Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 62 (2008) 106–109
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Significant differences in drug susceptibility among species in the *Candida parapsilosis* group

Eveline van Asbeck[a,b,c,d], Karl V. Clemons[a,b,c,*], Marife Martinez[a,b,c], Ann-Jay Tong[a,b,c], David A. Stevens[a,b,c]

[a]*Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center, San Jose, CA 95128, USA*
[b]*California Institute for Medical Research, San Jose, CA 95128, USA*
[c]*Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA 94305, USA*
[d]*Eijkman-Winkler Institute for Medical and Clinical Microbiology, University Medical Center Utrecht (UMCU), 3584 CX Utrecht, The Netherlands*

Received 7 January 2008; accepted 16 April 2008

Abstract

Candida parapsilosis family has 3 proposed species: *C. parapsilosis* sensu stricto, *Candida orthopsilosis*, and *Candida metapsilosis*. *C. parapsilosis* sensu stricto had significantly higher caspofungin (CAS) and anidulafungin MICs than *C. orthopsilosis* or *C. metapsilosis*; *C. metapsilosis* was least susceptible to fluconazole. *C. parapsilosis* sensu stricto more frequently displayed (37%) paradoxical growth in CAS ($P \leq 0.02$). These species susceptibility differences could affect therapeutic choices.

Keywords: *Candida parapsilosis*; Fluconazole; Caspofungin; Anidulafungin; Susceptibility

Candida parapsilosis is the 2nd or 3rd most frequently isolated yeast species from the blood in various global regions (Krcmery and Barnes, 2002; Levin et al., 1998; Levy et al., 1998; Pfaller and Diekema, 2002; Pfaller et al., 2002; Tortorano et al., 2004; van Asbeck et al., 2008; Weems et al., 1987). This species is particularly associated with bloodstream infections in very low birth weight neonates (Levy et al., 1998; Sarvikivi et al., 2005; van Asbeck et al., 2007) but is also frequently seen in catheter-associated candidemia and patients receiving intravenous hyperalimentation (Levin et al., 1998; Pfaller and Diekema, 2007; Weems et al., 1987). *C. parapsilosis* has previously been separated into 3 groups (I, II, and III or A, B, and C, respectively) by intergenic transcribed spacer region (ITS) region sequencing or randomly amplified polymorphic DNA (RAPD) methodologies (Lehmann et al., 1992; Lin et al., 1995; Tavanti et al., 2005; van Asbeck et al., 2007, 2008). Recently, Tavanti et al. (2005), owing to sufficient DNA sequence nonhomology, suggested that these 3 groups be reclassified as separate species, *C. parapsilosis* sensu stricto, *Candida orthopsilosis*, and *Candida metapsilosis*, respectively, which are, thus far, phenotypically indistinguishable.

Most systemic antifungal agents, including azoles, polyenes, flucytosine, and echinocandins, have recognized activity in the treatment of *C. parapsilosis* infections (Cappelletty and Eiselstein-McKitrick, 2007; Denning, 2003; Krcmery and Barnes, 2002; Pfaller et al., 2002, 2004). Caspofungin (CAS) and 2 recently introduced agents, micafungin and anidulafungin (ANI), belong to the echinocandin antifungal class, which has potent activity against *Candida* spp. both in vivo and in vitro (Cappelletty and Eiselstein-McKitrick, 2007; Denning, 2003; Pfaller et al., 2004; Pfaller et al., 2004, 2008). Of the *Candida* spp., concerns have been raised about resistance most notably with *C. parapsilosis* (Cappelletty and Eiselstein-McKitrick, 2007; Denning, 2003; Pfaller et al., 2004).

Turbid growth of some *Candida* spp. isolates has been observed in our laboratory, paradoxically, in some high

* Corresponding author. Division of Infectious Diseases, Santa Clara Valley Medical Center, San Jose, CA 95128, USA. Tel.: +1-408-998-4557; fax: +1-408-998-2723.
E-mail address: clemons@cimr.org (K.V. Clemons).

0732-8893/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.diagmicrobio.2008.04.019

concentrations of CAS, above the MIC (Stevens et al., 2004). The cause of this paradoxical growth remains unclear; however, compensatory up-regulation of chitin synthesis, a cell wall component, appears to be the mechanism of this phenomenon (Stevens et al., 2006).

The purpose of the present study was to determine the susceptibility patterns of the species *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis* to 3 antifungal agents. ANI was included to study a 2nd echinocandin. These data were analyzed to compare the susceptibility patterns of individual species. We also report here studies of paradoxical growth among these 3 closely related species.

