

# *Candida* species isolated from the vaginal mucosa of HIV-infected women in Salvador, Bahia, Brazil

## ABSTRACT

**Background:** Vulvovaginal candidiasis (VVC) is the second most common vaginal infection. HIV-infection is a risk factor for this infection. **Objective:** To determine the frequency of VVC and to describe the main *Candida* species isolated and their susceptibility to antifungal drugs in HIV-infected patients, compared to HIV-uninfected women in Salvador, Brazil. **Methods:** Cross-sectional study including a group of 64 HIV-infected women and 76 uninfected women, followed up at the AIDS reference center and at the Gynecological Clinic of *Escola Bahiana de Medicina e Saúde Pública* (Salvador, Bahia, Brazil). **Results:** Frequency of *Candida* spp. was higher in HIV-infected women (29.7%) than in HIV-uninfected controls (14.5%) ( $p = 0.02$ ). The *odds ratio* value for vulvovaginal candidiasis in HIV-infected patients was 2.6 (95% CI: 1.07 - 6.32  $p = 0.03$ ). *Candida albicans* was the most commonly isolated species in both HIV-infected (52.3%) and uninfected women (85.7%), followed by *C. parapsilosis* in 17.6% and 14.3%, respectively. In HIV-infected women, *C. glabrata*, *C. parapsilosis*, and a coinfection of *C. albicans* and *C. glabrata* were also identified. There was no significant difference between *Candida* species isolated from the vaginal mucosa of women with VVC and colonization of the vaginal mucosa of HIV-infected and HIV-uninfected women. One *C. glabrata* isolate from an HIV-infected patient was resistant to fluconazole and other two isolates exhibited a dose-dependent susceptibility. **Conclusion:** Our results confirm a higher frequency of *Candida* spp. isolated from the vaginal mucosa of HIV-infected women and a broader spectrum of species involved. Only *Candida glabrata* isolates showed decreased susceptibility to fluconazole.

**Keywords:** HIV; *Candida*; candidiasis, vulvovaginal.

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## INTRODUCTION

Vulvovaginal candidiasis (VVC) is one of the most frequent vaginal infections in the world.<sup>1,2</sup> It is estimated that 75% of women will have VVC at least once in their lifetime.<sup>3,4</sup> Clinical signs and symptoms of this infection include itching, white discharge, edema, and erythema of the vulva.<sup>4-6</sup> *Candida albicans*, a saprophyte present in the vaginal mucosa, is isolated in 80% of VVC cases.<sup>7,8</sup> Factors which predispose to VVC include: pregnancy, uncontrolled diabetes *mellitus*, corticosteroids or systemic/vaginal antibiotic therapy, HIV infection, and *Candida* vaginal colonization (CVC).<sup>9,10</sup>

The frequency of VVC caused by other *Candida* species, such as *Candida tropicalis*, *Candida glabrata*, and *Candida krusei* is increasing,

especially in HIV-infected women.<sup>5,8,11</sup> *C. albicans* and *C. glabrata* are responsible for the majority of VVC cases in HIV-infected women.<sup>10,12</sup> Although resistance to azoles antifungal therapy is rare in *C. albicans* isolates (1%), it is becoming commonplace among *C. glabrata* isolates (up to 15%) as well as among other non-*albicans* species.<sup>5,10,13,14</sup>

Few studies in Brazil have assessed the prevalence of VVC in HIV-infected women undergoing highly active antiretroviral therapy (HAART) treatment.<sup>15</sup> The aim of this study was to determine the frequency of VVC and to describe the main *Candida* species isolated in HIV-infected patients, compared to HIV-uninfected women in Salvador, Brazil. In addition, the susceptibility of *Candida* species to antifungal drugs was evaluated.

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We declare no conflict of interest.

## MATERIAL AND METHODS

### Study population and procedure

A group of sixty-four HIV-infected women receiving care at the AIDS reference center of Bahia (*Centro Especializado em Diagnóstico, Assistência e Pesquisa*, CEDAP) and 76 HIV-uninfected women from the Gynecology Clinic of *Escola Bahiana de Medicina e Saúde Pública* (Salvador, Bahia, Brazil) were included in this study, between May 2006 and May 2007.

Patients were sequentially enrolled at the moment of the medical appointment. Inclusion criteria were women aged more than 18 years old, sexually active, positive serology for HIV (HIV group) or negative (control group). Exclusion criteria were: pregnancy or *post-partum*, intra-vaginal treatment thirty days prior to vaginal collection, vaginal bleeding, sexual intercourse or vaginal douching within the 48 hours preceding vaginal sample collection.

