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BRIEF COMMUNICATION

EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON VAGINAL *Candida* spp. ISOLATION IN HIV-INFECTED COMPARED TO HIV-UNINFECTED WOMEN

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SUMMARY

Vulvovaginal candidiasis (VVC) in HIV-infected women contributed to the impairment of their quality of life. The aim of this study was to evaluate the effect of highly active antiretroviral therapy (HAART) use on the vaginal *Candida* spp. isolation in HIV-infected compared to HIV-uninfected women. This cross-sectional study included 178 HIV-infected (HIV group) and 200 HIV-uninfected women (control) that were studied at the Specialized Assistance Service (SAE) for sexually transmitted diseases (STD)/ AIDS of the city of Maringá, Brazil, from April 1 to October 30, 2011. The yeasts were isolated and identified by phenotypic and molecular methods. The *in vitro* antifungal susceptibility to fluconazole, itraconazole, nystatin and amphotericin B was tested by the reference microdilution method. Higher frequencies of total vaginal *Candida* spp. isolation were found in the HIV-infected group than in the control group. However, both groups showed a similar frequency of colonization and VVC. Although *C. albicans* was the most frequent and sensitive to azolics and polyenes in both HIV-infected and uninfected women, the emerging resistance of *C. glabrata* to amphotericin B in the HIV-infected women was observed. Although higher frequency of vaginal *Candida* spp. isolation had been observed in the HIV-infected than in HIV-uninfected women, colonization and VVC showed similar frequency in both groups, indicating that HAART appears to protect against vaginal colonization and VVC.

KEYWORDS: HIV; Vulvovaginal candidiasis; *Candida* spp.; Antiretroviral therapy.

INTRODUCTION

Vulvovaginal candidiasis (VVC) is a disease caused by the abnormal growth of yeast-like fungi in the mucosa of the female genital tract by members of the genus *Candida*[21]. These yeasts, in particular *C. albicans*, are well adapted to the human body, and are capable of colonizing it without producing signs of disease in conditions of physiological equilibrium[28]. However, under conditions that disrupt the delicate balance between the host and this commensal fungus, a parasitic relationship may occur, resulting in the development of infections termed candidiasis, including VVC[2]. For development of VVC, predisposing factors related to the host are very important, mainly being immunosuppressive diseases, such as HIV infection[13,15].

Because it strikes millions of women annually, causing great discomfort, interfering with sexual and affective relations and impairing work performance, VVC has been considered an important worldwide public-health problem[5,24]. In HIV-infected women, the impact of VVC on top of other medical complications that stem from the viral infection and its treatment certainly contributes to the impairment of their quality of life. It must still be considered that out of HIV-infected individuals worldwide (around 40 million), nearly half are women[16], raising concerns about VVC. In Brazil, HIV infection occurs in 0.5% of the population, with a trend toward expansion of the epidemic among women, from a ratio of 18.5 men:1 woman in the 1980s to 1.5:1 in 2004[2].

Use of the highly active antiretroviral therapy (HAART) has extended the life span of HIV-infected persons. However, some studies have reported that even for users of this continuous therapy opportunistic infections remain a serious problem[3,18]. Nevertheless, to the authors' knowledge, few studies have related vaginal *Candida* colonization and VVC in HIV-infected women to HAART use in different populations, have enrolled relatively few HIV-infected women[15], only treated specific clinical conditions as *Candida* colonization[14], lacked appropriate matched controls[9,15], or have been restricted to specialized subpopulations such as women with symptoms of vulvovaginitis[23]. There are some available

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studies that were performed before the broad use of HAART or in populations that do not regularly use this therapy[1,7,9,23,27].

The aim of this study was to evaluate the effect of HAART use on the vaginal *Candida* spp. isolation in two clinical conditions: colonization and VVC, in HIV-infected compared to HIV-uninfected women. In addition, the antifungal susceptibility of *Candida* species to the most commonly used antifungal drugs was evaluated.

MATERIAL AND METHODS

This was a cross-sectional study that included a group of 178 HIV-infected and 200 HIV-uninfected women, who were studied at the Specialized Assistance Service (SAE) for sexually transmitted diseases (STD)/AIDS in the city of Maringá, Brazil, from April 1 to October 30, 2011. Inclusion criteria were women having diagnoses of HIV/AIDS confirmed by two different methods (HIV group), or confirmed diagnosis as HIV-uninfected (control group). Exclusion criteria were: hysterectomized women, pregnant or postpartum women, women of less than 18 years of age, women with no history of sexual activity, women that had some degree of difficulty in understanding the study, women suffering vaginal bleeding, or women that had undergone sexual intercourse/vaginal douching within the 48 hours preceding collection of the vaginal sample.

