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Research article

DOI:10.2478/rrlm-2021-0012

Vaginal candidiasis in Konya area: Etiology, risk factors, virulence patterns, and antifungal susceptibility

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Abstract

Vulvovaginal candidiasis (VVC), a common genital tract infection, is known to affect millions of women worldwide. In this study, it was aimed to determine the prevalence, virulence, possible risk factors and antifungal susceptibility model of Candida species. Vaginal swab samples were taken from patients aged 18 years and older who presented to the gynecology outpatient clinic with signs and symptoms suggestive of vulvovaginitis. Demographic data were recorded using a questionnaire. Standard microbiological methods were used for the identification of the isolates. Broth microdilution method was used to determine the antifungal susceptibility of Candida isolates. Virulence factors of Candida strains were determined by performing proteinase, phospholipase, hemolytic and biofilm activity tests. Sequencing of the isolates identified as Candida were performed using ITS 1-4 primers. Vaginal discharge (OR: 3.365; 95% CI: 1.595-7.101), burning complaint (OR: 9.098; 95% CI: 2.284-36.232) and history of allergy (OR: 3.396; 95% CI: 0.968) were risk factors. The results showed that the most common isolated strain was Candida albicans (57%). It was found that the prevalence of C. glabrata remained at 26%, 44 of the C. albicans isolates presented proteinase, 35 had phospholipase, 47 had biofilm, and 47 had hemolytic activity. In this study, susceptible dose-dependent and resistant rates of all Candida strains were found for fluconazole as 9% and 16%, respectively. Host and organism-related factors should be considered in the clinical treatment of VVC, and continuous monitoring of changes in the prevalence of Candida species and susceptibility rates is required for effective antifungal therapy.

Keywords: *Candida vaginitis, genotypic identification, virulence factors, risk factors, antifungal susceptibility*

Received: 8th October, 2020; Accepted: 14th January 2021; Published: 16th February 2021

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Introduction

Vaginitis infections are one of the opportunistic infections in women and may present themselves with obvious symptoms or asymptomatic during routine examinations. Normal vaginal flora may vary depending on the pH of the environment, age, hormonal status, sexual activity, method of contraception, drugs used, antibiotics, and surgical interventions (1). *Candida* species are distributed widely both in living organisms and in a wide variety of environments such as hospitals (2,3). These species are members of the human microbiome. They are abundant in the genital area, urine, skin, oral cavity, scalp, nails, and mucosal surfaces of the respiratory and digestive tracts (2). In most women, they can reach the lower genital tract from the lower perianal region without signs of infection (4). Pathogenicity of these species is thought to be caused by some virulence factors such as protection from host defense mechanisms with filamentous forms, adhesion to host tissue, biofilm-forming capacity, production of phospholipases, which are tissue-destructive hydrolytic enzymes, lipase and hemolysin (5). Aspartyl proteinases and phospholipases, which are among these hydrolytic enzymes, are important enzymes secreted by *Candida* species in both the yeast phase and the hyphae phase and have been reported to induce tissue invasion (6). The most important factors causing *Candida* infections are changes in the host vaginal/vulvar environment. The infection that occurs in this case is called vulvovaginal candidiasis (VVC). This disease is particularly caused by *Candida* species in the absence of other bacterial agents. Considering that the most common cause of vaginal infections is vaginosis, VVC can be considered as the second most important cause of these infections (7). Vulvar or vaginal erythema, hyperemia, itching, pain, burning are the most common clinical signs of VVC. It is estimated that 10-15% of patients colonized with *Candida* are asymptomatic. Similarly, it is estimated that 70-75% of women colonized with *Candida* will experience VVC attacks during their lifetime, 50% of women who were initially infected will experience a second VVC, and 5-10% of all women will develop recurrent VVC (8). The factors that lead to VVC are generally increased sexual activity, high amount of estrogen or oral contraceptives, pregnancy, antibiotic use, diabetes, corticosteroid use, hormone replacement therapy, chemotherapy or vaginal douching (9). The most common *Candida* species causing this disease are *C. albicans*, *C. glabrata, C. parapsilosis*, *C. krusei and C. tropicalis*. Generally, there is only one type in etiology, but two or more types were detected in 1-10% of women with VVC. Coexistence of *C. albicans* and *C. glabrata* is more common when there is more than one *Candida* agent (10). Although significant therapeutic advances have been made in the treatment of this disease, the mechanisms that trigger VVC have unfortunately not yet been fully discovered. Therefore, it is considered appropriate to review the epidemiology of this disease, the specific risk factors and what is known about the virulence factors of *Candida* species. In this study, we aimed to determine the prevalence, virulence, possible risk factors and antifungal susceptibility model of *Candida* species.

