

FUNGEMIA CAUSED BY *Candida* SPECIES IN A CHILDREN'S PUBLIC HOSPITAL IN THE CITY OF SÃO PAULO, BRAZIL: STUDY IN THE PERIOD 2007-2010

Vanessa Kummer Perinazzo OLIVEIRA(1), Luciana da Silva RUIZ(1), Nélio Alessandro Jesus OLIVEIRA(2), Débora MOREIRA(1), Rosane Christine HAHN(3), Anely Salles de Azevedo MELO(4), Angela Satie NISHIKAKU(4) & Claudete Rodrigues PAULA(5)

SUMMARY

Candidemia remains a major cause of morbidity and mortality in the health care environment. The epidemiology of *Candida* infection is changing, mainly in relation to the number of episodes caused by species *C. non-albicans*. The overall objective of this study was to evaluate the frequency of yeasts of the genus *Candida*, in a four-year period, isolated from blood of pediatric patients hospitalized in a public hospital of the city of São Paulo, Brazil. In this period, yeasts from blood of 104 patients were isolated and, the identified species of *Candida* by phenotypic and genotypic methods were: *C. albicans* (39/104), *C. tropicalis* (25/104), *C. parapsilosis* (23/104), *Pichia anomala* (6/104), *C. guilliermondii* (5/104), *C. krusei* (3/104), *C. glabrata* (2/104) and *C. pararugosa* (1/104). During the period of the study, a higher frequency of isolates of *C. non-albicans* (63.55%) ($p = 0.0286$) was verified. In this study we verified the increase of the non-*albicans* species throughout the years (mainly in 2009 and 2010). Thus, considering the peculiarities presented by *Candida* species, a correct identification of species is recommended to lead to a faster diagnosis and an efficient treatment.

KEYWORDS: *Candida*; Candidemia; Pediatric.

INTRODUCTION

Candidemia is an important concern in the clinical medicine related to the public health²⁰, mainly because of the high mortality rates in children and adults, 30% and 50% respectively³⁸. The risk factors for the acquisition of this infection are similar in both ages and include a long-term hospitalization in an Intensive Care Unit, previous bacterial infection, central venous catheter use, parenteral nutrition and immunosuppression. The yeasts of the genus *Candida* represent the third/fourth pathogens responsible for the bloodstream infections^{10,11,24} and their epidemiology has been well studied in the United States and Europe, but not in Latin America. In Brazil, the incidence rates are fragmented when considering data from all regions of the country^{10,27}.

Although *C. albicans* remains the most frequent species of yeast isolated from bloodstream infections, over the last years the number of candidemias caused by non-*albicans* species has been increasing. Being that, some studies have reported that from 40% to 50% of the infections are caused by other species of *Candida*^{16,31,33}. Moreover, invasive infections caused by non-*albicans* species are more difficult to be treated due to its eventual innate or acquired resistance to antifungal agents. Therefore the treatment administration should be based on the species-level identification²³.

Many epidemiological studies have described the distribution

of the species, antifungal susceptibility and risk factors in the adult population^{10,33}. However, there are few studies about candidemia in the pediatric population^{30,34}. Some studies, done in hospitals from different geographic regions, report that the most frequent species that cause bloodstream infections in pediatric patients are *C. albicans* and *C. parapsilosis*^{16,28}. In adults, the most frequent isolated species are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*²⁸.

In Brazil, the most commonly isolated species in pediatric patients are *C. albicans*, *C. parapsilosis* and *C. tropicalis*. In adult patients the most common species are the same as in pediatric patients, except because of the species *C. tropicalis* that is the second most frequent species in adults¹⁰. This high isolation of *C. parapsilosis* in pediatric patients could be explained by the fact that this species is related to prematurity, presence of central venous catheters and the use of total parenteral nutrition³⁰.

Anatomical and physiological differences between the pediatric and adult patients change the susceptibility to infections caused by different species of *Candida*, which therefore influences the antifungal treatment approach, including issues related to the toxicity of the drugs, pharmacokinetic and dosage⁴¹.

Considering the existence of a restricted amount of data to guide the clinical decisions to child patients with invasive fungal infections when

(1) Department of Microbiology, Institute of Biomedical Science II, University of São Paulo (USP), São Paulo, SP, Brazil.

(2) Lilliehei Heart Institute, Dept. of Medicine, University of Minnesota, Minneapolis-MN, USA.

(3) Division of Infectious and Tropical Diseases, Federal University of Mato Grosso, MT, Brazil.

