ABSTRACT

Candida parapsilosis is an important non-albicans species responsible for invasive fungal infections and hospital acquired infections especially in critical care patients. C. parapsilosis complex has been named according to genetic bases into 3 different species C. parapsilosis sensu stricto, C. metapsilosis and C. orthopsilosis. This study was designed to describe the distribution and antifungal susceptibility profile of the three members of Candida parapsilosis complex among patients of intensive care units (ICUs) in Mansoura University Hospitals. Candida parapsilosis was identified by Analytic Profile Index (API) 20 C. C. parapsilosis sensu stricto, C. orthopsilosis and C. metapsilosis were recognized according to the secondary alcohol dehydrogenase (SADH) restriction pattern using BanI restriction enzyme. Antifungal susceptibility testing was performed by E test. A total of 68 C. parapsilosis isolates were included in this study. Sixty-two isolates (91.2%) were identified as C. parapsilosis sensu stricto, 4 (5.9%) were identified as C. orthopsilosis, and 2 isolates were identified as C. metapsilosis (2.9%). All isolates of the C. parapsilosis complex species were sensitive to amphotericin B. Fifty isolates (80.6%) of C. parapsilosis sensu stricto were susceptible to fluconazole; 7 isolates (11.3%) were susceptible-dose dependent (SDD) to fluconazole, and 5 isolates (8.1%) were resistant to fluconazole. Most of C. parapsilosis
sensu stricto isolates were sensitive to itraconazole 59 (95.2%). No itraconazole or fluconazole resistance were found among the C. metapsilosis and C. orthopsilosis isolates; there was single C. orthopsilosis isolate SDD to both itraconazole and fluconazole.

Keywords: Candida, C. parapsilosis, Fluconazole, Resistance, Antifungal.

INTRODUCTION

Candidiasis is a serious infection in hospitals worldwide, especially in intensive care units (ICUs) patients (1-2). Candidiasis can result from an endogenous colonization; however, hospital transmission and emergence of resistance to antifungal agents represent new and remarkable problems (3).

Although the main candidal species causing infections worldwide is still Candida albicans, there is an alarm from the increase of invasive infections caused by non-albicans species. Candida parapsilosis has emerged as the second most common causative agents of candidemia in Latin America, Asia (4-5) and in many European surveys (6-8). C. parapsilosis is considered one of the main causes of invasive fungal infections in USA especially in transplant patients (9).

Isolates of C. parapsilosis cannot be distinguished phenotypically. However, genetic analysis by randomly amplified polymorphic DNA revealed that C. parapsilosis complex is composed of three different species, originally they were designed group (I, II, and III). This designation is replaced later by C. parapsilosis sensu stricto, C. orthopsilosis and C. metapsilosis, for group (I, II, and II) respectively (10). This three genetically different species can be identified by restriction analysis of secondary alcohol dehydrogenase (SADH) gene which is present in all groups (11).

Candida infections are mostly treated with amphotericin B (AMB) and its lipid formulations (12-14). However, C. parapsilosis resistance to amphotericin B has been reported (15).

Fluconazole (FLU) is an effective and safe alternative option for treat-
ment of patients with candidemia (16-17) and in particular for candidemia caused by **Candida parapsilosis complex** (18). Several studies reported resistance in *C. parapsilosis* to fluconazole (19-20).

This study aimed at giving insight into the prevalence of the different *C. parapsilosis* complex species; *C. parapsilosis* sensu stricto, *C. metapsilosis*, and *C. orthopsilosis* and their distribution among patients of ICUs of Mansoura University Hospitals. Moreover, this study describes the susceptibility profile of these species to antifungal agents commonly used for treatment of candidal infections namely, AMB, FLU and Itraconazole (ITC).

**METHODS**

This cross sectional study was carried out including all patients aged ≥18 years with candidal infection during their hospital stay in ICUs during period extending from February 2013 to December, 2013 (11 months period). Our local ethical committee approved the protocol. Urine, respiratory samples, blood, and oral swabs were collected from cases with suspected candidal infections clinically.

Samples were collected and processed at the Medical Mycology unit and Microbiology Diagnostic and Infection Control unit in Medical Microbiology and Immunology department, Faculty of medicine, Mansoura University.

All media were prepared according to the manufacturer’s instructions. Processing of specimens was performed according to Koneman et al. (21).

*Candida* was identified according to colonial morphology on Sabouraud Dextrose Agar (SDA), Gram stained film, and non *albicans* Candida were differentiated from *Candida albicans* by germ tube test.

*Candida parapsilosis* was identified using API 20 C AUX (bioMérieux), according to the manufacturer’s instructions.

**DNA extraction.**

DNA Extraction Kit QIAamp was used to extract genomic DNA from Candida parapsilosis strains according the manufacturer’s instructions. The DNA obtained was finally suspended in 100 µl TE buffer and stored at -20°C until use.
PCR amplification and SADH gene restriction analysis.

