



Available online on 15.9.2020 at <http://ujpr.org>
Universal Journal of Pharmaceutical Research
 An International Peer Reviewed Journal

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Volume 5, Issue 4, 2020



RESEARCH ARTICLE

VULVOVAGINAL CANDIDIASIS PREVALENCE AMONG PREGNANT WOMEN IN DIFFERENT HOSPITALS IN IBB, YEMEN

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ABSTRACT

Background and aims: *Candida* species are opportunistic yeasts that affect the genital urinary tract that causes vulvovaginal candidiasis in the most females, particularly in developing countries. This work aims to find the prevalence of vulvovaginal candidiasis among pregnant women in Ibb, Yemen.

Material and methods: Two hundred and eighteen samples of females attending outpatient obstetrics and gynecology clinics were chosen from several hospitals (Al-Thawra, Maternity and Childhood Center, Maternal Reproductive Health Center, and Nasser General Hospital) located in Ibb, from December 2019 to March 2020. The laboratory works were performed in the microbiology department of Al-Thawra Hospital, Ibb. Vaginal swabs were cultivated on Sabouraud dextrose agar, then the species of *Candida* identification and anti-fungal sensitivity tests were performed regarding the standard procedure of microbiology.

Results: The findings revealed that out of 218 cases, 134 (61.5%) were infected with *Candida* species, and the rate of candidiasis was higher among women coming from rural areas than among women in urban areas; and in the age group 28-37 years. *C. albicans* (61.2%) were the most prevalent species followed by *C. tropicalis* (21.64%), *C. glabrata* (11.19%), and *C. kefyr* (5.97%). Susceptibility tests showed that the most isolated *Candida* species were sensitive to nystatin (94.8), fluconazole (91.04%), amphotericin B (88.80%), voriconazole (78.35%), clotrimazole (75.37%), ketoconazole (73.13%), and itraconazole (69.40%).

Conclusion: It can be concluded that *Candida* vaginitis is very common among pregnant women, and the tested anti-fungal agents continue to be effective against all isolates of *Candida* species. In contrast, there was a slight increase resistant against itraconazole, ketoconazole, clotrimazole, and voriconazole which raises concern about a rise in *Candida* species that are resistant to these drugs.

Keywords: *Candida albicans*, Susceptibility antifungal, Vulvovaginal candidiasis (VVC), Yemen.

Article Info: Received 27 June 2020; Revised 15 July; Accepted 12 August, Available online 15 September 2020



Cite this article-

Edrees WH, Al-Asbahi AA, Al-Shehari WA, Qasem EA. Vulvovaginal candidiasis prevalence among pregnant women in different hospitals in Ibb, Yemen. Universal Journal of Pharmaceutical Research 2020; 5(4):1-5.

DOI: <https://doi.org/10.22270/ujpr.v5i4.431>

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INTRODUCTION

Candida vaginitis, also often called vulvovaginal candidiasis, is a pathogen of the vagina caused by diverse types of *Candida* species^{1,2}. Vulvovaginal candidiasis (VVC) considers the second disease cause of symptomatic vaginal diseases after bacterial vaginitis^{1,3} and responsible for over 25% of infectious vaginitis^{4,5}. Almost 75% of women have VVC in their lifetime³. Also, it was documented that more than 40% of affected females will have two or more incidence of vulvovaginal candidiasis in the year^{6,7}. The clinical manifestations of vulvovaginal candidiasis are

representing on the itching, vaginal discomfort, an unpleasant burning feeling, and soreness that lead to whitish-yellowish vaginal discharge often with a curd-like appearance⁸. The frequency rates of *Candida* species among pregnant women with VVC various from the study area to another⁹. Some reports have documented that the *C. albicans* was ranged between 80-90% among women with acute VVC that caused by *Candida* sp., whereas other species of *Candida* are less frequently isolated^{1,10,11}. However, in the last 10 years, there are different species of non-albicans *Candida* that are *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C.*

lusitaniae, *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 resistance of *Candida* species to several antifungal drugs¹⁵. There are limited 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 with bacterial vaginosis in Hadhramout city. While there have been several studies that discussed oral candidiasis in Yemen in the past five years^{17,18,19}.

A study, in Sana'a, by Abdul-Aziz et al.,²⁰ found that the frequency of vaginal infection among reproductive-aged women was 37.6% of collecting samples. Also, the results showed that 27.2% by bacterial vaginosis, 6.6% by vulvovaginal candidiasis, and 0.9% by trichomonal vaginosis. To date, data on the prevalence of vulvovaginal candidiasis among women during pregnancy period and the rate of resistance to antifungal drugs for *Candida* species are not available for the city of Ibb, Yemen. So, the present study aimed to find the prevalence and types of *Candida* that cause vulvovaginal candidiasis among pregnant women and their sensitivity to antifungal drugs in Ibb, Yemen.