(4) Special Laboratory of Mycology, Federal University of São Paulo (UNIFESP).

(5) Department de Stomatologia, School of Dentistry, University of São Paulo (USP), São Paulo, SP, Brazil.

Correspondence to: Claudete Rodrigues Paula, PhD, Departamento de Estomatologia, Faculdade de Odontologia, Universidade de São Paulo (USP), Av. Prof. Lineu Prestes 2277, 05508-000 São Paulo, SP, Brasil. Phone/fax: 55.11.30917883. E-mail: crpmicol@uol.com.br

compared to adults with similar infections, this study had as its objective to evaluate the frequency of the yeast species isolated from blood during four years in a pediatric hospital in São Paulo, Brazil.

PATIENTS

From 2007 to 2010, yeasts isolated from blood of 104 patients, represented by children from five to 11 years old, were studied. Being that, each isolated represented a different episode of candidemia. The patients were hospitalized in a tertiary infant hospital with 90 hospital beds located in the city of São Paulo, Brazil.

MATERIAL AND METHODS

Isolate Process: The blood was seeded in a biphasic culture medium BACTEC (Difco[™], USA). Once the fungal growth was detected, the medium was seeded in Sabouraud dextrose agar added to chloramphenicol to the yeasts isolation, after that it was seeded in a CHROMagar[®] Candida Chromogenic Medium to ensure the purity of the isolates and then phenotypically identified.

Phenotypic identification: The conventional identification of all the isolates was performed according to the protocol of KURTZMAN *et al.*²⁰, using the germ tube test, microculture, auxanogram and zymogram. The phenotypic identification between *C. albicans* and *C. dubliniensis* was performed evaluating the colony color in a CHROMagar[®] medium, the thermotolerance³⁴ and the growth in a hypertonic medium³.

Genotypic identification: The molecular technique was used to the complex *C. parapsilosis* and for the differentiation of *C. albicans* and *C. dubliniensis*.

DNA extraction: The DNA extraction from the culture was carried out using the PrepMan[™] Ultra Sample Preparation reagent Quick Reference Card kit (Applied Biosystems, USA), according to the commercial protocol.

Differentiation of the complex *C. parapsilosis* (*C. parapsilosis sensu stricto*, *C. orthopsilosis*, *C. metapsilosis*): To the differentiation of the complex *C. parapsilosis* (*C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis*) a Ribosomal DNA ITS sequencing was performed³⁷. The amplification of the ITS-rDNA region was accomplished using the universal primers V9G / LS266¹³ and the Big Dye terminator 3.1 kit (Applied Biosystems, USA). The edition of the nucleotide sequences was performed and analyzed by comparing them with the sequences available in the GenBank using the BLASTn (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Differentiation between *C. albicans* and *C. dubliniensis*: It was carried out according to MANNARELLI & KURTZMAN²². The PCR was performed with two pairs of specific primers: one pair for *C. dubliniensis* (sense CDU2 and anti-sense NL4CAL) and another pair for *C. albicans* (sense CAL5 and anti-sense: NL4CAL). The presence or not of amplified fragments was visualized by using the agarose gel electrophoresis.

Quality control: To control the quality of the phenotypic and genotypic methods, the strain patterns used were: ATCC 64548 (*C.*

albicans), ATCC 777 (*C. dubliniensis*), *C. parapsilosis* (ATCC 22019), *C. orthopsilosis* (ATCC 96141) and *C. metapsilosis* (ATCC 96143).

Statistical analysis: Quantitative variables were analyzed by the unpaired Student's t test or Mann-Whitney (Prism version 5.0, GraphPad Software Inc., La Jolla, CA, USA). A *p* value < 0.05 was considered to be statistically significant.

RESULTS

During the period of the study, a total of 104 yeast strains were isolated from hemocultures. Figure 1 represents the total of yeasts isolated from blood per year.

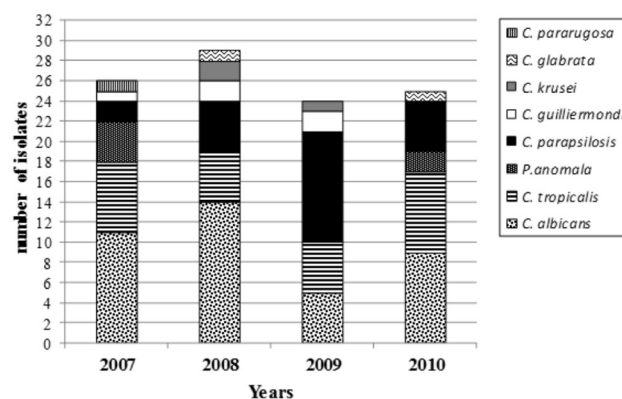


Fig. 1 - Number of strains and species isolated from blood of 104 pediatric patients, hospitalized in a children's public hospital, from 2007 to 2010.

