

Original article

The occurrence of vulvovaginal *Candida* species and their antifungal susceptibility pattern in HIV seropositive women in Ahvaz, Southwest Iran



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ARTICLE INFO

Keywords:

Vulvovaginal Candidiasis

HIV

Amphotericin B

Itraconazole

Caspofungin

ABSTRACT

Background: Vulvovaginal candidiasis (VVC) is a prevalent infection among HIV-seropositive women that still needs to be subjected to more researches. This study is aimed at determining the occurrence of vulvovaginal *Candida* species and their antifungal susceptibility pattern in HIV-seropositive women from the Behavioral Disease Counseling Center in Ahvaz, Iran.

Method: In this analytical cross-sectional study, 85 HIV-seropositive women with the mean age of 26.15 years (SD. 9.60) were evaluated. Vaginal swabs were collected from women to identify various *Candida* species through conventional methods and API 20 C AUX system. Antifungal susceptibility testing was done on specimens against Amphotericin B and Azole antifungals. Eventually, all data were analyzed by SPSS.

Results: The occurrence of vulvovaginal *Candida* species was 34.11% (29) among 85 HIV-seropositive women. Out of 29 *Candida* spp., 18 (62.06%) *C. albicans*, 6 (20.68%) *C. glabrata*, 4 (13.79%) *C. dubliniensis*, and 1 (3.44%) *C. krusei* were identified. There was no significant association between VVC and some medical variables such as antiretroviral therapy, birth control methods, underlying diseases, and CD4 count (PV > 0.05). However, there was a significant association between VVC and age and pregnancy (PV < 0.05). The most *Candida* spp. showed considerably resistant to Amphotericin B and Itraconazole while all species were susceptible to Caspofungin.

Conclusion: The high occurrence of *Candida* spp. (34.11%) among HIV-seropositive women is notable, with the highest frequency of *C. albicans*, *C. glabrata* and *C. dubliniensis*, respectively. Also, their notable resistance to Amphotericin B and Azole antifungals necessitates more effective treatments for VVC, particularly in pregnant women.

1. Introduction

Vulvovaginal candidiasis (VVC) is the most prevalent gynecological infections among childbearing women.¹ The frequency of vaginal *Candida* spp. was significantly higher among HIV-seropositive women than HIV-seronegative women.² Mucosal candidiasis (vaginal, oropharyngeal, and esophageal) is one of the most common infections among HIV-infected patients.^{3–5} Various behavioral and biological risk factors may predispose individuals to mucosal candidiasis.⁶ For example, risk factors including high sexual activity, pregnancy, diabetes,

and or using antibiotics can arise the risk of vaginal candidiasis.^{7,8} Moreover, cell-mediated immunodeficiency increases the risk of mucosal *Candida* colonization and candidiasis.^{8,9} The spectrum of Candidiasis is varied from asymptomatic colonization to symptomatic form. The low CD4 T-lymphocyte count has previously been shown as the main risk factor for the development of mucosal candidiasis.¹⁰ Despite the therapeutic effects of antiretroviral therapy (ART) on the AIDS-related infectious,¹¹ Candidiasis still remains a common opportunistic infection in HIV seropositive patients,¹² mostly because of increasing antibiotic resistance. The high prevalence of candidiasis (≥50%) was

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<https://doi.org/10.1016/j.cegh.2020.02.021>

Received 11 July 2019; Received in revised form 18 January 2020; Accepted 24 February 2020

Available online 26 February 2020

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reported among *HIV* positive patients in different regions,^{13–16} and *C. albicans* as the most common species.^{10,13,16,17}

Based on the researches in different regions of Iran, the prevalence of VVC and *Candida* spp. have been reported, for example Kermanshah (20%), Mazandaran (28.2%), Esfahan (54.6%), and Tehran (87.5%), respectively.^{18–21}

However, few data are available regarding the rates of vulvovaginal candidiasis and or *Candida* species among *HIV*-infected patients, especially from Iran. Furthermore, the increasing global risk of antibiotic-resistant infections particularly azole-resistant *Candida* species^{22,23} necessitate assessing their antifungal susceptibility pattern in *HIV*-infected patients. So, the present study report the occurrence of vulvovaginal *Candida* species and their antifungal susceptibility pattern in *HIV*-seropositive women from the Behavioral Disease Counseling Center in Ahvaz, Iran.

2. Materials and methods

In this descriptive-analytic and cross-sectional study, 85 *HIV*-seropositive women with the age range between 16 and 60 years (26.15 ± 9.60) were evaluated, who were referred to the Behavioral Disease Counseling Center in Ahvaz. This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences with Ethical Code: ajums.REC.1396.410. The informed consent forms were signed by all participants after explaining its contents.