The women signed the consent form to participate in the study, and completed a standardized questionnaire with information regarding symptoms of VVC. The HIV group also responded to a questionnaire regarding socio-demographic characteristics, obstetrical and gynecological history, and sexual behavior. Data regarding HIV infection, including the period of infection (years), $CD4^+$ T lymphocyte count, HIV viral load values, and HAART use were obtained from the medical records of each woman. A single health professional was responsible for contacting the women, administering the questionnaire, and collecting the vaginal sample. This research was approved by the Committee for Ethics in Research Involving Humans at the State University of Maringá, Paraná, Brazil (reports No. 185/2007 and No. 085/2011).

Vaginal samples were collected with sterile swabs and a disposable vaginal speculum, inoculated in sterile saline, and immediately seeded onto plates containing Sabouraud dextrose agar (SDA) (Difco, USA), with the addition of 100 mg/mL chloramphenicol, and incubated at 25 °C for up to five days. A pool of the colonies grown on each plate was subcultured on CHROMágar *Candida*® (Probac, France) to assure the purity of the isolates and to identify mixed cultures. Beginning with the pure culture, the yeasts were identified by classical phenotypic methods[11]. Additionally, the identification of yeasts was confirmed using matrix-assisted laser-desorption/ionization time-of-flight mass spectroscopy assay (MALDI TOF-MS). For MALDI TOF-MS identification, yeasts were prepared[26] and the measurements were performed[19] with Microflex LT mass spectrometer (Bruker Daltonics, Germany) using FlexControl software (version 3.0, Bruker Daltonics, Germany). The yeasts were stored in Sabouraud dextrose broth (SDB) (Difco, USA) with 10% glycerol at -20 °C.

Women were evaluated for the presence of clinical signs and symptoms of VVC by SAE doctors. *Candida* vaginal colonization was defined as culture positive for yeasts from women without signs and symptoms of VVC. Women with a positive culture were considered to have VVC if they reported at least two symptoms of this pathology (discharge, burning, vaginal itching, dysuria or dyspareunia), and signs of VVC reported by doctors[12].

The *in vitro* antifungal activity assay was performed for fluconazole (FLU, Pfizer Inc., NY, USA), itraconazole (ITRA, Janssen Pharmaceutical, NJ, USA), nystatin (NYST, Sigma Pharma, MO, USA) and Amphotericin B (AMB, Squibb Pharmaceutical, NJ, USA). All yeasts isolated were tested by means of the Clinical Laboratory Standards Institute reference broth microdilution method for fluconazol and itraconazol, with modifications for other drugs[4,5]. The minimum inhibitory concentration (MIC) for azoles was defined as the first well with a significant growth reduction (approximately 50%) compared to that of the positive control. In the case of NYST and AMB, the MIC was defined as the lowest concentration capable of inhibiting 90% of the growth[17]. The endpoints for antifungal agents: isolates with MIC between 16 and 32 µg/mL for FLU, 0.25 to 0.5 µg/mL for ITRA, and 8 to 32 µg/mL for NYST were considered as dose-dependent susceptibility (DDS). Isolates with an MIC ≤ 8 µg/mL for FLU, ≤ 0.125 µg/mL for ITRA, ≤ 4 µg/mL for NYST, and ≤ 1 µg/mL for AMB were susceptible (S). Those with an MIC ≥ 64 µg/mL for FLU, ≥ 1 µg/mL for ITRA, ≥ 64 µg/mL for NYST and ≥ 2 µg/mL for AMB were resistant (R).

The statistical analysis was performed using the STATA for Statistics and Data Analysis 9.1 software. All variables were expressed as absolute and relative frequencies. The frequencies of *Candida* spp. isolation from the vaginal mucosa, and also colonization and VVC, were calculated by the crude odds ratio (OR) with a 95% confidence interval (CI), and were evaluated between groups by the Chi-square test (χ^2) with Yates correction. A value of $p < 0.05$ was considered significant.

RESULTS

Figure 1 is an overview of the study and results.