Methods

Research population

This study was prospectively conducted in a tertiary university hospital over a 2-year period from April 2018 to April 2020.

The study protocol had the approval of the Ethics Committee of KTO Karatay University Medical Faculty (no. 41901325-050.99/date 21.02.2019). In the study, vaginal swab samples were taken from patients aged 18 years and older who presented to the gynecology outpatient clinic with signs and symptoms suggestive of vulvovagini-

tis. Demographic data were recorded using a questionnaire. Patients with yeast growth in culture and those with normal vaginal microflora were accepted as the case and control groups, respectively. The following individuals were not included in the study: pregnant women, those who did not want to participate in the study, those with a known history of gynecological cancer, patients with vaginal complaints who had been treated in the previous month, those who had had sexual intercourse in the last three days, girls with a hymen, those who had vaginal bleeding during the study and those who had menstrual period. Written informed consent was obtained from the patients included in the study, explaining the aim of the study. In line with the literature information obtained, the patients were asked 26 questions about demographic data, chronic disease history, gynecological history, sexual and menstrual behavior history, main complaints and other problems in a five-part form, which was prepared to identify specific risk factors.

Sampling

Two swab samples were taken from the posterior fornix and the lateral wall of the vagina with a sterile swab during the gynecological examination performed with a speculum for sampling. One of the swab samples taken from the patients was placed in a tube modified Stuart's medium and the other was placed in a 0.5 ml sterile saline tube. Before, each sample of vaginal swab was done by gram staining and examined microscopically. Direct examination was positive if it showed regular blastopores, as well as pseudopyphae and blastopores. Afterwards, the other swab was inoculated on Sabouraud Dextrose Agar (SDA, Merck, Germany) and then plates incubated at 37 °C for 48 h. After the incubation period, germ tube test and gram staining (Gram-positive budding cells with round or oval morphology were considered *Candida* positive), which are phenotypic methods, were applied to the yeast-looking colonies grown on the medium to determine the microorganisms that grew.

Germ tube test was used to differentiate *C. albicans, C. dubliniensis and C. africana* from other *Candida* species (11). Human serum was used to reproduce *Candida* species. It was observed that the cells incubated at 37 °C for 3 hours formed germ tubes detectable with KOH films.

In addition, in order to identificate the strains including chlamydoconidia formation, urease activity, growth at various temperatures; and carbohydrate assimilation patterns assays were performed. In addition, rapid identification test kits such as API 20C AUX system (Biomérieux, France) were used to identify the isolates.

In our study, the following criteria were used in the diagnosis of VVC, and the patient with at least one of them was diagnosed with VVC. These criteria are: appearance of budding yeast, pseudohyphae or hyphae in microscopic examination of wet-mount and Gram stained preparations; the presence of a large number of yeast in the culture ($>10^3$ CFU/ml suspension) and no yeast cells in microscopic examination, and the presence of symptoms and signs (vaginal discharge, vulvar and vaginal erthtema and pruritus, burning, inguinal pain) related to VVC in the patient were considered to be positive. A small number of yeast ($<10^3$ CFU/ml suspension) in the culture and no yeast in the microscopic examination were considered to be colonization and the patient was VVC negative (12).

Molecular diagnosis

DNA isolation from samples was performed with the "High Pure PCR Template Preparation Kit" (Roche Diagnostic, USA) according to the manufacturer's recommendations. Polymerase chain reaction (PCR) was performed by using ITS1 (forward) 5'-TCCGTAGGTGAACCTGCGG-3 'and ITS4 (reverse) 5'-TCCTCCGCTTATTGATATGC-3' (Sentebiolab, Turkey) primers. Based on a previously defined method, PCR reaction was carried out in 35 cycles following a 2 minute

initial denaturation at 95 °C in a thermal cycler device (BioRad, Italy) in 30 seconds, 2 minutes at 58 °C, and 1 minute at 72 °C, and lastly 5 minutes at 72 °C as the final cycle. Amplification products were run on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under UV light. Sequencing of PCR amplicons was performed by Sanger (capillary electrophoresis) sequencing. All amplicons were purified using the ExoSap-IT (Applied Biosystems, California, USA). The DNA fragments were sequenced using an automated DNA sequencer (ABI PRISM 377, Taiwan) with a BigDye Terminator kit (Applied Biosystems, Taiwan). Amplicons were sequenced using ITS1 and ITS4 primer.
Sequence analysis data were evaluated using the "National Center for Biotechnology Information (Bethesda, USA)" BLAST system (http://www.ncbi.nlm.nih.gov/BLAST/) and strains were identified at species level. The phylogenetic tree was created with MEGA 7.0 software (Fig. 1). The confidence levels of the monophyletic group were determined using bootstrap analysis (1,000 copies).

