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## Prevalence and Antifungal Susceptibility of *Candida* species Causing Vaginitis among Pregnant Women in Hajjah Governorate, Yemen

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#### **Abstract**

Background: Candida species, an opportunistic yeast, are the second commonest affecting the genitourinary tracts in females and causing vulvovaginal candidiasis. Aims: This study aimed to determine the occurrence of Candida species causing vulvovaginal candidiasis among pregnant women in Hajjah governorate, Yemen. Methods: A total of 50 vaginal swab specimens were collected and cultured on Sabouraud dextrose agar. The identification of Candida species and antifungal susceptibility testing was done according to standard microbiological procedures. Results: The results showed that 70.45% were positive for Candida species. It was found that the C. albicans was (68.3%) the most common species isolated while the non-Candida albicans was 32.26%. Also, it was recorded that the highest prevalence of Candida species was within-group aged 20-24 years. Similarly, the participant woman coming from rural areas had higher vulvovaginal candidiasis than from urban areas. A higher frequency of vulvovaginal candidiasis was observed among participated women with secondary a certificate, third trimester, paucipares (1-2 birth), and recurrent infection. Also, the pregnant women who complained of itching, burning, and discharge had a high rate of vulvovaginal candidiasis. Susceptibility tests revealed that the most isolated species of Candida were sensitive to fluconazole and nystatin as well as amphoteracin B. Conclusion: It can be concluded that vulvovaginal candidiasis is quite common in Yemen country with a high prevalence. Also, the fluconazole remains the effective agent against all isolates Candida species.

**Keywords:** Antifungal, *Candida* and *Non-Candida* albicans, Hajjah, pregnant Woman, Yemen

#### Introduction

Candida vaginitis is the infection of the vagina by several types of Candida species, also often called vulvovaginal candidiasis/candidosis<sup>1,2</sup>. Vulvovaginal candidiasis (VVC) considered to be the most common manifestation of genital candidiasis<sup>1,3</sup>. Also, the risk factors associated with an increased rate of VVC in pregnant women are immunologic alterations, increased

estrogen levels, and increased vaginal glycogen production mechanism<sup>3</sup>.

It is representing over 25% of infectious vaginitis<sup>4,5</sup>. 75% of women are affected by vulvovaginal candidiasis in their lifetimes<sup>3</sup>. Also, it was found that more than 40% of affected women will have 2 or more vulvovaginal candidiasis episodes<sup>6,7</sup>. Clinical manifestation of vulvovaginal candidiasis is pruritus, vaginal discomfort, burning, and

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soreness<sup>8</sup>.

The distribution of Candida sp. in vulvovaginal candidiasis cases differs widely depending on the geographical location and population studied<sup>9</sup>. Some reports have documented that among acute vulvovaginal women with candidiasis were caused by C. albicans that accounts for 80-90% of all vaginal candidiasis cases, whereas other species are less frequently isolated<sup>1,10,11</sup>.

However, in the last years, different species of *Candida* non-albicans are *C*. parapsilosis, C. glabrata, C. krusei, C. lusitaniae. *C*. tropicalis, 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 antifungals, especially against the vulvovaginal candidiasis lead increase the resistance of Candida species to several antifungal drugs<sup>15</sup>. Few reports 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, the frequency of vulvovaginal candidiasis was 61.5% reported among pregnant women in Ibb City<sup>17</sup>.

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 6.6% vulvovaginal vaginosis, by candidiasis, and 0.9% by trichomonal vaginosis. Also, In Sana'a city, the prevalence rate of VVC was 51.6% documented among pregnant women attending healthcare centers<sup>19</sup>.

Hajjah governorate is one of the Yemen governorates that lack data on the infectious diseases prevalent among the community particularly vulvovaginal candidiasis among pregnant women caused by *Candida* species and their

antifungal susceptibility pattern. In view of this, the aim of the current work aimed to determine the prevalence of species Candida that causes vulvovaginal candidiasis among pregnant women and antifungal susceptible patterns of isolated species to antifungals in Hajjah City, Yemen.

