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CLINICAL MICROBIOLOGY - RESEARCH PAPER

Distribution and antifungal susceptibility profiles of *Candida* species isolated from people living with HIV/AIDS in a public hospital in Goiânia, GO, Brazil

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Abstract

Oropharyngeal candidiasis (OPC) is the most common opportunistic fungal infection of the oral cavity and is a significant clinical problem, particularly in immunocompromised individuals, such as people living with HIV/AIDS (PLWHA). Although *Candida albicans* is the most frequent pathogen, at least 30 species capable of causing infection have been described. Identifying the infecting organism is necessary because the species respond differently to therapy, and antifungal susceptibility testing is important to determine the appropriate treatment. This study aimed to determine the epidemiological, clinical, and mycological profiles of OPC in hospitalized PLWHA. Clinical samples were collected from 103 PLWHA with suspected candidiasis admitted to the Hospital Estadual of Doenças Tropicais/Hospital Anuar Auad of Goiania, Goias, Brazil, for 14 months. *Candida* species were identified using phenotypic microbiological techniques and molecular analysis performed by PCR using species-specific primers. The antifungal susceptibility pattern of the isolates against the six antifungal agents was determined using the broth microdilution method. Here, female individuals were the most affected by OPC, presenting a higher risk of oral colonization by *Candida* spp. The main clinical manifestation was pseudomembranous candidiasis. The number of cases of candidiasis was 87.3% (90/103), with *C. albicans* being the most common species, followed by *C. tropicalis* and *C. glabrata*. In the susceptibility pattern, non-*albicans Candida* showed higher resistance to than *C. albicans*. The fast and accurate identification of *Candida* spp. is very important to identify therapeutic agents for the treatment of oral candidiasis in PLWHA.

Keywords *Candida* · Candidiasis · Antifungal agent · AIDS-related opportunistic infections · Epidemiology

Introduction

Oropharyngeal candidiasis (OPC) is characterized by overgrowth and successive tissue invasion by yeasts of the genus *Candida* in the oral mucosa [1]. The disease has been commonly found in immunocompromised individuals with severe and prolonged illnesses such as cancer, people living with HIV/AIDS (PLWHA), those with diabetes mellitus, solid organ transplant recipients, and those with prolonged therapy with broad-spectrum antibiotics [1, 2]. Despite the high efficiency of current antiretroviral therapies (ART), OPC remains a critical problem for residents of resource-limited settings and for those individuals who develop resistance to fungi [3, 4]. In PLWHA, OPC may develop recurrent and refractory episodes of infection [5–7]. Severe OPC grows with the advancement of HIV infection cases [8], providing prolonged hospital stays favoring the chances of disseminated infections [9].

Although *Candida albicans* is the main species responsible for OPC [1, 10–12], the importance of non-*albicans Candida* (NAC) species, such a s *C. glabrata*, *C. krusei*, *C.*

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tropicalis, *C. guilliermondii*, *C. kefyr*, and *C. parapsilosis* [13–15] in human diseases has been increasingly recognized. Although these species typically lack a range of virulence factors as those found in *C. albicans*, they stand out due to increased resistance to antifungal agents [16]. Antifungal resistance in *Candida* species has a negative impact on disease management being associated with ineffective clinical outcomes for patients during antifungal treatment, in addition to increasing healthcare costs [17]. Strain variation may promote pathogenesis through increased expression of virulence determinants affecting the nature of host immune system responses [18]. Therefore, it increases the importance of using antifungal susceptibility testing routinely to improve therapeutic decision-making and monitor the development of resistance.

This study aimed to determine the epidemiological, clinical, and mycological profiles of OPC in hospitalized PLWHA.

Material and methods

Patient characteristics and ethics statement

A cross-sectional study was conducted with samples of PLWHA hospitalized at the Hospital Estadual of Doenças Tropicais/Hospital Anuar Auad (HDT/HAA) with a clinical diagnosis of oral candidiasis from 2018 to 2019. All participants who agreed to participate in the study signed an informed consent form.

The following data were obtained in the interview: sociodemographic variables (age, sex, marital status, ethnicity), clinical (signs and symptoms of candidiasis) and predisposing factors. Data regarding the type of candidiasis, HIV diagnosis date, CD4 cell count and viral load, use of drugs, and possible associated diseases were collected from the medical records.

