



Previous Antibiotic Use and Its Influence on Antifungal Susceptibility of Candida Strains

Candida Türlerinin Antifungal Duyarlılığında Önceki Antibiyotik Kullanımının Önemi

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ABSTRACT

Aim: Antifungal drug resistance of Candida strains is a cause of recurrent or persistent vulvovaginal candidiasis (VVC). In order to prevent patient discomfort and to diminish the treatment cost of the disease, it is essential to identify the causative strain, determine the drug resistance status, and understand the mechanism of resistance.

Material and Method: Vaginal discharge specimens were collected from 300 patients. Sixty-five Candida albicans and thirty-five non-albicans species were identified. The susceptibility to voriconazole, fluconazole, and amphotericin-B was examined. Previous antibiotic use and its correlation with the antifungal drug resistance were studied.

Results: Fluconazole, voriconazole and amphotericin-B susceptibilities of Candida albicans strains were 92.3%, 86.2%, and 100% respectively. The non-albicans group's susceptibilities to these antifungals were 45.7%, 22.9%, and 85.7% respectively ($p=0.00$ for all). After nitroimidazole use, 92.8% of infections were caused by Candida albicans, and 7.2% of them non-albicans strains ($p=0.01$). All the infections occurred after macrolide use was non-albicans infections (100%). There were no detected candida albicans infections ($p=0.01$).

Conclusion: Since resistance to voriconazole, fluconazole, and amphotericin-B was more frequent in the non-albicans group, physicians should suspect from non-albicans strains for treatment-resistant VVC cases. Previous antibiotic use have not increased the amphotericin B, fluconazole and voriconazole resistance of both candida albicans and non-albicans strains. Recurrent infections which have no response to amphotericin B, fluconazole or voriconazole treatment may be due to a different mechanism other than antifungal resistance of Candida species. The decreased amount of lactobacilli in vaginal flora might be the reason for recurrent VVC.

Key words: vulvovaginal candidiasis; antifungal drug resistance; candidiasis management

ÖZET

Amaç: Candida suşlarının antifungal ilaç direnci, tekrarlayan veya inatçı vulvovajinal kandidiyazın (VVC) bir nedenidir. Hastanın rahat-sızlığını ve tekrarlayan VVC'nin ekonomik maliyetini önlemek için, nedensel suşu belirlemek, ilaç direnci durumunu belirlemek ve direnç mekanizmasını anlamak önemlidir.

Materyal ve Metot: Vajinal akıntı örnekleri 300 hastadan toplanmış, 65 Candida albicans ve 35 non albicans türü tespit edilmiş, vorikonazol, flukonazol ve amfoterisin B'ye duyarlılık araştırılmıştır. Önceki antibiyotik kullanımı ve suşların antifungal ilaç direnci ile ilişkisi araştırılmıştır.

Bulgular: Candida albicans suşlarının flukonazol, vorikonazol ve amfoterisin B duyarlılıkları %92,3, %86,2 ve %100 iken, non-albicans grubunun bu antifungallere olan duyarlılıkları %45,7, %22,9 ve %85,7 idi (hepsi için $p=0,00$). Nitroimidazol kullanımından sonra, enfeksiyonların %92,8'ine candida albicans, %7,2'sinin non-albicans suşları neden olmuştur ($p=0,01$). Makrolid kullanımından sonra ortaya çıkan tüm enfeksiyonların tamamında non-albicans türleri enfeksiyona neden olurken (%100) ve candida albicans nedenli enfeksiyon saptanmadı ($p=0,01$).

Sonuç: Non-albicans grubunda vorikonazole, flukonazol ve amfoterisin B direnci daha sık olduğu için olduğu için, tedaviye dirençli VVC olgularında klinisyenler non albicans suşlarından şüphelenmelidir. Önceki antibiyotik kullanımı, hem candida albicans hem de albicans olmayan suşların amfoterisin B, flukonazol ve vorikonazol direncini arttırmamaktadır. Amfoterisin B, flukonazol veya vorikonazol tedavisine cevap vermeyen tekrarlayan enfeksiyonlar, candida türlerinin antifungal direncinden farklı bir mekanizmaya bağlı olabilir. Vajinal florada azalmış laktobasil miktarı rekürren VVC'nin nedeni olabilir.