MATERIALS AND METHODS

Study Design and Data Collection

Two hundred and eighteen (218) samples were sampled from pregnant women presenting Obstetrics and Gynecology outpatient clinics in several 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 participated cases were interviewed and patient information was reported with intended questionnaires that include; demographic, age, medical history, and symptoms.

Sample Collection

The swab specimens were collected from female pregnant by the obstetrician. By using a sterile cotton swab, two wipes per patient, was carefully inserted into the upper part of the vagina. The samples were immediately transmitted to the diagnostic laboratory of the Microbiology Department of the AL-Thwrah Hospital for examination^{3,21}.

Examination of Specimen

Microscopic Examination

One swab of each case was subjected to direct examination by the wet mount. One drop of normal saline was transferred to each sample and mixed well and microscopically examined under 10x and 40x²².

Culture Methods

The second swab was cultivated on the surface of Sabouraud Dextrose Agar (SDA) (Oxide, UK) with chloramphenicol (250mg/l). The plates were incubated for 48 h at 37°C. The morphological features for the colony were studied and confirmed by detecting the budding characterization with pseudo-hyphae by using the Gram tube test²³.

Candida species Identification

The *Candida* species were identified according to morphological characterizations on the surface of culture medium, the formation of germ tube test, and carbohydrate assimilation as the following:

Germ Tube Test

A small part from a pure colony of *Candida* species was picked and transported into a sterile tube that contains 0.5ml serum of human, mixed well, and aerobic incubated for 2h at 37°C. Then, one drop of each serum was transported to a clean slide and scanned by a microscope with objective lenses (x40) to identify the existence of germ tubes that characterized with initially short hyphal²⁴.

Sugar Assimilation Test

From the overnight of yeast suspension culture, one mL was transferred to the basal carbohydrate-free medium (II) of molten agar cooled to 45°C and poured to petri dish plates. Saturated discs with 1% with sugar were placed on the solidified surface of plates and incubated for five days at 37°C. The occurrence of growth around each disc implies the carbohydrate assimilation of tested sugar. The glucose, sucrose, D-galactose, maltose, raffinose, lactose, trehalose, and xylose were used in the sugar assimilation test²⁵.

Antifungal Susceptibility Testing

The isolated *Candida* species were subjected to susceptibility antifungal agents by using a disc diffusion technique on the surface of Mueller Hinton agar. The used antifungal discs were Nystatin (100µg), Clotrimazole (50µg), Itraconazole (50µg), Ketoconazole (10mg), Voriconazole (10µg), Amphotericin B (50µg), and Fluconazole (100µg) (Himedia, India). The inhibition zone was measured after 48h of incubation at 37°C²⁶.

RESULTS

A hundred and thirty-four samples (61.5%) found positive growth in culture and 84 specimens (38.5%) recorded negative growth (Figure 1). Table 1 shows the prevalence of vulvovaginal candidiasis regarding patient's residents. It was observed that the women coming from the rural area had the highest rate (65%) while the patients coming from the urban area was 60.13%.

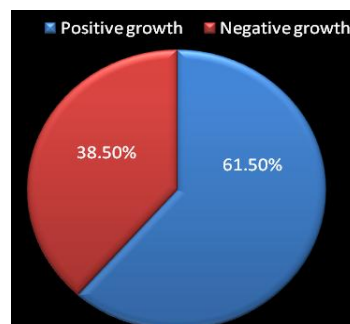


Figure 1: The type of growth in culture media

However, this work revealed that the highest rate of infection for first-time was 96/165 (58.18%), while the recurrent infection was 38/53 (71.69%). Table 2 shows that the highest prevalence of *Candida* infection was 73(54.48%) recorded in the group aged 28-37 years

and followed by a group aged between 38-47 years 49 (36.57%). While the lower rate of *Candida* infection was reported in the age group of 18-27 years 12(8.95%).

Table 1: Rate of candidiasis infection regard to resident

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)

The current study found that 96 (71.64%) of cases were reported as the first time infected with vulvovaginal candidiasis, while 38 (28.36%) of cases were recorded as the recurrent vulvovaginal candidiasis (Figure 2).

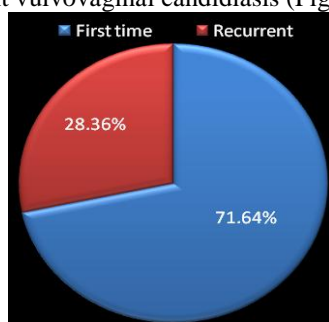


Figure 2: The first time and recurrent infection

In the present investigation, it was isolated four species of *Candida* from vaginal specimens and identified according to carbohydrate assimilation. *C. albicans* was (61.2%) the most predominant *Candida* species identified from specimens followed by a non-albicans species that are *C. tropicalis* 29(21.64%), *C. glabrata* 15(11.19%), and *C. kefyr* 8(5.97%) (Figure 3).

