Prevalence and antifungal susceptibility of Candida parapsilosis complex isolates collected from oral cavities of HIV-infected individuals

D. V. Moris,1 M. S. C. Melhem,2 M. A. Martins,2 L. R. Souza,1 S. Kacew,3 M. W. Szessz,2 L. R. Carvalho,4 M. V. Pimenta-Rodrigues,1 H. A. M. Berghs5 and R. P. Mendes1

1Departamento de Doenças Tropicais e Diagnóstico por Imagem, Faculdade de Medicina de Botucatu, UNESP, Botucatu, São Paulo State, Brazil
2Instituto Adolfo Lutz, São Paulo, Brazil
3Institute for Population Health, University of Ottawa, Ontario, Canada
4Departamento de Bioestatística, Instituto de Biociências, UNESP, Botucatu, São Paulo State, Brazil
5Fairport Ltda, São Paulo, Brazil

At present, few data are available on the prevalence and antifungal susceptibility of Candida parapsilosis complex isolates from HIV-infected individuals. The C. parapsilosis complex comprises three species, C. parapsilosis sensu stricto, C. metapsilosis and C. orthopsilosis. Fifteen of 318 Candida isolates were identified as members of the C. parapsilosis complex by PCR and restriction fragment length polymorphism (RFLP). The prevalence of C. parapsilosis complex isolates was 4.7%, 2.2% being identified as C. parapsilosis sensu stricto and 2.5% as C. metapsilosis, while no C. orthopsilosis was isolated. This is believed to be the first study that has identified isolates of C. metapsilosis obtained from the oral cavity of HIV-infected individuals.

Antifungal susceptibility tests indicated that all the isolates were susceptible to amphotericin B (AMB), fluconazole (FLC), ketoconazole (KTC), itraconazole (ITC), voriconazole (VRC) and caspofungin (CASPO). Although isolates of C. parapsilosis sensu stricto and C. metapsilosis were susceptible to FLC, isolates of C. metapsilosis showed a tendency for higher MICs (≥1.0 μg ml⁻¹). Based upon the frequency of candidiasis and the fact that certain isolates of the C. parapsilosis complex respond differently to FLC therapy, our data may be of therapeutic relevance with respect to susceptibility and potential resistance to specific antifungal agents. Our data suggest that C. metapsilosis can be a human commensal; its importance as a pathogen has yet to be confirmed.

INTRODUCTION

Candida parapsilosis is a well-known cause of nosocomial bloodstream infections, particularly among neonates and immunocompromised patients, and it is associated with candidaemia caused by intravascular and parenteral nutrition devices (Sarvikivi et al., 2005; Almirante et al., 2006; Pfaller & Diekema, 2002; 2007; Trofa et al., 2008). C. parapsilosis groups I, II and III were recently established as distinct species, which were classified by genotyping and designated C. parapsilosis sensu stricto, C. orthopsilosis and C. metapsilosis, respectively (Tavanti et al., 2005). Although the C. parapsilosis complex has been recognized as comprising distinct species, little is known about the transmission and infectivity of the two more rare species present within the complex, C. orthopsilosis and C. metapsilosis, while C. parapsilosis has now been recognized as a major human fungal pathogen, ranking as the second or third most frequently occurring cause of bloodstream infection in Europe, Canada and Latin America (Almirante et al., 2006; Pemán et al., 2005; Pfaller et al., 2008). Data on the frequency of isolation of C. orthopsilosis and C. metapsilosis have just started to be released (Odds et al., 2007; Tavanti et al., 2007; Kocsubé et al., 2007; Gomez-Lopez et al., 2008; Lockhart et al., 2008; Hensgens et al., 2009; Silva et al., 2009; Gonçalves et al., 2010; Ghannoun et al., 2010; Bonfetti et al., 2012). These retrospective epidemiological studies have been
undertaken to screen for \( C. \) orthopsilosis and \( C. \) metapsilosis among isolates previously identified as \( C. \) parapsilosis, showing that a small proportion of isolates actually belong to the species \( C. \) orthopsilosis or \( C. \) metapsilosis, with significant geographical variation.

