

The prevalence of vulvovaginal candidiasis in pregnant women attending several Hospitals in Ibb, Yemen

Abstract

Candida species are opportunistic yeasts affecting the genitourinary tracts which cause the vulvovaginal candidiasis in the most female especially in developing countries. This study aims to determine the prevalence of vulvovaginal candidiasis caused by *Candida* species causing among pregnant women in Ibb, Yemen. This study was carried out at the department of microbiology of AL-Kuwait University Hospital. 218 vaginal swabs were collected and cultured on Sabouraud dextrose agar. *Candida* species identification and antifungal susceptibility testing were determined according to standard microbiological methods. The results showed that out of 218 samples, 134(61.5%) were positive for *Candida* species. The pregnant woman coming from the rural areas had a higher rate of candidiasis disease than urban area women. Also, it was recorded that the highest prevalence of *Candida* species was within a group aged 28-37 years. The *C. albicans* (61.2%) was the most common species isolated followed by non-albicans species that are *C. tropicalis* (21.64%), *C. glabrata* (11.19%), and *C. kefyr* (5.97%). Susceptibility tests revealed that the most isolated species of *Candida* were sensitive to nystatin (94.8), fluconazole (91.04%), amphotericin B (88.8%), voriconazole (78.35%), clotrimazole (75.37%), ketoconazole (73.13%), and itraconazole (69.40%). It can be concluded that the vulvovaginal candidiasis is quite common in Yemen country with a high prevalence. Also, the tested antifungals remain an effective agent against all isolates of *Candida* species. In contrast, the slightly increase the resistance of *Candida* species to itraconazole, ketoconazole, clotrimazole, and voriconazole that commonly used antifungal is an alarming increase of vaginal candidiasis caused by antifungal-resistant *Candida* species.

Keywords: Antifungal, *Candida albicans*, Non-albicans *Candida*, Vaginitis, Yemen.

INTRODUCTION

Candida vaginitis is the infection of the vagina by several types of *Candida* species, also often called vulvovaginal candidiasis/candidosis^{1,2}. Vulvovaginal candidiasis (VVC) considered being the most common manifestation of genital candidiasis^{1,3}. It is representing over 25% of infectious vaginitis^{4,5}. 75% of women are affected by vulvovaginal candidiasis in their lifetimes³. Also, it was found that more than 40% of women that affected will have 2 or more vulvovaginal candidiasis episodes^{6,7}. Clinical manifestation of vulvovaginal candidiasis is pruritus, vaginal discomfort, burning, and soreness⁸.

The distribution of *Candida* sp. in vulvovaginal candidiasis cases differs widely depending on the geographical location and population studied⁹. Some reports have documented that among women with acute vulvovaginal candidiasis were caused by *C. albicans* that accounts for 80-90% of all vaginal candidiasis cases, whereas other species are less frequently isolated^{1,10,11}.

However, in last years, there are different species of *Candida* non-albicans that are *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. lusitanae*, *C. tropicalis*, *C. dubliniensis*, and *C. guilliermondii* isolated from vaginal samples^{12,13,14}. Misuse of antifungal drugs and lack of effective policies that control the use of antifungal, especially against the vulvovaginal candidiasis lead to increase the resistant of *Candida* species to several antifungal drugs¹⁵.

There are little reports that documented the prevalence of vaginitis in Yemen. A study by AL-Haik and Al-Haddad¹⁶ reported that 39.2% of pregnant women have been infected by bacterial vaginosis in Hadhramout city. Also, it was found that 11.1% of pregnant women attending primary healthcare in Sana'a city have been infected by Trichomonal vaginitis¹⁷.

A study, in Sana'a, by Abdul-Aziz *et al.*,¹⁸ revealed that the prevalence of vaginal infection between reproductive-aged women was 37.6% of collected samples. The results showed that 27.2% by bacterial vaginosis, 6.6% by vulvovaginal candidiasis, and 0.9% by trichomonal vaginosis.