The isolation of *C. albicans* in the children's public hospital studied was kept in the first two years, with the frequency of 42.3% in 2007 and 48.3% in 2008. In 2009 we had a decrease in the isolation rate (21.7%) followed by an increase in 2010 (36%). The isolation of *C. non-albicans* has always been higher than the one verified for *C. albicans* (*p* = 0.0286) and differently from these species, we had a considerable increase in the isolation rate for non-*albicans* species in the last two periods. The change in the numbers of isolates of *C. albicans* and non-*albicans* from 2007 to 2010 can be observed in the Figure 2.

In the four years of this study, the total frequency of identified species

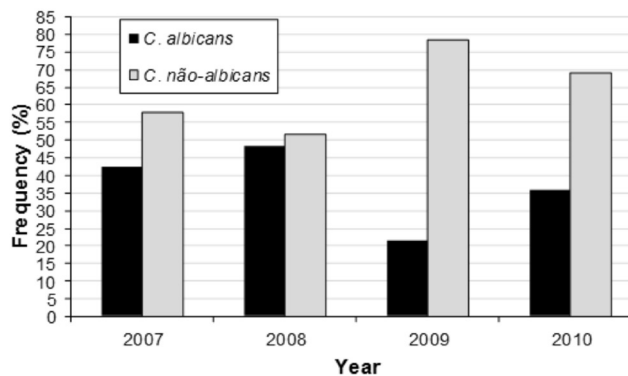


Fig. 2 - Frequency of isolates of *C. albicans* and non-*albicans* per year in a children's public hospital.

of *Candida* were: 37.5% of *C. albicans* (39/104), 24.03% of *C. tropicalis* (25/104), 22.11% of *C. parapsilosis* (23/104), 5.8% of *Pichia anomala* (6/104), 4.8% of *C. guilliermondii* (5/104), 2.88% of *C. krusei* (3/104), 1.92% of *C. glabrata* (2/104) and 0.96% of *C. pararugosa* (1/104). In the genotypic testing for differentiation molecular of *C. albicans* and *C. dubliniensis* and *C. parapsilosis* complex, the result was consistent with *C. albicans* and *C. parapsilosis* in 100% of the samples. The frequency of the species per year can be observed in Figure 1.

DISCUSSION

Candida albicans is an etiologic agent in about 40-60% of the bloodstream infections in pediatric patients^{14,28}. Studies have reported an increase in the rate of infections caused by non-*albicans* species⁶. In research, done in a pediatric hospital in the USA which had 97 patients studied, species of *Candida* (being the most frequent *C. albicans* and *C. parapsilosis*) were the most commonly isolated among fungemic patients, present in 91% of the cases. The species of *Candida* more commonly isolated from blood have changed along the time. In general, *C. albicans* represents 60% of the cases of isolates from blood between 1991 and 1996 and 48% of the isolates between 1997 and 2001, what was in accordance in the groups of ages¹. We emphasize in our study that 104 children with fungemia were observed in a shorter period than the study previously mentioned.

In another study performed in adults and pediatric patients with candidemia in a hospital in Porto Rico, the non-*albicans* species represented around 83% of the isolates; meanwhile *C. albicans* was responsible for 17% of the cases¹². In our study, *C. non-albicans* (62.5%) were also more isolated than *C. albicans* (37.5%). We noticed that in 2007 *C. albicans* represented 42.3% of the isolates, in 2008 we had a small increase of isolates of *C. albicans* (48.3%), but in 2009 and 2010 we had a decrease of isolates of these species, 21.7% and 36% respectively. The increase of the isolation rates of non-*albicans* species in patients with candidemia in Brazilian hospitals has been reported for a while^{9,31}. Different authors have also reported an increase in the incidence of candidemia caused by non-*albicans* species, represented by *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. tropicalis*. These authors affirmed that, however in the last years *C. albicans* has been responsible for more than a half of the cases of candidemias, the rates of infections caused by non-*albicans* species have slowly increased^{18,25,41}. In epidemiology, this could be associated with severe immunosuppression or illness, prematurity, exposure to broad-spectrum antibiotics, older patients and selective pressure exerted by prophylactic antifungals in patients at high risk of developing invasive fungal infections³⁵.