#### Materials and Methods Study design and area

This study is a cross-sectional study conducted at AL-Gumhorri hospital located in Hajjah city which is located 127 kilometers northwest of Sana'a capital of Yemen, at an elevation of about 1800 meters. The experimental work was performed at the microbiology laboratory at AL-Gumhorri hospital, Hajjah city.

## Ethics approval and consent to participate

This study was conducted after it was ethically reviewed and approved by the Ethical Review Board of the Department of Medical Microbiology, Faculty of Applied Sciences, Hajjah University. Also, written consents were obtained from voluntary participants.

#### **Data collection**

Prior to the specimen collection, the socio-demographic and health characteristics such as age, resident area, education status pregnancy status, pregnancy trimester, parity, recent use of antibiotics, infection frequency were collected by face- to- face interviews using a structured questionnaire. Also, clinical signs and symptoms of vaginal abnormalities such as itching, burning, and vaginal discharge were recorded.

#### Sample collection

Forty-four (44) swab specimens were collected from pregnant women seeking the Obstetrics and Gynecology department at AL-Gumhorri hospital

during the period between March to June 2021.

Vaginal swabs were collected from the pregnancy-woman patients by the obstetrician by using the moisten sterile cotton swab that was inserted carefully into the upper part of the vagina. All vaginal collected swabs were transferred immediately to the microbiology laboratory for examination<sup>3,20</sup>.

## **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  $40x^{21}$ .

#### **Culture methods**

The specimen cultured was individually the surface of on Sabouraud Dextrose Agar (SDA) (Himedia, India) supplemented chloramphenicol (250 mg/L)incubated aerobically at 37°C for 48 h. The colonies of Candida species were identified depending on morphological features on a culture medium and conformed by observing the budding characterization with pseudohyphae by using the Gram tube test germ tube formation<sup>22</sup>.

#### 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 microscope under high power (x40) to detect the presence of germ tubes that are short hyphal initials<sup>23</sup>.

#### **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 + 2% Glucose + 0.5 mcg/ml methylene blue dye medium. The antifungal discs used were Amphoteracin-B (50 µg), Fluconazole (25µg), and Nystatin (NS, 100 units) (Himedia, India). The inhibition zone was measured after 48h of incubation at  $37^{\circ}C^{24}$ .

#### Results Results

The socio-demographic characteristics of participated pregnant-woman are summarized in Table (1).

The result from the current study revealed that only 31(70.45%) specimens were showed positive culture media growth in and 13(29.55%) specimens were reported negative growth in culture media as shown in Figure (1).

Figure (2) shows the type of *Candida* species causing vaginitis among pregnant women. It was found that the *C. albicans* was 21(67.74%) while the non-*Candida albicans* was 10(32.26%) reported among pregnant women.

Table 2 shows that the highest prevalence of *Candida* infection was 12(38.71%) recorded within the age range of 20-24 years, followed by a group aged between 25-29 years (25.81%). Also, most vulvovaginal candidiasis is caused by *C. albicans* with a rate of 67.74%.

From the 31 positive isolates of *Candida* species, it was found that the highest prevalence of vulvovaginal candidiasis was among women from rural 10(76.92%) compared to women from urban 21(67.74%) areas as shown in Figure (3).

Table (1): Socio-demographic of participated pregnant-woman in study

Va	No. examined	Rate (%)			
Personal characterization					
	16-19	4	9.09%		
A go gwonn	20-24	13	29.56%		
Age group (in years)	25-29	12	27.27%		
(in years)	30-34	7	15.90%		
	35-39	8	18.18%		
Resident area	Rural	13	29.56%		
Resident area	Urban	31	70.45%		
	Illiterate	9	20.45%		
Educational	Primary	7	15.91%		
status	Secondary	16	36.37%		
	Graduate	12	27.27%		
Gestational	First trimester	12	27.27%		
trimester	Second trimester	11	25.0%		
trimester	Third trimester	21	47.73%		
D. 24	Paucipara (1-2 birth)	23	52.27%		
Parity	Multipara (>2 births)	21	47.73%		
Infection	First time	7	15.91%		
frequency	Recurrent	37	84.09%		
Recent Use of	Yes	39	88.64%		
antibiotics	No	5	11.36%		
Clinical signs and symptoms					
Itching	Yes	42	95.45%		
Turning	No	2	4.55%		
Rurning	Yes	42	95.45%		
Burning	No	2	4.55%		
Discharge	Yes	41	93.18%		
Discharge	No	3	6.82%		
Diabetes mellitus	Yes	0	0(0)		
Diabetes memtus	No	44	100%		

