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Major Article

Antifungal susceptibility and distribution of *Candida* spp. isolates from the University Hospital in the municipality of Dourados, State of Mato Grosso do Sul, Brazil

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ABSTRACT

Introduction: Hospital infections caused by *Candida* spp. are a leading cause of morbidity and mortality in hospitalized patients, particularly those that are critically ill or immunocompromised. In this study, the distribution of *Candida* species in isolates from the University Hospital of the Federal University at Grande Dourados and their *in vitro* susceptibility to antifungal drugs were analyzed. **Methods:** Yeasts were phenotypically identified using classical methodologies. Antifungal susceptibility tests to amphotericin B and fluconazole were performed using the broth microdilution technique. **Results:** A total of 50 *Candida* isolates were obtained from hospitalized patients during the study period. We analyzed yeast isolates from urine (n=31; 62%), blood (n=12; 24%), and tracheal secretions (n=7; 14%). The following *Candida* species were identified: *C. tropicalis* (n=21; 42%), *C. albicans* (n=18; 36%), *C. glabrata* (n=10; 20%), and *C. krusei* (n=1; 2%). Antifungal susceptibility tests demonstrated that *C. albicans* was susceptible to both antifungal agents. However, 31.2% of the non-*C. albicans Candida* isolates displayed dose-dependent susceptibility to fluconazole, and 3.1% were resistant to amphotericin B. **Conclusions:** In contrast to previous reports, our results indicated that *C. tropicalis* was the most commonly isolated yeast species among the hospital patients. The predominance of non-*C. albicans Candida* infections confirms the importance of species-level identification for implementing appropriate antifungal therapies.

Keywords: *Candida.* Candiduria. Candidemia. Amphotericin B. Fluconazole.

INTRODUCTION

Fungal hospital infections (HI) have increased worldwide in recent decades, which likely reflects advances in medical practices such as the increasing use of invasive procedures for diagnosis and treatment as well as the increasing number of immunocompromised patients at high risk for fungal infections[1].

According to Azie et al.[2], yeasts of the genus *Candida* are responsible for approximately 70% of all hospital-environment fungal infections. Therefore, *Candida* species are of enormous clinical importance because of the high frequencies at which they colonize and infect humans and cause diseases ranging from lesions in the mucous membranes and skin (superficial) to infections in body organs (and their resulting spread through the bloodstream, which characterizes invasive candidemia)[3].

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Candida spp. are commensal microorganisms that inhabit various sites in the human body (including the gastrointestinal and respiratory tracts) and comprise part of the vaginal and urethral microbiota[4]. These microorganisms can become pathogenic, however, as a result of alterations in host defense mechanisms or breakdown of anatomical barriers in the body – situations that are common in hospitalized patients who receive antibiotics or undergo frequent invasive procedures[5].

Hospital infections attributed to *Candida* spp. are responsible for increases in morbidity and mortality among hospitalized patients, particularly those that are critically ill or immunocompromised. The origins of these infections can be endogenous (due to yeast proliferation or dislocation) or exogenous (by transmission of microorganisms from the hospital environment through contact with health workers)[3].

Studies in Brazil that have focused on the epidemiology of infections caused by *Candida* species have reported that *C. albicans* is the most frequently isolated species of the genus. There have been, however, increases in HI caused by non-*C. albicans Candida* (NCAC) species such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis,* and *C. krusei*[6-8]. The occurrence of infections caused by these yeasts is relevant to HI vigilance because these micro-organisms have varying virulence attributes and profiles of antifungal drug sensitivity, including recent reports of resistance to the principal antifungal drugs currently administered in hospitals[9,10].

Amphotericin B and fluconazole are among the antifungal agents most widely used in treating systemic fungal infections. The former is an efficient polyene antifungal agent, although its use is limited due to its high degree of toxicity in humans. The latter compound is a triazole frequently prescribed to treat *Candida* spp. infections because of its excellent patient tolerance and minimal side effects[11], although a number of species of *Candida* display fluconazole resistance[12,13] and the growing worldwide use of this drug to treat candidemia is one of the principal causes of the recent increase in the prevalence of NCAC species[14].

