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Multicenter Evaluation of Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy-Based Method for Rapid Identification of Clinically Relevant Yeasts

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ABSTRACT Fourier transform infrared (FTIR) spectroscopy has demonstrated applicability as a reagent-free whole-organism fingerprinting technique for both microbial identification and strain typing. For routine application of this technique in microbiology laboratories, acquisition of FTIR spectra in the attenuated total reflectance (ATR) mode simplifies the FTIR spectroscopy workflow, providing results within minutes after initial culture without prior sample preparation. In our previous central work, 99.7% correct species identification of clinically relevant yeasts was achieved by employing an ATR-FTIR-based method and spectral database developed by our group. In this study, ATR-FTIR spectrometers were placed in 6 clinical microbiology laboratories over a 16-month period and were used to collect spectra of routine yeast isolates for on-site identification to the species level. The identification results were compared to those obtained from conventional biochemical tests and/or matrix-assisted laser desorption/ionization–time of flight mass spectrometry. Isolates producing discordant results were reanalyzed by routine identification methods, ATR-FTIR spectroscopy, and PCR gene sequencing of the D1/D2 and internal transcribed spacer (ITS) regions. Among the 573 routine clinical yeast isolates collected and identified by the ATR-FTIR-based method, 564 isolates (98.4%) were correctly identified at the species level, while the remaining isolates were inconclusive with no misidentifications. Due to the low prevalence of *Candida auris* in routine isolates, additional randomly selected *C. auris* ($n = 24$) isolates were obtained for evaluation and resulted in 100% correct identification. Overall, the data obtained in our multicenter evaluation study using multiple spectrometers and system operators indicate that ATR-FTIR spectroscopy is a reliable, cost-effective yeast identification technique that provides accurate and timely (~3 min/sample) species identification promptly after the initial culture.

KEYWORDS ATR, FTIR, infrared spectroscopy, attenuated total reflectance, clinical microbiology, *in vitro* diagnostics, multicenter evaluation, mycology, species identification, yeasts

Fungal infections affect over a billion people worldwide, resulting in an estimated 1.5 million deaths each year (1). It is also estimated that there are approximately 700,000 global cases of invasive candidiasis and over 220,000 cases of fungal disease due to *Cryptococcus neoformans* associated with HIV/AIDS complications (2, 3). Over the past few

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decades, there has been a reported increase in nosocomial candidiasis, with candidemia being associated with mortality rates of over 40% of those infected (4–6). Furthermore, *Candida* spp. in many developed countries are the 3rd or 4th leading cause of nosocomial bloodstream infections. These opportunistic microorganisms pose the greatest risk to the elderly population, neonates, and those who are immunocompromised (6). Although *Candida albicans* accounts for over 40% of all yeast infections, there is an increasing prevalence of infections due to *Candida* species other than *C. albicans*. For example, the increase in incidence of infections caused by *C. auris* is troublesome due to its ability to acquire antifungal resistance and the ease of person-to-person transmission, complicating treatment and/or resulting in poor patient outcomes (6–8).

Rapid identification of yeasts is necessary for appropriate patient care and to reduce the spread of antifungal resistance. Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) has revolutionized microbial identification in both bacteriology and mycology in the past decade due to its simplicity, rapidness, and reliability for identification of microorganisms to the species level relative to conventional biochemical techniques (9–12). It is also the first commercially available and widely accepted spectral fingerprinting technique for *in vitro* diagnostics. Although it has revolutionized clinical microbiology, the high initial capital cost associated with the technique, along with expenses for reagents, the requirement for additional pretreatment steps for fungi, disposable target plates, and maintenance, has hindered the spread to small and midsized laboratories of the technology (13).

Within the realm of microbiology, Fourier transform infrared (FTIR) spectroscopy has been widely applied for the investigation of cell metabolism, microbial identification, and strain typing (14–23). However, there is a lack of standardization and spectral database evaluation studies in the clinical setting. When coupled with the attenuated total reflectance (ATR) mode of spectral acquisition, FTIR spectroscopy becomes a rapid, reagent-free, and low-maintenance technique that does not require any sample preparation after culture nor heat-, drying-, or vacuum-related downtime.

