Clinical and Microbiological Characteristics of *Candida guilliermondii* and *Candida fermentati*

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Objectives: The *Candida guilliermondii* complex is a genetically heterogeneous complex of several phenotypically indistinguishable species, including *C. guilliermondii* and *Candida fermentati* (1). *C. guilliermondii* and *C. fermentati* cannot be distinguished by the commercial phenotypic and biochemical methods used in clinical practice (2). This complex is characterized by decreased susceptibility to echinocandin and azole antifungals (3,4), however, there have been limited studies reporting the epidemiological and clinical features of *C. guilliermondii* complex infections. This study aimed to clarify the clinical and microbiological characteristics of the *C. guilliermondii* complex.

Methods: Isolates were collected and re-identified by sequencing the internal transcribed spacer regions and restriction fragment length polymorphism of the intergenic spacer gene, confirming 27 *C. guilliermondii* and 12 *C. fermentati* isolates. Clinical information was reviewed retrospectively for all 39 patients. Susceptibility to eight antifungal agents was examined using a commercially prepared colorimetric microdilution panel, Sensititre YeastOne microtiter panel. Therapeutic effects of micafungin against *C. guilliermondii* clinical isolates were evaluated in a murine model of invasive candidiasis. We also explored differences in phenotype, including sensitivity to various stresses in vitro and resistance to candidacidal effect of murine macrophages, between *C. guilliermondii* and *C. fermentati*.

Results: Among all the clinical isolates, the majority (81.5%, n = 22) of *C. guilliermondii* strains, but none of the *C. fermentati* strains, were isolated from blood cultures (Table 1). Most *C. guilliermondii* and all *C. fermentati* isolates were susceptible to both azoles and echinocandins when evaluated by the *in vitro* microdilution test (Table 2). However, micafungin was significantly less effective against *C. guilliermondii* than against *Candida glabrata* in a mouse model of disseminated candidiasis (Figure 2). Phenotypic analyses revealed that *C. fermentati* strains were more sensitive to hydrogen peroxide than the *C. guilliermondii* strains. In addition, murine RAW 264 macrophages had higher candidacidal effects against *C. fermentati* than against *C. guilliermondii* (Figure 1).

Conclusion: This study demonstrates that *C. guilliermondii* and *C. fermentati* are closely related but have different microbiological and clinical characteristics, highlighting the importance of the accurate identification of these species.

Figure 1. Growth and killing assay in the presence of H₂O₂ and killing assay by RAW 264. (A) Serial dilutions of logarithmic-phase cells of *C. guilliermondii* and *C. fermentati* strains were spotted onto SC plates containing H₂O₂ at the indicated concentrations. (B) Logarithmic-phase cells of *C. guilliermondii* and *C. fermentati* were treated with 5 mM H₂O₂ for 4 h. The reduced viable cell counts of *C. guilliermondii* and *C. fermentati* were 1.1 ± 0.4 and 3.4 ± 0.5 -log cells/ml, respectively. There was a significant difference between the two groups (***P* < 0.001). (C) *Candida* cell suspensions were co-cultured with RAW 264 cells at 37°C for 4 h. Mean in vitro killing of *C. guilliermondii* and *C. fermentati* is expressed as the percent reduction of CFUs recovered from co-cultures compared with CFUs from control cultures (*Candida* cells without macrophages). The percent killing ratios of *C. guilliermondii* and *C. fermentati* were -0.6 ± 14.4 % and 24.4 ± 14.6 %, respectively. There was a significant difference between the two groups (***P* < 0.01).

References

(1) Vaughan-Martini A et al. FEMS Yeast Res.2005. (2) Lockhart SR et al. J Clin Microbiol.2009. (3) Chen CY et al. J Antimicrob Chemother. 2013. (4) Cheng JW et al. J Clin Microbiol. 2016.

Table 1. Patient characteristics associated with *C. guilliermondii* complex isolates

Characteristic	<i>C. guilliermondii</i> (n = 27)	<i>C. fermentati</i> (n = 12)	<i>P</i> value
Age (yr), mean ± SD	63.3 ± 18.7	61.2 ± 19.0	0.75
Male sex, no. (%)	20 (74.1)	7 (58.3)	0.46
Co-malignancies, no. (%)	18 (66.7)	10 (83.3)	0.45
Steroid use, no. (%)	11 (40.7)	8 (66.7)	0.18
Central venous catheter, no. (%)	22 (81.5)	9 (75.0)	0.68
In-hospital death, no. (%)	13 (48.1)	6 (50.0)	1.00
Antifungal therapy, no. (%)	12 (44.4)	9 (75.0)	0.10
Elevated β-D glucan, no. (%)	16 (64.0) ^a	2 (16.7)	0.01
Bloodstream isolates, no. (%)	22 (81.5)	0 (0)	< 0.0001
Cause of infection, no. (%)	24 (88.9)	0 (0)	< 0.0001

^a Serum β-D glucan was measured for 25 patients.

Table 2. In vitro susceptibility of 27 *C. guilliermondii* isolates and 12 *C. fermentati* isolates to eight antifungal agents as interpreted by breakpoints for echinocandins and epidemiological cutoff values for other agents

<i>C. guilliermondii</i> complex (no. of isolates)	MIC (μg/ml)		No. (%) of isolates by ECVs		No. (%) of isolates by CBPs		
	range	MIC ₅₀	WT	Non-WT	S	I	R
<i>C. guilliermondii</i> (27)							
Fluconazole	2 – >256	4	23 (85.2)	4 (14.8)			
Itraconazole	0.25 – >16	0.5	24 (88.9)	3 (11.1)			
Voriconazole	0.03 – 8	0.12	25 (92.6)	2 (7.4)			
Posaconazole	0.06 - 2	0.25	25 (92.6)	2 (7.4)			
Micafungin	0.25 – 1	0.5	27 (100)	0 (0)	27 (100)	0 (0)	0 (0)
Caspofungin	0.12 - 1	0.5	27 (100)	0 (0)	27 (100)	0 (0)	0 (0)
Anidulafungin	0.5 - 4	2	27 (100)	0 (0)	26 (96.3)	1 (3.7)	0 (0)
Amphotericin B	0.25 – 2	0.5	27 (100)	0 (0)			
<i>C. fermentati</i> (12)							
Fluconazole	2 – 8	4	12 (100)	0 (0)			
Itraconazole	0.25 – 0.5	0.25	12 (100)	0 (0)			
Voriconazole	0.06 – 0.25	0.12	12 (100)	0 (0)			
Posaconazole	0.03 – 0.25	0.12	12 (100)	0 (0)			
Micafungin	0.25 – 1	0.5	12 (100)	0 (0)	12 (100)	0 (0)	0 (0)
Caspofungin	0.25 - 1	0.5	12 (100)	0 (0)	12 (100)	0 (0)	0 (0)
Anidulafungin	0.5 - 2	1	12 (100)	0 (0)	12 (100)	0 (0)	0 (0)
Amphotericin B	0.5 – 2	1	12 (100)	0 (0)

Figure 2. Treatment efficacy of micafungin against *C. guilliermondii* clinical isolates and a *C. glabrata* wild-type strain in a mouse model of disseminated candidiasis. Immunocompromised mice were inoculated with *C. guilliermondii* NGS CG1, *C. guilliermondii* ACHCG213, and *C. glabrata* CBS138. Micafungin was administered at 4 mg/kg/day intraperitoneally for seven consecutive days. The number of cells recovered from the liver and bilateral kidneys is indicated for individual mice in the plots. NT indicates no treatment. Asterisks and NS indicate statistically significant differences (***P* < 0.001) and no significance (*P* > 0.05), respectively.