Exoenzymes Production and Antifungal Susceptibility of *Candida Species* Isolated from Pregnant Women with Vulvovaginitis

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Abstract: Background: Vulvo-vaginal candidiasis (VVC) remains one of the most common infections of the female genital tract. It has been estimated that up to 75% of women will have at least one episode of vaginal candidiasis during their lives. **The aim** of this study was to determine the frequency of *Candida species* isolation from pregnant women with VVC and to study antifungal susceptibility, phospholipase and proteinase production by the isolated *Candida species*. **Methods:** The study was conducted on 100 female patients complaining of symptoms of vulvovaginitis attending the Obstetrics and Gynecological Outpatient Clinic of Benha University Hospital from June 2011 to April 2012. The test group included 50 pregnant women in the third trimester of pregnancy, while the control group included 50 non-pregnant women in the childbearing period. Isolation and complete identification of *Candida species* was performed. Antifungal susceptibility testing was done by disc diffusion method and finally detection of proteinase & phospholipase exoenzymes production was performed. **Results:** Results showed significant higher number of positive cultures in pregnant women 56% (28/50) versus 34% (17/50) in non-pregnant women (p=0.026). *C. albicans* was the most common species associated with VVC (71.4% in pregnant women versus 64.7 % in non pregnant women). The results of in vitro antifungal susceptibility testing demonstrated that 100% of *Candida kefyr (C.kefyr),* 96.7% of *C.albicans*, 66.6% of *C.glabrata*, 50% of *C.tropicalis* were found to be sensitive to fluconazole. All *Candida species* tested were susceptible to voriconazole and nystatin (100%). There was insignificant difference in the number of phospholipase and proteinase producing isolates of *C. albicans* and *non albicans species* in pregnant and non pregnant women ($p< 0.05$). **Conclusion** VVC is more prevalent in pregnant women. The best approach for the diagnosis of VVC is to consider microscopic examination of vaginal secretion with culture. Due to its efficacy and low risk profile, nystatin remains the first line treatment for *Candida* infections during pregnancy. Phospholipase and proteinase activity do not have predominance in *Candida species* isolated from pregnant women with higher predominance in *C.albicans* than *non albicans species* in both groups.
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1. Introduction

Vulvo-vaginal candidiasis (VVC) remains one of the most common infections of the female genital tract. It is considered an important public health problem affecting millions of women worldwide every year. It has been estimated that up to 75% of women will have at least one episode of candidiasis during their lives, with 40% to 50% experiencing chronic recurring episodes. *C.albicans* is the most common etiological agent of VVC but there is little evidence of significant increase in infection rate due to the other *Candida species* such as *C. glabrata*, *C. krusie* and *C. tropicalis* **(1).**

While a number of factors are probably important in the pathogenesis of recurrent vulvo-vaginal candidiasis (RVVC), there are two main theories as to explain frequent recurrences: re-infection either from a sexual partner or a reservoir of yeasts in the gut and vaginal relapse due to incomplete eradication of the yeasts **(2).**

Candida infection in the vagina can cause a smelly, thick, white–yellow discharge that might be accompanied by itching, burning and swelling. It can also make walking, urinating or sex very painful. Since the symptoms of vaginal candidiasis are not specific, diagnosis cannot be made solely on clinical basis and should be confirmed by laboratory diagnosis **(3).**

A higher prevalence of VVC is seen in pregnant women compared with those who are not pregnant. Recurrence is also more common in pregnant women, and therapeutic responses are reduced **(4).**

Studies reinforce the need to evaluate the vulvo-vaginal infection by yeasts as a major cause of health impairment in women. The ability of some fungi to grow at 37°C, and to produce enzymes such as phospholipases and proteinases permit establishment of pathogenicity**(5).**

Proteinase production is important for increasing the ability of certain organisms to colonize

and penetrate the host tissue and evade the host immune system by degrading important proteins, such as immunoglobulins, complement proteins and cytokines. Phospholipases act by invading and causing tissue damage in host cells, rupturing the epithelial cell membranes and allowing the ends of hyphae to penetrate the cytoplasm**(6).**