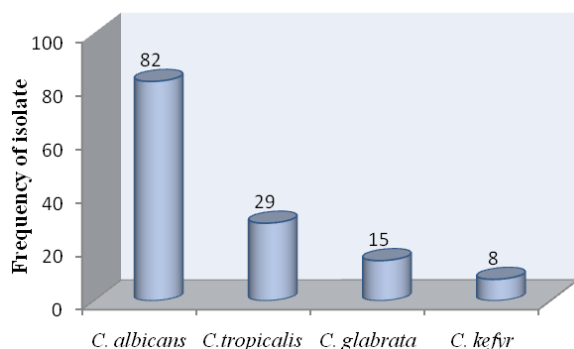


Figure 3: Distribution of isolated *Candida* species from pregnant

Table 2: Prevalence of vulvovaginal candidiasis related 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)

The susceptibility results revealed that 94.8% of identified *Candida* species were sensitive to nystatin. Fluconazole was the second drug with 91.04% susceptible 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 sensitivity profile 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 represents one of the most frequent gynecological disorders caused by an overgrowth of *Candida* species in the mucosa membrane of the genital tract in the female^{14,27}. In present results revealed that 62.5% of specimens were positive for *Candida* sp. growth in culture media while 38.5% of specimens were negative growth. The negative growth could be referred to as an infection caused by *Trichomonas vaginitis* or bacteria 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.,²⁸ in Sana'a, reported that 94% of vaginal samples of patients were positive growth for vulvovaginal candidiasis. In the current study, the women coming from rural areas had a higher rate (65%) of vaginitis than patients from the urban area. This finding in disagreement with Abdul-Aziz et al.,²⁰ who revealed that 88.44% of vaginitis was among reproductive-women residents in an urban area. The high occurrence of communicable vaginitis among rural women frequently refers to poor situations of healthcare, absence of health education, lower-income, and difficulty in medical treatment in time²⁹. However, the education status plays a significant role in contributing to the improvement of personal hygiene which lacks in the rural area and that may explain the variance in the frequency of infection between the urban area and rural area¹⁴. The results regarding age group showed that the higher rate of *Candida* species infection was most frequently noticed among the age of 28-37 years and this finding was similar to a study by Al-Karim et al.,³⁰ in Syria. A similar study in Ethiopia by Bitew and Abebaw¹⁴ recorded that the highest rate of vulvovaginal candidiasis was among age groups of 22-44 years. The current study found that 96 (71.64%) of cases were reported as the first time infected with vulvovaginal candidiasis, while 38 (28.36%) of cases were recorded as the recurrent vulvovaginal candidiasis. Most cases in the present work didn't have immunological diseases, so the first time exposure was highest and this finding was consistent with Sobel,³¹ in Nigeria. *C. albicans* is normal in a small amount that lives in the mucous membranes lining the genitals. It is the most responsible for vaginal candidiasis infection when occur disrupted in a healthy balance of

microorganisms through pregnancy or medication. The high level of estrogen in the body during pregnancy can upset the healthy balance of microorganisms and increase the likelihood of developing a vaginal infection by *Candida* species. Also, various medications such as steroids, antibiotics, hormone therapy, chemotherapy, and radiotherapy can increase the risk too. Additional factors, such as stress, genitals washing with soap, wearing synthetic and tight clothes, sweating and using panty liners or sanitary pads. The present investigation reported that the *C. albicans* was the most isolated of *Candida* species with 61.2% from pregnant women suffering from vaginal candidiasis symptoms and this result is consistent with the report of Al-mamari et al.,²⁸ who found that the 65.95% of isolated *Candida* species was *C. albicans*. Another study by Omar et al.,³⁵ in Egypt showed that *C. albicans* was the highest (78.3%) isolated species from infected women by vaginitis. In the last three decades, it has been observed that there was an increase in the percentage of vaginitis caused by species of *Candida* non-albicans. The present work revealed an increase in the frequency of non-albicans species considers as potential causes of vulvovaginal 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 a similar study by Babin et al.,³⁴ in Iran. The results of antifungal susceptibility, 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^{28,35,36,37}.

CONCLUSION

It can be concluded that vulvovaginal candidiasis is very common among pregnant women, and the tested anti-fungal agents continue to be effective against all isolates of *Candida* species. In contrast, there was a slight increase in resistance against itraconazole, ketoconazole, clotrimazole, and voriconazole which raises concern about a rise in *Candida* species that are resistant to these drugs.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTION

The manuscript was carried out, written, and approved in collaboration with all authors.

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