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Oral carriage of *Candida* species in HIV-infected patients during highly active antiretroviral therapy (HAART) in Belém, Brazil

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Objective. To identify the oral carriage of *Candida* spp in patients infected by human immunodeficiency virus (HIV) and the possible correlation with clinical characteristics.
Study Design. Mucosal swab samples collected from 246 patients who were infected by HIV, did not have oral candidiasis, and were being treated with highly active antiretroviral therapy were analyzed. Yeast colonies that developed were identified by using the VITEK 2 automated system.
Results. *Candida* yeasts were present in 41.87% of the samples, and *Candida albicans* was the most prevalent (32.52%). Other identified *Candida* species were *C tropicalis* (4.88%), *C parapsilosis* (2.85%), *C dubliniensis* (0.81%), and *C famata* (0.81%).
Conclusions. There was low rate of oral *Candida* carriage in patients infected by HIV who were on highly active antiretroviral therapy. A greater prevalence of *C albicans* than non-*albicans Candida* species was found at the species level. Prior candidiasis predicted the oral carriage of *C albicans*; however, it did not influence the carriage of non-*albicans* species. This is the first report of oral carriage of *C famata* in patients with HIV infection. (Oral Surg Oral Med Oral Pathol Oral Radiol 2015; 120:29-33)

Oral candidiasis is the most common fungal infection in immunocompromised patients, infecting approximately 90% of patients with acquired immunodeficiency syndrome (AIDS),[1] and the episodes are often recurrent.[2,3] *Candida albicans* is the leading species of yeast that causes candidiasis, and its treatment is based on antifungal drugs. Because of the high prevalence of candidiasis in patients infected by human immunodeficiency virus (HIV), there is an increase in the use of antifungal drugs to treat these infections. A significant increase in drug resistance has been observed, as a result of the increased prevalence of non-*albicans Candida* species, which reportedly do not respond to conventional antifungal therapy.[4-6] Because candidiasis is caused by *Candida* spp that normally colonize the mucosal surfaces, the risk of the development of non-*albicans* candidiasis may be related to its oral carriage, which has been incompletely characterized and may vary in different locations.

AIDS causes deterioration of the immune system and predisposes affected individuals to a number of complications, such as the development of cancer,[7] renal and cardiovascular diseases, lipodystrophy, changes in bone metabolism and vitamin D deficiency, and a variety of infectious diseases, such as pneumonia and tuberculosis.[8] Patients with HIV exhibit several oral manifestations, many of which reflect immunodeficiency. A very common finding in patients with HIV is oropharyngeal candidiasis.[9]

Candida spp is a family of saprophytic microorganisms, which, depending on predisposing factors, may become pathogenic and cause candidiasis.[3] There are several types of oral manifestations of candidiasis, including pseudomembranous, erythematous, hyperplastic, and mucocutaneous candidiasis and angular cheilitis.[6,10,11]

The treatment of oral candidiasis is widely discussed in the literature as a result of increasing drug resistance

Statement of Clinical Relevance

Patients infected by human immunodeficiency virus and have undergone highly active antiretroviral therapy show low rates of carriage of oral *Candida*, with predominance of the *Candida albicans* species. However, they may present with novel non-*albicans* species, such as the *C famata* identified in this Brazilian cohort.

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of various strains of *Candida*, especially in patients with HIV.[10,12] A small number of antifungal agents are used to treat candidiasis, including polyenes, such as nystatin and amphotericin B; azoles, including ketoconazole, itraconazole, and fluconazole; and newer azoles, such as voriconazole and posaconazole.[3,13] The development of azole antifungals was a great advance in the treatment of fungal infections, but their widespread use in the treatment of immunocompromised patients has increased the number of strains resistant to these antifungal agents, mainly the non-*albicans* *Candida* species.[14]

Since the spread of the AIDS epidemic in the early 1980s, the treatment for HIV infection has evolved considerably with the development of antiretroviral therapies. Highly active antiretroviral therapy (HAART) is now the gold standard for treating the disease and has increased the survival rates and improved the quality of life of patients with HIV.[15] HAART has reduced the occurrence of opportunistic infections, although candidiasis continues to affect many of these patients (30.1% to 37%).[16,17] These patients still have a lower prevalence (30.1%) compared with those patients who have not undergone HAART (69.1%).[16]

Given the large number of patients with HIV and the strong association with the development of candidiasis, as well as the increase in the morbidity and mortality among patients with HIV, this study aimed to identify the different species of *Candida* found in the oral cavity of patients on HAART who have not yet developed AIDS. Furthermore, we tested the relationship of the demographic and clinical characteristics, such as gender, previous episodes of candidiasis, and CD4+ cell count, which could act as predisposing factors for the oral carriage of *Candida* spp. The identification of *Candida* species colonizing the oral cavity and the possible predisposing factors may indicate a shift in the species composition and the trend of species currently found in the oral cavity of patients with HIV.

