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Short Communication

Clinical and laboratorial features of oral candidiasis in HIV-positive patients

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Abstract

Introduction: We describe the clinical and laboratorial features of oral candidiasis in 66 HIV-positive patients. **Methods:** Polymerase chain reaction-based techniques were performed for differentiation of *Candida* spp. isolated from patients at a public teaching hospital in Midwest Brazil. **Results:** Oral lesions, mainly pseudomembranous, were significantly related to higher levels of immunosuppression. Of 45 *Candida* isolates, 66.7% were *C. albicans*. Most of the isolates were susceptible to the antifungal drugs tested. **Conclusions:** Oral lesions were associated with higher immunosuppression levels. Lower susceptibility to antifungals by non-albicans isolates supports the importance of surveillance studies using susceptibility tests to aid in the treatment.

Keywords: *Candida* spp. HIV. Antifungal susceptibility.

Oral candidiasis is the most common opportunistic fungal infection in individuals infected with the human immunodeficiency virus (HIV) and is considered an independent predictor of immunodeficiency in patients with acquired immunodeficiency syndrome (AIDS)[1,2]. In addition, esophageal candidiasis is considered to be an AIDS-defining condition[3]. Clinical features of oral thrush and esophageal candidiasis comprise white oral patches; oral ulcers and painful swallowing may also be observed[4].

The *cluster of differentiation 4+* **(**CD4+) T-lymphocyte count in peripheral blood is an important marker of the patient's immune status and of their susceptibility to the development of opportunistic infections, such as oropharyngeal candidiasis[5]. Although *Candida albicans* is the most frequently-isolated species related to oral opportunistic infections, an increase in the frequency of oral infections caused by non-*albicans* species such as *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Candida krusei* and *Candida dubliniensis* has been observed in the last decade[1,2].

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Identification of the species is important for epidemiological reasons and for treatment purposes to ensure a better prognosis since some species present reduced susceptibility to azoles[2,4,5]. Therefore, the present study aimed to investigate the correlation between the presence of oral lesions caused by *Candida* spp. with the level of CD4+ T-lymphocyte in HIV-positive patients, and to determine the distribution and antifungal susceptibility of *Candida* species isolated from the oral cavities of HIV-positive patients, with and without lesions.

A total of 60 seropositive HIV patients [diagnosed by enzyme-linked immunosorbent assay (ELISA) and Western-blot] admitted at the University Hospital Maria Aparecida Pedrossian (UH-MAP), Mato Grosso do Sul, Brazil, were included in this study. The participants were recruited from December 2014 to July 2015 and data such as age, gender, hospitalization, the presence of oral lesions and CD4+ T-lymphocyte counts were obtained from their medical records. To compare the possible associations between the variables under study, the chi-square test and Fisher's exact test were applied, as appropriate. Statistical analyses were performed using the program Minitab, version 16 (Minitab Inc., State College, PA, USA).

Samples collected via oral swab were inoculated in Sabouraud-dextrose broth (Himedia, India). After 24h at 35ºC,

the samples were plated in Sabouraud-dextrose agar (Himedia, India) for 24h at 35ºC. Suggestive colonies of *Candida* spp. were then plated in CHROMagar *Candida* (Difco, USA) for 48h at 35°C for presumptive identification. For genotypic identification, genomic deoxyribonucleic acid (DNA) was extracted and purified using the YeaStar™ DNA Extraction Kit (Zymo Research, USA) in accordance with the manufacturer's instructions.

For the initial differentiation among *Candida* species, multiplex polymerase chain reaction technique (PCRm) was performed as described by Li et al.[6]. For the differentiation between *C. albicans* and *C. dubliniensis*, *C. parapsilosis* complex and *C. glabrata* complex, the following techniques were performed respectively: duplex PCR[7], PCR followed by restriction fragment length polymorphism[8] and PCRm[9]. All the primers used in the PCR techniques are described in **Table 1**.

The minimum inhibitory concentrations (MIC) of the antifungal agents fluconazole (Pfizer, Brazil), itraconazole (Sigma, USA), voriconazole (Sigma, USA) and amphotericin B (Sigma, USA) were determined using the broth microdilution method. ATCC strains (*C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019) were used as quality control. The MIC results were interpreted according to the Clinical and Laboratory Standards Institute breakpoints[10].

Ethical considerations

The study protocol was approved by the Research Ethics Committee of the Federal University of Mato Grosso do Sul. All patients who agreed to participate in this study signed a consent form. To our knowledge, this is the first study on oral candidiasis in the State of Mato Grosso do Sul, Midwest Region, Brazil.

The patients' ages ranged from 24 to 76 years, with a mean of 42.3 years, and with male predominance (75.8%). Similar to previous studies[2,4], the most affected age group was 24 to 40 years, which is the most sexually active population. The prevalence of male HIV-positive patients related to oropharyngeal candidiasis had been reported previously[4]. Patients' clinical, demographic and hospital admission data are summarized in **Table 2**.

