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Candida glabrata among *Candida* spp. from environmental health practitioners of a Brazilian Hospital

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ABSTRACT

The incidence of the species *Candida albicans* and non-*albicans Candida* was evaluated in a Brazilian Tertiary Hospital from the environment and health practitioners. In a 12-month period we had a total positivity of 19.65% of *Candida* spp. The most recurring non-*albicans Candida* species was *C. glabrata* (37.62%), generally considered a species of low virulence, but with a higher mortality rate than *C. albicans*. Subsequently, *C. parapsilosis* (25.74%) and *C. tropicalis* (16.86%) were the second and third most commonly isolated species. Considering the total samples collected from the emergency room and from the inpatient and the pediatric sector, 19.10% were positive for *Candida* spp., with the predominance of non-*albicans Candida* species (89.42%). The high percentage of positivity occurred in the hands (24.32%) and the lab coats (21.88%) of the health care assistants. No sample of *C. albicans* presented a profile of resistance to the drugs. All the non-*albicans Candida* species presented a decreased susceptibility to miconazole and itraconazole, but they were susceptible to nystatin. Most of the isolates were susceptible to fluconazole and amphotericin B. As expected, a high resistance rate was observed in *C. glabrata* and *C. krusei*, which are intrinsically less susceptible to this antifungal agent. The contamination of environmental surfaces by *Candida* spp. through hand touching may facilitate the occurrence of *Candida* infections predominantly in immunocompromised patients. In addition to that, the antifungal agents used should be carefully evaluated considering local epidemiologic trends in *Candida* spp. infections, so that therapeutic choices may be better guided.

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Introduction

The frequency of *Candida* spp. hospital infections has increased worldwide in recent years and it has been accompanied by a significant rise in morbidity and mortality. An important issue of public health involves the long time hospital stay due to the difficulty in the diagnosis, prevention, and treatment of invasive fungal infections.[1,2]

Candida spp. may cause serious nosocomial infections and it represents the fourth most frequent agent isolated from bloodstream infections in many regions. *Candida albicans* is the main species that causes hospital-acquired infections, although other species of non-*albicans Candida*, such as *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. lusitaniae* have shown an increased incidence of nosocomial infections.[3,4] While *C. albicans* remains the most common *Candida* species in infections, *C. glabrata*, generally considered a species of low virulence but with a higher mortality rate than *C. albicans*, has represented approximately 15–20% of all *Candida* infections in the United States and has been considered the most common non-*albicans Candida* isolated species.[5]

The hospital environment is inevitably a large reservoir of opportunistic pathogens, which may be transmitted to individuals in different ways. The modes of transmission and input port to hospital-acquired fungal infections vary according to the pathogen involved. *Candida* spp. infections are predominantly of an endogenous origin, but the cross-infection transmitted through the hands of health care practitioners or relatives, or even through hospital devices, has occurred constantly.[6] Furthermore, it is known that antifungal resistance is an important concern related to almost all main groups of pathogenic microorganisms, including the *Candida* species. The increasing use of prophylactic fluconazole in high-risk patients has played an important role in decreasing the incidence of *C. albicans* infections without affecting the incidence of infections caused by non-*albicans Candida* species, such as *C. glabrata* and *C. krusei*.[7]

An inappropriate antifungal therapy and the occurrence of resistant species may have an impact on the mortality rates. A correlation between reduced antifungal susceptibility in non-*albicans Candida* species and the use of antifungal prophylaxis have been suggested. Data about patterns of resistance of etiological agents are powerful tools to guide a prophylactic, preemptive, and empiric antifungal therapy.[4,8]

The aim of this study was to evaluate the incidence of *C. albicans* and non-*albicans Candida* isolated from the environment and from health practitioners to identify the hospital reservoirs of *Candida* spp. in a Brazilian tertiary-care hospital and to evaluate the susceptibility of the isolated samples to antifungal agents.

Materials and methods

Isolates

Samples from the environment and from health care practitioners were compiled throughout a 1-year period (2008–2009), from a tertiary care center with 140 beds that provides general and specialized assistance as well as surgical and intensive care. After the isolation, the pure cultures were stored at −20 °C in 15% glycerol.