SADH gene was amplified by PCR for confirmation of *C. parapsilosis*, the reaction was done as described previously by Tavanti et al. (11) using the following primers Fwd, 5'- GTTGATGCTGTGGATTGT-3' and Rev, 5'-CAATGCCAAATCTCCCAAA-3'. PCR reaction was done in a PTC-100™ instrument.

Isolates displaying SADH fragment sized of 716 bp were confirmed to be *Candida parapsilosis* complex and used in the study.

The products of PCR reaction were treated with the BanI enzyme (Thermo Fisher Scientific) in a tube containing 10 µl of the amplification products and 2 µL of BanI. The products of restriction reaction were detected by agarose gel electrophoresis. *Candida parapsilosis* species were distinguished as *C. parapsilosis sensu stricto*, *C. metapsilosis* and *C. orthopsilosis* according to the SADH restriction pattern. DNA bands were visualized using a UV transilluminator.

Antifungal susceptibility testing: was performed by E test (Liofilchem, Italy), and MIC results were interpreted according to the CLSI (22) guidelines.

RESULTS

This study enrolled 68 isolates of *Candida parapsilosis* complex as identified by API 20 C AUX (bioMérieux) and confirmed by PCR amplification of SADH gene.

For the *C. parapsilosis* complex, the amplified SADH fragment (716 bp) was cut by BanI restriction enzyme. According to the BanI restriction profile described before (11) isolates with single BanI restriction site (at position 196) were identified as *C. parapsilosis sensu stricto*, isolated with no restriction site were classified as *C. orthopsilosis* and isolates with three BanI restriction sites (at positions 96, 469, and 529) were identified as *C. metapsilosis*.

Distribution of *C. parapsilosis* complex species: The sex distribution and age groups of patients are presented in table (1). Prevalence of the *C. parapsilosis* complex species and their distribution in different clinical samples are described in table (2). About ninety percent (91.2%) of the isolates (62 isolates) were identi-
fied as *C. parapsilosis sensu stricto*. Four isolates representing (5.9%) were identified as *C. orthopsilosis*. Only two isolates representing (2.9%) were identified as *C. metapsilosis*. *C. parapsilosis sensu stricto* were detected in all types of collected clinical samples including blood. However, *C. orthopsilosis* and *C. metapsilosis* were isolated only from urine and mucosal samples.

**Antifungal susceptibility pattern:** All isolates were sensitive to AMB. Regarding fluconazole sensitivity, fifty isolates (80.6%) of *C. parapsilosis sensu stricto* were sensitive to FLU; 7 isolates (11.3%) were SDD to FLU, and 5 isolates (8.1%) were resistant to FLU. No azoles (fluconazole and itraconazole) resistance were detected among *C. metapsilosis* and *C. orthopsilosis* isolates; there was single *C. orthopsilosis* isolate SDD to both ITC and FLU.

### Table (1) Epidemiological features of patients

<table>
<thead>
<tr>
<th>Sex</th>
<th>NO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29 (42.6)</td>
</tr>
<tr>
<td>Female</td>
<td>39 (57.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age groups</th>
<th>NO (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD (min-max)</td>
<td>47.1±13.7 (18-68 years)</td>
</tr>
<tr>
<td>≥18 - ≤29</td>
<td>8 (11.8)</td>
</tr>
<tr>
<td>&gt;29 - ≤39</td>
<td>12 (17.6)</td>
</tr>
<tr>
<td>&gt;39-&lt;49</td>
<td>10 (14.7)</td>
</tr>
<tr>
<td>&gt;49-&lt;60</td>
<td>17 (25)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>21 (30.9)</td>
</tr>
</tbody>
</table>
Table (2): Distribution of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* in different clinical samples

<table>
<thead>
<tr>
<th>Clinical Sample</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. parapsilosis sensu stricto</em></td>
</tr>
<tr>
<td>Urine</td>
<td>29</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>17</td>
</tr>
<tr>
<td>Blood</td>
<td>6</td>
</tr>
<tr>
<td>Mucosal surface</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>62 (91.4)</strong></td>
</tr>
</tbody>
</table>

Table (3): Susceptibility profile of the three *Candida parapsilosis* spp. and their antifungal MIC range.

