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Original Article

Antifungal susceptibility and virulence factors of *Candida* species isolated in patients from Porto Velho, Rondônia, Brazilian Amazon

Suscetibilidade a antifúngicos e fatores de virulência de espécies de *Candida* isoladas em pacientes da Porto Velho, Rondônia, Amazônia Brasileira

C. B. Lovo[a], I. V. G. Ramos[b], L. D. Silva[b], N. C. Lopes[b], R. R. Durlacher[c,d], R. S. Rodrigues[c,e], C. C. Khuen[a], E. B. A. Souza[b,c], C. M. Carvalho[f] and N. B. Matos[a,b*]

[a]Universidade Federal de Rondônia – UNIR, Programa de Pós-graduação em Biologia Experimental – PGBIOEXP, Porto Velho, RO, Brasil
[b]Fundação Oswaldo Cruz Rondônia – FIOCRUZ, Departamento de Microbiologia, Porto Velho, RO, Brasil
[c]Centro de Pesquisa em Medicina Tropical – CEPEM, Porto Velho, RO, Brasil
[d]Centro de Medicina Tropical de Rondônia – CEMETRON, Porto Velho, RO, Brasil
[e]Instituto Oswaldo Cruz – IOC, Programa de Pós-graduação em Biologia Celular e Molecular - PGBCM, Rio de Janeiro, RJ, Brasil
[f]Universidade Federal do Acre – UFAC, Rio Branco, AC, Brasil

Abstract

Candida species normally colonize the human body in a commensal manner, however, under conditions of immunosuppression or host imbalance, they can cause opportunistic infections, including systemic forms, which are frequently associated with high mortality rates. This study evaluated the antifungal susceptibility profiles and virulence factors of *Candida* species isolated from patients in Porto Velho, Rondônia, Brazilian Amazon. A total of 31 isolates collected between April 2021 and March 2022 at the Medical Mycology Laboratory of the Tropical Medicine Research Center of Rondônia were analyzed. Identification was done by cultivation on CHROMagar and sequencing of the ITS region of rDNA. Susceptibility testing was conducted by the disk diffusion method using fluconazole, itraconazole, miconazole, amphotericin B, and nystatin. Hemolysin and phospholipase activities were also assessed. Most isolates were *C. albicans* (71%), followed by *C. tropicalis* (19.4%), *C. parapsilosis*, *C. duobushaemulonii*, and *Cyberlindnera jadinii* (3.2% each). The majority of samples came from sputum (64.5%), followed by skin scales (19.4%) and nails (9.7%). Candidiasis was more frequent in males (51.6%), especially between 40 and 59 years of age, with risk factors including comorbidities (71%), antibiotic use (61.2%), and invasive devices (58%). High resistance rates were observed to fluconazole (86.7%) and itraconazole (80%), with lower resistance to amphotericin B (13.3%) and nystatin (10%). *C. tropicalis* and *C. parapsilosis* exhibited concerning resistance profiles. All isolates produced hemolysin, while phospholipase activity was detected in *C. albicans* and *C. jadinii*. The associated mortality rate was 13%. These data emphasize the importance of local epidemiological surveillance to guide clinical management and prevention of candidiasis in the Amazon region.

Keywords: *Candida albicans*, fluconazole, phospholipase, Rondônia.

Resumo

Espécies do gênero *Candida* colonizam normalmente o organismo humano de forma comensal, mas, em situações de imunossupressão ou desequilíbrio do hospedeiro, podem causar infecções oportunistas, inclusive sistêmicas, associadas a altas taxas de mortalidade. Este estudo avaliou o perfil de suscetibilidade antifúngica e fatores de virulência de *Candida* isoladas de pacientes em Porto Velho, Rondônia, Amazônia Brasileira. Foram analisados 31 isolados coletados entre abril/2021 e março/2022 no Laboratório de Micologia Médica do Centro de Pesquisa em Medicina Tropical de Rondônia. A identificação utilizou cultivo em CHROMagar e sequenciamento da região ITS do rDNA. A suscetibilidade foi testada pelo método de difusão em disco para fluconazol, itraconazol, miconazol, anfotericina B e nistatina. Também foram avaliadas as atividades de hemolisina e fosfolipase. A maioria dos isolados foi de *C. albicans* (71%), seguida de *C. tropicalis* (19,4%), *C. parapsilosis*, *C. duobushaemulonii* e *Cyberlindnera jadinii* (3,2% cada). A maior parte das amostras veio de escarro (64,5%), depois escamas de pele (19,4%) e unhas (9,7%). A candidíase foi mais frequente em homens (51,6%), principalmente entre 40 e 59 anos, com fatores de risco como comorbidades (71%), uso de antibióticos (61,2%) e dispositivos invasivos (58%). Houve alta resistência ao fluconazol (86,7%) e itraconazol (80%), menor à anfotericina B (13,3%) e nistatina (10%). *C. tropicalis* e *C. parapsilosis* apresentaram perfis de resistência preocupantes. Todos os isolados produziram hemolisina, e a fosfolipase foi detectada em *C. albicans* e *C. jadinii*. A mortalidade associada foi de 13%. Esses dados reforçam a importância da vigilância epidemiológica local para orientar manejo clínico e prevenção da candidíase na Amazônia.

Palavras-chave: *Candida albicans*, fluconazol, fosfolipase, Rondônia.

*e-mail: najla.matos@fiocruz.br
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1. Introduction

Candida spp. colonize the human body as commensals. However, under certain host conditions, these fungi can behave as opportunistic pathogens, causing superficial mycoses or systemic infections, either acute or chronic (Rosa et al., 2009; Kullberg and Arendrup, 2015; Mba and Nweze, 2020). The development of the disease is associated with factors inherent to the microorganism as well as predisposing conditions of the host (Fisher et al., 2022). Factors such as advanced age, pregnancy, diabetes, malnutrition, immunodeficiencies, prolonged hospital stays, use of broad-spectrum antibiotics, and invasive medical devices favor the onset of infection (Pappas et al., 2003; Kullberg and Arendrup, 2015). Additionally, the production of extracellular enzymes such as phospholipase and hemolysin plays a crucial role in host cell adhesion and tissue invasion, contributing to pathogenesis (Canela et al., 2017).

Approximately 20 *Candida* species are recognized as causative agents of infections in humans, accounting for about 80% of systemic fungal infections in hospital settings (Dignani et al., 2003; Pfaller and Diekema, 2007). In Brazil, *Candida* infections represent the seventh leading cause of bloodstream infections in hospitalized patients, with candidemia incidence rates reaching 2.49 cases per 1,000 admissions in tertiary public hospitals (Colombo et al., 2006; Doi et al., 2016). Regarding mortality, multicenter studies in the country report rates higher than those observed in Europe and North America, exceeding 50% (Colombo et al., 2006; Doi et al., 2016).

The treatment of systemic candidiasis is essentially limited to polyenes, azoles, and echinocandins (Bhattacharjee, 2016). However, there is a progressive increase in azole resistance, both among *Candida* non-*albicans* species and in *C. albicans* itself, which makes the management of these infections an increasing challenge and contributes to higher mortality rates (Canela et al., 2017). According to the Centers for Disease Control and Prevention (CDC, 2022), infections caused by antifungal-resistant *Candida* species increased by 26% in 2020, possibly due to factors associated with the COVID-19 pandemic.

Despite invasive candidiasis being a growing problem in hospitals worldwide, studies addressing its incidence in Brazil are still scarce. The available research is mostly concentrated in the Northeast, Central-West, and Southeast regions of the country, with a notable lack of consolidated data regarding the occurrence of the disease in the Northern region.

This study aimed to describe the clinical-epidemiological characteristics, risk factors, species distribution, antifungal susceptibility, and virulence factors of *Candida* species isolated from patients with infections in Porto Velho, Rondônia, Brazil.

