CANDIDURIA IN A PUBLIC HOSPITAL OF SÃO PAULO (1999-2004): CHARACTERISTICS OF THE YEAST ISOLATES

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SUMMARY

The study involved 100 yeast isolates, obtained from urine samples provided by a Public Pediatric Hospital of São Paulo, Brazil, from 1999 to 2004. The most frequent species was Candida albicans, followed by C. tropicalis, C. glabrata and C. parapsilosis. In regard to virulence, 97% of the isolates showed index 3 for proteinase and 63% index 2 for phospholipase. The most frequent killer biotypes were 511 and 888.

KEYWORDS: Candiduria; Nosocomial infections; Epidemiological markers.

INTRODUCTION

In the last 20 years, there has been a significant rise in the occurrence of nosocomial infections due to Candida genus yeasts. Lately, this increase has been much more associated to urinary tract infections. The incidence of fungemia and urinary tract infections is gradually on the rise and is an important public health problem. About 10 to 15% of urinary tract hospital infections are due to Candida spp., and its prevalence is still increasing. Recent studies have shown that the rate of the urinary tract infection has increased from 0.9/1000 to 2.0/1000 patients.

At the end of the 1980s, in the United States, 7% of the total number of nosocomial urinary infections were caused by Candida species. A one-year yeast study conducted with 205 hospitalized patients, showed that, in cases of urinary infection, 22% of the isolates belonged to genus Candida.

Recent studies related to urinary infections caused by Candida spp. have focused on specific predisposing conditions, however, particularly for children, candiduria frequency, characteristics and implications are still unknown. Nevertheless, candiduria can be a precocious marker of a disseminated candidiasis, as demonstrated in a recent study of patients in a Surgical Intensive Care Unit precociously diagnosed with candiduria, who progressed to candidemia. Candiduria has developed in 1.3 - 10% of the patients who previously had funguria, and 0.4% of them evolved to death.

Many researches showed that candiduria cases not reflect a disseminated candidiasis but colonization or lower urinary tract infection. Often it is difficult to distinguish asymptomatic candiduria from bladder or renal infection. Urine cultures positive for Candida species have been noted in healthy men and women.

In the present study, 100 yeast isolates obtained from children with candiduria were identified, and their virulence factors (proteinase and phospholipase) and killer phenotypes were investigated.

MATERIAL AND METHODS

Source of yeasts: This study involved 100 isolates of yeasts obtained from confirmed cases of candiduria in children (zero to seven years) hospitalized in the period from 1999 till 2004 in a Public Pediatric Hospital in São Paulo City, SP, Brazil. This tertiary care hospital, mainly attending a low-income population, has 90 patient beds.

Inclusion criterion: To be considered urinary yeast infection, the yeasts have to be isolated from urine samples with counts superior to 10^4 UFC/mL for only one isolate and 10^5 UFC/mL for more than one isolate.

Yeasts identification: The yeasts were identified according to their macroscopic, microscopic and physiological characteristics, according to KURTZMAN & FELL. For the identification of Trichosporon genus, the classification recommended by GUEHO et al. was used.

Proteinase research: Proteinase enzyme research was carried out according to RUCHEL et al. The enzyme activity (PZ) was measured...
according to PRICE et al.35 (Pz = 1 - absence of enzyme activity = index 1; 1.0 < Pz ≥ 0.64 - positive enzyme activity = index 2; Pz < 0.64 - strongly positive enzyme activity = index 3). C. albicans pattern strain ICB-12A was used as a positive control.

**Phospholipase analysis:** Phospholipase enzyme testing was carried out according to PRICE et al.35. *C. albicans* pattern strain ICB-12A was used as a positive control.

**Susceptibility to killer toxins:** The *Candida* isolates’ susceptibility to killer toxins was investigated by the technique described by POLONELLI et al.36. Nine pattern strains of killer-toxin producers, from Parma University, Italy, were used as a control.

### RESULTS

The most frequent species were *Candida albicans*, 56.0%, followed by *C. tropicalis* (20.0%), *C. glabrata* (11.0%), *C. parapsilosis* (4.0%), *C. lusitaniae*, *C. guilliermondii*, *C. krusei* (2.0%) and *Trichosporon asahii* (3.0%).

In regard to the production of extracellular enzymes, 84.0% of all the isolates showed strongly positive activity for proteinase (index 3), and 40.0% of these presented the same index for phospholipase activity. Of the *C. albicans* isolates, 98.2% were proteinase producers and 17.8% did not present phospholipase activity. All the non-*albicans* species showed proteinase production. Of the 43 non-*albicans* isolates, 24 (55.8%) did not produce phospholipase. In regard to the three *Trichosporon asahii* isolates, it was observed that only one was a proteinase producer, while all of them did not produce phospholipase (Table 1).

The tests for susceptibility to killer toxins resulted in eight different biotypes. Of all 100 isolates, the most frequent biotypes were 511 (63.0%) and 888 (20.0%). The biotypes 513 and 555 were all *C. albicans*, while *T. asahii* revealed only biotype 888 (Table 2).