Members of the \( C. \) parapsilosis complex are normally susceptible to most antifungal compounds but previous studies reported decreased responsiveness to fluconazole (FLC) and caspofungin (CASPO) (Sarvikivi et al., 2005; Legout et al., 2006; Lockhart et al., 2008; van Asbeck et al., 2008; Gonçalves et al., 2010; Silva et al., 2009). It was also suggested that \( C. \) orthopsilosis and \( C. \) metapsilosis might be more susceptible to amphotericin B (AMB) and echinocandins than \( C. \) parapsilosis sensu stricto (Gomez-Lopez et al., 2008; van Asbeck et al., 2008; García-Effron et al., 2008) and that these differences in antifungal susceptibility could affect choice of therapy. It is, therefore, important to determine the distribution of these species in our environment, particularly in HIV-infected individuals, and to correlate genotypic variation in these species with virulence capabilities, as well as susceptibility to antifungal drugs.

Oropharyngeal Candida infections occur frequently in HIV-infected individuals who have lymphocyte counts of \(<400 \text{TCD}^4 \text{cells mm}^{-3}\). It should be noted that the presence of oral Candida is considered a biomarker associated with deterioration of the immune system and, in immunodeficiency disorders, can be indicative of a progressive disease (Vargas & Joly, 2002; Moris et al., 2008). To our knowledge, few studies have evaluated the prevalence of members of the \( C. \) parapsilosis complex in the oral cavity, relating to colonization or infection; in addition, no studies have been found specifically relating to colonization or infection of the oral cavity of HIV-infected individuals.

The aim of the present study was to: 1, distinguish the different species of \( C. \) parapsilosis complex isolated from the oral cavities of HIV-infected individuals using PCR and restriction fragment length polymorphism (RFLP); 2, characterize the \( C. \) parapsilosis complex clusters genotypically using random amplification of polymorphic DNA (RAPD); and 3, determine the susceptibility of the three different species of the \( C. \) parapsilosis complex to AMB, FLC, ketoconazole (KTC), itraconazole (ITC), voriconazole (VRC) and CASPO.

**METHODS**

Approval was obtained from the Comité de Ética em Pesquisa, Faculdade de Medicina de Botucatu, São Paulo State University (UNESP) and the Comité de Ética em Pesquisa com seres Humanos, Adolfo Lutz Institute. Informed patient consent was required for this study. Samples were collected from the oral cavity of HIV-positive individuals from June 2002 to July 2006. All patients with confirmed diagnosis of HIV-infection and who attended the Special Outpatient Clinic for Infectious and Parasitic Diseases or the Ward of Tropical Diseases of the Faculdade de Medicina de Botucatu, São Paulo State University (UNESP) were included in the study period.

**Fungal isolates.** Samples were collected for culture, independently of the clinical signs and symptoms of oral candidiasis, from the oral cavity of 214 HIV-infected individuals using swabs. The swabs were then placed on plates containing CHROMagar Candida medium at 35 °C for 48 h. Species identification was based on colony colour and morphology on CHROMagar, microscopic morphology on cornmeal agar (Oxoid) supplemented with 1% Tween 80 (Labsynth) (Kurtzman & Fell, 1998) and the use of the commercial system API 20 C AUX (bioMérieux) according to the manufacturer’s instructions. The isolates were stored in glycerol/water at −80 °C. All strains were subcultured on CHROMagar and incubated at 37 °C for 48 h to reconfirm their identification as a member of the \( C. \) parapsilosis complex.

A total of 318 Candida strains were isolated from the oral cavities of 214 HIV-infected individuals. The prevalence of \( C. \) albicans was 84.0% (267 isolates), \( C. \) glabrata was 5.7% (18 isolates), \( C. \) parapsilosis complex was 4.7% (15 isolates), \( C. \) tropicalis was 4.1% (13 isolates), \( C. \) dubliniensis was 0.9% (3 isolates); and \( C. \) krusei was 0.6% (2 isolates). Fifteen of the 318 Candida isolates from the oral cavities of HIV-infected individuals that were initially identified as \( C. \) parapsilosis by conventional morphological and physiological methods (Kurtzman & Fell, 1998) were subjected to molecular typing. The characteristics of the 15 HIV-infected individuals, from which members of the \( C. \) parapsilosis complex were isolated, are presented in Table 1.

