

ORIGINAL ARTICLE

Prevalence, Risk Factors and Antifungal Susceptibility of Vulvovaginal Candidiasis among Saudi Females

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ABSTRACT

Key words:
Prevalence,
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Background: Vulvovaginal candidiasis (VVC), is the most common fungal disease in normal healthy women. Understanding of the antifungal susceptibility patterns of pathogenic fungi is essential as resistance of some vaginal yeasts isolates to antifungal agents has been reported. **Objectives:** to determine the prevalence of VVC, associated risk factors and evaluate antifungal susceptibility and species (spp.) distribution in Saudi patients. **Methodology:** High vaginal swabs were collected from all patients suffering from vaginitis. Different *Candida* spp. were identified by Gram stain, germ tube test, growth on CHROM agar and API 20C AUX. Antifungal susceptibility test was done by disk diffusion method. **Results:** VVC represented 23.5%. Out of these patients 11% had recurrent vulvovaginal candidiasis (RVVC). *C. albicans* represented 82% whereas non *albicans* spp. represented 18%. In RVVC *C. albicans* represented 43% and non *albicans* spp. represented 57%. VVC was significantly presented with pruritus and associated with pregnancy, diabetes and age group from 30 to 39 year. RVVC was significantly presented with dysuria and/ or dysparonia. All isolates were sensitive to amphotericin B, while 94% were sensitive to nystatin. Over all sensitivity to fluconazole, voriconazole, ketoconazole and miconazole was 79.5%, 86%, 96.5% and 83% respectively. **Conclusion:** VVC prevalence represented 23.5% whereas RVVC was 11%. *C. albicans* is associated with VVC, whereas non *albicans* spp are associated with RVVC. Diabetes, pregnancy and age group (30 – 39) are considered as potential risk factors for VVC whereas antibiotics and contraceptive pills are not. Nystatin, amphotericin B and ketoconazole have the best antifungal activity against all spp.

INTRODUCTION

Vulvovaginitis is a condition characterized by irritation of vulva, vagina, or both. Vulvovaginal Candidiasis (VVC) is characterized by severe itching of vulva, abnormal vaginal discharge, erythema, edema of vulva, satellite lesions and accounts for approximately one-third of vaginitis cases.^{1,2}

Recurrent vulvovaginal candidiasis (RVVC) is defined as patients who experience more than three attacks of vulvovaginitis caused by *Candida* species (spp.) during the previous year.³ This condition may have serious health consequences, like chronic vulvovaginal pain syndrome.⁴

The prevalence of VVC is difficult to determine as it is clinically diagnosed and not confirmed by microscopic examination or culture. In addition, the widespread use of over-the-counter antimycotic drugs makes epidemiologic studies difficult to perform.⁵ VVC affects between 70 and 75% of adult women during their lifetime, among which approximately 40–50% will experience further episodes and 5% will develop RVVC⁶

The prevalence increases with age up to menopause. The disorder is uncommon in postmenopausal women and pre-pubertal girls.⁷ In addition to antibiotics, other risk factors for VVC include pregnancy, a history of VVC, oral hormones, either contraceptive or replacement therapy, diabetes mellitus and African American ethnicity.⁸ *Candida albicans* (*C.albicans*) is the frequently isolated spp. in most cases of VVC. However, other non-*albicans* spp. of *Candida* such as *C. glabrata* and *C. tropicalis* have also been found and implicated.⁹

The non-*albicans* spp. were shown to be important causative agents for recurrence and chronicity of the disease and many of them were resistant to common antifungal drugs¹⁰. Antifungal agents used for the treatment of VVC include imidazole, triazole and polyene¹¹.

The topical formulations of imidazole and triazole antifungals, are considered the therapy of choice during pregnancy¹² Notably, *C. albicans* is more susceptible to azoles, but non *C. albicans* spp. are more resistant, possibly due to the use of over the-counter azole agents¹³. Therefore, the use of nystatin for the treatment of

VVC is strongly recommended¹⁴. The majority of VVC respond within a few days to short course of topical or systemic treatment. However, women who experience RVVC, requires extensive therapies that entail multiple weeks of systemic treatment^{15,16}. The present study was designed to determine the prevalence of VVC, associated risk factors and evaluate antifungal susceptibility and spp. distribution in Saudi patients.

METHODOLOGY

The present cross sectional study took place in King Khaled general hospital, Saudi Arabia from June 2013 to May 2014. The study was performed on 1455 patients attending obstetric and gynecological clinics and suffering from symptoms suggestive of vaginitis. Two high vaginal swabs (HVS) were collected for each patient. The data collated included: age, pregnancy, presence of diabetes, use of contraceptive pills, use of antibiotics, number of vaginitis episodes and associated symptoms and signs.

