SHORT REPORT

High frequency of Candida parapsilosis on the hands of healthy hosts

L.A. Bonassolia, M. Bertoli, T.I.E. Svidzinski*

Universidade Estadual de Maringá, Hospital Universitário Regional de Maringá, Paraná, Brazil
Universidade Estadual de Maringá, Departamento de Análises Clínicas, Laboratório de Micologia Clínica, Avenida Colombo, 5790, Bloco J90, sala 11, 87020-900 Maringá, Paraná, Brazil

KEYWORDS
Candida parapsilosis; Hand carriage; Nosocomial infections; Virulence; Susceptibility

Summary  The presence of yeasts on the hands of 86 healthy hosts (62 hospital workers and 24 healthy members of the community with no hospital exposure) was investigated. A high rate of colonization was found (59.3%). Candida parapsilosis was the most frequently isolated species (51%), independent of the origin of the samples. The potential virulence and resistance to antifungals of the 26 C. parapsilosis isolates were determined.
All were proteinase producers and formed biofilms. The haemolytic activity was variable, with a predominance of total haemolysis of sheep erythrocytes. All isolates were susceptible to amphotericin B but two showed reduced susceptibility to fluconazole. Healthy people may be colonized by a species of yeast with a high capacity for adhesion to plastic surfaces, providing an infection risk to susceptible individuals.

Introduction

Candida species are now considered to be the fourth largest cause of systemic nosocomial infection. C. parapsilosis is probably the species that has had the largest increase in incidence since 1990, becoming the predominant agent of candidaemia in certain centres.

The patients most susceptible to C. parapsilosis infections are very-low-birthweight infants in neonatal intensive care units (NICUs) and immunocompromised patients, who generally require antibiotics and central venous catheters (CVCs) for long periods and frequently receive total parenteral nutrition (TPN).

The main source of hospital infections by yeasts is still the endogenous flora of the patient. However, the hands of healthcare workers (HCWs) are also considered to be important for colonization and infection, especially with C. parapsilosis.

The virulence of C. parapsilosis is associated
with its capacity for adhesion to plastic surfaces, and consequently to the development of candidaemia related to catheters.\textsuperscript{5,6} It is able to proliferate in high-concentration glucose solutions, and produces a large quantity of extracellular materials, forming extensive biofilms on the surface of a catheter, enabling the multiplication and permanence of the yeast and, consequently, its dissemination.\textsuperscript{6}

The aims of this study were to evaluate the rate of colonization by \textit{C. parapsilosis} on the hands of healthy hosts, the potential virulence and the antifungal susceptibility of the strains isolated.

**Methods**

**Samples**

Samples were collected from the hands of 86 individuals. Of these, 62 were health professionals working at Maringá Regional University Hospital in the NICU (\(N=21\)), laboratories (\(N=22\)) and the blood bank (\(N=19\)). The remainder were healthy members of the community (\(N=24\)) with no hospital exposure.

**Isolates**

Samples were collected during unannounced visits to the workplaces. Hands were washed in 20 mL of brain heart infusion broth (Difco) in sterilized plastic containers. After centrifugation, 20 \(\mu\)L of sediment was spread on CHROMagar \textit{Candida}\textsuperscript{®} (CHROMagar Company, Paris, France), and incubated at 25\(^\circ\)C for 48 h. After incubation, a semiquantitative evaluation of the colonies produced was performed.

**Identification**

The yeasts were identified by two methods: the classical biochemical method and the MicroScan\textsuperscript{®} rapid yeast identification panel (Dade Behring Inc, CA, USA).

**Proteinase production**

Proteinase was detected by the formation of an opaque halo of degraded protein around the colony. The enzymatic activity was measured by the ratio between the colony diameter and the colony diameter plus the precipitation zone.

**Biofilm formation**

Biofilm production was determined using the spectrophotometric method described by Shin \textit{et al.}\textsuperscript{7}

**Haemolytic activity**

A blood agar medium containing 7% glucose and 7% sheep blood was used to look for haemolysis,\textsuperscript{8} which was classified as absent, partial or total.

**Antifungal susceptibility testing**

The microdilution method in RPMI 1640 broth was used to test fluconazole and amphotericin B by the National Committee for Clinical Laboratory Standards’s method.\textsuperscript{9} The minimal inhibitory concentrations (MIC) of the antifungals against each \textit{C. parapsilosis} isolate were determined. For fluconazole, the MIC was the lowest concentration of antifungal capable of inhibiting \(\geq 50\%\) of microbial growth compared with the positive control, and for amphotericin B, the MIC was the lowest concentration that inhibited 100\% of the growth.

**Statistical analysis**

The chi-squared test was used to compare the rate of colonization by \textit{C. parapsilosis} with other yeasts on the hands of different healthy hosts.

**Results**

Of the people analysed, 59.3\% carried yeasts on their hands. \textit{C. parapsilosis} was the most common species isolated (51\%) (Table I).

Qualitatively, there was no significant difference in colonization between workplaces (\(P>0.05\)), but an important variation in the concentration of these micro-organisms, evidenced by the number of isolated colonies, was observed. Samples obtained from the NICU staff contained few yeasts (one to three colonies per plate), and more growth was observed in the other sectors (laboratories, blood bank and community). This was sometimes difficult to count due to confluent growth.

