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Characterization of *Candida* spp. isolated from the bloodstream in a reference hospital in Brazil: using MALDI-TOF/MS to identify unusual species of *Candida*

Caracterização de *Candida* spp. isoladas da corrente sanguínea em um hospital de referência no Brasil: usando MALDI-TOF/MS para identificar espécies incomuns de *Candida*

Caracterización de *Candida* spp. aislado del torrente sanguíneo en un hospital de referencia en Brasil: uso de MALDI-TOF/MS para identificar especies inusuales de Candida

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Abstract
Candidemia is a health problem with a high mortality rate. The aims of this study were to compare the identification of *Candida* spp. via traditional methodologies and mass spectrometry (MALDI-TOF/MS) in order to verify the antifungal susceptibility profile of these isolates and analyze the clinical evolution of patients. This was a two-year prospective cohort conducted at the Hospital de Clínicas de Uberlândia (HCU-UFU). *Candida* spp. were identified via VITEK®-2 and MALDI-TOF/MS. Antifungal susceptibility tests were conducted via VITEK®-2. Clinical-demographic data were assessed from medical records and variables were described as mean, standard deviation, frequency and percentage, and risk factors were evaluated using univariate and multivariate analysis. A total of 113 patients and 126 *Candida* spp. isolates were studied. *C. albicans* was most frequent (30%); MALDI-TOF/MS identified unusual species, including misidentification of *C. famata*. Most of the isolates (95%) were susceptible to the tested antifungals. Most patients were men (65%) and older than 60 years of age (45%); 85% of patients were treated with fluconazole. The 30-day mortality rate was 56%; patients who did not receive treatment and those who started later had a lower survival rate (p <0.0001). MALDI-TOF/MS was able to identify unusual *Candida* spp. Candidemia presented a high mortality rate; the correct identification of *Candida* spp. and the introduction of appropriate and timely therapy are essential for increasing patient survival.
Keywords: Candidemia; MALDI-TOF/MS; Antifungal agents; Mortality.

Resumo
A candidemia é um problema de saúde com alta taxa de mortalidade. Os objetivos deste estudo foram comparar a identificação de *Candida* spp. por meio de metodologias tradicionais e espectrometria de massa (MALDI-TOF/MS), verificar o perfil de suscetibilidade antifúngica desses isolados e analisar a evolução clínica dos pacientes. Esta foi uma coorte prospectiva de dois anos conduzida no Hospital de Clínicas de Uberlândia (HCU-UFU). Os isolados de *Candida* spp. foram identificados por meio de VITEK®-2 e MALDI-TOF/MS. Os testes de suscetibilidade antifúngica foram realizados via VITEK®-2. Os dados clínico-demográficos foram avaliados a partir de prontuários e as variáveis foram descritas como média, desvio padrão, frequência e porcentagem; os fatores de risco foram avaliados por meio de análise univariada e multivariada. Um total de 113 pacientes e 126 isolados de *Candida* spp. foram estudados. *C.*

albicans foi a mais frequente (30%); MALDI-TOF/MS identificou espécies incomuns, incluindo identificação errônea de *C. famata*. A maioria dos isolados (95%) foi suscetível aos antifúngicos testados. A maioria dos pacientes era do sexo masculino (65%) e com idade superior a 60 anos (45%); 85% dos pacientes foram tratados com fluconazol. A taxa de mortalidade em 30 dias foi de 56%; os pacientes que não receberam tratamento e os que iniciaram mais tarde apresentaram menor sobrevida (p<0,0001). MALDI-TOF/MS foi capaz de identificar espécies incomuns de *Candida* spp. A doença apresentou alta mortalidade; a correta identificação das espécies envolvidas e a introdução de terapia adequada e oportuna são essenciais para aumentar a sobrevida do paciente.
Palavras-chave: Candidemia; MALDI-TOF/MS; Antifúngicos; Mortalidade.

