ORIGINAL ARTICLE

DETECTION OF *CANDIDA* SPP. THAT CAUSES VULVOVAGINITIS IN WOMEN THAT USE CONTRACEPTIVE METHODS

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ABSTRACT

The aim: To determine the distribution of *Candida* spp. within different age groups and contraceptive methods in women with vulvovaginitis, as well as the susceptibility of *Candida* spp. to commonly used antifungals.

Materials and methods: High vaginal swabs were taken from 98 women aged 18 to 50 with vulvovaginitis who used contraceptives and attended the Women and Children Hospital in Al-Diwaniyah; after diagnosis of *Candida* species, the sensitivity of *Candida* spp. to some antifungals was studied.

Results: The results showed (43/98) women (43.87%) used IUD, (15/98) women (15.30%) used birth control pills, (7/98) women (7.14%) used an injection of contraceptive, (5/98) women (5.10%) used contraceptive suppositories, and (28/98) women (28.57%) did not use any contraceptives. *Candida* spp. was found in (48/83) specimens (57.831%) from women who used contraceptives and only (11/28) specimens (39.285%) from women who did not use contraceptives. Only (59/98) vaginal specimens tested positive for vaginal candidiasis, (28/59) isolates (47.457%) for *C. albicans*, then (16/59) isolates for *C. glabrata* (27.118%), (9/59) isolates (15.254%) for *C. tropicalis* and (6/59) isolates (10.169%) for *C. krusei*. Nystatin was the best treatment for all *Candida* spp. under study, and the MIC was 6.25, and the MFC was 50 for all antifungals and *Candida* species under study.

Conclusions: *C. albicans* was the most prevalent cause of vulvovaginal candidiasis, while *C. glabrata* was the most common non-albicans species in women aged 26 to 35; using an IUD was associated with an increased infection of vulvovaginal candidiasis, and nystatin was the most effective treatment.

KEY WORDS: vulvovaginitis, contraceptive, *Candida* spp., MIC, MFC

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INTRODUCTION

Since 1849, when Wilkinson reported the first infection with vaginal candidiasis and linked the presence of fungus to vaginal discharges, and discovered that vaginal thrush was the most common condition in women of childbearing age, vaginal thrush has been reported [1]. Various causes of vaginitis include bacteria, viruses, and parasites, but *Candida* spp. is the most common [2]. Yeasts have recently been identified as a significant source of vaginal infections in both the community and the hospital, candidiasis of the vulvovaginal tract, also known as VV candidiasis, is the second most common infection of the female genital tract, accounting for nearly 25% of all infectious diseases vaginitis [3-4]. VV candidiasis is a reasonably common infection that affects more than half of all women at some point in their life; of those who have had VV candidiasis, approximately 40-50 % experience further bouts of the infection [5]. *Candida* spp. is present in 20-50 % of otherwise healthy and symptom-free women during their premenopausal reproductive years, but its incidence drops drastically after women reach menopause [6-7]. It can become pathogenic and lead to candidiasis under situations like reduced immunity, prolonged antibiotic therapy, steroids and malnutrition, pregnancy and oral contraceptives, diabetes, and obesity [8]. *Candida* spp. coexists with *Lactobacillus* spp. in oral, GIT, and vaginal infections; in the absence of these essential bacteria, *Candida* spp. multiply and cause infections [9-10]. 80-90 % or more of the fungi found in women with acute VV candidiasis are caused by *C. albicans* [11-12].

Nonetheless, there appears to have been a tendency in the preceding year toward a higher prevalence of non-albicans *Candida* species in fungal cultures, such as *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* [13]. There aren't a lot of epidemiological data about where *Candida* spp. is found; they cause VV candidiasis, partly because there aren't enough standard microbiological tests [14]. Chemicals materials like polyenes and azoles that kill fungi have been used to treat fungal infections; still, the wide use of these antifungals has led to the development of strains that are resistant to different antifungals and side effects like high toxicity that affects the liver, kidneys, and immune system [15-16].

THE AIM

This study aimed to determine the distribution of *Candida* spp. by isolation and diagnosis of *Candida* spp. from women with vulvovaginitis who used contraceptives and attended the Women and Children Hospital in Al-Di-

waniyah from December 2021 to April 2022 and detected the susceptibility of *Candida* spp. to commonly used antifungals.

MATERIALS AND METHODS

SWABS COLLECTION

High vaginal swabs were taken from 98 patients aged 18 to 50 with vulvovaginitis. All specimens were taken from Women's General Hospital and Children in Al-Diwaniyah, Iraq. Specialized doctors gave clinical talks, and all patient's information was collected in a secure database; the specimens were collected using sterile cotton swabs and separated into two smears: one was inspected under a microscope right away for direct examination, while the other was usually transferred to the laboratory for culture.

DIAGNOSIS OF *CANDIDA* SPP.

DIRECT EXAMINATION

Vaginal swab specimens were examined by placing them on a clean slide, mounting them with a drop of KOH 10%, covering them with a coverslip, and gently worming the slide (but not boiling). *Candida* spp. was examined under a microscope; a gram stain was also used to determine the isolate's response to the colour and their forms, arrangement, and yeast budding form [17].