Our group, in previous work, has created and developed an ATR-FTIR spectroscopy-based microbial spectral profiling or "spectrotyping" technique (defined as the process of determining the differences between spectra obtained from microorganisms based on the absence or presence and relative intensities of infrared absorption bands) for rapid identification of clinically relevant yeasts (14). Spectrotyping entails the implementation of standardized culturing methods and well-defined ATR-FTIR spectral acquisition and preprocessing techniques to construct a spectral database of highly reproducible ATR-FTIR spectra of well-characterized isolates. Through spectrotyping of clinical yeast isolates, a database was created consisting of 65 species and 23 genera, and a multitier classification strategy was developed to classify an unknown yeast isolate based on its spectral similarity to isolates included in the database. This spectrotyping approach was subsequently evaluated centrally with 318 routine yeast isolates, resulting in 100% correct genus and 99.7% correct species identification (14).

The results of the latter proof-of-concept study warranted the undertaking of a multicenter evaluation study to confirm the performance of the ATR-FTIR spectroscopy-based yeast identification technique and serve as an initial step toward potential routine implementation. For this purpose, multiple ATR-FTIR spectrometers were furnished with the ATR-FTIR reference spectral database and classification models described above and were set up to automate all steps in the identification of an unknown yeast isolate beyond spectral acquisition (microbial sample deposition onto the sampling surface), producing an identification result within about a minute. Over a 16-month period, these ATR-FTIR systems for rapid on-site identification of routine yeast isolates were evaluated in 6 clinical microbiology laboratories, and the ATR-FTIR spectroscopy-based identification results were compared to the results of standard identification techniques (conventional biochemical tests, MALDI-TOF MS, and/or ribosomal DNA [rDNA] gene sequencing) implemented by the participating laboratories. A successful outcome of this multicenter study could pave the way for the accreditation of ATR-FTIR spectroscopy as an *in vitro* diagnostic device for yeast identification,

TABLE 1 Performance of centrally created ATR-FTIR spectral reference database in a multicenter evaluation study for the identification of clinical yeasts

Microorganism (previous name)	No. (%) of isolates				
	In database	Collected	With correct identification	Misidentified	With no identification[a]
Candida albicans	16	254	254 (100)	0 (0)	0 (0)
Candida auris	11	1	1 (100)	0 (0)	0 (0)
Candida dubliniensis	13	25	24 (96)	0 (0)	1 (4)
Candida (*Nakaseomyces*) *glabrata*	17	80	79 (98.8)	0 (0)	1 (1.25)
Candida metapsilosis	1	1	0 (0)	0 (0)	1 (100)
Candida orthopsilosis	9	4	3 (75)	0 (0)	1 (25)
Candida parapsilosis	12	90	90 (100)	0 (0)	0 (0)
Candida tropicalis	15	37	37 (100)	0 (0)	0 (0)
Clavispora (*Candida*) *lusitaniae*	10	25	25 (100)	0 (0)	0 (0)
Cryptococcus neoformans	9	2	2 (100)	0 (0)	0 (0)
Cyberlindnera jadinii (*Candida utilis*)	4	1	1 (100)	0 (0)	0 (0)
Kluyveromyces marxianus (*Candida kefyr*)	17	3	3 (100)	0 (0)	0 (0)
Meyerozyma caribbica (*Candida fermentati*)	5	1	1 (100)	0 (0)	0 (0)
Meyerozyma (*Candida*) *guilliermondii*	15	6	4 (66.7)	0 (0)	2 (33.3)
Naganishia (*Cryptococcus*) *diffluens*	2	1	1 (100)	0 (0)	0 (0)
Pichia cactophila (*Candida inconspicua*)	1	1	1 (100)	0 (0)	0 (0)
Pichia kudriavzevii (*Candida krusei*)	10	19	19 (100)	0 (0)	0 (0)
Rhodotorula mucilaginosa	3	2	2 (100)	0 (0)	0 (0)
Saccharomyces cerevisiae	13	2	2 (100)	0 (0)	0 (0)
Saprochaete clavata (*Geotrichum clavatum*)	1	1	0 (0)	0 (0)	1 (100)
Starmerella (*Candida*) *magnoliae*	1	1	0 (0)	0 (0)	1 (100)
Torulaspora delbrueckii (*Candida colliculosa*)	0	1	0 (0)	0 (0)	1 (100)
Trichosporon asahii[b]	4	2	2 (100)	0 (0)	0 (0)
Trichosporon (*Cutaneotrichosporon*) *dermatis*[b]	1	1	1 (100)	0 (0)	0 (0)
Trichosporon inkin[b]	1	1	1 (100)	0 (0)	0 (0)
Trichosporon (*Apiotrichum*) *mycotoxinivorans*[b]	1	1	1 (100)	0 (0)	0 (0)
Wickerhamiella (*Candida*) *pararugosa*	4	3	3 (100)	0 (0)	0 (0)
Wickerhamomyces anomalus (*Candida pelliculosa*)	2	5	5 (100)	0 (0)	0 (0)
Yarrowia (*Candida*) *lipolytica*	7	2	2 (100)	0 (0)	0 (0)
Total	205	573	564 (98.4)	0 (0)	9 (1.6)

