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Article

Distribution and antifungal susceptibility of *Candida* species isolated from clinical samples in southern Brazil

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Invasive infections caused by *Candida* species have been strongly associated with poor prognosis and high resistance rates to some antifungals. This study aimed to identify *Candida* species isolated from different anatomical sites and to describe their susceptibility profile to antifungals. Ninety-four clinical isolates of *Candida* were obtained from a Medical Laboratory of Santa Catarina/Brazil. Species identification was performed by MALDI-TOF MS. Susceptibility assays were performed as described by Clinical Laboratory Standard Institute (CLSI) microboth method. Among the analyzed samples, *C. albicans* was the pathogen most incident (59.9%) followed *by C. parapsilosis* complex (14.9%), *C. glabrata* complex (8.5%), and *C. tropicalis* (6.3%). 37 *Candida* strains were isolated from vaginal content (39.3%), 21 from the nail (22.4%), 8 from tracheal aspirates (8.5%), and 7 from urine (7.4%). Together, the *Candida* isolates presented decreased susceptibility to azole drugs, mainly to fluconazole and itraconazole. Amphotericin B showed sensibility in 95.7% of samples analyzed. Previous knowledge about etiology and antifungal susceptibility becomes indispensable to conduct an efficient treatment.

Keywords: Vulvovaginal candidiasis. *Candida albicans.* Antifungal agents. Antifungal Drug resistance. Azoles.

INTRODUCTION

Fungi can be considered as one of the major causes of human diseases, mainly in severely immunocompromised or hospitalized individuals, which can present underlying diseases, as Acquired Immunodeficiency Syndrome (AIDS), cancer, diabetes, and others (Firacative, 2020; Chang *et al.*, 2017).

In recent years, the opportunist fungal infections have been increased significantly, as well as a diversity of isolated fungi. The incidence of these infections affects approximately 1.6 billion individuals. The most common pathogens are *Candida* spp. and Aspergillus spp. These fungi cause more than 90% of all fungal infections (Beardsley *et al.*, 2018).

Invasive infections caused by genus *Candida* have been highly associated with severe infections with crude mortality of about 40-55% (Logan, Martin-Loeches, Bicanic, 2020). The spectrum of *Candida* infections is many heterogenic. These yeasts can be isolated from the oral cavity, gastrointestinal tract, and genital tract of healthy adults. However, under disadvantaged conditions, these microorganisms act as an opportunist agents and may be responsible for severe clinical manifestations, such as bloodstream infections and mucocutaneous lesions (Antinori *et al.*, 2016).

Candida albicans is the most common species in human candidiasis, however, it has been observed a significant increase in the number and importance of non-albicans *Candida* species (NAC) species, such as *Candida glabrata* and *Candida krusei* and *Candida*

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auris (Logan, Martin-Loeches, Bicanic, 2020). Many of these species' isolates are less susceptible to antifungals, such as fluconazole, posaconazole and voriconazole, which leads the treatment failure (Neves-Junior *et al*., 2015; Carvalho, Ramos, Barbedo, 2016; O'Brien *et al*., 2020).

Therapeutic failures may be evoked by the evolution of drug resistance. The molecular mechanisms associated with antifungal resistance include overexpression of membrane transporters, altered cell wall and ergosterol biosynthesis, the gain of function mutations in the transcription factors regulating membrane transporters, and ergosterol biosynthesis (Bhattacharya, Sae-Tia, Fries, 2020). In addition to the patient conditions, the set of virulence factors, as well as adhesins, proteinases production, phospholipases, and biofilms formation confer an increase in pathogenicity to these yeasts (Fuentefria *et al*., 2018; Nett, 2007; Sardi *et al*., 2013).

Strict surveillance of fungal pathogens, including antifungal resistance, remains inadequate. Many diagnostic laboratories, especially in low- and middle-income places, cannot perform routine identification. Clinicians need to have access to local epidemiology and reference susceptibility standards to guide treatment choices (Beardsley *et al*., 2018).

In this context, the objectives of this study were to evaluate *Candida* species, the anatomic sites, and susceptibility profile to the main antifungals employed in the therapeutic, by in ninety-four *Candida* isolates yeast strains obtained from a Clinical Analysis Laboratory of Santa Catarina/Brazil.

MATERIAL AND METHODS

Clinical isolates

Ninety-four clinical isolates of *Candida* genus were obtained from Santa Luzia Medical laboratory of Florianópolis/Brazil during October and December 2018. Standard strains of *C. albicans* were used (ATCC 18804). The Ethical Committee (Number 2.662.446) approved the study.