**Antifungal susceptibility testing.** Antifungal susceptibility testing was performed according to the European Committee on Antimicrobial Susceptibility Testing Edel 7.1 guidelines (EUCAST, 2008a). The employed antifungal agents were AMB, KTC, FLC, ITC, VRC and CASPO. Interpretative criteria for MIC breakpoints were used according to AFST-EUCAST guidelines (EUCAST, 2008a, 2008b, 2008c; Rodriguez-Tudela et al., 2010; http://www.euacast.org).

**Molecular identification of \( C. \) parapsilosis complex isolates.** \( C. \) parapsilosis ATCC 22019\(^T\), \( C. \) metapsilosis ATCC 96143 and \( C. \) orthopsilosis ATCC 96141\(^T\) were included as reference strains in this study. Each isolate was plated on Sabouraud glucose agar (Difco) and subcultured on CHROMagar and incubated at 37 °C for 2–3 h. Subsequently, this mixture was centrifuged at 15 000 \(g\) for 2–3 h. The supernatant was discarded and the pellet was resuspended in 1 ml of each lysis buffer (pH 8, Sigma), containing 40 μl lysis enzyme obtained from \( Trichoderma harzianum \) (10 mg ml\(^{-1}\) (Sigma), followed by incubation at 37 °C for 2–3 h. Subsequently, this mixture was centrifuged at 13 000 \(g\) for 10 min and the pellet was dissolved in lysis buffer containing 10 mM Tris/HCl (pH 8, Sigma), 10 mM EDTA (Sigma), 0.5% SDS (Labsynth), 0.01% N-laurylsarcosyl (Sigma) and 10 μg ml\(^{-1}\) proteinase K (Sigma). The mixture was then vortexed and incubated at 56 °C for 30 min. DNA was extracted by the phenol/ chloroform/isoamyl alcohol method and precipitated with 2-propanol according to Sambrook et al. (1989). After washing the pellet with 70% ethanol for 10 min at 10 000 \(g\), DNA was dissolved in 50 μl ultrapure water and frozen at −20 °C until use. DNA concentrations were determined using a NanoDrop1000 spectrophotometer (Thermo Fisher Scientific). For PCR and RAPD amplification, 1 μl of each DNA sample (−100 ng DNA) was used.

**PCR and RFLP.** The protocol used for PCR and RFLP was previously described by Tavanti et al. (2005). The secondary alcohol dehydrogenase (SADH) fragment was amplified for \( C. \) parapsilosis isolates. One pair of primers (Invitrogen) was chosen: sense (S1F: 5′-TGTTAGTCGTTG-CATTGTT-3′) and anti-sense (S1R: 5′-CAATGGCAATCTCCGAAA-3′). The amplification conditions were as follows: an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 1 min and elongation at 72 °C for 45 s, with a final extension step at 72 °C for 10 min. Amplified DNA
products were separated by electrophoresis on a 2% agarose gel in TBE buffer (89 mM Tris/HCl, 89 mM boric acid and 2 mM EDTA; pH 8) at 100 V for 35 min and a 100 bp DNA ladder was used as molecular marker (Invitrogen). The gel was stained with ethidium bromide (0.5 mg ml\(^{-1}\)) and DNA bands were visualized by UV transillumination (Mini Bis Pro, Bio-Imaging Systems). The amplified DNA products were loaded onto a 2% agarose gel in TBE buffer O (supplied with enzyme) and 2 μl PCR product, 2 μl 10× buffer O (supplied with enzyme) and 2 μl (40 U) BshNI. Digestion products were loaded onto a 2% agarose gel in TBE buffer and run at 100 V for 35 min with a 100 bp DNA ladder as a molecular marker (Invitrogen). The gel was stained with ethidium bromide (0.5 mg ml\(^{-1}\)) and DNA bands were visualized by UV transillumination (Bio-Imaging Systems).