[a]No identification is assigned when the triplicate spectra of the unknown isolate produce discordant results or when no acceptable match is present in the spectral database based on spectral similarity criteria described in the text.
[b]Species of the genus *Trichosporon* were identified only to the genus level by the ATR-FTIR spectroscopy-based method.

facilitating its acceptance and implementation in clinical settings for routine identification of both yeasts and bacteria.

MATERIALS AND METHODS

Samples. A total of 573 yeast isolates (17 genera and 29 species) (Table 1), obtained from routine clinical specimens (i.e., blood, urine, or respiratory specimens) onto agar culture media (i.e., Sabouraud dextrose agar [SAB], Sabouraud dextrose agar Emmons, or inhibitory mold agar), were tested in this multicenter study over a cumulative 16-month period. The majority of these isolates were tested on-site in the clinical microbiology laboratories at five hospitals (with the number of isolates indicated in parentheses): Centre Hospitalier Universitaire du Sherbrooke (CHUS) (n = 93), Centre Hospitalier Universitaire Sainte Justine (CHUSJ) (n = 51), Hôpital Maisonneuve-Rosemont (HMR) (n = 100), Centre Universitaire de Santé McGill (CUSM) (n = 100), and Centre Hospitalier de l'Université de Montréal (CHUM) (n = 97). The isolates tested at each study site were fresh routine isolates or came from frozen stock to facilitate batch testing and accumulation of approximately 100 isolates within a limited time frame. In addition, 132 isolates received by the provincial reference mycology laboratory Laboratoire de Santé Publique du Québec (LSPQ) for routine identification were included in the study.

Due to the low prevalence of *C. auris* (n = 1) among the routinely isolated yeasts in the multicenter study (but its high importance for accurate identification), it was necessary to obtain additional isolates of *C. auris*. Twenty-four well-characterized (by whole-genome sequencing and PCR) *C. auris* isolates (clades I to IV) were provided by the Centers for Disease Control and Prevention (CDC; Atlanta, GA).

Standard routine species identification and antimicrobial susceptibility testing. Isolates collected at CHUS, CUSM, CHUM, HMR, and LSPQ were routinely identified by MALDI-TOF MS (Vitek MS; bioMérieux, Marcy-l'Étoile, France) using the Vitek MS v3.2 clinical knowledge database, while isolates collected at CHUSJ were identified by Vitek 2 v8.01 (bioMérieux, Marcy-l'Étoile, France) or were sent to LSPQ for definitive identification. Confirmatory testing of supplemental isolates of *C. auris* (n = 24) received from

CDC was conducted by MALDI-TOF MS and by internal transcribed spacer (ITS) and D1/D2 rDNA gene sequencing at LSPQ.

Broth microdilution antimicrobial susceptibility testing following the Clinical and Laboratory Standards Institute guidelines (24, 25) was performed at LSPQ on selected isolates as part of routine laboratory procedures.

Sample preparation for ATR-FTIR spectral acquisition. Given that the FTIR spectra of microorganisms are sensitive to differences in the chemical composition of the agar culture media (26), the standardized sample preparation protocol employed in developing the reference spectral database (14) was adhered to in the present study. In accordance with this protocol, all isolates were cultured and/or subcultured onto Sabouraud dextrose agar (SAB: 40 g/liter dextrose, pH 5.6 $\pm$ 0.2 at room temperature) (BD Difco, Franklin Lakes, NJ) and were incubated at 30°C for 48 h prior to ATR-FTIR spectral acquisition.

