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Advances in Identification of Clinical Yeast Isolates by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based identification is being adopted by clinical laboratories for routine identification of microorganisms. To date, the majority of studies have focused on the performance and optimization of MALDI-TOF MS for the identification of bacterial isolates. We review recent literature describing the use of MALDI-TOF MS for the routine identification of a variety of yeasts and yeast-like isolates. Specific topics include the effect of optimized or streamlined extraction methods, modified scoring thresholds, expanded reference libraries, and the possibility of conducting antifungal susceptibility testing using MALDI-TOF MS.

Infections due to pathogenic yeasts and yeast-like organisms can range from mild irritation of mucosal surfaces to severe, invasive disease. Serious infections due to yeasts increased markedly in the early 1980s, as the use of immunosuppressive therapy following organ and bone marrow transplant and the emergence of HIV/AIDS increased the population vulnerable to these opportunistic pathogens. Mortality resulting from infection with yeasts such as *Candida* and *Cryptococcus* spp. peaked around 1990 in the United States with a rate of 0.5 to 0.6 per 100,000; however, *Candida* spp. continue to be the fourth leading cause of bloodstream infection with an associated crude mortality rate of 39.2% (1–3).

As with any infectious process, timely identification of the infecting organism and antimicrobial susceptibility testing play key roles in successful patient management. A recent population-based surveillance study conducted in two large U.S. cities demonstrated a shift in *Candida* species causing bloodstream infection (BSI). Whereas *Candida albicans* accounted for the majority of cases of candidemia in the early 1990s, the total proportion of candidemia caused by *C. albicans* fell to 38% by 2011 (4). This was accompanied by a rise in the proportion of candidemia caused by other species, including *Candida glabrata* (29%), *Candida parapsilosis* (17%), and *Candida tropicalis* (10%). Additionally, 1 to 2% of candidemia was attributed to *Candida krusei*. The increasing prevalence of species other than *C. albicans* can complicate empirical therapy choices due to the prevalence of antifungal resistance among these species. A 10-year study examining resistance trends in >200,000 isolates found resistance to fluconazole to be 15.7% (15.4 to 19.2%) in *C. glabrata*, 4.1% (3.6 to 4.5%) in *C. tropicalis*, and up to 79.3% (65.8 to 79.3%) in *C. krusei* (5). Of note, 59% of fluconazole-resistant *C. glabrata* isolates were also resistant to voriconazole, while only 9.2% of *C. krusei* isolates were resistant to both drugs. This is in contrast to fluconazole resistance in *C. albicans*, which remains <1.5% (5). In response to the increasing proportion of *Candida* infections by fluconazole-resistant organisms, guidelines put forth by the Infectious Disease Society of America (IDSA) favor the use of echinocandins for empirical therapy for candidemia; however, they recommend transitioning to fluconazole if the identified isolate is likely to be susceptible (6). Of particular interest are infections caused by *C. parapsilosis*, which demonstrate greatly elevated MIC_{50} and MIC_{90} to common echinocandins *in vitro* (4, 7). According to the most recent Clinical and Laboratory Standards Institute (CLSI) guidelines and species-specific breakpoints (8, 9), the majority of isolates with elevated MICs still fall into the "susceptible" category; however, in one case series, 38% of *C. parapsilosis* isolates had MICs to caspofungin that placed them in the "nonsusceptible" category (10). For these reasons, it is recommended that persons infected with *C. parapsilosis* should be closely monitored for clinical response or transitioned to therapy using an azole (6). In addition, although rare, other yeasts or yeast-like organisms, such as *Trichosporon*, *Rhodotorula*, *Saccharomyces*, and *Geotrichum* spp., have rarely been identified as the cause of bloodstream infections in patients with hematologic malignancies (11). Of note, a large percentage of these isolates demonstrate elevated *in vitro* MICs to common antifungal drugs, including fluconazole and caspofungin, although there are not established breakpoints for these organisms (11).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a soft ionization technique that generates a protein-based spectral profile or "fingerprint" that is unique to a given species. Analysis of bacteria can often be accomplished by direct application of the isolate to a target plate along with α-cyano-4-hydroxycinnamic acid or other matrix material. Excitation of the matrix material by a laser catalyzes charge transfer to the analyte and desorption from the target plate, thus producing charged particles of various sizes that are the basis for creation of spectral profiles. This method has been applied for the identification of a wide variety of bacterial isolates with great success. Comparisons of two commercially available instruments, the Bruker Biotyper (Bruker Daltonik, Bremen, Germany) and Vitek MS (bioMérieux, Marcy l'Etoile, France) have demonstrated similar performance for the identification of bacterial isolates. While some studies have found the Bruker Biotyper to provide a higher proportion of "high confidence" identifications (94.4% versus