To date, no data are available about the prevalence of vulvovaginal candidiasis among the pregnant women and the *Candida* species resistant to antifungal in Ibb City, Yemen. Therefore, this study aimed to determine the prevalence of *Candida* species that causes vulvovaginal candidiasis among pregnant women and the resistance of isolated species to antifungal in Ibb City, Yemen.

MATERIALS AND METHODS

Study Design and Data Collection

Two hundred and eighteen (218) specimens were collected from females attending Obstetrics and Gynecology outpatient clinics at different hospitals (AL-Thwrah, Maternity Center and Childhood, Reproductive Health Center for Mothers, Nasser General hospital) that situated in Ibb City, in the period from December 2019 to March 2020. All suspected cases were interviewed and patient information was recorded with intended questionnaires including; demographic, age, medical history, and symptoms.

Sample Collection

High vaginal swabs were taken from the patients by the obstetrician. A sterile cotton wool swab, two swabs of each patient, was inserted carefully into the upper part of the vagina. The samples were immediately transported to the laboratory of the Microbiology Department of the AL-Thwrah Hospital for examination^{3,19}.

Examination of Specimen

Microscopic Examination

The first swab was subjected to wet mount examination. One drop of normal saline was added to each sample and shaking vigorously and examined microscopically under 10x and 40x²⁰.

Culture Methods

The second swab was cultured on the surface of Sabouraud Dextrose Agar (SDA) (Oxide, UK) with and without chloramphenicol (250mg/L). The plates were incubated for 48 h at 37°C. The morphological features for the colony were studied and confirmed by observing the budding characterization with pseudohyphae by using the Gram tube test²¹.

Candida species Identification

Candida species were identified depending on morphological features on a culture medium, germ tube formation, and carbohydrate assimilation test as the following:

Germ Tube Test

A small portion was taken from a pure colony of *C. albicans* by sterile loop and inoculated into sterile tubes containing 0.5ml of human serum. The tubes were mixed and incubated aerobically for 2h at 37°C. One drop of each serum was transferred to a clean slide and examined by a microscope under high power (x40) to detect the presence of germ tubes that are short hyphal initials²².

Sugar Assimilation Test

The overnight of culture yeast suspension was added to the basal carbohydrate-free medium (II) of molten agar, cooled to 45°C, and poured to plates and left the plates to solidified. Discs saturated with 1% of sugar and placed on the surface of plates and incubated for five days at 37°C. The occurrence of growth around each disc indicates the carbohydrate assimilation of tested sugar. The glucose, Dgalactose, maltose, sucrose, lactose, raffinose, xylose, and trehalose were used in the sugar assimilation test²³.

Antifungal Susceptibility Testing

The isolated *Candida* species were subjected to susceptibility antifungal agents by using a disc diffusion method on the surface of Mueller Hinton agar. The antifungal the discs used were Ketoconazole (10mg), Clotrimazole (50µg), Itraconazole (50µg), Voriconazole (10µg), Fluconazole (100µg) Amphotericin B (50µg) and Nystatin (100µg) (Himedia, India). The inhibition zone was measured after 48h of incubation at 37°C²⁴.

RESULTS

The result from the current study revealed that the 218 vaginal swabs were collected from pregnant women (from urban and from rural area) who presented with genital manifestations. Only 134 samples (61.5%) were showed as positive growth in culture media and 84 samples (38.5%) were reported as negative growth in culture media as shown in Figure 1.

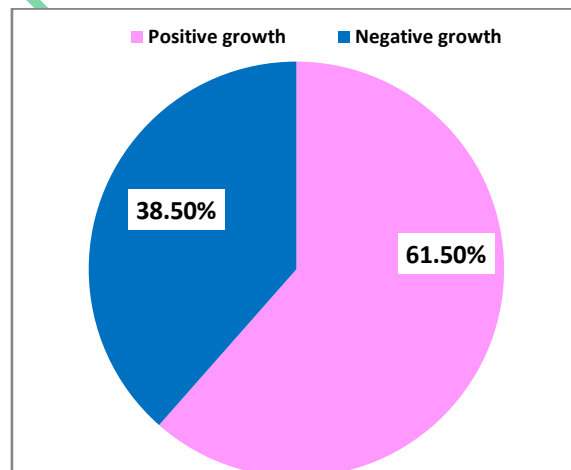


Figure 1: The type of growth in culture media

From the 134 positive isolates of *Candida* species, it was found that the highest prevalence of vulvovaginal candidiasis was among women from rural area 39(65%) compared to women from urban areas 95(60.13%) as shown in Table 1.

