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

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A B S T R A C T

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.5 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.1,8

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
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 ($p = 0.4808$).

**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., 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., 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
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. In other countries C. parapsilosis and C. tropicalis are also very frequent non-albicans Candida species. C. tropicalis is an important fungal pathogen in patients with neutropenia and/or with hematologic malignancies. C. parapsilosis is known for ability to form biofilm on medical devices, for their persistence in the nosocomial hospital environment, and for their propagation by the hands.

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

Some cases of candidemia may be caused by clusters of epidemiologically and genetically related strains and therefore may be potentially preventable. 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.

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. 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.

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. This species may present both innate and acquired resistance against antifungal drugs, due to its ability to modify ergosterol biosynthesis, mitochondrial function, or antifungal

### 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)                     | Flucanazole      | 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)                     | Flucanazole      | 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)                 | Flucanazole      | 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)                   | Flucanazole      | 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)                       | Flucanazole      | –       | –       | 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.
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 nystatin; 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 Bonfetti 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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References

1. Nucci M, Colombo AL. Risk factors of breakthrough candidemia. Eur J Clin Microbiol Infect Dis. 2002;21(3):209–211.
2. Tortorano AM, Peman J, Bernhardt H, et al. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol. 2004;23(4):317–322.
3. Chi HW, Yang YS, Shang ST, et al. Candida albicans versus non-albicans bloodstream infections: the comparison of risk factors and outcome. J Microbiol Immunol Infect. 2011;44(5):369–375.
4. Chow JK, Golan Y, Ruthazer R, et al. Factors associated with candidemia caused by non-albicans Candida species versus Candida albicans in the Intensive Care unit. Clin Infect Dis. 2008;46(8):1206–1213.
5. Colombo AL, Guimarães T. Epidemiologia das infecções hematogênicas por Candida spp. Rev Soc Bras Med Trop. 2003;36(5):599–607.
6. Ruiz LS, Sugizaki MF, Montelli AC, et al. Fungemia by yeasts in Brazil: occurrence and phenotypic study of strain isolated at the public Hospital, Botucatu, São Paulo. J Microl Medica. 2005;15(1):13–21.
7. Mar KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis. 2000;181(1):309–316.
8. Mario DAN, Denardi LB, Bandeira LA, et al. The activity of echinocandins, amphotericin B and voriconazole against fluconazole-susceptible and fluconazole-resistant Brazilian Candida glabrata isolates. Mem Inst Oswaldo Cruz. 2012;107(3):433–436.
9. Kurtzman CP, Fell JW. The Yeasts: A Taxonomic Study. 4th ed. Amsterdam: Elsevier; 1998.
10. Gales AC, Pfaller MA, Houston AK. Identification of Candida dubliniensis based on temperature and utilization of xylose and α-methyl-d-glucoside as determined with the API 20C AUX and Vitek YBC Systems. J Clin Microbiol. 1990;37:3804–3808.
11. Clinical and Laboratory Standards Institute (CLSI). Reference Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts. Approved Guideline–Second Edition (M44-A2). Wayne, PA: CLSI; 2009.
12. Storti LR, Pasquale G, Scomparim R, et al. Candida spp. Isolated from inpatients, the environment, and health practitioners in the Pediatric Unit at the Universitary Hospital of the Jundiaí Medical College, State of São Paulo. Rev Soc Bras Med Trop. 2012;45(2):225–231.
13. Martins-Dinis JN, Silva RA, Miranda ET, Mendes-Giannini MJ. Monitoramento de fungos anemófilos de leveduras em unidade hospitalar. Rev Saude Publica. 2005;39(3):398–405.
14. Hinrichsen SL, Falcão E, Vilella TAS, et al. Candida isolates in tertiary hospitals in northeastern Brazil. Braz J Microbiol. 2009;40:225–228.
15. Li L, Redding S, Dongari-Bagtzoglou A. Candida glabrata: an emerging oral opportunistic pathogen. J Dent Res. 2007;86(3):204–215.
16. Bonassoli JA, Bertoli M, Svidzinski TIE. High frequency of Candida parapsilosis on the hands of healthy hosts. J Hosp Infect. 2005;59:159–162.
17. Negri M, Silva S, Henriques M, Oliveira R. Insights into Candida tropicalis nosocomial infections and virulence factors. Eur J Clin Microbiol Infect Dis. 2012;31(7):1399–1412.
18. Estivill D, Arias A, Torres-Lana A, Carrillo-Muñoz AJ, Arabávlo MP. Biofilm formation by five species of Candida on three clinical materials Source Althia, Hospital Sant Joan de Déu, Manresa, Barcelona, Spain. J Microbiol Methods. 2011;86(2):238–242.
19. Kuhn DM, Chandra J, Mukherjee PK, Ghannoum MA. Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces. Infect Immun. 2002;70(2):878–888.
20. Van Asbeck EC, Huang YC, Markham AN, Clemons KV, Stevens DA. Candida parapsilosis fungemia in neonates: genotyping results suggest healthcare workers hands as source, and review of published studies. Mycopathologia. 2007;164(6):287–293.
21. Da Matta DA, de Almeida LP, Machado AM, et al. Antifungal susceptibility of 1000 Candida bloodstream isolates to 5 antifungal drugs: results of a multicenter study conducted in Sao Paulo, Brazil, 1995–2003. Diagn Microbiol Infect Dis. 2007;57(4):399–404.
22. Goldani LZ, Mario PSS. Candida tropicalis fungemia in a tertiary care hospital. J Infect. 2003;46(3):155–160.
23. Asmundsdóttir LR, Erlendsdóttir H, Haraldsson G, Guo H, Xu J, Gottfredsson M. Molecular epidemiology of candidemia.
evidence of clusters of smoldering nosocomial infections. 
Clin Infect Dis. 2008;47(2):17–24.

24. Diba K, Rhaimirad M, Makhdoomi K, Khorshidvand Z. 
Identification of Candida species isolated from hospital 
aquired infections cases and hospital indoor environments. 
Afr J Microbiol Res. 2012;6(2):4164–4168.

25. Pronovost P, Needham D, Berenholtz S, et al. An intervention 
to decrease catheter-related bloodstream infections in the 
ICUN. Engl J Med. 2006;355(26):2725–2732.

26. Bassetti M, Taramasso L, Nicco E, Molinari MP, Mussap M, 
Viscoli C. Epidemiology, species distribution, antifungal 
susceptibility and outcome of nosocomial candidemia in a 
tertiary care hospital in Italy. PLoS ONE. 2011;6(9):e24198.

27. Pemán J, Cantón E, Quindós G, et al. Epidemiology, species 
distribution and in vitro antifungal susceptibility of 
fungaemia in a Spanish multicentre prospective survey. J 
Antimicrob Chemother. 2012;67(5):1181–1187.

28. Ben-Ami R, Olshain-Pops K, Krieger M, et al. Antibiotic 
exposure as a risk factor for fluconazole-resistant Candida 
bloodstream infection. Antimicrob Agents Chemother. 
2012;56(5):2518–2523.

29. Córdoba S, Vivot W, Bosco-Borgeat ME, et al. Species 
distribution and susceptibility profile of yeasts isolated from 
blood cultures: results of a multicenter active 
laboratory-based surveillance study in Argentina. Rev Argent 
Microbiol. 2011;43(3):176–185.

30. Bonfietti LX, Szeszs MW, Chang MR, et al. Ten-year study of 
species distribution and antifungal susceptibilities of 
Candida bloodstream isolates at a brazilian tertiary hospital. 
Mycopathologia. 2012;174(5–6):389–396.