2. Material and Methods

2.1. Study design

The isolates analyzed in this study were obtained from patients treated at the Medical Mycology Laboratory of the Rondônia Tropical Medicine Research Center, between April 2021 and March 2022. This laboratory routinely receives samples from patients referred by public and private hospitals in Porto Velho, Rondônia, Brazil. All patients who had a positive diagnosis for candidiasis, confirmed by direct mycological examination, and who agreed to participate in the study were included. Samples that, despite being positive in the direct examination, did not show fungal growth in culture, as well as those with growth associated with contaminants, were excluded.

2.2. Yeast collection and isolation

The isolates analyzed in this study were obtained from skin and respiratory samples. Nail and skin scale samples were collected by scraping with a sterile scalpel or using a sterile swab moistened with saline solution (Lacaz and Porto, 2002). Tracheal aspirate, bronchoalveolar lavage and sputum samples were sent to the Medical Mycology Laboratory exclusively for microbiological analysis. All samples were subjected to direct mycological examination using 20% potassium hydroxide (KOH) solution and subsequently cultured on Sabouraud Dextrose Agar supplemented with chloramphenicol (100 mg/L). After fungal isolation and confirmation of the candidiasis diagnosis, the yeast isolates were cryopreserved at -80°C in Luria Bertani (LB) broth supplemented with 50% glycerol.

2.3. Clinical and epidemiological information

Clinical data from patients diagnosed with candidiasis were obtained through the review of medical records. Collected information included age, sex, comorbidities, risk factors, hospital unit of admission, and clinical outcome. Additionally, the anatomical origin of the biological samples from the isolates was recorded.

2.4. Species characterization

The isolates were initially identified using the selective medium CHROMagar Candida® (Difco). Molecular characterization was done using an approach based on polymerase chain reaction (PCR) amplification of the fungal rDNA ITS region, followed by genetic sequencing. Genomic DNA extractions were done using the DNeasy Ultra Clean Microbial Kit (Qiagen), according to the manufacturer's instructions.

The amplification of the ITS region of the rDNA was conducted in a 50 µL reaction mixture containing 4 µL of template DNA (1–20 ng/µL), 2.5 µL of each primer (0.5 mM) — ITS1 (5'-CCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), 3 µL of $MgCl_2$ (1.5 mM), 1 µL of dNTPs (0.2 mM), 5 µL of 1x PCR buffer, and 0.5 µL of Taq DNA polymerase (1 U/µL). The reaction was done in a thermocycler (Applied Biosystems, Thermo Fisher Scientific) with an initial denaturation at 95 °C for 2 minutes, followed by 35 amplification cycles (95 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute), and a final extension step at 72 °C for 7 minutes.

The amplified product was visualized on a 1% agarose gel and subjected to electrophoresis at 100 volts for 30 minutes, using the 100 bp ladder molecular weight marker (Synapse). PCR products approximately 600 to 700 bp in length were purified using the EasyPure® PCR Purification

Kit (Transgenbiotech). Sequencing was done on the Fiocruz Bahia platform, using the ABI 3100 Prism sequencer, series 1592-015 (Applied Biosystems).

Forward and reverse sequences were analyzed, and a consensus sequence was generated using BioEdit Sequence Alignment Editor software (version 7.0) with default settings. Sequences with a Phred quality score equal to or greater than 20 were considered valid, ensuring 99% confidence in base identification (Q20). Consensus reads with a threshold frequency above 95% were submitted to the BLAST algorithm and aligned against the database using the standard MEGABLAST configuration. *Candida* species were identified by sequence similarity, considering consensus sequences with identity equal to or greater than 99% and 100% coverage as valid.

Phylogenetic relationships were inferred using the Neighbor-Joining method (Saitou and Nei, 1987), and evolutionary distances were calculated by the Maximum Composite Likelihood method (Tamura et al., 2004). Evolutionary analyses were performed using MEGA11 software (Tamura et al., 2021). The reference sequences used were *C. albicans* NR125332.1 and *C. tropicalis* NR111250.1.

2.5. Antifungal susceptibility assays

Antifungal susceptibility was evaluated using the disk diffusion method, following the M44-P protocol (CLSI, 2009). All isolates were tested for susceptibility to fluconazole (25 µg), itraconazole (10 µg), miconazole (50 µg), amphotericin B (100 µg), and nystatin (100 IU), except for *C. duobushaemulonii*, which did not grow in this assay. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as quality control strains. The results were classified as susceptible (S), intermediate (I), or resistant (R).

2.6. Determination of hemolysin activity

Hemolytic activity was evaluated using an assay on sheep blood agar plates, as described by Mane et al. (2012), with readings taken after 48 hours of incubation. Results were expressed as the ratio between the diameter of the colony and the diameter of the translucent hemolysis halo (in mm). Pz values were classified as follows: Pz ≥ 1, negative activity; Pz < 0.99, positive activity; and Pz ≤ 0.64, strongly positive activity (Canela et al., 2017). Standard strains *C. albicans* ATCC 14053 and *C. parapsilosis* ATCC 22019 were used as positive and negative controls, respectively. The assay was done in triplicate and repeated at two independent time points (Canela et al., 2017).

2.7. Determination of phospholipase activity

Phospholipase activity was evaluated using the plate method containing egg yolk agar (Mane et al., 2012). The standard strains *C. albicans* ATCC 14053 and *C. parapsilosis* ATCC 22019 were used as positive and negative controls, respectively. The test was done in triplicate at two independent time points (Mane et al., 2012). The plates were incubated for five days, and phospholipase activity (Pz) was determined by the ratio between the colony diameter and the total diameter of the precipitation zone, with values classified as follows: Pz ≥ 1, negative activity; Pz < 0.99, positive activity; and Pz ≤ 0.64, strongly positive activity (Canela et al., 2017).

2.8. Statistical analyzes

The results were analyzed using Fisher's exact test or the chi-square test, with GraphPad Prism software version 8.0. p-values ≤ 0.05 were considered statistically significant.

2.9. Ethical aspects

This study was approved by the Research Ethics Committee of the Rondônia Tropical Medicine Research Center, under approval number 3.655.640. The authors declare that all procedures done are in accordance with the current ethical standards and the guidelines established by the competent authorities.

3. Results

During the study, a total of 31 clinical isolates of *Candida* spp. were analyzed, obtained from patients attended at the Medical Mycology Laboratory of the Tropical Medicine Research Center of Rondônia, Brazil. The most prevalent species was *Candida albicans* (71.0%), followed by *C. tropicalis* (19.4%), *C. parapsilosis* (3.2%), *C. duobushaemulonii* (3.2%), and *Cyberlindnera jadinii* (3.2%). Three isolates, initially identified on CHROMagar Candida medium as *C. albicans*, *C. tropicalis*, and *Candida* sp., were subsequently reclassified by molecular analysis as *C. parapsilosis*, *C. duobushaemulonii*, and *Cyberlindnera jadinii*, respectively. The phylogenetic analysis of the isolates is presented in Figure 1.

Sputum samples accounted for the majority of clinical specimens from which the isolates were obtained (64.5%), with a statistically significant difference ($p < 0.0001$), followed by skin scale samples (19.4%), nail scale samples (9.7%), bronchoalveolar lavage (3.2%), and tracheal aspirate (3.2%).

Candida albicans isolates were predominantly recovered from sputum samples (77.2%), followed by skin scales (18.2%) and tracheal aspirate (4.6%). *C. tropicalis* isolates were obtained from sputum (33.3%), skin scales (33.3%), nail scales (16.7%), and bronchoalveolar lavage fluid (16.7%). Additionally, *C. parapsilosis* (100%) and *C. duobushaemulonii* (100%) isolates were exclusively recovered from nail scale samples, while the *Cyberlindnera jadinii* isolate was obtained solely from a sputum sample (100%). The distribution of species according to the different types of biological material is shown in Table 1.

The demographic characteristics of the patients, the hospital care units, risk factors, and clinical outcomes are presented in Table 2. Candidiasis predominantly affected males (51.6%) compared to females (48.4%). Patients' ages ranged from 29 to 94 years, with the highest incidence observed in the 40 to 59 age group (51.6%). Most patients required hospitalization (32.2%) or admission to the intensive care unit (29.3%).