Table 1: Percentage of candidiasis infection according to area

Residence area	No. of examined	Positive (%)	Negative (%)
Urban	158	95 (60.13)	63(39.87)
Rural	60	39 (65)	21(35)
Total	218	134(61.5)	84 (38.5)

However, this study showed that the highest percentage for first-time infection was 96/165 (58.18%), while the recurrent infection was 38/53 (71.69%). Table 2 shows that the highest prevalence of *Candida* infection 73(54.48%) was recorded within the age range of 28-47 years, followed by a group aged between 38-47 years 49(36.57%). While the lowest prevalence of *Candida* infection was reported in the age group of 18-27 years 12(8.95%).

Table 2: The frequency of *Candida* vaginitis infections according to age

Age in years	Examined No. (%)	Positive <i>Candida</i> sp. No. (%)
18-27	35(16.1)	12 (8.95)
28-37	122(55.96)	73 (54.48)
38-47	61(27.98)	49 (36.57)
Total	218 (100)	134 (100)

In the present study, it was revealed that four species of *Candida* were isolated and characterized by vaginal samples according to carbohydrate assimilation. It was found that the *C. albicans* 82(61.2%) was the predominant *Candida* species isolated followed by a higher prevalence of non-albicans species like *C. tropicalis* 29(21.64%), *C. glabrata* 15(11.19%), and *C. kefyr* 8(5.97%) (Figure 2).

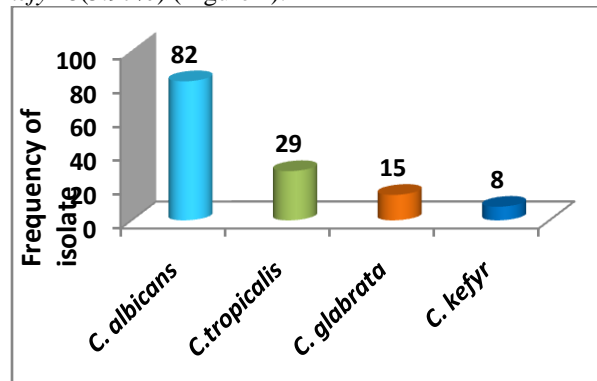


Figure 2: Distribution of isolated *Candida* species from pregnant

The susceptibility results revealed that the 94.8% of isolated *Candida* species were susceptible to nystatin. Fluconazole was the next effective drug with 91.04% sensitive followed by amphotericin B (88.8%), voriconazole (78.35%), clotrimazole 75.37%, ketoconazole 73.13%, and itraconazole 69.40%. Slightly resistance of antifungals was itraconazole, ketoconazole, clotrimazole, and voriconazole as shown in Table 3.

Table 3: Antifungal susceptibility pattern of isolated *Candida* sp.

Antifungals	Sensitivity (%)	Resistance (%)
Nystatin	127 (94.8)	7(5.2)
Fluconazole	122 (91.04)	12(8.96)
Amphotericin B	119(88.8)	15(11.2)
Voriconazole	105(78.35)	29(21.65)
Clotrimazole	101(75.37)	33(24.63)
Ketoconazole	98(73.13)	36(21.87)
Itraconazole	93(69.40)	41(29.60)

DISCUSSION

Vulvovaginal candidiasis is caused by the overgrowth of yeast in the mucosa membrane of the female genital tract and frequently diagnosed as a daily practice of gynecologist^{14,25}. Of 218 samples examined, 134 samples (62.5%) showed as positive growth in culture media and 84 samples (38.5%) were reported as negative growth. This negative culture cases may be infected with *Trichomonas vaginitis*. In Sana'a, Yemen, Abdul-Aziz *et al.*¹⁸ revealed that 37.6% was positive for vaginal infections among reproductive-age women. Also, Al-mamari *et al.*²⁶ in Sana'a, reported that 94% of vaginal samples of patients were positive growth for vulvovaginal candidiasis.