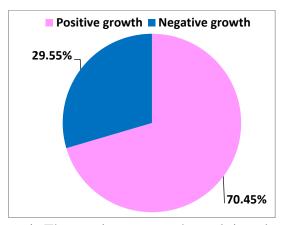


Figure 1: The specimens growth result in culture media

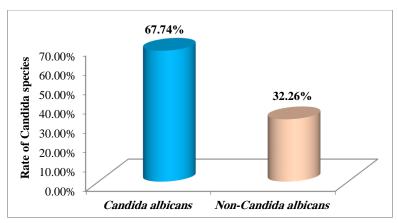


Figure 2: The rate of *Candida* species

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

Age group	Total tested	C. albicans	Non-Candida albicans	Total
(in years)	No. (%)	No. (%)	No. (%)	No. (%)
16-19	4 (9.09)	2(6.45)	0(0)	2(6.45)
20-24	13 (29.56)	8(25.81)	4(12.9)	12(38.71)
25-29	12(27.27)	5(16.13)	3(9.68)	8(25.81)
30-34	7(15.90)	3(9.68)	2(6.45)	5(16.13)
35-39	8(18.18)	3(9.68)	1(3.23)	4(12.9)
Total	44(100)	21(67.74)	10(32.26)	31(100)

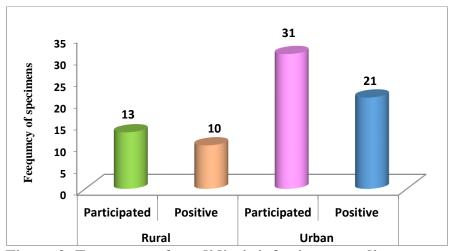


Figure 3: Frequency of candidiasis infection according to area

In the present study, women with secondary certificates were more affected than those patients with illiterate and primary school education. Similarly, vulvovaginal candidiasis was higher among the third trimester gestational age with 18(58.07%). Also, vulvovaginal candidiasis was higher slightly among paucipara mothers

(51.61%) compared to multipara (47.39%) study subjects. The highest rate of vulvovaginal candidiasis was 83.87% recorded for recurrent infection. The higher rate of vulvovaginal candidiasis was 90.32% found among women administered antibiotics (Table 3).

Table 3: Distribution of vulvovaginal candidiasis in relation to risk factors

		9			
Variables		Total	C.	Non-Candida	Total
		tested	albicans	albicans	
		No. (%)	No. (%)	No. (%)	No. (%)
	Illiterate	9(20.45)	5(16.13)	1(3.23)	6(19.35)
Educational					
	Primary	7(15.91)	1(3.23)	5(16.13)	6(19.35)
status	Secondary	16(36.37)	8(25.81)	3(9.68)	11(35.48)
	Graduate	12(27.27)	6(19.35)	2(6.45)	8(25.81)
Gestational	First trimester	12(27.27)	3(9.68)	4(12.9)	7(22.58)
	Second trimester	11(25.0)	5(16.13)	1(3.23)	6(19.35)
trimester	Third trimester	21(47.73)	12(38.71)	6(19.35)	18(58.07)
Dowitz	Paucipares (1-2 birth)	23(52.27)	9(29.03)	7(22.58)	16(51.61)
Parity	Multipara (> 2 births)	21(47.73)	11(35.48)	4(12.9)	15(47.39)
Infection	First time	7(15.91)	3(9.68)	2(6.45)	5(16.13)
frequency	Recurrent	37(84.09)	17(54.84)	9(29.03)	26(83.87)
Uses of	Yes	39(88.64)	18(58.06)	10(32.26)	28(90.32)
antibiotics	No	5(11.36)	2(6.45)	1(3.23)	6(19.35)

The present study showed that the most clinical signs and symptoms among pregnant women with vaginitis were itching (70.97%) burning (67.74%),

and discharge (61.29%) caused by C. *albicans* whereas all the participated patients were negative for diabetes mellitus as listed in the Table (4).