Virulence factors

Acid proteinase activity was investigated on agar plates containing bovine serum albumin (BSA) as defined by Staib (13). BSA agar plates were prepared by adding 1% BSA, 2% agar, 2% glucose, 0.05% $MgSO_4$ and 0.01% KH_2PO_4. 10 µl of the suspension prepared in cultures was inoculated into plates and allowed to incubate at 37 °C. Acid proteinase activity was assessed by halo formation around the colony at the end of the third day (14). Phospholipase activity was studied on egg yolk-containing agar plates as described by Price et al. (15). In the preparation of this medium, SDA medium, 1 M NaCl, 0.005 M $CaCl_2$ and 8% egg yolk were used. The egg yolk was centrifuged at 500xg for 15 min before adding to the medium. The supernatant was taken into a sterile tube and added to the medium when the temperature reached 50 °C after autoclaving. The prepared medium was then poured into the plates. The suspension prepared from the yeast colony was inoculated into 10 µl of medium and incubated at 37 °C. On the third day of incubation, white zone formation around the colony was evaluated in favor of phospholipase activity (16).
To determine the hemolytic activity of the isolated samples, the method described by Luo et al. (17) was used. After the isolated samples were incubated at 37 ° C for 24 h, the suspension inoculated in SDA was prepared in sterile saline at a rate of $1x10^8$ cells/ml. 10 µl of this suspension was taken and inoculated into SDA containing 7% sheep blood and 3% glucose. Plates were incubated in the presence of 5% CO_2 at 37 °C for 48 h. The distinct transparent zone formed around the inoculum area was accepted as positive hemolytic activity.
The modified tube adherence method was used to investigate the biofilm production potential. After 24-48 h incubation in SDA, a loopful colony was picked, inoculated into tubes containing Sabouraud broth (SB) with a final glucose concentration of 8% and incubated for 48 h at 37 °C. After incubation was completed, the contents of the tubes were emptied, washed twice with distilled water, and then stained with 1% safranine. After the tubes were dried in air, the presence of a colored film layer on the inner wall was evaluated as biofilm positive. Biofilm positivity was evaluated as weak positive (+), medium positive (++) and strongly positive (+++), according to the thickness of the layer formed (18).

Antifungal susceptibility test

For susceptibility experiments of the isolated sample, caspofungin (Sigma, China), amphotericin B (Sigma, Israel), fluconazole (Sigma, USA), ketoconazole (Sigma, China), voriconazole (Sigma, USA) and posaconazole (Sigma, USA) were

used as antifungals. The CLSI-recommended cut-off values in M27-S3 and M27-S4 were used to determine the antifungal susceptibility of the isolates (19, 20). In the absence of sufficient data to develop species-specific clinical limit values, the "epidemiological cut-off value (ECV)" was used to identify isolates that were less responsive to treatment. Epidemiological cut-off values were used to differentiate wild (not exposed to antifungal or acquired resistance mechanisms) and non-wild types (mutational or acquired resistant isolates) (21).

In U bottom shaped 96-well microplates, using two-fold serial dilutions, solutions varying in ranges of 0.015-8, 0.0313-16, 0.125-64, 0.0313-16, and 0.0313-16 μg/ml for caspofungin, amphotericin B, fluconazole, voriconazole and for posaconazole were prepared, respectively. *C. krusei ATCC 6258* and *C. parapsilosis ATCC 22019* strains were used as controls. At the end of 24 h of incubation at 37 ° C, the microplates were evaluated with the naked eye and the minimum inhibitory concentration (MIC) values were determined. The lowest antifungal concentration in the well where the growth showed a 50% reduction compared to the growth in the control well without antifungal was considered as the MIC value.

Statistical analysis

IBM SPSS v.25 was used for data analysis. Chi-square test, univariate analysis and Fisher's exact test were used to obtain quantitative statistical data. In addition, the relationship between dependent and independent variables was documented through bivariate and multivariate logistic regressions. Using these analyses, odds ratios (OR) and 95% confidence interval (CI) associated with risk factors were calculated. '*p*' values less than 0.005 were considered statistically significant.

Results

200 patients with signs and symptoms of vaginitis between April 2018 and April 2020 were included in the study. 100 patients with *Candida* growth in vaginal swab culture were included in the case group, and 100 patients with no growth in the culture were included in the control group. The ages of the patients included in the study ranged from 18 to 59, with a mean age of 33.7 ± 8.51. 180 (90%) of the participants were married. Basic demographic and clinical characteristics of the women in the case and control groups are presented in Table 1 and 2. When the basic characteristics of the women included in the study were examined, no significant difference was found between the case and the control groups ($p > 0.05$). When the basic clinical features were examined, a significant difference was found between the case and the control groups in terms of allergy history, discharge, itching, burning, and groin pain ($p < 0.05$) (Table 2). According to multivariate analysis results, risk factors for vaginitis were discharge (OR: 3.365; 95% CI: 1.595-7.101), burning complaint (OR: 9.098; 95% CI: 2.284-36.232), and history of allergy (OR: 3.396, 95% CI: 0.968-11.910) (Table 3).

