JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2009, p. 242–244 Vol. 47, No. 1
0095-1137/09/$08.00+0 doi:10.1128/JCM.01889-08

Identification and Susceptibility Profile of *Candida fermentati* from a Worldwide Collection of *Candida guilliermondii* Clinical Isolates[▽]

Shawn R. Lockhart,* Shawn A. Messer, Michael A. Pfaller, and Daniel J. Diekema

University of Iowa, Carver College of Medicine, Iowa City, Iowa 52242

Received 30 September 2008/Returned for modification 3 November 2008/Accepted 17 November 2008

***Candida fermentati* isolates make up a small percentage of the clinical isolates of the *Candida guilliermondii* complex and have a global distribution pattern. With the exception that the MICs of micafungin were significantly lower, the calculated average MICs for *C. fermentati* were not significantly different from those for *C. guilliermondii*.**

Candida species are now the fourth leading cause of nosocomial bloodstream infections in U.S. hospitals and have one of the highest mortality rates among nosocomial pathogens (20). *Candida guilliermondii* makes up only a small proportion of these bloodstream infection isolates, ~1 to 3%, depending upon the geographic region (14), but what makes these isolates of particular clinical importance is the decreased susceptibility of this species to fluconazole and the relatively high MICs of this species to the echinocandins (4, 10, 12, 13, 14). There are also a number of recent reports of high infection rates in pediatric unit patients, a particularly vulnerable population (8, 11).

Using the strength of molecular identification, there has been an increased interest in identifying cryptic species within larger species complexes such as *Candida orthopsilosis*, *Candida metapsilosis*, and *Lodderomyces elongisporus* in the *Candida parapsilosis* complex (6, 7, 18) and *Candida nivariensis* and *Candida bracarensis*, members of the *Candida glabrata* clade (2, 3). With the parental species of many of these complexes carrying a decreased susceptibility to antifungal drugs, it is important to identify these cryptic species to determine whether they carry an increased burden of antifungal resistance (2, 3, 6, 7). In that light, we screened a large global collection of *C. guilliermondii* isolates for the presence of *Candida fermentati*, a closely related species for which there is almost no clinical information (1, 5).

All of the yeast isolates submitted to the University of Iowa as part of the ARTEMIS Antifungal Surveillance Program were identified with the Vitek yeast identification system (bioMérieux, Durham, NC). *Candida guilliermondii* was morphologically distinguished from *C. famata* by detection of pseudohyphae on corn meal agar following 10 days of incubation at 25°C. All isolates identified as *C. guilliermondii* were subject to PCR with the primers RIBO-F (5′ACAGTTGGTC GAGGTGGTC3′) and RIBO-R (5′CCTGGGTTCCCAAG TAGTCA3′). The identification scheme used to differentiate

TABLE 1. Demographic and antifungal susceptibility data for the *C. fermentati* isolates in this study

Isolate	Country	Yr	Sample	MIC (μg/ml)[a]						
				FLUC	VORI	POSA	Ampho B	CASPO	ANID	MICA
F15	Australia	2002	Blood	4	0.06	0.25	0.19	0.25	2	0.5
F40	United States	2003	NA[b]	4	0.06	0.12	0.094	0.25	1	0.25
F44	United States	2003	Blood	4	0.12	0.25	0.38	0.5	1	0.5
F47	Colombia	2003	Peritoneal fluid	16	0.25	0.25	0.38	0.5	2	0.5
F79	Turkey	2003	Sputum	16	2	0.5	0.38	0.5	4	0.5
F110	Korea	2004	Blood	16	0.25	0.25	1	0.25	1	0.25
F133	United States	2004	Blood	4	0.12	0.25	0.25	0.25	1	0.25
F137	United States	2004	NA	16	0.25	0.5	0.5	0.5	1	0.5
F146	Venezuela	2005	Blood	8	0.12	0.25	0.38	0.5	2	0.5
F148	Brazil	2005	Blood	16	0.5	0.5	0.38	0.5	2	0.5
F154	United States	2006	Blood	2	0.25	0.25	0.19	1	2	0.25
F165	Canada	2007	Blood	2	0.12	0.12	0.19	0.5	1	0.25
F176	Czech Republic	2007	Joint fluid	2	0.06	0.12	0.38	0.12	0.5	0.12

[a] Abbreviations: FLUC, fluconazole; VORI, voriconazole; POSA, posaconazole; Ampho B, amphotericin B; CASPO, caspofungin; ANID, anidulafungin; MICA, micafungin.
[b] NA, data not available.

* Corresponding author. Present address: Mycotic Diseases Branch, Centers for Disease Control and Prevention, 1600 Clifton Rd., Mailstop G-11, Atlanta, GA 30333. Phone: (404) 639-2569. Fax: (404) 639-3546. E-mail: gyi2@cdc.gov.
▽ Published ahead of print on 26 November 2008.