Table 4: Clinical signs and symptoms of participating in a study

Variables		Total tested	Candida albicans	Non-Candida albicans	Total
		No. (%)	No. (%)	No. (%)	No. (%)
	Yes	42(95.45)	22(70.97)	9(29.03)	31(100)
Itching	No	2(4.55)	0(0)	0(0)	0(0)
Duming	Yes	42(95.45)	21(67.74)	8(25.81)	29(93.55)
Burning	No	2(4.55)	0(0)	2(6.45)	2(6.45)
	Yes	41(93.18)	19(61.29)	11(35.48)	30(96.77)
Discharge	No	3(6.82)	1(3.23)	0(0)	1(3.23)
Diabetes	Yes	0(0)	0(0)	0(0)	0(0)
mellitus	No	44(100)	21(67.74)	10(32.26)	31(100)

The antifungal susceptibility results revealed that between 90-100% of isolated *Candida* species were sensitive

to fluconazole. Also, amphotericin B was an effective drug with a rate between 45.45-55% and followed by

nystatin between 36.36-66.67%. Whereas, Non-Candida albicans showed resistance to amphoteracin B

(45.45%) and nystatin (33.33%) as shown in Table (5).

Table 5: Antifungal susceptibility profiles of isolated Candida sp.

Antifungals	Candida species					
	C. albicans	Non-Candida albicans				
Amphoteracin B						
Sensitive (%)	11 (55)	5(45.45)				
Moderate (%)	3 (15)	1(9.1)				
Resistant (%)	6 (30)	5 (45.45)				
Fluconazole	Fluconazole					
Sensitive (%)	18(90)	10(100)				
Moderate (%)	0(0)	0(0)				
Resistant (%)	2 (10)	0(0)				
Nystatin						
Sensitive (%)	8(36.36)	6(66.67)				
Moderate (%)	8(36.36)	0				
Resistant (%)	6(27.28)	3(33.33)				

#### **Discussion**

Vulvovaginal candidiasis is caused by the overgrowth of yeast in the mucosa membrane of the female genital tract and is frequently diagnosed as a daily practice of gynecologist<sup>14,25</sup>. In the present result, of 44 specimens examined in this study, it was found that 70.45% of specimens showed as positive growth for *Candida* sp. in the culture media whereas 29.55% of specimens were reported as negative growth. These negative culture cases maybe referred to as another causal of vaginitis. This result is slightly higher than the report by Edrees et al. 17 who found that the prevalence of VVC was 61.5% recorded among pregnant women in Ibb city, Yemen. In Egypt, Abbas et al., 26 reported that 60.8% of examined women were infected by vulvovaginal candidiasis.

However, the lower rate of VVC among

pregnant women was 51.6% found in Sana'a, Yemen, to according a study by Al-Rukeimi *et al*<sup>19</sup>. Another study by Abdul-Aziz *et al*.<sup>18</sup> recorded that the vaginal candidiasis among reproductiveaged women was 17.69%.

The incidence of VVC differs from one report to another according to health status. A study, in Lebanon, by Ghaddar et al 27 showed the 39% of examined pregnant woman was infected by Candida sp. The high rate of Candida sp. that found in the present study maybe refer to the socio-demographic factors such as status of patient characteristics, the immunity, use of the broad-spectrum antibiotics for treatment, and hormonal influences that contribute to influencing the prevalence of VVC among pregnant woman subjected to a study.

The result of this study was indicated that *C. albicans* 67.74% are responsible for the

greatest number of symptoms associated with vaginal candidosis. This finding is consistent with the several works reported by Edrees *et al.*<sup>17</sup> in Yemen, and Bitew and Abebaw<sup>14</sup> in Ethiopia and Nurat *et al.*,<sup>28</sup> in Nigeria.