It has been estimated that antifungal therapy was inappropriately prescribed in 54% of culture negative cases, such unnecessary use of antifungal therapy contributes to the development of antifungal resistance and the emergence of infections associated with other *Candida spp*. and other opportunistic fungi. There is evidence of an increased azole resistance among isolates of *Candida Species* isolated from women with VVC **(7).**

Due to the increased incidence of VVC, interest in the study of antibiotic susceptibility and virulence factors of these species has been intensified to establish strategies for the prevention and control of candidiasis. **The aim** of this study was to determine the frequency of *Candida species* isolation from pregnant women with vulvovaginitis and to study antifungal susceptibility, phospholipases and proteinase production by the isolated *Candida species.*

2. Patients and methods:

Patients

The study was conducted on 100 female patients attending the Obstetrics and Gynecological Outpatient Clinic of Benha University Hospital, Qalubia, Egypt, from June 2011 and April 2012. The test (pregnant) group included 50 pregnant women in the third trimester of pregnancy, while the control (non-pregnant) group included 50 non-pregnant women in the childbearing period. All women were complaining of symptoms of vulvovaginitis (pruritus, vulvar burning, urinary complaints and dyspareunia). After careful history taking and complete physical examination, ultrasound examinations were performed for pregnant women by one observer. Information about age, parity, gestational age in pregnant group, body mass index (BMI) was reported. Women with severe medical disorders, women who were currently menstruating, taking oral contraceptive pills, premature rupture of membrane, had taken a course of antibiotics or corticosteroids within the preceding 7 days or had vaginal douching during the previous 48 hours were excluded. Written informed consent was obtained from each participant.

Sampling

The patient was placed in lithotomy position and vaginal exposure was done through introduction of a non-lubricated sterile Cusco speculum. Double high vaginal swabs (HVS) were collected using sterile cotton tipped swabs and they were sent to the laboratory without delay.

Microscopic examination

One swab sample was examined microscopically (40x) after10% KOH preparation and staining with lactophenol cotton blue to detect the presence of budding yeast cells and pseudo-hyphae of *Candida species*.

Yeast isolation

The other swab sample was inoculated on Sabouraud's dextrose agar (SDA) supplemented with 0. 05g / L Chloramphenicol. After 48 hours incubation at 37ºC, cultures were examined for pasty, creamy and smooth white colonies of yeasts which were further identified.

Yeast identification

Yeast identification was done to each positive growth on SDA as follows:

a) Germ tube test (8).

The elongated daughter cells from the round mother cell without constriction at their origin were referred to as germ tubes, and constricted hyphae at the round mother cell were referred to as pseudohyphae. Germ tube is specific for *C. albicans.*

b) Cultivation on the selective medium Brilliance™ Candida Agar

Brilliance™ Candida Agar (oxoid: CM1002) is a selective differential medium for the rapid isolation and identification of clinically important *Candida species*.

All positive cultures on SDA were plated on Brilliance™ Candida Agar for identification of *non-albicans species*. Plates were incubated aerobically at 30°C and inspected for the growth of *Candida species* at 24, 48 and 72 hours.

Identification was based on colony color and morphology. Using this medium, we were able to identify the following individual species: *C. tropicalis* (wet, dark blue colonies), *C. krusei* (dry, irregular pink-brown), and *C. albicans* (green colonies,). *C.glabrata, C. kefyr, and C. parapsilosis* appear as a variety of beige/brown/yellow colors.

c) Sugar assimilation test (9)

It was performed to differentiate between *C. glabrata, C kefyr and C. parapsilosis* as they give the same colors (beige/brown/yellow) on Brilliance™ Candida Agar.

The query colonies on Brilliance™ Candida Agar were incubated each in tube containing two ml saline at room temperature for about 24 hrs to exhaust the carbohydrate reserves so that the sugar supplemented will be properly utilized and this rules out false negative results. A lawn culture of the pre incubated saline was made on the Yeast extract agar 01497(Sigma Aldrich) and the sugar disks (paper disks soaked in 1% (w/v) solutions of various

carbohydrates, glucose, maltose, sucrose, lactose) were put and incubated for 24-72 hrs at 25°C. Positive results showing enhanced growth around the disks were noted and tabulated. *C. glabrata* gives positive glucose and maltose assimilation test, *C. kefyr* gives positive glucose, sucrose and lactose sugar assimilation and *C. parapsilosis* is positive for glucose, maltose and sucrose. Isolates then were stored as suspensions in sterile 20% glycerol at -80°C until further processing.