Vaginal swab specimens were collected from women to identify various *Candida* species through conventional methods including culturing on the mediums of CHROMagar *Candida* or Cornmeal agar plus %1 Tween 80, Germ tube test and API 20 C AUX system.

1. Vulvovaginal *Candida* species

Three vaginal swabs were taken from the posterior vaginal fornix, and one of them was used for direct smear examination, and the others were aerobically inoculated on sabouraud dextrose agar (SDA; Zist Kavosh Iranian Company, IRAN) at 37 °C. In order to examination of vaginal candidiasis swabs, 10% Potassium hydroxide preparation (KOH preparation; Zist Kavosh Iranian Company, IRAN), Gram staining as well as microscopic evaluation were used. The second swab was cultured into SDA (Zist Kavosh Iranian Company, IRAN) and SDA with chloramphenicol (Zist Kavosh Iranian Company, IRAN), and then incubated for 48 h at 27 °C. The pure colonies were isolated and confirmed on Gram staining. For detecting *Candida* spp., isolated *Candida* colonies were subcultured onto differential CHROM agar *Candida* medium for 24 h at 37 °C (based on colony color of growing *Candida* spp.).

1.1. *Candida* Strains

The API 20 C AUX system (Bio merieux, France) was used for identification and confirmation of *Candida* species (based on manufacturer guidelines of kit). The strips were read after incubation at 30 °C for 48–72 h. Based on this method, *C. albicans* (green colonies), *C. dublinensis* (dark green colonies), *Candida glabrata* (purple colonies), *Candida krusei* (pink colonies) were detected.

2. The association between medical variables and vulvovaginal candidiasis

The characteristics and some medical variables of women were evaluated, including the usage history of antibiotics, antiretroviral therapy, history or presence of underlying diseases such as diabetes, CD4 count (less or greater than 200 cells/mm³) and duration of AIDS.

3. Antifungal susceptibility test

Antifungal susceptibility testing was done on specimens against various antifungals by broth microdilution assay and according to M27 A3 CLSI protocol.²⁴ An overnight culture of isolates was prepared in Sabouraud dextrose agar (Merck, Germany). A suspension of each isolate prepared and was adjusted to 0.5 McFarland standard. A 100 µL of each serial dilution of antifungals including, amphotericin B (Sigma - Aldrich, Germany) (16–0.062 µg/mL), caspofungin (Sigma - Aldrich, Germany) (4–0.031 µg/mL), itraconazole (Sigma - Aldrich, Germany) (16–0.125 µg/mL) and fluconazole (Serva, USA) (64–0.5 µg/mL) was inoculated into each well in 96 wells microplate. Then, 100 µL of diluted suspension was added into each well and incubated at 35 °C for 24–48 h. Minimum inhibitory concentration (MIC) rage, MIC₅₀, MIC₉₀ and MIC_{GM} were calculated.

4. Data analysis

All data were analyzed using SPSS ver. 22. The data related to qualitative and quantitative variables were respectively reported as frequency or percentage, and mean \pm standard deviation.

The primary assessment normal distribution of variables by Kolmogorov-Smirnov Test showed that none of them follows a normal distribution. So, the results and assumptions were eventually analyzed by Mann-Whitney *U* test and Chi-Square test with considering $PV < 0.05$ as statistically significant.

3. Results

The occurrence of vulvovaginal *Candida* species was 34.11% (29) among 85 *HIV* seropositive women. Out of 29 *Candida* spp., 18 (62.06%) *C. albicans*, 6 (20.68%) *C. glabrata*, 4 (13.79%) *C. dublinensis*, and 1 (3.44%) *C. krusei* were identified. There was no significant association between vulvovaginal candidiasis and some medical variables, including the usage history of antibiotics, antiretroviral therapy, birth control methods, history or presence of underlying diseases such as diabetes, CD4 count (less or greater than 200 cells/mm³) and duration of AIDS [Table 1].

However, there was a significant association between vulvovaginal candidiasis and age and pregnancy ($PV < 0.05$). In this regard, the mean age of *HIV*-infected women with candidiasis (24.68 ± 8.99) was considerably less than women without candidiasis (33.96 ± 15.59). The most *Candida* spp. showed considerably resistant to Amphotericin B and Itraconazole while all species showed antifungal susceptibility to Caspofungin. Although, *C. dublinensis* showed dose-dependent resistance to Itraconazole after 48 h growing, it was absolutely resistance after 72 h growing (Fig. 1).