This study was established in accordance with the guidelines and standards for research involving human beings (National Council Resolution No. 466/12 of December 12, 2012) and was approved by the Research Ethics Committee of the Hospital of the Clinics of Universidade Federal in Goias and of HDT/HAA, according to documents n.3.280.435 and n. 3.335.908, respectively.

Sample collection, culture, and identification of *Candida* species

The samples were obtained using sterile swabs from lesions present in the oral mucosa of PLWHA. The oral samples were placed in tubes containing 0.85% saline solution, cultured on Sabouraud dextrose agar (ASD, Difco Laboratories, Franklin Lakes, USA) plus chloramphenicol, and incubated at 35 °C for 48 h under aerobic conditions. After the incubation period, direct microscopy was performed to check for the presence of yeasts and pseudo-hyphae and posteriorly identified based on morphological characteristics. The production of germ tubes in fetal bovine serum of chlamydoconidium on cornmeal agar (Sigma, St. Louis, MO, USA) and carbohydrate assimilation tests (glucose, sucrose, galactose, xylose, cellobiose, raffinose, maltose, and trehalose) were performed according to the method described by Kurtzman et al. (2011) [19]. Yeasts were inoculated in a chromogenic medium for *Candida* (Kasvi, São José dos Pinhais, Brazil) to check mixed infection. After phenotypic identification, the yeasts were stored at − 20 °C for use in this study.

Molecular identification by polymerase chain reaction (PCR)

DNA extraction was performed according to McCullough et al. (2000)[20]. The PCR reaction described by Martínez et al. (2010) [21] consisted of a total volume of 25 µL, containing 2.5U of Taq DNA polymerase (Invitrogen Life Technologies, Waltham, USA), 2.5 mmol of each dNTP (Synapse, Selangor, Malaysia), and 2 µM of each species-specific primer (Invitrogen Life Technologies, Waltham, USA), plus 12 ng of DNA.

The primers used were *C. albicans* (5′-AAG TAT TTG GGA GAA GGG AAA GGG-3′; 5′-AAA ATG GGC ATT AAG GAA AAG AGC-3′), *C. glabrata* (5′-ACA TAT GTT TGC TGA AAA GGC-3′; 5′-ACT TTT TCT TAG TGT TCA GGA CTT CC-3′), *C. tropicalis* (5′-TGA TAG TTA GGA AAG ATC AGG TG-3′; 5′-AAC ATA TCC CAT GTG TGT GT-3′), *C. krusei* (5′-GAT TTA GTA CTA CAC TGC GTG A-3′; 5′-TCC TCC GCT TAT TGA TAT GC-3′), *C. dubliniensis* (5′-GAT ATT GGG AGA GGG AAA GAC C-3′; 5′-ACA GGG AAG TCG ATT CTT GC-3′), *C. kefyr* (5′-GCTCGTCTCTCCAGTGGACATA-3;5′-ACTCACTAC CAAACCCAAAGGT-3′), and *C. parapsilosis* (5′-AGG GAT TGC CAA TAT GCC CA-3′; 5′-GTG ACA TTG TGT AGA TCC TTG G-3′).

DNA amplification was performed using an initial cycle of 95 °C for 30 s, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 90 s, with a final step extension at 72 °C for 10 min. The amplified products were subjected to 1% agarose gel electrophoresis and photographed, and the resulting bands were analyzed according to their molecular weight. A PCR/Thermal Cycler (Bio-Rad PTC-100) was used to perform the test.

Antifungal susceptibility testing

The antifungal susceptibility test was performed according to the broth microdilution technique established by the

Clinical and Laboratory Standards Institute (CLSI) document M27-A3 [22]. The pure drug of fluconazole antifungals (Pfizer Central Research, Sandwich, UK), itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer Central Research, Sandwich, UK), miconazole (Pfizer Central Research, Sandwich, UK), amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), and nystatin (Bristol-Myers Squibb, Woerden, The Netherlands). These chemicals were used at the following concentrations: 0.125–64 µg/mL for fluconazole and 0.032–16 µg/mL for itraconazole, voriconazole, miconazole, amphotericin B, and nystatin. The test was performed in microtiter plates containing 96 wells, where serial dilutions of the antifungals were conducted in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO, USA). After dilution, the fungal inoculum was added to the final concentration of cells from $1–5 \times 10^3$ UFC/mL. The plates were incubated at 35 °C for 24–48 h. The results were analyzed using 16 µL of alamarBlue assay (Sigma Chemical Co., St. Louis, MO, USA), which allowed a better viewing of the minimum inhibitory concentration (MIC) [23]. All tests were performed in duplicate, and *C. parapsilosis* ATCC 22,019 was used as quality control.