Resumen
La candidemia es un problema de salud con una alta tasa de mortalidad. Los objetivos de este estudio fueron comparar la identificación de *Candida* spp. mediante metodologías tradicionales y espectrometría de masas (MALDI-TOF/MS), para verificar el perfil de susceptibilidad antifúngica de estos aislados y analizar la evolución clínica de los pacientes. Se trata de una cohorte prospectiva de dos años realizada en el Hospital de Clínicas de Uberlândia (HCU-UFU). *Candida* spp. fueron identificados usando VITEK®-2 y MALDI-TOF/MS. Las pruebas de susceptibilidad antifúngica se realizaron a través de VITEK®-2. Los datos clínico-demográficos se evaluaron a partir de las historias clínicas y las variables se describieron como media, desviación estándar, frecuencia y porcentaje; los factores de riesgo se evaluaron mediante análisis univariado y multivariado. Un total de 113 pacientes y 126 *Candida* spp. fueron estudiados. *C. albicans* fue la más frecuente (30%); MALDI-TOF/MS identificó especies inusuales, incluida la identificación errónea de *C. famata*. La mayoría de los aislamientos (95%) fueron sensibles a los antifúngicos probados. La mayoría de los pacientes eran hombres (65%) y mayores de 60 años (45%); El 85% de los pacientes fueron tratados con fluconazol. La tasa de mortalidad a los 30 días fue del 56 %; los pacientes que no recibieron tratamiento y los que lo iniciaron más tarde tuvieron menor supervivencia (p<0,0001). MALDI-TOF/MS pudo identificar especies poco comunes de *Candida* spp. La enfermedad tuvo una alta mortalidad; la identificación correcta de las especies involucradas y la instauración de una terapia adecuada y oportuna son fundamentales para aumentar la supervivencia de los pacientes.
Palabras clave: Candidemia; MALDI-TOF/MS; Antifúngicos; Mortalidad.

1. Introduction

Invasive infections caused by *Candida* species, especially candidemia, are a serious public health problem, being responsible for more than one million deaths/year worldwide (Almeida, Rodrigues & Coelho, 2019). Candidemia mainly affects immunocompromised transplanted patients who are using invasive devices, submitted to prolonged antimicrobial therapy and admitted to Intensive Care Units (ICU) (Al-Dorzi, et al., 2020) (Bassetti, et al., 2019) (Tsay, et al., 2020). The mortality rate due to candidemia can reach 70% (Pappas, et al., 2018), despite advances in diagnosis and adequate drug therapy.

Candida albicans is the species most frequently found in clinical isolates worldwide (Pote, et al., 2020). However, non-*albicans Candida* species (CNA) have been responsible for an increasing number of invasive yeast infections in recent years, mainly *C. glabrata*, *C. tropicalis*, *C. parapsilosis* complex and *C. krusei*; which, in association with *C. albicans*, make up more than 90% of clinical isolates from patients with candidemia (Delavy, et al., 2019) (Pappas, et al., 2018), (Pfaller, et al., 2019), (Pote, et al., 2020). The occurrence of candidemia caused by CNA draws attention due to the severity of this infection, and because some species are less susceptible to antifungals, which limits therapeutic options and can directly affect the increase in mortality (Pfaller, et al., 2019).

Laboratory diagnosis of candidemia is primarily performed via blood culture and tests are required to identify species and susceptibility to antifungals (Pappas, et al., 2018) in order to detect resistant isolates. However, it is described that the methodologies traditionally used for identifying yeasts, such as conventional phenotypic tests (biochemical tests, chromogenic media), are not able to correctly identify the cryptic species of *Candida* spp. (Fontecha, et al., 2019). An increase in the number of infections caused by unusual *Candida* species has been described in recent years (Tsai, et al., 2018). In addition, misidentified cases of these species have also been reported (Castanheira, et al., 2013). In this context, the use of mass spectrometry by desorption/ionization-time of flight (MALDI-TOF/MS) has improved the identification of fungal species in

clinical laboratories, considerably increasing the diagnosis accuracy (Cassagne, et al., 2016).

Despite the notable increase in candidemia in Brazilian hospitals, there are few reports about misidentification of *Candida* spp. Thus, this study aims to compare the identification of *Candida* spp. isolates using traditional methodologies and MALDI-TOF/MS, and to verify the antifungal susceptibility profile of the isolates. In addition, to analyze the clinical evolution of patients affected by the disease, including the use of inappropriate antifungal therapy.

2. Methodology

2.1 Study unit and patients

This was a two-year prospective cohort study conducted at the Hospital de Clínicas de Uberlândia at the Federal University of Uberlândia. It is a highly complex hospital, a reference in the Southeastern Brazilian region and has about 500 beds. All patients who had at least one episode of bloodstream infection caused by *Candida* spp. during the study period were included. Patients with more than one episode of the disease were counted once, being considered the first case of the infection.

2.2 Study design and data collection

Demographic and clinical data (gender, age, hospitalization sector, underlying disease/comorbidities, invasive devices such as a central catheter (CVC), urinary catheter, mechanical ventilation, probe in the digestive tract, parenteral nutrition, drugs for chronic use, surgical procedures, antibiotic treatment, antifungal treatment, duration of treatment and recurrence) of each patient were recovered from hospital medical records.

