Molecular characterization and antifungal susceptibility of the *Candida parapsilosis* species complex of clinical isolates from Monterrey, Mexico

ROGELIO DE J. TREVIÑO-RANGEL*, ELVIRA GARZA-GONZÁLEZ*, J. GERARDO GONZÁLEZ†, VIRGILIO BOCANEGRA-GARCÍA‡, JORGE M. LLACA† & GLORIA M. GONZÁLEZ*

*Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México, †Hospital Universitario, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México, and ‡Laboratorio de Medicina de Conservación, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamaulipas, México

Recently, it was proposed that the opportunistic yeast pathogen *Candida parapsilosis* was a complex composed of the following three species: *Candida parapsilosis* sensu stricto, *Candida orthopsilosis*, and *Candida metapsilosis*. A set of 344 clinical isolates of *Candida parapsilosis* from Monterrey, Mexico was re-identified by RFLP. Their antifungal susceptibility to fluconazole, caspofungin, anidulafungin and micafungin was determined using the Clinical and Laboratory Standards Institute M27-A3 protocol. *Candida parapsilosis* sensu stricto was the most frequent species, and was the only one which showed resistance to antifungals.

**Keywords** Candidosis, *Candida parapsilosis*, *Candida orthopsilosis*, *Candida metapsilosis*, antifungal susceptibility

Introduction

*Candida parapsilosis* has been reported as an important agent of fungemia in various geographic regions including Mexico [1–3]. In the clinical setting, this opportunistic yeast has been recovered from the hands of health-care workers [1], and is primarily associated with bloodstream infections in low-birth weight neonates, catheter-associated candidiasis and intravenous hyperalimentation [4,5]. *C. parapsilosis* is a complex of three species, i.e., *Candida parapsilosis* sensu stricto, *Candida orthopsilosis*, and *Candida metapsilosis* [6]. Recent studies reported a decreased susceptibility of *C. parapsilosis* to azoles and echinocandins which might become a cause of clinical concern [7,8].

The purpose of this study was to re-identify by RFLP a total of 344 clinical isolates of *C. parapsilosis* collected over a 10-year period (1999–2010) from Monterrey, Mexico, and to determine their antifungal susceptibility profiles to fluconazole (FLC), caspofungin (CAS), anidulafungin (ANI) and micafungin (MCF), using the Clinical and Laboratory Standards Institute (CLSI) method.

Materials and methods

Isolates were grown at 37°C for 24 h on potato dextrose agar (PDA) slants (Difco, Detroit, MI), and initially identified as *C. parapsilosis* through the use of API 20C AUX strips (bioMérieux, Mexico) and standard morphological methods. Details regarding the isolates are presented in Table 1.

In order to re-identify the isolates, they were subcultured on PDA slants and incubated at 37°C for 24 h. A 15-μl equivalent of each isolate was scraped from the plate and resuspended in 100 μl of sterile distilled water. The yeasts were then lysed by heating to 95°C for 10 min for DNA extraction.

The secondary alcohol dehydrogenase (*SADH*) gene was then amplified by PCR using the primers previously described [6]. Each 25-μl amplification mixture was composed of 2.5 μl of Tris HCl buffer (pH 8.8), 6 mM MgCl₂, 0.8 mM dNTPs,
0.35 pMol/μl of each primer, 0.03% BSA (Takara Bio Inc., Otsu, Shiga), 2.5 U of Taq DNA polymerase (Bioline, USA Inc., Boston, MA), and 7.5 μl of yeast lysate. The reaction consisted of a first cycle of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 50°C and 72°C, and a final step of 5 min at 72°C. PCRs were performed in PxE 0.2 Thermal Cycler (Thermo Electron Corporation).

The 716 bp PCR products were then digested with BanI (New England Biolabs, Country Road, Ipswich, MA) in a 20-μl volume containing 16.8 μl of the product and 10 U of BanI. The products were separated on a 2.5% agarose gel, and the strains were discriminated according to their SADH restriction patterns as follows: (1) C. parapsilosis sensu stricto has one BanI restriction site (generating two fragments, of 521 and 196 bp), (2) C. orthopsilosis has no restriction site, and (3) C. metapsilosis has three BanI restriction sites (releasing four fragments, of 370, 188, 93 and 60 bp) [6]. Type strains ATCC 22019, ATCC 96139, and ATCC 96144 were used as controls for C. parapsilosis sensu stricto, C. orthopsilosis, and C. metapsilosis, respectively.

The antifungal susceptibility testing of the strains was performed using the CLSI broth macrodilution method according to the M27-A3 document [9]. Stock solutions of FLC (Pfizer, Inc., Amboise, France), CAS (Merck, Rahway, NJ), ANI (Ben Venve, Northfield Road Bedford, OH), and MCF (Astellas Pharma, Inc., Tokyo, Japan) were prepared and diluted with RPMI 1640 medium with L-glutamine (Hardy Diagnostics, Santa Maria, CA). The final concentration of the antifungals ranged from 0.125–64 μg/ml for FLC and from 0.015–8.0 μg/ml for the echinocandins.

