Prevalence, Susceptibility Profile for Fluconazole and Risk Factors for Candidemia in a Tertiary Care Hospital in Southern Brazil

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Bloodstream infections caused by yeast, *Candida* spp, are quite important clinically and epidemiologically due to a high mortality rate and an increasing number of non-*albicans* species with a more resistant (differentiated susceptibility) profile. We examined species prevalence and susceptibility profile for fluconazole and the risk for nosocomial infections by *Candida* spp at the Hospital de Clínicas de Porto Alegre, a general tertiary care hospital in southern Brazilian, through a retrospective study, beginning with positive cultures of hospitalized patients. The distribution by species in 131 documented episodes was as follows: *Candida albicans* (45%), *C. parapsilosis* (24.4%), *C. tropicalis* (15.3%), *C. glabrata* (6.9%), *C. krusei* (4.6%) and 3.8% other species (*C. pelicullosa, C. guilliermondii, C. lusitaniae* and *C. kefyr*). The vast majority of samples (121– 92.4%) were susceptible to fluconazole; the resistant or dose-dependent sensitive samples included only *C. krusei* and *C. glabrata*. Blood diseases (leukemia, lymphoma), or neoplasias (solid tumors), were found in 35.0% of the candidemia episodes. We noted the previous use of antibiotics in 128 (97.7%) patients, with 79.7% using three or more antibiotics before the candidemia episode. Other risk factors included a central venous catheter in 94 (71.8%) and abdominal surgery in 32 (24.4%) patients. The overall mortality rate was 51.9%, which varied according to the underlying disease. We found that *C. albicans* was the most prevalent species, although the non-*albicans* species predominated. However, *in vitro* resistance to fluconazole was detected only among the species (*C. glabrata* and *C. krusei*) that tend to be resistant to the azolic compounds. Previous use of antibiotic and the use of a central venous catheter were the main risk factors among patients with candidemia.

Key Words: Fluconazole, candidemia, risks, Southern Brazil.

Bloodstream infections due to *Candida* have drawn considerable attention in several medical fields over the past few decades due to their increasing incidence, high general (57-61%) and attributed (38-49%) mortality rates [1-3], and the appearance of non-*albicans* species displaying a differentiated resistant susceptibility profile. *Candida* spp are responsible for around 80% of nosocomial fungal infections, and they are the fourth main cause of bloodstream infections in tertiary care hospitals in the United States [4,5]. In South America, a different profile has been observed in the distribution of species and the susceptibility profile [6,7].

The main risk factors for candidemia include the use of immunosuppressive drugs, and/or broad-spectrum antibiotic therapy, abdominal surgery, parenteral nutrition, hemodialysis and central venous catheter use [2,8-14]. Most often, *Candida albicans* is the main etiological species involved in hematogenic infections; however non-*albicans* species are increasingly reported as a cause of infection in patients with underlying diseases, such as hematological disorders, HIV infections and solid tumors, as these

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patients are exposed to various risk factors for the development of systemic fungal infection, due to specific therapies and differentiated procedures [10,15-17].

The epidemiology of systemic *Candida* infections varies in different regions, and frequently, even from one hospital to another within the same region [13,17]. Consequently, local studies are important in order to obtain relevant epidemiological data and the *Candida* susceptibility profile to anti-fungal drugs in order to assist in the management and treatment of hospitalized patients with *Candida* infection.

Our objectives were (1) to determine the prevalence of *Candida* species, (2) to examine the *in vitro* susceptibility to fluconazole and (3) to investigate the risk factors for candidemia in patients hospitalized at the Hospital de Clínicas de Porto Alegre, a tertiary care hospital located in the southern region of Brazil.

Material and Methods

We conducted a retrospective study to assess the prevalence of *Candida* species in blood cultures, the risk factors associated with candidemia and the *in vitro* susceptibility profile to fluconazole at the Hospital de Clinicas de Porto Alegre (HCPA), a general tertiary care hospital with 723 beds in the southern Brazil, from April 1998 to August 2004. We included only the C*andida* sp isolate obtained from the first episode of candidemia of each patient. The yeast was maintained in Sabouraud agar at room temperature before performing the susceptibility test. Blood samples were monitored and detected with Bactec® 9240 (Becton Dikinson, Sparks, USA), and sub-cultures were seeded in blood agar for subsequent identification on a mini-API with an ID-32C card (Biomérieux SA, Marcy-l'Etoile, France).