**RAPD analysis.** Amplification was carried out with the Ready-to-Go-RAPD Analysis Beads kit (GE Healthcare). Each reaction was performed by adding each DNA template and 25 pmol primer to a final reaction volume of 25 μl. The selection of primers was determined after testing six primer sequences for exclusive use in RAPD (GE Healthcare). The primers p1 (5’-GGTGGCGGGA-3’) and p2 (5’-GTTCGCCTCC-3’) were selected for *C. parapsilosis* and *C. metapsilosis* isolates. The amplification was performed in an automated thermal cycler (Progene) and consisted of an initial denaturation step at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 32°C for 1 min and extension at 72°C for 2 min, with a final extension step at 72°C for 10 min. DNA samples were tested in duplicate. Each amplification run contained a negative control (ultrapure water). Amplified DNA products were separated by electrophoresis on a 1.5% agarose gel in TBE buffer and visualized using the procedure as described earlier for PCR/RFLP. The band profiles in RAPD for *C. parapsilosis* and *C. metapsilosis* clinical isolates were compared to those for the reference strains *C. parapsilosis* ATCC 22019\(^7\), *C. metapsilosis* ATCC 96143 and *C. orthopsilosis* ATCC 96139\(^7\) were digested for 90 min with BshNI (Fermentas) in a 20 μl reaction volume containing 6 μl PCR product, 2 μl 10× buffer (supplied with enzyme) and 2 μl (40 U) BshNI. Digestion products were loaded onto a 2% agarose gel in TBE buffer and run at 100 V for 35 min with a 100 bp DNA ladder as a molecular marker (Invitrogen). The gel was stained with ethidium bromide (0.5 mg ml\(^{-1}\)) and DNA bands were visualized by UV transillumination (Bio-Imaging Systems).

**Analysis of data and dendrogram generation.** RAPD profiles were analysed by BioNumerics version 6.6 (Applied Mathcs). Gel images resulting from the use of the two different primers (p1, p2) were processed individually under two experiments (RAPD_p1, RAPD_p2) and pairwise similarity matrices were calculated using the Dice coefficient with 0% optimization and 1% tolerance. Individual dendrograms were generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm. A composite dataset was created with the two experiments having the same weight. A combined data matrix, a combined dendrogram was generated using the UPGMA algorithm. A similarity coefficient value (S\(_{AB}\)) of 1.00 (100%) indicated that the banding patterns for strain A were identical to those for strain B, S\(_{AB}\) values of 0.80–0.99 (80–99%) represented

---

### Table 1. Characteristics of the 15 HIV-positive individuals from which *C. parapsilosis* complex isolates were collected

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Individuals</th>
<th>Number of isolates (%)</th>
<th>Total (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>C. parapsilosis</em> sensu stricto</td>
<td><em>C. metapsilosis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>15</td>
<td>3 (20.0)</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>4 (26.7)</td>
<td>2 (13.3)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>7 (46.7)</td>
<td>8 (53.3)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Host–<em>Candida</em> relationship</td>
<td>15</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Colonization</td>
<td></td>
<td>5 (33.3)</td>
<td>5 (33.3)</td>
<td>10 (66.6)</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td>2 (13.3)</td>
<td>3 (20.1)</td>
<td>5 (33.4)</td>
</tr>
<tr>
<td>Virus infection stage</td>
<td>15</td>
<td>6 (40.0)</td>
<td>8 (53.0)</td>
<td>14 (93.0)</td>
</tr>
<tr>
<td>AIDS</td>
<td></td>
<td>1 (7.0)</td>
<td>0 (0.0)</td>
<td>1 (7.0)</td>
</tr>
<tr>
<td>non-AIDS</td>
<td></td>
<td>1 (6.7)</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>CD4+ T cell count (mm(^{-3}))</td>
<td>15</td>
<td>1 (6.7)</td>
<td>2 (13.3)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>CD4&lt;200</td>
<td></td>
<td>6 (40.0)</td>
<td>5 (33.3)</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>CD4&lt;1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4&gt;1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV viral load (copies.ml(^{-1}))‡</td>
<td>13‡</td>
<td>1 (7.7)</td>
<td>3 (23.1)</td>
<td>4 (30.8)</td>
</tr>
<tr>
<td>Undetectable</td>
<td></td>
<td>4 (30.8)</td>
<td>1 (7.7)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>&lt;10000</td>
<td></td>
<td>1 (7.7)</td>
<td>3 (23.0)</td>
<td>4 (30.7)</td>
</tr>
<tr>
<td>≥10000</td>
<td></td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>Prior fluconazole treatment</td>
<td>14‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>4 (28.6)</td>
<td>5 (35.7)</td>
<td>9 (64.3)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>Prior antifungal agents</td>
<td>14‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>4 (28.6)</td>
<td>5 (35.7)</td>
<td>9 (64.3)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>2 (14.3)</td>
<td>3 (21.4)</td>
<td>5 (35.7)</td>
</tr>
</tbody>
</table>