Because of the importance of identifying the *Candida* species involved in HI and their increasing resistance to antifungal agents, the present study analyzed the distribution of *Candida* species in the University Hospital of the Federal University at Grande Dourados and evaluated the yeasts' *in vitro* susceptibility to antifungal drugs.

METHODS

We evaluated clinical samples from patients being treated at the University Hospital of the Federal University at Grande Dourados, central-western Brazil, between June 2010 and June 2011. This hospital has 197 beds, including 32 in the intensive care unit (ICU). The following information about the patients was collected: sex, age, the hospital ward in which the patient was being treated when the infectious agent was identified, and mortality during hospitalization, whether attributable to *Candida* infection or not.

The clinical samples that were tested for *Candida* species included urine, blood, and tracheal secretions. The samples were cultured according to standard procedures described in the literature. Cultures from urine samples were considered significant at concentrations above 10^5 colony-forming units per milliliter (CFU/mL).

Yeasts were isolated and initially identified using CHROMagar *Candida*® (Difco®, Sparks, MD, *USA*). Isolates were definitively identified based on their microscopic, macroscopic, and biochemical characteristics as described by classical methodologies, including colony morphology, production of germinative tubes, micromorphological analyses, and carbohydrate assimilation and fermentation tests[15].

The susceptibility of the yeast isolates to antifungal agents was determined by the broth microdilution method, following the norms of the Clinical and Laboratory Standards Institute (CLSI), document M27-A3[16]. The antifungal agents tested were amphotericin B (Squibb Pharmaceutical, Princeton, NJ, USA) and fluconazole (Pfizer Inc, New York, NY, USA.).

The yeast isolates were pre-cultured in Sabouraud Dextrose agar (Difco®, Sparks, MD, *USA*)at 35 ºC for 24 hours. Suspensions were prepared in sterile saline solution (0.85%) with yeast concentrations adjusted to 0.5 to 2.5 x 10^3 CFU/mL. RPMI-1640 (Sigma-Aldrich®, St. Louis, MO, USA) culture media was used and was buffered with 3 morpholinopropanesulfonic acid (Sigma-Aldrich®, St. Louis, MO, USA) pH 7.0 and supplemented with 2% glucose.

Each microplate well (Nunclon, Roskilde, SN, DK) contained 100µL of inoculant and one of the 10 antifungal agent concentrations tested; the cultures were incubated at 35ºC for 48 hours. All tests were performed in triplicate. A standard strain of *C. parapsilosis* ATCC 22019 was used as a control in the tests. The microplates were analyzed using an Expert plus - ASYS® (Biochrom, Holliston, MA, USA) analyzer (at 490nm).

The minimum inhibitory concentration (MIC) for fluconazole was defined as the lowest concentration of that compound that could reduce fungal growth by 50%; the MIC of amphotericin B was defined as the lowest concentration of that compound that could impede any visible yeast growth when compared to positive controls (those without added drugs).

The cut-off levels of susceptibility to fluconazole were utilized according to the supplement M27-S3[17]: values of MIC $\leq$ 8 µg/mL indicated the yeast was susceptible, 16-32µg/mL indicated the yeast was susceptible dose-dependent (SDD), and $\geq$ 64 µg/mL indicated the yeast was resistant. This document did not consider amphotericin B, so the susceptibility references levels established by Yang et al. were used[18]: values of MIC $\leq$ 1 µg/mL indicated the yeast was susceptible, and levels $\geq$ 2 µg/mL indicated the yeast was resistant.

Ethical considerations

This research was approved by the Ethics Committee of the University Hospital of the Federal University at Grande Dourados (protocol 050/2010 – CEP/UFGD).

RESULTS

A total of 50 *Candida* isolates were obtained from hospitalized patients during the study period, with the following species distributions: *C. tropicalis* 42% (n = 21), *C. albicans* 36% (n = 18), *C. glabrata* 20% (n = 10), and *C. krusei* 2% (n = 1).

As observed in **Table 1**, yeast was more frequently isolated from women (70%) and ICU patients (58%). Case fatality rate was 18%. *Candida* infections were most common in patients older than 61 years (46%) and younger than one year (22%). Yeasts from tracheal secretions were mainly obtained from women (85.7%) aged older than 61 years (85.7%).