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88.8%) for all isolates tested and among nonfermenters (97.0% versus 89.5%), other studies have demonstrated better performance of the Vitek MS for identification of other subsets of bacteria, including anaerobes and viridans group streptococci (12–15). Regardless of the system used, the identification rate for routine clinical bacterial isolates using MALDI-TOF approaches 95%, and identification can be completed in less than 10 min and with a substantial savings in cost per identification (16–18). These results make the application of MALDI-TOF MS to the identification of clinical yeast isolates an enticing prospect.

One obstacle to the identification of yeast isolates using MALDI-TOF MS is the presence of a robust cell wall, which inhibits direct analysis of yeasts. Investigators have overcome this difficulty using a variety of preprocessing approaches aimed at liberating intracellular proteins that are the basis for MALDI-TOF MS identification. This minireview examines the current literature regarding the performance of MALDI-TOF MS for the identification of yeast isolates. Various methods for processing yeast isolates prior to MALDI-TOF MS analysis are highlighted. These include methods for analysis of yeast isolates cultured on solid medium and yeast strains isolated directly from positive blood cultures. We also review the impact of improved reference libraries and modified identification score thresholds on the final identification rate. Finally, we examine the prospect for rapid susceptibility testing of yeast isolates using MALDI-TOF MS.

CURRENT METHODS FOR THE IDENTIFICATION OF YEASTS AND YEAST-LIKE ORGANISMS

Current identification methods for yeasts and yeast-like organisms rely heavily on physical characteristics and biochemical properties of the isolate. Simple, rapid tests such as the germ tube test are often used to quickly and presumptively identify *C. albicans*. However, not all *C. albicans* isolates produce germ tubes, and other *Candida* species, most notably *C. tropicalis* and *C. dubliniensis*, can also form similar structures that may be misinterpreted as germ tubes (19). Similarly, a positive rapid assimilation of trehalose (RAT) test can presumptively identify *C. glabrata*, but several species, including *C. tropicalis*, can produce false-positive results if the test is performed incorrectly (20). Because of these limitations, rapid tests often must be confirmed using additional methods. Such methods rely on biochemical and carbohydrate utilization panels that are commercially available in manual (API ID 20C and API ID 32C) and semiautomated (Vitek ID YST and Phoenix Yeast ID) formats (bioMérieux, Marcy l'Etoile, France, and BD, Sparks, MD). These tests correctly identify 90 to 98% of common yeasts but may not identify other less common isolates, reaching only 60% correct identification in some studies (21, 22). Additionally, turnaround time (TAT) for these tests can be 24 to 72 h after the isolation of yeast in pure culture (21, 22). Because of intrinsic or predictable resistance patterns among some species of yeast, a more rapid identification method may be useful to treating physicians when considering antifungal therapy.

More-rapid methods for the identification of yeasts in clinical specimens include chromogenic medium and nucleic acid-based assays. Chromogenic media allow the presumptive identification of yeasts based upon differential utilization of chromogenic substrates added to the medium, resulting in a characteristically colored colony unique to each species. This method can be advantageous, since it enables presumptive identification of yeasts without isolation of colonies from a primary specimen plate. Additionally, chromogenic medium can aid in the identification of mixed-yeast cultures (23, 24). Direct plating of blood cultures containing yeast to chromogenic medium demonstrated no effect on the color of resulting colonies, suggesting that this method may be useful for the identification of yeast in positive blood cultures (23). However, the broad utility of chromogenic medium is limited by two key factors. First, these media generally are capable of discriminating a limited number of yeasts (e.g., *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *Saccharomyces*). Second, multiple species of yeasts can develop similar colors on these media, which can affect specificity (23, 24).

