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Original Article

Species identification and antifungal susceptibility of uncommon blood yeast isolates

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Abstract *Background/Purpose:* Accurate identification of *Candida* species is increasingly important in the era of emergence of *Candida auris*. We aimed to compare the identification performance of two matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems (Vitek MS and Bruker biotyper MS) and an oligonucleotide array

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Susceptibility;
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Yeast

for uncommon blood yeast isolates and demonstrate the susceptibilities among those isolates.
Method: *Candida* species isolates from blood culture other than *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* identified by biochemical methods were collected from multiple hospitals and further identified by an oligonucleotide array based on the internal transcribed spacer-1 (ITS-1) and ITS-2 sequences of the rRNA genes, Vitek MS and Bruker biotyper MS. The minimal inhibitory concentrations (MICs) of these clinical isolates were determined by the Sensititre YeastOne (SYO) system.
Results: Among 136 isolates, *Candida guilliermondii* was most common (52, 38.2%), followed by *C. lusitaniae* (13, 9.6%) and *C. haemulonii* (12, 8.8%). The oligonucleotide array, Vitek MS and Bruker biotyper MS correctly identified 89.7% (122), 90.4% (123), and 92.6% (126) of these isolates, respectively. Elevated minimal inhibitory concentrations (MICs) of fluconazole were observed for *C. haemulonii* (MIC_{90}: 256 mg/L), and *C. guilliermondii* (MIC_{90}: 16 mg/L) with 28.4% of uncommon *Candida* isolates with MIC $\geqq$ 8 mg/L.
Conclusions: For uncommon *Candida* species, the unmet need for current databases of two commercial MALDI-TOF MS systems is highlighted, and the oligonucleotide array may serve as a supplement.

Introduction

Candidemia is associated with a high attributable mortality rate (up to 30%) and substantial medical cost.[1] Due to diverse susceptibilities to antifungal agents and high mortality resulting from a delay in the initiation of antifungal therapy,[2] precise and rapid pathogen identification in patients with candidemia is essential. Although common *Candida* species, namely *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*, account for more than 90% of candidemia,[3] a variety of uncommon *Candida* species may cause candidemia[4—6] and some species have been reported to be less susceptible to current antifungal agents.[7]

Echinocandins have been recommended as target treatment of candidemia in current guidelines, but routine use of echinocandin might alter species distribution of invasive candidiasis and enhance selection pressure for resistance.[8] The appropriate antifungal treatment depends on rapid identification of *Candida* species. However, biochemical identification methods commonly used in clinical laboratories might misidentify *C. auris* as *C. haemulonii*, or *C. guilliermondii* as *C. famata*,[9,10] and incorrect identification may lead to inappropriate antifungal treatment.

Two commercial matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems, including Bruker MALDI-TOF Biotyper and bioMérieux Vitek MS, have demonstrated high accurate rate for identification of *Candida* species, but some discrepancies for uncommon *Candida* species were noted.[11—14] In addition to MALDI-TOF MS, an in-house oligonucleotide array based on the internal transcribed spacer-1 (ITS-1) and ITS-2 sequences of rRNA was able to identify 77 species of clinical relevant yeasts.[15] It has been used in diagnosis of invasive fungal infections as well.[16—18]

Because the numbers of uncommon yeast isolates in the studies mentioned above were limited and the identification performance of different identification modalities have not been compared, we aimed to compare the identification performance of several selected diagnostic methods or systems and determine the *in vitro* susceptibility of uncommon yeast isolates to antifungal agents.

Methods

Candida isolates

Between 2011 and 2016, yeast isolates were collected from blood cultures in several medical centers in Taiwan including National Taiwan University Hospital, National Cheng Kung University Hospital, Chi Mei Medical Center and Chi Mei Medical Center, Liouying Campus. *Candida* species other than *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. krusei* identified by biochemical methods were collected. In the patients who had two or more episodes of candidemia during the study period, only the first isolate was included. After gene sequencing for species identification, 136 isolates were included for further species determination by the oligonucleotide array, Vitek MS, and Bruker Biotyper MS.