In Gram stained smears, budding yeast cells (blastoconidia) and/or pseudohyphae showing regular points of constriction identified as *Candida spp*.

As a result of bidirectional DNA sequence analysis of the ITS1 and ITS4 regions of the isolates identified as *Candida* by phenotypic tests (germ tube test and gram staining), it was determined that they consisted of five different species. Accordingly, the most dominant species were *C. albicans* (57%), *C. glabrata* (26%), *C. kefyr* (12%), *C. krusei* (3%) and *C. dubliniensis* (2%). The evolutionary relationship between strains was evaluated by using the Neighbor-Joining method and the MEGA program. Phylogenetic analysis of the strains is shown in Figure 1.

Table 1. Basic demographic characteristics of the women included in the study.

Variables	*Candida* positive (>10^3 cfu/ml)	*Candida* negative (<10^3 cfu/ml)	Total	p
Contraception				
OCS	6 (75)	2 (25)	8	0.375
IUD	37 (45.1)	45 (54.9)	82	
Condom	10 (47.6)	11 (52.4)	21	
Other	47 (52.8)	42 (47.2)	89	
Personal allergic history				
Yes	12 (75)	4 (25)	16	0.037*
No	88 (47.8)	96 (52.2)	184	
Pregnancy				
Normal delivery	41 (46.1)	48 (53.9)	89	0.546
Caesarean section	28 (50.9)	27 (49.1)	55	
No birth	31 (55.4)	25 (44.6)	56	
Gravidity				
0	31 (55.4)	27 (46.6)	58	0.518
1	22 (53.7)	19 (46.3)	41	
2	24 (40.7)	35 (59.3)	59	
3	18 (58.1)	13 (41.9)	31	
4	2 (33.3)	4 (66.7)	6	
5	3 (60)	2 (40)	5	
Abortion				
0	80 (48.2)	86 (51.8)	166	0.313
1	12 (57.1)	9 (42.9)	21	
2	7 (77.8)	2 (22.2)	9	
3	1 (33.3)	2 (66.7)	3	
4	0 (0)	1 (100)	1	
Number of partner				
0	2 (40)	3 (60)	5	0.549
1	97 (50)	97 (50)	194	
2	1 (100)	0 (0)	1	
Complaint				
Vaginal discharge				
Yes	82 (55.4)	66 (44.6)	148	0.01*
No	18 (34.6)	34 (65.4)	52	
Vaginal pruritus				
Yes	35 (62.5)	21 (67.5)	56	0.27*
No	65 (45.1)	79 (54.9)	144	
Burning pain				
Yes	15 (83.3)	3 (16.7)	18	0.003*
No	85 (46.7)	97 (53.3)	182	
Inguinal pain				
Yes	23 (62.2)	14 (47.2)	37	0.101
No	77 (47.2)	86 (52.8)	163	
Erythema				
Yes	10 (71.4)	4 (28.6)	14	0.96
No	90 (48.4)	96 (51.6)	186	

Variables	Candida positive (>10^3 cfu/ml)	Candida negative (<10^3 cfu/ml)	Total	*p*
Thyroidism				
Hypothyroidism				
Yes	27 (50.9)	26 (49.7)	53	0.873
No	73 (49.1)	74 (50.3)	147	
Hyperthyroidism				
Yes	11 (73.3)	4 (26.7)	15	0.06
No	89 (48.1)	96 (51.9)	185	
Other chronic diseases				
Yes	10 (43.5)	13 (56.5)	23	0.506
No	90 (50.8)	87 (49.2)	177	
Renal failure				
Yes	0 (0)	1 (100)	1	0.316
No	100 (50.3)	99 (49.7)	199	
Lactation				
Yes	20 (62.5)	12 (37.5)	32	0.123
No	80 (47.6)	88 (52.4)	168	
Vaginal douching				
Once a day	48 (44.4)	60 (55.6)	108	0.131
Twice a day	18 (52.9)	16 (47.1)	34	
Three times a day	24 (66.7)	12 (33.3)	36	
Four times a day	5 (62.5)	3 (37.5)	8	
Five times a day	5 (35.7)	9 (64.3)	14	
Menopause				
Yes	8 (57.1)	6 (42.9)	14	0.579
No	92 (49.5)	94 (50.5)	186	
Antibiotic use in the last 4 weeks				
Yes	44 (55)	36 (45)	80	0.248
No	56 (46.7)	64 (53.3)	120	
Use of systemic steroid in the last 4 weeks				
Yes	0 (0)	1 (100)	1	0.316
No	100 (50.3)	99 (49.7)	199	
Surgical history				
Yes	37 (47.4)	41 (52.6)	78	0.562
No	63 (51.6)	59 (48.4)	122	
Perineal laceration				
Yes	23 (59)	16 (41)	14	0.212
No	77 (47.8)	84 (52.2)	186	
Vaginitis frequency				
1-4 times a year	95 (49.2)	98 (50.8)	193	0.248
More than 4 times a year	5 (71.4)	2 (28.6)	7	
Circumcision of partner				
Yes	99 (50.8)	96 (49.2)	14	0.174
No	1 (20)	4 (80)	186	