### DISCUSSION

The frequency of urinary tract infection caused by yeasts has increased greatly over the last two decades due to the greater prevalence of *Candida* species in clinical settings. The use of antibiotics and the increase in immunocompromised patients have contributed to the increased prevalence of *Candida* infections. The results of this study highlight the importance of understanding the characteristics of the yeast isolates and their potential for extracellular enzyme production and susceptibility to killer toxins.

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**Table 1**
Proteinase and phospholipase activity presented by 100 yeast isolates obtained from cases of candiduria in children hospitalized in a Public Hospital of São Paulo, Brazil (1999-2004)

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>PA (1n (%))</th>
<th>PPA (2n (%))</th>
<th>Enzymatic Indexes</th>
<th>PA (3n (%))</th>
<th>PPA (TOTAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> (56)</td>
<td>1 (1.8)</td>
<td>10 (17.8)</td>
<td>8 (14.2)</td>
<td>12 (21.4)</td>
<td>47 (83.9)</td>
</tr>
<tr>
<td><em>C. tropicalis</em> (20)</td>
<td>-</td>
<td>12 (60)</td>
<td>4 (20)</td>
<td>5 (25)</td>
<td>16 (80)</td>
</tr>
<tr>
<td><em>C. glabrata</em> (11)</td>
<td>-</td>
<td>6 (54)</td>
<td>-</td>
<td>2 (18)</td>
<td>11 (100)</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> (04)</td>
<td>-</td>
<td>3 (75)</td>
<td>-</td>
<td>1 (25)</td>
<td>4 (100)</td>
</tr>
<tr>
<td><em>C. lusitaniae</em> (02)</td>
<td>-</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1 (50)</td>
</tr>
<tr>
<td><em>C. krusei</em> (02)</td>
<td>-</td>
<td>2 (100)</td>
<td>-</td>
<td>2 (100)</td>
<td>2 (100)</td>
</tr>
<tr>
<td><em>C. guilliermondii</em> (02)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (100)</td>
<td>2 (100)</td>
</tr>
<tr>
<td><em>T. asahii</em> (03)</td>
<td>2 (67)</td>
<td>3 (100)</td>
<td>-</td>
<td>1 (33)</td>
<td>1 (33)</td>
</tr>
</tbody>
</table>

**Table 2**
Sensitivity to killer toxins of 100 isolates obtained from cases of candiduria in patients hospitalized in a Public Pediatric Hospital of São Paulo, Brazil (1999-2004)

<table>
<thead>
<tr>
<th>Species</th>
<th>511</th>
<th>513</th>
<th>555</th>
<th>587</th>
<th>812</th>
<th>887</th>
<th>888</th>
<th>111</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>46</td>
<td>01</td>
<td>-</td>
<td>01</td>
<td>01</td>
<td>-</td>
<td>05</td>
<td>02</td>
<td>56</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>14</td>
<td>-</td>
<td>01</td>
<td>03</td>
<td>-</td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>02</td>
<td>-</td>
<td>01</td>
<td>06</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>02</td>
<td>-</td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>04</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>01</td>
<td>-</td>
<td>01</td>
<td>02</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>02</td>
<td>-</td>
<td>-</td>
<td>02</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>01</td>
<td>-</td>
<td>-</td>
<td>01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>02</td>
</tr>
<tr>
<td><em>T. asahii</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>03</td>
<td>-</td>
<td>-</td>
<td>03</td>
</tr>
</tbody>
</table>

**Total** | 63 | 01 | 01 | 08 | 02 | 02 | 20 | 03 | 100 |

PA = proteolytic activity; PPA = phospholipase activity
of prolonged hospitalization, patients with advanced age, immunocompromised patients; use of antibiotics; prophylaxis by antifungal agents; use of urinary catheters; urinary tract surgical manipulation and, especially, longer stays in intensive care units. Catheterized patients are at special risk, since around 26% of the urinary tract infections are caused by fungus.

EMORI & GAYNES and JARVIS reported that 25% of the urinary tract infections are due to Candida genus yeasts. Candida albicans accounts for 40 to 65% of the fungi isolated from candiduria cases. However, there has been an increase in the incidence of other species, and recently it has been observed that the urinary tract is more frequently colonized by non-albicans species than are other sites. The non-albicans species include most notably C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, C. lusitaniae and Trichosporon spp.

A survey conducted from 1990 to 1991 of the aetiological agents in urine collected in a tertiary care hospital found a 34% rise in non-albicans species and a 11% decrease in C. albicans. KRCMÉRY & KOVACICOVÁ, in a 10-year study, also verified that although C. albicans was still the most frequently isolated species, there had been an increase of non-albicans species from 1% in 1991 to 46.3% in 1998. Candida albicans was identified in 61.6% of all the fungemia cases, followed by C. parapsilosis (9.9%), C. krusei (5.8%), C. tropicalis (4.1%) and C. glabrata (3.2%).