Gene sequencing of yeast isolates

The D1-D2 region of the 28S rRNA genes and ITS region of each isolate were amplified by polymerase chain reaction (PCR) and sequenced on a model 377 sequencing system (Applied Biosystems, Taipei, Taiwan). The DNA sequences were compared with uploaded sequences in the BLAST database. A threshold of $\geq$99% sequence identity was applied to determine the species. The fungus-specific primers ITS1 (5′-TCCGTAGGT GAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTT ATTGATATGCC-3′) were used to amplify the ITS region,[19] and the primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCG TGTTTCA AGACGG-3′) for the D1-D2 region.[20]

Oligonucleotide array

The species-specific probes in the oligonucleotide array were designed based on the ITS-1 and ITS-2 regions of the rRNA genes. The design process of the array and the sequences of the probes were published earlier.[15] The genomic DNA of targeted colonies was extracted first, and the ITS regions were amplified by the fungus-specific universal primers, ITS1 (5′-DIG-TCCGTAGGTGA ACCTGCGG-3′) and ITS4 (5′-DIG-TCCTCCGCTTATTGATATGC-3′). The amplified products encompassing ITS-1, ITS-2, and partial regions of the 18S and 28S rRNA genes, were hybridized with the species-specific probes as previously described.[15]

MALDI-TOF MS, Vitek MS system

First, each yeast isolate was applied directly onto a target slide composed of 48-spots and then lysed with 0.5 μl of 25% formic acid. Then, 1 μl of α-cyano-4-hydroxycinnamic acid matrix solution was applied to the spots after drying prior to Vitek MS (*BioMérieux*, Marcy-l'Étoile, France). Using Vitek MS (MS—ID version 3.0 knowledge base clinical use or *in vitro* devices [IVD]), the identification scheme produced a confidence value for each isolate. When an isolate with a confidence value of >99.9 was found, the identification result was retained. For the isolate without confirmed identification, the sample preparation was repeated and analyzed again. If the repeated analysis still failed to show matching species with adequate confidence values, the identification result will be recorded as "no matched species in the database".

MALDI-TOF MS, Bruker Biotyper system

A single colony of each yeast isolate was mixed with 300 μL of distilled water and 900 μL of ethanol in an Eppendorf tube. The sample was centrifuged at 13,000 rpm for 2 min, and the supernatant was discarded. The process of centrifugation and removal of supernatant was repeated. Twenty-five μL of 70% formic acid and 25 μL of 100% acetonitrile were added to the sample, followed by centrifugation at 13,000 rpm for 2 min. One μL supernatant was spotted to the target plate and was left for air dry at room temperature. Then 1 μL of α-cyano-4-hydroxycinnamic acid matrix solution was applied to the plate and dried at room temperature prior to mass spectrometry analysis. The mass spectra profiles were acquired by the Bruker Biotyper MALDI-TOF mass spectrometry (Bruker Daltonics, Billerica, MA) and were imported into the Biotyper software (MALDI Biotyper version 3.1, MBT 6903 MSP Library). According to the manufacture's instruction, the identification cut-off score was interpreted as followed. A score of ≥2.000 indicated species-level identification, between 1.700 and 1.999 genus-level identification, and <1.700 no identification. Like the process of using Vitek MS system, the sample preparation was repeated and the sample was analyzed again if the first analysis failed to show matching species.

Yeast isolates with discrepant identification results

The yeast species derived from gene sequencing was regarded as the reference species, and compared with those from the oligonucleotide array, Vitek MS system, or Bruker Biotyper MS system. Only accurate identification to the species level was regarded as "correct identification". Misidentification indicated that the species by one of three studied methods was inconsistent with that gene sequencing. However, when the identification result is "no matched species in the database", it implies the imperfect coverage of the database instead of a wrong result. Therefore, such a result would not be counted as "correct identification" nor "misidentification".

Antifungal susceptibility

Susceptibility to nine antifungal agents was determined by the broth microdilution method of the Sensititre YeastOne (SYO) system (part YO-10; TREK Diagnostic Systems, East Grinstead, U.K.) according to the manufacturer's instructions. In brief, we first prepared a working suspension of the organism containing approximately 1.5×10^3 CFU/mL in the SYO inoculum broth, and then the dried SYO panel was rehydrated by dispensing 100 μL of the working yeast suspension. After 24 h of incubation, the colorimetric MIC endpoint was read. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as the quality control strains. For *C. guilliermondii*, the susceptibility criteria for echinocandins were based on those of the Clinical and Laboratory Standards Institute.[21] For *Candida* species with available epidemiologic cutoff values (ECVs), non-wild-type isolates were those with MICs above the ECVs.[22,23] Applied epidemiological cutoff values and clinical breakpoints for antifungal agents by *Candida* species are listed in Table 1.