Table 2. Basic clinical characteristics of the women included in the study.

Variables	*Candida* positive (>10^3 cfu/ml)	*Candida* negative (<10^3 cfu/ml)	Total	*p*
Age				
18-29	44 (51.2)	42 (48.8)	86	0.214
30-39	41 (55.4)	33 (44.6)	84	
40-49	9 (32.1)	19 (67.9)	28	
50 >	6 (50)	6 (50)	12	
Marital status				
Married	88 (48.9)	92 (51.1)	180	0.315
Single	10 (55.6)	8 (44.4)	18	
Widowed-Divorced	2 (100)	0 (0)	2	
Education status				
Primary school	14 (56)	11 (44)	25	0.509
High school	31 (43.7)	40 (56.3)	71	
University	54 (53.5)	47 (46.5)	101	
Graduate	1 (33.3)	2 (66.7)	3	
Occupation				
House wife	55 (47.4)	61 (52.6)	116	0.470
Employee	42 (52.5)	38 (47.5)	80	
Student	3 (75)	1 (25)	4	
Tobacco use				
Yes	15 (53.6)	13 (46.4)	28	0.684
No	85 (49.4)	87 (50.6)	172	
Alcohol use				
Yes	2 (50)	2 (50)	4	0.999
No	98 (50)	98 (50)	196	
Common uses				
Toilets				
Yes	38 (50.7)	37 (49.3)	75	0.884
No	62 (49.6)	63 (50.4)	125	
Epilation center				
Yes	15 (53.6)	13 (46.4)	28	0.684
No	85 (49.4)	87 (50.6)	172	
Sauna				
Yes	2 (20)	8 (80)	10	0.52
No	98 (51.6)	92 (48.4)	190	
Turkish bath				
Yes	58 (55.8)	46 (44.2)	104	0.89
No	42 (43.8)	54 (56.2)	96	
Pad and underwear usage				
Menstrual pad	16 (38.1)	26 (61.9)	42	0.110
Daily pad	33 (47.8)	36 (52.2)	69	
Cotton underwear	51 (57.3)	38 (42.7)	89	

Table 3. Analysis of predictive factors for *Candida vaginitis* using multivariate logistic analyses.

Predictores	Multivariate analysis		
	OR	*p*	%95 CI
Vaginal discharge	3.365	0.001	1.595-7.101
Vaginal pruritus	1.469	0.27	0.753-2.866
Burning pain	9.098	0.002	2.284-36.232
Personal allergic history	3.396	0.05	0.968-11.910

When virulence factors of the detected isolates were examined, proteinase activity in 52, phospholipase activity in 38, biofilm activity in 79 and hemolytic activity in 95 of *Candida* species were detected. It was found that none of the *C. glabrata*, *C. kefyr* and *C. krusei* strains produced phospholipase. Proteinase, phospholipase, biofilm forming, and hemolytic activity properties of all isolates are shown in Table 4.

Amphotericin and caspofungin showed good activity against five *Candida* species. Resistance to fluconazole was detected in 15.8% of *C. albicans* and 26.9% of *C. glabrata* isolates. Resistance to voriconazole was detected in 7.1% of *C. albicans* isolates. Caspofungin resistance was detected in 5.3% of *C. albicans* and 7.7% of *C. glabrata* isolates. 37 of the *C. albicans*, 22 of the *C. glabrata*, 6 of the *C. kefyr* and 3 of the *C.*

Fig. 1. Phylogenetic tree representing the placement of the identified *Candida* species.

Table 4. Virulence activities of *Candida* species.