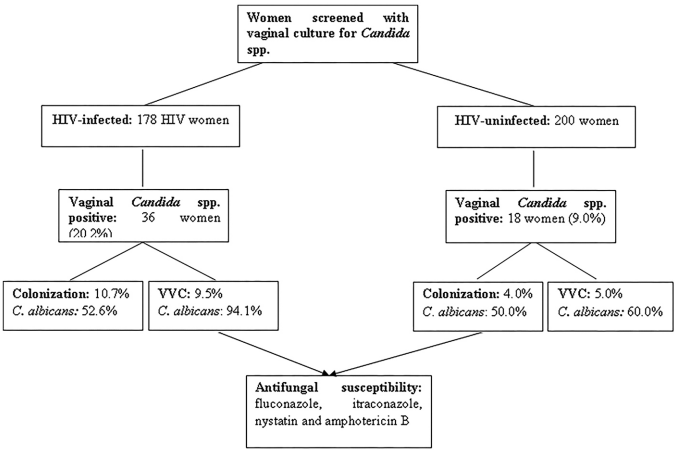

Table 1 describes the socio-demographic and clinical characteristics of the two groups. The median age of the HIV group was 41.24 ± 10.31 years, and for the control group it was 42.22 ± 14.14 years ($p > 0.05$). Considering all women studied, *Candida* spp. isolation from the vaginal

Table 1

Relationship between total *Candida* vaginal isolation, colonization and VCC, as well as the socio-demographic characteristics, obstetrical and gynecological history, sexual behavior and data regarding HIV of 178 HIV-infected women

Characteristics of *Candida* spp. in HIV-infected women	HIV-infected *n = 178*		Positive *Candida* isolation *n = 36*		*p*-value	Colonization *n = 19*		*p*-value	VVC *n = 17*		*p*-value
	n	(%)	*n*	(%)		*n*	(%)		*n*	(%)	
Age (years)											
15 to 30	28	15.7	10	27.8	0.4206	4	21.1	0.7867	6	35.3	0.2861
31 to 40	60	33.7	17	47.2	0.0286*	7	36.8	0.2285	10	58.8	0.0131*
≥ 41	90	50.6	9	25.0	0.1400	8	42.1	0.646	1	5.9	0.2103
Marital status											
married/cohabiting	93	52.2	19	52.8	0.9494	7	36.8	0.4337	12	70.6	0.2264
unmarried/non-cohabiting	85	47.8	17	47.2	0.9521	12	63.2	0.3204	5	29.4	0.4202
Education											
< 8 years	85	47.8	12	33.3	0.3414	5	26.3	0.3515	7	41.2	0.7375
≥ 8 years	93	52.2	24	66.7	0.1994	14	73.7	0.8782	10	58.8	0.6832
Family income											
< $ 240/month	25	14.0	4	11.1	0.8763	2	10.5	0.8911	2	11.8	0.9316
≥ $ 240/month	153	86.0	32	88.9	0.6519	17	89.5	0.6906	15	88.2	0.8139
Skin color											
white	109	61.2	18	50.0	0.3803	11	57.9	0.8311	7	41.2	0.3025
brown	50	28.1	12	33.3	0.7177	6	31.6	0.8582	6	35.3	0.7105
black	15	8.4	6	16.7	0.5635	2	10.5	0.9222	4	23.5	0.3957
yellow	4	2.2	0	0.0	-	0	0.0	-	0	0	-
Employ out side the home											
yes	98	55.1	23	63.9	0.4397	13	68.4	0.3652	10	58.9	0.8136
No	80	44.9	13	36.1	0.5500	6	31.6	0.5254	7	41.1	0.8467
Age of first sexual intercourse											
< 18	112	62.9	24	66.7	0.7329	15	78.9	0.2254	9	53.0	0.5527
≥ 18	66	37.1	12	33.3	0.8071	4	21.1	0.5201	8	47.0	0.584
Number of lifetime sexual partners											
< 10	129	72.5	25	69.4	0.7923	14	73.7	0.953	11	64.8	0.6127
≥ 10	49	27.5	11	30.6	0.8635	5	26.3	0.9545	6	35.2	0.7146
Number of deliveries											
< 3	87	48.9	17	47.2	0.8922	8	42.1	0.7135	9	53.0	0.8198
≥ 3	91	51.1	19	52.8	-	11	57.9	0.6708	8	47.0	0.8287
Period of HIV infection (years)											
< 10	131	73.6	25	69.4	0.6346	15	78.9	0.6576	10	58.9	0.3021
≥ 10	47	26.4	11	30.6	0.7579	4	21.1	0.8175	7	41.1	0.4101
HAART use											
yes	141	79.2	22	61.1	0.0670	13	68.4	0.3676	9	53.0	0.0724
no	37	20.8	14	38.9	0.1990	6	31.6	0.5588	8	47.0	0.1340
Current CD4+ T lymphocyte count											
< 200 cells/mm^3	15	8.4	6	16.7	0.5635	2	10.5	0.9222	4	23.5	0.3957
200 and 350 cells/mm^3	32	18.0	8	22.2	0.7873	5	26.3	0.6634	3	17.6	0.9863
> 350 cells/mm^3	131	73.6	22	61.1	0.2136	12	63.2	0.4401	10	58.9	0.3021
Current viral load											
< minimum limit copies/ml	104	58.4	11	30.6	0.0848	6	31.6	0.2001	5	29.4	0.2039
Minimum limit - 100,000 copies/mL	69	38.8	22	61.1	0.0726	11	57.9	0.2360	11	64.7	0.1134
> 100,000 copies/mL	5	2.8	3	8.3	0.7502	2	10.5	0.6904	1	5.9	0.8637

