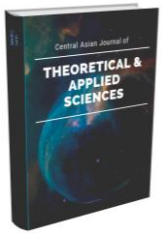

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Article

Antifungal Susceptibility Patterns in Candida Spp. Isolated From Vaginal Swabs of Pregnant Women in Iraq

Mhmood Abed Hamzah[1], Azhar Said Hendal[2], Faiza Jabbar Joda[3], Saif Mazeel Abed[4]

1. Ministry of Education, Al-Karkh 3
2. Open Educational College
3. Ministry of Education, Open College of Education, Iraq
4. Ministry of Health, Al-Muthana Health Directorate

* Correspondence: [1]mhmodalqaus@yahoo.com, [2]Azharsaidhendal@gmail.com, [3]faizajb1976@gmail.com, [4]Drsaifmazeel@gmail.com

Abstract: Vulvovaginal infections are one of the most important health issues faced by women of reproductive age. Most of the cases are caused by bacteria and fungi *Candida albicans*. The purpose of the study is to monitor the demographic pattern of vaginal infections in pregnant women and check the susceptibility of fungi against antifungal agents, which is of great concern in today's world with increasing drug resistance. One hundred vaginal swabs were collected from pregnant women in different areas of different cities, and the women were distributed in different age groups. The isolates were identified, and 30 fungal isolates of *Candida albicans* were subjected to antifungal susceptibility testing with antifungal agents such as *clotrimazole, amphotericin B, nystatin, itraconazole, fluconazole, and caspofengin*. MDR is the ability of an isolate to resist three or more of the most commonly used agents. The most important part of the study is the prevalence rate, which is 60% in the case of bacterial infections and 40% in the case of fungal infections. Demographic distribution: The highest affected groups were between the ages of 31-40, followed by the 18-30 age group, while the least affected were in the 41-60 age group. It was noted that the rural population was the highest affected by the disease. *Clotrimazole and Amphotericin* B had the highest sensitivity rates of 33.3%. Nystatin had an average effect of (26.7%), while Itraconazole had the least effectiveness of (3.3%), results showed total resistance (100%) to *fluconazole and caspofengin*. All Candida samples were resistant to three or more drugs, thus classified as multidrug-resistant.

Keywords: Antifungal Susceptibility, H.V.S, Candida spp, Pregnant Women.

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1. Introduction

Vaginal *Candida albicans* is one of the most common fungal infections in women across the world and presents a major health challenge, especially during pregnancy. Studies indicate that about 75% of women are affected by this disease at least once in their life, with a high percentage of them likely to suffer from recurrent infections [1]. The Candida yeast family is responsible for this infection. The Candida fungus lives harmlessly in the vagina, but when the body's natural balance is disrupted by changes such as hormonal changes, the use of antibiotics, diabetes, and changes to the immune system, the fungus becomes a disease-causing agent [2]. Pregnancy is one of the significant risk factors that increases susceptibility to infection. The increase in estrogen levels and glycogen in the vaginal tissue makes it a conducive environment for Candida growth, leading to a quick reproduction rate [3]. Even as Candida Albicans is identified as the common cause

of vaginal candidiasis, recent studies point to a significant increase and caused by other non-Albican Candida species such as *C. glabrata, C. krusei, and C. tropicalis*. The importance of non-Albican Candida lies in their low sensitivity or resistance to common anti-fungal drugs, as identified by [4]. The recent epidemiological change in the pattern of vaginal candidiasis to non-Albican Candida is a significant problem that has led to drug resistance in the effectiveness of current treatment protocols [5]. The use of drug classes such as azoles, polyenes, and echinocandins has not been effective due to the uncontrolled use of drugs, leading to resistance as seen in various studies on drugs such as ketoconazole, clotrimazole, and fluconazole. as noted in [6]. It is from this understanding that the significance of this research lies. The significance of this research lies in monitoring fungal susceptibility patterns for optimal treatment and for minimizing treatment failures. This is done by isolating and identifying different Candida albicans from vaginal swabs of pregnant women, determining the relative distribution of these species from the collected samples, and determining their susceptibility and resistance to these antifungal drugs.

2. Material and methods

Purification In this research, 100 vaginal swab samples were collected from the participants. The collection was done strictly adhering to sterile procedures. For this research, three specialized media were used for isolating different microorganisms. These media were include :

MacConkey agar was used for isolating Gram-negative bacteria, while blood agar was used for supporting both Gram-negative and Gram-positive bacterial growth. This medium also supported the monitoring of hemolytic activity.

Fungal media: Samples were cultured on Sabouraud dextrose agar medium and subjected to Candida fungus isolation. All the plates were subjected to normal temperature and atmospheric conditions. Microbial growth was observed periodically, and the required colonies were isolated. Antifungal Susceptibility Testing: All Candida isolates were subjected to vigorous antifungal susceptibility testing. These isolates were derived from vaginal swabs. Six antifungal agents were used in the susceptibility testing: clotrimazole, fluconazole, itraconazole, nystatin, amphotericin B, and caspofengin. Test Procedures according to [7]:

1. Methodology: Disk diffusion testing using appropriate culture media and internationally acceptable laboratory protocols .
2. Measurement and Evaluation: After the recommended period of incubation, the zone of inhibition formed around each antifungal agent was measured. \
3. Classification: Depending on the established interpretive criteria for each drug, the isolates were classified as sensitive, moderately sensitive, and resistant. Results were accurately recorded and analyzed to determine overall susceptibility patterns and the presence of MDR isolates.