Antifungal therapy was considered inadequate when the patient received antifungals that were resistant *in vitro*, when the treatment was started more than 48 hours after the infection diagnosis or when the antifungal treatment was less than five days, and also when the patient received no treatment (Tumbarello, et al., 2007).

2.3 Yeast identification and antifungal susceptibility test

The isolates were identified using three different techniques: profile of growth on CHROMagar™ *Candida* Medium chromogenic agar (Becton Dickinson, Germany), automated biochemical tests conducted on the VITEK®-2 equipment (bioMerieux, France) and MALDI-TOF/MS mass spectrometry (Bruker Daltonick, Germany). The BioTyper 3.1 software program (Bruker Daltonik, Germany) was used to capture and evaluate the mass spectra. The tests were done in triplicate; low quality spectra with discrepant peaks and those with a score <2.0 were excluded. *E. coli* (ATCC® 25922™), *C. albicans* (ATCC® 10231™), *C. parapsilosis* (ATCC® 22019™), *C. tropicalis* (ATCC® 750™), *C. glabrata* (ATCC® 15126™) and *C. krusei* (ATCC® 34135™) were used as controls and validation. The antifungal susceptibility test was carried out using the AST-YS07/AST-YS08 cards from the VITEK®-2 automated system (bioMerieux, France) which includes amphotericin B, caspofungin, fluconazole, micafungin and voriconazole. The criteria for interpreting the Minimum Inhibitory Concentrations (MIC) for classifying isolates into susceptible, intermediate or dose-dependent and resistant were in accordance with document Supplement M60- Performance Standards for Antifungal Susceptibility Testing of Yeasts of the Clinical and Laboratory Standards Institute (CLSI, 2017).

2.4 Statistical analysis

The agreement among the techniques used to identify the isolates was performed using the Kappa test (Rosner, 1995), considering the values described by Landis and Koch (1997). These values were: insignificant correlation (K <0); weak

correlation (K between 0 and 0.2); reasonable correlation (K between 0.21 and 0.4); moderate correlation (K between 0.41 and 0.6); strong/substantial correlation (K between 0.61 and 0.8) and almost perfect correlation (K between 0.81 and 1.0).

The quantitative variables were described as mean and standard deviation, whereas the qualitative variables were in frequency and percentage. Risk factors were evaluated by univariate logistic regression, as well as by multiple logistic regression, followed by the selection of variables using the Stepwise method (Hosmer & Lemeshow, 2004). All tests were 2-tailed, and a *p*-value <0.05 was considered statistically significant.

2.5 Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. This study was approved by Research Ethics Committee of the Federal University of Uberlândia, under the CAAE number: 65341817.0.0000.5152.

3. Results and Discussion

A total of 113 patients diagnosed with candidemia were evaluated during the study period. Four patients (3.5%) had more than one episode of bloodstream infection by *Candida* spp., and another four patients (3.5%) had two isolates per episode, totaling 126 isolates.

Of the 126 *Candida* spp. isolates, 41 were *C. albicans*; 27 *C. tropicalis*; 18 *C. glabrata*; 17 *C. parapsilosis* sensu stricto; 10 *C. krusei;* three *C. kefyr;* three *C. lusitaniae;* two *C. pelliculosa*; two *C. guilliermondii;* two *C. orthopsilosis* and one *C. dubliniensis*. The agreement between the two methodologies which were used to identify isolates was considered almost perfect (K=0.833) and significant (p<0.0001). Three isolates previously identified as *C. famata* via VITEK®-2 were reclassified via MALDI-TOF/MS as *C. albicans* (two isolates) and *C. parapsilosis* sensu stricto (one isolate). Moreover, the identification of the two isolates of *C. orthopsilosis* (previously identified as *C. parapsilosis* by VITEK®-2) was only possible via MALDI-TOF/MS.

Most of the isolates were susceptible to all tested antifungals. In addition, 114 (90.5%) of the 126 isolates of *Candida* spp. studied presented susceptibility to fluconazole. Only 12 isolates (9.5%) presented resistance to fluconazole; 10 of these were *C. krusei* (7.9%), one was *C. parapsilosis* and one was *C. guilliermondii*. Table 1 shows the *Candida* spp. antifungal susceptibility profile.