Identification of the clinical isolates

Strains identified in the medical laboratory by MALDI-TOF mass spectrometry technique. MALDI-TOF MS – VITEK MS (bio Mérieux). Software (Myla™), the database for analysis (bio Mérieux). Isolates colonies with the growth of the least 48 hours were selected for the study and a small amount of the isolates were transported to the analysis. The sample was inoculated in the circle with dimensions standards by the manufacturer in circular movements. The yeasts were lysed with formic acid and posteriorly 0.5 µL of formic acid was added to the smear. After drying, 1µL of the matrix was added over the lysed sample. The matrix with acid formic and three strains of known yeast was used as negative and positive controls, respectively.

Susceptibility assay

The antifungal susceptibility testing followed the methodology described by CLSI – protocol M27-A3 (Clinical and Laboratory Standards Institute – CLSI, 2008). The fungus was a culture of sabouraud dextrose agar medium. The inoculum was resuspended in saline solution and the absorbance was adjusted in a spectrophotometer at 530 nm to obtain equivalence transmittance 1x106 a 5x106 UFC/mL. The culture medium used for broth microdilution was Gibco™ RPMI 1640 Medium (RPMI) buffered with MOPS final concentration of 0,165 mol/L. In 96-well plates were added the medium, fungal inoculum, and antifungal. RPMI broth plus fungal inoculum of each isolate was used as positive control and RPMI broth was employed as a negative control. The assay was performed in triplicate. After incubation for 24 h at 37°C the Minimal Inhibitory Concentration (MIC) was determined. The MIC for Amphotericin B is the lowest concentration that inhibits 90% of fungal growth, while for azole agents, MIC is defined as the lowest concentration that inhibits 50% of fungal growth. It was considered fungal growth in the wells that occurred turbidity. Fluconazole, itraconazole, ketoconazole and amphotericin B were tested. The concentration of each antifungal followed the CLSI protocol (M27-S4 (2012)).

RESULTS AND DISCUSSION

Among the ninety-four strains of *Candida* included in the study, were obtained during October 2018 and December 2018. Among the analyzed sample, 59.6% were identified as *C. albicans*, 14.9% *C. parapsilosis* complex, 8.5% *C. glabrata* complex, 6.3% *C. tropicalis*, 3.1% *C. krusei*, 2.1% *C. guilliermondii* complex, 2.1% *C. dubliniensis*, 1.1% *C. lusitanea*, 1.1% *C. lipolitica* and 1.1% *C. kefir* (Table I).

Table I – Distribution of Candida isolates according to the clinical isolation sites

Candida strains	Vaginal	Nail	Sputrum	Tracheal aspirates	Urine	Blood	Abdom. fluid	Bronchial lavage	Othrers	Total N°/%
C. albicans	34 (60.7)	04 (7.1)	04 (7.1)	04 (7.1)	03 (5.3)	01 (1.8)	01 (1.8)	01 (1.8)	04 (7.1)	56 (59.6)
C. dubliniensis			01 (50)					01 (50)		02 (2.1)
C. glabrata	01 (12.5)	01 (12.5)		01 (12.5)	04 (50)		01 (12.5)			08 (8.5)
C.guilliermondii		02 (100)								02 (2.1)
C. kefir						01 (100)				01 (1.1)
C. krusei				01 (33.3)		01 (33.3)	01 (33.3)			03 (3.1)
C. lipolitica		01 (100)								01 (1.1)
C. lusitanea							01 (100)			01 (1.1)
C. parapsilosis	02 (14.3)	08 (57.1)		01 (7.1)					03 (21.4)	14 (14.9)
C. tropicalis		05 (83.4)		01 (16.6)						06 (6.3)
Total N° %	37 (39.3)	21 (22.4)	05 (5.4)	08 (8.5)	07 (7.4)	03 (3.2)	04 (4.3)	02 (2.1)	07 (7.4)	94 (100)

Abdomin. fluid: Abdominal fluid; *C guilliermondii* - *C. guilliermondii* complex*; C. glabrata – C. glabrata* complex*; C. parapsilosis*- *C. parapsilosis* complex

Candida albicans was the species identified as incident in this study, followed by C. *parapsilosis* complex, *C. glabrata* complex and *C. tropicalis*. This yeast is widely distributed in the nature, occurring in diverse habitats, unlike other species of the genus that have limited distribution (Santana *et al.*, 2013).

Sadeghi *et al.*, (2018) emphasize the growing importance of the involvement of non-albicans *Candida* species in the etiology of candidiasis. *C. parapsilosis* complex and *C. glabrata* complex were the predominant species involved in the confirmed cases of candidiasis. In Chile, a progressive increase in infections caused by NAC

has also been observed, with *C. parapsilosis* complex as species most frequent, followed by C. *tropicalis* and *C. glabrata* complex (Nucci *et al.*, 2010).

Our study corroborates another one carried out in Brazil, where *C. albicans* was isolated as responsible for the majority of the cases, followed by *C. tropicalis*, *C. parapsilosis* complex and *C. glabrata* complex (Sardi *et al.*, 2013).