COLONIAL MORPHOLOGY

All isolates were cultured on (SDA) medium to find pure colonies of *Candida* spp. the plates were kept in an incubator at 30°C for 24-48 hours so that their shape, size, colour, and consistency could be studied; the isolates were then put onto CHROM agar-Candida medium and cultured at 37°C for 24-72 hours in the look of bright green colonies (a typical colour of *C. albicans*) [18].

LACTOPHENOL COTTON BLUE EXAMINATION

A little piece of the colony was cut and placed on a clean slide with a drop of lactophenol cotton blue; the slide was then covered with a coverslip to look for budding Candida cells under a light microscope [19].

GERM TUBE FORMATION TEST

The yeast cells were suspended in 0.5ml of human serum in a tiny tube (the serum was obtained by blood centrifugation at 1500 rpm for 15 minutes), and the tubes were incubated at 37°C for 2-3 hours; yeast species may begin to develop germ tubes if the incubation time exceeds 3 hours, a drop of cultured serum was placed on a slide, covered with a coverslip, and inspected under a microscope for germ tubes [20].

PURIFICATION OF CANDIDA SPP.

Colonies with proven *Candida* spp. the status was streaked on (SDA) medium and incubated for 2 days at 30-35°C to obtain an isolate; this isolate was streaked across the plate for 1-2 days at 35 °C before parafilm was used to protect it from contamination, and stored in the refrigerator at 4°C.

ANTIFUNGAL SENSITIVITY TEST

The sensitivity of *Candida* spp. to some antifungals (Nystatin, Metronidazole, Clotrimazole, and Ketoconazole) was studied using Sabouraud dextrose broth to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC), using the Broth dilution method which includes:

PREPARATION OF INOCULUM

The inoculum was made from a 24-hour (SDA) medium culture with yeast cells adjusted to $1x10^5$cell/ml.

ANTIFUNGAL SOLUTIONS

The antifungal was used as a pure powder, and the solutions were made by dissolving 0.003 gm of each in 10 ml of (DMSO), then preparing the other concentrations; after organizing the first concentration of 100 µg/ml, a series of antifungal concentrations ranging from 50 to 0.05 µg/ml were prepared. We used 12 tubes, each with 2 ml of (SDB) medium; in the first tube, we added 2 ml of antifungal to get the first concentration of 50 µg/ml, and then 2 ml from the first tube were moved to the second tube, then from the second tube to the third tube, and so on until the last tube, where the previous 2 ml were thrown away, positive (broth and inoculum) and negative (broth and antifungal) controls were also prepared, each tube was inoculated with 0.05 ml of *Candida* spp., suspension and incubated for 48 hours at 30°C, and after the incubation period, each tube was thoroughly mixed, and the growth was compared to positive and negative controls. The MIC concentration was the lowest in which no growth was recorded. MFC was evaluated by culturing 0.01ml from each tube, which showed no change. Positive control was cultivated on (SDA) medium without antifungal for 48 hours at 30°C; after sub-culturing, MFC was the lowest concentration that produced negative consequences [21].

STATISTICAL ANALYSIS

A statistical analysis system called SAS (2008) was used to compare the percentages of the data in this study. The Chi-square test was done, and the least significant differences (LSD) test was used to compare means.

RESULTS

DIVISION OF PATIENTS ACCORDING TO CONTRACEPTIVE METHODS

Table I shows that 43 out of 98 women (43.87%) used an intrauterine device (IUD), 15 out of 98 women (15.30%)

Table I. The percentage value of contraceptive users.

Methods of Contraceptive users	No. of specimens	Percentage [%]
IUD	43	43.87
The pill	15	15.30
Injection	7	7.14
Suppositories	5	5.10
Control	28	28.57
Total	98	-

Table II. Division of patients according to vulvovaginitis caused by *Candida* spp.

Specimens groups	Positive results		Negative results		Chi-square value
	No. of specimens	Percentage [%]	No. of specimens	Percentage [%]	
Test group n= 83	48	57.831	35	42.168	2.573 ns
Control group n= 28	11	39.285	17	60.714	3.754 *
Chi-square value	-	0.583 ns	-	0.294 ns	

* ($p<0.05$), ns= non-significant.

Table III. Division of vulvovaginitis in women according to age groups.

Age groups (Year)	No. of specimens	Percentage [%]
18-25	27	27.551
26-35	40	40.816
36-45	21	21.428
≥ 46	10	10.204
Chi-square value	-	5.534 *

*($p<0.05$)

used birth control pills, 7 out of 98 women (7.14%) used an injection of contraceptive, 5 out of 98 women (5.10%) used contraceptive suppositories, and 28 out of 98 women (28.57%) were the control group (does not used any contraceptives).

DIVISION OF PATIENTS ACCORDING TO VULVOVAGINITIS CAUSED BY *CANDIDA* SPP.