Anahtar kelimeler: vulvovajinal kandidiyazis; antifungal ilaç direnci; kandidiyazis tedavisi

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Introduction

Vulvovaginal candidiasis (VVC) which is characterized by vulvovaginal itching and irritation, thick vaginal discharge, edema on the vulvar region, vulvar swelling and external dysuria elucidates one-third of total vaginitis cases, and it is the second most common cause of vaginitis after bacterial vaginosis^{1,2}. *Candida albicans* is the causative microorganism for approximately 90% of vaginal candidiasis, and the remaining 10% occurs as a consequence of colonization with *Candida glabrata*, *Candida parapsilosis*, or other uncommon non-*albicans* species³. Predisposing factors for VVC include diabetes mellitus, altered immune system function, increased estrogen levels, and antibiotic use⁴. The relationship between previous antibiotic use and VVC could be explained via bactericide effect of antibiotics on lactobacillus species in the vaginal ecosystem. Lactobacilli which produce lactic acid and maintain the acidity of the vagina (pH: 4–4.5) are protective bacteria against vulvovaginal infections through their ability to produce peroxidase which further stimulates vaginal epithelial cells to produce antimicrobial peptides^{5,6}. It is revealed that *Lactobacillus* species are susceptible to most of the antibiotic groups such as nitroimidazoles, B lactams, macrolides, fluoroquinolones, or aminoglycosides⁷. Broad spectrum antibiotic use decreases the number of *Lactobacillus* species and increases the risk of VVC development⁸.

Candida vaginitis is divided into two groups named as uncomplicated and complicated based on their response to antifungal drug therapy. 80–90% of uncomplicated cases go under resolution after treatment while complicated infections which are characterized by four or more episodes of disease per year become chronic symptomatic disease or relapse rapidly after the first attack^{9,10}. Relapsing disease decreases the patients' quality of life, increases the cost of antifungal drug therapy for countries, and causes complications such as risk for miscarriage or newborn colonization in pregnant population^{11,12}. Each *candida* species demonstrate different levels of resistance and susceptibility to different antifungal drugs¹³. In order to decrease the rate of recurrent infections and provide sufficient resolution, it is essential to determine the causative *candida* strains and choose the antifungal drug which that species is susceptible.

In this study, the effects of previous antibiotic use on the susceptibility of *Candida albicans* and

non-*albicans* species to Amphotericin B, Fluconazole and Voriconazole are investigated to establish an effective treatment for *candida* vaginitis.

Material and Method

In these two years of retrospective study, data was conducted at the VM Medical Park Kocaeli Hospital Department of Obstetrics and Gynecology between 2015 and 2017. The local ethical committee approved the study. An informed consent form was signed by each of the patients, and they filled a questionnaire about age, body mass index, systemic diseases, current infections, gravidity, and parity. Age spectrum of 300 Turkish women that were included in this study was between twenty and fifty-three. Vaginal discharge specimens were collected during routine gynecologic examination by patients with vulvovaginitis complaints. Specimens were incubated at the Sabouraud dextrose agar. After the identification of infectious agents, patients were divided into two groups as infected by *Candida albicans* and non-*albicans*.

Susceptibility of *Candida* species isolates to each antifungal agent was determined to utilize sixty-four great AST-YS07 (Biomerieux Inc, Hazelwood, MO) cards. The inoculum suspensions for VITEK 2 (Biomerieux Inc, Hazelwood, MO) were prepared in sterile saline at turbidity equal to 2.⁰ Mcfarland standard, as measured using a DensiCheck instrument (bioMerieux). The standardized fungal suspension was placed in a VITEK 2 cassette along with a sterile polystyrene test tube and the AST-YS07 card containing serial dilutions of each antifungal agent tested. Following loading of the cassette, dilutions of the fungal suspensions and card filling were performed automatically by the VITEK 2 microbial ID/AST test up to the system. The AST-YS07 cards were incubated at 35C for seventeen hours.