VVC, vulvovaginal candidiasis; *$p < 0.05$ was considered significant.

mucosa and VVC was significantly associated with the 31 to 40 years of age group ($p = 0.0286$ and $p = 0.0131$, respectively). For the groups studied, only the control group showed association with age, since vaginal yeast colonization was significantly associated with the 31 to 40 years of age group ($p = 0.0185$). Data regarding HIV infection, including the period of HIV infection, $CD4^+$ T lymphocyte count, HIV viral load values and correct use of HAART were not significantly associated with *Candida* spp. total isolation, colonization or VVC.

The HIV infected group showed a higher frequency of colonization than VVC (52.8% and 47.2%, respectively, $p = 0.05$), and the control group showed more frequent VVC than colonization (55.5% and 44.4%, respectively; $p = 0.01$). However, comparing the two groups, *Candida* spp. total vaginal isolation was more frequent in the HIV group (n = 36/178, 20.2%) than in the control group (18/200, 9.0%; $p = 0.003$). For clinical conditions, the HIV group showed a similar frequency of vaginal colonization (19/178, 10.7%) (OR = 2.9; 95% CI 0.91-9.6; $p = 0.1072$) and VVC (17/178, 9.5%) (OR = 1.879; 95% CI 0.8657-1.994; $p = 0.4057$) to the control group (n = 8/200, 4.0%; n = 10/200, 5.0%, respectively) (Table 2).

With respect to yeast species in the present study, *C. albicans* was the most frequently isolated in both the HIV (n = 26, 72.2%; $p = 0.02$) and control groups (n = 10, 55.6%; $p = 0.05$). With respect to yeast species in different clinical conditions, the HIV group showed more *C. albicans* than the control in VVC ($p = 0.007$; OR 3.1; 95% CI 1.24-9.05) and in colonization ($p = 0.005$; OR 2.9; 95% CI 0.91-10.87). In the HIV group, the following non-*albicans* species were identified: *C. glabrata* (n = 7), *C. parapsilosis* (n = 2) and *C. rugosa* (n = 1). In the control group, the following were identified: *C. glabrata* (n = 5), *C. parapsilosis* (n = 2) and *C. tropicalis* (n = 1). Thus, in both groups, *C. glabrata* was the second most common yeast isolated (19.4% and 27.8%, for the HIV and control groups, respectively) (Table 2).

Table 3 shows the interpretation of the antifungal activity results for drugs as well as for susceptibility, dose-dependent susceptibility or resistance. In general, the *C. albicans* isolates showed no resistance to the antifungal agents tested for both the HIV and the control group. The results for amphotericin B were 100% sensitivity. For nystatin, the results showed elevated rates of vaginal isolates of *C. albicans* and non-*albicans* species with dose-dependent susceptibility. Of the non-*albicans* species identified in the HIV group, only *C. glabrata* showed resistance, one (4.8%) to FLU, two (9.5%) to ITRA, and another two (9.5%) to AMB. In the control group, two non-*albicans* yeasts (23.3%) showed resistance to FLU and one (6.7%) to ITRA; the two resistant species found in this group were *C. glabrata* and *C. tropicalis.*