Another method for the rapid identification of yeast is peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH). A number of FDA-cleared assays based on PNA FISH technology for the detection of yeasts are available and have been evaluated (25–27). An advantage of PNA FISH compared to chromogenic medium is that it does not rely on growth of the isolate for presumptive identification. This enables the identification of select species of yeast directly from positive blood cultures in as little as 2 h with 97.5% to 100% sensitivity (25, 26). While more specific than chromogenic medium, PNA FISH is also limited in the number of species that can be identified, and cross-reactivity of probes with other species of yeast has been reported (25).

APPLICATION OF MALDI-TOF MS TO THE IDENTIFICATION OF YEASTS IN THE CLINICAL LABORATORY

Identification of plate-grown isolates (tube-based extraction methods). The first attempts to identify yeast isolates using MALDI-TOF MS were performed using pure isolates cultured on solid medium. This primarily involved culture of yeast on Sabouraud dextrose agar for 48 h at 30°C prior to MALDI-TOF MS analysis, although it should be noted that extended incubation to 96 h and culture on alternative solid media, including blood, chocolate, inhibitory mold agar (IMH), and chromogenic medium did not significantly affect final identification results (28–30). Unlike analysis of bacteria, preprocessing of the yeast isolates was required to extract fungal proteins. The protein extraction method used to process yeast isolates for MALDI-TOF MS was adapted directly from established methods used to identify difficult bacterial isolates. Specifically, 1 to 5 colonies of an isolate were inactivated in 75% ethanol, pelleted, and then suspended in a 1:1 mixture of 70% formic acid and acetonitrile. The resulting supernatant was then analyzed by MALDI-TOF MS. This method provided good-quality protein spectra and resulted in genus or species level identification for 92.5% to 98.2% of all yeast isolates and a correct identification rate of 99.3% to 100% (29, 31, 32) (Table 1). The best performance was observed with *Candida* spp., for which 96.0% to 100% of isolates were identified with 100% accuracy (29, 31, 32). Application of this method to non-*Candida* yeast-like isolates (*Trichosporon*, *Geotrichum*) was considerably less successful, resulting in identification of only 41.2% to 61.9% of isolates (31, 32). This shortcoming reflected the absence of reference spectra for these genera or species in the MALDI-TOF MS library rather than inadequate processing, because (i) identification of isolates present in the library reached >99%, and (ii) addition of spectra from type strain isolates corresponding to unidentified strains resulted in correct identification upon reanalysis (31, 32).

TABLE 1 Methods for analysis of isolated yeast colonies

Method	Study enrollment	Positive ID (%)[a]	Correct ID (%)[a]	Reference
Tube-based extraction (Cultured for 48 h at 30°C on SAB. One to 5 colonies were extracted using 75% EtOH, followed by suspension in a 50/50 mixture of 70% formic acid and ACN.)[b]	267 yeast isolates (250 *Candida* spp.)	247/267 (92.5)	247/247 (100)	Marklein et al. (32)
	1,192 yeast isolates (1,007 *Candida* spp.)	1,171/1,192 (98.2)	1,163/1,171 (99.3)	Bader et al. (31)
	241 yeast isolates (193 *Candida* spp.)	225/241 (93.4)	225/225 (100)	Dhiman et al. (29)
On-plate extraction (Cultured for 48 h at 30°C on SAB. One colony was transferred directly to MALDI-TOF MS analysis plate and overlaid with 70% or 25% formic acid.)	167 yeast isolates	135/167 (80.8)	135/135 (100)	Van Herendael et al. (34)
	90 yeast isolates (71 *Candida* spp.)	86/90 (95.6)	89/90 (98.9)	Theel et al. (33)
	192 yeast isolates (182 *Candida* spp.)	185/192 (96.3)	184/185 (99.5)	Iriart et al. (35)

[a] ID, identification. Number of isolates identified to the genus or species level (numerator) according to defined score interpretation guidelines out of the total number of isolates tested (denominator).
[b] EtOH, ethanol; ACN, acetonitrile.

Identification of plate-grown isolates (on-plate extraction methods). Two key modifications have been examined to further optimize the identification of yeast isolates cultured on solid medium. The first aimed to streamline preprocessing steps necessary for protein extraction prior to MALDI-TOF MS analysis, and the second aimed to increase the percentage of isolates identified to the species level.