4. Discussion

Many studies have reported the prevalence of candidiasis among women from different regions. Nurat et al.'s study reported that the prevalence of VVC was 25% among asymptomatic pregnant women in Nigeria. In this regard, the prevalence of candidiasis in women in the age range of 20–29 years (33.8%) was more than those in the age range of 30–39 years (24.3%).¹⁷ In another study, the prevalence of VVC was reported 30% in Nigeria²⁵ and 30.7% in Jamaica.²⁶

Beigi et al.'s study revealed that asymptomatic VVC was about 5–30% in Ohio, USA.²⁷ A high rate of VVC was reported in women in the age range of the 20–29 years in many studies,^{17,28} which was consistent with our findings (24.68 ± 8.99 years), probably due to drug usage and or contraceptives. Spinillo et al. reported a high rate of Candidiasis among married women between 30 and 45 years with frequent sexual activity, reproductive history, and taking contraceptives.²⁹ Our findings showed that the rate of pregnancy is significantly more frequent among women with VVC. In this regard, Dias et al.'s study was reported the frequency distribution of 92.3% *C. albicans*, 3.3% *C. krusei*, 2.2% *C. glabrata*, 1.1% *C. parapsilosis* and 1.1%

Table 1
The statistically association between vulvovaginal candidiasis and some medical variables.

Medical Variables	HIV-infected women with candidiasis (n = 29)	HIV-infected women without candidiasis (n = 29, randomly selected)	P Value
Duration of AIDS (month)	29.17 ± 17.59	31.34 ± 16.79	0.5646 ^{MWW}
AGE (year)	24.68 ± 8.99	33.96 ± 15.59	0.0298 ^{MWW*}
CD4 > 200 cells/mm ³	430.68 ± 364.69	455.86 ± 377.15	0.9566 ^{MWW}
CD4 ≤ 200 cells/mm ³	424.65 ± 359.39	433.96 ± 364.49	0.8825 ^{MWW}
The usage history of			
_ Antibiotics	17 (58.62%)	22 (75.86%)	0.1619 ^c
_ Birth control pills	2 (6.89%)	4 (13.79%)	0.3885 ^c
_ Antiretroviral therapy	20 (68.96%)	25 (86.20%)	0.1154 ^c
_ Contraception (Birth control methods)	16 (55.17%)	17 (58.62%)	0.7909 ^c
History or presence of			
_ Underlying diseases	5 (17.24%)	9 (31.03%)	0.2197 ^c
_ Pregnancy	17 (55.17%)	1 (3.44%)	< 0.0001 ^{c ***}
_ Diabetes	4 (13.79%)	2 (6.89%)	0.3885 ^c

MWW: Mann-Whitney *U* test.

C: Chi-square test.

*PV < 0.05 is considered as statistically significant value.

C. tropicalis among pregnant women with VVC in Brazil.³⁰ Moreover, other studies were reported *C. albicans* as the most prevalent isolate from VVC in HIV-infected and uninfected women (80–90%).^{31,32}

Oliveira et al. reported a higher frequency of *Candida* spp. among HIV-infected women (29.7%) than controls (14.5%) in Brazil. Based on their findings, *C. albicans* was the most frequent isolated species in both HIV-infected (52.3%) and uninfected women (85.7%), followed by *C. parapsilosis* in 17.6% and 14.3%, respectively. However, the frequency of *Candida* spp. colonization and VVC were similar in both groups. Only the isolate of *C. glabrata* has shown resistant to fluconazole while others were susceptible to Amphotericin B, Voriconazole, Flucytosine, and Caspofungin³³.

In regard to the frequency of *C. albicans*, our findings were in consistent with the mentioned studies. Our study reported the frequency distribution of 18 (62.06%) *C. albicans*, 6 (20.68%) *C. glabrata*, 4 (13.79%) *C. dubliniensis*, and 1 (3.44%) *C. krusei* among HIV seropositive women with VVC. The most *Candida* spp. showed considerable resistant to Amphotericin B and Itraconazole but antifungal susceptibility to Caspofungin. Although, there are similar evidences of Azole antifungal resistance in *Candida* spp in other studies too,^{33–35} a considerable resistant to Amphotericin B was rarely reported. Hence, our findings emphasize on the use of non- Amphotericin B therapy in addition to non-azole therapy.

According to Badiie et al.'s study in Shiraz, *C. albicans* (50%) followed by *C. glabrata* (21.4%), *C. dubliniensis* (13.3%), *C. krusei* (9.8%), *C. kefyr* (3.1%), *C. parapsilosis* (1.6%), and *C. tropicalis* (0.8%) were the most prevalent isolated species from mucosal sites in HIV positive patients. All species showed notable sensitivity to amphotericin B, nystatin, voriconazole, ketoconazole and caspofungin while some isolates were resistance to fluconazole and itraconazole.³⁶ Their findings were significantly similar to our findings. However, the notable resistant to Amphotericin B was a rare issue in our study.

The frequency of *Candida* spp colonization in HIV-infected women from United States and Italy was in a higher range (81%–92%),^{37,38} compared to our finding (34.11%). Such variation could be due to the variation in the immune status of people in various regions or other Candidiasis-related-risk factors. So, in the present study we also assessed the association between vulvovaginal candidiasis and some medical variables. In this regard, our findings showed a significant association between VVC and age and pregnancy (PV < 0.05) while there was no significant association between CD4 count, the usage history of antibiotics, antiretroviral therapy, birth control methods, history or presence of underlying diseases, and duration of AIDS (PV > 0.05).