Study realized in Northeastern Brazil demonstrated prevalence of 60% of NAC isolated from infections in a public hospital (Silva *et al.*, 2019). The frequency of different *Candida* species in clinic isolates differs with the geographic location and patient conditions (Beardsley *et al.*, 2018).

Current trends show a significant increase in the number and importance of NAC species, such as *C. tropicalis*, *C. parapsilosis* complex, and *C. krusei*. The hypothesis for this fact may be related to the indiscriminate use of azoles antifungals (Carvalho, Ramos, Barbedo, 2016).

Regarding anatomical sites, the yeasts were isolated mainly from vaginal content (39.3%), nail (22.4%), tracheal aspirates (8.5%) and urine (7.4%). The other sites were not observed at high frequency. In isolated strains of vaginal content, *C. albicans* was incident species, representing 91.8% of yeast identified, followed by *C. parapsilosis* complex with 5.4% and *C. glabrata* complex with 2.7% (Table I).

Candida vaginitis is a universally important disease with wide-reaching effects on the overall physical and mental health of women. *Candida* vaginitis is a complicated disease, whose symptoms are governed by the intersection of host physiology, fungal biology, and the immunological response (Willems *et al.*, 2020). *Candida albicans* is responsible for 80 to 90% of cases, followed by *C. glabrata* complex, *C. tropicalis*, *C. krusei* and *C. parapsilosis* complex (Alvares, Svidzinski, Consolaro, 2007). A study with asymptomatic pregnant women showed *C. albicans* as predominant species and *C. tropicalis* was the NAC isolated most frequently (Mushi, Mmole, Mshana, 2019).

Sasikala and Udayasari (2018) observed five different species in vaginal content samples: *C. albicans*, *C. krusei*, *C. glabrata* complex, *C. tropicalis* and *C. guilliermondii* complex. *Candida albicans* adheres to the vaginal epithelial cells in higher number comparing to other species. It could be explaining the high frequency of isolates of this species.

The nail was the second most affected anatomical site with 21 (22.4%) of the total samples analyzed. Among the analyzed species, *C. parapsilosis* complex presented as incident followed by *C. tropicalis*, *C. albicans*, *C. guilliermondii* complex, *C. glabrata* complex and *C. lipolitica.*

Onychomycosis is a fungal infection of the nail, causing discoloration and thickening of the affected nail plate, and is the most common nail infection worldwide (Gupta *et al.*, 2020). *Candida* genus has emerged as important agents and frequent in many cases of superficial infections. A retrospective study evaluated the nail mycological examinations and showed *C. tropicalis* and *C. krusei* species as most prevalent (Fay *et al.*, 2019). Similar results to our study were observed by Fich *et al.* (2014) in nail samples: *C. parapsilosis* complex was identified as the majority, followed by *C. guilliermondii* complex and *C. albicans.*

Candida spp. were isolated from 8 to 8.5%) samples of tracheal aspirates. In this anatomical site, *C. albicans* was the species predominantly isolated. The presence of yeast in the respiratory tract can be an important risk factor for pulmonary infections. A previous study shows that *C. albicans* was the most incident species (66.7%) isolated from tracheobronchial samples and NAC species identified were *C. glabrata* complex and *C. tropicalis* (Ferreira, Yatsuda, Pini, 2019).

Candida species were observed in 7 (7.4%) urine samples. The species isolated from this anatomic site were *C. glabrata* complex and *C. albicans*. Candiduria is common in hospitalized patients and most of them are asymptomatic. The main risk factors are diabetes mellitus, residential urinary catheters, and the use of broad-spectrum antibiotics, urinary obstruction, and admission in intensive care units. Urinary tract infections by *Candida* spp. can be caused by hematogenous dissemination after candidemia or retrograde via the urethra (Odabasi, Mert, 2020).

Studies in Turkey, Brazil, and India have registered the prevalence of NAC in candiduria as much higher than

older studies in Europe and North America (Toner *et al*., 2016). The increase in NAC species is a worrisome factor because the ability to acquire azole resistance is an emblematic characteristic of the pathogenic fungus *C. glabrata* complex (Pais *et al*., 2020).

The incidence of infections caused by NAC species has increased dramatically in recent years, however, in vitro susceptibility data are scarce. Of the samples analyzed in our study, 59.6% were identified as *C. albicans* and 40.4% as NAC.

Analyzing the susceptibility profile to fluconazole, itraconazole, ketoconazole, and amphotericin B is possible to affirm that the yeasts demonstrated better sensibility to amphotericin B for in vitro assays: 95.7% of isolates were sensitive (Table II). Despite this, in clinical practice, this antifungal should be administrated with caution and reserved only cases of resistance to other drugs due toxicity risk, in particular conventional amphotericin B deoxycholate (Demitto *et al*., 2012; Wang *et al*., 2021).