The standard tube-based protein extraction process can be completed in 5 to 15 min, but for laboratories with a high volume of fungal cultures, processing individual isolates "on demand" is cumbersome and batch processing slows turnaround time, abrogating one of the main advantages of MALDI-TOF MS. Further, added steps required for tube-based extraction complicate sample traceability and increase the potential for error. Three groups of investigators examined the possibility of on-plate specimen preparation to simplify analysis. Rather than immersing yeast isolates in ethanol, vortexing, and extracting proteins in a mixture of formic acid and acetonitrile, a single yeast colony was transferred directly to the MALDI-TOF MS analysis plate and overlaid with 25% or 70% formic acid. Following a short drying period, matrix material was added, and the isolate was subjected to MALDI-TOF MS. This method reduced the processing time to as little as 3 to 5 min, reduced consumables, and eliminated extra specimen labeling and manipulation steps. The genus or species identification rate using on-plate processing was 80.8% to 82.5% when the Bruker Biotyper system was used, which is 12% to 15% lower than obtained using the standard tube-based protein extraction method (33, 34). In contrast, 95.8% of isolates analyzed using the Vitek MS gave acceptable genus or species identification when processed using the on-plate method (35). Importantly, although fewer isolates were successfully identified using the Bruker Biotyper with on-plate processing, the correct identification rate was still 98.9% to 100% (33, 34).

Modification of confidence score thresholds. Determination of whether an isolate identification is classified as "reliable," "low discrimination," "genus level," "species level," or "unidentified" is largely based upon manufacturer-driven breakpoints commonly referred to as "confidence scores." The Vitek MS uses a standard confidence score method based on 0 to 100% confidence. Confidence scores of >90% are coded in green, scores of 85 to 90% are coded in yellow, and scores of < 85% are coded in white. Any identification coded as green (>90%) is considered to be "reliable." The Bruker Biotyper uses a scoring method that generates identification confidence scores ranging from 0 to 3.0. Confidence scores of >2.0 are considered secure species level identification, scores of 1.7 to 2.0 are considered genus-only identification, and scores of <1.7 are considered unreliable identification. These breakpoints have been found to be overly conservative by several studies. For example, two recent studies using the Bruker Biotyper have demonstrated that lowering the "secure species" identification threshold from the manufacturer-recommended 2.0 to 1.8 increased species level identifications from 87% to 92% to >99% of isolates while preserving 100% accuracy of identifications (29, 36).

Establishment of a lower threshold for identification acceptance can have a significant impact when using on-plate processing. In one study using the Bruker Biotyper, 100% of isolates with lower confidence scores indicating "genus-only" identification were also found to have correct species level identification (34). Using a reduced score threshold increased the identification rate for on-plate specimen preparation from 80.8% to 97.6%, which is equivalent to the identification rate using standard tube-based extraction methods (34). A similar result was obtained by Theel et al., who increased the successful on-plate analysis rate using the Bruker Biotyper from 81.1% to 95.6% by lowering the "secure genus" threshold from a score of 1.7 to 1.5 (33). The necessity to modify manufacturer-recommended score thresholds may be less important if using the Vitek MS system. Even when using an on-plate processing method, only 3.7% of 192 isolates did not meet the criteria for acceptable identification scores (35).

Identification of yeast isolates directly from positive blood cultures. Emergence of *Candida* spp. other than *C. albicans* as a significant cause of fungemia has complicated empirical antifungal therapy. Current guidelines for the management of candidemia recommend changing empirical therapy from an echinocandin to fluconazole if the identified yeast is likely to be susceptible to fluconazole (e.g., *C. albicans*). However, if the isolate is identified as *C. glabrata* or *C. parapsilosis*, the recommendation is not to switch because of noted resistance to fluconazole in these species (6). Accurate identification of yeast isolates directly from positive blood culture could aid in selection of appropriate antifungal therapy 24 to 48 h earlier than identification based on subculture of the isolate. Additionally, studies using other rapid identification methods such as PNA FISH have demonstrated significant reduction in antifungal therapy cost based on more timely identification of *Candida* species in positive blood cultures (37). MALDI-TOF MS analysis of yeast isolates directly from blood culture poses additional challenges compared to analysis from solid medium subculture. Intracellular proteins from yeasts must still be liberated for successful identification; however,

