Antifungal susceptibility patterns of vulvovaginal Candida species among Pregnant and Non Pregnant Women

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Abstract

Objectives: Determination of different Candida species present in vagina of pregnant and non-pregnant women with recurrent vaginal candidiasis, and the susceptibility patterns of vaginal Candida to antifungal drugs.

Patients & Methods: This study included 93 women with recurrent clear clinical picture of Candida vaginitis. Divided equally into 3 groups: 31 pregnant women in the third trimester, 31 non-pregnant women in the period of fertility using combined pills and 31 non-pregnant women not using contraception. Two high vaginal swabs were taken by sterile cotton wool swabs soaked in sterile saline. One swab was examined by Gram stain and the second swab was streaked onto a plate of sabouraud dextrose agar (SDA) with chloramphenicol. Identified Candida species were tested for susceptibility to antifungal drugs (Fluconazole, Itraconazole, Caspofungin, Voriconazole, Econazole and Nystatin) by disc diffusion method.

Results: In this study, Candida albicans and Candida glabrata were the most common species isolated from vaginal specimen followed by Candida krusei. There was a statistically significant increase in the percentage of Candida krusei among non-pregnant not using oral contraception group than other groups. The resistance to Econazole, fluconazol and Itraconazole were significantly increased in C. non albicans more than their resistance in C.albicans.

Conclusion: Although Candida albicans was the most prevalent species causing vaginal candidiasis in pregnant and non-pregnant using contraception women, candida non albicans are also incriminated in high percentage, e.g. C.glabrata, and C. krusei. Both C. albicans and C. non albicans were highly sensitive to voriconazole and Caspofungin in contrast to other drugs tested.

Keywords: Candida, antifungal drugs, susceptibility, Vulvovaginal Candidiasis.

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INTRODUCTION

When Candida is found in the vagina without immunosuppression or mucosal damage, it is typically not accompanied by any symptoms and is therefore called colonization. Vulvovaginal Candidiasis (VVC), as opposed to asymptomatic colonization, is characterized by inflammatory signs and symptoms when Candida species are present but no other pathogenic cause is present. Ten years ago, VVC was divided into simple and complex cases; this division has now been adopted and recognized globally (1).

An antifungal drug is a type of pharmaceutical fungicide that is used to treat and prevent dangerous systemic infections such cryptococcal meningitis, ringworm, athlete's foot, and candidiasis (thrush). These medications are typically only accessible with a prescription from a doctor, while some are over-the-counter (OTC) (2).

According to studies, the most common species responsible for pregnant women's vaginal candidiasis were Candida albicans. Most vaginal Candida species isolates showed a high level of azole drug susceptibility. In contrast to azole medications, topical nystatin had a propensity to decrease the sensitivity of certain isolates (3).

It has also been noted that non-albicans species, particularly Candida krusei, are becoming more resistant to medications. This is the case with the exception of itraconazole. With the exception of topical nystatin, the continued use of antifungal medications for the treatment of vaginal candidiasis in pregnant women is supported by the observed susceptibility of vaginal Candida species to azole medicines. To find any new medication resistance, however, ongoing monitoring of antifungal resistance to Candida species is necessary (4).

AIM OF WORK

To determine the different Candida species present in the vagina of pregnant and non-pregnant women, and the susceptibility patterns of vaginal isolates of Candida to antifungal drugs.

PATIENTS AND METHODS

This observational cross-section study was conducted at the Obstetrics and Gynecology department of Benha University Hospitals after receiving approval by the Research Ethical Committee of Benha Faculty of Medicine with the code RC 42-1-2024. Written informed consent was obtained from all participants prior to commencing the study. This study enrolled 93 women divided into 3 groups.

- Group 1: Pregnant women in the 3rd trimester (n=31).
- Group 2: Non-pregnant women in the fertility period using combined pills (n=31).
- Group 3: Non-pregnant women not using contraception (n=31).

Inclusion criteria:

1. Female patients with recurrent clear clinical picture of Candida vaginitis.
2. Age of female patients within the range of 20 to 38 years.