Breakpoints for fluconazole, voriconazole, and itraconazole were interpreted as suggested in documents M27-S3 and M60 [22, 24], whereas those for miconazole, amphotericin B, and nystatin were interpreted according to different authors[4, 25–27]. The sensitivity profile of the isolates to antifungals was classified as sensitive (S), dose-dependent sensitivity (S-DD), intermediate (I), or resistant (R).

Data management and statistical analysis

The participants' sociodemographic and clinical information were recorded into a database using the REDcap® platform and analyzed using the IBM SPSS® statistical program (version 25.0). To compare the proportion of clinical variables of the individuals, the chi-square or Fisher's exact test was used to categorize all variables according to the statistical parameters. A *t*-test was performed for continuous variables. For all tests, statistical differences were considered significant if the *p*-value was < 0.05.

Results

Association of demographics data of patients and OPC

Individual demographic data are summarized in Table 1. This study included 103 PLWHA with clinical signs and symptoms of OPC who were hospitalized at the HDT/HAA between 2018 and 2019. Most participants (69.9%) were male, with ages ranging from 20 to 69 years, with a mean age of 40 years (SD = ± 10.4 years). Most (78.0%) declared that they were single. Among the 103 oral samples collected from PLWHA, 92 (89.3%) individuals had positive cultures recovered after growth on ASD medium.

The main types of lesions found in the oral mucosa were pseudomembranous candidiasis in 65.0% (67/103) and erythematous candidiasis in 9.7% (10/103) of cases. Mixed lesions were observed in 16 participants (15.5%), with pseudomembranous candidiasis associated with others (Table 2).

The average duration of HIV infection was 4 years, with 25% of the participants in the first month of infection by the virus. Positive cultures for *Candida* spp. were higher in the first years of infection ($p = 0.021$; Table 3).

Antiretroviral therapy had not yet been initiated by 35 (34.7%) participants, and 66 (65.3%) had received the therapy at some moment, being active or irregular. The rate of positivity culture in naive participants was 100% ($p = 0.014$; Table 3).

Among the 93 individuals, 81 (87.1%) had values lower than 200 CD4 cells/mm^3. It was observed that 59 (60.8%) patients had more than 100,000 HIV copies/mL (Table 3).

Table 1 Demographic characteristics of HIV-positive patients

Characteristics	Total	Positive culture	Negative culture	*p*-value
Gender	$n = 103$	92 (89.3)	11 (10.7)	-
Female	31 (30.1)	31 (100.0)	-	**0.031**
Male	72 (69.9)	61 (84.7)	11 (15.3)	
Age (years)	$n = 103$	$n = 92$	$n = 11$	
Mean ± SD	40.0 ± 10.4	40.0 ± 10.8	39.9 ± 7.0	0.968
Minimum–maximum	20–69	20–69	25–48	-
Civil status	$n = 91$	$n = 80$	$n = 11$	
Single	71 (78.0)	62 (87.3)	9 (12.7)	-
Married	8 (8.8)	6 (75.0)	2 (25.0)	-
Divorced	5 (5.5)	5 (100.0)	-	-
Widower	7 (7.7)	7 (100.0)	-	-

Fisher's exact test ($p < 0.05$)

Table 2 Clinical forms of oropharyngeal candidiasis found in people living with HIV/AIDS

Clinical forms of candidiasis	*n*	%
Pseudomembranous	67	65.0
Erythematous	10	9.7
Pseudomembranous + angular cheilitis	7	6.8
Pseudomembranous + erythematous	6	5.8
Angular cheilitis	6	5.8
Hyperplastic	4	3.9
Pseudomembranous + hyperplastic	3	2.9

Regarding the signs and symptoms presented by the participants, 57 (55.3%) reported experiencing changes in taste. The statistical analysis of these signs and symptoms associated with the positivity of the culture was not significant ($p = 0.338$; Table 3).

It was found that 70 (68.0%) participants had previously used antifungal agents, with fluconazole being the most used (80.0%). Although not statistically significant, a high percentage of positive cultures for yeasts (88.6%) was observed in participants who used antifungal agents (Table 3).

Among the risk factors found, only the use of illicit drugs was significant ($p = 0.006$; Table 4) in relation to the positivity of the culture.