This result is higher than reported by Al-Rukeimi *et al.*<sup>19</sup> in Sana'a and lower than study by Omar *et al.*,<sup>29</sup> in Egypt who found that the *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 rate of vaginitis caused by non-albicans species of *Candida*. The present study showed the increase in the frequency of non-albicans species as potential causes of vaginal candidiasis. The higher rate of non-*Candida albicans* was reported by Nurat *et al.*<sup>28</sup> and Al-Rukeimi *et al.*<sup>19</sup>.

The highest frequency of *Candida* species infection in this study was most commonly seen among the 20-24 years age group and this result is in agreement with Al-Rukeimi *et al.*<sup>19</sup> in Sana'a who documented that the highest VVC was among the age group of 20-24 years,. Also, in a similar study carried out in Nigeria by Nurat *et al.*<sup>28</sup> who observed the age of 20-29 years old had the highest rate of candidiasis.

In a different study by Edrees *et al.*,<sup>17</sup> showed that the highest prevalence of *Candida* infection was 54.48% recorded in the group aged 28-37 years.

The highest distribution of vaginitis (76.92%) cases in the current study were found among women coming from the rural area compared to women from the urban (67.74%) area. This result in agreement with several reports that documented the higher rate of VVC among pregnant women living in rural area<sup>17,18</sup>.

The high rate 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 time<sup>26</sup>. 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 area<sup>14</sup>.

The highest rate of VVC in this work was 35.48% recorded among participated women who have secondary school certificate. This result is in disagreement with different reports that noticed the high rate of VVC was found the illiterate woman<sup>14, 19</sup>. Educational status plays an important role in increasing awareness about personal hygiene and reduces the transmission of disease.

However, the VVC was higher slightly among paucipara mothers compared to multipara mothers. Similarly, vulvovaginal candidiasis was higher among the third trimester gestational age with 18(58.07%). This result is similar to the report by Sangaré *et al.*, 30 different from results reported by Al-Rukeimi *et al.* 19; who observed that pregnant woman with the first trimester had the rate of VVC.

The present study showed that the high frequency of isolated *Candida* was 83.87% reported among women with recurrent vulvovaginal candidiasis infection. These results in disagreement with Abruquah,<sup>31</sup>.

This result revealed that the highest rate of VVC among pregnant women administered antibiotics. Vulvovaginal candidiasis frequently follows the use of vaginal or systemic antibiotics <sup>32</sup>. Antibiotics alter the normal flora of the vagina and thus allow overgrowth of *Candida* sp. After antibiotic use, the increase in vaginal colonization with *Candida* sp.,<sup>33</sup>.

In the current study, the frequency of isolated *Candida* sp. among pregnant

women who complained of itching, burning, and discharge were 100%, 93%, and 93.55%, respectively, which was the highest percentage. This study similar to results by Falahati *et al.*, <sup>34</sup> who showed that the frequency of isolated *Candida* among women who complained of discharge was 82.1%, itching 62.7%, and burning 49.3%. However, it was observed that all participated women free from diabetes mellitus.

In the antifungal susceptibility results, it was reported that the highest sensitivity of antifungals against isolated Candida species was fluconazole, amphotericin B, and nystatin. In contrast, non-Candida albicans showed resistance to amphoteracin B (45.45%) and nystatin (33.33%). This finding is in agreement with the work of Bitew and Abebaw<sup>14</sup> and Edrees et al., <sup>17</sup>. The most concern in Yemen is representing on the availability of antibiotics and easy to purchase from drug store without prescription by physician<sup>35-37</sup>. Therefore, antifungal susceptibility testing is very necessary to determine the effective antifungal for VVC among pregnant women without complications on both maternal and fetal health<sup>38-40</sup>.

#### **Study Limitations**

The limitation of this study representing the use of conventional mycology techniques which depend on the phenotypic identification technique that is known to be insufficient to differentiate between *Candida* species. Therefore, the identification was limited to differentiate only between *Candida albicans* and non-*Candida albicans*.

#### Conclusion

In conclusion, vaginal infections are very common in our region and have a high frequency. It was found that *C. albicans* was the predominant isolated species from pregnant-woman. All isolates were susceptible to fluconazole and nystatin. This is the first report on the types of *Candida* sp., causing vaginal candidiasis

and their antifungal susceptibility patterns in Ha Yemen.

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