ATR-FTIR spectral acquisition. Three ATR-FTIR spectrometers (Cary 630; Agilent Technologies, Santa Clara, CA) were utilized in the present study and were furnished with the reference spectral database previously created for identification of clinically relevant yeasts, consisting of spectra acquired on another Cary 630 ATR-FTIR spectrometer. Spectra were acquired in the range of 4,000 to 650 cm^{-1}, with a spectral resolution of 8 cm^{-1} and 64 co-added scans. The spectral acquisition protocol entailed collection of a new background spectrum prior to each sample spectrum in order to compensate for fluctuations in ambient humidity. Then, 1 to 2 isolated colonies were picked from the culture plate with a disposable 1-μL loop and directly deposited onto the clean ATR sampling surface (diamond) of the spectrometer. Following spectral acquisition, the sample was wiped from the ATR sampling surface with a 70% ethanol-soaked tissue. Care was taken to ensure that the surface was completely dry before a new background spectrum was collected. For each isolate, triplicate spectra were collected from three independent colonies (from the same culture plate). Spectral acquisition time was approximately 60 s (30 s for background scans and 30 s for sample scans) per replicate. Subsequent automated spectral preprocessing and identification of the isolate were completed within 10 s, resulting in a total analysis time of roughly 3 min per isolate (3 replicates).

ATR-FTIR spectroscopy-based yeast identification. Species identification by ATR-FTIR spectroscopy was achieved by employing the ATR-FTIR spectral database previously created for identifying clinically relevant yeasts (14) (referred to herein as the "reference database"). The reference database consists of the spectra of 263 reference strains (made available by LSPQ) and encompasses 65 species belonging to 23 clinically relevant genera of yeasts (see Table S1 in the supplemental material); each spectrum in this database is an average of triplicate spectra of the reference strain. It should be noted that species of the genus *Trichosporon* were identified only to the genus level by the ATR-FTIR spectroscopy-based method due to low representation of individual species within the database but adequate representation at the genus level ($n = 7$).

Identification of an unknown yeast isolate was achieved by a sequential multitier pairwise search of the reference database to find the best match to its ATR-FTIR spectrum (14). The best match is defined as the spectrum in the database that has the highest spectral similarity to the spectrum of the unknown isolate within the spectral range of 1,480 to 980 cm^{-1}, as measured by the cosine similarity metric in an n-dimensional spectral data space, where a value of 1 represents a perfect match. The result is deemed acceptable and reported only if the following criteria are met: (i) the cosine similarity of the spectrum of the unknown isolate to the best match reference spectrum is $>$0.7, and (ii) the Euclidean distance in the n-dimensional spectral data space between the spectrum of the unknown isolate and the best match reference spectrum, compared to the standard deviation of the distances between the reference spectrum, which is an average of three replicate spectra, and each of these replicate spectra, is less than 3 standard deviations. If either criterion is not met, then the result is deemed unacceptable and reported as "inconclusive." Results are exported as a spreadsheet with the following information per spectrum: spectrum filename, top match, cosine similarity, and the standard deviation of the distance between the reference spectrum and the test spectrum. No high-, medium-, or low-confidence cutoff values were determined. Additionally, different species (or genera) identification from each of the triplicate spectra collected from one strain (grown on one SAB plate) was also considered inconclusive (i.e., no identification is reported) and possibly indicating the presence of a contaminating microorganism.

Inconclusive identification results were addressed by reculturing the isolate and reacquiring the ATR-FTIR spectra along with reanalyzing the isolate by Vitek MS (bioMérieux, Marcy-l'Étoile, France). If results remained inconclusive, definitive identification was achieved at the LSPQ by PCR rDNA gene sequencing of the D1/D2 (NL1 and NL4 primers [27]) and ITS regions (ITS1 and ITS4 primers [28]) from the same reculture plates.

