THE PREVALENCE OF VULVOVAGINAL CANDIDIASIS IN PREGNANT WOMEN ATTENDING SEVERAL HOSPITALS IN SANA’A, YEMEN

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ABSTRACT

Objective: Candida species are opportunistic yeasts affecting the genitourinary tract which causes theyuvovaginal 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 Sana’a City, Yemen.

Methods: This study was carried out at the department of microbiology of AL-Kuwait University Hospital. 250 vaginal swabs were collected and cultured on Sabouraud dextrose agar. Candida species identification and antifungal susceptibility testing were determined according to standard microbiological methods.

Results: The results showed that out of 250 samples, 63(25.2%) were positive for Candida species. It was found that the Calbicans (68.3%) was the most common species isolated followed by non-albicans species that are C. tropicalis (20.6%), C. glabrata (6.3%), and C. kefer (4.8%). Also, it was recorded that the highest prevalence of Candida species was within group aged of 36-46 year. Susceptibility tests revealed that the most isolated species of C. albicans were sensitive to nystatin (95%), miconazole was the next effective drug with 64% sensitive followed by amphotericin B (29%) and clotrimazole (24%). But only 6% of the isolates were sensitive to fluconazole.

Conclusion: It can be concluded that the vulvovaginal candidiasis are quite common in Yemen country with a high prevalence. Also, the nystatin remains the effective agent against all isolated of Candida species. In contrast, the increase resistance of Candida species to fluconazole that commonly used antifungal is an alarming increase of vaginal candidiasis caused by antifungal-resistant Candida species.

Keywords: Antifungal, Candidaalbicans, non-albicans Candida, vaginitis, Yemen.

INTRODUCTION

Candida vaginitis is the infection of vagina by several type of Candida species, also often called vulvovaginal candidiasis/candidosis. Vulvovaginal candidiasis (VVC) considered to be the most common manifestation of genital candidiasis. It is representing over 25% of infectious vaginitis. It is reported that 75% of women are affected by vulvovaginal candidiasis in their lifetimes. Also, it was found that more than 40% of women that affected will have 2 or more vulvovaginal candidiasis episodes. Clinical manifestation of vulvovaginal candidiasis is pruritus, vaginal discomfort, burning, and soreness. The distribution of Candida sp. in vulvovaginal candidiasis cases differs widely depending on the geographical location and population studied. 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. However, in last year’s, there are different species of Candida non-albicans that are C. parapsilosis, C. glabrata, C. krusei, C. lusitaniae, C. tropicalis, C. dubliniensis, and C. guilliermondii isolated from vaginal samples. Misuse of antifungal drugs and lack of effective polices that control the use of antifungal especially against the vulvovaginal candidiasis lead to increase resistant of Candida species to several antifungal drugs.
vaginitis in Yemen. A study by Al-Haik and Al-Haddad 16 reported that among 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 vaginitis 17. 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 Sana’a City, Yemen.

Therefore, this study aimed to determine the prevalence of Candida species that causes vulvovaginal candidiasis among pregnant women and resistance of isolated species to antifungal in Sana’a City, Yemen.

MATERIALS AND METHODS

Study design and sample collection

Two hundred and fifty (250) specimens were collected from females attending Obstetrics and Gynecology outpatient clinics at different hospitals (AL- Kuwait, AL-Thwrah, AL-Gumhorri and Matnah) that located in Sana’a City, in the period from September 2018 to August 2019. All suspected cases were interviewed and patient information was recorded with intended questionnaires including: demographic, age, medical history, and symptoms. 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- Kuwait 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 40x 20.

Culture methods

The second swab was cultured on surface of Sabouraud Dextrose Agar (SDA) (Himedia, India) with and without chloramphenicol (250mg/L). The plates were incubated for 48 h at 37°C. The morphological features for colony were studied and confirmed by observing the budding characterization with pseudoforms by using the Gram tube test. The growth of bacteria on the SDA plates without chloramphenicol was purified on Blood agar and MacConkey agar and identified by biochemical test 21.