TABLE 2 Methods for analysis of yeast isolates directly from blood culture

Method	Study enrollment	Positive ID[a] (%)	Correct ID[a] (%)	Reference
Centrifugation of 4 ml of blood culture to remove leukocytes followed by centrifugation of supernatant to collect yeast (no detergent added). Extracted using 75% EtOH followed by suspension in 50/50 mixture of 70% formic acid and ACN.[b]	18 positive cultures (all *Candida* spp.)	1/18 (5.6)	1/1 (100)	Ferreira et al. (42)
Centrifugation of 1 to 8 ml of blood culture followed by pure water wash and addition of detergent (e.g., 0.1% SDS, 5% saponin, 0.1% Tween 80). Washed with pure water and extracted as described above.	346 positive cultures (344 *Candida* spp.) 36 positive cultures (all *Candida* spp.) 31 positive cultures (all *Candida* spp.)	316/346 (91.3) 36/36 (100) 31/31 (100)	316/316 (100) 36/36 (100) 31/31 (100)	Spanu et al. (40) Marinach-Patrice et al. (39) Ferroni et al. (38)
Centrifugation of 1 ml of blood culture followed by pure water wash and Sepsityper processing. Extracted as described above.	42 positive cultures (41 *Candida* spp.)	42/42 (100)	42/42 (100)	Yan et al. (43)

[a] ID, identification. Number of isolates identified to the genus or species (numerator) according to defined interpretation guidelines out of the total number of isolates tested (denominator).
[b] EtOH, ethanol; ACN, acetonitrile.

yeast proteins must also be separated from blood components (erythrocytes, leukocytes, and serum proteins), which present as spectral contaminants that can interfere with spectrum and peak matching.

Seeded bottle and prospective clinical studies have utilized various methods to further isolate and purify yeast proteins for MALDI-TOF MS analysis. Successful methods include an initial wash in pure water followed by the addition of a mild detergent (e.g., 0.1% SDS, 5% saponin, 0.1% Tween 80) to selectively lyse residual erythrocytes and leukocytes. This enables the separation of these components from the yeast isolate via simple centrifugation and wash steps (38–40). Omission of either the initial wash or the addition of detergent prior to MALDI-TOF MS analysis results in identification of yeasts and yeast-like organisms in as few as 5.6% of cultures (41–43). When appropriate wash and detergent lysis steps are performed, identification of *Candida* spp. directly from blood culture ranged from 91.3% to 100% (Table 2). The majority of blood cultures that failed to generate a positive identification using this methodology were either polymicrobial or contained an organism density of $<10^4$ CFU/ml (40), which is below the established threshold of 5.9×10^5 CFU/ml necessary for MALDI-TOF MS analysis (43). A commercially available kit for specimen processing (Sepsityper; Bruker, Billerica, MA) was also evaluated for preparation of samples directly from blood culture. This method resulted in correct species identification of 100% of isolates tested, including 45 *Candida* spp. and 1 *Cryptococcus neoformans* isolate (43).

Narrowing the range of peaks considered for identification to those in the 5,000- to 7,400-Da mass range can also aid spectral analysis and identification. This effectively removes from analysis any peaks corresponding to residual blood components in the sample, which can interfere with spectral best-match algorithms. Using this method, 48 strains representing 6 *Candida* spp. were correctly identified to the species level in a seeded bottle study (39). Narrowing the mass range used for MALDI-TOF MS analysis has also been used to improve identification scores and the overall identification rate for blood cultures containing bacteria (41).

Creation of expanded reference libraries. Identification of yeast and other microorganisms using MALDI-TOF MS is based on acquisition of a quality protein spectrum from an unknown isolate and comparison of this spectrum to a reference library of defined spectra. Therefore, one limiting factor of this technology is the breadth and depth of the reference library used. If there are no spectra present for a given genus or species, unknown isolates of that genus or species will produce low scores or unacceptable identifications. A current weakness of commercially available reference libraries is that they contain a limited number of spectra from rare, non-*Candida* species yeast. This is evidenced by studies indicating identification rates of only 41.2% to 82.5% for non-*Candida* species isolates (29, 31, 32). Genera with particularly poor performance include *Aurobasidium*, *Cryptococcus*, *Pichia*, and *Geotrichum*. Not surprisingly, these genera were either underrepresented or completely absent from the reference library used for identification in these studies (31, 32). Addition of spectra obtained from type strains corresponding to unidentified isolates is often sufficient to correct this deficiency. This approach resulted in identification of 20/20 and 3/3 isolates that failed initial analysis in two independent reports (32, 36). These data highlight the importance of inclusion of well-characterized "type strains" into reference libraries, provided the type strains are representative of currently circulating and clinically important isolates.