Sample collection

The samples were collected from three different sources in the hospital environment: surfaces of hospital departments (including emergency room, inpatient sector, and pediatric sector), hands and lab coats of health care practitioners, totaling 445, 37 and 32 samples in each source respectively. Samples were collected with sterile swabs soaked in physiological solution supplemented with 0.5 g/l chloramphenicol and placed in brain-heart infusion (BHI) broth supplemented with 0.5 g/l chloramphenicol. Samples from the lab coats of health care professionals were collected by pressing a Petri dish, measuring 4 cm in diameter and containing BHI agar supplemented with 0.5 g/l chloramphenicol, on the frontal part of the lab coats, about 5 cm proximal to the pockets. All plates were incubated at 35 °C for 48 h.

Sample identification

An analysis of the growth in a chromogenic culture medium (CHROMagar Candida®) facilitated the determination of purity of colonies and the identification of *Candida* spp. Macromorphological/micromorphological analysis and physiological tests such as zymograms and auxanograms were performed to confirm the results of the chromogenic culture medium.[9,10]

Determination of the pattern of response to antifungal drugs

30 samples of *Candida* spp. were pre-selected according to their best growth pattern and thereby antifungal tests were conducted with these 30 pre-selected samples of *Candida* spp. through disk diffusion methodology and the results were interpreted as susceptible (S), susceptible dose-dependent (SDD), or resistant (R), based on documents M44-A2.[11] The susceptibility profile of *Candida* spp. was analyzed with the antifungal drugs fluconazole (25 μg disk), amphotericin B (100 μg disk), nystatin (100 IU disk), itraconazole (10 μg disk), miconazole, and ketoconazole (50 μg discs). *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) were used as test controls. The evaluation of response profiles of *Candida* spp. to different antifungal agents was performed in triplicate and on different days.

Statistical analysis

The Chi-square test or the statistic of Fisher was applied to evaluate the significance of differences in the frequency distribution of the isolates. Differences with $p < 0.05$ were considered significant.

Results

514 samples were collected from the hospital environment and from health care practitioners, of which 445 were

Table 1 – Incidence of *Candida* species among isolates from the environment and the staff of a Brazilian tertiary hospital.

Candida species *n* (%)	Collection sites			
	Hospital departments	Hands	Lab coats	Total
C. glabrata	30 (35.29)	4 (44.44)	4 (57.14)	38 (37.62)
C. parapsilosis	22 (25.99)	3 (33.33)	1 (14.28)	26 (25.74)
C. tropicalis	16 (18.82)	1 (11.11)	–	17 (16.83)
C. albicans	9 (10.58)	–	1 (14.28)	10 (9.90)
C. krusei	6 (7.05)	1 (11.11)	–	7 (6.93)
C. lusitaniae	1 (1.17)	–	1 (14.28)	2 (1.98)
C. famata	1 (1.17)	–	–	1 (0.99)

n, number of isolates.

collected from surfaces, 37 from hands, and 32 from lab coats. Among the collected samples, 101 samples (19.65%) were positive for *Candida* spp. Only 10 (9.90%) were identified as *C. albicans* and 91 (90.10%) were grouped as non-*albicans Candida*. The difference of distribution was considered highly significant ($p < 0.0001$) (Table 1). The positivity according to the collection sites was 19.10% of surfaces of hospital departments, 24.32% of hands, and 21.88% of lab coats of health care practitioners. Thus, the prevalence of *Candida* spp. was not significantly different ($p > 0.05$) among collection sites. The frequency distribution of the isolates according to the species shows a highly significant difference ($p < 0.0001$). *C. glabrata* was the predominant species (37.62%), followed by *C. parapsilosis* (25.74%), *C. tropicalis* (16.83%), *C. albicans* (9.90%), *C. krusei* (6.93%), *C. lusitaniae* (1.98%) and *C. famata* (0.99%) (Table 1). Among the isolates obtained from hands, no isolate of *C. albicans* was found and the most frequent species was *C. glabrata* (44.44%). A prevalence of non-*albicans Candida* species was also observed in isolates from lab coats of health care practitioners and surfaces of hospital departments, being *C. glabrata* the most frequent species with 57.14% and 37.62%, respectively. However, the frequency in the distribution of the isolates of *C. glabrata* among the collection sites showed no significant difference ($p = 0.4808$).