Most patients presented one or more risk factors associated with the development of candidiasis, with comorbidities being the most frequent cause (71.0%),

Table 1. Distribution of *Candida* species according to biological material of origin.

Candida species	n	Clinical specimen				
		AT	ES	NS	Sputum	BL
Candida albicans	22	1 (4.6%)	4 (18.2%)	0 (0.0%)	17 (77.2%)	0 (0.0%)
Candida tropicalis	6	0 (0.0%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	1 (16.7%)
Candida parapsilosis	1	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
Candida duobushaemulonii	1	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)
Cyberlindnera jadinii	1	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)
Total	31	1 (3.2%)	6 (19.4%)	3 (9.7%)	20 (64.5%) *	1 (3.2%)

AT: tracheal aspirate; ES: epidermal scales; NS: nail scales; BL: bronchoalveolar lavage.

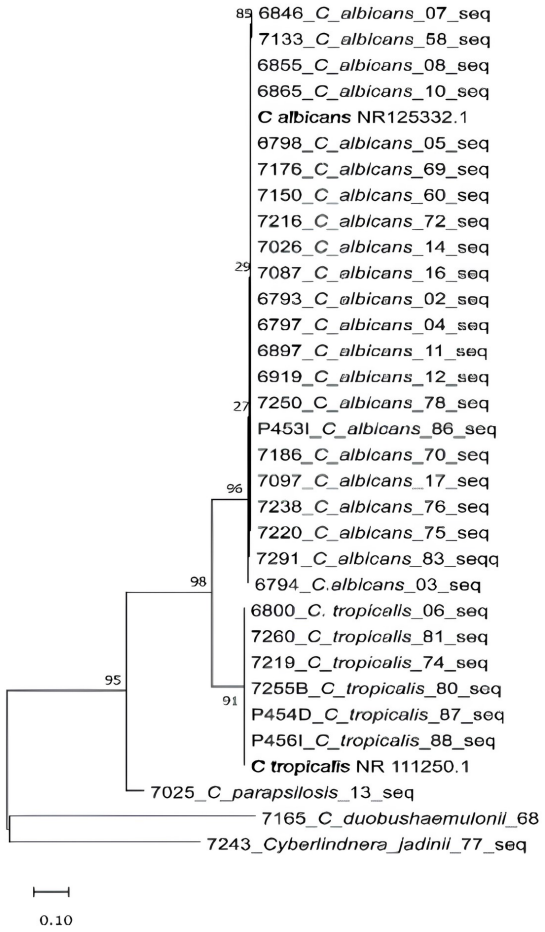

Figure 1. Phylogenetic tree based on ITS sequences demonstrating the clustering of 31 clinical isolates of *Candida* spp.

Table 2. Demographic characteristics, hospital unit, risk factors and outcome of cases diagnosed with candidiasis at the Rondônia Tropical Medicine Research Center, Rondônia, Brazil.

Variable	n	%
Sex		
F	15	48.4%
M	16	51.6%
Age		
0-19	0	0.0%
20-39	7	22.5%
40-59	16	51.6%
60-79	6	19.4%
80-99	2	6.4%
Hospital unit		
Ambulatory	12	38.5%
Hospital internment	10	32.2%
ICU	9	29.3%
Risk factors		
Underlying diseases	22	71.0%
ICU	9	29.3%
Antibiotic therapy	19	61.2%
Use of catheter	18	58.0%
Outcome of cases		
Hospital discharge	17	54.8%
Unit Evasion/Transfer	10	32.2%
Death	4	13.0%

F: female; M: male; ICU: intensive care unit.

followed by antibiotic use (61.2%), catheter use (58.0%), and admission to the intensive care unit (29.3%).

Pulmonary diseases were the most prevalent comorbidities (48.4%), followed by COVID-19 (19.3%), kidney diseases (19.3%), HIV (9.7%), cardiovascular diseases (9.7%), substance abuse (9.7%), American tegumentary leishmaniasis (9.7%), diabetes mellitus (6.5%), and pregnancy (6.5%). A smaller number of patients presented less frequent conditions such as histoplasmosis (3.2%), brucellosis (3.2%), systemic lupus erythematosus (3.2%), malnutrition (3.2%), syphilis (3.2%), and a history of transplantation (3.2%).

Regarding invasive devices, most patients required peripheral venous access (58.0%), followed by indwelling urinary catheter (32.2%), nasogastric tube (32.2%), central venous access (22.5%), orotracheal intubation (16.1%), tracheostomy (12.9%), and Shilley catheter (9.6%).

As for clinical outcomes, most patients were discharged from the hospital (54.8%), one patient was transferred to another unit, nine patients abandoned follow-up care (32.2%), and four patients died (13.0%).

The *Candida duobushaemulonii* isolate tested negative for phospholipase production and did not grow in antifungal susceptibility assays nor in hemolysin activity determination. For this reason, it was excluded from the statistical analyses of the performed tests.

The results of the *in vitro* antifungal susceptibility tests are shown in Table 3. Of the 30 *Candida* spp. isolates evaluated, most demonstrated resistance to fluconazole (86.7%) and itraconazole (80.0%), while a smaller proportion showed resistance to amphotericin B (13.3%) and nystatin (10.0%).

The majority of *Candida albicans* isolates showed susceptibility to amphotericin B (95.5%) and nystatin (100%). Regarding miconazole, all *C. albicans* isolates exhibited intermediate susceptibility. Additionally, these isolates demonstrated high resistance rates to fluconazole (81.8%) and itraconazole (77.3%). All *Candida tropicalis* isolates were resistant to fluconazole and itraconazole (100%). Resistance to amphotericin B (33.3%) and nystatin (33.3%) was also observed in this species. The *C. parapsilosis* isolate showed complete resistance (100%) to miconazole, amphotericin B, and nystatin, with statistically significant results for amphotericin B ($p < 0.0095$) and nystatin ($p < 0.0023$). The *Cyberlindnera jadinii* isolate exhibited complete resistance (100%) to miconazole, fluconazole, and itraconazole.

The results of the virulence factor assays are presented in Table 4. Hemolytic activity was detected in all isolates analyzed. However, although all species produced hemolysin, the activity levels in *C. albicans*, *C. tropicalis*, and *Cyberlindnera jadinii* were relatively higher compared to *C. parapsilosis*. Phospholipase activity was detected only in *C. albicans* and *Cyberlindnera jadinii* isolates, with no statistically significant difference between them.

4. Discussion

Opportunistic fungal infections range from superficial to systemic and predominantly affect immunocompromised patients, often resulting in high mortality rates (Bongomin et al., 2017). The majority of candidiasis cases are caused by *Candida albicans* (Sudbery, 2011; Pappas et al., 2018; Bouglita et al., 2022). However, in recent years, infections caused by *C.* non-*albicans* species have been on the rise and may collectively account for up to 50% of candidiasis cases (Chen et al., 2021).

Despite the observed trend toward increasing infections by *C.* non-*albicans* species (Odds et al., 2007; Horn et al., 2009), the distribution of *Candida* species varies widely across studies, largely depending on factors such as hospital settings, geographic location, and the clinical profiles of patients. Consequently, prevalence rates differ according to the specific context of each study (Canela et al., 2017). This variability underscores the critical need for localized research to better guide treatment strategies for candidiasis.

Table 3. Antifungal susceptibility of *Candida* spp. fluconazole, itraconazole, miconazole, amphotericin B and nystatin.