Table II shows that *Candida* spp. was found in 48 out of 83 specimens from women (57.831%) who used contraceptives, but only 11 out of 28 specimens from women (39.285%) who didn't use contraceptives.

DIVISION OF PATIENTS WITH VULVOVAGINITIS ACCORDING TO AGE GROUPS

A total of 98 women, ranging in age from 18 to 50, were examined, and their ages were categorized into the following four groups: 18-25, 26-35, 36-45, and 46 and above. There were 27 patients with vulvovaginitis symptoms in the 18-25 age group in percentage (27.551%), 40 in the 26-35 age group in percentage (40.816%), 21 in the 36-45 age group in percentage (21.428%), and 10 in the 46 years and above age group in percentage (10.204%). Additionally, the results showed significant differences in the distribution of vulvovaginitis between age groups (Table III).

DIAGNOSIS OF *CANDIDA* SPP.

After a direct microscopic examination and determining the morphological characteristics of the colonies and growth in the CHROM agar-Candida medium and testing the formation of the germ tube, only 59 of the 98 vaginal specimens tested positive for vaginal candidiasis, 28 isolates for *C. albicans* with the percentage of (47.457%), then 16 isolates for *C. glabrata* with the percentage of (27.118%), 9 isolates for *C. tropicalis* with the percentage of (15.254%), and 6 isolates for *C. krusei* with the percentage of (10.169%) (Table IV).

SENSITIVITY OF *CANDIDA* SPP. TO ANTIFUNGAL

The results showed Nystatin's Minimum Inhibitory Concentration (MIC) for all *Candida* spp. under study was 6.25. The MIC for the antifungal Metronidazole was 6.25

Table IV. *Candida* species isolated from women under study.

Candida spp.	No. of isolates	Percentage [%]	No. of germ tube	Colony colour on CHROM Agar, texture
C. albicans	28	47.457	28	Light green ,smooth
C. glabrata	16	27.118	-	Pink to cream
C. tropicalis	9	15.254	-	Blue-pink
C. krusei	6	10.169	-	Pink-white border
Total	59	100		
Chi-square value	-	6.532 *		

*($p<0.05$)

Table V. MIC and MFC for antifungal against *Candida* spp. under study

Candida spp.	Antifungal [µg/ml]							
	Nystatin		Metronidazole		Clotrimazole		Ketoconazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans	6.25	50	12.5	50	12.5	50	12.5	50
C. glabrata	6.25	50	12.5	50	12.5	50	12.5	50
C. tropicalis	6.25	50	6.25	50	12.5	50	12.5	50
C. krusei	6.25	50	12.5	50	12.5	50	12.5	50

for *C. tropicalis* but 12.5 for other *Candia* spp. The MIC for the antifungals Clotrimazole and ketoconazole was also 12.5 for all *Candida* spp. under study. The Minimum Fungicidal Concentration (MFC) was 50 for all antifungals and *Candida* species (Table V).

DISCUSSION

Over half of all women will encounter vulvovaginal candidiasis infection at some point in their lives, and of those women, roughly 40-50 % will have additional bouts of the condition [22]. This study's positive and negative test group results weren't significantly different. Positive and negative control group results differed significantly. No significant differences were found between positive and negative effects in the test and control groups, consistent with Cetin *et al.* [23], who found *Candida* spp. in 44.2% of contraceptive users and 37.9% of non-users. Candidiasis is becoming more common in women between the ages of 25 and 35 because of pregnancy, antibiotics, and birth control; as glycogen levels rise, vaginal epithelial cells produce more estrogen, which increases the number of lactic acid bacteria that break down glycogen, which lowers vaginal pH. and become a good place for *Candida* spp. to spread and grow [24]. In other reports, Ghaddar *et al.* [25] and Ignjatovi *et al.* [26] looked into women with VV symptoms and found that *C. albicans* were high numbers among *Candida* spp. In this study, the species distribution was affected by the low number of non-albicans species caused by antifungals [27]. The concentration of antifungals plays an essential role in treating fungal infections; 50 (µg/ml) and higher concentrations are considered fungicidal [28]. The MIC was affected by differences in medium, temperature, and incubation duration; Nystatin demonstrated high sensitivity (99%) compared to Clotrimazole and Fluconazole against *Candida* spp. isolates in vitro, suggesting it could be a suitable antifungal drug for resistant infections [29-30]. Azoles have two effects on living cells; the first is an effect on cytochrome P_{450}, which stops the formation of ergosterol by removing 14-α methyl sterol from the plasma membrane. The second is an antifungal effect that destroys membranes by directly interfering with lipid membranes; a change in the sterol biosynthesis pathway often causes resistance [31].

CONCLUSIONS

C. albicans was the most common cause of vulvovaginitis candidiasis, and the most frequent non-albicans species seen to cause vulvovaginal candidiasis in women aged 26 to 35 was *C. glabrata*, and using an IUD was associated with an increased infection of vulvovaginal candidiasis. The most successful treatment for candidiasis was nystatin, an antifungal.

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Conflict of interest:

The Authors declare no conflict of interest.

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