	Proteinase				Phospholipase				Hemolysis			Biofilm		
	Negative	2+	3+	4+	Negative	2+	3+	4+	α	β	g	Negative	Weak	Moderate	Strong
C. albicans (57)	13	10	16	18	22	1	10	24	11	43	3	10	38	7	2
C. glabrata (26)	23	1	1	1	26	0	0	0	10	16	0	6	14	2	0
C. kefyr (12)	11	0	1	0	9	0	0	3	5	5	2	2	7	1	2
C. krusei (3)	0	0	0	0	3	0	0	0	3	0	0	3	0	0	0
C. dubliniensis (2)	1	1	0	0	2	0	0	0	1	1	0	0	1	0	1

krusei isolates were evaluated as wild strains according to the epidemiological cut-off values of ketoconazole. 18 strains of *C. albicans*, 2 strains of *C. glabrata*, 6 strains of *C. kefyr* and 2 strains of *C. krusei* were evaluated as wild strains according to the epidemiological cut-off values of posaconazole. *C. dubliniensis* strains were evaluated as non-wild strains according to the epidemiological cut-off values of amphotericin, ketoconazole, and fluconazole (Table 5).

Table 5. Antifungal susceptibility rates of *Candida* species.

Activities of antifungal agents by species	MIC Range	MIC_{50}	MIC_{90}	Sensitive n (%)	Sensitive Dose Dependent n (%)	Intermediate n (%)	Resistant n (%)	Wild Type n (%)	Non-Wild Type n (%)
C. albicans (n=57)									
Amphotericin B	0.03-1	0.25	1	57 (100)	-	-	0	-	-
Ketoconazole	0.03-4	0.5	4	-	-	-	-	37	20
Fluconazole	0.125-64	2	16	39 (68.4)	9 (15.8)	-	9 (15,8)	-	-
Posaconazole	0.03-2	0.5	2	-	-	-	-	39	18
Voriconazole	0.03-1	0.125	0.5	43 (75.4)	-	10 (17.5)	4 (7,1)	-	-
Caspofungin	0.015-4	0.015	0.015	54 (94.7)	-	-	3 (5,3)	-	-
C. glabrata (n=26)									
Amphotericin B	0.03-1	0.25	0.5	26 (100)	-	-	0	-	-
Ketoconazole	0.03-2	0.5	1	-	-	-	-	22	4
Fluconazole	0.25-64	8	32	19 (73.1)	-	-	7 (26.9)	-	-
Posaconazole	0.03-2	0.5	2	-	-	-	-	24	2
Voriconazole	0.03-1	0.125	0.5	-	-	-	-	22	4
Caspofungin	0.015-4	0.015	0.015	24 (92.3)	-	-	2 (7.7)	-	-
C. kefyr (n=12)									
Amphotericin B	0.25-1	0.25	0.5	12 (100)	-	-	0	-	-
Ketoconazole	0.03-4	0.5	2	-	-	-	-	2	10
Fluconazole	0.25-16	2	16	-	-	-	-	8	8
Posaconazole	0.03-2	0.25	1	-	-	-	-	6	6
Voriconazole	0.03-1	0.125	1	-	-	-	-	9	3
Caspofungin	0.015-4	0.015	0.015	-	-	-	-	10	2
C. krusei (n=3)									
Amphotericin B	0.125-0.25	0.125	0.125	3 (100)	-	-	0	-	-
Ketoconazole	0.5-2	0.5	0.5	-	-	-	-	3	0
Fluconazole	0.25-4	0.25	0.5	-	-	-	-	-	-
Posaconazole	0.125-0.5	0.25	0.25	-	-	-	-	2	1
Voriconazole	0,25-0,5	0.25	0.25	3 (100)	-	-	0	3	0
Caspofungin	0,015-0,015	0.015	0.015	-	-	-	-	3	0
C. dubliniensis (n=2)									
Amphotericin B	0.25-0.5	-	-	-	-	-	-	-	2
Ketoconazole	0.5-1	-	-	-	-	-	-	0	2
Fluconazole	0.5-2	-	-	-	-	-	-	0	2
Posaconazole	0.06-0.5	-	-	-	-	-	-	-	-
Voriconazole	0.06-0.125	-	-	-	-	-	-	-	-
Caspofungin	0.015-0.015	-	-	-	-	-	-	-	-

Discussion

VVC is caused by the pathogenesis of *Candida* species in the genital tract mucosa and its incidence has increased significantly in recent years (2). The most common *Candida* species associated with VVC are *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* and *Saccharomyces cerevisiae* may also cause infection. Most of these mixed infections are due to the association between *C. albicans* and *C. glabrata* (2). In the present study, 57% of *C. albicans* and 26% of *C. glabrata* were isolated. These data were found to be consistent with the results of all studies reporting the prevalence of *C. albicans* varying between 33.3% and 97% (22,23). Recurrent VVC (RVVC) was encountered in 5% of cases (5), when *C. glabrata* was most frequently isolated in 3% of cases. The second species responsible of RVVC was *C. aibicans* in 2% of cases. *C. glabrata* was the most common type of NAC in the current study. According to most reports, *C. glabrata* is the most common type of NAC, accounting for half to two-thirds of NAC vaginitis (24,25).