Antifungal		*Candida* species				
		Candida albicans **(n = 22)**	***Candida tropicalis*** **(n = 6)**	***Candida parapsilosis*** **(n = 1)**	***Cyberlindnera jadinii*** **(n = 1)**	**Total (n = 30)**
MCZ	S	5 (22.3%)	1 (17%)	0 (0.0%)	0 (0.0%)	6 (20.0%)
	I	17 (77.2%)	5 (83%)	1 (100%)	0 (0.0%)	23 (76.7%)
	R	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100%)	1 (3.3%)
FLU	S	3 (13.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)
	I	1 (4.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.3%)
	R	18 (81.8%)	6 (100%)	1 (100%)	1 (100%)	26 (86.7%)
ICZ	S	1 (4.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (3.3%)
	I	4 (18.2%)	0 (0.0%)	1 (100%)	0 (0.0%)	5 (16.7%)
	R	17 (77.3%)	6 (100%)	0 (0.0%)	1 (100%)	24 (80.0%)
ANF	S	21 (95.5%)	4 (66.7%)	0 (0.0%)	1 (100%)	26 (86.7%)
	I	1 (4.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	0 (0.0%)	2 (33.3%)	1 (100%) *	0 (0.0%)	4 (13.3%)
NYS	S	22 (100%)	4 (66.7%)	0 (0.0%)	1 (100%)	27 (90.0%)
	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	R	0 (0.0%)	2 (33.3%)	1 (100%) *	0 (0.0%)	3 (10.0%)

MCZ: miconazole; FLU: fluconazole; ICZ: itraconazole; ANF: amphotericin B; NYS: nystatin.

Table 4. Virulence in *Candida* spp. isolated from patients seen at the Research Center for Tropical Medicine in Rondônia, Rondônia, Brazil.

***Candida* species**	**Virulence factors**	**Negative**	**Positive**	**Strongly positive**
Total	**Hemolysin**	**0 (0.0%)**	**2 (6.7%)**	**28 (93.3%)**
(n = 30)	**Phospholipase**	**20 (66.7%)**	**8 (26.6%)**	**2 (6.7%)**
Species	**Virulence factors**	**Negative**	**Positive**	**Strongly positive**
Candida albicans	Hemolysin	0 (0.0%)	1 (4.5%)	21 (95.5%)
(n = 22)	Phospholipase	13 (59.1%)	7 (31.8%)	2 (9.1%)
Candida tropicalis	Hemolysin	0 (0.0%)	0 (0.0%)	6 (100%)
(n = 6)	Phospholipase	6 (100%)	0 (0.0%)	0 (0.0%)
Candida parapsilosis	Hemolysin	0 (0.0%)	1 (100%)	0 (0.0%)
(n = 1)	Phospholipase	1 (100%)	0 (0.0%)	0 (0.0%)
Cyberlindnera jadinii	Hemolysin	0 (000%)	0 (0.0%)	1 (100%)
(n = 1)	Phospholipase	0 (0.0%)	1 (100%)	0 (0.0%)

Our study in Rondônia, Brazil, confirmed that *C. albicans* remains the predominant species associated with candidiasis, followed by *C. tropicalis* and *C. parapsilosis*. Similar findings have been reported in other regions of Brazil, where *C. albicans* is consistently identified as the main causative agent (Doi et al., 2016; Braga et al., 2018; Medeiros et al., 2019; Vieira de Melo et al., 2019). Among *C.* non-*albicans* species, *C. tropicalis* and *C. parapsilosis* are commonly isolated in bloodstream infections, with their prevalence varying by geographic region (Hinrichsen et al., 2009; Nucci et al., 2010).

The species *Cyberlindnera jadinii*, also identified in this study, is the teleomorph of *Candida utilis*, a yeast generally considered to have low virulence and regarded as an emerging opportunistic pathogen, occasionally linked to candidiasis development (Gaisne et al., 2018; Mohzari et al., 2021). Despite its typically low virulence, human infections caused by *C. jadinii* have been associated with invasive disease (Gaisne et al., 2018; Mohzari et al., 2021). These infections are usually susceptible to azole antifungals (Sreelekshmi et al., 2021), which contrasts with the resistance patterns observed in the present study.

This study also reported *Candida duobushaemulonii* as a causative agent of candidiasis. This species, along with *C. pseudohaemulonii*, *C. haemulonii*, and *C. vulturna*, forms the *C. haemulonii* complex (Gade et al., 2020). These fungi are phylogenetically related to the multidrug-resistant pathogen *Candida auris* and are frequently misidentified, especially in laboratories lacking genomic sequencing capabilities (Hou et al., 2016).

Since its initial identification in 2012 (Cendejas-Bueno et al., 2012), *C. duobushaemulonii* infections have been reported worldwide, including in Brazil (Ramos et al., 2014; Boatto et al., 2016; Almeida Junior et al., 2016). Recognized as an emerging opportunistic pathogen, *C. duobushaemulonii* has been isolated from both superficial and systemic infections. It warrants special attention due to its typically reduced susceptibility to polyenes, azoles, and echinocandins (Cendejas-Bueno et al., 2012; Frías-De-León et al., 2019).

The incidence of candidiasis can vary significantly across geographic regions and hospital settings, influenced by multiple factors including healthcare practices and patient clinical profiles (Canela et al., 2017). For example, population-specific characteristics, such as the marked increase in acquired immunodeficiency syndrome cases in northern Brazil (BRASIL, 2020), may impact disease incidence, given that immunosuppressive conditions are well-established risk factors for candidiasis development (Pappas et al., 2003).

In this study, the majority of patients presented with underlying comorbidities (71.0%) and required hospitalization (61.5%) either in general wards or intensive care units. Common risk factors contributing to candidiasis include advanced age, broad-spectrum antibiotic use, invasive surgical procedures, medical device utilization, ICU stays, diabetes, and pregnancy. Notably, older patients tend to have an increased risk of mortality associated with candidiasis (Yakut et al., 2021).

Based on the analysis of clinical and epidemiological data, this study found that candidiasis affected men more frequently than women, corroborating findings from previous studies conducted in Brazil (Doi et al., 2016; Oliveira et al., 2021). Additionally, patients aged 40 to 59 years were the most affected, consistent with other reports indicating a mean attributable age range between 40 and 56 years among candidiasis cases (Horn et al., 2009; Doi et al., 2016; Oliveira et al., 2021). Regarding the origin of isolates by clinical specimen type, Magalhães et al. (2015) and Khadka et al. (2017) reported that *Candida* spp. were predominantly recovered from urine and sputum samples, findings similar to the present study, which recovered 64.5% of *Candida* isolates from sputum.

The significant rise in antimicrobial resistance, combined with the notable cytotoxic effects associated with many antifungal agents, has greatly limited their use (Muhaj et al., 2022). Therapeutic failures are often multifactorial, linked to patient-specific factors, microbial characteristics, and the properties of the antifungal agents themselves (Jensen et al., 2015; Fisher et al., 2018; Fisher et al., 2022). Therefore, the selection of antifungal treatment must take into account risk factors, the patient's clinical history, the

infecting species, and the antifungal susceptibility profile (Alcazar-Fuoli and Mellado, 2014).

Consequently, assessing the antifungal susceptibility profile is crucial for monitoring the emergence of resistance and guiding effective therapeutic strategies to successfully restore patients' clinical condition in cases of candidiasis (Canela et al., 2017).

In the present study, the *C. duobushaemulonii* isolate failed to grow on both sheep blood agar and Sabouraud Dextrose Agar after standardization in saline solution, which prevented the determination of its antifungal susceptibility profile. Further investigations are needed to identify the factors contributing to this lack of growth, especially since similar studies have reported successful growth and susceptibility testing under these conditions (Ramos et al., 2018).

The *in vitro* analyses conducted revealed a marked reduction in the efficacy of the tested triazole antifungals against *Candida* spp. isolates, with 86.7% showing resistance to fluconazole and 80.0% to itraconazole. These findings align with previous reports indicating fluconazole resistance in approximately 75% of *Candida* isolates (Bhattacharjee, 2016) and decreased susceptibility to azoles in 57.6% of isolates, particularly among *C.* non-*albicans* species (Mattos et al., 2017).