We used the broth microdilution technique in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) document M-27 A2 [18] for quantitative determination of susceptibility to fluconazole. Fluconazole was supplied by the manufacturer (Pfizer Inc, New York, NY, USA), prepared at a concentration of 5,120 μg/mL and kept at –20º C. The medium employed was RPMI-1640, with L-glutamine, without bicarbonate (Gibco Invitrogen Corporation, USA) and buffered with morpholinopropanosulfonic acid (MOPS) 0.165 M at pH 7.0. Two successive subcultures were made from the stored samples on Sabouraud agar before conducting the susceptibility test.

The microdilution technique was performed in a multi-well plate, containing RPMI-MOPS; the plate was incubated at 35º C for 48 hours. The concentrations of fluconazole varied from 0.125 μg/mL to 64 μg/mL. The cell concentration reading was conducted by visual comparison of the turbidity of the test well with the turbidity of the positive control well without fluconazole, and the Minimal Inhibitory Concentration (MIC) was established as the lowest concentration of the antifungal agent that inhibited yeast growth. The MIC was interpreted according to the breakpoints suggested by document M27-A2 from NCCLS, and reported as "Resistant" (MIC = 64 μg/L), "Dose-Dependent Sensitive - DDS" (MIC between 16 and 32 μg/L) and "Sensitive" (MIC 8 = μg/L). Quality control was performed with strains of *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Medical records were retrospectively reviewed starting 30 days before the positive result in blood culture in order to look for risk factors, and the outcome was followed until the death of the patient or resolution of candidemia.

A database was created with the following variables: age, gender, location of hospitalization, underlying disease, previous use of antibiotics, chemotherapy, antifungal agents, corticosteroids, total parenteral nutrition, neutropenia, presence of central venous catheter, surgery, mechanical ventilatory support, clinical symptoms (fever, hypothermia, hypotension, eye lesions and skin lesions) and presence of *Candida* sp in other sites. The statistical analysis was performed with the SPSS 9.0 software through descriptive analysis for relative frequency and prevalence.

Consultation of the patients' medical records was performed in accordance with the term of commitment for data usage at the "Serviço de Arquivo Médico e Informações em Saúde" (Service of Medical Archives and Health Information). The study protocol was approved by the Institutional Ethics Committee and the resources for the development of the project were obtained from the Fundo de Incentivo a Pesquisa e Eventos do Hospital de Clinicas de Porto Alegre (FIPE-HCPA).

Results

Characteristics of patients

A total of 131 blood samples collected from 77 male and 54 female patients from June 1998 to July 2004 that yielded *Candida* spp were included. The age of patients ranged from nine days to 91 years, with a mean of 33.6 and a median of 28 years. Twenty-one (16%) patients were under one year of age at the time of the candidemia episode. During the episodes, the patients were located in the following areas: 53 patients hospitalized in medical units (40.4%), 31 in adult intensive care units (ICUs) (23.7%), 21 in pediatric intensive care units (16%), 18 in oncology units (13.8%), 5 in neonatology units (3.8%), 2 in the emergency sector (1.5%) and 1 in the bone marrow transplant unit (0.8%). Among the episodes observed, 10 (7.6%) were identified within 48 hours of hospitalization, indicating community infection according to CDC definitions, while 121 (92.4%) cases of candidemia were considered as nosocomial infections.

Etiology and susceptibility profile

Candida albicans (45%) was the most prevalent species, followed by *C. parapsilosis* (24.4%) and *C. tropicalis* (15.3%) (Table 1). The vast majority of isolates (121 – 92.4%) of *Candida* were susceptible to fluconazole (MIC ≤ 8 μg/mL); 2 μg/mL and 4 μg/mL inhibited 90% (MIC 90) of *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, respectively.

Only 4 out of 131 isolates (3%) of *Candida* spp proved to be resistant (MIC ≥ 64 μg/L) to fluconazole. These isolates were identified as *C. krusei*, which characteristically presents intrinsic resistance to fluconazole. Additionally, 6 isolates (2 *C. krusei* and 4 *C. glabrata*) that were dose-dependent sensitive to fluconazole were identified.