Table II – Susceptibility profile of isolates from *Candida* spp. to antifungals

N°	*Candida* strains	Amphotericin B			Ketoconazole			Fluconazole			Itraconazole		
		S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)
56	*C. albicans*	98.2		1.8	26.8	69.6	3.6	62.5	10.7	26.8	23.2	51.8	25.0
02	*C. dubliniensis*	100			50	50		100			50.0	50.0	
08	*C. glabrata*	87.5		12.5		100			62.5	37.5		12.5	87.5
02	*C.guilliermondi*	100				100		50.0		50.0			100
01	*C. kefir*	100				100		100			100		
03	*C. krusei*	100				100				100			100
01	*C. lipolitica*	100				100		100					100
01	*C. lusitanea*	100				100		100				100	
14	*C. parapsilosis*	85.7		14.3	7.1	85.7	7.1	85.7		14.3	28.6	21.4	50
06	*C. tropicalis*	100			16.6	66.6	16.6	50.0	16.6	33.3	16.6	50	33.3
01	*C.albicans**	100				100		100			100		

**ATCC strains; C guilliermond - C. guilliermondii* complex*; C. glabrata – C. glabrata* complex*; C. parapsilosis- C. parapsilosis* complex*;* S-susceptible; SDD- Susceptible dose dependent; R- resistant.

In general, in our study, the isolates presented decreased susceptibility to azole antifungals (Table II). A study performed to Midwestern of Brazil also demonstrated an increase in the percentage of isolates resistant to azole: 57.6% of the *Candida* spp. samples showed decreased susceptibility (Mattos *et al*., 2017). A previous study suggested that long-term treatment with fluconazole might induce mutations that lead to drug resistance and, consequently, treatment failure (Peron *et al*., 2016). Zida *et al*., (2017) observed the highest rates of resistance to fluconazole, itraconazole, and ketoconazole when clinical isolates were tested.

In our study, most strains were SDD (sensitive dose-dependent) for ketoconazole, indicating low efficacy for treatment. This antifungal is effective main in deep infections (Ellah *et al*., 2019). For non-albicans *Candida* species was noted greater resistance to itraconazole, where 87.5% of *C. glabrata* complex

strains, 50% of *C. parapsilosis* complex 33.3% of *C. tropicalis* and 100% of *C. krusei*, *C. guilliermondii* complex and *C. lipolitica* presented resistance of this antifungal.

Studies with resistance to itraconazole demonstrate that empiric treatment can lead to failure treatment, especially if administrated against *C. tropicalis*. This yeast has become increasingly prevalent in the world and is already the second yeast most frequently isolated in Brazil (Nunes *et al*., 2011).

A recent study in Chine shows that the rate of resistance to itraconazole was the highest in all *Candida* species, followed by fluconazole, voriconazole and 5-fluorocytosine (Zeng *et al*., 2019).

Candida albicans strains showed resistance for both fluconazole (26.8%) and itraconazole (25%). It is noteworthy that most of *C. albicans* strains were isolated from vaginal content (60.7%) (Table I).

From results obtained with *C. albicans* strains isolated from vaginal content (Table III) is possible to observe high resistance to fluconazole (35%) and itraconazole (29.4%). Besides this, high rates of SDD to fluconazole (14.7%), ketoconazole (76.5%) and itraconazole (52.9%) also observed.

Table III – Susceptibility profile of isolates of *Candida albicans* collected from vaginal content to antifungals\

Susceptibility	Amphotericin B	Ketoconazole	Fluconazole	Itraconazole
Susceptible	100%	20.6%	50%	17.6%
Susceptible dose dependent		76.5%	14.7%	52.9%
Resistant		2.9%	35.3%	29.4%

Resistance mainly to fluconazole has increased considerably in the past decade in women with recurrent vaginal candidiasis. Studies verify that almost all the women diagnosed with fluconazole-resistant *C. albicans* had previously used this drug (Marchaim *et al*., 2012).

Azole resistance rates are highly variable and can be influenced by medical prescription patterns to treatment and prophylaxis, in addition to resistance acquired in different regions (Lirio *et al*., 2019).

The high rate of resistance observed, suggest the necessity to associate laboratory tests with a clinical diagnostic to reduce the empiric treatments which can contribute to the development of resistance (Brandolt *et al*., 2017; Denning *et al*., 2018).

Together, the data obtained here demonstrated that *C. albicans* was the most found species. However, an increase in the appearance of other NAC species can be observed. The anatomical site most affected by fungal infections is the vagina, as the growth of yeast in samples of vaginal content occurred more frequently. In addition, the yeast analyzed showed higher resistance to the antifungal azois. The increase in fungal infections with resistance to conventional therapies is more frequent, showing the importance of the development of new drugs and adequate management of patients.

DECLARATION OF INTEREST

The authors report no conflicts of interest.

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