-TOF MS method alone. However, the MALDI-TOF MS method required only 1 to 2 simple testing protocols and provided objective results for the identification of nearly all yeast isolates and half of the mycobacteria isolates. This method can help in the standardization of laboratory testing, which is critical for strengthening laboratory services and systems in limited-resource settings.

In our study, the species-level identification rates for *Candida* species for the two MS systems were comparable to those in recent studies (25, 27). However, the identification rate for non-*Candida* yeasts was lower than that in another study performed in Europe (27). This may be due to the differences in the protein extraction steps used in the two studies, and further investigations should be focused on the proper extraction method needed for non-*Candida* yeasts.

Also, with the subjective determination of some phenotypic tests, such as the germ tube test for rapid yeast identification, *Candida tropicalis* or *C. dubliniensis* might be misinterpreted as *C. albicans* due to the presence of structures similar to the germ tube chlamydospores in *C. tropicalis* and *C. dubliniensis* (31–33). Through our study, we found that *C. albicans* was easily differentiated from *C. tropicalis* and *C. dubliniensis* by using the MALDI-TOF MS method. The turnaround time used for differentiating these *Candida* species was found to be around 3 h, which was much shorter than the germ tube analysis or ITS molecular method, which take 1 to 2 days.

For mycobacterial identification, the species-level identification rate in our study (62.8%) was much lower than the rate demonstrated in another study published in the United States (93.8%) (17). As our study tested a wide collection of species, including a number of rare NTM (e.g., *M. novocastrense*, *M. parascrofulaceum*, and *M. tusciae*), which the U.S. study did not include, it is therefore reasonable that the species-level identification rate was lower in our study.

Although 16S rRNA gene sequencing has generally been used for the routine identification of NTM, there are still some medically important NTM, such as *M. abscessus* and *M. chelonae* species, which were not differentiated using this method (34). Extra working days for the comparison of their antimicrobial susceptibility patterns are needed to differentiate these two species (35). In concordance with other studies, the MALDI-TOF MS method can be used to clearly differentiate these two species (17). For some NTM (*M. nebraskense*, *M. paraffinicum*, and *M. tusciae*) for which the Bruker Biotyper system generated incorrect identifications, we found that these species were not covered by the spectrum database of the Bruker identification system. This should be the major reason that these species were not identified correctly by the Bruker system. The other misidentified *Mycobacterium* species (*M. novocastrense* and *M. parascrofulaceum*) included in this study were not mentioned in other similar studies (17, 24, 36). These identification failures may be due to the highly conserved ribosomal protein among these species and also their limited reference spectra in the Bruker spectrum database.

On the other hand, this study demonstrated that culture from either LJ or Middlebrook 7H10 solid medium can be used for MALDI-TOF MS analysis. This was also confirmed by another study with a similar approach (17). However, from our practical experience, it is more convenient to use culture from Middlebrook 7H10 agar for MS analysis, as the colonies that grow on it can be easily picked out for sample preparation.

For species in the *M. tuberculosis* and *M. avium* complexes, we found that the 16S rRNA gene sequencing and MALDI-TOF MS methods could not generate species-level identification, showing that the MALDI-TOF MS method does not currently show an advantage at the subcomplex level of differentiation.

Other than identification rate, the turnaround time is also important. The use of the ethanol-formic acid extraction method provided a definitive identification for the majority of mycobacterium and yeast isolates within 3 h, whereas the Vitek II semiautomated rapid identification system required >24 h for biochemical reaction incubation. Our study results were similar to other studies that confirmed that the identification of yeast and mycobacteria by the MALDI-TOF MS method can significantly shorten the turnaround time by 24 by 72 h compared to the use of phenotypic tests or sequencing (20, 22).

Bruker Biotyper versus bioMérieux Vitek MS. In comparison to previous studies evaluating the performance of MALDI-TOF MS on yeast species-level identification (20, 21), our study demonstrated that the Biotyper (73.0%) and Vitek MS (75.0%) systems achieved a high level of species-level identification, especially for *Candida* species (Biotyper, 81.0%, versus Vitek MS, 86.1%). For other yeast-like fungi, both systems only identified <50% of the strains in the cohort, while the Biotyper system (47.6%) was observed to give slightly better performance than the Vitek MS (33.3%). The largest discrepancy between the two systems was found for *C. neoformans* identification, for which the Biotyper system gave a slightly higher percentage of species-level identification than the Vitek MS system. Through this study, we observed that the identification for yeast was either no identification due to the absence of reference spectra for a species or at least genus-level identification on the Biotyper and Vitek MS systems, showing the high specificity of both systems.

A direct-comparison study of the two MALDI-TOF MS systems for mycobacterial identification was limited, and most of the previous studies focused on using the Biotyper system only (24, 36, 37). From our experience, we found that only the Biotyper system generated reliable identification of various *Mycobacterium* species cultured from solid-phase medium like Lowenstein-Jensen or Middlebrook 7H10 medium, whereas the Vitek MS failed. The spectra from Vitek MS were found to have comparable quality to that of the Bruker spectra; however, no identification was generated by the Vitek MS. The actual reason for this is unclear; how-

ever, this may be related to the type of culture medium (such as mycobacterium growth indicator tube [MGIT] culture broth) that was used when the manufacturer developed the Vitek MS mycobacterium spectrum database. These media may contain other nutrient substances that can interfere with the pattern of reference spectra.

In summary, the MALDI-TOF MS method provides a standardized working protocol for the identification of yeast and mycobacteria. The quick turnaround time and expandability of MALDI-TOF MS identification databases demonstrate that it is suitable as a first-line test for the identification of yeast and mycobacteria in the clinical microbiology laboratory.

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