Antifungal susceptibility testing:

Disk diffusion (DD) testing of fluconazole, voriconazole and nystatin was performed as described previously **(10)** and in CLSI document M44-A **(11).**

Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue dye was prepared according to the manufacturer recommendation.

Preparation of inocula

Inocula were prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24-hour-old culture of *Candida species*. Colonies were suspended in 5 ml of sterile saline (8.5 g/L NaCl). The resulting suspension was vortexed for 15 seconds and its turbidity was visually adjusted to that of a 0.5 McFarland standard producing a yeast stock suspension of 1 x 10^6 to 5 x 10^6 cells per ml. Inoculation of the dried agar plates using a sterile cotton swab dipped into suspension was performed by streaking the swab over the entire agar surface.

Anti-fungal disks, fluconazole 25µg (Oxoid) CT1806B, voriconazole 1µg (Oxoid) CT1807B and nystatin 100 Units (Oxoid) CT0073B were dispensed onto the surface of the inoculated agar plate. The plates were inverted and placed in an incubator at 35° C and examined after 24 hours. The zone of inhibition was measured and the result was recorded as susceptible (S), susceptible dose dependent (SDD) and resistant (R) **(11).**

Detection of exoenzymes production:

Preparation of inocula (6):

The inocula of yeast cells were prepared from stock cultures and incubated for 18 hours at 37ºC in Brain Heart Infusion (Oxoid Ltd) and turbidity was visually adjusted to that of a 0.5 McFarland standard producing a yeast stock suspension of 1 x 10^6 to 5 x 10^6 cells per ml.

Detection of protinase production was performed according to Júnior et al. (6)

Preparation of media:

The test medium consisted of plates containing bovine serum albumin (BSA) agar. Sixty millilitres of a solution containing 0.04g $MgSO_4.7H_2O$, 0.5g K_2HPO4, 1g NaCl, 0.2g yeast extract, 4g glucose and 2g bovine serum albumin (BSA) (Sigma - Aldrich). The pH was adjusted to 4 and the solution was sterilized by filtration and then mixed with 140ml of sterile molten agar.

Seeding:

10 µl of suspension of yeast culture at a density of 10^6 yeast /ml was inoculated onto a 1% BSA agar plate. The plates were incubated at 37°C for 5 days. Before incubation, the BSA-agar was slightly opaque. The plates were observed daily for subsequent clearing due to hydrolysis by the acid proteinases of the fungi. Staining with 1.25% amidoblack (Sigma - Aldrich) for 15 min was performed on the fifth day followed by washing with 90% (v/v) methanol/water destaining solution. Clear zones around the disks, could not be stained with amidoblack indicated proteinase activity.

Interpretation:

Proteinase activity was measured and calculated according to the method described by **Price *et al.* (12)**. Activity zone (ZA) was calculated as the ratio of the colony diameter to the colony plus the clear zone of proteolysis. ZA equal to or greater than 1.0 detects no proteinase activity, ZA lower than 1.0 detects proteinase activity.

Detection of phospholipase was performed according to Price et al. (12).

Preparation of media:

The test medium is SDA supplemented with 1 M sodium chloride, 0.005 M calcium chloride and 2% egg yolk. All components except egg yolk were sterilized at 121°C for 20min. Egg yolk was added to cooled medium at (45-50°C), mixed and dispensed in plates.

Seeding:

An aliquot (10µl) of the yeasts suspension was inoculated on the centre of test medium which was then incubated at 37°C for four days to check and measure the formation of an opaque halo around the colony.

Interpretation:

Activity zone (ZA) was calculated by dividing colony diameter by the sum of the colony diameter and size of precipitation zone. ZA equal to or greater than 1.0 detects no phospholipase activity, ZA lower than 1.0 detects phospholipase activity.