Results

The species distribution of 136 blood yeast isolates identified by the reference method is shown in Table 2. *C. guilliermondii* was most common (52 isolates, 38.2%), followed by *C. lusitaniae* (13, 9.6%) and *C. haemulonii* (12, 8.8%). The isolate numbers of correct identification and misidentification by the oligonucleotide array, Bruker Biotyper MS and Vitek MS are also listed in this table. All three methods demonstrated good identification performance. The accurate identification rate of the oligonucleotide array, Vitek MS and Bruker Biotyper MS was 89.7%, 90.4%, and 92.6%, respectively. However, there were 12 isolates (8.8%), 11 isolates (8.1%), and 7 isolates (5.1%) matching no species in the database by using the oligonucleotide array, Vitek MS and Bruker Biotyper MS, respectively. There were 20 isolates not classified to uncommon *Candida* species after gene sequencing, including 7 isolates identified as common *Candida* species and 13 isolates identified as non-*Candida* species. Then, when we focused on isolates of uncommon *Candida* species, the oligonucleotide array, Vitek MS, and Bruker Biotyper MS system could identify correctly 90.5%, 93.1% and 92.2% of 116 isolates.

Table 1 Epidemiological cutoff values and clinical breakpoints for antifungal agents by *Candida* species.

Species	Antifungal agents (μg/ml)								
	Amphotericin B	Flucytosine	Anidulafungin	Caspofungin	Micafungin	Fluconazole	Itraconazole	Posaconazole	Voriconazole
C. guilliermondii									
ECV	2	1	4	2	2	16[a]	1[a]	1[a]	0.5[a]
CBP									
Susceptible	—	—	≤2	≤2	≤2	—	—	—	—
Intermediate	—	—	4	4	4	—	—	—	—
Resistant	—	—	≥8	≥8	≥8	—	—	—	—
C. lusitaniae									
ECV	2	0.5	2	1	0.5	4[a]	0.5[a]	0.12[a]	0.03[a]
C. pelliculosa									
ECV	—	—	—	0.12	—	4	—	2	0.25
C. dubliniensis									
ECV	2	0.5	0.12	0.12	0.12	1[a]	0.25[a]	0.12[a]	0.01[a]

[a] Some ECV of azoles in *Candida* speceis were updated based on reference 23. Method-Dependent Epidemiological Cutoff Values for Detection of Triazole Resistance in *Candida* and *Aspergillus* Species for the Sensititre YeastOne Colorimetric Broth and Etest Agar Diffusion Methods. *Antimicrob Agents Chemother* 2018; 63(1):e01651-18.
ECV: epidemiological cutoff value; CBP: clinical breakpoints.
All ECVs were based on reference 22 except values with footnote "a". Reference 22:Progress in antifungal susceptibility testing of Candida spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. *J Clin Microbiol* 2012; 50(9):2846—2856.

There were 12 (8.8%) isolates misidentified by biochemical identification. In contrast, the oligonucleotide array, Vitek MS, and Bruker Biotyper MS system misidentified 1.5% (2 isolates), 1.5% (2) and 2.2% (3) of 136 isolates, respectively. Those isolates misidentified by one method could be identified correctly by the other two tools (Table 3). In addition to one isolate of *C. guilliermondii*, there were two isolates of *Lodderomyces elongisporus* and *Trichosporon capitatum*, respectively, misidentified as *C. sake* by the biochemical reactions. Similarly, there were several *Candida* species, *Rhodotorula rubra*, and *Trichosporon montevideense* were misidentified. For the oligonucleotide array, a *C. fabianii* and a *C. glabrata* isolate were misidentified as *Hansenula saturnus* and *C. globosa*. For two MALDI-TOF MS systems, the misidentified species were *C. guilliermondii*, *C. intermedia*, and *C. antarctica* by the Bruker Biotyper MS system, and *C. haemulonii* and *C. metapsilosis* by the Vitek MS system.