When constructing a library, it is important to include not only a wide variety of different species but also several strains of each species, since strain-to-strain variability within a species can affect MALDI-TOF MS identification (44, 45). Species represented by multiple strains in a reference library account for this intraspecies variability and yield higher-quality identification results. Pinto et al. observed high confidence scores with little variance for well-represented species, including *C. albicans* and *C. glabrata*, while

Candida nivariensis, which had only 2 entries in the reference library, generated lower scores with larger standard deviations (45).

Supplemental spectral libraries have been created to improve the identification of specific groups of bacteria, including *Mycobacterium*, *Streptococcus*, and *Pseudomonas*. Creation of supplemental libraries is relatively simple and involves collecting 20 to 30 replicate spectra from each isolate desired to be added to a library. These libraries can be queried independently or can be integrated into existing libraries. This approach has been applied to *Cryptococcus* spp., which is a weakness of one commercially available library. Spectra from 26 type and reference strains representing seven species and two subspecies of *Cryptococcus* were created as a supplemental library which was then merged with a standard reference library. Addition of these strains increased species level identification of clinical isolates from 58.4% to 100% and raised the average confidence score from 2.02 to 2.26 compared to the original library (46). It also allowed discrimination between *C. neoformans* subspecies *grubii* and *neoformans* in 98.8% of cases. This highlights the resolving power of MALDI-TOF MS provided adequate spectra are available in a reference library.

Antimicrobial susceptibility testing. The rapid identification of yeast isolates can aid in selection of empirical antifungal therapy based upon predictable resistance profiles of some yeasts; however, for isolates with variable susceptibility patterns, antifungal susceptibility testing (AST) must be conducted for a definitive result. Current CLSI and EUCAST guidelines recommend the use of broth microdilution (BMD) methods as a means to establish an MIC. This requires a pure culture of the isolate to be tested on Sabouraud dextrose agar (SAB) or potato dextrose agar (PDA). A suspension of the isolate is then diluted to allow inoculation of $\sim 1 \times 10^3$ CFU to each well of a microtiter plate containing doubling dilutions of a given antifungal agent. Following 24 h of incubation, the MIC for each isolate/antifungal combination is determined as the concentration of the drug that results in a 50% decrease in culture turbidity compared to the turbidity of the control well (47). This method is widely used, but results can be subjective, depending on manual inspection for fungal growth, and it also requires a minimum of 24 to 72 h from initial isolation of the organism before results are available.

Resistance to azole class drugs, including fluconazole is achieved by alteration of the target (ergosterol biosynthesis 11 [ERG11]) or upregulation of efflux pumps (CDR and MDR). Generally, an increase in expression of MDR pumps is specific to resistance to fluconazole, while increased expression of CDR pumps or alteration of the EGR11 target confers class-wide resistance (48). Likewise, resistance to echinocandins is mediated by mutations in genes encoding 1,3-β-D-glucan synthase (FKS1, FKS2, and FKS3), the target of this class of drugs (48). Because MALDI-TOF MS analysis is based on the spectral analysis of proteins, differences in the levels of expression of certain proteins can sometimes be detected through careful examination of raw spectra, computerized algorithms, or by using virtual gel analysis. For this method to be effective, the difference in protein expression needs to be dramatic, or in the case of protein modification, the protein needs to be highly abundant so it is discernible above other "background" proteins expressed in each cell.

In a novel study by Marinach et al. (49), the protein profiles of 16 *C. albicans* strains with defined genetic backgrounds were examined when the strains were grown in the presence of increasing concentrations of fluconazole (FCZ). The peaks obtained from a given strain cultured at various FCZ concentrations were compared to the same strain grown at high FCZ concentrations (128 μg/ml) and in the absence of FCZ. Using a statistical method, these data were used to determine the lowest drug concentration at which changes in the protein spectrum became statistically significant. This concentration was termed the minimum profile change concentration (MPCC). A comparison of the MPCC to standard MIC based on broth microdilution revealed 94% agreement within a one doubling dilution range (49). Using the same methodology, De Carolis and colleagues evaluated the ability of MALDI-TOF MS to detect differences in protein profiles in *C. albicans*, *C. glabrata*, *C. krusei*, and *C. parapsilosis* in response to increasing concentrations of caspofungin. The authors demonstrate >94% (32/34) categorical agreement between the MALDI-TOF method and BMD (50).