Table 1: MIC values (mcg/mL) presented by the *Candida* species bloodstream infection from the patients hospitalized

		C. albicans (41)	*C. tropicalis* (27)	*C. glabrata* (18)	*C. parapsilosis* (17)	*C. krusei* (10)	Other * (13)
Fluconazole	**MIC Range**	0.5-1.0	0.5-1.0	1.0-4.0	0.5-64	4-16	0.5-32
	MIC50	1.0	1.0	2.0	0.5	8	1.0
	MIC90	1.0	1.0	4.0	4	16	4.0
	GM[a]	0.83	0.87	2.72	1.3	10.5	1.13
Voriconazole	**MIC Range**	0.12	0.12	0.12-4.0	0.12-1.0	0.12-0.25	0.12-4.0
	MIC50	0.12	0.12	0.25	0.12	0.12	0.12
	MIC90	0.12	0.12	0.25	0.25	0.12	0.12
	GM[a]	0.12	0.12	0.23	0.14	0.12	0.15
Amphotericin B	**MIC Range**	0.25-1.0	0.25-0.5	0.25-16.0	0.25-1.0	0.25-2.0	0.25-1.0
	MIC50	0.5	0.5	0.5	0.5	0.5	0.5
	MIC90	1.0	0.5	1.0	1.0	2.0	1.0
	GM[a]	0.61	0.38	0.58	0.42	0.87	0.26
Micafungin	**MIC Range**	0.06-0.5	0.06	0.06-8.0	0.06-2.0	0.06-2.0	0.06-5.0
	MIC50	0.06	0.06	0.06	0.5	0.12	0.12
	MIC90	0.06	0.06	0.06	1.0	2.0	0.5
	GM[a]	0.06	0.06	0.07	0.56	0.21	0.16
Caspofungin	**MIC Range**	0.12-0.25	0.12-0.25	0.12-8	0.25-1.0	0.25-2.0	0.12-0.5
	MIC50	0.25	0.25	0.25	0.5	0.5	0.25
	MIC90	0.25	0.25	0.5	1.0	2.0	0.5
	GM[a]	0.20	0.18	0.35	0.48	0.5	0.4

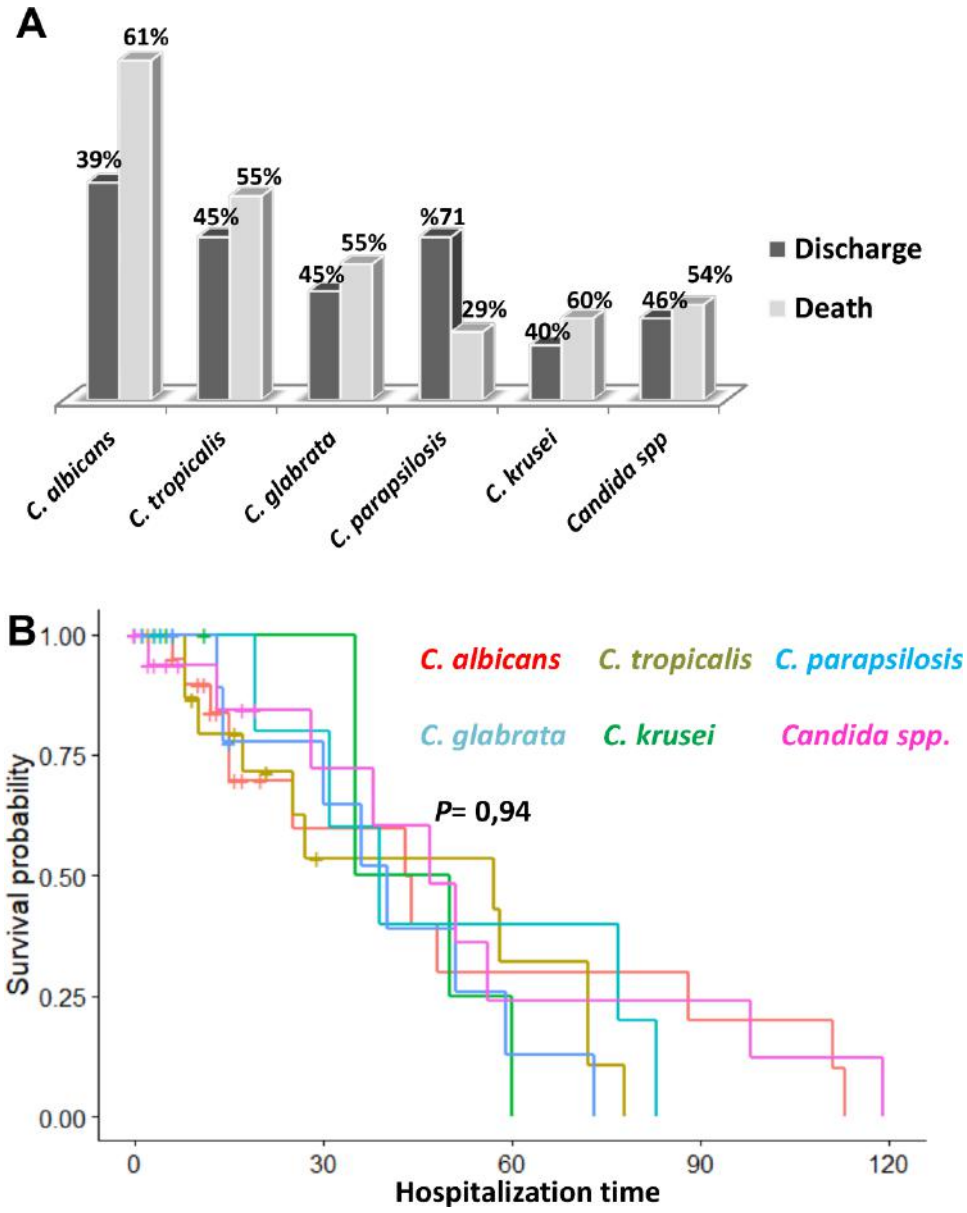
Note: [a] Geometric mean; MIC50: concentration of antifungal that inhibits the growth of 50% of the isolates. MIC90: concentration of antifungal that inhibits the growth of 90% of the isolates. *Other species: *Candida kefyr* (3), *Candida lusitaniae* (3), *Candida guilliermondii* (2), *Candida orthopsilosis* (2), *Candida peliculosa* (2), *Candida dubliniensis* (1). Source: Authors.