The study was approved by the Institutional Research Boarding of the *Fundação Oswaldo Cruz* Bahia (Fiocruz - Bahia). All patients signed an informed consent form prior to admission.

### Specimen collection

Demographic and clinical data were obtained by specific standardized data collection forms. CD4+ T lymphocytes count and HIV viral load values were obtained from medical records. Vaginal samples were collected with sterile swabs, during gynecological examination. Samples were cultured on Sabouraud's agar (BD-Difco), incubated at 35°C for 48 h, and a direct examination was performed. Yeasts isolates were sub-cultured on CHROMagar *Candida* (Beckton Dickinson) in order to identify *C. albicans* and mixtures of yeasts. All *C. albicans* isolates were screened with Bichro-Dubli® (Fumouze) in order to identify *C. dubliniensis*. Other *Candida* species were identified with ID32CAux® (BioMérieux). Minimal inhibitory concentrations (MICs) were determined by the E-Test® method with *C. parapsilosis* ATCC 22019 as control. After 24 hours of incubation, an elliptical zone of inhibition was produced and the point at which the ellipse meets the strip gives a reading for the MIC of the drug.

*Candida* vaginal colonization was defined as culture isolation of yeasts from asymptomatic women. Women with a positive culture were considered to have VVC if they reported symptoms (vaginal itching, burning, and/or discharge) at the time of the medical appointment.

### Statistical analysis

Frequencies of *Candida* spp. of HIV-infected women were compared to those of the control group by using *t* tests for continuous variables and Chi-square tests or Fisher's exact test for categorical variables. A mul-

tiple logistic regression model was used to evaluate the prediction capacity of each independent variable in the occurrence of the expected condition. Unadjusted *odds ratios* (ORs) were initially calculated to screen for inclusion in the multivariate model; variables that exhibited at least moderate association ( $p = 0.10$ ) with the outcome in the presence of these design variables were considered for inclusion in the final models. Statistical analysis was done with the SPSS (Statistical Package for the Social Sciences) 13.0.

## RESULTS

The median age of HIV-infected patients was similar to that of the uninfected control group. Age at time of first sexual intercourse was lower in HIV-infected women ( $16.2 \pm 4.0$  years) compared to the control group ( $17.5 \pm 3.9$  years) [ $p = 0.05$ ]. The number of lifetime sexual partners and the frequency of reported sexually transmitted diseases were significantly higher in the HIV-infected group, compared to uninfected patients. Thirty-four HIV-infected women (56.3%) were treated with HAART. The mean CD4+ T lymphocytes count was  $644 \pm 551$  cells/mm<sup>3</sup> and mean HIV viral load was  $3.9 \pm 4.3$  log<sub>10</sub> copies/mL.

*Candida* spp. isolation from the vaginal mucosa was more frequent in HIV-infected patients (29.7%), compared to uninfected women (14.5%) ( $p = 0.02$ ). However, the frequency of VVC and *Candida* spp. colonization were similar in both HIV-infected and uninfected women ( $p = 0.95$ ) (Table 1). The *odds ratio* value for the presence of *Candida* spp. in vaginal mucosa of HIV-infected patients was 2.6 (95% CI: 1.07 – 6.32,  $p = 0.03$ ), after adjusting for variables (Table 2).

*Candida* spp. identification was possible in 24 vaginal samples (17 from HIV-infected patients and 7 from uninfected women) (Table 3). *C. albicans* was the most frequent species isolated in both HIV-infected (52.9%) and uninfected women (85.7%). In HIV-infected women were also identified the following species: *C. glabrata*, *C. parapsilosis*, *C. dubliniensis* and a coinfection of *C. albicans* and *C. glabrata*. In the HIV-uninfected group, the only species isolated were *C. albicans* and *C. parapsilosis*.

In both HIV-infected and uninfected women, there was no significant difference observed in the frequency of a particular species of *Candida* isolated from the vaginal mucosa and the presence of VVC, nor was there a difference in the frequency of a particular species of *Candida* colonizing the vaginal mucosa (Table 4).

Only one out of 24 yeast isolates, an isolate of *C. glabrata* from an HIV-infected patient, was resistant to fluconazole (MIC > 256 mg/L). Two *C. glabrata* isolates exhibited a dose-dependent susceptibility to fluconazole (MIC = 24 and 48 mg/L) (Table 5).