Candida non-*albicans* species generally exhibit lower susceptibility to azole antifungals (Enoch et al., 2017), with *C. tropicalis* notably demonstrating emerging resistance to this drug class (Wang et al., 2021). For instance, in the United States, while *C. albicans* maintains a low fluconazole resistance rate, *C. tropicalis* shows significantly higher resistance levels (Berkow and Lockhart, 2017). Consistent with these observations, the *C. tropicalis* isolates examined in this study exhibited a clear trend toward resistance to both fluconazole and itraconazole.

Fungi of the genus *Candida* express multiple virulence factors, including morphogenetic transitions, phenotypic switching, and secretion of extracellular enzymes, among other mechanisms (D'Enfert et al., 2021; Lopes and Lionakis, 2022), which synergistically act to promote adhesion, colonization, and tissue invasion during the infectious process. Specifically, during adhesion to epithelial cells and subsequent colonization, the release of hydrolytic enzymes facilitates active penetration into host tissues (Naglik et al., 2011; Canela et al., 2017). Among these enzymes, hemolysin and phospholipase are key hydrolytic proteins involved in extracellular matrix degradation and modulation of the host immune response, thereby favoring deep tissue invasion by pathogens (Mba and Nweze, 2020; Weissman et al., 2021).

In this study, all *Candida* isolates analyzed demonstrated hemolytic activity regardless of species, corroborating previously reported data in the literature (Nouraei et al., 2020; Chen et al., 2021). It is noteworthy that Mroczynska and Brillowska-Dabrowska (2021) also reported reduced hemolytic activity in *C. parapsilosis*, which is consistent with the findings observed here.

Phospholipase activity was detected in *C. albicans* and *Cyberlindnera jadinii* isolates, aligning with previous studies (Yagmur et al., 2016). Although *C. albicans* generally exhibits higher levels of secretion of this enzyme (Vieira de Melo et al., 2019; El-Houssaini et al., 2019; El-Kholy et al., 2021), *C.* non-*albicans* species also possess the capacity to produce phospholipase, representing a significant virulence factor for their pathogenicity (Sharma et al., 2017; Erum et al., 2020; Nouraei et al., 2020).

In the clinical context of candidiasis, characterization of species distribution, epidemiological patterns, and antifungal susceptibility profiles is essential for defining effective therapeutic strategies. Such information is fundamental for understanding microbial resistance mechanisms, controlling hospital outbreaks, and formulating targeted local public health policies, thereby contributing to improved clinical prognosis of patients (Pfaller et al., 2019; Taei et al., 2019).

This study highlights the importance of local epidemiological surveillance, taking into account patient heterogeneity, variability in species distribution, and emerging trends in antimicrobial resistance. Such data are essential to guide optimized, evidence-based therapeutic interventions tailored to the specific clinical and microbiological context.

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Data Availability Statement

The entire data set that supports the results of this study was published in the article itself.

References

ALCAZAR-FUOLI, L. and MELLADO, E., 2014. Current status of antifungal resistance and its impact on clinical practice. *British Journal of Haematology*, vol. 166, no. 4, pp. 471-484. http://doi.org/10.1111/bjh.12896. PMid:24749533.

ALMEIDA JUNIOR, J.N., ASSY, J.G., LEVIN, A.S., DEL NEGRO, G.M., GIUDICE, M.C., TRINGONI, M.P., THOMAZ, D.Y., MOTTA, A.L., ABDALA, E., PIERROTI, L.C., STRABELLI, T., MUNHOZ, A.L., ROSSI, F. and BENARD, G., 2016. *Candida haemulonii* complex species, Brazil, January 2010-March 2015. *Emerging Infectious Diseases*, vol. 22, no. 3, pp. 561-563. http://doi.org/10.3201/eid2203.151610. PMid:26891028.

BERKOW, E.L. and LOCKHART, S.R., 2017. Fluconazole resistance in *Candida* species: a current perspective. *Infection and Drug*

Resistance, vol. 10, no. 1, pp. 237-245. http://doi.org/10.2147/IDR.S118892. PMid:28814889.

BHATTACHARJEE, P., 2016. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. *Current Medical Mycology*, vol. 2, no. 2, pp. 20-27. http://doi.org/10.18869/acadpub.cmm.2.2.5. PMid:28681016.

BOATTO, H.F., CAVALCANTI, S.D., DEL NEGRO, G.M., GIRÃO, M.J., FRANCISCO, E.C., ISHIDA, K. and GOMPERTZ, O.F., 2016. *Candida duobushaemulonii*: an emerging rare pathogenic yeast isolated from recurrent vulvovaginal candidiasis in Brazil. *Memorias do Instituto Oswaldo Cruz*, vol. 111, no. 6, pp. 407-410. http://doi.org/10.1590/0074-02760160166. PMid:27304096.

BONGOMIN, F., GAGO, S., OLADELE, R.O. and DENNING, D.W., 2017. Global and multi-national prevalence of fungal diseases-estimate precision. *Journal of Fungi*, vol. 3, no. 4, pp. 57. http://doi.org/10.3390/jof3040057. PMid:29371573.

BOUGLITA, W., RABHI, S., RAICH, N., BOUABID, C., BELGHITH, C., SLIMANI, O., HKIMI, C., GHEDIRA, K., KARESS, R.E., GUIZANI-TABBANE, L., ATTIA, L. and RABHI, I., 2022. Microbiological and molecular screening of *Candida* spp. isolated from genital tract of asymptomatic pregnant women. *Journal of Medical Microbiology*, vol. 71, no. 9. http://doi.org/10.1099/jmm.0.001589. PMid:36126092.

BRAGA, P.R., CRUZ, I.L., ORTIZ, I., BARREIROS, G., NOUÉR, S.A. and NUCCI, M., 2018. Secular trends of candidemia at a Brazilian tertiary care teaching hospital. *The Brazilian Journal of Infectious Diseases*, vol. 22, no. 4, pp. 273-277. http://doi.org/10.1016/j.bjid.2018.07.008. PMid:30118654.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde, 2020 [viewed 8 June 2023]. *Boletim epidemiológico. HIV/Aids 2020* [online]. Ministério da Saúde. Available from: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/boletins-epidemiologicos/especiais/2020/boletim-hiv_aids-2020-internet.pdf

CANELA, H.M.S., CARDOSO, B., VITALI, L.H., COELHO, H.C., MARTINEZ, R. and FERREIRA, M.E.D.S., 2017. Prevalence, virulence factors and antifungal susceptibility of *Candida* spp. isolated from bloodstream infections in a tertiary care hospital in Brazil. *Mycoses*, vol. 61, no. 1, pp. 11-21. http://doi.org/10.1111/myc.12695. PMid:28940753.

CENDEJAS-BUENO, E., KOLECKA, A., ALASTRUEY-IZQUIERDO, A., THEELEN, B., GROENEWALD, M., KOSTRZEWA, M., CUENCA-ESTRELLA, M., GÓMEZ-LÓPEZ, A. and BOEKHOUT, T., 2012. Reclassification of the *Candida haemulonii* complex as *Candida haemulonii* (*C. haemulonii* group I), *C. duobushaemulonii* sp. nov. (*C. haemulonii* group II), and *C. haemulonii* var. *vulnera* var. nov.: three multiresistant human pathogenic yeasts. *Journal of Clinical Microbiology*, vol. 50, no. 11, pp. 3641-3651. http://doi.org/10.1128/JCM.02248-12. PMid:22952266.

CENTERS FOR DISEASE CONTROL AND PREVENTION – CDC, 2022 [viewed 18 May 2023]. *COVID-19: U.S. impact on antimicrobial resistance, special report 2022* [online]. Atlanta: Centers for Disease Control and Prevention, Department of Health and Human Services. Available from: https://www.cdc.gov/drugresistance/pdf/covid19-impact-report-508.pdf

CHEN, J., HU, N., XU, H., LIU, Q., YU, X., ZHANG, Y., HUANG, Y., TAN, J., HUANG, X. and ZENG, L., 2021. Molecular epidemiology, antifungal susceptibility, and virulence evaluation of *Candida* isolates causing invasive infection in a tertiary care teaching hospital. *Frontiers in Cellular and Infection Microbiology*, vol. 11, pp. 721439. http://doi.org/10.3389/fcimb.2021.721439. PMid:34604110.