Statistical analysis:

The collected data were analyzed using SPSS version 16 software. Data were presented as numbers and percentages. □ Z ″ test for 2 variables and ″ χ^2 □ (Chi square) test for more than two were used as tests of significance. P value of <0.05 was considered statistically significant.

3. Results

Study population:

A total of 50 pregnant and 50 non pregnant women were recruited to the study. All had symptoms and signs suggestive of VVC. Demographic characteristic data of both pregnant and non pregnant groups as regard age, parity, BMI, frequency of the symptoms and the gestational age in pregnant group are illustrated in table1.

Isolation and Identification of *Candida* species:

Results obtained from microscopic examination of vaginal swabs stained with lactophenol cotton blue showed that 48% (24/50) were positive in pregnant women versus 26% (13/50) in non-pregnant women. The difference between the two groups was statistically significant (p=0.022). There was a significant higher number of positive cultures on SDA in pregnant women 56% (28/50) versus 34% (17/50) in non-pregnant women (p=0.026).

On comparing the results of microscopic examination of vaginal discharge and culture on SDA, VVC was diagnosed in 48 %(24/50) versus 56 %(28/50) in pregnant women and 26 %(13/50) versus 34% (17/50) in non pregnant women. This difference did not reach a significant level (p=0.42 and 0.38, respectively). These results are illustrated in table 2.

C. albicans was the most common species associated with vulvo -vaginitis (71.4% in pregnant women versus 64.7 % in non pregnant women), followed by *C. glabrata*(14.3% versus 11.7%), *C.tropicalis*, (7.1% versus 11.7%), *C.krusie* (3.6% versus 5.9%) and *C.kefyr* (3.6% versus 5.9%). The differences of *candida species* distribution between the two groups were not statistically significant (p = 0.951). These results are illustrated in table 3.

Antifungal susceptibility testing:

The results of in vitro antifungal susceptibility of *Candida* isolates by disc diffusion method are shown in table (4). 100% of *C.kefyr*, 96.7% of *C.albicans*, 66.6% of ***C.****glabrata*, 50% of *C.tropicalis* were found to be sensitive to fluconazole, while 100% of *C.Krusie* were found to be resistant to it. All *Candida species* tested were susceptible to voriconazole and nystatin (100%).

Exoenzymes production:

There was insignificant difference in the number of proteinase producing isolates of *C. albicans* (p = 0.94) and *non albicans species* (p= 0.64) in pregnant and non pregnant women. There was also insignificant difference in the number of phospholipase producing isolates of *C. albicans* (p = 0.64) and *non albicans species* (p =1) in pregnant and non pregnant women. The number of proteinase positive isolates of *C. albicans* was higher than that of the *non-C. albicans* isolates(64.5% versus 42.8%), and the number of phospholipase positive isolates of *C. albicans* was higher than that of the *non-C. albicans* isolates (77.4% versus 50%) but these differences did not reach significant levels (p = 0.066 and 0.17 respectively). These results are shown in tables 5&6

Table (1): Demographic characteristics of the study groups

Variables	Pregnant group (n=50)	Non pregnant group (n=50)
Age (Y)	29.3 ± 10.1 (19-39)	28.8 ± 9.5 (20-39)
Parity	2.6 ± 0.5	2.4 ± 0.7
BMI (kg /m^2)	23.8 ± 4.1	24.5 ± 4.6
Gestational age (W)	*10.2±2.1*	-
Frequency of symptoms		
Pruritus	48 (96%)	45 (90%)
Discharge	34(68%)	32(64%)
Dyspareunia	21 (42%)	22 (44%)
Dysuria	16(32%)	19(38%)

BMI: Body mass index

Table (2): shows results of microscopic examination and culture of high vaginal swab

	Pregnant women	Non pregnant women	*p* value
Positive microscopic examination	24/50 (48%)	13/50 (26%)	0.022*
Positive culture	28/50 (56%)	17/50 (34%)	0.026*
p value	0.42**	0.38**	-