Recent studies have reported that *C. tropicalis* is one of the species that most frequently causes urinary tract infections and candidemia, especially in patients with cancer²⁶.

Recent studies evinced that the increase of *C. parapsilosis* incidence may be explained by some relevant factors such as parenteral nutrition and the use of central venous catheters, and this is explained by the fact that this species has the facility to form biofilm^{2,19}. However, possible sources of infections and routes of transmission of infections caused by *C. parapsilosis* are not always easily identified and in some cases the epidemiology of the infections remains undercover⁵. Therefore, the epidemiological relevance of this study should be highlighted considering that *C. parapsilosis* was

one of the most frequently isolated species and had our special attention.

Comparing nowadays with the 1980's, we can notice that, at present, there is a higher frequency of species *albicans* causing bloodstream infections as relevant pathogens like *C. glabrata* in the USA. Meanwhile, in Europe, Canada and Latin America, *C. parapsilosis* and *C. tropicalis* are the most frequent species responsible for causing bloodstream infections²⁸.

The increase of fungemias caused by non-*albicans* species like *C. glabrata* and *C. krusei*, is really relevant to the therapy. *C. krusei* has an inherent resistance to fluconazole and *C. glabrata* is relatively resistant to fluconazole, but fluconazole is the most frequently chosen antifungal drug to be used in this cases.

C. guilliermondii has also been isolated in our study. However, infections caused by *C. guilliermondii* are not common. They occurred in patients with cancer, hematological neoplasias as nosocomial infections by the use of central venous catheter having a high mortality rate⁴⁰.

Blood, skin, nails, tissues and, less frequent urine and genital tract, represent the most common places where the fungi have been more frequently isolated as a possible pathogen³⁶.

Regarding the *P. anomala* strains, it is highlighted in a previous study that it had already been isolated from a child patient in a pediatric hospital³⁰. PASQUALOTTO *et al.*³², in another study carried out in a pediatric hospital in Brazil, from October 2002 to December 2004, an outbreak of *P. anomala* had been detected. Seventeen patients had developed fungemia due to this species and molecular studies demonstrated that this outbreak was caused by just one strain. This yeast was not isolated, neither from the health professionals' hands nor from the environment.

In India, a hospital outbreak caused by *P. anomala* in a 23-month period (from April 1996 to February 1998), that had the spread from the fungi on the health professionals' hands, led to the commission of the hospital infection control to have a strict and educational control to improve the hand washing. Analyzing the potential risk factors to infections by *P. anomala* in children, we verified that some of these factors are: prematurity, very low-weight, a long-term hospitalization⁸, the use of central venous catheters, total parenteral nutrition, lipid emulsion, and the previous use of antimicrobial and other invasive procedures⁴.

Based on the finding of this study and the literature, we could notice the relevance of studying nosocomial infections cases by yeasts, especially from the genus *Candida*. We verified the increase of the non-*albicans* species throughout the years (mainly in 2009 and 2010). The non-*albicans* species cases have been increasing year after year in these nosocomial infection cases and concerning all these data, it is highlighted a correct species-level identification to lead to a fast diagnosis and efficient treatment.

RESUMO

Fungemia por espécies de *Candida* em Hospital Pediátrico da cidade de São Paulo, Brasil: estudo no período de 2007 a 2010

Candidemia permanece como a maior causa de morbidade e mortalidade em ambiente hospitalar. A epidemiologia de infecções por

Candida vem se alterando, principalmente em relação ao número de episódios causados por espécies não-*albicans*. Este estudo teve como objetivo avaliar a frequência, em um período de quatro anos, de leveduras do gênero *Candida* isoladas de sangue de pacientes pediátricos internados em hospital público da cidade de São Paulo, Brasil. Neste período foram isoladas leveduras de sangue de 104 pacientes, e as espécies de *Candida* identificadas, por métodos fenotípicos e genotípicos, foram: *C. albicans* (39/104), *C. tropicalis* (25/104), *C. parapsilosis* (23/104), *Pichia anomala* (6/104), *C. guilliermondii* (5/104), *C. krusei* (3/104), *C. glabrata* (2/104) e *C. pararugosa* (1/104). Em todo período do estudo foi observada maior frequência de isolamento de *C. não-albicans* (63,55%) ($p = 0,0286$). Neste estudo verificou-se aumento das espécies não-*albicans* ao longo dos anos (principalmente em 2009 e 2010), assim, ressalta-se que correta identificação em nível de espécie é recomendável, para que isso acarrete diagnóstico rápido e tratamento eficaz.

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