CLINICAL AND LABORATORY STANDARDS INSTITUTE – CLSI, 2009 [viewed 10 May 2023]. *M44-P. Method for antifungal disk diffusion susceptibility testing of yeasts* **[online]**. Clinical and Laboratory Standards Institute. Available from: https://clsi.org/shop/standards/m44/

COLOMBO, A.L., NUCCI, M., PARK, B.J., NOUÉR, S.A., ARTHINGTON-SKAGGS, B., MATTA, D.A., WARNOCK, D. and MORGAN, J., 2006. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. *Journal of Clinical Microbiology*, vol. 44, no. 8, pp. 2816-2823. http://doi.org/10.1128/JCM.00773-06. PMid:16891497.

D'ENFERT, C., KAUNE, A.K., ALABAN, L.R., CHAKRABORTY, S., COLE, N., DELAVY, M., KOSMALA, D., MARSAUX, B., FRÓIS-MARTINS, R., MORELLI, M., ROSATI, D., VALENTINE, M., XIE, Z., EMRITLOLL, Y., WARN, P.A., BEQUET, F., BOUGNOUX, M.E., BORNES, S., GRESNIGT, M.S., HUBE, B., JACOBSEN, I.D., LEGRAND, M., LEIBUNDGUT-LANDMANN, S., MANICHANH, C., MUNRO, C.A., NETEA, M.G., QUEIROZ, K., ROGET, K., THOMAS, V., THORAL, C., VAN DEN ABBEELE, P., WALKER, A.W. and BROWN, A.J.P., 2021. The impact of the Fungus-Host-Microbiota interplay upon *Candida albicans* infections: current knowledge and new perspectives. *FEMS Microbiology Reviews*, vol. 45, no. 3, pp. 1-55. http://doi.org/10.1093/femsre/fuaa060. PMid:33232448.

DIGNANI, M.C., SOLOMKIN, J.S. and ANAISSI, E.J., 2003. Candida. In: E. ANAISSE, M.R. MCGINNIS and M.A. PFALLER, eds. *Medical mycology*. Philadelphia: Churchill Livingstone, pp. 295-239.

DOI, A.M., PIGNATARI, A.C., EDMOND, M.B., MARRA, A.R., CAMARGO, L.F., SIQUEIRA, R.A., DA MOTA, V.P. and COLOMBO, A.L., 2016. Epidemiology and microbiologic characterization of nosocomial candidemia from a Brazilian National Surveillance Program. *PLoS One*, vol. 11, no. 1, e0146909. http://doi.org/10.1371/journal.pone.0146909. PMid:26808778.

EL-HOUSSAINI, H.H., ELNABAWY, O.M., NASSER, H.A. and ELKHATIB, W.F., 2019. Correlation between antifungal resistance and virulence factors in *Candida albicans* recovered from vaginal specimens. *Microbial Pathogenesis*, vol. 128, no. 1, pp. 13-19. http://doi.org/10.1016/j.micpath.2018.12.028. PMid:30562603.

EL-KHOLY, M.A., HELALY, G.F., EL GHAZZAWI, E.F., EL-SAWAF, G. and SHAWKY, S.M., 2021. Virulence factors and antifungal susceptibility profile of *C. tropicalis* isolated from various clinical specimens in Alexandria, Egypt. *Journal of Fungi*, vol. 7, no. 5, pp. 351-353. http://doi.org/10.3390/jof7050351. PMid:33947158.

ENOCH, D.A., YANG, H., ALIYU, S.H. and MICALLEF, C., 2017. The changing epidemiology of invasive fungal infections. *Methods in Molecular Biology*, vol. 1508, pp. 17-65. http://doi.org/10.1007/978-1-4939-6515-1_2. PMid:27837497.

ERUM, R., SAMAD, F., KHAN, A. and KAZMI, S.U., 2020. A comparative study on production of extracellular hydrolytic enzymes of *Candida* species isolated from patients with surgical site infection and from healthy individuals and their co-relation with antifungal drug resistance. *BMC Microbiology*, vol. 20, no. 368, pp. 1-12. http://doi.org/10.1186/s12866-020-02045-6. PMid:33272216.

FISHER, M.C., ALASTRUEY-IZQUIERDO, A., BERMAN, J., BICANIC, T., BIGNELL, E.M., BOWYER, P., BROMLEY, M., BRÜGGEMANN, R., GARBER, G., CORNELY, O.A., GURR, S.J., HARRISON, T.S., KUIJPER, E., RHODES, J., SHEPPARD, D.C., WARRIS, A., WHITE, P.L., XU, J., ZWAAN, B. and VERWEIJ, P.E., 2022. Tackling emerging threat of antifungal resistance to human health. *Nature Reviews. Microbiology*, vol. 20, no. 1, pp. 557-571. http://doi.org/10.1038/s41579-022-00720-1. PMid:35352028.

FISHER, M.C., HAWKINS, N.J., SANGLARD, D. and GURR, S.J., 2018. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science*, vol. 360,

no. 6390, pp. 739-742. http://doi.org/10.1126/science.aap7999. PMid:29773744.

FRÍAS-DE-LEÓN, M.G., MARTÍNEZ-HERRERA, E., ACOSTA-ALTAMIRANO, G., ARENAS, R. and RODRÍGUEZ-CERDEIRA, C., 2019. Superficial candidoses by *Candida duobushaemulonii*: an emerging microorganism. *Infection, Genetics and Evolution*, vol. 75, no. 103960, pp. 1-5. http://doi.org/10.1016/j.meegid.2019.103960. PMid:31325612.

GADE, L., MUÑOZ, J.F., SHETH, M., WAGNER, D., BERKOW, E.L., FORSBERG, K., JACKSON, B.R., RAMOS-CASTRO, R., ESCANDÓN, P., DOLANDE, M., BEN-AMI, R., ESPINOSA-BODE, A., CACERES, D.H., LOCKHART, S.R., CUOMO, C.A. and LITVINTSEVA, A.P., 2020. Understanding the emergence of multidrug-resistant *Candida*: using whole-genome sequencing to describe the population structure of *Candida haemulonii* species complex. *Frontiers in Genetics*, vol. 11, pp. 544. http://doi.org/10.3389/fgene.2020.00554. PMid:32587603.

GAISNE, R., JEDDI, F., MORIO, F., LE CLERC, Q.C., HOURMANT, M., BLANCHO, G., GIRAL, M., CANTAROVICH, D., DANTAL, J. and VILLE, S., 2018. *Candida utilis* fungaemia following endoscopic intervention on ureteral stent in a kidney transplant recipient: case report and a review of the literature. *Mycoses*, vol. 61, no. 8, pp. 594-599. http://doi.org/10.1111/myc.12766. PMid:29575315.

HINRICHSEN, S.L., FALCÃO, E., VILELLA, T.A., RÊGO, L., LIRA, C., ALMEIDA, L., MARTINS, M., ARAÚJO, C., DUARTE, M. and LOPES, G., 2009. Candida isolates in tertiary hospitals in northeastern Brazil. *Brazilian Journal of Microbiology*, vol. 40, no. 2, pp. 325-328. http://doi.org/10.1590/S1517-83822009000200021. PMid:24031366.

HORN, D.L., NEOFYTOS, D., ANAISSIE, E.J., FISHMAN, J.A., STEINBACH, W.J., OLYAEI, A.J., MARR, K.A., PFALLER, M.A., CHANG, C.H. and WEBSTER, K.M., 2009. Epidemiology and Outcomes of Candidemia in 2019 Patients: Data from the Prospective Antifungal Therapy Alliance Registry. *Clinical Infectious Diseases*, vol. 48, no. 12, pp. 1695-1703. http://doi.org/10.1086/599039. PMid:19441981.