**Z test was used (2.28, 2.21)* ***Z test was used (0.8, 0.87)*

Table (3): Candida species isolated by vaginal culture

Candida species	Pregnant women		Non pregnant women		Total	
	No.	% of isolates	No.	% of isolates		
C.albicans	20	(71.4%)	11	(64.7%)	31	(68.9%)
C.glabrata	4	(14.3%)	2	(11.7%)	6	(13.4%)
C.tropicalis	2	(7.1%)	2	(11.7%)	4	(8.9%)
C.krusie	1	(3.6%)	1	(5.9%)	2	(4.4%)
C.kefyr	1	(3.6%)	1	(5.9%)	2	(4.4%)
Total	28	(100%)	17	(100%)	45	(100%)

χ^2 test was used =0.63, p value= 0.951

Table 4: Percentage of antifungal susceptibility of *candida species* by disk-diffusion method

Candida species	Antifungal agents								
	Fluconazole			Voriconazole			Nystatin		
	S	SDD	R	s	SDD	R	S	SDD	R
	SIZ ≥ 19	SIZ 15-18	SIZ ≤14	SIZ ≥ 17	SIZ 14-16	SIZ ≤13	SIZ >10	SIZ -	SIZ <10
C. albicans 31	30(96.7 %)	1(3.3%)	0	31 (100%)	0	0	31(100%)	0	0
C. glabrata 6	4(66.6%)	1(16.7%)	1(16.7)	6(100%)	0	0	6(100%)	0	0
C. tropicalis 4	2(50%)	2(50%)	0	4(100 %)	0	0	4(100%)	0	0
C. krusie 2	0	0	2(100%)	2(100 %)	0	0	2(100%)	0	0
C. kefyr 2	2(100%)	0	0	2(100%)	0	0	2(100%)	0	0

R, resistant; S, susceptible; S-DD: susceptible dose-dependent.

Table (5) Phospholipase and acid proteinase activity of isolated *Candida species*

Candida species	Positive Phospholipase activity	Positive Proteinase activity
C.albicans 31	24 (77.4%)	20 (64.5%)
C.glabrata 6	2 (33.3%)	3 (50%)
C.tropicalis 4	3 (75%)	2(50%)
C.krusie 2	1 (50%)	1(50%)
C.kefyr 2	1 (50%)	0(0%)
Total 45	31(68.9%)	26(57.8%)

Table (6) Number and percentage of isolated *Candida spp* showing positive phospholipase and acid proteinase activity

		Pregnant women	Non pregnant women	Total	P value
Positive Proteinase activity	*C. albicans*	13/20 (65%)	7/11 (63.6%)	20/ 31(64.5%)	0.94
	non C. albicans	3/8 (37.5%)	3/6 (50%)	6/ 14(42.8%)	0.64
	p value	0.18	0.58	0.17	----------
Positive Phospholipase activity	*C. albicans*	16/20 (80%)	8/11 (72.7%)	24/31(77.4%)	0.64
	non C. albicans	4/8 (50%)	3/6 (50%)	7/ 14 (50%)	1.0
	p value	0.11	0.34	0.066	----------

Z test was used

4. Discussion

Candida species is an important cause of wide spectrum of diseases including vulvovaginitis. Vulval itching and soreness were the only symptoms predictive of VVC, but overall symptoms are non-specific, confirming the findings that presence of VVC cannot be definitely identified by clinical criteria **(2, 13).**

VVC can be diagnosed by direct microscopic examination and vaginal culture. The microscopic examination of the clinical material is rapidly performed and may identify the presumptive etiologic agent, but vaginal culture is indispensable to confirm the diagnosis **(14).** Our results clearly demonstrated significant increase in the number of positive microscopic findings in pregnant women (48%, 24/50), compared to (26%, 13/50) in non-pregnant women. The difference between the two groups is statistically significant (p= 0.022). These results agree with those reported by **Babić and Hukić (15)**.

It is important to confirm the mycological findings by culture of *Candida species* in diagnosis of VVC. In this study, on comparing the results of microscopic examination of vaginal discharge and culture, VVC was diagnosed in 48% versus 56% in pregnant women and 26% versus 34% in non pregnant women. This difference did not reach a significant level (p=0.42 and 0.38, respectively).

