

**Antifungal susceptibility
pattern of Candida spp. isolated
from vaginal discharge of
pregnant women**

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<https://doi.org/10.33976/IUGNS.28.1/2020/07>

Abstract:

Vaginal candidiasis is common fungal infections among pregnant women. The aim of this study is to determine antifungal susceptibility pattern of Candida spp. isolated from vaginal discharge and to investigate possible risk factors associated with Candida infection. One hundred high vaginal swabs were collected from pregnant women complaining from abnormal discharge from various clinics in Gaza city, during 2019. Samples were plated on Sabouraud Dextrose Agar supplemented with chloramphenicol. Candida isolates were identified to the Genus level based on cultural and morphological characteristic. The susceptibility to seven antifungals was determined using disc diffusion method. Demographic, environmental, behavioral and other possible risks factors associated with vaginal candidiasis were recorded through an interview with patients before collecting the vaginal swabs and after obtaining a verbal consent. Data analysis was done using the SPSS software. Candida spp. was isolated from 43% of vaginal swabs of pregnant women with discharge. Variations existed in the susceptibilities of the different Candida isolates to antifungals. Amphotericin B the most effective with 0% resistance followed by itraconazole and nystatin with 9.4% resistance. Resistance to miconazole, ketoconazole, voriconazole and fluconazole was 11.6%. Statistically significant symptoms associated with positive Candida spp. cultures among pregnant women included; redness of vaginal area, secretions, yeast-like smell, urinary urgency, low sexual desire, itching and irritation ($p \leq 0.05$). No statistical significance difference with regard to burning urinary sensation and painful intercourse. Candida spp. was isolated from 43% of vaginal swabs from pregnant women. Risks factors should be minimized by patient education and routine testing of pregnant women.

Keywords:

Antifungal resistance
Candidiasis, Gaza, Palestine,
Pregnant women, Vaginal
Discharge.

1. Introduction:

Candida species are ubiquitous, ranging from free saprophytes through endo-symbionts of animals, to pathogens in many animals and in humans. Some of the pathogenic species are of medical importance, especially *Candida albicans*. However, the prevalence of other non-*albicans Candida* species as human pathogens is on the rise worldwide (1).

Vulvovaginal candidiasis (VVC) is considered as the second most common cause of vaginal infections, after bacterial vaginosis that is reported at gynaecological clinics (2). An estimated cost of diagnosis and treatment of VVC which take into consideration, loss of productivity and absenteeism in the U.S. is about \$1 billion yearly (2). *Candida albicans* is the most frequent causative agent in about 80 – 90% of VVC infections even though other non *albicans Candida* species are increasingly reported (2).

VVC is the most common gynecological ruling among women of child-bearing age (3). Among sexually active women, 75% have at least once experienced symptomatic VVC, which usually presents as soreness, burning, itching and abnormal curd-like vaginal discharge (4, 5). Pregnancy was the commonest factor (55%) followed by usage of broad-spectrum antibiotics (8%) (6).

Recommendations for vulvovaginitis diagnosis included speculum examination, and microscopic examination of slide from vaginal exudates to look for protozoan (*Trichomonas vaginalis*), bacterial (*Gardnerella vaginalis*) or fungal (*Candida* spp.) agents, followed by vaginal fluid specimen culture to confirm the diagnosis. Otherwise, the confirmatory diagnosis based on culture is not routinely performed nor generally advised in many regions, because all procedures are expensive and time consuming (7).

Many reports from around the world reports increasing frequency of *Candida* resistance to common antifungals. The main objective of this study is to determine the prevalence of vaginal candidiasis among pregnant women with abnormal discharge and their resistance profile to antifungals.

2. Methods

2.1 Sample collection and culture

A total of 100 high vaginal swab samples were collected from symptomatic and asymptomatic pregnant women aging between (18-49) years attending ante-natal clinics during the period from December 2018 to June 2019. Samples were transport to the Microbiology laboratory at the Islamic University of Gaza within 2 hours and were inoculated onto the surface of Sabouraud Dextrose Agar (SDA) with chloramphenicol, then incubation on 37 °C for 48 hours.