Candida spp. identification

- **Microscopic examination of HVS:** A saline suspension was prepared by putting one HVS into a tube containing 0.5 ml of sterile saline. A drop was mixed with 10% potassium hydroxide (wet mount), examined microscopically for the presence of yeast, pseudohyphae and budding.
- **Culture:** The second HVS was streaked on Sabouraud dextrose agar and incubated at 37°C for 48 hour(h). *Candida* identification was based on
 1. Gram stained smears from suspected creamy white colonies showing budding yeast.
 2. Germ tube: A colony of yeast is emulsified in 0.5 ml of serum and incubated at 35°C for 3 h. A drop of the serum is examined microscopically for the formation of germ tubes which was considered a definitive identification of *C. albicans*.⁽¹⁷⁾
 3. Growth on CHROM agar: After inoculation on to CHROM agar (Saudi Prepared Media Laboratory, Riyadh, KSA), the plates were incubated for 24-48 h. at 30°C. The color change developed on the medium was used for the presumptive identification of *C. tropicalis*, *C. krusei*, and *C. albicans* by the production of chromogen pigments (green: *C. albicans*, blue: *C. tropicalis* and rose: *C. krusei*)¹⁷.
 4. Biochemical analysis was done with API 20C AUX (BioMérieux, France). as directed by the manufacturer, and results were recorded after the strips had been incubated at 30°C for 72 h.

Antifungal susceptibility test:

The isolates were tested by disk diffusion method using Muller-Hinton agar supplemented with 2%

glucose and 0.5 µg/ml of methylene blue.¹⁹ The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of 0.5 McFarland standard.¹⁹ The following antifungal disks were used: fluconazole (FCA, 25µg), voriconazole (VCZ,1µg), ketoconazole (KET,50 µg), miconazole (MCZ,50 µg), amphotericin B (AB,100 µg) and nystatin (NY,100 IU) (BioRad, France). The plates were incubated at 35°C and inhibition zone diameters were measured after 24 and 48 h especially for *C. glabrata*. Inhibition zones were interpreted according to manufacturer instructions as shown in table (1)^{18,19}

Table 1: Inhibition zones for antifungal discs

Antifungal agent	Susceptible	Susceptible dose dependant	Resistant
Fluconazole	≥19	15–18 mm	≤14
Voriconazole	≥17	14-16 mm	≤13
Ketoconazole	>20	10-20 mm	<10
Miconazole	>20	10-20 mm	<10
Amphotericin B	>10		≤10
Nystatin	>10		≤10

Statistical analysis:

Data were analyzed by SPSS 19 (Statistical Package for Social Science; release 19.0). The relationship between risk factors and microbiological results were compared. Yates-corrected chi-square, odds ratio and confidence interval were used and a P-value < 0.05 was considered as significant.

RESULTS

Out of 1455 patients with vulvovaginitis, 340 (23.5%) patients showed VVC. Among these (340) patients, only 37 (11.0%) had RVVC. In patients with VVC the most frequently isolated spp. was *C. albicans* 278 (82%) whereas in RVVC, *C. albicans* represented only 16 (43%) (Table 2). The predominant presentation in VVC was pruritus with or without other symptoms (28%, 50%). (Table 3) While the most common presentation for RVVC was Dysuria and/ or dysparonia associated with other symptoms(67.5%) (Table 4). There was significant correlation between pregnancy (P=0.004), diabetes (P=0.019) and age group (30-39)year (P=0.041) and VVC as shown in Table 5. All *Candida* isolates were sensitive to AB. The overall sensitivity to FCA, VCZ, KET, MCZ, and NY was 79.5%,86%,96.5%.83% and 94% respectively (Table 6)

Table 2: distribution of different Candida spp. in VVC and RVVC

<i>Candida spp.</i>	<i>VVC</i>		<i>RVCC</i>	
	No	%	No	%
<i>C. albicans</i>	278	82%	16	43%
<i>C. parapsilosis</i>	7	2%	4	11%
<i>C. glabrata</i>	31	9%	10	27%
<i>C. krusei</i>	11	3%	5	13.5%
<i>C. tropicalis</i>	13	4%	2	5.5%
Total	340	100%	37	100%