Excluding 7 isolates identified as common *Candida* species and 13 isolates identified as non-*Candida* species, there were 116 uncommon *Candida* species further examined for the susceptibility. When we employed the previously proposed clinical breakpoint (≧ 8 mg/L) of fluconazole for *Candida* species,[24] there were 28.4% (n = 33) fluconazole-resistant isolates. The MICs of uncommon *Candida* species which has more than 10 isolates are shown in Table 4, and the other species with less number of isolates are listed in Supplemental Table 1. Of 52 *C. guilliermondii* isolates, <4% were resistant to echinocandins, and non-wild types for azoles accounting for less than 10%. *C. haemulonii* (12 isolates) and *C. norvegensis* (5) exhibited high fluconazole MICs (*C. haemulonii*: MIC_{50}/MIC_{90} 16/256 mg/L; *C. norvegensis*: MIC_{50}/MIC_{90} 32/64 mg/L). In contrast, 13 *C. lusitaniae* isolates were wild-types for all antifungal agents, but all 5 *C. dubliniensis* isolates were non-wild type for voriconazole. All seven *C. pelliculosa* isolates were wild type for posaconazole and voriconazole, but two (28.6%) were non-wild type isolates for fluconazole. Four *C. intermedian* isolates and two *C. phangngaensis* isolates exhibited low MICs of fluconazole (MIC ≤ 2 mg/L).

Discussion

In our study, the oligonucleotide array, Vitek MS, and Bruker Biotyper MS systems were able to identify most uncommon yeast blood isolates, but there was approximately 10% of isolates which were not identified correctly. Furthermore, there were more than a quarter of uncommon *Candida* isolates exhibiting elevated MICs of fluconazole (i.e., 28.4% isolates with MICs ≧ 8 mg/L). These misidentified species might lead to suboptimal treatment. Our findings highlight the need for continuing improvement of species identification to guide antifungal therapy for invasive infection caused by uncommon yeast species.

The identification for uncommon *Candida* species by biochemical methods seems unreliable because 12 (8.8%) isolates were misidentified as other species. Each *Lodderomyces* and *Rhodotorula* isolate has been misidentified as *Candida* species, and some common *Candida* species, e.g., *C. albicans*, were misidentified as uncommon *Candida*

Table 2 Number of isolates of correct identification and misidentification by oligonucleotide array, Vitek MS and Bruker Biotyper.

Final identification	Number of isolates (%)	Correct identification (number of isolates)			Misidentification (number of isolates)		
		Oligonucleotide array	Vitek MS	Bruker Biotyper	Oligonucleotide array	Vitek MS	Bruker Biotyper
Candida guilliermondii	52(38.2)	52	52	48	0	0	1
Candida lusitaniae (*Clavispora lusitaniae*)	13(9.6)	13	13	13	0	0	0
Candida haemulonii	12(8.8)	12	11	12	0	1	0
Candida pelliculosa	7(5.1)	7	6	6	0	0	0
Lodderomyces elongisporus	6(4.4)	6	6	6	0	0	0
Candida dubliniensis	5(3.7)	5	5	5	0	0	0
Candida norvegensis (*Pichia norvegensis*)	5(3.7)	5	5	5	0	0	0
Candida intermedia	4(2.9)	4	4	3	0	0	1
Candida lipolytica (*Yarrowia lipolytica*)	4(2.9)	4	4	4	0	0	0
Candida orthopsilosis	4(2.9)	0	4	4	0	0	0
Candida parapsilosis	3(2.2)	3	3	3	0	0	0
Cryptococcus curvatus	3(2.2)	3	0	3	0	0	0
Candida albicans	2(1.5)	2	2	2	0	0	0
Candida metapsilosis	2(1.5)	0	0	2	0	1	0
Candida phangngaensis	2(1.5)	0	0	0	0	0	0
Candida rugosa	2(1.5)	2	2	2	0	0	0
Candida antarctica (*Trichosporon oryzae*)	1(0.7)	0	0	0	0	0	1
Candida catenulata	1(0.7)	1	1	1	0	0	0
Candida fabianii (*Hansenula fabianii*)	1(0.7)	0	0	1	1	0	0
Candida glabrata	1(0.7)	0	1	1	1	0	0
Candida pararugosa	1(0.7)	0	1	1	0	0	0
Candida tropicalis	1(0.7)	1	1	1	0	0	0
Pichia ohmeri	1(0.7)	1	1	1	0	0	0
Rhodotorula rubra	1(0.7)	1	0	0	0	0	0
Trichosporon capitatum	1(0.7)	0	1	1	0	0	0
Trichosporon montevideense	1(0.7)	0	0	1	0	0	0
Total	136	122	123	126	2	2	3
%		89.7	90.4	92.6	1.5	1.5	2.2