2.2 Antifungal susceptibility testing

Antifungal susceptibility testing was undertaken by the disk diffusion method. Fluconazole disk (10 µg), itraconazole (10 µg), voriconazole (10 µg), amphotericin B (10 µg), nystatin (100 IU), and Ketokonazole antifungal discs were applied on MHA as recommended by the Clinical Laboratory Standard Institute (CLSI). The plates were incubated in ambient air at 35°C and read after 24 hours. Interpretation of all antifungal susceptibility (susceptible S, intermediate I and resistant R) was done according to CLSI standards (8).

3. Results.

The study sample comprised of swabs from 100 pregnant woman attending Gaza clinics, aged between 18-49 years (Table 1).

Table 1: Distribution of study subjects according age group location, and residency, (n=100)

Variable	Category	%
Age	18-28	69
	29-38	25
	≥39	6
Governorate	Gaza	63
	Middle and South	8
	North Gaza	29
Residence	City	75
	Camp	25

Culturing results indicated that 43/100 of the pregnant woman were positive for *Candida* spp. with an overall prevalence 43%. As shown in table (2) the highest

vaginal *Candida* positive cultures was among the 18-28 age group with a prevalence of 46.4%, but no significant difference among different age groups (P value = 0.1). Residence and the number of rooms in the house showed statistically significant difference (P value =0.02 and 0.03 respectively).

Table 2: Distribution of *Candida* positive culture distributed by age, income, residence and room number

Risk factors		Positive N (%)	Negative N (%)	p-value
Age	18-28	32 (46.4)	37 (53.6)	0.13
	29-38	7 (28.0)	18 (72.0)	
	≥ 39	4 (66.7)	2 (33.3)	
Income	<1000	20 (37.7)	33 (62.3)	0.1
	1000-1600	14 (41.2)	20 (58.8)	
	≥ 1700	9 (69.2)	4 (30.8)	
Residency	City	37 (49.3)	38 (50.7)	0.02
	Camp	6 (24.0)	19 (76.0)	
Room number	One	9 (52.9)	8 (47.1)	0.03
	Two	15 (44.1)	19 (55.9)	
	Three	8 (25.8)	23 (74.2)	
	Four	11 (68.8)	5 (31.3)	
	≥5	0 (0)	2 (100)	

The results of antifungal susceptibility testing is illustrated in table (3). No resistance was found against amphotericin B. Resistance to Ketoconazole,

fluconazole, miconazole and voriconazole was 11.6%, while lower resistance was found against itraconazole and nystatin which was 9.3% as shown table (3).

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

Antifungal drug	R		S	
	N	%	R	%
Ketokonazole	5	11.6	38	88.4
Fluconazole	5	11.6	38	88.4
Amphotericin B	0	0.0	43	100
Itraconazole	4	9.3	39	90.7
Nystatin	4	9.3	39	90.7
Miconazole	5	11.6	20	88.4
Voriconazole	5	11.6	38	88.4

Prior use of antibiotics (during the last 6 months from infection) did not show significant difference (p=0.1) despite the fact that higher rate of infection (51.2%) was found among those who took antibiotics compared to only 37.3% among those who did not. Previous UTI (p=0.4), taking cortisol or its derivatives (p=0.6), use of contraceptives (p=0.7), the number of live births (P=0.2), and abortion (p=0.5) did not show statistically significant differences. While, previous Vaginitis (p=0.03), carbohydrates consumption (p<0.01), sleep anxiety (p <0.01), general mode (p= <0.01), using tight underwear (p=0.01), and wearing tight outfit (p= <0.01) were found to be statistically significant risk factors. (Table 4).

Table 4: Possible risk factors associated with vulvovaginal candidiasis

Possible Risk factors		Positive N (%)	Negative N (%)	p-value
Prior antibiotic use (last 6 months)	Yes	21 (51.2)	20 (48.8)	0.1
	No	22 (37.3)	37 (62.7)	
Previous UTI	Yes	24 (47.1)	27 (52.9)	0.4
	No	19 (38.8)	30 (61.2)	
Previous Vaginitis	Yes	29 (52.7)	26 (47.3)	0.03
	No	14 (31.1)	31 (68.9)	
Cortisol/derivatives	Yes	2 (33.3)	4 (66.7)	0.6
	No	41 (43.6)	53 (56.4)	
Use of contraceptive	Yes	18 (40.9)	26 (44.6)	0.7
	No	25 (44.6)	31 (54.4)	
Number –live- births	None	17 (54.8)	14 (45.2)	0.2
	1-2	15 (39.5)	23 (60.5)	
	≥ 3	11 (35.5)	20 (64.5)	
Abortion	0	33 (54.8)	39 (54.2)	0.5
	1	6 (35.3)	11 (64.7)	
	2	2 (25)	6 (75.0)	
	3	1 (50)	1 (50)	
	5	1 (100)	0 (0.0)	