In the present study, the highest rate of VVC was seen in women aged 18-29, a finding consistent with several studies (23, 24, 26). The underlying mechanism is thought to be through sexual activity and increasing amounts of estrogen produced in this age group, which encourages yeast adhesion and penetration into the vaginal mucosa (8).

In multivariate analysis, the first risk factor was that patients with vaginal discharge who were more prone to vaginal candidiasis (adjusted OR: 3.365, 95% CI: 1.595-7.101). Some authors reported that vaginal discharge is a risk factor in VVC cases (26, 27). The second risk factor was vaginal pruritus (adjusted OR: 9.098, 95% CI: 2.284-36.232), and like with other authors, a correlation was found between VVC and vaginal itching in the present study (26, 28). In contrast, some studies have failed to show a link between VVC formation and vaginal pruritus (29-31). The third risk factor was found to be personal allergic history (adjusted OR: 3.396, 95% CI: 0.968-11.910). Only 1% of these patients reported using oral contraceptive pills. However, in the present study, as in many studies, no relationship was found between personal allergic history and VVC (29-31).

In the present study, proteinase activity was found positive in 49 (49%) of 100 *Candida* strains isolated. Proteinase positivity was observed in 44 (77.2%) of 57 *C. albicans* strains and 5 (11.6%) of 43 non-albicans *Candida* strains. Yamamoto et al. (32) examined 222 *Candida* strains and found that all 134 *C. albicans* strains (100%) and 24 (27.2%) of 88 non-albicans *Candida* strains secreted proteinase. Fotedar, Al-Hedaithy (33) detected proteinase activity in 41 (79%) of 52 *C. albicans* strains and 28 (32%) of 87 non-albicans *Candida*.

In this study, phospholipase activity was detected in 35 (61.4%) of 57 *C. albicans* strains and 3 (6.4%) of 43 non-albicans *Candida* strains. Fotedar and Al-Hedaithy (33) detected phospholipase activity in all 52 *C. albicans* strains (100%), while no phospholipase activity was detected in any of the 42 non-albicans *Candida* strains. Udayalaxmi and D'Souza (34) found phospholipase activity in 21 (52.5%) of 40 *C. albicans* strains isolated from genitourinary system samples and 5 (11.9%) of 42 non-albicans *Candida* strains.

In the present study, 43 (75.4%) of the *C. albicans* strains and 22 (46.8%) of the non-albicans *Candida* strains were found to exhibit beta-hemolytic activity. Luo et al. (16) reported that *C. albicans* strains exhibited beta-hemolysis in hemolytic activity studies. Yenişehirli et al. (17) reported that 147 *C. albicans* strains isolated from various clinical samples exhibited beta hemolysis.

In the present study, biofilm activity of *C. albicans* (82.4%) strains was higher than non-albicans strains (68%). Some studies have reported that *C. albicans* strains have higher biofilm acti-

vity than non-albicans strains (16, 35). However, there are also studies reporting higher biofilm activity of non-albicans *Candida* strains (36,37). Evaluation of susceptibility to *in vitro* vaginal *Candida* isolates is important because of the increased recovery of isolates that show natural or acquired resistance to antifungals. In the present study, microdilution method was used to determine the susceptibility of *Candida* strains isolated from vaginal samples to amphotericin B, ketoconazole, fluconazole, voriconazole, posaconazole, and caspofungin and MIC values of these antifungals were determined.

In the present study, all *Candida* strains were determined to be susceptible to amphotericin B. Kalkanci et al. (38) did not detect resistance to amphotericin B in any *Candida* strain isolated from patients with vaginitis. Gamarra et al. (39) investigated the antifungal susceptibility of yeasts that cause vulvovaginitis, and they found no amphotericin B resistant strains (0.04-0.12 μg/mL).

In the present study, 39 (68.4%) of the *C. albicans* strains were susceptible to fluconazole, 9 (15.8%) were dose-dependent susceptible, and 9 (15.8%) were resistant. It was determined that 7 (26.9%) of the *C. glabrata* strains were resistant, 8 (75%) of the *C. kefyr* and 2 (100%) of the *C. dubliniensis* strains were not wild type. Seneviratne et al. (37) found fluconazole resistance as 31.7% in all strains and reported that 40% of *C. albicans* were resistant. Different data are available in the literature regarding fluconazole resistance. Some researchers stated that the rate of resistance to this antifungal agent was 46.4% (40). In another study, it was reported that the species with the highest resistance was *C. albicans* (41). In this study, the mean MIC of *Candida* species for fluconazole was found between 0.5 and 64, and resistance was higher in non-albicans species.