<table>
<thead>
<tr>
<th>Species (no. of isolates)</th>
<th>Antifungal agent</th>
<th>MIC (mg/ml) Range</th>
<th>Mean</th>
<th>MIC 90</th>
<th>MIC 50</th>
<th>No (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. parapsilosis sensu stricto</em> (62)</td>
<td>AMB</td>
<td>0.03-1 0.21</td>
<td>0.5 0.125</td>
<td>62 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FLC</td>
<td>2-64</td>
<td>1.3 32</td>
<td>8</td>
<td>50 (80.6)</td>
<td>7 (11.3%)</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>0.05-0.25</td>
<td>0.15 0.25</td>
<td>0.125</td>
<td>59</td>
<td>3 (4.8%)</td>
</tr>
<tr>
<td><em>C. orthopsilosis</em> (4)</td>
<td>AMB</td>
<td>0.006-0.5 0.19</td>
<td>0.5 0.09</td>
<td>4 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FLC</td>
<td>2-32</td>
<td>1.2 3.2</td>
<td>6</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>0.125-0.5</td>
<td>0.34 0.5</td>
<td>0.375</td>
<td>3</td>
<td>1 (25%)</td>
</tr>
<tr>
<td><em>C. metapsilosis</em> (2)</td>
<td>AMB</td>
<td>0.25</td>
<td>- -</td>
<td>2 (100%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FLC</td>
<td>0.5-8</td>
<td>4.3</td>
<td>-</td>
<td>2 (100%)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TIC</td>
<td>0.25-1</td>
<td>0.63</td>
<td>-</td>
<td>2 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

S: susceptible
SDD: susceptible dose dependant
R: resistant

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DISCUSSION

Three different species of \textit{C. parapsilosis complex} have previously been recognized according to genetic background namely; \textit{C. parapsilosis sensu stricto, C. metapsilosis} and \textit{C. orthopsilosis} \cite{11,23}.

In this study, \textit{C. parapsilosis sensu stricto} represents (91.2\%) of all isolated \textit{C. parapsilosis} strains. \textit{C. orthopsilosis} and \textit{C. metapsilosis} represent (5.9\% and 1.5 \%) respectively. \textit{C. parapsilosis sensu stricto} was the only member of the complex that was isolated from blood samples of the ICUs patients.

This result agrees with results of other studies like Silva et al. and GE et al. \cite{24-25}. This augments the assumption that main member of \textit{C. parapsilosis complex} responsible for hematogenous infections is \textit{C. parapsilosis sensu stricto}. The other two members (\textit{C. orthopsilosis} and \textit{C. metapsilosis}) are responsible for other infections like urinary tract infections and mucosal infections.

The higher prevalence of \textit{C. parapsilosis sensu stricto} may be due to its higher capacity for persistence in the hospital environment which may help its transmission to patients. \cite{26-27} And/or may be explained the its capacity to express virulence determinants more than the other two species \cite{28-29} (e.g) adherence to host cells, the ability to form biofilm, and several hydrolytic enzymes production, such as phospholipases, lipases, and proteases \cite{30}.

\textit{C. parapsilosis sensu stricto} was the only species of the complex that can form biofilms \cite{31,32}. Tavanti et al. \cite{33} found that most of \textit{C. parapsilosis sensu stricto} strains are proteinase producers, the higher producers being recovered from blood and mucosal specimens.

The isolation frequency of the three species of \textit{C. parapsilosis} complex is variable throughout the world. In almost all studies, \textit{C.parapsilosis sensu stricto} is the most common isolated species. However, the prevalence and distribution of the three species is variable. This distribution may vary according to socioeconomic conditions of the affected patients population in different countries and cities throughout the world. For example, \textit{C.parapsilosis sensu stricto}
incidence varies from (95.6%) in Kuwait (34) to (64.5%) in China (35). Also elevated incidence of *C. metapsilosis* (10–35.5%) was found in studies performed in hospitals from China (35-36) and in Hungary (37) compared to other countries. On the other hand, *C. orthopsilosis* has higher incidence about (9%) in other studies like Bonfetti et al. (38) in Brazil.

Antifungal susceptibility tests were performed by E test to itraconazole, fluconazole and amphotericin B. All *C. parapsilosis sensu stricto*, *C. metapsilosis* and *C. orthopsilosis* isolates were susceptible to amphotericin B. The *C. parapsilosis sensu stricto* MIC50 and MIC90 of AMB was 0.125 µg/ml and 0.5 µg/ml respectively. This result agrees with most of studies before that found the *C. parapsilosis* MIC50 and MIC90 average values range from 0.13 to 1 µg/ml and from 0.5 to 1 µg/ml, respectively (39-42).

Regarding azole antifungal agents, about eighty percent of *C. parapsilosis sensu stricto isolates* were susceptible to fluconazole, (11.3%) were SDD and (8.1%) were resistant. About ninety five percent of the isolates were sensitive to Itraconazole and (4.8%) were SDD. No FLU-resistant or ITC resistance was detected among *C. metapsilosis* and *C. orthopsilosis* isolates. Only one isolate of *C. orthopsilosis* were SDD to Fluconazole and Itraconazole (MIC 32 µg/ml and 0.5 respectively).