Possible Risk factors		Positive N (%)	Negative N (%)	p-value
Carbohydrates consumption	Large	22 (95.7)	1 (4.3)	0.00
	Moderate	16 (59.3)	11 (40.7)	
	Small	2 (6.7)	28 (93.3)	
	Very small	3 (15.0)	17 (85.0)	
Number of current wives	1	43 (43.4)	56 (56.6)	0.3
	2	0 (0)	1 (100)	
Husband is smoker	Yes	23 (46.0)	27 (54.0)	0.5
	No	20 (40)	30 (60)	
Sleep anxiety	Yes	33 (67.3)	16 (32.7)	0.00
	No	10 (19.6)	41 (80.4)	
Mode usually	Normal	5 (31.3)	11 (68.8)	0.00
	Stable	5 (15.2)	28 (84.8)	
	Worried	23 (60.5)	15 (39.5)	
	Nervous	10 (76.9)	3 (23.1)	
Cottony underwear	Yes	36 (43.9)	46 (56.1)	0.6
	No	7 (38.9)	11 (61.1)	
Tight underwear	Yes	33 (64.7)	18 (35.3)	0.00
	No	10 (24.4)	39 (75.6)	
Tight outfit	Yes	29 (45.3)	24 (54.7)	0.01
	No	14 (29.8)	33 (70.2)	

All subjects (100%) reported having at least one of the eleven-vulvovaginal symptoms in the last week prior to attending the clinic. Analysis of the results using the chi square test showed statistically significant results with each of the following symptoms; itching and irritation ($p<0.01$), lower sexual desire ($p=0.01$), urinary

urgency ($p=0.03$), secretions with yeast-like smell ($p<0.01$), redness of the vagina area ($p<0.01$), thick and white/creamy secretions ($p<0.01$), and Thin milky secretions ($p<0.01$). While burning sensation during urination ($p=0.5$), blood during intercourse ($p=0.2$) and painful intercourse ($p=0.08$) did not show statistically significant differences as shown in table (5).

Table 5: The association between prevalence of vaginal candida Infection self-reported symptoms.

Symptom		Positive N (%)	Negative N (%)	p-value
Burning sensation during urination	Yes	26 (40.6)	38 (59.4)	0.5
	No	17 (47.2)	19 (52.8)	
Itching and irritation	Yes	35 (71.4)	14 (28.6)	0.0
	No	8 (15.7)	43 (84.3)	
Lower sexual desire	Yes	14 (66.7)	7 (33.3)	0.01
	No	29 (36.7)	50 (63.3)	
Painful intercourse (dyspareunia)	Yes	10 (62.5)	6 (37.5)	0.08
	No	33 (39.3)	51 (60.7)	
Blood during intercourse	Yes	1 (100)	0 (0)	0.2
	No	42 (42.4)	57 (57.6)	
Urinary urgency	Yes	26 (54.2)	22 (45.8)	0.03
	No	17 (32.7)	35 (67.3)	
Secretions smell Yeast like	Yes	23 (92.0)	2 (8.0)	0.00
	No	20 (26.7)	55 (73.3)	
Thick and white/creamy	Yes	27 (84.4)	5 (15.6)	0.00

Symptom		Positive N (%)	Negative N (%)	p-value
secretions	No	16 (23.5)	52 (76.5)	0.00
	Yes	36 (78.3)	10 (21.7)	
Thin milky secretions	No	7 (13)	47 (87)	0.00
	Yes	27 (75)	9 (25)	
Redness of the vagina area	No	16 (25)	48 (75)	0.00
	Yes	27 (75)	9 (25)	

4. Discussion

This present study investigated the prevalence of vaginal candidiasis among pregnant women with symptoms of vaginal infection attending different gynecological clinic over the period of five months in a different area in Gaza city.