Table 3: Common symptoms and signs associated with vulvovaginitis, VVC

<i>Symptoms and signs</i>	<i>Vulvovaginitis</i>		<i>VVC</i>		<i>Chi square</i>	<i>P value</i>
Pruritus alone	90	8%	170	50%	309.2	0.000
Discharge alone	215	19%	50	15%	3.3	0.07
Discharge & Pruritus	62.5	69.5%	95	28%	122.7	0.000
Dysuria and/ or dyspareunia associated with other symptoms	115	10.5%	25	7%	2.2	0.130
Total	1115	100%	340	100%		

Table 4: Common symptoms and signs associated with RVVC

<i>Symptoms and signs</i>	<i>VVC</i>		<i>RVVC</i>		<i>Chi square</i>	<i>P value</i>
Pruritus alone	170	50%	8%	3	23.58	< 0.0001
Discharge alone	50	15%	5.5%	2	2.43	0.119033
Discharge & Pruritus	95	28%	19%	7	1.38	0.240101
Dysuria and/ or dyspareunia associated with other symptoms	25	7%	67.5%	25	112.92	<0.0001
Total	340	100%	100%	37		

Table 5: Clinical characteristics of outpatients with VVC

<i>Clinical characteristics</i>	<i>Vulvovaginitis (1115)</i>		<i>VVC (340)</i>		<i>95% CI</i>	<i>OR</i>	<i>Chi square</i>	<i>P value</i>
	No	%	No	%				
Age								
20-29	306	27.5%	98	29%	0.811-1.413	1.071	0.183	0.699
30-39	386	34.5%	139	41%	1.010-1.688	1.306	4.165	0.041
40-49	308	27.6%	79	23%	0.591-1.063	0.793	2.350	0.125
More than 49	115	10.4%	24	7%	0.407-1.065	0.660	2.829	0.093
Diabetes								
Diabetic	739	66%	249	73%	1.053-1.841	1.392	5.472	0.019
Non diabetic	376	34%	91	27%				
Antibiotic (6weeks)								
Users	647	58%	197	58%	0.773-1.284	0.966	0.000	1.000
Non users	468	42%	143	42%				
Contraceptive pills								
Non Users	917	82%	268	79%	0.909-1.701	1.244	1.795	0.180
users	198	18%	72	21%				
Pregnancy								
Pregnant non	220	20%	43	13%	1.178-2.454	1.698	8.357	0.004
pregnant	895	80%	297	87%				

*OR: odds ratio *95% CI: 95% confidence interval

Table 6: Antifungal susceptibility of different *Candida* spp. (number and percentage of sensitive isolates)

<i>Antifungal</i>	<i>C. albicans</i>		<i>C. parapsilosis</i>		<i>C. glabrata</i>		<i>C. krusei</i>		<i>C. tropicalis</i>		<i>Total</i>	
	No	%	No	%	No	%	No	%	No	%	No	%
Fluconazole												
S	246	88.5%	7	100%	5	16%	0	0%	12	92%	270	79.5%
SDD	7	2.5%	0	0%	3	9%	1	9%	0	0%	11	3.2%
R	25	9%	0	0%	23	74%	10	91%	1	8%	59	17.3%
Voriconazole												
S	238	85.5%	6	86%	28	90%	8	73%	12	92%	292	86%
SDD	11	4%	0	0%	0	0%	0	0%	1	8%	12	3.5%
R	29	10.5%	1	14%	3	10%	3	27%	0	0%	36	10.5%
Ketoconazole												
S	269	97%	7	100%	30	97%	11	100%	11	85%	328	96.5%
SDD	0	0%	0	0%	0	0%	0	0%	1	7.5%	1	0.3%
R	9	3%	0	0%	1	3%	0	0%	1	7.5%	11	3.2%
Miconazole												
S	228	82%	5	71%	27	87%	10	91%	13	100%	283	83%
SDD	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
R	50	18%	2	29%	4	13%	1	9%	0	0%	57	17%
Amphotericin B												
S	278	100%	7	100%	31	100%	11	100%	13	100%	340	100%
R	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Nystatin												
S	258	93%	7	100%	30	97%	10	91%	13	100%	318	94%
R	20	7%	0	0%	1	3%	1	9%	0	0%	22	6%