TABLE 2. Geometric mean MICs and statistical significance of differences between *C. guilliermondii* and *C. fermentati* MICs

Species or parameter	MIC (μg/ml) or *P* value						
	Fluconazole	Voriconazole	Posaconazole	Amphotericin B	Caspofungin	Anidulafungin	Micafungin
C. guilliermondii	3.17	0.07	0.22	0.18	0.46	1.60	0.57
C. fermentati	6.13	0.18	0.25	0.31	0.38	1.38	0.35
P value	0.079	0.13	0.46	0.088	0.056	0.362	0.0001

between *C. guilliermondii* and *C. fermentati* was described by Lan and Xu (5). Briefly, a single fragment of the riboflavin synthetase gene (*RIBO*) was amplified. In two separate reactions, the fragment was individually cleaved with the restriction enzyme HgaI or HincII. The presence of cleavage, as well as the size of the cleavage fragments, was used to differentiate between *C. guilliermondii* and *C. fermentati*. Only isolates that had one of the two outlined cleavage patterns were included in the analysis. *P* values were determined with the Student *t* test.

Of the isolates banked between 2001 and 2007, 149 were identified as *C. guilliermondii* complex isolates and 13 (8.7%) of those isolates were further identified as *C. fermentati*. The *C. fermentati* isolates originated from North America, South America, Europe, Asia, and Australia (Table 1). Previous *C. fermentati* isolates have been reported from India, the United Kingdom, Japan, and Brazil (8, 17, 19). There have been no clinical or surveillance reports of *C. fermentati* and very few of *C. guilliermondii* isolates from Africa, but this may be due to the paucity of information from that region. Eight of the 11 isolates for which epidemiological data were available were bloodstream isolates, similar to the 78% of *C. guilliermondii* isolates that were from bloodstream infections. While 80% of the patients with a *C. fermentati* infection were ≥40 years old, only 38% of the *C. guilliermondii* isolates came from patients who were ≥40 years old. Only 10% of the *C. fermentati* isolates were from a pediatric population, while 29% of the *C. guilliermondii* isolates were from pediatric patients.

Antifungal susceptibility testing was performed on all of the isolates by broth microdilution for fluconazole, voriconazole, posaconazole, caspofungin, anidulafungin, and micafungin and by Etest for amphotericin B as outlined in Clinical and Laboratory Standards Institute document M27-A2 and as previously described (9, 15) All MICs were determined visually. Echinocandin MICs were determined at 24 h, while azole and amphotericin B values were determined at 48 h. None of the 13 *C. fermentati* isolates were resistant to fluconazole, but 38% of them were susceptible dose dependent (SDD). The geometric mean MIC of fluconazole was higher than the geometric mean for the 136 *C. guilliermondii* isolates included in this study (Table 2), but the difference did not reach statistical significance. In a large global study of *C. guilliermondii* isolates, Pfaller and colleagues (14) found that 14% were SDD and 11% were fully resistant (although *C. fermentati* isolates were not distinguished in this study), and in a recent report from India of five isolates of *C. fermentati* from the oral cavities of human immunodeficiency virus patients, the reported geometric mean MIC of these isolates for fluconazole was 8 μg/ml, with one SDD isolate and one fully resistant isolate (19). There was one *C. fermentati* isolate SDD to voriconazole. Breakpoints for *Candida* spp. to posaconazole have not yet been established, but the differences between *C. fermentati* and *C. guilliermondii* were not statistically significant.

According to the recently recommended breakpoint value of 2 μg/ml for the echinocandins (16), only a single *C. fermentati* isolate with an anidulafungin MIC of 4 μg/ml fell outside of the susceptible range for any of the echinocandins tested. By comparison, 4% of the *C. guilliermondii* isolates were nonsusceptible to caspofungin, 11.8% were nonsusceptible to anidulafungin, and none were nonsusceptible to micafungin. It is interesting that the geometric mean MIC for *C. fermentati* and micafungin was statistically significantly lower than the geometric mean MIC for *C. guilliermondii*. Although the geometric mean MIC of the *C. fermentati* isolates against amphotericin B was higher than that for the *C. guilliermondii* isolates, all of the values fell below 0.5 μg/ml.

In conclusion, *C. fermentati* isolates are not significantly different enough from *C. guilliermondii* isolates in terms of antifungal susceptibility to warrant routine identification in the clinical microbiology laboratory. However, given the high average MICs of fluconazole reported here and elsewhere (19) and the ease with which *C. fermentati* and *C. guilliermondii* can be distinguished, this is a species for which further surveillance is warranted.

The findings and conclusions of this article are ours and do not necessarily represent the views of the CDC.