In the present study, the isolation rate of *Candida* spp. from pregnant women was 43%. The highest rate was among 18-28 age group with a prevalence of 46.4%. These results are higher than those observed in previous reports on vaginal *Candida* spp. (33.8%) in women in the age range of 20–29 years, followed by (24.3%) those in the age range of 30–39 years, and the overall prevalence of VVC was 25% (9). Other researchers reported lower results than ours including 17% obtained from Malaysia (10), 15-20% in Taiwan (11), 14% in Ibadan (12), 30% in Nigeria (13), 37% in Turkey (14) and 38% in Hyderabad (15). While our results were lower than (55.4%) those obtained in Cameroon (16) and even very much lower than (92%) in a report from Libya (17). Feyi (18) in Tanzania and Menza *et al* (19) in Kenya reported prevalence rates in agreement with our result; they reported that, prevalence of vaginal candidiasis among pregnant women were 42.9% , 42.7% respectively.

The high prevalence of vaginal candidiasis may due to many different reasons include; suppression of the immune system due to the pregnancy as it is among the contributing factors of vaginal candidiasis (20), extended and misuse of antibiotics which leads to the destruction of good and beneficial bacteria resulting to reduction of vaginal immunity could have also contributed to the increase of the prevalence of the infection (21, 22).

Virulent factors of *Candida* spp. like dimorphism, phenotypic switching, protease and phosphatase which enhance its attachment to human epithelium play an importance role in their high occurrence. High incidence rate also may due to increased physiological changes, estrogen and rich glycogen content of the vaginal mucosa thereby providing an adequate supply of utilizable sugar that favor *Candida albicans* growth during pregnancy (17).

In this study, only 5 (11.6%) of *Candida* spp. was resistant to fluconazole, this is higher than studies conducted in United States and Brazil were all *C. albicans* vaginal isolates were susceptible to fluconazole (23), and zero resistance Malaysia (10). A study in Argentina (24) showed a slightly higher result (13.46%).

Resistance of *Candida* isolates to nystatin was 9.3%. This higher than previously reported in pregnant woman (only 0.61%) in Peshawar (25). In our study miconazole and itraconazole was resistant in 5 and 4 (11.6%, 9.3%) respectively of isolates. In an 8-years study, fluconazole resistance increased from 2.4% to 55.4% (2006–2012), but the rate dropped to 8.9% in 2013. Considering miconazole and itraconazole, resistance increased from 2.4% and 7.1% to 59.8% and 58.9% (2006–2013), respectively (26).

Age, level of education, income, and residency as possible risk factors for vulvovaginitis were investigated in the present study. We did not find associations between some socio-demographic characteristics and the prevalence of vulvovaginal candidiasis. Among socio-demographic characteristics, age seems to be not an important factor in the overall occurrence of vulvovaginal candidiasis. Out of 43 patients with vulvovaginal candidiasis, 32 (46.4%) patients were in their 18-28 of life. Our result was comparable with previous studies (27, 28).

The study of Janković *et al.* (29), showed that vulvovaginal candidiasis was statistically associated with continual wearing of tight underwear, a finding which is consistent with our result ($p<0.01$).

In the present study, the presenting complaints in 71.4% of positive patients reported itching and irritations, 75% Redness of the vagina area, 84.4% suffered from thick and white/creamy secretions, 92% Secretions smell Yeast like, 66.7% lower sexual desire, and 54.2% Urinary urgency. These is comparable to the prevalence rate obtained from Pakistan (25). This most likely owes to raised level of hormones during pregnancy, which is an energy source for *Candida* growth (6). pregnancy resulted significantly associated to asymptomatic infection (30). In other study, 39.5% of pregnant women had symptoms of VV (31).

Discharge was the most symptoms were noticed in pregnant followed by itching while odor and burning are present lowest percentages in all subjects (17). The frequency of vaginal candidiasis during pregnancy was found to be 38%, in which 27% were symptomatic and 11% were asymptomatic group (15).

As this is the first study that investigated *Candida* among pregnant women, the authors were not able to compare the results of this study with local studies. Other studies should be conducted, to include larger sample and covers the entire Gaza strip. Gynecology clinics are recommended to perform culture and sensitivity to provide an appropriate antifungal. The concerned authorities should work closely to monitor the use of therapeutic antifungals to minimize drug resistance. Education of women on genital hygiene and regular screening for candidiasis in antenatal program is highly recommended.

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