DISCUSSION

Vulvovaginal candidiasis (VVC), particularly the recurrent form, is a multifactorial disease that affects 75% of women at least once during their lifetime. In addition 50% of these women will also suffer a single recurrence¹⁵. Knowledge of *Candida* spp. causing VVC is important for clinicians, as non *albicans* spp. often fail first-line treatment²⁰ Therefore, rapid and specific detection and identification of *Candida* spp. will help to choose the suitable antifungal and improve patient care¹⁷ The exact incidence of VVC is unknown as it is not a reportable disease, often diagnosed without confirmatory tests and treated with over-the-counter medications.²¹ In the present study VVC represented 340 (23.5%) of all vulvovaginitis cases. Similar findings were also reported by Essel et al. and Hedayati et al. who found that *Candida* spp. represented 25.5%, and 28.2% respectively.^{22,23} Higher^{24,25} or lower²⁶ incidence were reported by other studies. Out of these 340 patients 37(11%) suffered from RVVC. This is higher than both Janković et al. and Ringdahl who reported it to be 6.3% and 5% respectively^{3,6} and lower than Hedayati et al., who reported it to be 24.2%.²³ In an international survey made in 5 European countries and the United States, The rate of VVC ranged between 29% and 49% while that of RVVC was 9%.⁷ Eckert et al. attributed this variation due to inaccuracies in pathogen detection, mismanagement, drug resistance,

incomplete therapeutic course, self-treatment, lack of appropriate health habits and intestinal infestation²⁷.

Our study showed that *C. albicans* was the most frequent spp. isolated (82%). This result is in agreement with other studies which reported that *C. albicans* was the most frequent spp. isolated in VVC^{10,26,28,29}. Some authors attributed this to the greater ability of *C. albicans* to adhere to vaginal mucosa, which is the primary step in establishment of a fungal infection.²³ On the other hand, in RVVC non *albicans* spp. were more prevalent than *C. albicans* which represented only (43%) as the non-*albicans* spp. were shown to be important causative agents for recurrence and chronicity of the disease and many of them were resistant to common antifungal drugs¹⁰. Similarly, Ramsay et al reported that in RVVC, the likelihood of having a non *albicans* spp. increases.³⁰

In the present study VVC was significantly associated with pruritus whereas the main complain in RVVC was Dysuria and/ or dysparonia associated with other symptoms. This is similar to other studies who reported pruritus with or without vaginal discharge and vaginal erythema to be the most common symptom in VVC^{10,22,27}

Based on our findings, all *Candida* spp. were sensitive to AB. Several studies have similarly reported that AB was the most effective drug against vaginal *Candida* isolates.^{18,31,32} Overall NY sensitivity was 94%. This finding is in agreement with other studies

carried out in Uganda, Argentina and China^{19,33,34} The Chinese study reported that the excellent antifungal activity of NY was due to the unique mechanism of changing cell membrane permeability and relatively low-frequency use in clinical setting.³⁴

The overall sensitivity to FCA was 79.5%. Ninety one percent and 74% of *C. krusi* and *C. glabrata* isolates were resistant to FCA respectively. This result was expected due to intrinsic resistance of this spp. to FCA³⁴ Whereas *C. albicans* showed 9% resistance only. This result is less than that reported by Wang et al., who reported that 16% of *C. albicans* were resistant to FCA³⁴ and more or less similar to that reported by ElFeky et al (10.5% resistance) and Al-akeel et al.^{18,17}

The overall sensitivity to VCZ was 86% which is similar to other studies reported from Egypt¹⁸ and less than that reported from Uganda¹⁹. For KET and MCZ the overall sensitivity was 96.5% and 83% respectively. Al-akeel et al.³⁴, reported that all yeast-isolates, were sensitive to KET.¹⁷ Wang et al. reported the susceptibility to MCZ to be less than the present study (73%)

In the present study VVC was significantly associated with diabetes (P value, 0.02) and pregnancy (P value, 0.004) as it is believed that the high estrogen levels and high glycogen content in vaginal secretions during pregnancy provide an excellent carbon source and enhances the adherence of yeast cells to the vaginal mucosa³⁵. It is also well documented by other studies that pregnancy and diabetes mellitus increases the rate of vaginal colonization and infection with *Candida*^{36,37}. Also, VVC was more frequently associated with the age group (30-39) (P=0.041) which was attributed by some authors to the active behavioural sexual relations in this age group¹⁷. On the other hand, there was no significant correlation between VVC and previous antibiotic use or contraceptive pills. This can be explained by lack of use of contraceptive pills as the first method of contraception. Some studies showed results contrary to our results^{8,38} whereas other studies showed similar results and explained this discrepancy to be due to differences in size of studied women subjects, duration and types of used contraceptives or antibiotics, as well as methods of yeast-detection¹⁷

CONCLUSION

VVC prevalence represented 23.5% whereas RVVC was 11%. *C. albicans* is associated with VVC, whereas non *albicans* spp are associated with RVVC. Diabetes, pregnancy and age group (30–39) are considered as potential risk factors for VVC whereas antibiotics and contraceptive pills are not. Nystatin, amphotericin B and ketoconazole have the best antifungal activity against all spp.

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