Most of the patients were male (65%; 74/113), and the most affected were those over the age of 60 (45%; 41/113) for both genders. The infection occurred on average after 23 days of hospitalization, and most patients presented candidemia after 8 days of using indwelling medical devices. The hospital sectors with the highest number of infections by *Candida* spp. were the ICUs (51%; 57/113), followed by the clinical (25%; 28/113) and surgical (24%; 27/113) units. In addition, the average length of stay in the ICU was 47 days (range 5 to 127 days). The most frequent comorbidities and invasive devices were previous abdominal surgery (38%), neoplasia (27%) and systemic arterial hypertension (20%), presence of CVC (90%), probe in the digestive tract (73%), bladder catheter (64%), mechanical ventilation (57%), prolonged parenteral nutrition (55%), previous use of antimicrobials (96%), and digestive tract surgery (44%).

Antifungal treatment was empirically performed in 85% (96/113) of the patients, preferably with fluconazole (800 mg in loading dose; after 400 mg/day) as monotherapy (66%; 63/96). Echinocandins were used to treat 37% of patients, mainly for those who had infection caused by *C. krusei* and *C. glabrata* infection, 22% of them in monotherapy, and 16% in combination with fluconazole. The length of antifungal treatment was 12 days (mean). In addition, 15% (17/113) of the patients did not receive any type of treatment, since most of them died before the laboratory diagnosis of the infection.

The distribution of *Candida* species and the outcome of the disease were also studied; among the patients who died, *C. albicans* was the predominant species (61%). It was also found that those who had infection with *C. krusei* more rapidly progressed to death than other patients; however, there was no statistical difference in patient survival according to *Candida* species (Figure 1).

Figure 1. Distribution of *Candida* spp. in relation to death (**A**) and Kaplan-Meier curve in relation to the death/discharge groups, considering the length of hospital stay (**B**).



Note: *Candida* spp.: *C. kefyr*; *C. orthopsilosis*; *C. lusitaniae*; *C. guilliermondii*; *C. pelliculosa*; *C. dubliniesis*. Source: Authors.

The 30-day mortality rate due to candidemia was 56%, and age proved to be an independent risk factor (p=0.0195). The univariate analysis of risk factors associated with mortality showed three significant variables: age over 60 years (p=0.0355), admission to the ICU (p=0.0493) and the presence of the bladder catheter (p=0.0078). On the other hand, antifungal treatment lasting more than 14 days was a protective factor for patients' survival (p=0.0000). Table 2 summarizes the main clinical and epidemiological characteristics of patients affected by the disease.

Table 2: Clinical and epidemiological characteristics of patients with candidemia.