Underlying disease or condition

The cases of candidemia were categorized into 14 types of underlying conditions or diseases. Patients with hematological diseases (leukemia, lymphoma) and neoplasias (solid tumors) comprised 35% of the candidemia episodes (Table 2). *Candida* sp were found in the group of patients with bone marrow (n = 5) and solid organ transplantation (n = 2); these patients constitute a significant risk group for opportunistic infections.

Risk factors

Among the 131 patients included in the study, only three had not used antibiotics previously. Considering the 128 patients with previous antibiotics use, we observed that 26 (20.3%) had used one to two antimicrobial drugs, 56 (43.8%) used three to five antimicrobial drugs and 46 (35.9%) used five or more antimicrobial drugs previous to the episode of candidemia. It was found that 94 patients (72%) had been treated with vancomycin and 63 patients (48%) with (48%); several groups of antimicrobial drugs (penicillins, aminoglycosides, carbapenems, quinolones and sulfonamides) had been used in a smaller proportion. There was previous use of antifungal agents by three patients (2.3%), one patient was using ketoconazole, one patient was using fluconazole and one patient used amphotericin B.

Among the 131 patients assessed, 50 patients underwent surgery within 30 days before the episode of candidemia, and abdominal surgery was the most common procedure (24.4%) (Table 3). The use of a short or long-term central venous catheter (intra-cath®, Hickman®, port-a-cath®, swan ganz, double-lumen) was observed in 94 (71.8%) of the patients.

Table 1. Distribution of species and susceptibility profile of *Candida* spp. (n =131) to fluconazole

Species	N (%)	MIC range*	MIC_{50}	MIC_{90}	Susceptible N (%)	DDS** N (%)	Resistant N (%)
C. albicans	59 (45)	0.12-4.0	0.5	2.0	59 (100)	0	0
C. parapsilosis	32 (24.4)	0.12-4.0	0.5	2.0	32 (100)	0	0
C. tropicalis	20 (15.3)	0.25-4.0	1.0	4.0	20 (100)	0	0
C. glabrata	9 (6.9)	0.5-32	8.0	32.0	5 (55)	4 (45)	0
C. krusei	6 (4.6)	32-64	64.0	64.0	0	2 (33)	4 (67)
Others ***	5 (3.8)	0.125-8.0	0.125	8.0	5 (100)	0	0

* µg/mL. **DDS: dose-dependent susceptibility. *** *C. pelicullosa* (2), *C. guilliermondii* (1), *C. lusitaniae* (1), *C. kefyr* (1). MIC = minimum inhibitory concentration.

Table 2. Location and underlying disease or condition of patients with candidemia

Characteristics	Patients (N=131)	%
Male/female	77/54	58.8 / 41.2
Age	33.6 years*	
Location		
Adult ICU	31	23.7
Medical hospitalization	53	40.4
Oncology	18	13.8
Emergency	2	1.5
Neonatology	5	3.8
Pediatric ICU	21	16.0
BMT	1	0.8
Subjacent disease or condition		
Hematological diseases	26	19.8
Neoplasias (solid tumors)	20	15.2
Respiratory tract diseases	14	10.7
Gastrointestinal tract diseases	14	10.7
Premature babies	11	8.4
Endocrinopathy	9	6.9
AIDS	8	6.0
Transplantation of bone marrow and solid organs	7	5.5
Cardiovascular diseases	6	4.5
Neurological diseases	5	3.8
Infectious diseases	4	3.1
Genetic syndromes	3	2.3
Nephropathy	3	2.3
Rheumatological diseases	1	0.8

* Range: 9 days-91 years; IC = Intensive Care Unit; BMT = Unit for bone marrow transplant.