*P>0.05 for detectable vs undetectable using Fisher’s exact test.
†Two patients did not have their HIV viral load evaluated when the oral swabs were collected.
‡In one case there was no information about previous intake of antifungal compounds.
highly similar but non-identical strains and $S_{AB}$ values $<0.80$ indicated unrelated strains (Soll, 2000).

**Statistical analyses.** Differences in antifungal MIC distributions were examined using the Mann–Whitney U test. Comparison of frequencies was carried out by Fisher’s exact test. The criterion for significance was set at $P<0.05$. All statistical analyses were performed using Statistical Analysis System version 9.2 (SAS Institute).

**RESULTS**

**Molecular identification of C. parapsilosis complex isolates**

The SADH gene was successfully amplified in all clinical isolates from 15 of the HIV-infected individuals, as well as the reference strains, and a single sharp band of $\sim 720$ bp was observed in all samples confirmed by PCR as belonging to *C. parapsilosis*. Digestion of PCR products by the restriction enzyme BshNI resulted in three different band patterns. Isolates were molecularly distinguished as *C. parapsilosis sensu stricto*, *C. orthopsilosis* or *C. metapsilosis* as follows: *C. parapsilosis sensu stricto* isolates possessed one BshNI restriction site, which revealed two bands at positions 521 and 196 bp; *C. orthopsilosis* isolates had no restriction sites, which yielded one band at position 716 bp; and *C. metapsilosis* isolates possessed three BshNI restriction sites, which revealed four bands at positions 370, 188, 93 and 60 bp (Fig. 1). Seven (46.7%) out of the 15 *C. parapsilosis* complex isolates from HIV-infected individuals were identified as *C. parapsilosis sensu stricto* and eight (53.3%) were identified as *C. metapsilosis*, whereas no *C. orthopsilosis* isolates were found.

![BshNI restriction digestion of secondary alcohol dehydrogenase (SADH) PCR products. M, 100 bp ladder. SADH fragment PCR amplification (product of 716 bp): lane 1, C. parapsilosis ATCC 22019$^1$; lane 2, C. metapsilosis ATCC 96143; lane 3, C. orthopsilosis ATCC 96141$^2$; lane 4, isolates from the oral cavity of HIV-infected patients. BshNI restriction digestion of SADH PCR product: lane 5, C. parapsilosis ATCC 22019$^1$; lane 6, C. metapsilosis ATCC 96143; lane 7, C. orthopsilosis ATCC 96141$^2$; lanes 8 and 9, respective C. parapsilosis sensu stricto and C. metapsilosis isolates from the oral cavity of HIV-infected patients.](image)

**Prevalence of members of the C. parapsilosis complex**

The prevalence of members of the *C. parapsilosis* complex was 4.7% (15 of 318 isolates from 214 HIV-positive individuals). *C. parapsilosis sensu stricto* occurred with a prevalence of 2.2%, while *C. metapsilosis* had a prevalence of 2.5%. No strains of *C. orthopsilosis* were isolated from oral swabs of the 214 HIV-infected individuals. The characteristics of the 15 HIV-positive individuals, from which *C. parapsilosis* complex strains were isolated are presented in Table 1. The prevalence of *C. parapsilosis sensu stricto* was generally higher ($0.05<P<0.10$) in patients with viral loads $<10,000$ HIV copies mm$^{-3}$ (30.8%).