Features	Total (n= 113) n; (%)	Outcome		Statistical analysis			
				Univariate		Multivariate	
		DISCHARGE (n= 50) n; (%)	DEATH (n= 63) n; (%)	OR (IC 95%)	*p* value	OR (IC 95%)	*p* value
Gender	39; (35)	18; (36)	21; (33,3)	0,89 (0,41-1,94)	0,7672		
Age: mean (SD)	50,7	42,82 (24,58)	57,10 (21,62)	1,03 (1,01-1,05)	0,0025*	1,03 (1,0-1,10)	0,0195*
0-18 years	16 (14)	10 (20)	6 (9,5)	0,42 (0,14-1,25)	0,1198		
19-59 years	46 (41)	23 (46)	23 (36,5)	0,68 (0,32-1,44)	0,3086		
>60 years	51 (45)	17 (34)	34 (54)	2,28 (1,06-4,90)	0,0355*		
Hospital length of stay (all patients): mean (SD)	51,9	75,16 (37,36)	32,95 (26,98)	0,96 (0,94-0,98)	0,0000		
Patients addmited in ICU	57 (50)	20 (40)	37 (58,7)	2,13 (1,02-4,54)	0,0493*		
ICU length of stay (mean of days)	47,15	31,58 (44,51)	17,6 (21,31)	0,99 (0,97-0,99)	0,0352		
Comorbidities							
HIV/AIDS	1 (0,8)	1 (2,4)	0	1,00 (-)	0,9913		
Chronic kidney failure	3 (2,6)	1 (2,4)	2 (3,4)	1,40 (0,12-16,7)	0,7849		
Digestive tract alteration (non-surgical)	4 (3,5)	2 (4,9)	2 (3,4)	0,68 (0,09-5,07)	0,7102		
Systemic arterial hypertension	23 (20)	8 (19,5)	15 (25,4)	1,41 (0,53-3,51)	0,4907		
Diabetes Mellitus	11 (10)	4 (9,8)	7 (11,9)	1,25 (0,34-4,56)	0,7407		
Heart disease	12 (11)	4 (9,8)	8 (13,6)	1,45 (0,41-5,18)	0,5665		
Neoplasia	31 (27)	11 (26,8)	20 (33,9)	1,40 (0,58-3,36)	0,4530		
Previous abdominal surgery	43 (38)	14 (34,1)	29 (49,2)	1,86 (8,82-4,25)	0,1379		
Chronic medication							
Immunosuppressant	6 (5)	3 (13,6)	3 (7,1)	0,49 (0,09-2,64)	0,4047		
Chemotherapy	6 (5)	2 (9,1)	4 (9,5)	1,05 (0,18-6,25)	0,9550		
Invasive devices							
Central venous catheter	102 (90)	46 (92)	56 (88,9)	0,70 (0,19-2,52)	0,5810		
Bladder catheter	72 (64)	25 (50)	47 (74,6)	2,94 (1,33-6,49)	0,0078*		
Mechanical Ventilation	65 (57)	25 (50)	40 (63,4)	1,39 (0,57-3,39)	0,4741		
Digestive Tract Probe (NG / NE)	83 (73)	35 (70)	48 (76,1)	0,59 (0,14-2,43)	0,4635		
Digestive Tract Ostomy	10 (9)	3 (6)	7 (11,1)	1,70 (0,41-7,05)	0,4635		
Parenteral nutrition	62 (55)	25 (50)	37 (58,7)	1,42 (0,67-3,31)	0,3550		
Surgical procedures during hospitalization							
Digestive Tract Surgery	51 (44)	19 (38)	31 (49,2)	1,58 (0,74-3,36)	0,2347		
Urinary Tract Surgery	11 (10)	5 (10)	6 (9,5)	0,95 (0,27-3,31)	0,9324		
Antifungal treatment	96 (85)	49 (98)	47 (74,6)	0,06 (0,01-0,47)	0,0074		
Time of treatment- mean (SD)	11,8 (5,97)	15 (4,44)	6,11 (6,27)	0,75 (0,67-0,83)	0,0000		
Treatment $\geq$ 14 days	54 (56)	45 (90)	9 (14,2)	0,02 (0,01-0,08)	0,0000	0,01 (0,0-0,06)*	0,0000*
Onset time $\leq$ 48 hours after diagnosis of infection	92 (96)	47 (94)	45 (71,4)	1,91 (0,17-21,8)	0,6010		
Use of antimicrobials prior to infection							
Antibiotic	109 (96)	47 (94)	62 (98,4)	3,96 (0,4-39,26)	0,2400		
Antibiotic use time - mean (SD)	13,31 (8,29)	13,83 (8,68)	12,92 (7,94)	0,99 (0,94-1,03)	0,5634		
Antibiotic use $\geq$ 14 days	52 (48)	24 (48)	28 (44,4)	0,85 (0,40-1,81)	0,6707		
Antifungal	14 (12)	7 (14)	7 (11,1)	0,77 (0,25-2,35)	0,6440		
Recurrence		2 (4)	2 (3,2)	0,80 (0,11-5,89)	0,8266		

Note: OR: Odds Ratio; SD: Standard deviation; NG: Nasogastric; NE: Nasoenteric; *: Statistical significance. Source: Authors.