RESULTS

In this study, a total of 573 routine clinical yeast isolates were identified in 6 clinical microbiology laboratories by the ATR-FTIR spectroscopy-based method developed in previous work (14). This multicenter evaluation entailed the transfer of the ATR-FTIR spectral database created in the latter work to 3 ATR-FTIR spectrometers that had not been used to acquire any of the spectra in the database. The results of this study demonstrate that matching the spectra acquired on different instruments and by different operators against the spectra in the database resulted in 100% correct identification of all isolates at the genus level and a total of 564/573 isolates (98.4%) correctly identified at the species level (Table 1). The 9 remaining isolates were not identified (i.e., no satisfactory spectral match was found in the reference spectral database). Reculturing the isolates a second time and their analysis by MALDI-TOF MS and ATR-FTIR spectroscopy did not alter

the outcome. Further analysis by rDNA/gene sequencing was carried out and was in concordance with the MALDI-TOF MS results. Among the 9 isolates for which species identification based on ATR-FTIR spectroscopy was inconclusive, 4 isolates belonged to 4 different species (*Candida metapsilosis*, *Saprochaete clavata* [*Geotrichum clavatum*], *Starmerella* [*Candida*] *magnoliae* and *Torulaspora delbrueckii* [*Candida colliculosa*]) that were underrepresented in the reference spectral database (i.e., represented by <5 isolates) (Table 1). The 5 remaining isolates belonged to 4 species (*C. dubliniensis* [*n* = 1], *C. glabrata* [*n* = 1], *C. orthopsilosis* [*n* = 1], and *Meyerozyma guilliermondii* [*n* = 2]) that had higher spectral representation in the reference spectral database (>9 isolates per species). It should be noted that 13 isolates were correctly identified even though they belonged to species that were nominally underrepresented (<5 isolates per species) in the reference spectral database, namely, *Cyberlindnera jadinii* (*Candida utilis*), *Naganishia* (*Cryptococcus*) *diffluens*, *Pichia cactophila* (*Candida inconspicua*), *Rhodotorula mucilaginosa*, *Wickerhamiella* (*Candida*) *pararugosa*, and *Wickerhamomyces anomalus* (*Candida pelliculosa*).

In addition to the 573 isolates tested in the multicenter evaluation study, a supplementary set of 24 *C. auris* isolates was acquired to evaluate the performance of the ATR-FTIR-based method for the identification of *C. auris*. All 24 isolates were correctly identified to the species level by matching their ATR-FTIR spectra against the reference spectral database.

DISCUSSION

Employing the previously constructed ATR-FTIR spectral database representing 65 yeast species from 23 genera, this multicenter evaluation study resulted in 98.4% correct species identification, with no misidentification at the genus or species level and 1.6% of isolates reported as inconclusive at the species level. While inconclusive results were obtained for 4 isolates belonging to species that are underrepresented in the reference spectral database (<5 isolates per species), 5 isolates belonging to species better represented in the reference spectral database (>9 isolates/species) were also not identified rather than being misidentified. The latter finding illustrates the need for augmentation of the spectral database with additional spectra of certain species that likely exhibit a larger spectral variability stemming from a broader range of phenotypic/metabolomic diversity and highlights the limitation of selecting an arbitrary minimum number of isolates that must be included in the database to adequately represent a given species. Species that have minimal fluctuations in their biochemical composition under specified growth conditions will be well represented with fewer isolates in the reference spectral database, while those that have a larger fluctuation in their metabolome/biochemical composition may require a larger number of isolates to be included in the spectral database. For example, *C. glabrata* is well known for its great intraspecies diversity (29); although *C. glabrata* is represented in the spectral reference database by a relatively large number of isolates, (*n* = 17) and 75/76 (98.8%) *C. glabrata* isolates were correctly identified in the present study, 1 isolate of *C. glabrata* was not identified due to insufficient spectral similarity to the *C. glabrata* isolate that was the best match in the reference spectral database (cosine similarity to best match = 0.304). Based on visual observations, the unidentified *C. glabrata* strain, relative to those represented in the reference spectral database, exhibited slower growth (smaller colonies after incubation for 48 h at 30°C). Furthermore, through broth microdilution, the isolate in question was found to be resistant to fluconazole (MIC = 64 μg/ml), while the *C. glabrata* isolates employed in the creation of the reference spectral database were all susceptible-dose dependent to fluconazole (or nonresistant). Both of these findings may have impacted the spectral profile of the isolate and resulted in no identification due to this isolate being phenotypically atypical relative to the *C. glabrata* isolates in the reference spectral database.