Identification of *Candida* species using conventional method and PCR

Among the 103 samples collected, we obtained 92 positive cultures. However, two different species were isolated in four individuals, totaling 96 clinical isolates. The precise identification fungal isolates at the species level was as follows: 70 *C. albicans* (74.5%), nine *C. tropicalis* (9.6%), seven *C. glabrata* (7.5%), three *C. krusei* (3.2%), two *C. parapsilosis*

Table 3 Clinical characteristics of HIV-positive patients

Characteristics	Total	Positive culture	Negative culture	*p*-value
HIV diagnosis time	***n* = 102**			
≤ 1 year	52 (51.0)	50 (96.2)	2 (3.8)	**0.021****
> 1 year	50 (49.0)	41(82.0)	9 (18.0)	
Antiretroviral therapy	***n* = 101**			
Naive	35 (34.7)	35 (100.0)	-	**0.014***
Active and irregular	66 (65.3)	56 (84.8)	10 (15.2)	
Cell T CD4 count (cells/mm^3)	***n* = 93**			
≤ 200 cells	81 (87.1)	73 (90.1)	8 (9.9)	0.149*
> 200 cells	12 (12.9)	9 (75.0)	3 (25.0)	
Viral charge (HIV)	***n* = 97**			
≤ 100,000 copies	38 (39.2)	31 (81.6)	7 (18.4)	0.104*
> 100,000 copies	59 (60.8)	55 (93.2)	4 (6.8)	
Signals and symptoms	***n* = 103**	**92 (89,3)**	**11 (10,7)**	
Oral burning	54 (52.4)	47 (87.0)	7 (13.0)	0.531*
Change in taste	57 (55.3)	49 (86.0)	8 (14.0)	0.338*
Oral mucosa pain	49 (47.6)	42 (85.7)	7 (14.3)	0.343*
Food aversion	45 (43.7)	38 (84.4)	7 (15.6)	0.204*
Use of antifungals				
Previous use of antifungal	70 (68.0)	62 (88.6)	8 (11.4)	0.162**
No previous use of antifungal	33 (32.0)	30 (90.9)	3 (9.1)	0.999*
Fluconazole	56 (80.0)	50 (89.3)	6 (10.7)	
Itraconazole	1 (1.4)	1 (100.0)	-	
Amphotericin B	3 (4.3)	3 (100.0)	-	
Nystatin	2 (2.9)	2 (100.0)	-	
Fluconazole, amphotericin B, and itraconazole	1 (1.4)	1 (100.0)	-	
Fluconazole and amphotericin B	3 (4.3)	3 (100.0)	-	
Fluconazole and nystatin	3 (4.3)	2 (66.7)	1 (33.3)	
Fluconazole and ketoconazole	1 (1.4)	-	1 (100.0)	

*Fisher's exact test ($p < 0.05$), **chi-square test ($p < 0.05$)

Table 4 Risk factors found for oropharyngeal candidiasis in people living with HIV/AIDS

Risk factors		Total	Positive culture	Negative culture	*p*-value
		$n=103$			
	Tuberculosis	16 (15.5)	14 (87.5)	2 (12.5)	0.655[a]
1	Cigarettes	44 (44.0)	38 (86.4)	6 (13.6)	0.455[b]
	Dental prosthesis	29 (28.4)	26 (87.7)	3 (10.3)	0.928[b]
	Use of broad spectrum antibiotics	81 (87.1)	73 (90.1)	9 (9.9)	0.612[a]
	Diabetes mellitus	8 (7.8)	8 (100.0)	-	0.586[a]
	Cancer	4 (3.9)	3 (75.0)	1 (25.0)	0.396[a]
	Recent surgeries	10 (10.4)	9 (90.0)	1 (10.0)	0.878[b]
	Illicit drugs	19 (19.4)	13 (68.4)	6 (31.6)	0.006[a] *
	Comorbidity	48 (46.6)	43 (89.6)	5 (10.4)	0.936[b]

[a]Fisher's exact test ($p<0.05$), [b]chi-square test ($p<0.05$)

(2.1%), two *C. dubliniensis* (2.1%), and one *C. kefyr* (1%). In addition, 4.3% (4/94) of PLWHA were co-infected as follows: *C. albicans* and *C. tropicalis* (2/4), *C. albicans* and *C. glabrata* (1/4), and *C. albicans* and *C. Krusei* (1/4). Two isolates were phenotypically identified as *C. tropicalis* but there was no DNA amplification during the PCR reaction using species-specific primers. These two isolates were sequenced to verify the true identity of the microorganism. The PCR products were purified using a DNA purification kit (Invitrogen, Germany) and sequenced using the ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) primers in a reaction ABI Prism™ Bigdye™ terminator cycle reaction kit (Applied Biosystems, USA). After analyzing the sequences in GenBank, the isolates were identified as *Lodderomyces elongisporus*.

