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

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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* 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; Bonfietti 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; Garcia-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 TCD4+ 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 Comitê 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.eucast.org).

**Molecular identification of C. parapsilosis complex isolates.** C. parapsilosis ATCC 22019T, C. metapsilosis ATCC 96143 and C. orthopsilosis ATCC 96141 were used as reference strains in this study. Each isolate was plated on Sabouraud glucose agar (Difco) and incubated at 30 °C for 24 h. The yeast cells were then transferred to a microcentrifuge tube containing 1 ml 50 mM EDTA (pH 8), vortex mixed and centrifuged at 13 000 g for 15 min. The supernatant was discarded and the pellet was resuspended in 200 µl 50 mM EDTA (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 2–3 h. DNA was extracted using the phenol/chloroform/isooamyl alcohol method and precipitated with 2-propa- nol 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’-GTGATGTCTGTTG-GATTGT-3’) and anti-sense (SIR: 5’-CAATGGCCAAATCTCCGAA-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
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>
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<tr>
<td></td>
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<td><strong>C. parapsilosis sensu stricto</strong></td>
<td><strong>C. metapsilosis</strong></td>
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<tr>
<td></td>
<td></td>
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<td>No.</td>
<td></td>
</tr>
<tr>
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<td>3 (20.0)</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
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<td>4 (26.7)</td>
<td>2 (13.3)</td>
<td>6 (40.0)</td>
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<td></td>
<td>2 (13.3)</td>
<td>3 (20.1)</td>
<td>5 (33.4)</td>
</tr>
<tr>
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<td>8 (53.3)</td>
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<td>3 (21.4)</td>
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<tr>
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<td></td>
<td>4 (28.6)</td>
<td>5 (35.7)</td>
<td>9 (64.3)</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.

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

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 SADH fragments from all C. parapsilosis clinical isolates and from reference strains C. parapsilosis ATCC 22019, C. metapsilosis ATCC 96143 and C. orthopsilosis ATCC 96139 were digested for 90 min with BshNI (Fermentas) in a 20 μl reaction containing 6 μl PCR product, 2 μl 10 x 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’-GGTCGCGGAA-3’) and p2 (5’-GTTCGTCGCC-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 and C. metapsilosis ATCC 96143.

Analysis of data and dendrogram generation. RAPD profiles were analysed by BioNumerics version 6.6 (Applied Math). 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 (SAB) of 1.00 (100 %) indicated that the banding patterns for strain A were identical to those for strain B, SAB values of 0.80–0.99 (80–99 %) represented
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 ~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 yielded 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.

![Image](https://www.microbiologyresearch.org/download/1/1671-1761.png)

**Fig. 1.** 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; lane 2, C. metapsilosis ATCC 96143; lane 3, C. orthopsilosis ATCC 96141; lane 4, isolates from the oral cavity of HIV-infected patients. BshNI restriction digestion of SADH PCR product: lane 5, C. parapsilosis ATCC 22019; lane 6, C. metapsilosis ATCC 96143; lane 7, C. orthopsilosis ATCC 96141; lanes 8 and 9, respective C. parapsilosis sensu stricto and C. metapsilosis isolates from the oral cavity of HIV-infected patients.

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 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). 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 (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.
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 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 C. parapsilosis sensu stricto (2.2%), while eight isolates were identified as C. metapsilosis (2.5%). The dendrogram in Fig. 2 shows that isolates of the species C. parapsilosis sensu stricto shared high SAB values, suggesting a high level of genetic similarity. The genomic similarity among isolates of C. parapsilosis sensu stricto has been demonstrated previously by using various molecular techniques (Kocsubé 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 C. parapsilosis sensu stricto isolates (Lasker et al., 2006) demonstrated 30 different microsatellite genotypes from 42 isolates. The genetic heterogeneity of 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 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. C. parapsilosis sensu stricto (previously known as C. parapsilosis group I) was identified in 78 (89.7%) samples, followed by C. orthopsilosis (previously group II), identified in eight (9.2%) samples, and 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 C. parapsilosis sensu stricto (64.3%) and C. orthopsilosis (35.7%), but no C. metapsilosis was found at this site. However, in one sample isolated from pharyngeal mucosa, C. metapsilosis was identified. These findings differ from our results, which showed prevalence rates of 46.7% for C. parapsilosis sensu stricto and 53.3% for 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). Kocsubé et al. (2007) determined the genetic variability of C. parapsilosis complex isolates from 20 samples taken from six different anatomical sites of patients hospitalized in two Hungarian hospitals; the prevalent species was C. parapsilosis sensu stricto (90%), followed by C. metapsilosis (10%). One sample, isolated from the throat, was identified as C. metapsilosis, whereas no 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 C. parapsilosis sensu stricto was identified in 160 (94.7%) samples, C. orthopsilosis in five (2.9%) samples and 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 C. parapsilosis sensu stricto and 5.8% to C. metapsilosis.

The above-mentioned studies show a higher occurrence of C. parapsilosis sensu stricto (55.8–94.7%) within the C. parapsilosis complex, which accounted for 46.7% of samples in the present study. The clinical relevance of C. orthopsilosis and C. metapsilosis as aetiological factors in candidiasis development cannot be assessed in the present study due to the small number of oral cavity samples.

Fig. 2. Dendrograms generated for oral isolates of C. parapsilosis sensu stricto (n=7) and C. metapsilosis (n=8) from HIV-infected individuals with asymptomatic Candida carriage. Reference strains are given in bold.
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.

### Susceptibility testing

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

Sensitivity to AMB was confirmed in previous studies from MICs ≤1 μg ml⁻¹ (Lin et al., 1995; Gomez-Lopez et al., 2008; Gonçalves et al., 2010; Kocsubé et al., 2007). It is of note that Silva et al. (2009) reported two cases out of 160 isolates of C. parapsilosis sensu stricto that developed resistance to AMB and Lockhart et al. (2008) reported that the MICs of AMB for C. metapsilosis were lower than those of C. parapsilosis sensu stricto. C. metapsilosis isolates displayed a tendency to have diminished susceptibility to FLC, compared with C. parapsilosis sensu stricto with median MICs of 1.5 and 0.5 μg ml⁻¹, respectively, which are agreement with those observed by van Asbeck et al. (2008); Gonçalves et al. (2010) and Hensgens et al. (2009) also found higher MICs for C. metapsilosis than C. parapsilosis sensu stricto. Our results with KTZ confirmed those of Lin et al. (1995) and Hensgens et al. (2009), showing all isolates to be susceptible to this antifungal compound. Several authors reported sensitivity of C. parapsilosis complex isolates to ITC (Gomez-Lopez et al., 2008; Gonçalves et al., 2010; Kocsubé et al., 2007), which was also noted in our study; this was not the case for one out of four C. metapsilosis isolates, which showed a dose-dependant susceptibility (DDS) to this azole compound, as reported by Gonçalves et al. (2010). The majority of the isolates were susceptible to VRC in the present study, which was also found in other studies (Gomez-Lopez et al., 2008; Gonçalves et al., 2010; Kocsubé et al., 2007).
et al., 2007); however, Silva et al. (2009) noted resistance to VRC in one of 160 C. parapsilosis sensu stricto isolates. Sensitivity to CASPO was previously reported for C. parapsilosis sensu stricto (2008). A naturally occurring proline-to-alanine amino acid change was identified, which may be associated with geographical or regional patterns. The sensitivity of the different species that constitute the C. parapsilosis complex to antifungal compounds needs to be elucidated to better understand geographical 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