Implementation of the ATR-FTIR-based method must include a highly consistent standard operating procedure, in particular, the use of a consistent growth medium and incubation parameters such as time and temperature (14, 30). The use of standardized culture media and growth conditions is therefore required to achieve the high specificity and sensitivity reported in this study. As such, end users of the method would have to make changes in their routine workflow to conform to the growth media and conditions utilized

in the construction of the reference spectral database, which clearly limits the practical utility of the method. Accordingly, future work should address expanding the reference spectral database to include spectra of reference isolates grown on different culture media or constructing additional culture-medium-specific spectral databases to enhance the robustness and flexibility of this identification method. Alternatively, laboratories may construct their own databases with the use of commercially available software packages such as OMNIC 9 (Thermo Fisher Scientific, Inc., WI) in combination with multivariate data analysis software such as TQ Analyst (Thermo Fisher Scientific, Inc., WI) or Unscrambler 11 (Aspen Tech, MA).

The spectral reference database employed in the present study is dynamic as additional spectra of clinical yeasts can be added by users, which may serve to enhance representation of species diversity by including spectra of isolates acquired from various geographical locations (31–33). Likewise, the commercially available MALDI-TOF MS reference databases are constructed with mass spectral profiles of microorganisms representing intraspecies diversity (i.e., typical strains, atypical strains, strains isolated from different regions, and strains isolated from different sources), as well as microorganisms cultured on several culture media and grown under different atmospheric conditions (34, 35).

The results of this study delineate further the performance of the first rapid ATR-FTIR-based method for accurate identification of clinical yeasts. The use of a highly standardized ATR-FTIR-based method has been evaluated in a multicenter study demonstrating the reliability of ATR-FTIR spectroscopy for species-level identification of clinically relevant yeasts. Importantly, this successful outcome was contingent on the transferability of the ATR-FTIR reference spectral database from the instrument on which it was created to 3 other spectrometers employed in 6 locations to acquire the spectra of all the isolates tested in this study. The spectral database proved to be directly transferable, indicating that the spectral preprocessing routines compensated for any instrument-to-instrument variability in spectral quality. In addition, spectral quality was independent of the ambient conditions at each location (temperature and humidity fluctuations) as the result of collection of a new background spectrum immediately prior to every acquisition of a spectrum of a sample. It may be noted that 3 different technicians acquired spectra by following the sample preparation and spectral acquisition protocols; spectral preprocessing and species identification were automated.

This is the first study to demonstrate the robustness of the constructed reference database to achieve >98% correct identification using multiple ATR-FTIR spectrometers at 6 clinical microbiology laboratories and with multiple operators. Accordingly, the ATR-FTIR spectroscopy-based method should be considered for further validation and diagnostic method accreditation as it offers a new reagent-free, cost-effective means of species identification within minutes after initial culture. Additionally, it is highly affordable and may be used as an alternative (without compromising accuracy of results) to costly molecular techniques and biochemical techniques for small and midsize laboratories. The use of ATR-FTIR spectroscopy in high-throughput laboratories may necessitate the development of a multisample ATR-FTIR spectroscopy accessory. Recently, such an accessory has become available from PIKE Technologies (WI) and Specac (Kent, United Kingdom) to facilitate batch testing by ATR-FTIR spectroscopy. Automated FTIR instrumentation offering a computer-controlled X-Y stage allowing for analysis of 96 microbial samples in transmission mode is commercially available (IR Biotyper, Bruker GmbH, Bremen, Germany). This system has found use in microbiology for microbial strain typing (23, 36) but is not suitable for ATR-FTIR spectral acquisition.

The next steps are to construct spectral databases for use in the identification of clinically relevant bacteria, develop safe methodologies (i.e., working under the biosafety cabinet or microorganism inactivation protocols) to include mycobacteria and dimorphic fungi (or other risk group 3 microorganisms) in reference spectral databases, and pursue accreditation of the ATR-FTIR-based method for yeast identification as an *in vitro* diagnostic device, facilitating its acceptance and implementation in a clinical setting.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.03 MB.