Exclusion criteria:

1. Women who did not consent and those who reported a history of vaginal bleeding, diabetic, hypertensive and thyroid problems.
2. Women used antifungal treatment, vaginal douches, lubricant or any local vaginal medication for at least 24 hours before sampling.

Methods

A- History taking:

Each patient was subjected to full history taking which involve name, age, last normal

menstrual period, gravidity&parity state, residence, occupation and past history of preterm labor.

B-Sampling procedure:

Women were selected and recruited into the study after a health talk about the study which was given by the principal investigator or the nurse on duty. Two high vaginal swabs were taken one after the other by inserting a sterile vaginal speculum into the vagina, and then a sterile cotton wool swab soaked in sterile saline was inserted into the posterior vaginal fornix and rotated gently before withdrawing. Each swab was inserted back into the tube from which it was taken. The tube containing the swab was labelled with the patient study number and date.

C- Microscopic examination:

One swab was examined by Gram stain to give the preliminary results to the clinician for patient management.

D- Cultivation and isolation of yeast

The second swab was streaked onto a plate of sabouraud dextrose agar (SDA) (Difco laboratories, USA) with chloramphenicol and incubated at 37°C for 48 hours. Pure yeast colonies (pasty, yellow-white) were isolated, and a Gram stained film was prepared.

E-Subculture and species identification:

Subculture of preserved yeasts was done twice on SDA with chloramphenicol medium, and inoculated plates were incubated at 37°C for 18 to 24 hours. Identification of Candida was then carried out by the germ tube test and subcultured on chromoagar media.

F-Antifungal susceptibility testing:

Antifungal susceptibility testing was done by disc diffusion method, Antifungal discs ;Fluconazole (FLU)(25µg), Itraconazole (ITC) (10µg), Caspofungin (CAS) (5 mg) and Voriconazole(Vo) (1µg), Econazole(ECN) (10µg) and Nystatin (NY) (100 IU) (LIOFILCHEM s.r.i –Italy) were used. Mueller-Hinton Agar supplemented with 2%w/v glucose and 0.5µg/ml methylene blue dye was used as recommended by CLSI document M44A.

Statistical analysis:

Data were entered checked and analyzed using SPSS version 22

RESULTS

Candida albicans (40, 43 %) and Candida glabrata (39, 41.9 %) were the most common species isolated from vaginal specimen followed by Candida krusei (14, 15.1 %). There was statistical significance increase in the percentage of Candida krusei (32.2 %) among non-pregnant not using contraception methods group than other groups Table (1). There were statistical significant differences in sensitivity of Candida albicans to different antifungal drugs with increased sensitivity to Caspofungin&Voriconazole, increased resistance to Econazole (67.5%), Fluconazole (55%), and increased percentage of dose dependent to Itraconazole (40%) and Nystatin(52.5%) Table (2). That there were statistical significant differences in sensitivity of Candida krusei to different antifungal drugs with increased sensitivity to Caspofungin(100%) & Voriconazole (71.4%), increased resistance to Econazole, Fluconazole (100% each) &Itraconazole (71.4%) and increased percentage of dose dependent to and Nystatin (71.4%) Table (3). There were statistical significance differences in sensitivity of Candida glabrata to different antifungal drugs with increased sensitivity to Caspofungin (76.9%) &Voriconazole(84.6%), increased resistance to Fluconazole, (66.7%) Econazole (84.6%) &Itraconazole (53.8%) and increased percentage of dose dependent to Nystatin (69.2%) Table (4). There was statistical significance increase in Econazole and fluconazol resistance in Candida krusei (100% each) compared to Candida glabrata (84.6% & 66.7% respectively) and Candida albicans (67.5% & 55% respectively). Also,