Table 3 *Candida* and other yeast isolates with misidentification by biochemical identification, oligonucleotide array, Vitek MS, and Bruker Biotyper MS system (isolate No.).

Final identification	Biochemical identification	Oligonucleotide array	Vitek MS	Bruker Biotyper
C. guilliermondii	*C. sake* (1)	-	-	*C. parapsilosis* (1)
C. haemulonii		-	*C. krusei* (1)	-
C. parapsilosis	*C. norvegensis* (1), *C. famata* (1)	-	-	-
C. metapsilosis	*Kodamaea ohmeri* (1)	-	*Blastomyces dermatitdis* (1)	
Lodderomyces elongisporus	*C. sake* (2)	-	-	-
C. intermedia		-	-	*C. haemulonii* (1)
C. albicans	*C. dubliniensis* (1)	-	-	-
C. phangngaensis	*C lipolytica* (1)	-	-	-
C. antarctica (*Trichosporon oryzae*)	*C. lipolytica* (1)	-	-	*Pseudozyma aphidis* (1)
C. fabianii (*Hansenula fabianii*)		*Hansenula saturnus* (1)	-	-
C. glabrata		*C. globosa* (1)	-	-
Rhodotorula rubra	*C. haemulonii* (1)	-	-	-
Trichosporon capitatum	*C. sake* (1)	-	-	-
Trichosporon montevideense	*C. guilliermondii* (1)	-	-	-

Table 4 In vitro susceptibility of blood isolates of uncommon *Candida* species which has more than 10 isolates.

Species	AMB	5FC	AFG	CAS	MFG	FLC	ITC	POS	VRC
C. guilliermondii (n = 52)									
MIC_{50} (mg/L)	0.5	≦0.06	1	0.375	0.5	4	0.5	0.25	0.12
MIC_{90} (mg/L)	1	0.12	2	2	1	16	1	1	0.5
MIC range (mg/L)	≦0.12–2	≦0.06–0.25	≦0.015- > 8	≦0.008–8	≦0.008- > 8	≦0.12- >256	≦0.015- >16	≦0.008- >8	≦0.008–8
No. (%) of isolates by CBPs									
Susceptible	–	–	50 (96.2)	50 (96.2)	50 (96.2)	–	–	–	–
Intermediate	–	–	1 (1.9)	0	1 (1.9)	–	–	–	–
Resistant	–	–	1 (1.9)	2 (3.9)	1 (1.9)	–	–	–	–
No. (%) of isolates by ECVs									
Wild type	52 (100)	52 (100)	51 (98.1)	50 (96.2)	50 (96.2)	48 (92.3)	50 (96.2)	51 (98.1)	48 (92.3)
Non-wild type	0	0	1 (1.9)	2 (3.9)	2 (3.9)	4 (7.7)	2 (3.9)	1 (1.9)	4 (7.7)
C. lusitaniae (n = 13)									
MIC_{50} (mg/L)	0.5	0.06	0.25	0.25	0.06	0.5	0.06	0.03	0.008
MIC_{90} (mg/L)	1	0.06	0.25	0.5	0.12	1	0.12	0.03	0.015
MIC range (mg/L)	≦0.12–1	≦0.06–0.12	≦0.015–0.25	≦0.008–0.5	≦0.008–0.12	≦0.12–2	≦0.015–0.25	≦0.008–0.12	≦0.008–0.03
No. (%) of isolates by ECVs									
Wild type	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)	13 (100)
Non-wild type	0	0	0	0	0	0	0	0	0
C. haemulonii (n = 12)									
MIC_{50} (mg/L)	2	≦0.06	0.12	0.12	0.12	16	0.5	0.375	0.25
MIC_{90} (mg/L)	2	0.12	0.12	0.25	0.25	256	16	8	8
MIC range (mg/L)	≦0.12–4	≦0.06–0.12	≦0.015–0.25	≦0.008–0.25	≦0.008–0.25	≦0.12- > 256	≦0.015- > 16	≦0.008- > 8	≦0.008–8

