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Short Communication

# Variability in the clinical distributions of *Candida* species and the emergence of azole-resistant non-*Candida albicans* species in public hospitals in the Midwest region of Brazil

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## Abstract

**Introduction**: Incidence and antifungal susceptibility of *Candida* spp. from two teaching public hospitals are described. **Methods**: The minimum inhibitory concentrations of fluconazole, voriconazole, itraconazole, and amphotericin B were determined using Clinical Laboratory Standard Institute broth microdilution and genomic differentiation using PCR. **Results**: Of 221 *Candida* isolates, 50.2% were obtained from intensive care unit patients; 71.5% were recovered from urine and 9.1% from bloodstream samples. *Candida parapsilosis sensu stricto* was the most common candidemia agent. **Conclusions:** We observed variations in *Candida* species distribution in hospitals in the same geographic region and documented the emergence of non-*C. albicans* species resistant to azoles.

**Keywords:** Candidiasis. Candidemia. Epidemiology.

*Candida* spp. are microorganisms that can cause infections ranging from superficial to systemic infections and are considered the main agents of fungal infections in hospitalized patients. The consequences of invasive candidiasis are severe for both the patient and the institution owing to prolonged hospitalization and increased mortality[1].

Although *Candida albicans* species are the most frequently isolated, the epidemiology of *Candida* infections is changing, with increased incidence of non-*Candida albicans* (NCA) species[1,2,3].

The choice of treatment for candidiasis should be based on the *Candida* species and infection site. In addition, knowledge of the local antifungal susceptibility is of great importance to ensure better patient prognosis.

This study investigated the incidence of *Candida* isolates and their antifungal susceptibility. We performed a prospective study in two public teaching hospitals located in Mato Grosso do Sul State, Brazil, namely University Hospital Maria Aparecida Pedrossian (UH-MAP) and University Hospital of the Federal University of Grande Dourados (UH-FUGD), from March 2013 to March 2014.

This study included *Candida* spp. isolates obtained from different clinical specimens. If patients had more than one isolate of the same species, only the first sample was considered. Data regarding patient age, sex, and hospital units were obtained from the computerized system of each hospital.

The minimum inhibitory concentrations (MICs) of fluconazole, voriconazole, itraconazole, and amphotericin B were determined by using the Clinical Laboratory Standards Institute (CLSI) broth microdilution (BMD) method. For quality control and reproducibility of the tests, American Type Culture Collection (ATCC) strains (*C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) were included. The MICs were interpreted according to the proposed CLSI breakpoints[4].

Genomic deoxyribonucleic acid (DNA) was extracted and purified using a commercial YeaStar DNA Extraction Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. For the first differentiation between species, multiplex polymerase chain reaction (PCRm) was performed as described by Li *et al*.[5]. The primers used were CL (*Candida lusitaniae*): GTTAGGCGTTGCTCCGAAAT; CP (*Candida parapsilosis* complex): GGCGGAGTATAAAGTAATGGATAG; CT (*Candida tropicalis*): AAGAATTTAACGTGGAAACTTA; CGU

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(*Candida guillermondii*): GTATTGGCATGGGTAGTACTG; CA (*Candida albicans*): TCAACTTGTCACACCAGATTATT3; CK (*Candida krusei*): GAT TTAGTACTACACTGCGTGA; CGL (*Candida glabrata*): CACGACTCGACACTTTCTAATT.

For differentiation between *Candida albicans* and *Candida dubliniensis* isolates, duplex PCR was performed as described by Ahmad *et al*.[6]. The primers used were CALF: TGGTAAGGC-GGGATCGCTT + CALR: GGTCAAAGTTTGAAGATATAC; and CDUF: AAACTTGTCACGAGATTATTTTT + CDUR: AAAGTTTGAAGAATAAAATGGC for *C. albicans* and *C. dubliniensis*, respectively.

Differentiation of the *C. parapsilosis* complex was performed by PCR-restriction fragment length polymorphism (RFLP) as described by Tavanti *et al*.[7]. The primers used were S1F: GTTGATGCTGTTGGATTGT; S1R: CAATGCCAAATCTCCCAA.

The *C. glabrata* complex was differentiated by using PCRm in accordance with the study published by Romeo *et al*.[8]. The primers used were UNI-5.8 (universal reverse primer): ACCAGAGGGCGCAATGTG; GLA-F (*Candida glabrata*): CGGTTGGTGGGTGTTCTGC; NIV-F (*Candida nivariensis*): AGGGAGGAGTTTGTATCTTTCAAC; BRA-F (*Candida bracarensis*): GGGACGGTAAGTCTCCCG.