**RAPD analysis**

The presence of intra-specific differences was investigated by RAPD analysis between the seven *C. parapsilosis sensu stricto* and eight *C. metapsilosis* isolates obtained from the oral cavities of 15 HIV-infected individuals. The dendrogram, based on $S_{AB}$ values, generated with *C. parapsilosis* ATCC 22019$^2$ and *C. metapsilosis* ATCC 96143 as reference strains (Fig. 2), showed that the seven isolates identified as *C. parapsilosis sensu stricto* and the eight isolates identified as *C. metapsilosis* had high coefficients of similarity within their respective species groups. Five isolates of *C. parapsilosis sensu stricto* shared an $S_{AB}$ value of 100% when compared with each other and an $S_{AB}$ value of 81.9% when compared with the type strain of the species (ATCC 22019$^2$). However, two isolates of *C. parapsilosis sensu stricto* shared an $S_{AB}$ value of 73.7% when compared with the other isolates in this species and an $S_{AB}$ value of 79.3% when compared with the type strain ATCC 22019$^2$ (Fig. 2). The eight *C. metapsilosis* isolates were grouped in three clusters with $S_{AB}$ values ranging from 83.7 (compared with the reference strain ATCC 96143) to 96.4% between members of this species (Fig. 2).

**Antifungal susceptibility testing**

All tested organisms grew after 24 h of incubation. The results of susceptibility tests to AMB, FLC, KTC, ITC, VRC and CASPO for the seven *C. parapsilosis sensu stricto* and eight *C. metapsilosis* isolates are presented in Table 2. All isolates were susceptible to the antifungal agents tested. The only significant difference in susceptibility between species was observed for isolates of *C. parapsilosis sensu stricto*, which showed a tendency toward a higher sensitivity to FLC than *C. metapsilosis*, with median MICs of 0.5 and 1.5 µg ml$^{-1}$, respectively.

**DISCUSSION**

It is not possible to differentiate between species of the *C. parapsilosis* complex by using phenotypic methods alone. In order to distinguish between *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis* isolates, Tavanti et al.
(2005) proposed the use of restriction polymorphism analysis of the SADH gene, which encodes a secondary alcohol dehydrogenase and is common to all three species. To our knowledge, few studies have evaluated the prevalence of these three novel species in cases of infection or colonization, while no studies have been found which focus on the prevalence of these species in the oral cavities of HIV-infected individuals. In the current study, the prevalence of \textit{C. parapsilosis} complex isolates was 4.7 % in HIV-infected individuals. The amplification of the SADH gene by PCR and the genetic profile generated by BshNI restriction enzyme and RFLP analysis, indicated that seven isolates belonged to the species \textit{C. parapsilosis sensu stricto} (2.2 %), while eight isolates were identified as \textit{C. metapsilosis} (2.5 %). The dendrogram in Fig. 2 shows that isolates of the species \textit{C. parapsilosis sensu stricto} shared high SEB values, suggesting a high level of genetic similarity. The genomic similarity among isolates of \textit{C. parapsilosis sensu stricto} has been demonstrated previously by using various molecular techniques (Kocsubi\'e et al., 2007; Lehmann et al., 1992; Tavanti et al., 2005, 2007; Tay et al., 2009). However, an investigation of the size polymorphisms of loci harbouring microsatellite repeat sequences in \textit{C. parapsilosis sensu stricto} isolates (Lasker et al., 2006) demonstrated 30 different microsatellite genotypes from 42 isolates. The genetic heterogeneity of \textit{C. metapsilosis}, as reflected by RAPD analysis in this study, has been demonstrated by other investigators (Iida et al., 2005; Lasker et al., 2006; Tavanti et al., 2005, 2007; van Asbeck et al., 2008; Tay et al., 2009).