there was highly statistical significant increase in Itraconazole resistance among Candida krusei (71.4%) and Candida glabrata (53.8 %) compared to Candida albicans (30%). There was statistical significant increase in Voriconazole resistance in Candida krusei (28.6%) compared to Candida glabrata (15.4%) and Candida albicans (7.5%). Finally, there was statistical significant increase in Caspofungin resistance in Candida glabrata (23.1 %) compared to Candida krusei (0%) and Candida albicans (7.5%) Table (5). There was statistical highly significant increase in Econazole resistance in Candida non albican (88.7%) more than C. albicans species (67.5%). Also, there was statistical highly significant increase in Itraconazole resistance in Candida non albicans species (58.5%) more than C. albicans species (30%). There was statistical non-significant increase in Fluconazole resistance in Candida non albican (75.5%) more than C. albicans species (55%). Finally, there was statistical highly significant increase in voriconazole resistance in Candid non- albicans species (18.9%) more than C. albicans species (7.5%) Table (6).

Table (1): Candida species among the studied groups:

Candida species(n)	Non pregnant using Contraception methods (n=31)		Non pregnant not using Contraception methods (n=31)		Pregnant in the third trimester (n=31)		χ^2	P
	No	**%**	**No**	**%**	**No**	**%**		
Candida albicans(40)	15	48.4	9	29	16	51.6		
Candida krusei(14)	4	12.9	10	32.2	0	0	**13.5**	**0.009 ****
Candida glabrata(39)	12	38.7	12	38.7	15	48.4		

χ^2: Chi square test **: Highly Significant (P<0.01) n:number

Table (2): Susceptibility of Candida albicans to antifungal drugs.

Variable	**Candida albicans (n=40)**	
	No	**%**
Nystatin:		
Dose dependent	21	52.5
Resistant	12	30
Sensitive	7	17.5
Econazole:		
Dose dependent	6	15
Resistant	27	67.5
Sensitive	7	17.5
Itraconazole:		
Dose dependent	16	40
Resistant	12	30
Sensitive	12	30
Fluconazole:		
Dose dependent	14	35
Resistant	22	55
Sensitive	4	10

Voriconazole:		
Dose dependent	6	15
Resistant	3	7.5
Sensitive	31	77.5
Caspofungin:		
Resistant	3	7.5
Sensitive	37	92.5
P	**<0.001****	

**: Very highly Significant (P<0.001)

Table (3): Susceptibility of Candida krusei to different antifungal drugs.

Variable	Candida Krusei (n=14)	
	No	**%**
Nystatin:		
Dose dependent	10	71.4
Sensitive	4	28.6
Econazole:		
Resistant	14	100
Itraconazole:		
Resistant	10	71.4
Sensitive	4	28.6
Flucanazole:		
Resistant	14	100
Voricanazole:		
Resistant	4	28.6
Sensitive	10	71.4
Caspofngin:		
Resistant	0	0
Sensitive	14	100
P	**<0.001****	

**: Very highly Significant (P<0.001)

Table (4) : Susceptibility of Candida glabrata to antifungal drugs in all studied groups.

Variable	Candida glabrata (n=39)	
	No	**%**
Nystatin:		
Dose dependent	27	69.2
Resistant	6	15.4
Sensitive	6	15.4
Econazole:		
Resistant	33	84.6
Sensitive	6	15.4
Itraconazole:		
Dose dependnat	6	15.4
Resistant	21	53.8
Sensitive	12	30.8

Fluconazole: Dose dependnat Resistant Sensitive	 10 26 3	 25.6 66.7 7.7
Voriconazole: Resistant Sensitive	 6 33	 15.4 84.6
Caspofungin: Resistant Sensitive	 9 30	 23.1 76.9
P	**<0.001****	

**:Very highly Significant (P<0.001)

Table (5): Difference in susceptibility between three species of Candida to antifungal drugs.