HOU, X., XIAO, M., CHEN, S.C., WANG, H., CHENG, J.W., CHEN, X.X., XU, Z.P., FAN, X., KONG, F. and XU, Y.C., 2016. Identification and antifungal susceptibility profiles of *Candida haemulonii* Species Complex Clinical Isolates from a Multicenter Study in China. *Journal of Clinical Microbiology*, vol. 54, no. 11, pp. 2676-2680. http://doi.org/10.1128/JCM.01492-16. PMid:27535688.

JENSEN, R.H., ASTVAD, K.M., SILVA, L.V., SANGLARD, D., JØRGENSEN, R., NIELSEN, K.F., MATHIASEN, E.G., DOROUDIAN, G., PERLIN, D.S. and ARENDRUP, M.C., 2015. Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in *Candida albicans* orchestrated by multiple genetic alterations. *The Journal of Antimicrobial Chemotherapy*, vol. 70, no. 9, pp. 2551-2555. http://doi.org/10.1093/jac/dkv140. PMid:26017038.

KHADKA, S., SHERCHAND, J.B., POKHREL, B.M., PARAJULI, K., MISHRA, S.K., SHARMA, S., SHAH, N., KATTEL, H.P., DHITAL, S., KHATIWADA, S., PARAJULI, N., PRADHAN, M. and RIJAL, B.P., 2017. Isolation, speciation and antifungal susceptibility testing of *Candida* isolates from various clinical specimens at a tertiary care hospital, Nepal. *BMC Research Notes*, vol. 10, no. 1, pp. 218. http://doi.org/10.1186/s13104-017-2547-3. PMid:28646915.

KULLBERG, B.J. and ARENDRUP, M.C., 2015. Invasive candidiasis. *The New England Journal of Medicine*, vol. 373, no. 15, pp. 1445-1456. http://doi.org/10.1056/NEJMra1315399. PMid:26444731.

LACAZ, C.S. and PORTO, E., 2002. Leveduras de interesse médico. In: C.S. LACAZ, E. PORTO, J.E.C. MARTINS, E. M. HEINS-VACCARI and N.T. MELO, eds. *Tratado de micologia médica*. 9. ed. São Paulo: Sarvier, cap. 6, pp. 183-202.

LOPES, J.P. and LIONAKIS, M.S., 2022. Pathogenesis and virulence of *Candida albicans*. *Virulence*, vol. 13, no. 1, pp. 89-121. http://doi.org/10.1080/21505594.2021.2019950. PMid:34964702.

MAGALHÃES, Y.C., BOMFIM, M.R., MELÔNIO, L.C., RIBEIRO, P.C., COSME, L.M., RHODEN, C.R. and MARQUES, S.G., 2015. Clinical significance of the isolation of *Candida* species from hospitalized patients. *Brazilian Journal of Microbiology*, vol. 46, no. 1, pp. 117-123. http://doi.org/10.1590/S1517-838246120120296. PMid:26221096.

MANE, A., GAIKWAD, S., BEMBALKAR, S. and RISBUD, A., 2012. Increased expression of virulence attributes in oral *Candida albicans* isolates from human immunodeficiency virus-positive individuals. *Journal of Medical Microbiology*, vol. 61, no. Pt 2, pp. 285-290. http://doi.org/10.1099/jmm.0.036269-0. PMid:21959206.

MATTOS, K., RODRIGUES, L.C., OLIVEIRA, K.M.P., DINIZ, P.F., MARQUES, L.I., ARAUJO, A.A. and CHANG, M.R., 2017. 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. *Journal of the Brazilian Society of Tropical Medicine*, vol. 50, no. 6, pp. 843-847. http://doi.org/10.1590/0037-8682-0163-2017. PMid:29340465.

MBA, I.E. and NWEZE, E.I., 2020. Mechanism of *Candida* pathogenesis: revisiting the vital drivers. *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 39, no. 10, pp. 1797-1819. http://doi.org/10.1007/s10096-020-03912-w. PMid:32372128.

MEDEIROS, M.A.P., MELO, A.P.V., BENTO, A.O., SOUZA, L.B.F.C., NETO, F.A.B., GARCIA, J.B., ZUZA-ALVES, D.L., FRANCISCO, E.C., MELO, A.S.A. and CHAVES, G.M., 2019. Epidemiology and Prognostic factors of nosocomial candidemia in Northeast Brazil: a six-year retrospective study. *PLoS One*, vol. 14, no. 8, e0221033. http://doi.org/10.1371/journal.pone.0221033. PMid:31437188.

MOHZARI, Y., AL MUSAWA, M., ASDAQ, S.M.B., ALATTAS, M., QUTUB, M., BAMOGADDAM, R.F., YAMANI, A. and ALDABBAGH, Y., 2021. *Candida utilis* and *Stenotrophomonas maltophilia* causing nosocomial meningitis following a neurosurgical procedure: a rare co-infection. *Journal of Infection and Public Health*, vol. 14, no. 11, pp. 1715-1719. http://doi.org/10.1016/j.jiph.2021.10.004. PMid:34700290.

MROCZYNSKA, M. and BRILLOWSKA-DABROWSKA, A., 2021. Virulence of clinical *Candida* isolates. *Pathogens*, vol. 10, no. 4, pp. 466-478. http://doi.org/10.3390/pathogens10040466. PMid:33921490.

MUHAJ, F.F., GEORGE, S.J., NGUYEN, C.D. and TYRING, S.K., 2022. Antimicrobials and resistance part II: antifungal, antivirals, and antiparasitics. *Journal of the American Academy of Dermatology*, vol. 86, no. 6, pp. 1207-1226. http://doi.org/10.1016/j.jaad.2021.11.065. PMid:35122895.

NAGLIK, J.R., MOYES, D.L., WÄCHTLER, B. and HUBE, B., 2011. *Candida albicans* interactions with epithelial cells and mucosal immunity. *Microbes and Infection*, vol. 13, no. 12-13, pp. 963-976. http://doi.org/10.1016/j.micinf.2011.06.009. PMid:21801848.

NOURAEI, H., PAKSHIR, K., ZARESHAHRABADI, Z. and ZOMORODIAN, K., 2020. High detection of virulence factors by *Candida* species isolated from bloodstream of patients with candidemia. *Microbial Pathogenesis*, vol. 149, pp. 104574. http://doi.org/10.1016/j.micpath.2020.104574. PMid:33075515.

NUCCI, M., QUEIROZ-TELLES, F., TOBÓN, A.M., RESTREPO, A. and COLOMBO, A.L., 2010. Epidemiology of opportunistic fungal infections in Latin America. *Clinical Infectious Diseases*, vol. 51, no. 5, pp. 561-570. http://doi.org/10.1086/655683. PMid:20658942.

ODDS, F.C., BOUGNOUX, M.E., SHAW, D.J., BAIN, J.M., DAVIDSON, A.D., DIOGO, D., JACOBSEN, M.D., LECOMTE, M., LI, S.Y., TAVANTI, A., MAIDEN, M.C., GOW, N.A. and D'ENFERT, C., 2007. Molecular phylogenetics of *Candida albicans*. *Eukaryotic Cell*, vol. 6, no. 6, pp. 1041-1052. http://doi.org/10.1128/EC.00041-07. PMid:17416899.

OLIVEIRA, C.S., COLOMBO, A.L., FRANCISCO, E.C., DE LIMA, B., GANDRA, R.F., DE CARVALHO, M.C.P., CARRILHO, C.M.D.M., PETINELLI, R., PELISON, M., HELBEL, C., CZELUSNIAK, G., PAZ MORALES, H.M., PEROZIN, J.S., PINHEIRO, R.L., COGNIALLI, R., BREDA, G.L. and QUEIROZ-TELLES, F., 2021. Clinical and epidemiological aspects of Candidemia in eight medical centers in the state of Parana, Brazil: Parana Candidemia Network. *The Brazilian Journal of Infectious Diseases*, vol. 25, no. 1, pp. 101041. http://doi.org/10.1016/j.bjid.2020.11.006. PMid:33370563.