REFERENCES

1. **Bai, F.-Y.** 1996. Separation of *Candida fermentati* comb. nov. from *Candida guilliermondii* by DNA base composition and electrophoretic karyotyping. Syst. Appl. Microbiol. **19:**178–181.
2. **Bishop, J. A., N. Chase, S. S. Magill, C. P. Kurtzman, M. J. Fiandaca, and W. G. Merz.** 2008. *Candida bracarensis* detected among isolates of *Candida glabrata* by peptide nucleic acid fluorescence in situ hybridization: susceptibility data and documentation of presumed infection. J. Clin. Microbiol. **46:**443–446.
3. **Borman, A. M., R. Petch, C. J. Linton, M. D. Palmer, P. D. Bridge, and E. M. Johnson.** 2008. *Candida nivariensis*, an emerging pathogenic fungus with multidrug resistance to antifungal agents. J. Clin. Microbiol. **46:**933–938.
4. **Cuenca-Estrella, M., L. Rodero, G. García-Effrón, and J. L. Rodriguez-Tudela.** 2002. Antifungal susceptibilities of *Candida* spp. isolated from blood in Spain and Argentina, 1996-1999. J. Antimicrob. Chemother. **49:**981–987.
5. **Lan, L., and J. Xu.** 2006. Multiple gene genealogical analyses suggest divergence and recent clonal dispersal in the opportunistic human pathogen *Candida guilliermondii*. Microbiology **152:**1539–1549.
6. **Lockhart, S. R., S. A. Messer, M. A. Pfaller, and D. J. Diekema.** 2008. Geographic distribution and antifungal susceptibility of the newly described species *Candida orthopsilosis* and *Candida metapsilosis* in comparison to the closely related species *Candida parapsilosis*. J. Clin. Microbiol. **46:**2659–2664.
7. **Lockhart, S. R., S. A. Messer, M. A. Pfaller, and D. J. Diekema.** 2008. *Lodderomyces elongisporus* masquerading as *Candida parapsilosis* as a cause of bloodstream infections. J. Clin. Microbiol. **46:**374–376.
8. **Medeiros, E. A., T. J. Lott, A. L. Colombo, P. Godoy, A. P. Coutinho, M. S. Braga, M. Nucci, and M. E. Brandt.** 2007. Evidence for a pseudo-outbreak of *Candida guilliermondii* fungemia in a university hospital in Brazil. J. Clin. Microbiol. **45:**942–947.
9. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth microdilution antifungal susceptibility testing of yeast, 2nd

ed. Approved standard M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.
10. **Ostrosky-Zeichner, L., J. H. Rex, P. G. Pappas, R. J. Hamill, R. A. Larsen, H. W. Horowitz, W. G. Powderly, N. Hyslop, C. A. Kauffman, J. Cleary, J. E. Mangino, and J. Lee.** 2003. Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. Antimicrob. Agents Chemother. **47:**3149–3154.
11. **Pemán, J., M. Bosch, E. Cantón, A. Viudes, I. Jarque, M. Gómez-García, J. M. García-Martínez, and M. Gobernado.** 2008. Fungemia due to *Candida guilliermondii* in a pediatric and adult population during a 12-year period. Diagn. Microbiol. Infect. Dis. **60:**109–112.
12. **Pfaller, M. A., L. Boyken, R. J. Hollis, J. Kroeger, S. A. Messer, S. Tendolkar, and D. J. Diekema.** 2008. In vitro susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. J. Clin. Microbiol. **46:**150–156.
13. **Pfaller, M. A., L. Boyken, R. J. Hollis, S. A. Messer, S. Tendolkar, and D. J. Diekema.** 2006. In vitro susceptibilities of *Candida* spp. to caspofungin: four years of global surveillance. J. Clin. Microbiol. **44:**760–763.
14. **Pfaller, M. A., D. J. Diekema, M. Mendez, C. Kibbler, P. Erzsebet, S. C. Chang, D. L. Gibbs, and V. A. Newell.** 2006. *Candida guilliermondii*, an opportunistic fungal pathogen with decreased susceptibility to fluconazole: geographic and temporal trends from the ARTEMIS DISK antifungal surveillance program. J. Clin. Microbiol. **44:**3551–3556.
15. **Pfaller, M. A., D. J. Diekema, S. A. Messer, L. Boyken, and R. J. Hollis.** 2003. Activity of fluconazole and voriconazole determined by broth microdilution, disk diffusion, and Etest methods against 1,586 recent clinical isolates of *Candida* species: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. J. Clin. Microbiol. **41:**1440–1446.
16. **Pfaller, M. A., D. J. Diekema, L. Ostrosky-Zeichner, J. H. Rex, B. D. Alexander, D. Andes, S. D. Brown, V. Chaturvedi, M. A. Ghannoum, C. C. Knapp, D. J. Sheehan, and T. J. Walsh.** 2008. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. J. Clin. Microbiol. **46:**2620–2629.
17. **San Millán, R. M., L. C. Wu, I. F. Salkin, and P. F. Lehmann.** 1997. Clinical isolates of *Candida guilliermondii* include *Candida fermentati*. Int. J. Syst. Bacteriol. **47:**385–393.
18. **Tavanti, A., A. D. Davidson, N. A. R. Gow, M. C. J. Maiden, and F. C. Odds.** 2005. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. J. Clin. Microbiol. **43:**284–292.
19. **Umamaheswari, K., and T. Menon.** 2008. *Candida fermentati* from HIV patients in Chennai, South India. Int. J. Infect. Dis. **12:**e153–e154.
20. **Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond.** 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. **39:**309–317.