Susceptibility tests

All the samples were susceptible to nystatin and most *Candida* isolates were susceptible to fluconazole, with exception of isolates of *C. krusei* and *C. glabrata* that are intrinsically less susceptible to this antifungal agent. All isolates of *C. krusei* were classified as resistant to fluconazole (Table 2). In relation to amphotericin B the isolates of *C. albicans* and non-*albicans Candida* were susceptible, except one isolate of *C. krusei* that presented intermediate susceptibility. No sample of *C. albicans* presented resistance to evaluated drugs. All isolates of *C. albicans* were susceptible to amphotericin B, ketoconazole and nystatin, but in relation to itraconazole, most of them were intermediate. All the non-*albicans Candida* isolates presented some resistance or decreased susceptibility to miconazole and itraconazole, but all of them were susceptible to nystatin (Table 2).

Discussion

Hospital-acquired infections caused by yeasts represent a persistent public health problem and a frequent complication among patients admitted to the hospital. *Candida* spp. has been the most frequently isolated agent, which corresponds to approximately 80% of the hospital-acquired fungal infections that cause death to 12% to 60% of the patients who develop candidemia.[5]

We had a positivity of 19.65% for *Candida* spp. among the samples isolated from the emergency room and from the inpatient and pediatric sectors, hands and lab coats of health care practitioners during the 12-month period of collection and our results were similar to those obtained by Storti et al.,[12] which found a positivity of 19.20%. The non-*albicans Candida* species were predominant (90.10%) and were represented by *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. lusitaniae* and *C. famata*.

In the research, a high rate of non-*albicans Candida* was found among the health care practitioners including hands and lab coats (85.72%) and all the isolates of *Candida* spp. from the hands were non-*albicans Candida*. Among the results obtained by Storti et al.,[12] only one isolate of hands of a health care assistant was of *C. albicans* and the incidence of *Candida* spp. found among the practitioners was 15.7%, a lower average than those previously verified by other studies including ours. Our results disagree with those obtained by Martins-Diniz et al.,[13] in which 23% of positive samples obtained from staff members correspond to *C. albicans* and 19% correspond to other species. In the work cited, 66 positive samples of yeast were isolated, and 46 of these samples were positive for *Candida* spp.

The predominance of non-*albicans Candida* species (89.42%) was observed among the positive samples for *Candida* spp. from the surfaces of hospital departments with prevalence of *C. glabrata* (37.62%). This species was responsible for 57.14% of the isolates from lab coats, 44.44% from hands of the health care practitioners, and 35.29% from hospital departments. Our results contrast with other studies, which did not identify *C. glabrata* as one of most isolated species in Brazil.[12,14] *C. glabrata* is considered a common commensal in gastrointestinal and genitourinary tracts, but it can turn into an opportunistic fungal pathogen in immunocompromised patients.[15]

Table 2 – Antifungal susceptibility profile of *Candida* species isolated from the environment and staff of a Brazilian Tertiary Hospital.

Candida species (number of isolates)	Antifungal agent	Classification (% of isolates)		
		S	SDD	R
C. glabrata (8)	Fluconazole	25.0	25.0	50.0
	Amphotericin B	100.0	–	–
	Miconazole	25.0	62.5	12.5
	Itraconazole	62.5	37.5	–
	Ketoconazole	100.0	–	–
	Nystatin	100.0	–	–
C. albicans (6)	Fluconazole	66.6	33.3	–
	Amphotericin B	100.0	–	–
	Miconazole	50.0	50.0	–
	Itraconazole	33.3	66.6	–
	Ketoconazole	100.0	–	–
	Nystatin	100.0	–	–
C. parapsilosis (6)	Fluconazole	83.3	16.7	–
	Amphotericin B	100.0	–	–
	Miconazole	16.7	50.0	33.3
	Itraconazole	16.7	50.0	33.3
	Ketoconazole	100.0	–	–
	Nystatin	100.0	–	–
C. tropicalis (6)	Fluconazole	100.0	–	–
	Amphotericin B	100.0	–	–
	Miconazole	66.6	16.7	16.7
	Itraconazole	16.7	50.0	33.3
	Ketoconazole	83.3	16.7	–
	Nystatin	100.0	–	–
C. krusei (4)	Fluconazole	–	–	100.0
	Amphotericin B	75.0	–	25.0
	Miconazole	–	50.0	50.0
	Itraconazole	–	75.0	25.0
	Ketoconazole	100.0	–	–
	Nystatin	100.0	–	–

S, susceptible; SDD, dose-dependent susceptible; R, resistant.