Candidemia is the fourth leading cause of nosocomial bloodstream infection (Wan Ismail, et al., 2020), generally associated with longer hospitalization, high medical costs and with mortality rates ranging from 19.6 to 67% of cases (Zhang, et al., 2019).

Candida albicans was isolated in 30% of the cases; 70% of candidemia episodes were due to CNA, mainly to *C. tropicalis*, *C. glabrata*, *C. parapsilosis* sensu stricto and *C. krusei*. These data agree with the international (Al-Dorzi, et al., 2020) (Bassetti, et al., 2019) (Pfaller, et al., 2019) (Poissy, et al., 2020) (Tsay, et al., 2020), and with Brazilian literature (Doi, et al., 2016) (Khouri, et al., 2016) (Marins, et al., 2018) (Medeiros, et al., 2019). Regional differences, as well as the

complexity of each hospital service, can influence the predominance of *Candida* species (Menezes, et al., 2015). Studies indicate that *C. albicans* is one of the species most associated with mortality (Hirayama, et al., 2020) (Kadosh & Mundodi, 2020); however, there was no statistical difference regarding mortality and the *Candida* species evaluated in this study. It is important to highlight that those patients who had infection due to *C. krusei* died earlier than those who had infection with other species. According to Kronen et al. (2018), patients with infection from *C. krusei* have a higher mortality rate when compared to patients with infection by other CNA, although this may be a greater reflection of the severity of the underlying disease of these patients than being due to microorganism virulence. Studies of systemic candidiasis in murines corroborates this, which have shown that *C. krusei* exhibits less virulence than *C. albicans* (Gómez-Gaviria & Mora-Montes, 2020).

Candida spp. are routinely identified via traditional phenotypic tests that may not correctly recognize their many species (Ambaraghassi, et al., 2019). MALDI-TOF/MS presents 100% accuracy for yeast identification, becoming a new gold standard which is fast and safe for identifying *Candida* species (Xie, et al., 2019). The present study showed an almost perfect correlation between MALDI-TOF/MS and VITEK®-2; however, a misidentification was found in three isolates classified as *C. famata* via VITEK®-2 and the two isolates belonging to the "*psilosis*" complex. Karapetsa et al. (2019) related that sepsis caused by *C. famata* is a rare event in immunocompetent patients and some authors have questioned if this species can really be considered a pathogen (Toubas & Depaquit, 2012). According to Ghaith et al. (2021), *C. famata* can be associated with infections in humans and the incorrect identification of this species could lead to a misdiagnosis and misinterpretation of antifungal susceptibility, bringing harm to the prognosis and treatment of patients. In addition, they claim that traditional methods are not effective for identifying this species, and MALDI-TOF/MS is the ideal tool for this purpose. Huang et al. (2021) also described that the use of conventional methods for identifying *Candida* spp. can result in high misidentification rates of unusual species, such as *C. famata*. Consequently, inconsistency in phenotypic tests or the presence of unusual *Candida* species must be confirmed by more sensitive and specific methods, such as MALDI-TOF/MS (Kim, et al., 2014). In our study, it is important to highlight that although the discrepancy in identifying the three isolates of *C. famata* was verified, these patients did not present a worse clinical evolution. It is also described that biochemical tests do not have the ability to distinguish cryptic species from the "*psilosis*" complex; therefore, these yeasts are correctly identified only via molecular and proteomic techniques (De Carolis, et al., 2014).