These preliminary reports suggest that the use of MALDI-TOF MS for susceptibility testing of yeast isolates may be a viable alternative to the standard BMD method in some circumstances. Potential advantages include the elimination of BMD read subjectivity and a reduction in incubation time required for valid results to as little as 15 h. However, additional studies are needed to assess the efficacy and reproducibility of this method using larger numbers and more diverse yeast species as well as additional antifungal agents.

CONCLUSION

MALDI-TOF MS has emerged as a promising new method for the identification of clinical yeast and yeast-like isolates. The use of either standard tube-based or on-plate extraction methods provide a definitive identification for the majority of yeast isolates within 10 to 40 min with only 5 min of hands-on time (29, 32). This allows significantly more rapid identification of clinical isolates compared to routine biochemical identification methods, which in turn may aid selection of the most appropriate therapy and reduce overall cost of care. A recent study using PNA FISH to identify yeast isolates directly from positive blood culture broths demonstrated an average savings of $1,837 per patient based on early differentiation of *C. albicans* from other *Candida* spp. and resulting modification of antifungal therapy (37). Another major advantage cited by all current studies is the rare incidence of incorrect identification reported when the identification score was within the "acceptable" category (17, 20, 21, 24). Isolates that generated poor-quality spectra and species not present in the reference library received low identification scores and were categorized as "unacceptable identification." This is in contrast to methods such as Vitek 2 or RapID Yeast which incorrectly identify up to 2.5% of isolates based on a limited number of biochemical reactions or deficiency in the reference databases (39).

Preparation for MALDI-TOF MS analysis, including protein extraction, requires only minimal, inexpensive reagents that equate to approximately $0.50 per identification (29). While the initial cost of a MALDI-TOF MS instrument can be significant ($180,000 to $250,000), low reagent cost and high identification rate significantly reduce the per isolate cost for identification of yeast isolates compared to current methods (29). For laboratories using low-cost manual biochemical tests such as the API 20C or API 32C (~$7/test), the time until return on investment (ROI) can be as little as 2 to 3 years (assuming 20 to 30 identifications/day) if used exclusively for the identification of yeast isolates. Of course, the utility of MALDI-TOF MS extends beyond yeast iden-

tification to include bacterial and mycobacterial identification which demonstrate similar cost benefits (18, 51).

MALDI-TOF MS identification is based on obtaining and comparing the unique protein profile of an isolate to a reference library using spectral pattern matching. This approach allows discrimination between closely related species or subspecies which cannot be easily differentiated using biochemical tests (e.g., *C. albicans/dublinensis*, *C. parapsilosis/orthopsilosis/metapsilosis*) (10). This can aid in epidemiologic studies and may have implications for antifungal therapy (10). However, it is important to note that the capability of MALDI-TOF MS to successfully identify a wide variety of isolates and discriminate between closely related species is directly related to the breadth and depth of the reference library used for comparison, i.e., identification is limited only by the size of the reference library (31, 32, 36). A benefit of most MALDI-TOF MS identification platforms is the ability to supplement the reference library if specific deficiencies are identified. This enables the platform to be flexible to accommodate regional variations in prominent clinical isolates or the addition of emerging pathogens through the addition of these strains to a library. Indeed, a weakness of all studies presented in this minireview is the limited number of non-*Candida* species isolates included in each study, which ranges from 0 to 21% of all isolates analyzed. This leaves a significant area for further research and evaluation of MALDI-TOF MS capabilities, as well as development of more comprehensive reference libraries. Finally, protein profile analysis using MALDI-TOF MS may provide a novel method for determining the antifungal susceptibility of some species/drug combinations.

The combined characteristics of rapid turnaround time, accurate identification, and low per isolate cost associated with MALDI-TOF MS identification of yeast isolates have the potential to positively impact patient care and laboratory efficiency. However, large, standardized, multicenter studies are required before the impact and utility of MALDI-TOF MS for the clinical lab can be fully assessed.

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