Aslam *et al.* (16) found that high vaginal swab culture revealed VVC in 48% pregnant women as compared to 38% on Grams stain / KOH preparation. Similar results (46.6% versus 43%) were documented by **Donders *et al.* (17)** and **Levett (18).**

The difference in sensitivity of direct microscopy could be explained by difference in yeast concentration in vaginal secretions. Direct microscopy is reliable only if the infection is fairly heavy. Therefore, vaginal swab culture before starting antifungal therapy becomes essential for elucidation of diagnosis of VVC **(19).**

Based on culture, this study showed a higher isolation rate of *Candida species* in pregnant women (56%, 28/50), compared to (34%, 17/50) in non-pregnant women. This difference is statistically significant (p= 0.026). **Babić and Hukić (15)** reported higher positive cultures (46.8%, 95/203) in pregnant women, compared to non-pregnant women (25.4%, 62/244). Also **Dias *et al.* (20)** found higher

rates of occurrence of VVC in pregnant (44.8%) than non-pregnant (34.5%).

During pregnancy, due to high levels of reproductive hormones there is greater amount of glycogen in the vagina, which further acts as a good source of carbon required for *Candida* growth. It was demonstrated that the affinity of vaginal epithelial cell adherence of *Candida* increases and immunoglobins in vaginal secretions decreases due to estrogen. Progesterone has suppressive effects on the anti-*Candida* activity of neutrophils and formation of yeast blastospores are increased by these hormones. These factors contribute to increased vulnerability of pregnant women to vaginal candidiasis **(16, 21).**

In this study, *C. albicans* was the most common species associated with vulvovaginitis (71.4% in pregnant women versus 64.7 % in non pregnant women), followed by *C. glabrata*(14.3% versus 11.7%), *C.tropicalis*, (7.1% versus 11.7%), *C.krusie*(3.6% versus 5.9%) and *C.kefy* (3.6% versus 5.9%). These results are comparable to **Kalkanci *et al.* (22)** who reported similar results.

A higher percentage found for *C. albicans* (90.4%) in the article published by **Garcia Heredia *et al.* (23),** and (92.3%) in the article published by **Dias *et al.* (20)** for pregnant patients and 72.9% in non pregnant patients. However **Us and** Cengiz, **(24)** reported a lower percentage of *C. albicans* species (53.2%) in pregnant patients.

The first step in establishing yeast infection is binding to the vaginal mucosa. It seems that *C. albicans* is more adhesive than other non-*C. albicans species*. This could be considered as the main cause of predominance of *C. albicans* **(14).**

A high prevalence of non *C. albicans species* was achieved in the study of **Mohanty *et al.* (25)** who found that the overall percentage of non-*C.albicans* vaginitis was 64.8%. These non *C.albicans* yeasts are relatively non pathogenic but ultimately get selected and start appearing more frequently because of the widespread abuse of antifungals, use of single oral or topical dose, and long term maintenance regimens of oral azoles **(19).**

Antifungal susceptibility testing in our study revealed that (96.7%) of *C. albicans* isolates tested were fluconazole susceptible, though 1 of 31(3.3%) isolates was fluconazole S-DD. However, *C. krusei isolates were* 100% resistant to fluconazole**. Dias *et al.* (20)** found 96% of *C albicans* isolates were susceptible to fluconazole, which is similar to the percentage obtained in the present study.

No fluconazole resistance was identified among 75 *C. albicans* vaginal isolates from symptomatic women by **El- Din *et al.* (2)** in England. **Akortha *et al.* (26)** reported a high fluconazole susceptibility rate (95.7%) in *C. albicans* and a 100% resistance rate was observed for *C. krusei*, which is consistent with our findings.

A high percentage (66.7%) of the *C. glabrata* vaginal isolates in the current study was fluconazole susceptible, (16.7%) were susceptible-dose dependent and few resistant isolates (16.7%) were detected. These results are similar to fluconazole resistance in bloodstream isolates described by **Pfaller and Diekema, (27)** who reported similar results.

Development of resistance by *C. albicans* is described almost exclusively in strains of mucosal isolates in AIDS patients after long-term therapy with fluconazole. Increased resistance to fluconazole has been reported in oral, esophageal and urinary *Candida* isolates, but this has not been commonly observed in genital tract isolates **(20)**.