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REFERENCES

1. Bongomin F, Gago S, Oladele RO, Denning DW. 2017. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi 3:57. https://doi.org/10.3390/jof3040057.
2. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. 2012. Hidden killers: human fungal infections. Sci Transl Med 4:165rv13. https://doi.org/10.1126/scitranslmed.3004404.
3. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A, Boulware DR. 2017. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis 17:873–881. https://doi.org/10.1016/S1473-3099(17)30243-8.
4. Richardson M, Lass-Flörl C. 2008. Changing epidemiology of systemic fungal infections. Clin Microbiol Infect 14:5–24. https://doi.org/10.1111/j.1469-0691.2008.01978.x.
5. Lamoth F, Lockhart SR, Berkow EL, Calandra T. 2018. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother 73:i4–i13. https://doi.org/10.1093/jac/dkx444.
6. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. 2018. Invasive candidiasis. Nat Rev Dis Primers 4:18026. https://doi.org/10.1038/nrdp.2018.26.
7. Castanheira M, Messer SA, Rhomberg PR, Pfaller MA. 2016. Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). Diagn Microbiol Infect Dis 85:200–204. https://doi.org/10.1016/j.diagmicrobio.2016.02.009.
8. Chowdhary A, Sharma C, Meis JF. 2017. Candida auris: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog 13:e1006290. https://doi.org/10.1371/journal.ppat.1006290.
9. Gautam V, Sharma M, Singhal L, Kumar S, Kaur P, Tiwari R, Ray P. 2017. MALDI-TOF mass spectrometry: an emerging tool for unequivocal identification of non-fermenting Gram-negative bacilli. Indian J Med Res 145:665–672. https://doi.org/10.4103/ijmr.IJMR_1105_15.
10. Stefaniuk E, Baraniak A, Fortuna M, Hryniewicz W. 2016. Usefulness of CHROMagar Candida medium, biochemical methods—API ID32C and VITEK 2 compact and two MALDI-TOF MS systems for Candida spp. identification. Pol J Microbiol 65:111–114. https://doi.org/10.5604/17331331.1197283.
11. Murray PR. 2012. What is new in clinical microbiology—microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William Beaumont Hospital Symposium on Molecular Pathology. J Mol Diagn 14:419–423. https://doi.org/10.1016/j.jmoldx.2012.03.007.
12. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, Manuel R, Brown CS, Candida auris Incident Management Team. 2018. Candida auris: a review of the literature. Clin Microbiol Rev 31:e00029-17. https://doi.org/10.1128/CMR.00029-17.
13. Seng P, Rolain J-M, Fournier PE, La Scola B, Drancourt M, Raoult D. 2010. MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol 5:1733–1754. https://doi.org/10.2217/fmb.10.127.
14. Lam LM, Dufresne PJ, Longtin J, Sedman J, Ismail AA. 2019. Reagent-free identification of clinical yeasts by use of attenuated total reflectance Fourier transform infrared spectroscopy. J Clin Microbiol 57:e01739-18. https://doi.org/10.1128/JCM.01739-18.
15. Quilès F, Accoceberry I, Couzigou C, Francius G, Noël T, El-Kirat-Chatel S. 2017. AFM combined to ATR-FTIR reveals Candida cell wall changes under caspofungin treatment. Nanoscale 9:13731–13738. https://doi.org/10.1039/c7nr02170d.
16. Costa FS, Silva PP, Morais CL, Arantes TD, Milan EP, Theodoro RC, Lima KM. 2016. Attenuated total reflection Fourier transform-infrared (ATR-FTIR) spectroscopy as a new technology for discrimination between Cryptococcus neoformans and Cryptococcus gattii. Anal Methods 8:7107–7115. https://doi.org/10.1039/C6AY01893A.
17. Silva S, Tobaldini-Valerio F, Costa-de-Oliveira S, Henriques M, Azeredo J, Ferreira EC, Lopes JA, Sousa C. 2016. Discrimination of clinically relevant Candida species by Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR). RSC Adv 6:92065–92072. https://doi.org/10.1039/C6RA16769A.
18. Essendoubi M, Toubas D, Lepouse C, Leon A, Bourgeade F, Pinon J-M, Manfait M, Sockalingum GD. 2007. Epidemiological investigation and typing of Candida glabrata clinical isolates by FTIR spectroscopy. J Microbiol Methods 71:325–331. https://doi.org/10.1016/j.mimet.2007.09.018.