5. Conclusion

The high occurrence of vulvovaginal *Candida* spp. (34.11%) among HIV seropositive women in Ahvaz (Southwest Iran) is notable, with the highest frequency of *C. albicans* (62.06%), followed by *C. glabrata* (20.68%) and *C. dubliniensis* (13.79%). The considerable resistance of all *Candida* spp to Amphotericin B and Azole antifungals necessitates more effective treatment programs to reduce the rate of this infection in HIV-infected women, particularly in pregnant women.

Funding

This study was funded by Ahvaz Jundishapur University of Medical Sciences (No: OG-94170).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee and with the 2008 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences with Ethical Code: ajums.REC.1396.417.

Authors' contributions

Study concept and design: Mehran Varnasiri, Shokrolah Salmanzadeh, Ali Zarei Mahmoudabadi.

Sampling and conducting the experiments: Ali Zarei Mahmoudabadi, Marzieh Halvaezadeh, Simin Taghipour, Shahla Molavi.

Data analysis and interpretation of the results: Mehran Varnasiri, Seyed Mohammad Alavi*, Khojasteh Hoseiny Nezhad, Parastoo Moradi Choghakabodi.

Drafting of the manuscript: Seyed Mohammad Alavi, Khojasteh H. Nezhad, Parastoo Moradi Choghakabodi.

Critical revision of the manuscript: Mehran Varnasiri, Seyed Mohammad Alavi, Khojasteh H. Nezhad, and Parastoo Moradi Choghakabodi.

Declaration of competing interest

Dr. Mehran Varnasiri and Dr. Seyed Mohammad Alavi has received research grants from Ahvaz Jundishapur University of Medical

<i>Candida</i> species	Antifungals	MIC range	MIC ₅₀	MIC ₉₀	MIC _{GM}	R
<i>Candida albicans</i> (18 isolates)	Amphotericin B	= 8	= 8	= 8	32	18(100%)
	Caspofungin	0.062 - 2	1	2	0.7	
	Itraconazole	2 - =8	2	=8	3.03	
	Fluconazole	0.5 - = 64	16	= 64	10.55	
<i>C. glabrata</i> (6 isolates)	Amphotericin B	0.0625 - 8				
	Caspofungin	0.031 - 0.25				
	Itraconazole	2 - = 16				
	Fluconazole	4 - = 64				
<i>C. dubliniensis</i> (4 isolates)	Amphotericin B	= 8				4(100%)
	Caspofungin	0.25 - 1				
	Itraconazole	2 - = 16				
	Fluconazole	0.5 - 2				
<i>C. krusei</i> (1 isolate)	Amphotericin B	= 16				1(100%)
	Caspofungin	2				
	Itraconazole	4				
	Fluconazole	= 64				
All isolates (29 isolates)	Amphotericin B	0.0625 - = 8				
	Caspofungin	0.031 - 2				
	Itraconazole	2 - = 16				
	Fluconazole	0.5 - = 64				

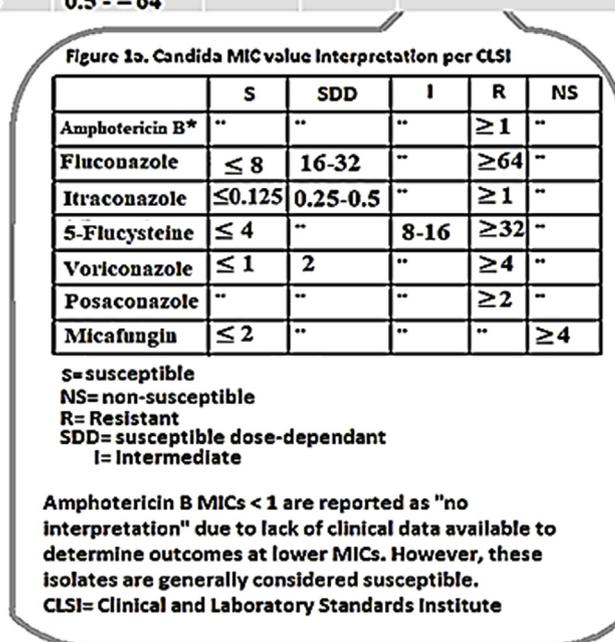


Fig. 1. Antifungal susceptibility of *Candida* specimens against various antifungals.

Sciences. Other coauthors report no conflicts of interest relevant to this article.

Acknowledgments

We would like to thank the Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences for their support. This study was a MD thesis (Mehran Varnasiri) supported by a grant (No: OG-94170) from the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

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