According to a previous study, variations in the clinical aspects of oral candidiasis have been associated with the progression of HIV infection as the CD4+ T-lymphocyte counts decrease[11]. In our study, 16 (24.2%) out of 66 patients presented oral lesions with the following features:

TABLE 1: Primers used in the molecular identification of *Candida* species.

Primer	Sequence (5' → 3')	Reference
CL	GTTAGGCGTTGCTCCGAAAT	Li et al.[6]
CP	GGCGGAGTATAAAGTAATGGATAG	
CT	AAGAATTTAACGTGGAAACTTA	
CGU	GTATTG GCA TGG GTA GTA CTG	
CA	TCAACTTGTCACACCAGATTATT3	
CK	GAT TTAGTACTACACTGCGTG A	
CGL	CACGACTCGACACTTTCTAATT	
CALF	TGGTAAGGCGGGATCGCTT	Ahmad et al.[7]
CALR	GGTCAAAGTTTGAAGATATAC	
CDUF	AAACTTGTCACGAGATTATTTTT	
CDUR	AAAGTTTGAAGAATAAAATGGC	
S1F	GTTGATGCTGTTGGATTGT	Tavanti et al.[8]
S1R	CAATGCCAAATCTCCCAA	
UNI-5.8S	ACCAGAGGGCGCAATGTG	Romeo et al.[9]
GLA-f	CGGTTGGTGGGTGTTCTGC	
NIV-f	AGGGAGGAGTTTGTATCTTTCAAC	
BRA-f	GGGACGGTAAGTCTCCCG	

CL: *Candida lusitaniae*; **CP**: *Candida parapsilosis complex*; **CT**: *Candida tropicalis*; **CGU**: *Candida guillermondii*; **CA**: *Candida albicans*; **CK**: *Candida krusei*; **CGL**: *Candida glabrata* species-specificity primers; **CALF** + **CALR** and **CDUF** + **CDUR**: species-specificity primers pairs for *Candida albicans* and *Candida dubliniensis* identification, respectively; **S1F** and **S1R**: primer sequences used for identification of species of *Candida parapsilosis complex*; **UNI-5.8S**, **GLA-f**, **NIV-f** and **BRA-f**: primer sequences used for identification of species of *Candida glabrata complex*.

TABLE 2: Distribution of 66 HIV-positive patients according to demographic characteristics, unit of attendance, oral lesions, and immunological evaluation

Variable	Number	Percentage	*p* value*
Gender			< 0.001
male (M)	50	75.8	
female (F)	16	24.2	
Age (years)			< 0.001
24 – 40	36	54.5	
41 – 60	25	37.9	
> 60	5	7.6	
Attendance unit			<0.001
infectious and parasitic diseases/ward	47	71.2	
day-care hospital	17	25.8	
adult intensive care	2	3.0	
Presence of oral lesions			< 0.001
yes	16	24.2	
no	50	75.8	
Type of oral lesions			0.14
pseudomembranous	8	50.0	
erythematous	3	18.8	
chronic multifocal	3	18.8	
angular cheilitis	2	12.5	
Correlation: species *vs* type of interaction			0.15
Infection			
C. albicans	10	33.3	
non-albicans *Candida*	3	20.0	
Colonization			
C. albicans	20	66.7	
non-albicans *Candida*	12	80.0	
Correlation: species *vs* lesion form			0.50
C. albicans			
pseudomembranous	5	83.3	
erythematous	2	66.7	
C. tropicalis			
pseudomembranous	1	16.7	
erythematous	1	33.3	
TCD4⁺ lymphocytes count (cells/µL)			0.03
≤200	42	63.6	
>200	24	36.4	

HIV: human immunodeficiency virus; ***C.***: C*andida;* **TCD4+:** cluster of differentation 4+ T-cell counts**;** *Fisher's exact test.

TABLE 3: *In vitro* antifungal susceptibility of *Candida* species isolated from the oral cavities of HIV-positive patients.

***Candida* species**	**Antifungal agent**	**MICs in µg/mL**	
		MIC range	**MIC50/90***
C. albicans (n= 300)	Fluconazole	0.125 - 0.500	0,125/0,25
	Itraconazole	0.003 - 0.125	0,015/0,06
	Voriconazole	0.015 - 0.06	0,015/0,015
	Amphotericin B	0.06 - 0.5	0,5/0,5
C. tropicalis (n=6)	Fluconazole	0.125 - 0.500	0,5/0,5
	Itraconazole	0.015 - 0.06	0,06/0,060
	Voriconazole	0.015	0,015/0,015
	Amphotericin B	0.5 - 1	0,5/0,5
C. krusei (n=4)**	Fluconazole	-	-
	Itraconazole	0.015 - 0.125	0,03/0,03
	Voriconazole	0.015 - 0.06	0,03/0,03
	Amphotericin B	0.5 - 1.0	1/1
C. glabrata sensu stricto (n=2)	Fluconazole	4.0 - 8.0	
	Itraconazole	0.03 - 0.250	
	Voriconazole	0.06 - 0.125	
	Amphotericin B	1	
*C. parapsilosis sensu stricto (*n=2)	Fluconazole	0.125 - 0.5	
	Itraconazole	0.015- 0.06	
	Voriconazole	0.015	
	Amphotericin B	0.06 - 0.5	
C. dubliniensis (n=1)	Fluconazole	0.125	
	Itraconazole	0.015	
	Voriconazole	0.015	
	Amphotericin B	0.06	