Table 2 shows that the species most commonly isolated from urine was *C. tropicalis* (38.8%), followed by *C. albicans* (29%), *C. glabrata* (29%), and *C. krusei* (3.2%). Among the isolates derived from blood, *C. albicans* (66.6%) was predominant, whereas *C. tropicalis* was the yeast species most (71.4%) frequently isolated from tracheal secretions.

The MICs for amphotericin B varied from 0.125 to 16µg/mL and from 0.25 to > 64µg/mL for fluconazole. The MICs capable of inhibiting between 50% and 90% of the growth of isolates of each species are listed in **Table 3**.

All of the *C. albicans* isolates were susceptible to amphotericin B, and 94.5% were susceptible to fluconazole. Among the samples of *C. tropicalis*, only one displayed resistance to amphotericin B (MIC=16µg/mL), and one was SDD to fluconazole

TABLE 1 - Characteristics of the patients from which positive clinical samples of *Candida* spp. were obtained.

Characteristics	Clinical samples[a] urine n = 31	%	blood n = 12	%	tracheal secretions n = 7	%	Total[a] %
Gender							
male (n = 15)	9	29.0	5	41.6	1	14.3	30.0
female (n = 35)	22	71.0	7	58.4	6	85.7	70.0
Age[b]							
≤ 1 (n = 11)	4	13.0	7	58.4	-	-	22.0
2 - 20 (n = 4)	-	-	4	33.3	-	-	8.0
21 - 40 (n = 4)	3	9.7	-	-	1	14.3	8.0
41 - 60 (n = 8)	8	25.7	-	-	-	-	16.0
≥ 61 (n = 23)	16	51.6	1	8.3	6	85.7	46.0
Treatment site							
ICU (n = 29)	17	54.8	7	58.4	5	71.4	58.0
GW (n = 20)	14	45.2	4	33.3	2	28.6	40.0
IMC (n = 1)	-	-	1	8.3	-	-	2.0
deaths (n = 9)	7	22.5	2	16.6	-	-	18.0

M: male; F: female; ICU: intensive care unit; IMC: intermediate care unit; GW: general ward. [a]values expressed as percentages; [b]ages are given in years.

TABLE 2 - Distribution of *Candida* species in clinical samples obtained from hospital patients.

Species	Clinical samples[a] urine n	%	blood n	%	tracheal secretions n	%	total n	%
Candida tropicalis	12	38.8	4	33.4	5	71.4	21	42.0
Candida albicans	9	29.0	8	66.6	1	14.3	18	36.0
Candida glabrata	9	29.0	-	-	1	14.3	10	20.0
Candida krusei	1	3.2	-	-	-	-	1	2.0
Total	31	62.0	12	24.0	7	14.0	50.0	100.0

[a]numbers of isolates and percentages.

(MIC=16µg/mL). The other isolates were susceptible to both antifungal drugs. All isolates of the species *C. glabrata* and *C. krusei* were susceptible to amphotericin B, but exposure to fluconazole revealed high MICs among these isolates **(Table 3)**.

DISCUSSION

Fungal infections are frequent complications for hospitalized patients. *Candida* spp. infections have increased in the last few decades, particularly those caused by NCAC species, indicating the importance of laboratory diagnoses for the correct identification of the species involved and the initiation of timely and adequate treatment[3].

The data presented here included the factors of age, sex, and the hospital units in which the patients were infected. The data were similar to those of previous studies. Furnaleto et al.[12] observed that infections caused by *Candida* spp. were more frequent in older people (> 61 years), those less than one year old, and among ICU patients[19,20].

Hospital infections are more frequent and can often be more serious in older patients due to factors related to aging itself, the underlying disease, and hospitalization. The death rate from candidemia, however, is highest among young patients[21]. The occurrence of *Candida* spp. infections in small children often involves the colonization of their mucous membranes or skin, which puts them at risk for invasive illnesses caused by changes in their host-parasite equilibrium[22]. Infections caused by *Candida* among female patients (principally candiduria) are often associated with the presence of *Candida* spp. in the vaginal microbiota. The high infection percentages of ICU patients is directly related to their exposure to risk factors for fungal infection, including previous use of antibiotics, invasive

TABLE 3 - Antifungal susceptibility profile of *Candida* species isolated from clinical samples and variations in the MIC, MIC_{50}, and MIC_{90} of the isolates of *Candida* spp.