3. Results and Discussion

The distribution of microbial isolates

The distribution of microbial isolates in 100 sample, according to age groups showed that bacterial infections represented the highest proportion of cases, accounting for approximately 60% of the total samples. Candida species were the second most frequently detected microorganisms, representing about 40% of the total cases. The age group of 31-40years recorded the highest number of cases for both bacterial and Candida infections compared with the other age groups. The number of infections was slightly lower in the 18–30 years age group, although bacterial isolates remained more frequent than Candida.

In the older age groups (41–50 and 51–60 years), the number of detected cases was lower compared with the younger groups as in table (1).

Table 1. The distribution of microbial isolates.

Age	Candida 40% positive	Bacteria 60 % postive
18- 30	15	25
31- 40	16	20
40-50	4	9
51-60	5	6

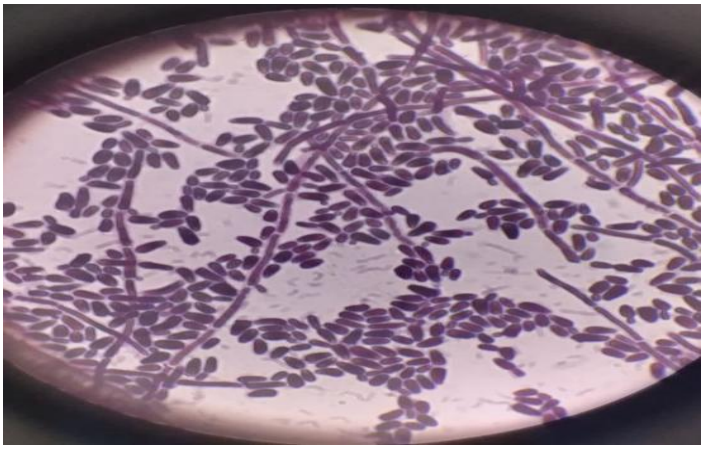

Figure 1. Candida spp with Gram stain.

The current investigation showed that among the high vaginal swabs taken from the women under study, bacterial infections accounted for the largest percentage of microbial isolates (60%) compared to Candida species (40%). This result is in line with a study by Workowski et al., which found that because bacterial vaginosis and other bacterial pathogens are so common in women of reproductive age, bacterial infections are frequently more common than fungal infections in vaginal microbiota [8]. Because pathogenic bacteria can multiply more easily than fungal species due to changes in vaginal pH and disturbance of normal vaginal flora, the prevalence of bacterial isolates may be explained.

In a similar vein, the current results are consistent with a study by Sobel that found that, despite the prevalence of vulvovaginal candidiasis, bacterial infections such bacterial vaginosis continue to account for a greater percentage of vaginal infections in various clinical settings [9] This is due to the diverse nature of the microbes that cause vaginal infections, along with behavioral and environmental factors such as personal hygiene habits, antibiotic use, and hormonal fluctuations. The results showed that the 31–40 age group is most susceptible to bacterial and Candida fungal infections. This is consistent with [10], that women in this age group are most prone to vaginal infections due to increased sexual activity, hormonal fluctuations, and physiological changes that lead to an imbalance in the vaginal microbiome. In contrast, there is a decrease in infection rates in older age groups (41–60 years), which is consistent with the study by [11]. The risk of acquiring an infection also decreases with the cessation of reproductive life, owing to the fall in estrogen levels and the vaginal environment, which includes a decrease in glycogen content in epithelial cells, thereby inhibiting the growth of pathogens such as Candida. The high rate of isolation of Candida (40%) is consistent with the findings of [12], which reported that this fungus is one of the most common vaginal infections in pregnant

women. This is because hormonal, glycogen, and immune system changes in pregnant women provide an ideal medium for the growth of this fungus.

The demographic distribution of vaginal infections also indicated that there is a marked difference in the urban and rural areas. In the younger age group (18-30 years), bacterial infections were more common than fungal infections in both urban and rural areas. In the middle age group (31-40 years), the maximum number of infections was reported, with a significant advantage in rural areas. However, in the older age group (41-60 years), the number of infections in both urban and rural areas is low. In most age groups, bacterial infections were more common than fungal infections, with variations in geographical distribution. This suggests that certain groups in rural areas were more susceptible, highlighting the influence of geographic location and age as factors affecting the spread of this infection within the studied population according to [13].

Table 2. The demographic distribution of microbial isolates.