HIV: human immunodeficiency virus; ***C.***: C*andida;* **MIC:** minimum inhibitory concentration as defined by the CLSI; **CLSI**: Clinical and Laboratory Standards Institute. *MIC50 and MIC90: MIC at which 50% and 90% of the isolates were inhibited. ***C. krusei* is intrinsically resistant to fluconazole, independent of the MIC result obtained *in vitro*.

pseudomembranous (8, 50%), erythematous (3, 18.7%), chronic multifocal (3, 18.7%) and angular cheilitis (2, 12.5%). Candidiasis with pseudomembranous lesion as observed in most cases in our study is consistent with the findings of other authors[2,11].

There was no significant correlation between species and type of host-parasite relationship and between species and clinical presentation of oral lesions **(Table 2)**. However, the number of cases was small and these results should be re-evaluated in a larger number of patients.

Among the 16 individuals with oral lesions, 15 (93.7%) had CD4+ T-lymphocyte counts ≤ 200 cells/µL ($p < 0.05$) showing that oral candidiasis is a highly predictive marker of immunosuppression, as previously reported[5,11]. Despite having no apparent lesion, 32 (48.5%) patients had *Candida* spp. isolated from their oral cavities, demonstrating colonization. Previous studies have shown that in Brazil, the rate of colonization has ranged from 50.4% to 62.0% in this group of patients[1,12,13]. The use of antibiotics and oral prostheses are some of the predisposing factors for oral colonization by *Candida* spp.[1].

Among the 45 *Candida* spp. isolates, *C. albicans* (30, 66.7%) was prevalent in relation to non-albicans species (15, 33.3%), confirming the results of previous studies[1,2,4,13-15]. The following non-albicans species were identified: *C. tropicalis* (6, 13.3%), *C. krusei* (4, 8.9%), *C. parapsilosis sensu stricto* (2, 4.4%), *C. glabrata sensu stricto* (2, 4.4%) and *C. dubliniensis* (1, 2.2%).

Studies carried out in São Paulo showed that among non-albicans isolates, *C. glabrata* was the most frequently-isolated species from the saliva of HIV-infected patients without clinical lesions[13] while in other cities of the Southeastern region of Brazil such as São José dos Campos-SP and Uberlândia-MG, the most commonly isolated non-albicans species from oropharyngeal candidiasis were *C. tropicalis*[15] and *C. parapsilosis*[1], respectively. This fact shows that the distribution of non-albicans isolates from the oral cavity varies from region to region

To assist the clinician in making therapeutic decisions and to optimize treatment, clinical microbiologists should identify *Candida* to the species level, especially in HIV-positive patients, in whom non-albicans species are being increasingly recognized to cause serious infections[4].

With the advent of molecular biology techniques, it was possible to differentiate species such as *C. albicans* and *C. dubliniensis*, *C. parapsilosis* complex and *C. glabrata* complex. Although *C. dubliniensis* has been associated with oral candidiasis in HIV infected patients worldwide, in our study, as in other Brazilian studies[12,13], the prevalence rate of this species was considered low.

The *in vitro* susceptibility profiles of the *Candida* species studied are shown in **Table 3**. *Candida albicans, C. tropicalis, C. krusei, C. parapsilosis sensu stricto* and *C. dubliniensis* presented susceptibility to all antifungal agents tested. Two isolates of *C. glabrata sensu stricto* showed dose-dependent susceptibility to fluconazole and one dose-dependent susceptibility to itraconazole.

The pattern of antifungal resistance also varies with geographical region. In contrast to previous studies carried out in other Brazilian regions[12,14], non-albicans species isolated from patients living in Mato Grosso do Sul State, showed a high susceptibility to the antifungal agents tested.

In conclusion, individuals with high levels of immunosuppression are more susceptible to the development of oral candidiasis. The presence of oral lesions caused by *Candida* spp., mainly of the pseudomembranous form, can act as an indirect marker of immunosuppression in HIV-positive patients.

In addition, *Candida* spp. are commonly found among HIV-positive patients with and without oral lesions. Although *C. albicans* is the most frequent species isolated in the oral mucosa, non-albicans isolates represent a relevant percentage of isolates. Despite the good *in vitro* susceptibility, the lower susceptibility to azoles by non-albicans isolates supports the importance of surveillance studies using susceptibility tests to achieve better results in the pharmacological treatment of oropharyngeal candidiasis.

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Conflict of interest

The authors declare that there is no conflict of interest.

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