MATERIALS AND METHODS

Samples for mycology were collected by using oral mucosal swabs from 246 patients with HIV (confirmed by enzyme-linked immunosorbent assay and Western blot analysis) treated at a specialized center for treatment of infectious and parasitic diseases (URE-DIPE) in Belém, Brazil. This unit provides care to patients with HIV from across the state of Pará, northern Brazil. All patients were informed of the study objectives, and informed consent forms were signed. This study was approved by the Ethics Committee in Research of the Institute of Health Sciences, Federal University of Pará (ICS-UFPA), protocol number 120/09 CEP-ICS/UFPA. All patients who agreed to take part in this study underwent oral clinical examination by 1 examiner (experienced in special care dentistry), who assessed the patients for signs and symptoms of oral candidiasis, according to the following criteria: burning sensation, buccal pain, dysphagia, erythema with or without soreness, white patches, redness, fissuring, and soreness at the angle of the mouth. Adult patients over 18 years of age who were on HAART and did not show clinical signs or symptoms of oral candidiasis or the active phase of AIDS were included in this study.

Isolation and identification of *Candida* species

Samples were collected by using sterile swabs from the buccal mucosa of the oral cavity, plated in duplicate in 15 × 160 mm test tubes, and sealed with hydrophobic buffer containing 5 mL of sabouraud dextrose agar with chloramphenicol (Merck KGaA, Darmstadt, Germany). All samples were processed in the Laboratory of Bacteriology and Mycology of the Instituto Evandro Chagas IEC/SVS/MS.

Yeast colonies that developed a moistened appearance and white-yellowish color were kept in the mycology unit at room temperature for 15 days. *Candida* species were identified by using the automated Vitek 2 system and Vitek 2 ID-YST card for identifying clinically important yeasts and yeast-like organisms (BioMérieux, Grenoble, France).

Statistical analysis

Data were analyzed with the use of BioEstat 5.0 software (BioEstat Software, Belém, PA). Multiple logistic regression was used to analyze correlation between three different groups (*Candida* spp, *Candida albicans*, and non-*albicans* *Candida*) and the following predisposing factors: previous candidiasis, $CD4^{+}$ count <200, and gender.

RESULTS

Of the 246 patients with HIV included in this study, 46.34% were female and 53.66% male. Patients' ages ranged between 18 and 66 years, and the average age was 39 years. Of the strains that were grown, 103 were of the genus *Candida*; the majority of them were *C albicans* (77.67%). Among the non-*albicans* species in the oral cavity, *C tropicalis* (52.17%), *C parapsilosis* (30.43%), *C dubliniensis* (8.7%), and *C famata* (8.7%) were identified (Table I).

We tested whether gender, CD4+ cell count, and prior occurrence of candidiasis were related to the presence or absence of *Candida* in the oral cavity. Multiple logistic regression analysis was performed to assess this association. Patients who had a previous candidiasis infection showed twofold higher odds for

Table I. Identification of *Candida* species found in oral cavity of 246 patients infected by human immunodeficiency virus (HIV) in treatment with highly active antiretroviral therapy (HAART)

Description	*Species*	*No.*	*%*
General sample	Not found	143	58.13
	Candida albicans	80	32.52
	C tropicalis	12	4.88
	C parapsilosis	7	2.85
	C dubliniensis	2	0.81
	C famata	2	0.81
Total	-	246	100
Candida spp	*C albicans*	80	77.67
	Candida non-*albicans*	23	22.33
Total	-	103	100
Candida non-*albicans*	*C tropicalis*	12	52.17
	C parapsilosis	7	30.43
	C dubliniensis	2	8.70
	C famata	2	8.70
Total	-	23	100

carriage of *Candida* spp and *C albicans*. Statistically significant differences were not found between carriage of non-*albicans* species of *Candida* and any of the variables studied (Table II).

DISCUSSION

Oral carriage of *Candida* species is relatively common in individuals with HIV, affecting approximately 62% to 67% of them.[18-20] Our results showed that 41.87% of the samples collected from the oral cavities of patients with HIV, grew *Candida* colonies. Of these, approximately 77.67% were *C albicans*, which was more frequent than the reported prevalence in the literature (32% to 62%).[10,18,19] Our results suggest a low prevalence of oral cavity colonization by *Candida* spp (41.87%) as well as by non-*albicans Candida* species (9.35%).

The advent of HAART has allowed for the suppression of viral replication to very low levels and a partial recovery of CD4+ cells in patients with HIV, which has consequently reduced opportunistic infections.[20,21] The low prevalence of oral carriage of *Candida* spp found in this study may reflect the fact that the Brazilian government currently guarantees free and universal access to HAART for all patients with HIV.[22] Despite the relatively low level of asymptomatic oral carriage of *Candida* in our study (41.87%), the prevalence is, nevertheless, greater than the average (34.4%) reported in a healthy population not infected by HIV.[23] This study showed that patients who had previous candidiasis have a high probability of asymptomatic oral carriage of *Candida* spp and *C albicans*.