It is of interest that \textit{C. metapsilosis} was reported to be rarely present in urine, skin and blood samples of patients admitted to care units of different hospitals as well as isolates from obtained worldwide collections (Enger et al., 2001; Tavanti et al., 2005, 2007; de Toro et al., 2011).

Enger et al. (2001) identified 89 clinical isolates collected at 15 different sites from patients from Europe, Asia and USA by means of Cp3-13-specific probe hybridization. \textit{C. parapsilosis sensu stricto} (previously known as \textit{C. parapsilosis} group I) was identified in 78 (89.7 %) samples, followed by \textit{C. orthopsilosis} (previously group II), identified in eight (9.2 %) samples, and \textit{C. metapsilosis} (previously group III), identified in one (1.1 %) sample. The evaluation included 14 (15.7 %) isolates from the oral cavity, which were identified as \textit{C. parapsilosis sensu stricto} (64.3 %) and \textit{C. orthopsilosis} (35.7 %), but no \textit{C. metapsilosis} was found at this site. However, in one sample isolated from pharyngeal mucosa, \textit{C. metapsilosis} was identified. These findings differ from our results, which showed prevalence rates of 46.7 % for \textit{C. parapsilosis sensu stricto} and 53.3 % for \textit{C. metapsilosis} in the oral cavity isolates. It is of note that all the individuals included in the current study were HIV-positive, whereas no information regarding the origin of the isolates was provided in the study of Enger et al. (2001). Kocsubi\'e et al. (2007) determined the genetic variability of \textit{C. parapsilosis} complex isolates from 20 samples taken from six different anatomical sites of patients hospitalized in two Hungarian hospitals; the prevalent species was \textit{C. parapsilosis sensu stricto} (90 %), followed by \textit{C. metapsilosis} (10 %). One sample, isolated from the throat, was identified as \textit{C. metapsilosis}, whereas no \textit{C. orthopsilosis} was detected. Silva et al. (2009) studied 169 samples isolated from nine different anatomical sites of patients hospitalized in a tertiary care hospital in Portugal and found that \textit{C. parapsilosis sensu stricto} was identified in 160 (94.7 %) samples, \textit{C. orthopsilosis} in five (2.9 %) samples and \textit{C. metapsilosis} in four (2.4 %) samples. Of the 69 mucosal samples isolated in the study by Silva et al. (2009), 94.2 % corresponded to \textit{C. parapsilosis sensu stricto} and 5.8 % to \textit{C. metapsilosis}.

The above-mentioned studies show a higher occurrence of \textit{C. parapsilosis sensu stricto} (55.8–94.7 %) within the \textit{C. parapsilosis} complex, which accounted for 46.7 % of samples in the present study. The clinical relevance of \textit{C. orthopsilosis} and \textit{C. metapsilosis} as aetiological factors in candidiasis development cannot be assessed in the present study due to the small number of oral cavity samples.
Table 2. MICs of the six antifungals against 15 C. parapsilosis complex isolates tested according to the EUCAST procedure using the Mann–Whitney test.

<table>
<thead>
<tr>
<th>Species</th>
<th>AMB</th>
<th>FLC</th>
<th>KTC</th>
<th>ITC</th>
<th>VRC</th>
<th>CASPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. parapsilosis sensu stricto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=7)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MIC (µg·ml⁻¹)</td>
<td>0.015</td>
<td>0.03</td>
<td>0.06</td>
<td>0.12</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>P-value</td>
<td>0.40</td>
<td>0.185</td>
<td>0.500</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
</tr>
</tbody>
</table>

The present study demonstrated that 53.3% of the 15 oral cavity isolates from HIV-infected individuals, initially identified as C. parapsilosis sensu stricto, were, in fact, C. metapsilosis. Thus, it is possible that in previous studies, correlation of data from particular samples with C. parapsilosis infection may, in fact, have been associated with C. metapsilosis or C. orthopsilosis. Our study revealed a trend of higher prevalence of C. parapsilosis sensu stricto colonization in patients with viral loads of <10,000 HIV copies ml⁻¹, which, on its own, is difficult to explain. However, none of the reviewed literature analysed this variable, thus precluding any comparisons.

