AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Identification and Antifungal Susceptibility Profile of *Candida guilliermondii* and *Candida fermentati* from a Multicenter Study in China

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With molecular sequencing as a gold standard, the Vitek MS, Bruker Biotyper MS, and Vitek-2 Compact systems correctly identified 92.7%, 97.0%, and 15.2% of 164 *Candida guilliermondii* isolates, respectively, and none of 8 *C. fermentati* isolates. All of the isolates showed high susceptibility to echinocandins, but some *C. guilliermondii* isolates showed low azole susceptibility.

Invasive candidiasis (IC) is a public health threat worldwide and is considered a major cause of infection-related morbidity and mortality (1, 2). The incidence of candidemia due to *Candida guilliermondii* is low, ranging from 1% to 3%, depending on the geographic region (3, 4). However, the few available reports on *C. guilliermondii* infections indicate that these organisms are associated with poor clinical outcomes, which warrants further investigation (5–9).

C. guilliermondii complex is a genetically heterogeneous group of phenotypically indistinguishable yeast species, including *C. guilliermondii*, *C. fermentati*, *C. carpophila*, and *C. xestobii*. Accurate and timely identification of *C. guilliermondii* complex isolates to the species level, including the associated antifungal susceptibility profiles, is essential for guiding clinical decisions (8). However, there is limited information on the performance of phenotypic methods like the Vitek-2 Compact (bioMérieux, France), and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems in the identification of species within the *C. guilliermondii* complex in China.

C. guilliermondii complex isolates ($n = 172$) collected from 40 hospitals in 18 provinces of China, under the China Hospital Invasive Fungal Surveillance Net (CHIF-NET) program (2010 to 2014), were studied. We evaluated the performance of the Vitek-2 Compact (bioMérieux, France) and two MALDI-TOF MS systems, including the Vitek MS system (IVD Knowledgebase version 2.0; bioMérieux) and the Bruker Autoflex Speed TOF/TOF MS system (with Biotyper version 3.1 software; Bruker Daltonics, USA) in the identification of *C. guilliermondii* complex isolates. Sequencing of the ribosomal DNA (rDNA) internal transcribed spacer (ITS) region was used as the gold standard (10).

In vitro susceptibilities of the isolates to nine antifungal drugs were determined by the Sensititre YeastOne YO10 (Thermo Scientific, USA) system as per the manufacturer's instructions. MIC values were interpreted according to the CLSI document for echinocandins (11) and epidemiological cutoff values (ECVs) for the other agents (12). This study was approved by the relevant Human Research Ethics Committee (S-263).

Isolates were derived from 172 patients (117 males and 55 females), with an average age of 54 ± 23.1 years. Detailed information on the origin of the isolates (location and specimen type) is available in Table 1. The majority of the isolates were from surgical department (36.6%) and intensive care unit (ICU) (32.6%); patients admitted in these departments are more likely to have serious underlying disease or cancer or to be immunocompromised, which may be risk factors for infections (8, 13). Overall, *C. guilliermondii* complex isolates represented 1.7% (164/9,673) of all the yeast isolates for the 5-year study period and specifically accounted for 2.6% (106/4,122) of all yeasts from blood cultures.

TABLE 1 Distribution of 172 *C. guilliermondii* complex isolates by department and specimen type

Distribution	No.	%
Department		
Surgery department	63	36.6
Intensive care unit	56	32.6
Medical department	31	18.0
Outpatient and emergency department	11	6.4
Other	11	6.4
Specimen type		
Blood culture	111	64.5
Ascetic fluid	17	9.9
Catheter	13	7.6
Pus	11	6.4
Other[a]	20	11.6

[a] Includes cerebrospinal fluid, pleural fluid, bile, and bronchoalveolar lavage fluid.