Table 3. Risk factors, clinical signs and symptoms in patients with candidemia

Risk factors	Patients (n=131)	%
Previous use of antibiotics	128	97.7
Number of antibiotics (1-2)	26	20.3
Number of antibiotics (3-5)	56	43.8
Number of antibiotics (>5)	46	35.9
Central venous catheter	94	71.8
Corticosteroids	87	66.4
Mechanical ventilatory support	64	48.9
Neutropenia	37	28.2
Chemotherapy	31	23.7
Total parenteral nutrition	33	25.2
Abdominal surgery	32	24.4
Non-abdominal surgery	18	13.7
Other sites/materials with *Candida* spp		
Urinary tract	19	14.5
Catheter tip	17	13
Oral cavity	14	10.7
Pulmonary (Biopsy)	3	2.3
Others (ascitis fluid, feces, cerebrospinal fluid)	5	3.8
Clinical symptoms		
Fever	114	87
Skin lesions	17	13.1
Hypotension	13	8.4
Hypothermia	7	4.6
Eye lesions	6	3.9
Death	68	51.9

Clinical manifestations and mortality

In addition to the detection of *Candida* sp in the blood, we observed *Candida* sp in urine (19 patients), the oral cavity (17 patients), catheter tips (14 patients) and in a lung biopsy (3 patients).

Fever was the main clinical symptom observed in patients (87%), and more specific symptoms for candidemia, including skin lesions and eye lesions were described in 17 (13.1%) and 6 (3.9%) patients, respectively.

Sixty-eight patients (51.9%) died within a period of up to 30 days after the episode of candidemia. This overall mortality rate varied according to the underlying disease or condition, reaching levels up to 100% in diabetic patients, in contrast with 28.6% for patients with transplantation of bone marrow or solid organs.

The mortality rates varied for the patients with different species of *Candida*, being highest for *C. glabrata* (78%), and *C. tropicalis* (60%). The mortality rate was around 50% for the remaining species (*C. albicans, C. parapsilosis* and *C. krusei*)

Discussion

The distribution of species of *Candida* obtained from the bloodstream varies according to the

geographic area and the population on which the studies are performed, probably due to variations in the endogenous microorganisms of the patients [19]. We examined patients in a tertiary care hospital in southern Brazil; these included adult and pediatric patients, with different underlying diseases or clinical conditions. We observed a high proportion (40.4%) of patients hospitalized in medical units, despite their unstable conditions; probably many of these patients should had been hospitalized in intensive care units (ICU), but due to the shortage of available beds in ICU Brazilian tertiary care hospitals these patients are often transferred or allocated to non-ICU units, as suggested by Colombo et al. [10].

Along with the increase in the incidence of infections by *Candida* spp, there has been a trend towards an increase in non-*albicans* species [4]. In the United States, where several studies were performed assessing prevalence and epidemiological surveillance, *C. albicans* was reported at a frequency of around 50% (38% to 79.4%) in most institutions [19-21]. Among non-*albicans* species, the most prevalent included *C. glabrata*, *C. tropicalis* and *C. parapsilosis*. We highlight the prevalence of *C. glabrata*, a species relatively resistant to azolic compounds (10-21%), a phenomenon that appears to be less frequent in South American hospitals, perhaps due to less use of azolic drugs for prophylaxis [10,19,21,22]. In a study performed in nine tertiary hospitals in Australia, Slavin, found a pattern similar to that of the United States, with 56% *C. albicans*, 16% *C. parapsilosis* and 5% *C. krusei*, *C. glabrata* and *C. tropicalis* [15]. In Canada, the frequency of *C. albicans* was slightly higher, ranging between 53 and 74% in some series [6,19]. In Europe, the incidence of *C. albicans* ranges from 49% and 59%, according to the epidemiological surveillance programs EORTC and SENTRY, respectively [21,23], reaching 70% in some hospitals [19].

We found a prevalence of 45% for *C. albicans*; this figure is the same as that found in other series from South America and specifically from Brazil [6,7,16,24,25]. In an epidemiological study made in six Brazilian tertiary hospitals, Colombo found a small difference between *C. albicans* and non-*albicans* species, with a predominance (63%) of non-*albicans* species [10]. The second-most prevalent species was *C. parapsilosis* (24.4%), a species with an acknowledged ability to develop in solutions containing glucose, to produce biofilm, and to colonize skin in association with the use of a central venous catheter [9,17]. The frequency of occurrence of *C. parapsilosis* is higher in children and premature newborns who are hospitalized in ICUs; however, with lower mortality when compared with *C. albicans* [26,27]. The explanation for this effect may be related to the lower virulence of *C. parapsilosis* in animal models, its inability to adhere to and penetrate the endothelium of human cells and its susceptibility to phagocytosis [27, 28]. Though *C. parapsilosis* is associated with the use of a central venous catheter, there are controversies on whether the catheter should be removed or not. Some authors suggest that the central venous catheter must be removed whenever possible, since it is associated with treatment failure and higher mortality, while others prefer to try anti-fungal therapy without removing the catheter [27, 29].