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 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 22.

Sugar assimilation test

The overnight of culture yeast suspension was added to 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 day at 37°C. The occurrence of growth around each disc indicates the carbohydrate assimilation of tested sugar. The glucose, D-galactose, maltose, sucrose, lactose, raffinose, xylose and trehalose were used in the sugar assimilation test 23.

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), Miconazole (10µg), Itraconazole (50µg), Voriconazole (10µg), Fluconazole (100µg), Amphotericin B (50µg) and Nystatin (100µg). The inhibition zone was measured after 48h of incubation at 37°C 24.

Statistical analysis

The statistical analysis was performed by using program version 20 SPSS (Statistical package for social Science). Percentage and Chi-square test were used to evaluate the degree of the significance with 95% confidence (p<0.05).

Figure 1: The sample positive and negative growth in culture media

RESULTS

The result from the current study revealed that the 250 vaginal swabs were collected from pregnant women who presented with genital manifestations.

Table 1: The types of microorganisms isolated from vaginal swabs samples

<table>
<thead>
<tr>
<th>Type of isolation</th>
<th>Number of sample</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidiasis</td>
<td>63</td>
<td>34.06</td>
</tr>
<tr>
<td>Bacterial vaginitis</td>
<td>122</td>
<td>65.94</td>
</tr>
<tr>
<td>Total</td>
<td>185</td>
<td>100</td>
</tr>
</tbody>
</table>
Only 185 samples (74%) were showed as positive growth in culture media and 65 samples (26%) were reported as negative growth in culture media as shown in Figure 1. This study revealed that an overall isolation rate of candidiasis was 34.06% and 65.94% were bacterial vaginitis from vaginal swabs as listed in Table 1. From the 63 positive isolates of Candida species, it was found that the highest prevalence of vulvovaginal candidiasis was among women from urban (80.20%) compared to women from rural areas (19.80%) as shown in Figure 2.

![Figure 2: Percentage of candidiasis infection according area](Image)

Also, this study showed that the highest percentage for first time infection was 52/193 (26.9%), while the recurrent infection was 11/57 (19.3%). Table 2 shows that the highest prevalence of Candida infection (71.43%) was recorded within the age range of 36-46 year, followed by group aged between 26-35 year (26.99%).

<table>
<thead>
<tr>
<th>Age interval</th>
<th>Total tested</th>
<th>Positive Candida species</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-25</td>
<td>17</td>
<td>6.8</td>
<td>1.58</td>
</tr>
<tr>
<td>26-35</td>
<td>183</td>
<td>73.2</td>
<td>71.43</td>
</tr>
<tr>
<td>36-46</td>
<td>50</td>
<td>20</td>
<td>26.99</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

While the lowest prevalence of Candida infection was reported in age group of 16-25 years (1.58%). In the present study, it was revealed that four species of Candida were isolated and characterized by vaginal samples according to sugar assimilation test. It was found that the C. albicans (68.3%) was the predominant Candida species isolated followed by a higher prevalence of non-albicans species like C. tropicalis (20.6%), C. glabrata (6.3%), and C. kefyr (4.8%) (Table 3).

This study showed that the most clinical symptoms among women with vaginitis and frequency of Candida isolated were burning, discharge, and itching (Table 4).

The susceptibility results revealed that the 95% of isolated Candida species were susceptible to nystatin. Miconazole was the next effective drug with 64% sensitive followed by Amphotericin B (29%), Clotrimazole (24%), Itraconazole (16%), Ketoconazole (14%) and Voriconazole (11%). Only 6% of the Candida isolates were sensitive to Fluconazole as shown at Table 5.