In our study, 36 (36%) of *Candida* strains for ketoconazole were non-wild type. Chong et al. (42) found that 87.5% of isolates that cause vaginitis were resistant to ketoconazole. In another study investigating the resistance of three *C. albicans*, three *C. glabrata* strains and one *Candida spp.*, it was reported that the susceptibility of these strains to ketoconazole was 96.9% (43).

In the present study, 43 (75.4%) of the *C. albicans* strains were susceptible to voriconazole, 10 (17.5%) were moderately susceptible, and 4 (7.1%) were resistant. Pesewu et al. (44) reported that 47 (71%) of the *C. albicans* species were resistant to voriconazole. They found 49 (83%) of the *C. glabrata* and 3 (10%) of the *C. krusei* and 18 (73%) of the *C. tropicalis* species to be non-wild type (MIC > ECV). In addition, a moderate sensitivity to 1.1% voriconazole was determined in *C. parapsilosis* isolates. The rate of resistance to voriconazole was investigated by Fothergill et al. (45) who reported that resistance rates to voriconazole increased in all *Candida* species compared to previous resistance rates, and this increase was statistically significant, especially in *C. glabrata* species (previous resistance rate: 6.1%, new resistance rate: 18.4%, $p < 0.0001$).

In the present study, it was determined that 18 (31.6%) *Candida*, 2 (7.7%) *C. kefyr* and 6 (50%) *C. kefyr* strains were not wild type for posaconazole. In one study, all *C. albicans*, *C. glabrata* and *C. kefyr* strains, 3.5% of *C. parapsilosis* strains and 1.9% *of C. krusei* strains were determined as wild type for posaconazole (46). In another study, 11 (32%) of the *C. albicans* strains, 5 (12%) of the *C. glabrata* strains and 5 (10%) of the *C. krusei* strains were determined to be wild type (47).

In the current study, 54 (94.7%) of the *C. albicans* strains were susceptible to caspofungin, 3 (5.3%) were resistant, 24 (92.3%) of the *C. glabrata* strains were susceptible and 2 (7%, 7) were resistant. Etiz et al. (48) found that 16 (20%) of *C. albicans*, 5 (3%) of *C. tropicalis* and 8 (5%) of *C. glabrata* strains were resistant to caspofungin among the non-albicans species evaluated.

Gültekin et al. (14) reported that all 46 *Candida* spp. strains were susceptible to caspofungin. In a study by Pfaller et al. (49) the rate of resistance to caspofungin was 12.5% in *C. krusei*.

Limitations of the study

The most important limiting factor regarding the results obtained is that the data were collected through a tertiary healthcare institution, thus the sampling was small. Therefore, it is not possible to generalize the results of the study to the whole universe. In addition, the composition of *Candida* species that infect women in each geographic region may also vary, as the sociodemographic characteristics of the study area and consequently potential risk factors may differ. Despite these limitations, this study in women of reproductive age in Turkey provide significant information about the patterns and risk factors of VVC infection. According to the results, it was concluded that more studies should be done on *C. albicans* and NAC.

Conclusion

Considering the ages of the patients in our study, it was observed that VVC affected women of reproductive age more frequently. *C. albicans* is the most dominant species in patients with VVC, followed by *C. glabrata*. In the current study, it was found that discharge, burning complaints, and allergies were associated with the development of VVC. In addition to proteinase and phospholipase enzymes, which are considered to be important virulence factors of *C. albicans*, beta-hemolysis and the ability to produce biofilms may also play a role in pathogenesis. In antifungal susceptibility tests, it was determined that there was a little increase in the rate of resistance to fluconazole among *Candida* strains. Since the epidemiology of vulvovaginal candidiasis changes over time and varies with geographic locations, it is thought that continuous monitoring of changes in the prevalence of *Candida* species and susceptibility rates is required to guide empirical treatment. Studies on VVC will provide a better understanding of this infection and contribute to the determination of new targets for more effective therapeutic approaches to *Candida* strains that are the causative agents of VVC.

Authors' contribution

S.T. (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing –review & editing);
I.H.K (Supervision; Validation; Visualization; Writing – original draft; Writing –review & editing);
J.E.H. (Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Visualization);
E.G.M. (Conceptualization; Data curation; Formal analysis; Investigation);
F.E.T. (Data curation; Formal analysis; Investigation; Methodology; Resources);
I.D.K. (Supervision; Validation; Visualization; Writing – original draft; Writing –review & editing)

Conflict of interests

None to declare.

Acknowledgments

The authors would like to thank to Scientific Research Council of Gaziantep University, for the financial support (Project No: FEF.DT.19.16).

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