All 26 \textit{C. parapsilosis} isolates produced proteinase, with 84.6\% showing high enzymatic activity and the others showing intermediate activity. They also produced biofilm. Twenty-two isolates were moderately positive (+3) and four (15.4\%) were strongly positive (+4).\textsuperscript{7}

Haemolytic activity was variable. Total and partial haemolysis were observed with 16 (61.5\%)
and nine (34.6%) isolates, respectively. Only one strain did not exhibit any haemolytic activity.

Amphotericin B had good activity, inhibiting 100% of the strains with MIC ≤ 1 μg/mL. Most isolates were susceptible to fluconazole, with an average MIC of 5.42 μg/mL (MIC<sub>90</sub> = 4 μg/mL and MIC<sub>90</sub> = 8 μg/mL). However, two isolates from hospital environments (laboratory and NICU) showed reduced susceptibility with MICs of 16 and 32 μg/mL, respectively.

**Discussion**

A high rate of yeast colonization on the hands of healthy hosts has been reported previously, and *C. parapsilosis* was the most commonly isolated species.<sup>4,10</sup>

The lower concentration of yeasts in NICU staff can be attributed to the strict application of hygienic hand disinfection. This is not common practice in the other sections of the hospital.

*C. parapsilosis* is an important pathogen as it implies the possibility of nosocomial transmission of fungaemia by the hands of HCWs.<sup>10,11</sup> In a Brazilian multi-centre study, Colombo *et al.*<sup>12</sup> demonstrated that nosocomial candidaemias are caused predominantly by non-*albicans* *Candida* species, and *C. parapsilosis* was the most frequently isolated species. This was also found by Matsumoto *et al.*,<sup>3</sup> who isolated yeasts from the blood and catheters of paediatric inpatients.

We studied 26 skin isolates of *C. parapsilosis* and found that all of them were proteinase producers. Bernardis *et al.*<sup>5</sup> also reported production of high concentrations of proteinase by skin isolates of *C. parapsilosis*.

Biofilms were produced by all the isolates when grown in a glucose-containing solution. Most isolates were moderately positive as were those described by Bernardis *et al.*<sup>5</sup> In contrast, Pfaller *et al.*<sup>13</sup> found that most skin isolates were weakly positive.

The capacity of the *Candida* species to produce biofilms in vitro may reflect their potential to cause fungaemia related to CVCs in patients receiving TPN.<sup>7,13</sup> The micro-organisms in biofilms also become more resistant to treatment than those in the planktonic form.<sup>6</sup>

Research with invasive isolates of *C. parapsilosis* (blood and catheter) has confirmed the association between pathogenicity and the extensive production of biofilm.<sup>6,7</sup> Shin *et al.*<sup>7</sup> compared biofilm production between species of *Candida* isolated from systemic infections and from other sources. They observed that *C. parapsilosis* strains isolated from blood produced significantly more biofilm than those from elsewhere. Luo *et al.*<sup>8</sup> demonstrated that some species of *Candida* produced one or more types of haemolysin in vitro, but *C. parapsilosis* had no haemolytic activity. In contrast, our study showed variable haemolytic activity, with a predominance of total haemolysis in sheep erythrocytes. The function of haemolysin in relation to virulence in yeasts is not clear. Further studies are needed to investigate whether these haemolytic factors facilitate the dissemination of the micro-organisms.

The *C. parapsilosis* isolates were inhibited by low concentrations of amphotericin B (MIC<sub>90</sub> = 1 μg/mL), which is in agreement with previous studies. Two isolates had reduced susceptibility to fluconazole, signalling a possible need for a higher dose of this agent. In contrast, Colombo *et al.*<sup>12</sup> reported low resistance of *C. parapsilosis* to fluconazole.

Our results confirmed that *C. parapsilosis* is the most frequently isolated yeast on the hands of healthy people, including NICU workers who frequently work with sick infants, increasing the risk of development of an invasive disease.

Healthy people working in places unrelated to hospitals also carry yeasts on their hands with the same potential virulence, and which therefore offer the same risk of infection. This information should be considered when preventive measures are established. Attention to the

<table>
<thead>
<tr>
<th>Sector</th>
<th>Total number of people</th>
<th>Number (%) of people with some type of yeast</th>
<th>Number (%) of people with <em>C. parapsilosis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood bank</td>
<td>19</td>
<td>11 (57.9)</td>
<td>5/11 (45.5)</td>
</tr>
<tr>
<td>Laboratory</td>
<td>22</td>
<td>15 (68.2)</td>
<td>7/15 (46.7)</td>
</tr>
<tr>
<td>NICU</td>
<td>21</td>
<td>13 (61.9)</td>
<td>7/13 (53.8)</td>
</tr>
<tr>
<td>Community</td>
<td>24</td>
<td>12 (50.0)</td>
<td>7/12 (58.3)</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>51 (59.3)</td>
<td>26/51 (51.0)</td>
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</tbody>
</table>

NICU, neonatal intensive care unit.
colonization of hands should not be restricted to high-risk units such as NICUs, but should also include other sections of hospitals and even families, babysitters and people that work with susceptible patients.

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References