The MICs were recorded after 24 h of incubation with all antifungals, and the susceptibility breakpoints were those previously established by Pfaller et al. [10,11], in which a MIC of <2 μg/ml was considered as susceptible, while a MIC of >8 μg/ml indicated resistance. Type strains C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were used for quality control.

### Results

The distributions of the species that comprise the C. parapsilosis complex are detailed in Table 1. Three hundred and eleven isolates (90.4%) were identified as C. parapsilosis sensu stricto, while 29 (8.4%) and 4 (1.2%) proved to be C. orthopsilosis and C. metapsilosis, respectively. C. parapsilosis sensu stricto was recovered from all types of biological samples, particularly associated with blood. C. orthopsilosis and C. metapsilosis were primarily isolated from blood and skin isolates, respectively.

The in vitro antifungal susceptibilities of the C. parapsilosis isolates are summarized in Table 2. The MIC90 of C. parapsilosis sensu stricto and C. orthopsilosis to FLC was 8 μg/ml for both species. On the other hand, C. parapsilosis sensu stricto and C. orthopsilosis showed similar susceptibility patterns to CAS (MIC90 1 μg/ml for C. parapsilosis sensu stricto, and 0.5 μg/ml for C. orthopsilosis), as well as to ANI and MCF in that the MIC90 of 2 μg/ml was found with both species. Since there were so few isolates of C. metapsilosis, its MIC90 and MIC90 were not calculated for any of the antifungals. Resistance to tested antifungals was detected only among C. parapsilosis sensu stricto isolates.

### Discussion

The C. parapsilosis species distribution we found is similar to that reported in other studies [12,13], except we noted a slightly higher frequency of C. orthopsilosis than C. metapsilosis [14,15]. This can probably be attributed to differences in their geographic distribution patterns. C.
C. parapsilosis species complex of clinical isolates from Monterrey, Mexico.

**Declaration of interest:** The authors have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**


---

**Table 2. In vitro susceptibility of Candida parapsilosis sensu stricto, Candida orthopsilosis, and Candida metapsilosis to four antifungals.**

<table>
<thead>
<tr>
<th>Species (no. of isolates)</th>
<th>Antifungal agent</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
<th>%R*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. parapsilosis</em> (311)</td>
<td>Fluconazole</td>
<td>0.125–64</td>
<td>1</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.125–8</td>
<td>0.5</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.06–8</td>
<td>1</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>0.06–8</td>
<td>1</td>
<td>2</td>
<td>3.2</td>
</tr>
<tr>
<td><em>C. orthopsilosis</em> (29)</td>
<td>Fluconazole</td>
<td>0.25–2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.125–2</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.5–2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Micafungin</td>
<td>0.25–2</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>C. metapsilosis</em> (4)</td>
<td>Fluconazole</td>
<td>0.125–0.5</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Caspofungin</td>
<td>0.5–1</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Anidulafungin</td>
<td>0.25–0.5</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

*R*, resistant (MIC ≥ 8 µg/ml).

parapsilosis sensu stricto was the most frequently isolated species of the complex, being particularly common in blood samples, which is linked to the fact that it is a human commensal frequently isolated from hands [16,17]. To date, the prevalence and distribution of the species in the *C. parapsilosis* complex in cases of superficial candidiasis is not clear. In our study, we found that *C. parapsilosis* sensu stricto was the most prevalent species involved in these and disseminated infections. *C. orthopsilosis* and *C. metapsilosis* were both rarely isolated from superficial lesions as reported in other studies [12,18]. Our findings are similar to those recently reported by Feng et al. [19].

*C. parapsilosis* sensu stricto and *C. orthopsilosis* showed similar susceptibility profiles to echinocandins in contrast to the results from the study by Silva et al. [15]. Despite the high echinocandin MICs of the three species of the complex, *C. parapsilosis* sensu stricto isolates were less susceptible in *vitro* to CAS and ANI than *C. orthopsilosis* and *C. metapsilosis* which is similar to the findings previously reported by Cantón et al. [20].

Although there are some surveys that report excellent *in vitro* activity of echinocandins and reveal no evidence of resistance among clinical isolates of *C. parapsilosis* [7,21], the echinocandin susceptibility profiles obtained in this study do not match those findings. Recent reports have suggested that *C. parapsilosis* is less susceptible to echinocandins [15,22]. On the other hand, we detected 14 strains of *C. parapsilosis* sensu stricto which were resistant to FLC. These results may have important clinical implications and deserve the implementation of local surveillance programs to monitor the antifungal susceptibility patterns of these clinically important organisms.

This is the first paper to date that provides local-level information on the distribution and antifungal susceptibilities of