We analyzed factors that could influence the oral carriage of *Candida* in patients with HIV. The most important factor was a prior infection by *Candida*. This was the unique statistically significant predisposing factor present in this study, although a low count of CD4+ cells was an important contributor to increase the probability of oral *Candida* carriage. A CD4+ cell count less than 200 cells/μL is considered a predisposing factor for candidiasis.[24] However, our results indicate that only a low CD4+ cell count in patients on HAART is not associated to high risk of oral *Candida* carriage.[18,20]

We hypothesized that patients who had had previous episodes of candidiasis would be more prone to oral carriage of non-*albicans Candida* due to the selection mechanisms and resistance of the yeast species. However, we found that patients who had previous candidiasis were more prone to oral carriage of *C albicans* than the non-*albicans Candida* species. Furthermore,

Table II. Multiple logistic regression for oral carriage of *Candida* in patients infected by human immunodeficiency virus (HIV) in treatment with highly active antiretroviral therapy (HAART)

	Candida *spp*				
Predisposing factors	*RC (b)*	*SE*	*OR*	*CI 95%*	*P*
Previous candidiasis	0.6558	0.2790	1.9267	1.12-3.33	.0187
CD4+ <200	0.4257	0.3311	1.5307	0.80-2.93	.1985
Male	−0.1396	0.2641	0.8697	0.52-1.46	.5972
Predisposing factors	*Candida albicans*				
Previous candidiasis	0.6992	0.2992	2.0120	1.12-3.62	.0195
CD4+ <200	0.4791	0.3526	1.6146	0.81-3.22	.1742
Male	−0.2049	0.2861	0.8147	0.47-1.43	.4738
Predisposing factors	non-*albicans Candida*				
Previous candidiasis	0.5034	0.4730	1.6544	0.65-4.18	.2872
CD4+ <200	0.2867	0.5633	1.3321	0.44-4.02	.6107
Male	0.0297	0.4566	1.0302	0.42-2.52	.9481

RC, regression coefficient; *SE*, standard error; *OR*, odds ratio; *CI*, confidence interval.

the presence of species of non-*albicans Candida* was not influenced by prior infection or any other variable studied. Only three patients who presented with oral carriage of non-*albicans Candida* had had previous episodes of candidiasis and remembered their treatment. Two of them who had used nystatin, showed oral carriage of *C tropicalis* and *C parapsilosis*, and the third, who reported the use of fluconazole, demonstrated oral carriage of *C parapsilosis*.

The results of this study demonstrate that oral carriage of non-*albicans Candida* species in patients with HIV is lower than that reported in the literature. These data may reflect 2 factors. The first is free and universal access to HAART offered by the Brazilian government, which ensures a gold standard treatment to the whole population. The second factor is the lack of uniformity in the treatment of candidiasis.

Despite the prevalence of oral carriage of *C albicans* seen in this study, 23 cultures (9.35%) grew non-*albicans Candida* species. Among these, *C tropicalis* was the most common (12 cases), which was followed by *C parapsilosis*, *C dubliniensis*, and *C famata*. Colonization by species of *C tropicalis*, *C parapsilosis*, and *C dubliniensis* is a relatively common finding, with prevalence varying by study location.[10,25,26] Unexpectedly, we found two cases of *C famata*, which is a rare human pathogen that is commonly found in natural sources or in processed food products.[27] These two cases were found in men aged 31 and 38 years who had never had a previous episode of candidiasis.

C famata belongs to the group of flavinogenic yeasts that can oversynthesize riboflavin when they are iron deficient. Some strains of *C famata* belong to the most flavinogenic organisms, and these have long been used for the industrial production of riboflavin.[27] In a multicentric study on the epidemiology of candidiasis in Latin America, 672 cases of candidiasis were studied, and only a single case of *C famata* was found.[25] Another study with 300 Turkish children found only 2 cases of oral *C famata* in a specific 6-8-year-old age group.[28] We found a rare colonization of *C famata*, the first to be reported in patients infected by HIV according to the literature. Studies have shown that *C famata* is less sensitive to fluconazole and itraconazole and has intermediate susceptibility to amphotericin B and that posaconazole and voriconazole are the most effective inhibitors of *C famata* strains.[29]

We found that the main species of *Candida* in the oral cavity of asymptomatic patients with HIV (*C albicans*, *C tropicalis*, *C parapsilosis*, and *C dubliniensis*) accounted for 98.06% of all yeasts grown. According to the literature, these species are associated with low resistance to the main antifungal drugs (ketoconazole, itraconazole, and amphotericin B),[19,20,25,29] and this suggests that empirical therapy with any of these 3 drugs in this population should prove to be efficient and effective treatment.

Therefore, we conclude that there was a low carriage of *Candida* in the oral cavity of patients with HIV and a higher prevalence of *C albicans* than non-*albicans* species of *Candida* in comparison with similar studies. These findings may be the result of the free and universal access to HAART for AIDS treatment in Brazil. The previous occurrence of candidiasis is a predisposing factor for the oral carriage of *C albicans* but not the carriage of non-*albicans Candida* species. This suggests that previous candidiasis does not shift the species composition of the oral cavity to more aggressive and drug-resistant non-*albicans Candida* species in the studied population. According to the consulted literature, this was the first report of carriage of *C famata* in the oral cavity of patients with HIV.

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