### Table 3: Distribution of Candida species according to sugar assimilation test

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>GIU</th>
<th>SAC</th>
<th>LAC</th>
<th>TRE</th>
<th>RAF</th>
<th>GAL</th>
<th>MAL</th>
<th>Xyl</th>
<th>GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


The incidence of vulvovaginal candidiasis differs from one report to another. In this study, 34.06% of samples showed vaginal candidiasis infection and 65.94% were bacterial vaginitis. In a similar study, in Sana’a, by Abdul-Aziz et al. recorded that the bacterial vaginitis was 76.92% reported among reproductive-aged women, while the vaginal candidiasis was 17.69%. In Egypt, Abbas et al. reported that the 60.8% of examined women were infected by vulvovaginal candidiasis, 37.1% were infected by bacterial vaginitis, and 2.1% by trichomoniasis. Another study by Bitew and Abebaw reported that only 41.4% of vagina samples were positive growth.

The highest distribution of vaginitis (80.20%) cases in the current study werefound in urban areas. This result in agreement with Abdul-Aziz et al. who observed that the 88.44% of vaginitis was among reproductive-aged women resident in urban area.

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

**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. Of 250 samples examined, 185 samples (74%) showed as positive growth in culture media and 65 samples (26%) were reported as negative growth. This negative culture cases may be infected with T. vaginitis. In Sana’a, Yemen, Abdul-Aziz et al. revealed that the 37.6% was positive for vaginal infections among reproductive-age women. Also, Almamari et al. in Sana’a, reported that the 94% of vaginal samples of patients were positive growth for vulvovaginal candidiasis.
present study showed that the frequency of isolated *Candida* among women with vulvovaginal candidiasis for the first time was 52 (82.54%), while recurrent vulvovaginal candidiasis infection was 11 (17.46%).

**Table 4: Clinical symptoms of vaginosis between positive and negative candidiasis cases**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Total</th>
<th><em>Candida</em> isolated</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>202</td>
<td>80.8</td>
<td>54.2</td>
</tr>
<tr>
<td>No</td>
<td>48</td>
<td>19.2</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td>Burning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>228</td>
<td>91.2</td>
<td>61</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>8.8</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>203</td>
<td>81.2</td>
<td>55</td>
</tr>
<tr>
<td>No</td>
<td>47</td>
<td>18.5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100</td>
<td>63</td>
</tr>
</tbody>
</table>

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 Sobi32 in Nigeria.

**Table 5: Sensitivity of isolated *Candida* sp. to tested antifungal drugs.**

<table>
<thead>
<tr>
<th>Antifungals</th>
<th>Number of affected <em>Candida</em></th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nystatin</td>
<td>60</td>
<td>95</td>
</tr>
<tr>
<td>Miconazole</td>
<td>40</td>
<td>64</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

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 transplantation3 and leukemia3 this may be depended 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* 43(68.3%) are responsible for the greatest number of symptoms associated with the vaginal candidiasis. This finding is in agreement with the work of Al-mamari et al.,34 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 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 percentage 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. It was found that the *C. tropicalis* (20.6%), *C. glabrata* (6.3%), and *C. kefyr* (4.8%) 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 the all isolates were highly sensitive to Nystatin and this finding is in agreement with the work of Al-mamari et al.26 and Mahmoudabadi et al.35. In addition, in this study of 63 positive cases, *Candida* species were sensitive to Miconazole and this finding in agreement with work of Al-mamari et al.26 and Abruquah36. This study revealed that the most of *Candida* species showed higher resistance to Fluconazole which may be due to frequent use of these drugs as therapeutic alternatives to Amphotericin B. Azole antifungal agents are easy for administration and are less toxic9,26,32.

**CONCLUSION**

In conclusion, vaginal infections are very common in the region and have a high frequency. It was found that *C. albicans* (68.3%) was the predominant isolated species followed by *C. tropicalis*. All isolates were susceptible to nystatin and fluconazole. This is the first report on the types of *Candida* sp., causing vaginal candidiasis and their antifungal susceptibility patterns in Yemen.

**AUTHOR’S CONTRIBUTION**

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

**CONFLICTS OF INTEREST**

There are no any conflicts of interest.

**REFERENCES**