**Table 1. Demographic characteristics and frequency of *Candida* spp. in HIV-infected and HIV-uninfected patients**

	HIV-infected group (n = 64)		HIV-uninfected group (n = 76)		p
	n		n		
Age (years) <sup>#</sup>	64	30.4 ± 6	76	28.7 ± 6	0.07
<b>Marital status (n, %)</b>					
Married/cohabiting	34	53.1	47	61.8	0.29
Unmarried/non-cohabiting	30	46.9	29	38.2	0.29
<b>Education (n, %)</b>					
< 8 years	40	62.5	26	34.2	0.001
> 8 years	24	37.5	50	65.8	0.001
<b>Family income (n, %)</b>					
< \$ 240/month	48	75	34	44.7	< 0.001
≥ \$ 240/month	16	25	42	55.3	< 0.001
<b>Sexual and obstetric history</b>					
Age of first intercourse <sup>#</sup>	64	16.2 ± 4.0	76	17.5 ± 3.9	0.05
No. of partners	64	8.3 ± 13.4	76	3.6 ± 4.3	< 0.01
STD history (n, %)	26	40.6 %	10	13.2	< 0.01
No. of deliveries	64	1.7 ± 1.2	76	1.3 ± 1.2	0.02
Users of HAART	38	59.4%	-	-	NA
T CD4+ count	64	644 ± 551	-	-	NA
Viral load	64	3.9 ± 4.3	-	-	NA
Vaginal <i>Candida</i> spp. (n)	19	29.7	11	14.5	0.02
<i>Candida</i> spp. colonization	5	26.3	3	27.3	0.95
VVC	14	73.7	8	72.7	0.95

SD, standard deviation; NA, not applicable. STD, sexually transmitted disease. HAART, highly active antiretroviral therapy; CD4+ cells/mm<sup>3</sup>; viral load: log<sub>10</sub> copies/mL. p < 0.05,  $\chi^2$  test, Student's *t* test.

<sup>#</sup> mean ± SD

**Table 2. Adjusted odds ratio for *Candida* spp. presence in HIV infected women**

Variables	Adjusted OR	IC 95%	p
HIV infection	2.61	1.07-6.32	0.03
Age	1.00	0.92-1.08	0.98
Years of schooling	0.89	0.78-1.02	0.1
Family income	0.97	0.7-1.33	0.86
First sexual intercourse	1.02	0.91-1.14	0.73
Condom use	0.82	0.27-2.43	0.72
Number of sexual partners	0.99	0.95-1.04	0.9
STD history	1.00	0.36-2.75	0.99

Adjusted variables: HIV infection, age, years of schooling, family income, first sexual intercourse, condom use, number of sexual partners, STD history. OR, odds ratio. p < 0.05. Multiple logistical regression.

## DISCUSSION

This study assessed for the first time the prevalence of VVC and the main *Candida* species found in vaginal mucosa of HIV-infected women, in Salvador-Bahia, Brazil. The main finding of our study was the higher frequency of *Candida* spp. in vaginal mucosa of HIV-infected women than HIV-uninfected, but the frequency of VVC was similar in both groups. These results were expected since the majority of the HIV-infected patients enrolled in this study were on HAART and had CD4+ T-cell counts higher than 500 cells/mm<sup>3</sup>. Indeed, the risk of colonization with *Candida* species in HIV-infected women with CD4+ T-cell counts below 200 cells/mm<sup>3</sup> is three times higher, compared to immunocompetent HIV-infected or HIV-uninfected women.<sup>16,17</sup> Similar prevalence of VVC (23.5%) was found in patients from a cohort of 458 HIV-infected patients under antiretroviral therapy followed in Rio de Janeiro-Brazil. However, the authors did not identify species of *Candida* and there was no

Table 3. Frequency of *Candida* species isolated from the vaginal mucosa of HIV-infected and uninfected women

Isolated species	HIV-infected women n = 17		HIV-uninfected women n = 7		p = 0.56
	n	%	n	%	
<i>C. albicans</i>	9	52.9	6	85.7	
<i>C. parapsilosis</i>	3	17.6	1	14.3	
<i>C. glabrata</i>	3	17.6	0	0	
<i>C. dubliniensis</i>	1	5.9	0	0	
<i>C. albicans</i> and <i>C. glabrata</i>	1	5.9	0	0	

p < 0.05,  $\chi^2$  test.