All *candida species* isolated in this study were susceptible to voriconazole. This new triazole exhibits greater potency and spectrum than fluconazole. The activity of this drug extends to some fluconazole resistant strains of *Candida* including *C. glabrata or C. krusei* **(28).**

All *Candida species* were susceptible to nystatin in our study and these results agree with **Fan and Liu, (29)** who detected no nystatin resistance among *C.albicans* and *non-albicans* isolates.

Due to its efficacy and low risk profile, nystatin remains the first line treatment for *Candida* infections in the first pregnancy trimester. Recent investigations demonstrate comparable or higher susceptibility of *Candida species* for topical nystatin compared to clotrimazole, itraconazole, fluconazole, miconazole or terbinafine. Nystatin remains an efficient, safe and economic option in the treatment of VVC infections during pregnancy (**30).**

Phospholipase and proteinase activities are considered to play important roles in the pathogenesis of fungi. It was reported that these enzymes act as virulence factors that contribute to host tissue invasion by digesting proteins such as hemoglobin, and collagen, and can also degrade cell membranes **(31).**

In this study, There was insignificant difference in the number of proteinase producing isolates of *C. albicans* (p= 0.94) and *non albicans species* (p = 0.64) in pregnant and non pregnant women. There was also insignificant difference in the number of phospholipase producing isolates of *C. albicans* (p = 0.64) and *non albicans species* (p =1) in pregnant and non pregnant women. The number of proteinase positive isolates of *C. albicans* was higher than that of the *non-C. albicans* isolates(64.5% versus 42.8%), and the number of phospholipase positive isolates of *C. albicans* was higher than that of the *non-C. albicans* isolates(77.4% versus 50%) but these

differences did not reach significant levels (p = 0.066 and 0.17 respectively).

In their study **Oksuz *et al.* (32),** reported that the frequencies of phospholipase and proteinase activity in *C. albicans* were 53 and 56.7%, respectively, while for *C. non-albicans* isolates, the values obtained were 17 and 43.9%. **Tay *et al.* (33)** found insignificant difference in the expression levels of proteinase among the *Candida* isolates studied (p= 0.272), but the phospholipase activity of *C. albicans* was significantly higher than that of the *non-C. albicans* isolates (p = 0.003).

Mohandas and Ballal, **(34)** reported that 46.93% of *C. albicans* isolates and 42% of non-*Candida albicans* isolates produced phospholipase. The proteinase-producing capacity of *Candida non albicans* (50.45%) was less than that of *C. albicans* (67.34%). The interspecies variation in the amount of proteinase produced varied significantly (p <0.05).

In contrast to these results, **Júnior *et al.* (6)** found that *C. tropicalis* was the species with the highest number of positive isolates for phospholipase (91.7%). Statistically significant differences were observed in relation to production of phospholipases among species (p <0, 0001). Regarding the production of acid proteinase, the isolates of *C. parapsilosis* tested presented a larger number of producers (69.2%) among the species analyzed and the percentage of proteinase producing isolates did not differ statistically ($\chi 2$=1.9, p =0.5901).

Analysis of these results indicates marked differences in phospholipase and acid proteinase production between *Candida spp*. isolates from different sources. It was suggested that the pathogenicity of *Candida* might be related to the site of infection **(6).**

Although phospholipases and proteinases of *C. albicans* are considered important virulence factors, not all pathogenic strains of *Candida* produce proteinase and phospholipase as virulent factors. The virulence of *Candida species* is attributed not to a single factor but to a combination of several factors, like proteinase, phospholipase, biofilm production, mycelium formation and susceptibility to antimycotics **(35, 36).**

Conclusion

VVC is more prevalent in pregnant women. The best approach for diagnosis of VVC is to consider microscopic examination of vaginal secretion with culture which is valuable not only for the accurate diagnosis of VVC but also to avoid indiscriminate use of antifungal agents and to prevent development of resistant *Candida species*. Due to its efficacy and low risk profile, nystatin remains the first line treatment for *Candida* infections during pregnancy. Phospholipase and proteinase activity do not have predominance in *Candida species* isolated from pregnant women with higher predominance in *C.albicans* than *non albicans species* isolated in both groups.

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