19. Toubas D, Essendoubi M, Adt I, Pinon J-M, Manfait M, Sockalingum GD. 2007. FTIR spectroscopy in medical mycology: applications to the differentiation and typing of Candida. Anal Bioanal Chem 387:1729–1737. https://doi.org/10.1007/s00216-006-1005-1.
20. Lecellier A, Gaydou V, Mounier J, Hermet A, Castrec L, Barbier G, Ablain W, Manfait M, Toubas D, Sockalingum G. 2015. Implementation of an FTIR spectral library of 486 filamentous fungi strains for rapid identification of molds. Food Microbiol 45:126–134. https://doi.org/10.1016/j.fm.2014.01.002.
21. Taha M, Hassan M, Essa S, Tartor Y. 2013. Use of Fourier transform infrared spectroscopy (FTIR) spectroscopy for rapid and accurate identification of yeasts isolated from human and animals. Int J Vet Sci Med 1:15–20. https://doi.org/10.1016/j.ijvsm.2013.03.001.
22. Naumann A. 2015. Fourier transform infrared (FTIR) microscopy and imaging of fungi, p 61–88. *In* Dahms TES, Czymmek KJ (ed), Advanced microscopy in mycology. Springer, New York, NY.
23. Novais Â, Freitas AR, Rodrigues C, Peixe L. 2019. Fourier transform infrared spectroscopy: unlocking fundamentals and prospects for bacterial strain typing. Eur J Clin Microbiol Infect Dis 38:427–448. https://doi.org/10.1007/s10096-018-3431-3.
24. CLSI. 2017. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-ED4. Clinical and Laboratory Standards Institute, Wayne, PA.
25. CLSI. 2018. Performance standards for antifungal susceptibility testing of yeasts. Approved standard M60-ED1. Clinical and Laboratory Standards Institute, Wayne, PA.
26. Lasch P, Naumann D. 15 September 2006. Infrared spectroscopy in microbiology. Encyclopedia of analytical chemistry: applications, theory and instrumentation. https://doi.org/10.1002/9780470027318.a0117.
27. Kurtzman C, Robnett C. 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5'end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 35:1216–1223. https://doi.org/10.1128/jcm.35.5.1216-1223.1997.
28. White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols 18:315–322.
29. Carreté L, Ksiezopolska E, Gómez-Molero E, Angoulvant A, Bader O, Fairhead C, Gabaldón T. 2019. Genome comparisons of Candida glabrata serial clinical isolates reveal patterns of genetic variation in infecting clonal populations. Front Microbiol 10:112. https://doi.org/10.3389/fmicb.2019.00112.
30. Pebotuwa S, Kochan K, Peleg A, Wood BR, Heraud P. 2020. Influence of the sample preparation method in discriminating Candida spp. using ATR-FTIR spectroscopy. Molecules 25:1551. https://doi.org/10.3390/molecules25071551.
31. Carranza L. 2012. Standardization and internal validation of a bacteria identification method utilizing focal-plane-array Fourier transformed infrared spectroscopy. McGill University, Canada.
32. Wehrli PM, Lindberg E, Svensson O, Sparén A, Josefson M, Dunstan RH, Wold AE, Gottfries J. 2014. Exploring bacterial phenotypic diversity using factorial design and FTIR multivariate fingerprinting. J Chemometrics 28:S681–S686. https://doi.org/10.1002/cem.2588.
33. Ribeiro da Cunha B, Fonseca LP, Calado CR. 2019. A phenotypic screening bioassay for Escherichia coli stress and antibiotic responses

based on Fourier-transform infrared (FTIR) spectroscopy and multivariate analysis. J Appl Microbiol 127:1776–1789. https://doi.org/10.1111/jam.14429.
34. bioMerieux—Diagnostics. 2020. VITEK MS mass spectrometry microbial identification system: comprehensive, regularly-updated database. https://www.biomerieux-diagnostics.com/vitekr-ms-0#VITEK%C2%AE%C2%A0SOLUTIONS:%20Complete%20traceability%20and%20flexibility.
35. USFDA. 2018. K181412: Vitek MS, clinical mass spectrometry microorganism identification and differentiation system. https://www.accessdata.fda.gov/cdrh_docs/pdf18/K181412.pdf.
36. Hu Y, Zhou H, Lu J, Sun Q, Liu C, Zeng Y, Zhang R. 2021. Evaluation of the IR Biotyper for Klebsiella pneumoniae typing and its potentials in hospital hygiene management. Microb Biotechnol 14:1343–1352. https://doi.org/10.1111/1751-7915.13709.