Data were analyzed in SPSS 20.0 program. In order to compare the results, it was established the p values of the variants by using independent sample t-test and chi square in SPSS 20.0 program.

Results

Demographic characteristics of the study are indicated in Table 1. When age, body mass index, presence and recurrence of urinary tract infections, the existence of other infections and previous antibiotic use in last three months were considered, p values demonstrate that

there was no significant difference between *Candida albicans* and non-*albicans* groups.

Susceptibility and resistance profiles of *Candida albicans* and non-*albicans* pathogens to the antifungal drugs Fluconazole, Voriconazole, and Amphotericin B were investigated, and results were indicated in Table 2. Since p values determined the significant difference between two groups ($p=0.001, 0.001, 0.001$), susceptibility to fluconazole, voriconazole, and amphotericin B was greater in *Candida albicans* group, and resistance to these antifungal drugs was significantly higher in the non-*albicans* group.

Previously used antibiotics were categorized under seven groups, such as nitroimidazoles, Beta-lactams, tetracyclines, macrolides, aminoglycosides, and fluoroquinolones. Previous use of Beta-lactams, tetracyclines, aminoglycosides, and fluoroquinolones did not affect the distribution of *Candida albicans* and non-*albicans* infections ($p>0.05$) (Table 3). However, *Candida albicans* infections were significantly higher compared to non-*albicans* infection after the use of nitroimidazole antibiotics ($p=0.01$), and non-*albicans* infections were significantly higher after use of macrolide group antibiotics ($p=0.01$).

Effect of previous antibiotic use in the last three months on the susceptibility of *Candida* species to fluconazole, voriconazole and amphotericin B was investigated (Table 4). When fluconazole and voriconazole were considered, there was not any significant difference between antibiotic use present and absent groups ($p=0.09$ and 0.44 , respectively). However, amphotericin B usage eradicated all infections in patients used antibiotics in the last three months ($p=0.04$). It suggests that previous antibiotic use does not affect the fluconazole and voriconazole resistance in *Candida* species.

Discussion

Antifungal drug resistance of *Candida albicans* and non-*albicans* species were discussed by some studies^{14–17}. Fluconazole, itraconazole and amphotericin B resistance of the *Candida* species were searched from patients who had vaginitis symptoms; itraconazole and amphotericin B were effective against all *Candida* strains while fluconazole was not¹⁷. Besides, it is showed that *Candida albicans* strains are more susceptible to fluconazole comparing to non-*albicans* species¹⁵. Some research suggests that antifungal drug resistance is statistically significantly

Table 1. Demographic variables of *Candida albicans* and non *albicans* groups

	Candida albicans n=65	Non-albicans n=35	p value
Age	34.48±6.43	34.00±5.98	0.74
BMI	25.86±4.18	24.70±3.32	0.20
Urinary tract infection	22 (33.8%)	15 (42.8%)	0.42
Recurrent urinary tract infections two or more	10 (15.3%)	8 (22.8%)	0.51
Respiratory tract infections	6 (9.2%)	4 (10.3%)	0.90
Previous antibiotic use in last three months	30 (46.1%)	12 (34.2%)	0.42

BMI: body mass index.