Antifungal susceptibility testing

A total of 8.5% (8/94 isolates) of *Candida* species were resistant to fluconazole, 19.1% (18/94) to itraconazole, 5.3% (5/94) to voriconazole, and 15.9% (15/94) to miconazole (Table 5). In the polyene derivatives, 2.1% (2/94) of the isolates showed resistance to amphotericin B, whereas all isolates were sensitive to nystatin (Table 5).

Non-*albicans Candida* showed a resistance of 29.7% whereas in *C. albicans*, this resistance was 21.2%. Antifungal susceptibility profiles and the variation in MIC, MIC_{50}, and MIC_{90} of each *Candida* species are shown in Table 5.

Discussion

In recent decades, the increased incidence of fungal infections has been associated with congenital or acquired immunodeficiency [4].Oral candidiasis during HIV infection is a common infection in PLWHA [9, 28, 29]. Although some studies have investigated this issue in Brazil [4, 30, 31], in the state of Goias, there is no evidence of the prevalence of *Candida* species and oral candidiasis in the PLWHA in the last decade. Thus, in this study, PLWHA who were admitted to the HDT/HAA were evaluated by physicians for possible clinical signs of OPC.

The finding of 87.3% of participants with OPC found in 103 PLWHA in this study confirms the importance of OPC in these individuals. In Brazil, in the last decade, this index varied between different regions. The highest rates were found by Miguel et al. (2018) [30] in Rio de Janeiro (100%), Terças et al. (2017) [4] in Maranhão (83%), and Spalanzani et al. (2018) [31] in Mato Grosso do Sul (75%). Other investigators have found lower rates of OPC in Brazil, as Goulart et al. (2018) [32] also in Mato Grosso do Sul with a rate of 39.6%, Ribeiro et al. (2015) [33] in Pará (41.8%), and Grazziotin et al. (2015) [34] in Rio Grande do Sul (48.9%). The observed differences in indices show the importance of epidemiological studies of fungal infections, essentially OPC.

Here, it is worth noting that OPC was diagnosed more frequently in women than in men. Reinhardt et al. (2018) explain that women are more likely than men to seek medical attention for symptoms of *Candida* infection. Although not statistically significant, in this study, elderly individuals had higher mean colonization rates. The increased risk of colonization above 45 years was found by Goulart et al. (2018) [32], which justified that the frequent use of dentures in middle-aged and elderly patients increases the risk of colonization and infection by *Candida* spp. in these age groups (Table 1).

This study found a significant relationship between the time of HIV diagnosis and fungal infection. Individuals in the first year of viral infection were more likely to develop OPC. This occurrence is possibly due to the fact that many individuals have not yet started antiretroviral therapy. This treatment can have a direct effect on *Candida* spp. virulence by inhibiting fungal-secreting aspartyl proteinases [29].