Among the 172 *C. guilliermondii* complex isolates, 164 (95.3%) isolates were confirmed as *C. guilliermondii* and 8 as *C. fermentati* by ITS gene sequencing. Identification results obtained by the Vitek-2 Compact and the two MALDI-TOF MS systems are shown in Table 2. By using an acceptable confidence value of 99.9% for the Vitek MS system and an identification score of

Received 4 May 2016 **Returned for modification** 23 May 2016
Accepted 25 May 2016
Accepted manuscript posted online 1 June 2016
Citation Cheng J-W, Yu S-Y, Xiao M, Wang H, Kudinha T, Kong F, Xu Y-C. 2016. Identification and antifungal susceptibility profile of *Candida guilliermondii* and *Candida fermentati* from a multicenter study in China. J Clin Microbiol 54:2187–2189. doi:10.1128/JCM.00938-16.
Editor: D. W. Warnock, University of Manchester
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TABLE 2 Performance of Vitek MS, Bruker Biotyper MS and Vitek-2 Compact systems compared with ITS gene sequencing for the identification of 164 *C. guilliermondii* isolates

Identification system	No. (%) of isolates			
	Correct identification to species level	Correct identification to genus level	No identification (invalid result)	Misidentification
Vitek MS system	152 (92.7)	7 (4.3)	3 (1.8)	2 (1.2)
Bruker Biotyper MS system	159 (97.0)[a]	0 (0)	5 (3.0)	0 (0)
Vitek-2 Compact system	25 (15.2)	122 (74.4)[b]	0 (0)	17 (10.4)[c]

[a] 130 isolates with identification scores of ≥2.0 and 29 isolates with identification scores of 1.7 to 1.99.
[b] Low discrimination between *C. famata* and *C. guilliermondii*.
[c] Misidentified as *C. famata*.

≥1.700 for the Bruker Biotyper MS system for correct identification of *C. guilliermondii* to the species level, the Vitek MS and Bruker Biotyper MS systems performed better than the Vitek 2 Compact system, accurately identifying 92.7% and 97.0% of this species, compared to 15.2% for the Vitek 2 Compact system ($P < 0.01$). All 3 systems failed to identify any of the 8 *C. fermentati* isolates due to noncoverage of these species in the respective databases. To improve identification accuracy of this species in the future, we have added mass spectral profiles (MSPs) of the 8 *C. fermentati* isolates to the Bruker system.

The antifungal susceptibilities of the 172 isolates to nine antifungal agents are shown in Table 3. All three echinocandins exhibited good antifungal activity against the 164 *C. guilliermondii* isolates (97% to 99.4%). For azoles, itraconazole showed the highest susceptibility rate (96.9%), followed by posaconazole (95.7%), voriconazole (90.2%), and fluconazole (89.0%). Sixteen (9.7%) isolates were cross-resistant to azoles, including five (3.0%) that were of the non-wild-type (WT) phenotype to all four azoles tested. Moreover, 93.9% and 100% of the isolates were assigned to be WT by ECVs to 5-flucytosine and amphotericin B, respectively. The eight *C. fermentati* isolates were all susceptible to the three echinocandins by breakpoints (BPs) and belonged to the WT for the other agents by ECVs.

In the present study, 2.6% of all candidemia cases were caused by *C. guilliermondii*, which is similar to findings elsewhere (1 to 3%) (3, 4). In addition, a rise in the rate of isolation of *C. guilliermondii* was observed in the last 3 years (2012 to 2014) of the CHIF-NET program, which ultimately reached 3.7% in 2014. This sharp rise in the isolation rate of this organism, especially in bloodstream infections, is a cause for concern which warrants further surveillance. Previous studies indicated that *C. fermentati* strains constitute about 9% of all species in the *C. guilliermondii* complex (14, 15), which disagrees with our study in which only 4.7% (8/172) were detected.