Fluconazole-resistance has been reported in clinical isolates of *C. parapsilosis sensu stricto* around the world (43-48).

We observed only one *C. orthopsilosis* isolate was SDD to fluconazole and itraconazole (MIC 32 µg/ml and 0.5 respectively). However, because of the small number of isolates belonging to the new species, this study may not present a complete picture about the antifungal susceptibility pattern of *C. orthopsilosis*, and *C. metapsilosis*.

This study has some limitations. First, the current study did not investigate possible risk factors for *C. parapsilosis* infections. Furthermore, the study did not search the virulence factors of *C. parapsilosis* complex and the differences of virulence fac-
tors between members of the complex that may increase the prevalence of *C. parapsilosis sensu stricto* infections among these patients. So, further studies are required to discuss these factors.

**Conclusion**

*C. parapsilosis sensu stricto* represent majority of *C. parapsilosis complex* causing infections in ICUs patients. AMB retains its activity against the members of the *C. parapsilosis complex*. There is an alarming of azoles resistance in the members of the complex especially fluconazole.

**REFERENCES**


5- Ma CF, Li FQ, Shi LN, Hu YA, Wang Y, Huang M, Kong QQ (2013) : Surveillance study of species distribution, antifungal susceptibility and mortality of nosocomial candidemia in a tertiary care hospital in Chi-


20- Sarvikivi E, Lyytikäinen O, Vol. 43, No. 1 & 2 Jan. & April, 2014


22- CLSI (2012) : Reference method for broth dilution antifungal susceptibility testing of yeasts; 4th informational supplement. CLSI document M27-S4. Clinical and Laboratory Standards Insti-


MANSOURA MEDICAL JOURNAL
الملخص العربي

أنواع وحساسية ميكروب الكانديدا باراسليوبسزم لضادات الفطريات

في مرضى العناية المركزية بمستشفى جامعة المنصورة.

بحث مقدم من / غادة السعيد مشالي
قسم الميكروبيولوجيا الطبية والمناعة
كلية الطب-جامعة المنصورة

ميكروب الكانديدا باراسليوبسزم هو واحد من الأنواع الكانديدة الهامة المسؤولة عن العدوى الفطرية الغازية وعندما تستجدي المكتبة، والإسهال في المرضى الروتيني الحريجة، وقد تم إعادة تسمية مجمع ميكروب الكانديدا باراسليوبسزم وفقًا لقواعد وراثية لثلاثة أنواع سانيستركلتو، أورتوسيوبسزم و مياتابسليم.

وقد صممت هذه الدراسة لوصف توزيع وخصائص حساسية إعضاء مجمع الكانديدا باراسليوبسزم لمضادات الفطريات بين المرضى من وحدات العناية المركزية في مستشفى جامعة المنصورة. وقد تم التعرف على إعضاء مجمع ميكروب الكانديدا باراسليوبسزم بواسطة مؤشر الملف التحليلي API20 C وفقًا لخصائص قطع جين باستخدام انزيم القطع و تم إجراء اختبار الحساسية لضادات الفطريات عن طريق اختبار E.

وقد تم عزل 88 عزلة ميكروب الكانديدا باراسليوبسزم نتائج 12 عزلة من نوع سانيستركلتو، وشكل نسبة (91.2%) واربع عزلات من نوع أورتوسيوبسزم بنسبة (7.9%)، عزلتين من نوع مياتابسليم بنسبة (2.9%)، جميع العزلات كانت حساسة لعقار الأمفوتيريسين. وب، ما زيادة عن ثمانين في المئة (80%) من العزلات من نوع سانيستركلتو كانت حساسة لعقار الفلوكونازول و (80.6%) العزلات (11.2%) ذو حساسية متغيرة على حسب الجرعة و حمض عزلة الفلوكونازول (0.8%) كانت حساسة لعقار الاتراكونازول، و معظم العزلات (97.9%) كانت حساسة لعقار الاتراكونازول. ثم يتم عزل اي عزلات من التنوع أورتوسيوبسزم و مياتابسليم مقاومة لعقار الفلوكونازول و الاتراكونازول في هذه الدراسة، كما كانت هناك عزلة واحدة من التنوع أورتوسيوبسزم ذو حساسية متغيرة على حساب الجرعة لعقار الفلوكونازول و الاتراكونازول.

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و نستخلص من هذا البحث أن نوع سانسيستريكتو يشكل غالبية مجمع ميكروب الكانديدا بارابسلوبسنز المسبب للعدوى في مرضى العناية المركزة و لازال عقار امفورتريسين يحتفظ بفاعليتة ضد جميع أنواع مجمع ميكروب الكانديدا بارابسلوبسنز و لكن هناك زيادة في مقاومة الميكروب لعقارات الأتروازات خاصة عقار فلوكونازول.