	Amphotericin B					Fluconazole					
	MIC values (μg/mL)					MIC values (μg/mL)					
	S	R	range/	MIC_{50}	MIC_{90}	S	SDD	R	range/	MIC_{50}	MIC_{90}
Species[a]			GM						GM		
Candida tropicalis (21)	20	1	0.125 - 16	0.5	1	20	1	0	0.25 - 16	1	2
	-	-	0.65	-	-	-	-	-	0.93	-	-
Candida albicans (18)	18	0	0.25 - 0.5	0.25	0.5	17	1	0	0.25 - 32	0.25	0.5
	-	-	0.41	-	-	-	-	-	0.39	-	-
Candida glabrata (10)	10	0	0.25 - 1	0.5	1	0	8	2	16 - >64	32	32
	-	-	0.70	-	-	-	-	-	29.34	-	-
Candida krusei (1)	1	0	1	-	-	0	1	0	32	32	32
	-	-	-	-	-	-	-	-	-	-	-
Total (50)	49	1	-	-	-	37	11	2	-	-	-

S: susceptible; SDD: dose-dependent susceptible; R: resistant; GM: geometric mean; MIC: minimum inhibitory concentration; MIC_{50} and MIC_{90}: lowest concentration capable of inhibiting growth in 50% and 90% of the isolates, respectively. [a]number of isolates.

procedures such as the use of central venous catheters, prolonged hospitalization periods, and weakness due to the underlying causes for their original hospitalization[23].

Studies have indicated an apparent decrease in the infection rates of *C. albicans* in recent decades but an increase in infections caused by NCAC species[8], which corroborates the results of the present study, in which *C. albicans* represented only 36% of the isolates, whereas NCAC species (*C. tropicalis*, *C. glabrata*, and *C. krusei*) represented the majority of isolates (64%). These results are similar to other recent studies conducted in central-western Brazil. Yamamoto et al.[20] reported a prevalence of *C. albicans* of 39% among isolates in Cuiabá, State of Mato Grosso, and other reports from the same country have indicated a predominance of NCAC species over *C. albicans* in clinical samples isolated from hospitalized patients[12,24].

According to our results, *C. tropicalis* was the most frequently isolated species in hospitalized patients (42%), mainly from urine, in contrast to the results of studies in other parts of the world, where *C. albicans* is usually predominant among hospital infections. Among urinary tract fungal infections, *C. tropicalis* is the second or third most frequently isolated *Candida* species in urine cultures[25,26].

In the present study, *C. albicans* was the most (66.6%) frequent species among the cases of candidemia, similar to reports from the United States, Europe, and Brazil[27,28,29]. However, Chang et al.[19] reported a prevalence of *C. albicans* of 45.8% among analyzed candidemia infections at the University Hospital of the State of Mato Grosso do Sul. In Brazil and in our study, several studies have indicated *C. tropicalis* as the second most frequent isolate in cases of candidemia among hospitalized patients[12,20,24]. *C. tropicalis* is the fourth most frequent *Candida* species isolated in cases of candidemia in the United States[30] and the third most frequent isolate in Spain[8] and Asia[6]. The ability to detect bloodstream infections caused by *Candida* spp. is limited because bloodstream infections do not have any specific symptomatology and produce only low positive results in blood cultures. These limitations facilitate the spread of yeast to other organs, resulting in the worsening of the clinical situation of the patient, prolonged administration of medication, and high hospital costs[13]. Cases of candidemia were more frequently observed in children younger than one year.

A high sensitivity of *C. albicans* to antifungal agents was observed, corroborating other published works[6,12,31]. As observed in **Table 3**, among the NCAC species (n=32), 31.2% of the isolates demonstrated SDD to fluconazole, with 3.1% being resistant to amphotericin B (which corroborates published reports of high resistance of these species to antifungal agents)[9,13]. None of the isolates of *C. glabrata* were susceptible to fluconazole, although 80% were SDD and 20% were resistant, in agreement with the findings of other authors who described the low sensitivity of this species to triazol[32,33].

Therefore, to adequately treat yeast infections in hospital environments and to control and prevent HI, the identification of *Candida* isolates at the species level is extremely important, and antifungal sensitivity tests are necessary.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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