Variable	Candida albicans (n=40)		Candida krusei (n=14)		Candida glabrata (n=39)		χ^2	P
	No	%	No	%	No	%		
Nystatin: Dose dependent Resistant Sensitive	 21 12 7	 52.5 30 17.5	 10 4 0	 71.4 28.6 0	 27 6 6	 69.2 15.4 15.4	**7.48**	**0.11 NS**
Econazole: Dose dependent Resistant Sensitive	 6 27 7	 15 67.5 17.5	 0 14 0	 0 100 0	 0 33 6	 0 84.6 15.4	**11.90**	**0.02***
Itraconazole: Dose dependnat Resistant Sensitive	 16 12 12	 40 30 30	 0 10 4	 0 71.4 28.6	 6 21 12	 15.4 53.8 30.8	**13.66**	**0.008 ****
Fluconazole: Dose dependnat Resistant Sensitive	 14 22 4	 35 55 10	 0 14 0	 0 100 0	 10 26 3	 25.6 66.7 7.7	**9.45**	**0.05***
Voriconazole: I Resistant Sensitive	 6 3 31	 15 7.5 77.5	 0 4 10	 0 28.6 71.4	 0 6 33	 0 15.4 84.6	**11.60**	**0.02***
Caspofungin: Resistant Sensitive	 3 37	 7.5 92.5	 0 14	 0 100	 9 30	 23.1 76.9	**6.71**	**0.04***

χ^2: Chi square test **: Highly Significant (P<0.01)

*:Significant (P<0.05) NS: Non significant (P>0.05)

Table (6): Difference in susceptibility between Candida albicans and Candida non albicans to antifungal drugs.

Variable	Candida albicans (n=40)		Candida non albicans (n=53)		χ^2	P
	No	%	No	%		
Nystatin: Dose dependent Resistant Sensitive	21 12 7	52.5 30 17.5	37 10 6	69.8 18.9 11.3	5.23	0.07 NS
Econazole: Dose dependent Resistant Sensitive	6 27 7	15 67.5 17.5	0 47 6	0 88.7 11.3	**9.86**	**0.007** **
Itraconazole: Dose dependnat Resistant Sensitive	16 12 12	40 30 30	6 31 16	11.3 58.5 30.2	**11.93**	**0.003** **
Fluconazole: Dose dependnat Resistant Sensitive	14 22 4	35 55 10	10 40 3	18.8 75.5 5.7	4.30	**0.11** NS
Voriconazole: I Resistant Sensitive	6 3 31	15 7.5 77.5	0 10 43	0 18.9 81.1	**10.10**	**0.006** **
Caspofungin: Resistant Sensitive	3 37	7.5 92.5	9 44	17 83	1.82	0.18 NS

χ^2: Chi square test **: Highly Significant (P<0.01) NS: Non significant (P>0.05)

DISCUSSION

In women who are still in the reproductive age, vulvovaginitis, or inflammation of the vulva and vagina, is typically caused by infectious organisms; approximately one-third of cases are caused by Candida vulvovaginitis (5).

Candida vulvovaginitis is a result of an infection with Candida species, most frequently Candida albicans, which causes inflammatory alterations in the vulval and vaginal epithelium. Many women have Candida as part of their regular flora, and it is frequently asymptomatic. Consequently, in order for Candida vulvovaginitis to occur, there must be Candida in the vagina or vulva in addition to discomfort, itching, dysuria, or inflammation (6).

Candida albicans is an endogenous commensal that is dimorphic and has been identified as the causative cause of most female lower reproductive tract infections. Even though they are less common than Candida albicans, other non-Candida species including Candida glabrata and Candida krusei can nonetheless result in serious opportunistic infections and show increased resistance to some antifungal drugs (7).

Our findings from this investigation indicate that Candida albicans significantly outnumbered Candida species that are not albicans. About 43% were made up of C. albicans, followed by C. glabrata (41.9%) and C. krusei (15.1%). The total prevalence of Candida species other than Candida albicans

(57%) was nearly equal to that Candida albicans (43%) and is somewhat comparable to the findings of Do Ngoc et al. (2019), who reported a very high proportion of non-albicans Candida species (60%) with Candida glabrata being almost as frequent as Candida albicans. Candida tropicalis accounted for 10.84 percent of all isolated cases, with C. krusei coming in second (8.43%) (8).

Additionally, our findings align with a study that reported a high prevalence of Candida albicans in China, with Candida glabrata, Candida tropicalis, and Candida parapsilosis following closely behind. Comparing isolated Candida species in Iran to non-albicans species, C. albicans was the most common species (9).