During the study period, 10,680 and 8,542 patients were hospitalized in UH-MAP and UH-FUGD, respectively. A total of 221 *Candida* species were evaluated. Of these, 164 were isolated from patients admitted to UH-MAP while 57 were isolated from those admitted to UH-FUGD. These represent rates of 15.35 and 6.67 per 1,000 admissions in UH-MAP and UH-FUGD, respectively. The incidence of candidemia in UH-MAP was 1.40 per 1,000 hospital admissions (20, 11.7%). In UH-FUGD, the incidence was 0.58 per 1,000 admissions [5 (8.6%)]. Of the patients admitted to UH-MAP and UH-FUGD, 1,035 (9.7%) and 84 (1%) were considered critically ill patients, respectively.

The increased incidence of fungal infections observed in recent years has been associated with the increased use of invasive devices, transplantation, and extensive surgeries, among other medical procedures[1,9]. In our study, the difference in incidence rates observed between the two hospitals may be related to the higher number of critically ill patients admitted to UH-MAP compared to UH-FUGD.

The age of the patients with candidiasis ranged from 1 day to 98 years, with those ≥60 years most often affected by *Candida* infection. Most of the patients were women [122 (56%)] and were hospitalized in intensive care units (ICUs) [111 (50.2%)]. Elderly patients, as observed in our study, are at high risk of fungal infections due to the reduced immunity and increased incidence of chronic diseases associated with advancing age[10]. In addition, ICU admission is considered a risk factor for fungal infections because of the severity of cases and the frequent use of invasive devices[1,9]. **Table 1** shows the patient demographic characteristics, species distribution, and clinical specimens from which *Candida* isolates were obtained in the two hospitals.

Of the 221 *Candida* isolates, 78 (35.3%) were *C. albicans* and 143 (64.7%) were NCA, including 58 (35.4%) *C. albicans* and 106 (64.6%) NCA species from UH-MAP and 20 (35.1%) *C. albicans* and 37 (64.9%) NCA from UH-FUGD.

*Candida* spp. were isolated from 11 different clinical specimens **(Table 1)**, mainly urine [158 (71%)], blood samples [20 (9.1%)], and tracheal aspirate [20 (9.1%)].

**TABLE 1:** Demographic characteristics, species distribution and clinical specimens of *Candida* isolation according to the hospitals.

| Variables | UH-MAP | | UH-FUGD | | Total | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| **Age group** | | | | | | |
| 0–28 days | 1 | 0.6 | 2 | 3.5 | 3 | 1.4 |
| 29 days to ≤1 year | - | - | 1 | 1.8 | 1 | 0.5 |
| >1–12 years | 6 | 3.7 | - | - | 6 | 2.7 |
| 13–18 years | 4 | 2.4 | 1 | 1.8 | 5 | 2.3 |
| 19–59 years | 55 | 33.5 | 20 | 35.1 | 75 | 33.9 |
| ≥60 years | 98 | 57.8 | 33 | 57.9 | 131 | 59.3 |
| **Sex*** | | | | | | |
| female | 88 | 54 | 34 | 61.8 | 122 | 55 |
| male | 75 | 46 | 21 | 38.2 | 96 | 44 |
| **Hospital unit** | | | | | | |
| intensive care unit | 76 | 46.3 | 35 | 61.4 | 111 | 50.2 |
| emergency room | 34 | 20.7 | 21 | 36.9 | 55 | 24.9 |
| medical clinic | 38 | 23.2 | 1 | 1.8 | 39 | 17.7 |
| surgical clinic | 16 | 9.8 | - | - | 16 | 7.2 |
| **Specimens** | | | | | | |
| urine | 119 | 72.6 | 39 | 68.4 | 158 | 71.5 |
| blood | 15 | 9.1 | 5 | 8.8 | 20 | 9.1 |
| tracheal aspirate | 12 | 7.3 | 8 | 14 | 20 | 9.1 |
| catheter tip | 2 | 1.2 | 4 | 7 | 6 | 2.7 |
| surgical site | 5 | 3.1 | - | - | 5 | 2.3 |
| skin scraping | 3 | 1.8 | 1 | 1.8 | 4 | 1.8 |
| abdominal aspirated | 2 | 1.2 | - | - | 2 | 0.9 |
| biopsy | 2 | 1.2 | - | - | 2 | 0.9 |
| vaginal aspirate | 2 | 1.2 | - | - | 2 | 0.9 |
| pleural fluid | 1 | 0.6 | - | - | 1 | 0.5 |
| bone fragment | 1 | 0.6 | - | - | 1 | 0.5 |
| ***Candida* species** | | | | | | |
| *albicans* | 58 | 35.4 | 20 | 35.1 | 78 | 35.3 |
| *tropicalis* | 53 | 32.3 | 21 | 36.9 | 74 | 33.5 |
| *glabrata stricto sensu* | 27 | 16.5 | 8 | 14.0 | 35 | 15.8 |
| *parapsilosis stricto sensu* | 19 | 11.6 | 3 | 5.3 | 22 | 9.9 |
| *krusei* | 4 | 2.4 | 3 | 5.3 | 7 | 3.2 |
| *guilliermondii* | 2 | 1.2 | 1 | 1.8 | 3 | 1.4 |
| *orthopsilosis* | 1 | 0.6 | - | - | 1 | 0.5 |
| *lusitaniae* | - | - | 1 | 1.8 | 1 | 0.5 |