Ninety-five clinical isolates identified as *C. parapsilosis* by routine laboratory methods (Tortorano et al., 2004) were used in these studies. These isolates came from Europe (23 isolates), Asia (8), North America (44), Latin America (9), and the Middle East (11) and were obtained from the following clinical sources: 50 isolates were blood or central venous catheter isolates (29 *C. parapsilosis* sensu stricto, 19 *C. orthopsilosis*, and 2 *C. metapsilosis*), 41 were from other body sites (21 *C. parapsilosis* sensu stricto, 11 *C. orthopsilosis*, and 9 *C. metapsilosis*), and 3 (2 *C. parapsilosis* sensu stricto and 1 *C. metapsilosis*) were from health care workers' hands. The site of 1 isolate (*C. parapsilosis* sensu stricto) was not recorded. Each isolate tested was from a unique individual.

Molecular typing of all isolates was performed by RAPD analysis using the RPO2 primer (5′-GCGATCCCCA-3′) (Tavanti et al., 2005; van Asbeck et al., 2007, 2008). Fifty-three strains of *C. parapsilosis* sensu stricto, 30 strains of *C. orthopsilosis*, and 12 strains of *C. metapsilosis*, defined by their RAPD profiles, were included in this study.

The antifungals tested were CAS, fluconazole (FLC), and ANI. Susceptibility was determined using a broth macrodilution method, using an 80% turbidity end point (Clinical and Laboratory Standards Institute, 2002). The entire group of isolates, or a sampling strategy using randomly selected subsets, was tested. All isolates tested against ANI were included in the sample of isolates tested against CAS. The range of 2-fold dilution tested was 0.5 to 64 μg/mL for FLC, 0.39 to 50 μg/mL for CAS, and 0.03 to 16 μg/mL for ANI.

Statistical analysis of the antifungal susceptibility test results was done by nonparametric Kruskal–Wallis analysis of variance followed by a Dunn's test for multiple comparisons or a Mann–Whitney *U* test. Differences in paradoxical effect were determined by χ^2. A *P* value of <0.05 was considered significant.

We analyzed the in vitro activities of CAS, FLC, and ANI against *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis* (Table 1 and Fig. 1). *C. parapsilosis* sensu stricto isolates were significantly less susceptible in vitro to CAS ($P < 0.001$ and $P < 0.01$, respectively) or ANI ($P < 0.001$ for both) than were isolates of *C. orthopsilosis* or *C. metapsilosis*. However, *C. metapsilosis* isolates were less susceptible to FLC than *C. orthopsilosis* or *C. parapsilosis* sensu stricto ($P < 0.05$ or <0.001, respectively). Why *C. parapsilosis* sensu stricto is less susceptible to CAS or ANI than *C. metapsilosis* and *C. orthopsilosis* remains unknown, but these interspecies differences might be explained by structural difference in the cell wall components, a reduced affinity for the glucan synthase protein complex or variation in the regulatory network of this complex. The differences in FLC susceptibility may also reflect differing affinity of azoles for the key ergosterol-synthesizing enzyme, 14-demethylase, or other enzymes in this pathway.

Although the purpose of this study was to compare the 3 species *within* the dilution ranges of each of 3 drugs, looking, in addition, *across* drugs, we note *C. parapsilosis* sensu stricto was significantly more susceptible to CAS than to ANI ($P < 0.001$). This echinocandin difference could explain differences in susceptibility among echinocandins attributed to *C. parapsilosis* as a family (Marco et al., 1998; Pfaller et al., 2008) and differences in outcome of this family in clinical trials of candidiasis (Mora-Duarte et al., 2002; Reboli et al., 2007).

These species differences could explain susceptibility patterns previously attributed to *C. parapsilosis*; for example, relative resistance among *Candida* spp. to echinocandins (Cappelletty and Eiselstein-McKitrick, 2007; Denning, 2003; Pfaller et al., 2004). They could also explain variations in the species' global distribution (van Asbeck et al., 2008). Patterns of use of antifungal agents could be an explanation of the emergence of more resistant non-albicans *Candida* spp. (Garber, 2001; Pfaller and Diekema, 2002, 2007; Pfaller et al., 2002). Species susceptibility profiles provide important information for the

Table 1
Susceptibility of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* to FLC, ANI, and CAS

Species	MIC (μg/mL)											
	No.[a]	FLC			No.[a]	ANI			No.[a]	CAS		
		Range	Median	GM		Range	Median	GM		Range	Median	GM
C. parapsilosis	53	≤0.5 to 64	1	1.07	15	1–4	2	2	19	≤0.39 to 1.6	≤0.39	0.54
C. orthopsilosis	30	≤0.5 to 64	2	2	28	≤0.03 to 4	0.38	0.38	29	≤0.39	≤0.39	≤0.39
C. metapsilosis	12	2–8	4	3.36	12	0.13–0.5	0.25	0.30	12	≤0.39	≤0.39	≤0.39

GM = geometric mean.
[a] Number of isolates tested.