The Vitek-2 Compact system performed poorly in the identification of the 164 *C. guilliermondii* isolates, with only 15.2% of

TABLE 3 *In vitro* susceptibility of 172 isolates of *C. guilliermondii* and *C. fermentati* to nine antifungal agents as interpreted by breakpoints for three echinocandins and epidemiological cutoff values for other agents

C. guilliermondii complex (no. of isolates)	MIC(mg/liter)			No. (%) of isolates by ECVs[a]		No. (%) of isolates by BPs[b]		
	Range	MIC_{50}	MIC_{90}	WT	non-WT	S	I	R
C. guilliermondii (164)								
Fluconazole	1–>256	2	16	146 (89.0)	18 (11.0)			
Itraconazole	0.06–>16	0.12	0.5	159 (96.9)	5 (3.1)			
Voriconazole	0.015–>8	0.06	0.25	148 (90.2)	16 (9.8)			
Posaconazole	0.015–>8	0.12	5	157 (95.7)	7 (4.3)			
5-Flucytosine	≤0.06–>64	0.06	0.06	154 (93.9)	10 (6.1)			
Caspofungin	0.06–>8	0.25	1			163 (99.4)	0 (0)	1 (0.6)
Micafungin	0.06–>8	0.25	1			161 (98.2)	1 (0.6)	2 (1.2)
Anidulafungin	0.06–>8	1	2			159 (97.0)	2 (1.2)	3 (1.8)
Amphotericin B	0.12–1	0.25	0.5	164 (100)	0 (0)			
C. fermentati (8)								
Fluconazole	1–4			8 (100)	0 (0)			
Itraconazole	0.06–0.5			8 (100)	0 (0)			
Voriconazole	0.015–0.12			8 (100)	0 (0)			
Posaconazole	0.015–0.25			8 (100)	0 (0)			
5-Flucytosine	≤0.06			8 (100)	0 (0)			
Caspofungin	0.03–0.5					8 (100)	0 (0)	0 (0)
Micafungin	0.03–0.5					8 (100)	0 (0)	0 (0)
Anidulafungin	0.03–2					8 (100)	0 (0)	0 (0)
Amphotericin B	0.12–0.5			8 (100)	0 (0)			

[a] Epidemiological cutoff values (ECVs) were referred to CLSI M59 (11).
[b] Breakpoints (BPs) were referred to CLSI M27 (12).

isolates correctly identified to the species level, which is similar to the results of previous studies (16, 17). Furthermore, there was also a low discrimination rate of 74.4% (122 isolates) between *C. famata* and *C. guilliermondii*, and 10.4% (17 isolates) was misidentified as *C. famata*. The misidentification of *C. guilliermondii* may result in inappropriate treatment, especially given the high antifungal resistance associated with this species (18). Although there are differences between the two MALDI-TOF MS systems that affect spectra quality and hence identification scores and accuracy, both the Vitek MS and Bruker Biotyper MS systems exhibited high accuracy rates for identification of *C. guilliermondii* isolates, which is in agreement with our previous studies (19). The two MALDI-TOF systems may be effective tools for rapid routine identification of *C. guilliermondii* in the clinical microbiology laboratory, especially when labor and cost factors are considered.

In the present study, fluconazole showed the lowest activity against *C. guilliermondii* compared with that of the other azoles, with a WT rate of 89.0%, which is higher than the data reported from the global ARTEMIS DISK Antifungal Surveillance Program (75%) (3). Some of our isolates were a little less susceptible to the nine antifungal agents than in another study in Taiwan (96% to 100%) (13). Although cross-resistance to azoles has been reported sporadically in the literature (7–9), 9.7% of the isolates in the present study showed cross-resistance to azoles, which is an important consideration for antifungal therapy. Although the majority of the isolates were susceptible to echinocandins, the MICs for *C. guilliermondii* have been observed to be much higher than those for other common *Candida* species (14, 15).

The study is limited by the lack of representation of all species within the *C. guilliermondii* complex and the small number of *C. fermentati* isolates analyzed. Furthermore, not all *C. guilliermondii* complex species are covered in the Vitek MS and Bruker databases, which can affect identification accuracy. Performance may be improved by adding MSPs into the current databases.

ACKNOWLEDGMENTS

We thank all of the laboratories that participated in the CHIF-NET program in 2010 to 2014.

This work was supported by Research Special Fund for Public Welfare Industry of Health (Grant 201402001).

We declare no conflicts of interest.

FUNDING INFORMATION

This work, including the efforts of Ying-Chun Xu, was funded by Research Special Fund for Public Welfare Industry of Health (201402001).

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