**UH-MAP:** University Hospital Maria Aparecida Pedrossian; **UH-FUGD:** University Hospital of the Federal University of Grande Dourados. *The sex of patients less than 28 days old was not included in the computerized system of hospitals.

**TABLE 2:** Susceptibility to antifungals of *Candida* species according to hospitals.

| Antifungal | UH-MAP | | | | | UH-FUGD | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MIC range | MIC50/90* | Number by category | | | MIC range | MIC50/90* | Number by category | | |
| | | | S | SDD | R | | | S | SDD | R |
| ***C. albicans*** | | | | | | | | | | |
| FLU | 0.12 – 16 | 0.25/0.5 | 53 | 2 | 3 | 0.25 – 4 | 0.25/1 | 19 | 1 | - |
| VOR | 0.015 – 0.25 | 0.015/0.06 | 56 | 2 | - | 0.03 – 0.06 | 0.03/0.06 | 20 | - | - |
| ITRA | 0.015 – 1 | 0.03/0.125 | 53 | 4 | 1 | 0.03 – 0.5 | 0.125/1 | 13 | 7 | - |
| AMB | 0.015 – 1 | 0.5/1 | 58 | - | - | 0.03 – 0.5 | 0.03/0.5 | 20 | - | - |
| ***C. tropicalis*** | | | | | | | | | | |
| FLU | 0.05 – 16 | 0.25/2 | 49 | 1 | 3 | 0.25 – 4 | 1/1 | 20 | 1 | - |
| VOR | 0.015 – 1 | 0.03/0.25 | 47 | 5 | 1 | 0.03 – 2 | 0.06/0.125 | 17 | 2 | 2 |
| ITRA | 0.015 – 1 | 0.06/1 | 39 | 11 | 3 | 0.03 – 0.5 | 0.06/0.125 | 18 | 3 | - |
| AMB | 0.125 – 1 | 1/0.5 | 53 | - | - | 0.03 – 0.5 | 0.03/0.5 | 21 | - | - |
| ***C. glabrata sensu stricto**** | | | | | | | | | | |
| FLU | 0.12 – 64 | 4/16 | - | 26 | 1 | 0.25 – 32 | 16/16 | - | 8 | - |
| VOR | 0.015 – 2 | 0.06/0.25 | - | - | - | 0.03 – 1 | 0.5/1 | - | - | - |
| ITRA | 0.015 – 8 | 0.25/0.5 | 8 | 17 | 2 | 0.03 – 8 | 1/8 | 2 | 1 | 5 |
| AMB | 0.03 – 1 | 0.5/1 | 27 | - | - | 0.03 – 0.5 | 0.03/0.5 | 8 | - | - |
| ***C. parapsilosis sensu stricto*** | | | | | | | | | | |
| FLU | 0.12 – 16 | 1/8 | 15 | - | 4 | 0.25 | - | 3 | - | - |
| VOR | 0.015 – 0.25 | 0.015/0.125 | 16 | 3 | - | 0.03 | - | 3 | - | - |
| ITRA | 0.015 – 1 | 0.03/0.125 | 16 | 2 | 1 | 0.03 – 0.25 | - | 2 | 1 | - |
| AMB | 0.125 – 1 | 0.5/1 | 19 | - | - | 0.03 – 0.25 | - | 3 | - | - |
| ***C. krusei****** | | | | | | | | | | |
| FLU | 2 – 8 | - | - | - | - | 1 – 32 | - | - | - | - |
| VOR | 0.06 – 0.125 | - | 4 | - | - | 0.03 – 0.5 | - | 3 | - | - |
| ITRA | 0.125 – 0.5 | - | 2 | 2 | - | 0.25 – 0.5 | - | - | 3 | - |
| AMB | 0.5 – 1 | - | 4 | - | - | 0.03 | - | 3 | - | - |
| ***C. guilliermondii*** | | | | | | | | | | |
| FLU | 1 – 2 | - | 2 | - | - | 0.25 | - | 1 | - | - |
| VOR | 0.03 | - | 2 | - | - | 0.06 | - | 1 | - | - |
| ITRA | 0.125 | - | 2 | - | - | 0.03 | - | 1 | - | - |
| AMB | 0.25 – 0.5 | - | 2 | - | - | 0.5 | - | 1 | - | - |
| ***C. orthopsilosis*** | | | | | | | | | | |
| FLU | 2 | - | 1 | - | - | - | - | - | - | - |
| VOR | 0.06 | - | 1 | - | - | - | - | - | - | - |
| ITRA | 0.06 | - | 1 | - | - | - | - | - | - | - |
| AMB | 0.25 | - | 1 | - | - | - | - | - | - | - |
| ***C. lusitaniae*** | | | | | | | | | | |
| FLU | - | - | - | - | - | 1 | - | 1 | - | - |
| VOR | - | - | - | - | - | 0.03 | - | 1 | - | - |
| ITRA | - | - | - | - | - | 0.125 | - | 1 | - | - |
| AMB | - | - | - | - | - | 0.5 | - | 1 | - | - |