AMB, amphotericin B; 5FC, flucytosine; AFG, anidulafungin; CAS, caspofungin; MFG, micafungin; FLC, fluconazole; ITC, itraconazole; POS, posaconazole; VRC, voriconazole; ECVs, epidemiological cutoff values; CBPs, clinical breakpoints.

species. The unreliability of conventional phenotypic methods was consistent with a previous report, in which most of isolates previously identified as *C. famata* by the Vitek 2 biochemical system were was re-identified as *C. tropicalis* and *C. albicans* by the ITS sequencing.[25] The misidentification of uncommon *Candida* species may not only result in treatment failure but also carry a risk of outbreak of resistant pathogens such *C. auris*.[6] Our findings emphasize the possibility of identification inaccuracy of commercial identification systems using biochemical methods when an uncommon yeast species is reported, and the requirement of other tools for species confirmation.

The numbers of uncommon yeast species in previous surveys comparing identification performance of two MALDI-TOF MS (Vitek MS and Bruker Biotype MS) systems were usually limited.[11,13,14] However, Wang et al. included 53 *C. guilliermondii* isolates, which were correctly identified by the Bruker Biotyper MS system.[12] In contrast, the Bruker Biotyper MS system with the same version of database failed to identify four (7.7%) of 52 *C. guilliermondii* isolates in our study. More clinical isolates are needed to determine the identification performance of MALDI-TOF MS for *C. guilliermondii*.

It is noteworthy that three *Cryptococcus curvatus* isolates were not accurately identified by the Vitek MS system, despite of the inclusion of *C. curvatus* in the database. In addition, one *C. guilliermondii* isolate was misidentified as *C. parapsilosis* by the Bruker Biotyper MS system, and one *C. metapsilosis* isolate as *Blastomyces dermatitdis* by the Vitek MS system in our study. Of note, the oligonucleotide array, Vitek MS, and Bruker Biotyper MS systems will not misidentify any single isolate at the same time. This finding suggests one of these diagnostic tools may serve as a supplement for the other two. Even though the oligonucleotide array took a longer turnaround time (8 h) than two MALDI-TOF MS systems, the low cost makes it as an alternative tool when the other modality is not available.

Our *C. dubliniensis* and *C. lusitaniae* isolates exhibited low MICs of fluconazole, which was in line with previous studies.[26,27] Likewise, our *C. haemulonii* isolates exhibited high MICs for fluconazole.[9,28] On the other hand, we revealed high MICs for fluconazole among *C. guilliermondii* isolates, as noted previously.[27,29] However, some reports showed higher fluconazole susceptibility for *C. guilliermondii* isolates.[30,31] This discrepancy may result from geographic variation and underscore the importance of antifungal resistance surveillance in different areas.

There were several limitations in this study. First, though the included clinical isolates were collected from multiple hospitals in Taiwan, our results should not be generalized to other countries. However, due to the scarcity of uncommon yeast isolates, the inclusion of more than one hundred of clinical isolates in our study can add valuable information to clinical practices. Second, the identification performance of MALDI-TOF MS systems might vary for different versions of database. The database of Vitek MS we used was IVD, but we did not evaluate the Vitek MS database for research only (RUO). Third, the biochemical methods used in different hospitals were not uniform during the whole study period. For example, Vitek Yeast Biochemical Card had been used in the early years and then was changed to Vitek 2 YST ID Card. The specific biochemical method in each hospital and the timing of method change were not available. Finally, antifungal susceptibility was determined by the SYO system, not the reference CLSI method. However, the agreement of both methods was recognized to be 96%.[32]

In summary, the conventional phenotypic method failed to identify uncommon yeast species in some occasions, and some uncommon species, such as *C. guilliermondii* and *C. haemulonii*, exhibited elevated MICs of azole in our study. The inability to identify uncommon yeast species may be supplemented by the oligonucleotide array, Vitek MS, or Bruker Biotyper MS system.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.01.009.