Table 5 In vitro susceptibility for yeasts against six antifungal agents

Species (*n*)	Antifungal agent	Range	MIC_{50}	MIC_{90}	*S*	S- DD	*R*
C. albicans(70)	Fluconazole	0.12–64	0.5	2	65	3	2
	Itraconazole	0.03–16	0.25	1	34	27	9
	Voriconazole	0.03–4	0.03	0.12	64	5	1
	Miconazole	0.03–16	1	8	63	-	7
	Amphotericin B	0.03–2	0.5	1	69	-	1
	Nystatin	0.06–2	1	2	70	-	-
C. tropicalis(9)	Fluconazole	0.5–16	1	64	7	-	2
	Itraconazole	0.06–16	0.25	2	3	4	2
	Voriconazole	0.03- 16	0.03	8	6	-	3
	Miconazole	8–16	16	16	2	-	7
	Amphotericin B	0.25–1	0,5	0.5	9	-	-
	Nystatin	0.5–2	0.5	2	9	-	-
C. glabrata(7)	Fluconazole	0.25–64	2	8	-	6	1
	Itraconazole	0.03–16	0.25	1	4	-	3
	Voriconazole	0.03–4	0.25	0.5	6	-	1
	Miconazole	0.03–16	0.25	8	6	-	1
	Amphotericin B	0.12–2	0.25	1	6	-	1
	Nystatin	0.5–2	1	2	7	-	-
C. krusei(3)	Fluconazole	16–32	16	16	-	-	3
	Itraconazole	0.12–16	1	1	-	-	3
	Voriconazole	0.03–0.5	0.03	0.03	3	-	-
	Miconazole	0.5–4	2	2	3	-	-
	Amphotericin B	0.25–0.5	0.5	0.5	3	-	-
	Nystatin	0.5–1	1	1	3	-	-
C. dubliniensis(2)	Fluconazole	0.06–0.5	-	-	2	-	-
	Itraconazole	0.03–0.25	-	-	-	2	-
	Voriconazole	0.03	-	-	2	-	-
	Miconazole	0.06–0.25	-	-	2	-	-
	Amphotericin B	0.12–0.25	-	-	2	-	-
	Nystatin	0.5	-	-	2	-	-
C. kefyr(1)	Fluconazole	0.5	-	-	1	-	-
	Itraconazole	0.5	-	-	-	1	-
	Voriconazole	0.03	-	-	1	-	-
	Miconazole	0.03	-	-	1	-	-
	Amphotericin B	0.25	-	-	1	-	-
	Nystatin	2	-	-	1	-	-
C. parapsilosis(2)	Fluconazole	0.5–2	-	-	2	-	-
	Itraconazole	0.06–1	-	-	1	-	1
	Voriconazole	0.03–0.25	-	-	1	1	-
	Miconazole	0.25–2	-	-	2	-	-
	Amphotericin B	0.06–0.25	-	-	2	-	-
	Nystatin	0.5–2	-	-	2	-	-
Lodderomyces elongisporus(2)	Fluconazole	0.125–0.25	-	-	2	-	-
	Itraconazole	0.06–0.12	-	-	2	-	-
	Voriconazole	0.03–0.06	-	-	2	-	-
	Miconazole	0.03–0.06	-	-	2	-	-
	Amphotericin B	0.03–0.12	-	-	2	-	-
	Nystatin	0.25–1	-	-	2	-	-

MIC_{50}, concentration capable of inhibiting the growth of 50% of the isolates; MIC_{90}, concentration capable of inhibiting 90% of the isolates; *S*, susceptible; *SDD*, susceptible dose dependent; *R*, resistant

The high viral load (> 100,000 copies of the virus) in the majority of the participants and a low number of CD4 T lymphocytes (≤ 200 cells/mm^3) showed that most participants with OPC were in a severe state of immunosuppression. CD4 T lymphocytes are necessary to protect the oral cavity against infection by this commensal microbe [32, 36, 37].

All the risk factors studied can lead to individual immunosuppression, providing a greater chance of OPC. When comparing the risk factors with the presence of yeasts in the oral mucosa, high rates of positivity were observed. These factors were found by other authors [9, 29, 38]. However, the only statistically significant risk factor found in this study was the use of illicit drugs. The oral health condition is determined by the quality and lifestyle of individuals. In this way, carelessness with hygiene together with the interaction of the chemicals that make up the drugs have the ability to influence the oral ecosystem creating a favorable environment for the proliferation of yeasts [39].

Identifying *Candida* spp. is important in epidemiological studies and can help in therapy and clinical decision-making. In this study, *C. albicans* was the predominant species, but we verified the presence of non-*albicans Candida* in 25.5% of the samples, showing that other agents are increasing. *C. albicans* constitutes the majority of fungal species isolated from the oral cavity, which may be due to its several virulence factors, such as adhesins for adherence to host tissues, biofilm formation, and secretion of hydrolytic enzymes [40]. In addition, *C. albicans* can change its morphology, converting itself from a single-celled yeast to pseudo-hyphae or hyphae. Hyphae growth plays an important role in tissue invasion and resistance to phagocytosis [40]. According to Zuza-alves et al. (2017) [41], *C. tropicalis* also shares these virulence factors and was the second most isolated species in our study.

The coexistence of two or more clinical species together, as evidenced in our study, has also been reported by other authors [4, 36, 42]. The co-infection of two or three different *Candida* spp. in the PLWHA population may contribute to the azole resistance profile, causing recurrence of oral infection in these individuals [43]. Oropharyngeal infections associated with *C. glabrata* tend to be more severe and refractory to treatment than candidiasis caused by *C. albicans* alone [5, 32, 36].