Broth microdilution is the reference methodology for antifungal susceptibility testing (CLSI, 2017); however, it has no routine applicability in most clinical microbiology laboratories, as it is extremely laborious (Pfaller, et al., 2013). Therefore, commercial tests, such as the semi-automatic system VITEK®-2 (bioMérieux, France), can be used to predict the antifungal susceptibility of *Candida* spp. (da Matta, et al., 2017). In this study, excluding the cases of intrinsic resistance of *C. krusei* to fluconazole, it was possible to only observe a small percentage of isolates (5%) resistant to antifungal agents tested, especially *C. krusei* and *C. glabrata*. These data are consistent with international surveillance studies (Pfaller, et al., 2019). Resistance to antifungals available for treating candidemia can still be considered an uncommon event for *Candida* spp.; however, resistant microorganisms have been reported. In this context, antifungal resistance should be monitored given that infections caused by resistant microorganisms can lead to higher mortality from candidemia (da Matta, et al., 2017) (Hart, et al., 2019).

Most of the patients affected by the disease were male and aged over 60 years, which is consistent with the literature data (Poissy, et al., 2020) (Toda, et al., 2019). Although the difference in candidemia incidence by gender has not yet been evidenced (Poissy, et al., 2020), Arroyo-Mendonza et al. (2020) suggested that testosterone may reduce resistance to systemic infection from *C. albicans*; a fact which may explain the higher incidence of the disease in male patients.

The higher number of cases in the ICU in this study was probably due to the number of risk factors associated with patient care in these units (Papas, et al., 2018). The breaking of natural host defenses (surgical manipulation of the digestive

tract, multiple invasive devices, and use of immunosuppressive drugs, prolonged parenteral nutrition) provides translocation of fungal skin, mucous membranes or intestine into the bloodstream (Poissy, et al., 2020), and if the immune response is compromised, yeast proliferates, leading to candidemia (Zhai, et al., 2020).

The therapeutic management of candidemia should be initially treated with echinocandins (Bienvenu, et al., 2020) (Roilides, et al., 2019) (Roilides, et al., 2020) (Tashiro, et al., 2020). Herein, 85% of patients received antifungal treatment, in most cases with fluconazole, mainly in monotherapy. Most patients who did not receive the treatment died before the laboratory diagnosis of infection. The administration of echinocandins as the first choice therapy was performed in only 20% of patients, probably due to the high cost of these when compared to the cost of azole derivatives (Choi, et al., 2019) (Marins, et al., 2018). According to Colombo et al. (2013), fluconazole can be an alternative therapy for clinically stable patients with mild conditions who have not been exposed to prophylaxis regimens with azoles and who are admitted to medical services with a low incidence of infections caused by *C. glabrata* and *C. krusei.* However, medical services that have an incidence rate greater than 10% of isolates resistant to fluconazole should not use this medication before identification of the *Candida* species (Colombo, et al., 2013).

Mortality rate due to candidemia was 56%, with age over 60 years, presence of a bladder catheter, and admission to the ICU constituting the risk factors associated with a worse clinical outcome 30 days after the diagnosis of the infectious process. Studies have shown that this infection can have varying mortality rates, from 12% to 70% (Bassetti, et al., 2019) (Medeiros, et al., 2019) (Pappas, et al., 2018) (Tsay, et al., 2020), which reflects the severity of cases of this disease. Studies have indicated high mortality rates in Brazil due to candidemia, from 43% to 72.2% (Alves, et al., 2020) (Doi, et al., 2016) (Khouri, et al., 2016). It is important to note that 26% (17/63) of the patients who died in the present study did not receive antifungal treatment, since the death occurred before the infection diagnosis. Therefore, early diagnosis and treatment are essential for managing the disease, since there is a higher mortality rate when the treatment is not done properly (Pappas, et al., 2018).

This study has some limitations, since the data were collected in a relatively short period of time (2 years) and correspond to the reality of candidemia cases in only one University Hospital among many others in Brazil. However, clinical-epidemiological studies can facilitate adopting preventive measures, especially in relation to invasive procedures and indwelling devices, thus contributing to reduce the incidence of the disease. Knowing that the bloodstream infection by *Candida* spp. is common in tertiary-level hospitals, the correct speciation of these yeasts, early introduction of antifungal treatment and monitoring of antifungal resistance should all be encouraged in order to reduce the morbidity and mortality of candidemia.

4. Conclusion

MALDI-TOF/MS is a methodology option that presents precision for the correct identification of *Candida* spp. isolates, especially when unusual *Candida* species, such as *C. famata*, are identified via traditional methodologies. Antifungal susceptibility tests are necessary to identify resistant isolates. Furthermore, these tests must be carried out in order to introduce adequate antifungal therapy and to detect possible hospital outbreaks. This study has evidenced the severity of candidemia, a disease which presents high mortality. Thus, the correct identification of the species involved and the institution of appropriate and timely drug therapy treatments are fundamental for the correct management of the disease, contributing to increased patient survival.

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