**Susceptibility testing**

Finally, all C. metapsilosis isolates were found to be susceptible to AMB, FLC, KTC, ITC, VRCZ and CASPO.

Sensitivity to AMB was confirmed in previous studies from literature, where most isolates were susceptible to AMB, FLC, KTC, ITC, VRC and CASPO. However, none of the reviewed literature analysed this variable, thus precluding any comparisons.

analysed (Enger et al., 2001; Tavanti et al., 2005; van Asbeck et al., 2008; Kocsubé et al., 2007; Ghannoum et al., 2010; Hensgens et al., 2009). However, it is conceivable that, although occurring less frequently, there are infections in which C. orthopsilosis and C. metapsilosis are present. In our study, the eight C. metapsilosis isolates were identified as colonizers. Hensgens et al. (2009) identified 20 C. metapsilosis isolates out of 395 C. parapsilosis complex isolates; however, these were not confirmed as cases of colonization or infection. Ghannoum et al. (2010) identified one isolate colonizing the oral cavity of a healthy individual. This suggests that C. metapsilosis could be a human commensal, though its importance as a pathogen has yet to be confirmed.

The present study demonstrated that 53.3% of the 15 oral cavity isolates from HIV-infected individuals, initially identified as C. parapsilosis sensu stricto, were, in fact, C. metapsilosis. Thus, it is possible that in previous studies, correlation of data from particular samples with C. parapsilosis infection may, in fact, have been associated with C. metapsilosis or C. orthopsilosis. Our study revealed a trend of higher prevalence of C. parapsilosis sensu stricto colonization in patients with viral loads of <10,000 HIV copies ml⁻¹, which, on its own, is difficult to explain. However, none of the reviewed literature analysed this variable, thus precluding any comparisons.

http://jmm.sgmjournals.org
et al., 2007); however, Silva et al. (2009) noted resistance to VRC in one out of 160 
C. parapsilosis sensu stricto
isolates. Sensitivity to CASPO was previously reported
in eight out of 160 
C. parapsilosis sensu stricto
isolates by Silva et al. (2009); however, Silva et al. (2009) demonstrated that 61 of 160 
C. parapsilosis sensu stricto
isolates were resistant to CASPO. Lockhart et al. (2008) and van Asbeck et al. (2008)
also observed that higher MICs of CASPO were detected for 
C. parapsilosis sensu stricto than for 
C. metapsilosis. In our study, there were no marked differences in MICs between
strains. Therefore, the resistance of 
C. parapsilosis sensu stricto to VRC and CASPO that was observed by Silva et al.
(2009) may be associated with a geographical or regional pattern. The sensitivity of the different species that constitute the 
C. parapsilosis complex to antifungal compounds needs to be elucidated to better understand
genotypical susceptibility differences in the prevalence of these different
species and the impact this has on treatment, especially for immunosuppressed
patients. In addition, distinction between species within the complex may be important in
determining whether noted differences in MICs are associated with differences in clinical outcomes.

REFERENCES


Candida parapsilosis, 
Candida orthopsilosis and 


Candida parapsilosis, 
Candida orthopsilosis, and 


Iida, S., Imai, T., Oguri, T., Okuzumi, K., Yamanaka, A., Moretti-Branchini, M. L., Nimishima, K. & Mikami, Y. (2005). Genetic diversity of the internal transcribed spacers (ITS) and 5.8S rRNA genes among the clinical isolates of 
Candida parapsilosis in Brazil and Japan. Nippon Ishinkin Gakkai Zasshi 46, 133–137.


Candida orthopsilosis and 
Candida metapsilosis in comparison to the closely related species 


Odds, F. C., Hanson, M. F., Davidson, A. D., Jacobsen, M. D., Wright, P., Whyte, J. A., Gow, N. A. & Jones, B. L. (2007). One year prospective survey of 