The development of resistance among *Candida* spp is a phenomenon that has increased during the past few years, especially in patients who have used fluconazole for prophylaxis and in neutropenic cancer patients [30]. This phenomenon is more evident in the United States, where this approach has been adopted for a longer time than in South America [10]. This aspect has been evidenced in studies of HIV-positive patients who were colonized by fluconazole-resistant *Candida* sp in the oral epithelium, which was associated with the use of this antifungal agent as prophylaxis for oral candidiasis. We confirm the high susceptibility of *Candid*a spp in South America [7,16,24]. Pfaller et al. described high susceptibility to fluconazole in isolates of *C. parapsilosis* and *C. tropicalis* (98.9 to 100%) obtained in Canada and South America, demonstrating that *Candida* species from the southern hemisphere are generally more susceptible than those from the northern hemisphere [6].

However, the pathogenic yeasts can develop complex mechanisms of resistance to antifungal drugs

[30,31]; this probably will lead to an increase in resistant isolates in the Brazilian tertiary care hospitals, where there is increasing use of antifungal prophylaxis, especially in patients with hematological diseases and in bone marrow and solid organ transplants [16].

In the investigation of the underlying diseases associated with candidemia, our findings were similar to those described from other centers [8-10,13,19]. Though cancer patients are more frequently affected, some groups of patients without degenerative disease are highly susceptible due to exposition to various risk factors, such as invasive procedures during hospitalization [2,8,9,11,14,23].

When they examined episodes of candidemia in tertiary hospitals, Fraser et al. observed that 92% of the patients who developed candidemia had received antibiotics previously and 62% had been treated with at least four antimicrobial drugs. In our study, 97.7% of the patients with candidemia received previous antibiotic therapy, and 79.7% had used three or more anti-microbial drugs before the episode [32].

In a prospective study, analyzing data from six intensive care units in the United States, the National Epidemiology of Mycoses Survey (NEMIS) found abdominal surgery and total parenteral nutrition to be independent risk factors [11]. In our study, abdominal surgery was the most frequent surgical intervention, performed in 32 of 50 patients who underwent surgery. On the other hand, the use of total parenteral nutrition was observed in only 25.2% of the patients included in our study. Due to the variability of underlying diseases or conditions for which surgical processes and parenteral nutrition would not be indicated, it is possible that the percentage of these risk factors has been underestimated. The objective of our study was not to determine risk factors for candidemia, but to analyze the risk factors originally described in the literature for hematogenic candidiasis.

The clinical significance of *Candida* sp in the urine (found 14.5% of the patients with candidemia in our study) is controversial. This finding usually poses a therapeutic problem, since the presence of yeast can indicate contamination or colonization [33]; but it should not be ignored, especially in high-risk patients [33,34]. We understand that this finding must be reported for clinical interpretation, provided that proper procedures for the collection and transportation of urine to the laboratory have been used.

Clinical symptoms of candidemia are not specific, and there are few reports in clinical practice about the involvement of the different organs in cases of candidemia. We observed involvement of the skin in 13.1% of patients and eye lesions in 3.9%, the latter being an uncommon manifestation in neutropenic patients [17].

Breakthrough candidemia (BT) occurs mainly in patients in the ICU, undergoing neutropenic and/or under corticosteriod treatment. The three patients who were identified in our study had underlying diseases, which were leukemia and Aids; the third was a bone-marrow-transplant patient. Consequently, these patients had antifungal prophylaxis, which is a risk factor for developing candidemia by resistant species, or BT [35].

Despite the predominance of species susceptible to fluconazole, the general mortality of patients with candidemia in our study was 51.9%, demonstrating that epidemiological studies are important for monitoring these infections.

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