Table 2. Fluconazole, voriconazole, and amphotericin B resistance and susceptibility status of *Candida albicans* and non *albicans* groups

		Candida albicans n=65	Non albicans n=35	p value
Fluconazole	Susceptibility n=76	60 (92.3%)	16 (45.7%)	0.001
	Resistance n=24	5 (7.7%)	19 (54.3%)	
Voriconazole	Susceptibility n=64	56 (86.2%)	8 (22.9%)	0.001
	Resistance n=36	9 (13.8%)	27 (77.1%)	
Amphotericin B	Susceptibility n=95	65 (100%)	30 (85.7%)	0.001
	Resistance n=5	0 (0.0%)	5 (14.3%)	

Table 3. Previous use of nitroimidazoles, Beta lactams, tetracyclines, amphenicoles, macrolides, aminoglycosides, and fluoroquinolones in candida albicans and non albicans groups

	Candida albicans n=65	Non albicans n=35	P value	Total
Nitroimidazole	13 (92.8%)	1 (7.2%)	0.01	14
Beta lactam	20 (76.9%)	6 (23.1%)	0.21	26
Tetracycline	4 (100%)	0 (0%)	0.13	4
Macrolide	0 (0%)	3 (100%)	0.01	3
Aminoglycoside	3 (100%)	0 (0%)	0.19	3
Fluoroquinolone	4 (66.6%)	2 (33.4%)	1	6
Total	44	12		

Table 4. Previous antibiotic use effects on fluconazole, voriconazole and amphotericin B susceptibility in Candida albicans and non albicans strains

		Previous antibiotic use in last three months		p value
		Not present	Present	
Fluconazole	Susceptible	39 (51.3%)	37 (48.7%)	0.09
	Resistant	17 (70.8%)	7 (29.2%)	
Voriconazole	Susceptible	34 (53.1%)	30 (46.9%)	0.44
	Resistant	22 (61.1%)	14 (38.9%)	
Amphotericin B	Susceptible	51 (53.7%)	44 (46.3%)	0.04
	Resistant	5 (100%)	0 (0%)	

higher in non-albicans candida species^{14,16}. Therefore, it is essential to identify the causative pathogen for VVC to provide sufficient treatment and prevent recurrence. For non-albicans species, amphotericin B is a more efficient drug of choice comparing to itraconazole and fluconazole, which are commonly used antifungal agents in clinical practice¹⁶. Our study results also support this literature.

In order to diagnose VVC, although culture is not a required work up, it plays an important role to identify non-albicans strains for effective management of disease in persistent or recurrent infections¹. Non-albicans strains which colonize in vaginal flora after the loss of lactobacilli due to previous antibiotic use are one of the criteria to describe complicated infections¹⁸. As the non-empirical pathogen aided drug therapy is going to decrease recurrence of these complicated infections, identifying the suspected strain for resistant infections after antibiotic use is a useful option to decrease infection frequency.

Correlation between previous antibiotic use and VVC prevalence is essential to understand expectant vaginal yeast infection and choice of treatment. The literature supports that antibiotic use is increasing

the VVC in patients with vaginitis symptoms¹⁹. There are not enough studies in the literature that compares which antibiotic groups are more suspicious for VVC. Only a few data searched a specific antibiotic group about VVC frequency, it is suggested that cephalosporins carry the risk for further VVC, and norfloxacin use for urinary tract infection is not a predisposing factor for VVC^{20,21}. The correlation between previous antibiotic use and antifungal drug resistance development by Candida species is an unenlightened topic.

Our study results support that the effect of previous antibiotic use on antifungal resistance in candida strains is not statistically significant. At that point, recurrent candida infections after antibiotic use may be related to a different mechanism other than antifungal drug resistance, such as changes in vaginal flora with a decrease in lactobacilli amount.

Limitations of this study include an insufficient number of patients, treating patients only with antifungal drugs and not to add lactobacilli increasing agents to treat, and having limited information of patients about risk factors of recurrent VVC such as pregnancy, diabetes mellitus, or immunosuppression.

Since resistance to voriconazole, fluconazole, and amphotericin B was more frequent in the non-albicans group, physicians should suspect from non-albicans strains for treatment-resistant VVC cases. Previous antibiotic use did not affect the fluconazole and voriconazole resistance of both candida albicans and non-albicans strains. Recurrent infections that do not respond fluconazole or voriconazole treatment may be due to a different mechanism other than antifungal resistance of Candida species. Decreased amount of lactobacilli in vaginal flora might be the reason for recurrent VVC.

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