C. parapsilosis and *C. tropicalis* were the next most commonly isolated species, responsible for 25.74% and 16.86% of the isolates from all sources analyzed, respectively. Brazilian reports have pointed these species as the main agents isolated among non-*albicans Candida* species.[14,16] In other countries *C. parapsilosis* and *C. tropicalis* are also very frequent non-*albicans Candida* species.[13] *C. tropicalis* is an important fungal pathogen in patients with neutropenia and/or with hematologic malignancies.[17] *C. parapsilosis* is known for ability to form biofilm on medical devices,[18,19] for their persistence in the nosocomial hospital environment, and for their propagation by the hands.[16]

Environmental sources are more commonly implicated in infections caused by *C. parapsilosis*, when compared with others *Candida* species[20] and their importance has been highlighted in previous studies.[6,21,22] According to our results, *C. krusei*, *C. lusitaniae* and *C. famata* had the lowest rate of isolation, as also reported in another study.[23]

Some cases of candidemia may be caused by clusters of epidemiologically and genetically related strains and therefore may be potentially preventable.[23,24] Although most candidemia cases occur due to a pre-existent colonization in the patient, it may also be acquired through manipulation and direct contact made by the hands of health care practitioners.[25]

The increased use of invasive medical procedures, as well as the prophylactic and empirical use of antifungal drugs, especially those of azolic derivation, has been responsible for the emergence of non-*albicans Candida* species.[26] The antifungal susceptibility tests may be used in guiding treatment of candidiasis, especially in situations where there is failure in the initial empirical treatment.

In the present study, most of the isolates were susceptible to fluconazole. Resistant isolates of *C. albicans* were not found. However, considerable levels of resistance were observed among the isolates of non-*albicans Candida*. As expected, a high resistance rate was observed in *C. glabrata* and *C. krusei*, which are intrinsically less susceptible to this antifungal agent. This profile of susceptibility was also observed in Péman et al.[27]

Studies have shown that patients submitted to the prophylactic fluconazole are more susceptible to colonization and infection by *C. glabrata* because the exposure to subtherapeutic concentrations of fluconazole may result in resistance.[8,28] This species may present both innate and acquired resistance against antifungal drugs, due to its ability to modify ergosterol biosynthesis, mitochondrial function, or antifungal

efflux. This resistance allows overgrowth in relation to susceptible species and may contribute to the recent emergence of infections by *C. glabrata* in chronically immunocompromised individuals.[15]

All *C. krusei* and *C. glabrata* isolates were susceptible to ketoconazole and nistatin; most of them were also susceptible to amphotericin B, with the exception of 25% of isolates of *C. krusei*, which presented resistance to this drug. These results are consistent with other studies.[12,29]

In our results, a resistance to the azoles Miconazole and Itraconazole of up 33.3% in *C. parapsilosis* and *C. tropicalis* was observed (Table 2), while in the study of Bonfietti et al.[30] all the isolates of *Candida* spp. exhibited a high susceptibility to itraconazole. Our findings of susceptibility of *C. tropicalis* to fluconazol are consistent with a previous Brazilian study which reported susceptibility of all isolates of this species.[22]

Our data emphasize the importance of continuing surveillance programs to evaluate the trends of *Candida* species, including critical species, like *C. glabrata*, and their resistance profiles to antifungal drugs commonly used in medical practice. It is worth noting that voriconazole and caspofungin have been recently included as therapeutic choices in the candidemia treatment. The resistance profiles for these drugs must be continuously monitored, and the results must be added to a national databank for empiric therapeutic approaches. Among the preventive measures, the active environmental surveillance and strict application of cleaning procedures should be implemented in order to prevent cross-infections and the onset of hospital outbreaks.

Conflicts of interest

The authors declare no conflicts of interest.

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