**UH-MAP:** University Hospital Maria Aparecida Pedrossian; **UH-FUGD:** University Hospital of the Federal University of Grande Dourados; **MIC:** minimum inhibitory concentration as defined by Clinical Laboratory Standard Institute; **S:** susceptible; **SDD:** susceptible dose dependent; **R:** resistant; ***C.:*** *Candida;* **FLU**: fluconazole; **VOR:** voriconazole; **ITRA;** itraconazole; **AMB:** amphotericin B. *MIC50 and *MIC90: MIC at which 50% and 90% of the isolates were inhibited. ***Candida glabrata* does not have breakpoints for voriconazole because the data were insufficient to demonstrate the in vitro correlation with the clinic. **** *Candida krusei* was intrinsically resistant in vivo to fluconazole, independent of the minimum inhibitory concentration.

Despite being tertiary and teaching hospitals located in the same region, the two hospitals showed differences in the incidence of *Candida* infection-causing species **(Table 1)**.

In UH-MAP, the main agent of candiduria was *C. albicans* [47 (39.5%)], whereas in UH-FUGD, it was *C. tropicalis* [15 (38.5%)]. The presence of *Candida* spp. in the urine may indicate infection or colonization of the urinary tract. In hospitalized patients, the detection of *Candida* as a colonizing agent has clinical relevance because, in immunocompromised patients, it may be a risk factor for candidemia[11].

Unlike previous studies that reported *C. albicans* as the main species of candidemia in Latina American medical centers[3,12], our study showed that NCA species were most commonly isolated from blood cultures [19 (95%)].

*Candida parapsilosis sensu stricto* was the main cause of candidemia in UH-MAP [6 (40%)]. In UH-FUGD, no difference was observed in the number of species isolated from blood culture. In a recent review[3] *C. parapsilosis sensu stricto* was identified as the main NCA species causing candidemia in 25 of 40 studies. In six studies, this species was more prevalent than *C. albicans*, similar to the observation in the present study. *Candida parapsilosis* complex is an important agent of candidemia due to their ability to form biofilms and adhere to plastic surfaces such as central venous catheters that are frequently used in critically ill patients[13].

Previous studies[2,12,14,15] indicate that *Candida* isolates of various species were susceptible to amphotericin B. In contrast, 132 (57.6%) *Candida* spp. isolates had decreased susceptibility to azole drugs. Of these, 40 (17.5%) and 11 (17.5%) isolates were considered susceptible dose-dependent (SDD) and resistant to fluconazole, respectively. Regarding itraconazole, 53 (23.1%) isolates were considered SDD while 13 (5.7%) were resistant; finally, 12 (5.2%) and 3 (1.3%) isolates were SDD and resistant to voriconazole, respectively. The results of *in vitro* assessment of the susceptibility to antifungal drugs according to *Candida* species are shown in **Table 2**.

Compared with the scarce data from previous studies conducted in the Midwest region of Brazil[12,14,15], our results show an increase in the percentage of isolates resistant to antifungal azoles.

A previous study suggested that prolonged fluconazole treatment may induce fluconazole resistant mutations and, consequently, treatment failure[2].

In this study, we verified that *Candida* spp. is important agents of infection in hospitalized patients. Despite affecting all age groups, the most affected were adults and elderly patients admitted to the ICU.

We showed differences in the distributions of *Candida* species causing candiduria and candidemia in tertiary teaching hospitals within the same region. We also documented the emergence of azole drug resistance, mainly in NCA species.

### Ethical considerations

Descriptive statistics were used to characterize the variables. The study was approved by the Research Ethics Committee of the Federal University of Mato Grosso do Sul, under the registration number CAAE: 30746214.3.0000.0021

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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