PAPPAS, P.G., LIONAKIS, M.S., ARENDRUP, M.C., OSTROSKY-ZEICHNER, L. and KULLBERG, B.J., 2018. Invasive candidiasis. *Nature Reviews Disease Primers*, vol. 4, pp. 18026. http://doi.org/10.1038/nrdp.2018.26.

PAPPAS, P.G., REX, J.H., LEE, J., HAMILL, R.J., LARSEN, R.A., POWDERLY, W., KAUFFMAN, C.A., HYSLOP, N., MANGINO, J.E., CHAPMAN, S., HOROWITZ, H.W., EDWARDS, J.E., DISMUKES, W.E. and NIAID MYCOSES STUDY GROUP, 2003. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. *Clinical Infectious Diseases*, vol. 37, no. 5, pp. 634-643. http://doi.org/10.1086/376906. PMid:12942393.

PFALLER, M.A. and DIEKEMA, D.J., 2007. Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical Microbiology Reviews*, vol. 20, no. 1, pp. 133-163. http://doi.org/10.1128/CMR.00029-06. PMid:17223626.

PFALLER, M.A., DIEKEMA, D.J., TURNIDGE, J.D., CASTANHEIRA, M. and JONES, R.N., 2019. Twenty Years of the SENTRY antifungal Surveillance Program: results for *Candida* species from 1997-2016. *Open Forum Infectious Diseases*, vol. 6, no. 1, suppl. 1, pp. S79-S94. http://doi.org/10.1093/ofid/ofy358. PMid:30895218.

RAMOS, L.S., FIGUEIREDO-CARVALHO, M.H., BARBEDO, L.S., ZICCARDI, M., CHAVES, A.L., ZANCOPÉ-OLIVEIRA, R.M., PINTO, M.R., SGARBI, D.B., DORNELAS-RIBEIRO, M., BRANQUINHA, M.H. and SANTOS, A.L., 2014. *Candida haemulonii* complex: species identification and antifungal susceptibility profiles of clinical isolates from Brazil. *The Journal of Antimicrobial Chemotherapy*, vol. 70, no. 1, pp. 11-15. http://doi.org/10.1093/jac/dku321. PMid:25134720.

RAMOS, R., CACERES, D.H., PEREZ, M., GARCIA, N., CASTILLO, W., SANTIAGO, E., BORACE, J., LOCKHART, S.R., BERKOW, E.L., HAYER, L., ESPINOSA-BODE, A., MORENO, J., JACKSON, B.R., MORAN, J., CHILLER, T., DE VILLARREAL, G., SOSA, N., VALLABHANENI, S. and RED NACIONAL DE VIGILANCIA EPIDEMIOLOGICA EN MICROBIOLOGIA CLINICA, 2018. Emerging Multidrug-resistance *Candida duobushaemulonii* infections in Panama Hospitals: importance of laboratory surveillance and accurate identification. *Journal of Clinical Microbiology*, vol. 56, no. 7, pp. 1-6. http://doi.org/10.1128/JCM.00371-18. PMid:29695521.

ROSA, F.G., GARAZZINO, S., PASERO, D., DI PERRI, G. and RANIERI, V.M., 2009. Invasive candidiasis and candidemia: new guidelines. *Minerva Anestesiologica*, vol. 75, no. 7-8, pp. 453-458. PMid:19078900.

SAITOU, N. and NEI, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, vol. 4, no. 4, pp. 406-425. http://doi.org/10.1093/oxfordjournals.molbev.a040454. PMid:3447015.

SHARMA, Y., CHUMBER, S.K. and KAUR, M., 2017. Studying the prevalence, species distribution, and detection of in vitro production of phospholipase from *Candida* isolated from cases of invasive candidiasis. *Journal of Global Infectious Diseases*, vol. 9, no. 1, pp. 8-11. http://doi.org/10.4103/0974-777X.199995. PMid:28250619.

SREELEKSHMI, T.S., NINAN, M.M., PREMANAND, A., CHACKO, A., SAHNI, R.D. and MICHAEL, J.S., 2021. *Candida utilis*: a rare cause of septicemia in children. *Access Microbiology*, vol. 3, no. 10, pp. 000281. http://doi.org/10.1099/acmi.0.000281. PMid:34816095.

SUDBERY, P.E., 2011. Growth of *Candida albicans* hyphae. *Nature Reviews. Microbiology*, vol. 9, no. 10, pp. 737-748. http://doi.org/10.1038/nrmicro2636. PMid:21844880.

TAEI, M., CHADEGANIPOUR, M. and MOHAMMADI, R., 2019. An alarming rise of non-*albicans Candida* species and uncommon yeasts in the clinical samples: a combination of various molecular techniques for identification of etiologic agents. *BMC Research Notes*, vol. 12, no. 779, pp. 1-7. http://doi.org/10.1186/s13104-019-4811-1. PMid:31783903.

TAMURA, K., NEI, M. and KUMAR, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 30, pp. 11030-11035. http://doi.org/10.1073/pnas.0404206101. PMid:15258291.

TAMURA, K., STECHER, G. and KUMAR, S., 2021. MEGA 11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, vol. 38, no. 7, pp. 3022-3027. http://doi.org/10.1093/molbev/msab120. PMid:33892491.

VIEIRA DE MELO, A.P., ZUZA-ALVES, D.L., DA SILVA-ROCHA, W.P., FERREIRA CANÁRIO DE SOUZA, L.B., FRANCISCO, E.C., SALLES DE AZEVEDO MELO, A. and MARANHÃO CHAVES, G., 2019. Virulence factors of Candida spp. obtained from blood cultures of patients with candidemia attended at tertiary hospitals in Northeast Brazil. *Journal de Mycologie Médicale*, vol. 29, no. 2, pp. 132-139. http://doi.org/10.1016/j.mycmed.2019.02.002. PMid:30876744.

WANG, B., HE, X., LU, F., LI, Y., WANG, Y., ZHANG, M., HUANG, Y. and XIA, J., 2021. *Candida* Isolates from Blood and Other Normally Sterile Foci from ICU Patients: determination of epidemiology, antifungal susceptibility profile and evaluation of associated risk factors. *Frontiers in Public Health*, vol. 9, pp. 779590. http://doi.org/10.3389/fpubh.2021.779590. PMid:34858938.

WEISSMAN, Z., PINSKY, M., DONEGAN, R.K., REDDI, A.R. and KORNITZER, D., 2021. Using genetically encoded sensors to probe the mechanisms of heme uptake and homeostasis in *Candida albicans*. *Cellular Microbiology*, vol. 23, no. 2, e13282. http://doi.org/10.1111/cmi.13282. PMid:33104284.

WHITE, T.J., BRUNS, T.D., LEE, S.B. and TAYLOR, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A. INNIS, D.H. GELFAND, J.J. SNINSKY and T.J. WHITE, eds. *PCR protocols: a guide to methods and applications*. New York: Academic Press, pp. 315-322.

YAGMUR, G., SAV, H., ZIYADE, N., ELGORMUS, N., SEN, S., AKKOYUN BILGI, E., ATAN, Y., BUYUK, Y. and KIRAZ, N., 2016. Evaluation of virulence factors and antifungal susceptibility in yeast isolates from postmortem specimens. *Journal of Forensic Sciences*, vol. 61, no. 4, pp. 1000-1006. http://doi.org/10.1111/1556-4029.13089. PMid:27364280.

YAKUT, N., KEPENEKLI, E., ERGENC, Z., BARAN, E. and CERIKCIOGLU, N., 2021. Antifungal susceptibility, species distribution and risk factors associated with mortality of invasive candidiasis in children in Turkey: a six-year retrospective, single-centre study. *Journal of Medical Mycology*, vol. 31, no. 1, pp. 101082. http://doi.org/10.1016/j.mycmed.2020.101082. PMid:33249314.