In addition to *Candida* species, two species of *Loddero-myces elongisporus* were found, identified by DNA sequencing, causing infection in the oral mucosa in PLWHA. This species is an emerging pathogenic yeast associated with bloodstream infections, including fungal endocarditis and catheter infections [44]. This yeast, rarely found to cause infection, can be misidentified by commercial yeast identification systems such as VITEK 2 as *C. parapsilosis* [45]. This misidentification is common due to the physiological similarities and phylogenetic relatedness between these species [46]. The CHROMagar® chromogenic test, routinely used in the laboratory, shows *L. elongisporus* as blue colored colonies, which can be easily confused with *C. tropicalis*. There are no reports in the literature about *L. elongisporus* species causing infections in the oropharyngeal region and little is known about their virulence attributes or their environmental niche. However, some factors have been pointed out to the increase in its occurrence, such as prolonged hospitalization time, mainly in intensive care units, administration of multiple broad-spectrum antibiotics, use of life support systems, and prolonged use of intravascular catheters [47]. The detection of this yeast is important so that it is possible to follow its evolution, since it is possible for this yeast to take advantage of the selection pressure created by the prophylactic and therapeutic use of antifungal agents, resulting in increased colonization and invasive infection [48]. The lack of accurate identification and little experience in the management of these rare fungal infections may pose future diagnostic and therapeutic challenges, which could lead to higher mortality rates.

The use of antifungal agents as prophylactic therapy is high in hospital settings. In this study, most individuals (88.6%) who used one or more types of antifungal agents, including fluconazole, itraconazole, nystatin, and amphotericin B, had OPC (Table 2). Fluconazole is generally considered the drug of choice for the treatment and prophylaxis of OPC infections in PVWHA [49].

Although most *Candida* spp. are susceptible to azoles and polyenes, the antifungal susceptibility varies significantly between *C. albicans* and NAC [5]. Among the *Candida* spp. isolated, *C. albicans* was more sensitive to azoles compared to NAC species. This agrees to the fact that many NAC species are resistant to these antifungals, particularly fluconazole and itraconazole[49]. The high rate of resistance to itraconazole and miconazole found in this study is possibly due to cross-resistance to fluconazole, since all azoles exert the same mechanism of action. Fluconazole was routinely administered to PLWA with clinical symptoms of candidiasis without prior testing of in vitro antifungal sensitivity. Thus, the increase number of resistant *Candida* spp. may occur because of prolonged or constant treatment with azole antifungals.

Similar to the results of Alves et al. (2006) [50] who showed that 96% of isolates from PLWHA were susceptible to amphotericin B here was found that 98% of isolates were susceptible to this drug. Despite being a long-lasting antifungal agent, resistance to amphotericin B is still rare. This drug binds to ergosterol in the fungal membrane, disrupting the membrane structure and function, thereby exhibiting fungicidal activity [51]. In this study, all isolates were sensitive to nystatin. In 2014, the World Health Organization recommended that topical therapy with nystatin suspension or lozenges would be an alternative to oral fluconazole for

the treatment of OPC in PLWHA [52, 53]. Although not routinely used, this drug may be effective for the treatment of OPC.

The results obtained in this study are important because they show a variable resistance profile among the different strains and also the need to identify the *Candida* spp. involved in the infection. Therefore, the precise identification of the species and knowledge of the antifungal profile are important in the treatment.

In summary, although *C. albicans* remains the most common agent of OPC in PLWHA, the other *Candida* species and their resistance to antifungal agents deserve attention in the epidemiology of OPC in Brazil, particularly in the state of Goias. The incidence of resistant *Candida* species and co-infection with other species in OPC increases the potential risk of complicated infections. Amphotericin B and nystatin are not routinely used but may be suggested for the treatment of patients who are refractory to treatment with other drugs. Identifying clinical samples and determining the minimum concentration to inhibit the isolate help predict the appropriate therapy for PLWHA.

Author contribution Freitas, V.A.Q., experimental part and writing of the work. Santos, A. S.: responsible for in vitro susceptibility. Zara, A. L. S. A.: responsible for statistical data. Costa, C. R.:, Godoy, C. S. M., and Soares, R. B. A.: assistance in data collection. Ataídes, F. S.: assistance in the experimental part. Silva, M. R. R.: writing and reviewing the work.

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Declarations

Competing interests The authors declare no competing interests.

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