Our findings indicate that there was a significant predominance of Candida albicans over non-albicans Candida species in pregnant women. These findings are consistent with other studies that found Candida albicans to be the most common species causing vaginal candidiasis in pregnant women (4). Additionally, it was shown that Candida albicans predominated species in pregnant women with Candida vaginitis, accounting for 69.23 percent, followed by Candida glabrata (23.07%) and Candida andida tropicalis (7.69%) (10).

Knowing the antifungal susceptibility patterns of pathogenic fungus is essential for selecting the right treatment for mycoses. Testing for antifungal susceptibility can also be used to estimate the efficacy of antifungals, guaranteeing a positive course of therapy, tracking the emergence of drug resistance, and evaluating the therapeutic potential of novel drugs (11).

Treatment options for vulvovaginal candidiasis include polyene antifungals like amphotericin B and nystatin as well as azoles like fluconazole, ketoconazole, itraconazole, voriconazole, and clotrimazole. But the best medications for treating vaginal candidiasis in both pregnant and non-pregnant women are topical nystatin and azole antifungal medications (fluconazole, ketoconazole, itraconazole, and clotrimazole) (12).

Based on our findings, it was shown that Candida albicans was much more resistant to fluconazole (55%) and econazole (67.5%) and more susceptible to the antifungal medications casofungin (92.5%) and variconazole (77.5%).

Concerning Candida species other than Candida albicans, C. krusei and C. Glabrata showed susceptibility to antifungal drugs such as capsofungin (100%, 76.9% respectively) and voriconazole (71.4%, 84.6% respectively), but resistance to fluconazole (100% & 66.7% respectively), econazole (100%, 84.6% respectively), and itraconazole (71.4%, 53.8% respectively). All Candida species showed increased percentage of dose dependent sensitivity to Nystatin (52.2% for C. albicans, 71.4% for C. krusie and 69.2% for C. glabrata).

Both Candida albicans and non-Albicans (C. krusie, C. glabrata) exhibited considerable resistance to fluconazole (55%, 100%, and 66.7%, respectively). Regarding econazole, C. krusie (100%) and C. glabrata (84.6%) demonstrated greater resistance than C. albicans (67.5%). Furthermore, C. krusie and C. glabrata had higher levels of resistance to itraconazole (71.4% and 53.8%, respectively) than did C. albicans (30%). Additionally, resistance to voriconazole began to emerge, and it was more common in C. non albicans (28.6% in C. krusie and 15.4% in C. glbrata) than in C. albicans (7.5%).

The findings align with the findings of Satora et al. (2023), who reported that the isolates of Candida found in the vagina were extremely resistant to fluconazole. Conversely, another study revealed that C. albicans had a very high susceptibility to fluconazole (96%) (12).

A study using in vitro antifungal susceptibility analysis revealed that 41.7% of the C. krusei isolates were resistant to fluconazole.

Furthermore, a resistance to fluconazole has been observed in Candida glabrata. Furthermore, 100% resistance to itraconazole was demonstrated by C. glabrata and C. krusei. There was little resistance to nystatin and voriconazole (13).

The majority of the non-Candida albican species, including Candida krusei and Cadida albican, were shown to be susceptible to itraconazole, which is in contrast to our findings (11).

Consequently, fungal infections are frequently difficult to treat; antifungal medications must be used carefully to prevent further resistance development, and they can only be taken as directed by a physician.

CONCLUSION

In contrast to Candida non albicans species, which showed greater resistance to Itraconazole, the majority of vaginal Candida species isolates showed low susceptibility to the azole medications (Fluconazole, Econazole, and Itraconazole). Furthermore, susceptibility to the drug increased with increasing dosage. There was also a trend to find that topical nystatin, particularly at higher doses, was more effective at suppressing both Candida albicans and non-Albicans. Unlike other medicines examined, voriconazole and capsaicin showed a significant degree of sensitivity for both Candida albicans and Candida non albicans.

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