Age Group	Candida Urban	Candida Rural	Bacteria Urban	Bacteria Rural	Total Urban	Total Rural
18–30	7 (23.33%)	8 (26.67%)	12 (20%)	13 (21.67%)	14 (15.56%)	16 (17.78%)
31–40	6 (20%)	10 (33.3%)	9 (15%)	11 (18.33%)	15 (16.67%)	21 (23.33%)
40–50	2 (6.67%)	2 (6.67%)	5 (8.33%)	4 (6.67%)	7 (7.78%)	6 (6.67%)
51–60	4 (13.33%)	1 (3.33%)	3 (5%)	3 (5%)	7 (7.78%)	4 (4.44%)

The results obtained from the study indicated that the distribution of microbial isolates varies according to geographic location and age group, with bacteria being the most prevalent when compared with Candida fungi. This is in agreement with the study done in Yemen, which stated that vaginal candidiasis is the second most prevalent when compared with bacteria, which is the leading cause of vaginitis, as reported by [14].

The 31-40 age group was the most prevalent in the study, as was noted by other studies that indicated that the 25-35 age group is the most susceptible to microbial infections to various physiological factors that occur during the reproductive period, such as hormonal changes, pregnancy, and increased sexual activity, which have a direct effect on the vaginal microbiome, as reported by [15].

The rural-urban disparity, the study noted that demographic differences were observed between the rural and urban population, with the rural population showing the highest rates of microbial infections. This difference, according to previous studies, is related to a variety of socioeconomic factors, most notably the lack of access to healthcare services and awareness of reproductive health practices, in addition to an important factor: the difference in personal hygiene practices. As for the decrease in the rates of infection in the older age group, the results indicate that there is a decrease in the rates of infection in women in the 41-50 and 51-60 age groups. This is in accordance with epidemiological studies that indicate that there is a decrease in the rates of vaginal infections in women after the end of the reproductive period. This decrease is related to hormonal factors, most notably the decrease in estrogen levels with age, which leads to a change in the nature of the vaginal environment and decreases the ability of microbes to colonize and grow, as mentioned in [16].

The antifungal susceptibility

Sensitivity tests performed on 30 Candida fungal isolates showed marked variation in response to antifungal agents. The results were Clotrimazole and amphotericin B showed the highest sensitivity rates, with each reaching 33.3% (10 out of 30 isolates). [17], showed moderate activity at 26.7% (8 out of 30 isolates), while itraconazole had very low activity, with only one isolate (3.3%) showing sensitivity. The results indicate that all

isolates (100%) exhibited complete resistance to fluconazole and caspofengin. This high level of resistance is mainly attributed to the widespread and excessive use of antifungal drugs, which has led microbes to develop defense mechanisms, such as modifications in ergosterol biosynthesis pathways, increased activity of efflux pumps to eliminate the drug, or genetic mutations affecting the drug's biological targets within the fungal cell [18]. Therefore, all isolates in this study were classified as multidrug-resistant. (MDR), as shown in Table (3).

Table 3. Antifungal Sensitivity and MDR for Candida spp.

Antifungal Agent	Sensitive Isolates (n)	Percentage (%)
Clotrimazole	10	33.3
AP (Amphotericin)	10	33.3
NS (Nystatin)	8	26.7
IT (Itraconazole)	1	3.3
FLU (Fluconazole)	0	0
CAS (Caspofungin)	0	0
MDR (≥3 drugs)	30	100

The response of Candida isolates to antifungal agents is highly variable. Clotrimazole and amphotericin B showed the highest sensitivity rates (33.3%), followed by nystatin (26.7%), while itraconazole exhibited the lowest efficacy (3.3%). These results are consistent with the study by [19], which found that vaginal Candida isolates are more susceptible to polyenes than azoles. This is attributed to the mechanism of action of polyenes, which bind to ergosterol in the fungal cell membrane, leading to its rupture and death. The moderate sensitivity to nystatin is consistent with the study by [20]. It should be noted that the variation in results is often due to differences in the distribution of fungal species and the development of resistant strains in certain populations. Another significant observation was the complete resistance (100%) to fluconazole and caspofengin. Fluconazole resistance is increasingly reported worldwide and is related to the overuse or prolonged administration of this class of drugs. This can cause mutations in the genes encoding the ergosterol synthesis pathway (ERG11) or overexpression of ectopic pumps [21] Resistance to caspofengin could also be related to FKS genes encoding the cell wall structure of the pathogen [22].

Multi-Drug Resistance (MDR) Phenomenon, all the isolates in the study were found to be multidrug-resistant (MDR) isolates, which are resistant to more than one type of drug. This observation is similar to the study by [20], which suggests that the overuse of drugs and the lack of adherence to the full regimen of drugs can cause the development of such resistant strains.

4. Conclusion

The study concludes that bacterial infections remain the leading cause of vaginal infections in pregnant women, with a concerning proportion of Candida infections. Age (especially in the 31–40 age group) and geographic location (rural areas) have been shown to play a pivotal role in determining prevalence rates. Several clinical recommendations emphasize continuous monitoring. Given the high rates of multidrug resistance, particularly global resistance to fluconazole and caspofen, periodic monitoring of fungal susceptibility patterns is crucial. Clinicians should rationalize the use of antifungals and rely on laboratory tests to determine local susceptibility patterns before prescribing treatment. This will help avoid failure of standard treatment protocols and limit the spread of resistant strains.

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