In the present study, C. albicans was the species most frequently isolated, representing 56% of all the yeasts. However, other species were isolated and the second-most frequent was C. tropicalis (20%). C. glabrata (11%). During the period from 1998 to 1999, WEINBERGER et al. studied 751 patients with candiduria and found that C. albicans was the most common species, with an incidence of 56.4%, followed by C. tropicalis (19%) and C. glabrata (15%). Other recent studies have also observed that C. albicans is still the most commonly isolated species, followed by C. tropicalis. Lately, studies have shown that C. glabrata is the second most isolated species in candiduria cases.

Significant geographic variations on the etiological pattern of invasive Candida spp. infections have been reported in various countries. In North America there is a predominance of C. glabrata among non-albicans species, in South America, however, C. parapsilosis and C. tropicalis are the predominant ones.

These data have been confirmed by statistical studies performed in South America that have shown the relevance of invasive infections due to C. parapsilosis and C. tropicalis. The reasons for this inversion of the species’ distribution pattern have not yet been completely elucidated, but may be related to the microorganisms virulence potential and resistance to antifungals.

For the Candida genus, one of the most studied virulence factors is the production of extracellular enzymes, such as the secreted aspartyl proteinase (SAPs) that hydrolyse peptide bonds, along with the phospholipases, which hydrolyse phospholipides.

Various studies have demonstrated that Candida albicans and other species are proteinase and phospholipase producers, underscoring the role that these enzymes play as virulence determinants, regardless of the site from which they are isolated. According to IBRAHIM et al., Candida albicans seems to be the only Candida species which produces phospholipase in vitro. That study also evaluated the ability of various Candida spp. species to produce phospholipase, and found that while 79% of the 41 C. albicans isolates produced this enzyme, this was not the case for any of the C. tropicalis, C. glabrata or C. parapsilosis isolates. MATSUMOTO et al. studied the production of phospholipase in Candida obtained from catheter and blood, found that while 87.5% of the C. albicans isolates produced this enzyme, only three non-albicans isolates did. RUIZ et al. observed phospholipase production in 48.3% of C. albicans isolates and in 2.7% of the non-albicans ones.

In the present study, a higher phospholipase activity was also observed for C. albicans (82%), in comparison to non-albicans (41%) and T. asahii (33%) isolates. This enzyme may also be used as a marker in the diagnosis of candidiasis.

In regard to proteinase, the enzymatic activity of C. albicans isolates ranged from 62.5% to 100%. Experimentally, mutants deficient in terms of proteinase production seem to be less virulent to animals than their proteolytic relatives.

The literature has demonstrated distinct levels of proteinase production by different C. albicans isolates and different Candida species. Moreover, a correlation has been demonstrated between the level of proteinase production and virulence by species, in descending order: C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. glabrata and C. guilliermondii.

SILVA et al. isolated C. albicans from the oral mucosa of patients with AIDS and verified proteinase production in 100% of the isolates. MATSUMOTO et al. studied 80 Candida isolates obtained from blood and catheter and observed that 81.3% presented proteolytic activity. A study performed by RUIZ et al. demonstrated a strong proteinase activity in Candida species: 100% for C. albicans and 97.8% for non-albicans, isolated from blood. In the present study, 98% of the C. albicans and 100% of non-albicans species presented strong proteinase activity.

Among the 100 isolates of the present study eight different killer biotypes were found. The biotype most frequently encountered was 511 (63% of all the isolates), representing 82.1% of the Candida albicans and 70.0% of the Candida tropicalis. Biotype 888, the second most frequent, was observed in Candida glabrata (54.5%) and Candida parapsilosis (50.0%).

RUIZ et al. demonstrated six killer biotypes, while POLONELLI et al. and MATSUMOTO et al. registered, respectively, 25, 23 and seven different biotypes. These differences might be related to the variety of anatomical sites from which the yeast samples were taken. Moreover, some studies have found a lower number of killer biotypes than were encountered in these studies.

The most frequent biotypes, 511 and 888, were also prevalent in
89.6% of the Candida species isolated from the blood of hospitalized patients, so they are not restricted only to the urine.

According to MORACE et al., the use of the killer system for differentiating isolates of pathogenic yeast species can be a useful method for dealing with the nosocomial infections caused by these microorganisms.

RESUMO

Candiduria em hospital público de São Paulo (1999-2004): características das leveduras isoladas

Estudou-se 100 amostras de leveduras, isoladas de urina, provenientes de Hospital Público Infantil de São Paulo, Brasil, no período de 1999-2004. A espécie mais freqüente foi Candida albicans, seguida de C. tropicalis, C. glabrata e C. parapsilosis. Em relação à virulência, 97% dos isolados apresentaram índice 3 para proteína e, 63% índice 2 para fosfolipase. Os bioótipos “killer” mais freqüentes foram o 511 e 888. Os bioótipos “killer” mais freqüentes foram o 511 e 888.

REFERENCES


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