Table 4. Frequency of vulvovaginal candidiasis and colonization with *Candida* species from the vaginal mucosa of HIV-infected and uninfected women

<i>Candida</i> species	HIV-infected n = 17		HIV-uninfected n = 7	
	VVC	Colonization	VVC	Colonization
	n	n	n	n
<i>C. albicans</i>	7	2	5	1
<i>C. parapsilosis</i>	2	1	1	0
<i>C. glabrata</i>	1	2	0	0
<i>C. dubliniensis</i>	1	0	0	0
<i>C. albicans</i> + <i>C. glabrata</i>	1	0	0	0

VVC, vulvovaginal candidiasis. p < 0.05,  $\chi^2$  test, Fisher's exact test.

Table 5. Minimal inhibitory concentrations (mg/L) of *Candida* spp.

<i>Candida</i> species n = 25	MIC median, mg/L, (min-max)				
	Amphotericin B	Flucytosine	Fluconazole	Voriconazole	Caspofungin
<i>C. albicans</i> (n = 16)	0.22 (0.094-0.38)	0.5 (0.023- > 32)	0.25 (0.094-0.5)	0.008 (0.004-0.032)	0.11 (0.064-0.5)
<i>C. dubliniensis</i> (n = 1)	0.064	0.004	0.094	0.006	0.19
<i>C. glabrata</i> (n = 4)	0.5 (0.38-0.5)	0.007 (0.006-0.008)	36 (8- > 256)	0.285 (0.064-1)	0.19 (0.19-0.38)
<i>C. parapsilosis</i> (n = 4)	0.38 (0.25-0.38)	0.023 (0.012-0.023)	0.285 (0.19-0.5)	0.007 (0.006-0.016)	0.56 (0.25-1)

uninfected-control group.<sup>15</sup> The prevalence of *Candida* spp. isolated from the vaginal mucosa is variable. In Italy, 16.8% of HIV-infected women have *Candida* spp. in vaginal mucosa,<sup>10</sup> while in the United States 35% of HIV-infected women had vaginal positive culture for *Candida* spp. (EUA).<sup>18</sup> Such variation could be explained by differences in the immune status of patients involved or by the presence of additional risk factors to *Candida* infection, such as a decreased number of CD4+ T-cells or concomitant antibiotic therapy.<sup>18</sup>

There is scant information about the frequency of non-*albicans* species in vaginal mucosa of HIV-infected women in Brazil. In the present study, *C. albicans* was the most frequent species isolated from the vaginal mucosa in both HIV-infected and uninfected women. In other countries such as Italy and the United States, the frequency was higher ranging from 81% to 92%, compared to that found in our study (52.9%).<sup>10,11,18</sup> In HIV-uninfected patients, our data was similar to other studies performed in Brazil, which describe the prevalence of *C. albicans* isolated in vaginal samples ranging from 60% to 80%<sup>19,20</sup> and in other countries where the prevalence of *C. albicans* in vaginal samples ranges between 80% to 90%.<sup>1,11,21,22</sup> A higher diversity of non-*albicans* species was found in HIV-infected patients compared to uninfected controls. *C. glabrata* was the most frequent non-*albicans* species isolated in HIV-infected patients, followed by *C. parapsilosis*.

In accordance with other studies, there was no relationship found between symptoms and isolated yeast species.<sup>23</sup> No correlation could be established between the level of CD4+ T-cell counts, the amount of HIV viral load, and the frequency of VVC in HIV-infected patients. These results were expected because of the observational nature of our study and the small sample size.

Regarding the susceptibility of *Candida* species to antifungal drugs, isolated yeasts were susceptible to Amphotericin B, Flucytosine, Voriconazole, and Caspofungin. Only three isolates had dose-dependent susceptibility or resistance to fluconazole, all of these were *C. glabrata* present in the vaginal mucosa of HIV-1-infected patients. These data are in agreement with other studies<sup>23-25</sup> that described azole refractory vaginitis caused by non-*albicans* *Candida*, particularly by *C. glabrata*.<sup>23</sup> Non-*albicans* *Candida* infections frequently require the use of non-azole therapy, including topical therapy with boric acid, Flucytosine, or Amphotericin B.<sup>24,26</sup>

In summary, our study found higher prevalence of *Candida* spp. in vaginal mucosa of HIV-infected patients than uninfected women. Thus, these patients could benefit from a periodic gynecological examination and VVC screening, even when undergoing HAART treatment. Although the species of *C. albicans* is the most frequent, it is evident the emergence of non-*albicans* *Candida* species, including *C. glabrata* with intrinsic resistance to azoles. Therefore, alternative agents to treat VVC caused by *C. glabrata* should be considered.

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