The highest distribution of vaginitis (65%) cases in the current study were found in rural areas. This result in disagreement with Abdul-Aziz *et al.*¹⁸ who observed that 88.44% of vaginitis was among reproductive-aged women residents in an urban area. The high occurrence rate of infectious vaginitis among rural women mostly refers to poor conditions of medical care, absence of health education, lower-income, and difficulty in timely medical treatment²⁷.

The highest frequency of *Candida* species infection in this study was most commonly seen among 36-46 years age group and this result is an agreement with Al-Karim *et al.*²⁸ in Syria. Also, Bitew and Abebaw¹⁴ in Ethiopia documented that the highest vulvovaginal candidiasis was among the 22-44 years age group

The present study showed that the frequency of isolated *Candida* among women with vulvovaginal candidiasis for the first-time was 96 (71.64%), while recurrent vulvovaginal candidiasis infection was 38(28.36%). Most patients in this study did not have immunity disease so the highest percentage was with the first time of exposure according to specialist doctors. This result is an agreement with Sobel,²⁹ in Nigeria.

This study analyzed the predisposing factors of vaginal candidiasis from positive *Candida* isolated; pregnancy has been the most frequently associated risk factor (33%). This high percentage due to the increased amount of glycogen in the vagina and high levels of estrogen hormones. It provides a good source of carbon, which favors the growth of *Candida* species³⁰, and lowest occur with kidney transplantation (3) and leukemia (2) this may be dependent on the type of sample at this study. The result of this study is in agreement with the work of Abu Baker³¹ and Babin *et al.*³².

In this study, the frequency of isolated *Candida* among women complained of burning, discharge and itching were (27%) which was the highest percentage. This study has differed with Falahati *et al.*,³³ who showed that the frequency of isolated *Candida* among women complained of discharge was 55 (82.1%), itching 42 (62.7%), and burning 33 (49.3%).

The result of this study was indicated that *C. albicans* 82(61.2%) are responsible for the greatest number of symptoms associated with vaginal candidiasis. This finding is in agreement with the work of Al-mamari *et al.*²⁶ who found that the 65.95% of isolated *Candida* species was *C. albicans*. Another study by Omar *et al.*,³⁴ in Egypt who found that *C. albicans* was the highest (78.3%) isolated species from infected women by vaginitis.

During the last three decades were noticed that the increase in the percentage of vaginitis caused by non-albicans species of *Candida*. The present study showed an increase in the frequency of non-albicans species as potential causes of vaginal candidiasis. It was found that *C. tropicalis* (1.64%), *C. glabrata* (11.19%), and *C. Kefyr* (5.97%) were recorded in this study. This finding was supported by Babin *et al.*,³² in Iran.

In the antifungal susceptibility results, it was reported that all isolates were susceptible to tested antifungals. The highest sensitivity of antifungals against isolated *Candida* species was nystatin (94.8), fluconazole (91.04%), amphotericin B (88.8%), voriconazole (78.35%), clotrimazole (75.37%), ketoconazole (73.13%), and itraconazole (69.40%). Whereas slight resistance of antifungals was itraconazole, ketoconazole, clotrimazole, and voriconazole as reported against isolated *Candida* species. These findings are in agreement with the work of researchers that observed that most isolated *Candida* species were susceptible to tested antifungals^{26,35,36,37}.

CONCLUSION

In conclusion, vaginal infections are very common in our region and have a high frequency. It was found that *C. albicans* (61.5%) was the predominant isolated species followed by *C. tropicalis*. All isolates of *Candida* species were susceptible to tested antifungal. This is the first report on the types of *Candida* sp., causing vaginal candidiasis and their antifungal susceptibility patterns in Yemen.

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