DISCUSSION

In the present study, *Candida* spp. total vaginal isolation was significantly more frequent in the HIV group than in the control group. However, a similar frequency of colonization and VVC in the HIV and

Table 2
Frequencies of *Candida* spp. vaginal total isolation, colonization and vulvovaginal candidiasis (VVC) in HIV-infected and uninfected women in southern Brazil

Candida species	HIV-infected group n = 178						HIV-uninfected group n = 200					
	Total isolation		Colonization		VVC		Total isolation		Colonization		VVC	
	n	%	n	%	n	%	n	%	n	%	n	%
C. albicans	26	72.2	10	52.6	16	94.1	10	55.6	4	50.0	6	60.0
Non-*albicans* species	10	27.8	9	47.4	1	5.9	8	44.4	4	50.0	4	40.0
Total	36	100.0	19	52.7	17	47.3	18	100.0	8	44.4	10	55.5

Table 3
Interpretation of the results for minimal inhibitory concentration (MIC) for antifungal drugs in vaginal yeasts from HIV-infected (n = 36, *C. albicans* = 26) and HIV-uninfected groups (n = 18, *C. albicans* = 10)

Antifungals	HIV-infected group												HIV-uninfected group											
	C. albicans						non-*albicans* species						*C. albicans*						non-*albicans* species					
	S		DDS		R		S		DDS		R		S		DDS		R		S		DDS		R	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
FLU	24	92.3	2	7.7	-	-	4	71.4	5	23.8	1	4.8	10	100.0	-	-	-	-	1	33.3	5	43.4	2	23.3
ITRA	25	96.1	1	3.9	-	-	8	90.5	-	-	2	9.5	9	90.0	1	10.0	-	-	-	-	7	93.3	1	6.7
NYST	25	96.1	1	3.9	-	-	8	66.7	2	33.3	-	-	3	30.0	7	70.0	-	-	6	76.7	2	23.3	-	-
AMB	26	100.0	-	-	-	-	8	90.5	-	-	2	9.5	10	100.0	-	-	-	-	8	100.0	-	-	-	-

FLU = fluconazole; ITRA = itraconazole; NYST = nistatyn and AMB = amphotericn B; S (susceptible): isolates with MICs ≤ 8 µg/mL for FLU; ≤ 0.125 µg/mL for ITRA; ≤ 4 µg/mL for NYST; ≤ 1 µg/mL for AMB. DDS (dose-dependent susceptibility): isolates with MIC between 16 and 32 µg/mL for FLU; ≤ 0.25 to 0.5 µg/mL for ITRA; ≤ 8 to 32 µg/mL for NYST. R (resistant): Isolates with MIC ≥ 64 µg/mL for FLU; ≥ 1 µg/mL for ITRA; ≥ 64 µg/mL for NYST; ≥ 2 µg/mL for AMB.

control groups was shown, which is consistent with the results of the most HIV-infected women enrolled in this study that showed excellent control of the infection. Further, the data regarding HIV infection were not associated with *Candida* spp. isolation from the vaginal mucosa, colonization or VVC.

The results found in this study differ from those described in other populations that also had good control of HIV infection. In these studies, the frequency of VVC in the HIV group was similar to the control group, but the frequency of colonization was higher[7,15]. Also, for vaginal *Candida* colonization, the results differ from those in which the risk of colonization in HIV-infected women with $CD4^+$ T-cell counts below 100-200 cells/mm^3 was three or four times higher, compared to immunocompetent HIV-infected or HIV-uninfected women[1,7].

The results are encouraging, since HAART seems to protect against vaginal colonization and VVC, and some investigators have reported a relationship between vaginal *Candida* colonization and VVC with heterosexual transmission of HIV. Recent research has shown that women with vaginal yeasts were more likely to acquire HIV, and the condition may contribute more to the HIV epidemic than previously thought[27]. It was shown that treatment of VVC in HIV-infected women can reduce genital shedding of HIV RNA and DNA by 3.2 and 3 times, respectively[17].

For the HIV and control groups, *C. glabrata* was the second most common yeast isolated, in agreement with other studies[1,15]. Older studies described higher rates of vaginal colonization with non-*albicans* species in HIV-infected than in uninfected women[7,17]. With respect to yeast species in different clinical conditions, the HIV group showed more *C. albicans* than the control group in VVC and in colonization, similar to other studies[1]. In some studies, *C. albicans* was related to symptoms in HIV-infected women[22], but in others no relationship between symptoms and the yeast species isolated[15] was found.

In relation to antifungal susceptibility, *C. albicans* showed no resistance to the antifungal agents tested for both the HIV and the control group and 100% of sensibility for amphotericin B. These results reinforce previous findings showing that amphotericin B is an excellent and highly efficacious therapeutic option for vaginal *C. albicans* including in HIV-infected women[8]. For nystatin, the results of this study are in accordance with others that have shown elevated rates of vaginal isolates with dose-dependent susceptibility, and also some resistance[5]. Nystatin has been used for several decades as one of the principal treatments for vaginal *Candida* spp. in Brazil[23]. This history may partly explain the elevated dose-dependent susceptibility rate observed in both groups studied. Large-scale use of fluconazole began more recently, which may also partly explain the better therapeutic activity of this drug against *C. albicans* found in the present study.

Non-*albicans* species showed resistance to fluconazole and itraconazole. In general, these results are not surprising since the management of women with non-*albicans* species, mainly *C. glabrata,* is difficult because of the lower sensitivity of non-*albicans* species to both azoles[6,25] and polyenes[10]. To the authors' knowledge, this is one of the first studies to show amphotericin B resistance in *C. glabrata* isolated from HIV-infected women. The importance of the results in relation to antifungal susceptibility is confirmed by the report which found no trials that addressed treatment of VVC in HIV-infected women[20]. However, there is a need to evaluate drugs and drug regimens for VVC treatment and prophylaxis in HIV-infected women.

In conclusion, this study found higher frequencies of total vaginal *Candida* spp. isolation in the HIV-infected women with prolonged HAART use, in relation to HIV-uninfected. However, a similar frequency of colonization and VVC in the HIV and control groups was shown. Thus, the results are encouraging, since HAART seems to protect against vaginal colonization and VVC. Although *C. albicans* was the most frequent and sensitive to azolics and polyenes in both HIV-infected and uninfected women, the emerging resistance of *C. glabrata* to amphotericin B in the HIV-infected women studied was observed. If this proves to be correct, implementing routine culture identifications of vaginal *Candida* spp. in HIV-infected women could help in guiding treatment, assisting in care, and improving the quality of life of these patients. Once the resistance of *C. glabrata* to amphotericin B is detected, and this yeast is intrinsically resistant to azoles, it is important to have knowledge of their involvement in VVC of HIV-positive women.

RESUMO

Efeito da terapia anti-retroviral altamente ativa no isolamento vaginal de *Candida* spp. em mulheres infectadas por HIV comparado às não infectadas

Candidíase vulvovaginal (CVV) em mulheres infectadas pelo HIV contribuiu substancialmente para a diminuição da sua qualidade de vida. O objetivo deste estudo foi avaliar o efeito do uso de terapia anti-retroviral altamente ativa (HAART) no isolamento de *Candida* spp. vaginais em mulheres HIV positivas comparado às não infectadas por HIV. Este estudo transversal incluiu 178 mulheres infectadas pelo HIV (grupo HIV) e 200 mulheres não infectadas (grupo controle) acompanhadas no Serviço de Assistência Especializada (SAE) para as doenças sexualmente transmissíveis (DST)/AIDS da cidade de Maringá/Brasil, de 1 abril a 30 de outubro de 2011. As leveduras foram isoladas e identificadas por métodos fenotípicos e moleculares. A susceptibilidade *in vitro* aos antifúngicos fluconazol, itraconazol, nistatina e anfotericina B foi avaliada pelo método de referência de microdiluição. Nós encontramos maior frequência de isolamento vaginal total de *Candida* spp. no grupo HIV do que no grupo controle. Entretanto, foi observada frequência similar de colonização e CVV entre os dois grupos. Apesar de *C. albicans* ser a mais frequente e sensível a azólicos e polienos em mulheres infectadas pelo HIV e não infectadas, foi detectada emergente resistência de *C. glabrata* a AMB nas mulheres infectadas pelo HIV. Embora tenha sido observada maior frequência de isolamento vaginal de *Candida* spp. nas mulheres infectadas pelo HIV do que nas não infectadas, colonização e CVV apresentaram frequência similar em ambos os grupos, o que indica que HAART parece proteger contra colonização vaginal e CVV.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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