Vaginal and oral use of probiotics as adjunctive therapy to fluconazole in patients with vulvovaginal candidiasis: A clinical trial on Iranian women

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Abstract

Background and Purpose: Vulvovaginal candidiasis is considered the second most prevalent gynecologic infection among women and one of the main reasons for referring to a gynecologist. During recent decades, probiotic usage has been defined as one of the therapeutic regimens for vaginal candidiasis management, but these findings were controversial. The current study was conducted to determine the effect of fluconazole plus vaginal and oral probiotics supplementation on clinical and mycological improvement of vaginal candidiasis concomitant with antifungal susceptibility of Candida species to fluconazole.

Materials and Methods: This double-blind, randomized, placebo-controlled trial was conducted on 76 women with vaginal candidiasis admitted to Naghavi and Imam Reza Gynecology Clinics in Kashan, Central Iran, from July 2017 to March 2020. Patients were diagnosed according to vaginal candidiasis symptoms and positive culture for Candida species. The patients were divided into two groups; one of them received fluconazole plus vaginal and oral probiotics, while the other one received fluconazole with placebo. The clinical and mycological findings were recorded before and after the treatment. In vitro, the fluconazole susceptibility test was determined by the microdilution method according to the Clinical and Laboratory Standards Institute (M27-A3) for the baseline Candida isolates.

Results: Based on the findings, 35 days after the intervention, a significant reduction was reported in vaginal candidiasis symptoms in the probiotics supplementation group. Although probiotics supplementation therapy was a better mycological cure, compared to the fluconazole with the placebo group, this difference was not significant (68.4% vs. 46.9%, P=0.184). Exclusion of resistant and susceptible dose-dependent strain in the regression model demonstrated a significant reduction in positive culture probiotics in the supplementation group.

Conclusion: Oral and vaginal supplementation with probiotics for 4 weeks played a significant role in the elimination of vaginal candidiasis symptoms. Adjustment of clinical and mycological responses with drug resistance patterns of patients could open a promising horizon for probiotics consumption as a complementary treatment.

Keywords: Fluconazole, Probiotic, Vulvovaginal candidiasis

Introduction

Fungi are the main ingredients of the human microbiome [1] and are related to approximately 1.7 billion superficial fungal infections (SFIs) and 1.5 million mortality due to invasive fungal infections [2]. While invasive fungal infections have been the focus of medical mycologists [2], SFIs are sometimes neglected and considered as mild and easily treatable cases. Vulvovaginal candidiasis (VVC) is one of the most prevalent manifestations of SFIs [2, 3], and it is
estimated that nearly 75% of women have a history of vaginal candidiasis during childbearing [4]. More importantly, near to 5% of women have recurrent vulvovaginal candidiasis (RVVC) which is defined as at least four episodes of infection during 1 year, despite antifungal therapy [5].

It has been estimated that approximately 138 million women suffer from RVVC annually, and this number is predicted to increase to 158 million by 2030 [3]. This disease can severely affect the quality of life of patients and impose a significant financial burden on them, which exceeds 14.39 billion USD in developed countries [3]. Since the treatment is costly and time-consuming, in poor communities, where access to physicians and insurance coverage is more limited, the cases are treated by over-the-counter and traditional products.

Overuse of antibiotics, pregnancy, cystic fibrosis, and diabetes are among the predisposing factors for RVVC and VVC [3]. In numerous studies, Candida albicans are indicated as the dominant species (70-90%), while a minority of infections are attributed to non-albicans Candida species, such as C. glabrata, C. tropicalis, C. parapsilosis, C. kefyr, C. guilliermondii, and C. krusei which are naturally more resistant to azoles [6, 7]. Symptoms of VVC include physical complaints, such as vaginal discharge, burning urination, vulvar pruritus, dyspareunia, irritation, and psychological problems that harm sexual desire which could lead to the destruction of marital life [8, 9].

Despite different predisposing factors for VVC, its pathogenesis has been controversial up to now. Vaginal microbial flora plays an important role in improving vaginal health and preventing vaginal infections [10, 11]. Therefore, any disturbance among vaginal microbiota may play a remarkable role in facilitating Candida species growth [12, 13]. The VVC is treated by numerous intravaginal and oral antmycotic agents, but the cure rates are different according to vaginal candidiissis forms (complicated or uncomplicated) that naturally have different predisposing and causative agents [14]. Overall, the success rate for the treatment of acute vaginal candidiasis is approximately 80% and different antifungal agents are equally effective [5].

Fluconazole (Diflucan), as a triazole, is one of the first-line treatments for VVC that act through inhibition of ergosterol synthesis, alteration of cellular membranes, and increase of membrane permeability in Candida species [15, 16]. Fluconazole in both oral and topical form has demonstrated a good efficacy and safety profile for the treatment of candidiasis [16]. C. albicans was reported to be the most common agent of VVC [6, 17]. However, in recent decades, the pattern of Candida species has changed, accordingly, there has been an increase in the role of non-C. albicans Candida species as an etiological agent of VVC. This change has been influenced by the widespread use of broad-spectrum antibiotics and the over-the-counter use of antmycotic agents [18].

The majority of non-C. albicans species have an intrinsic resistance or low susceptibility to some antmycotic agents; therefore, failure of treatment in fungal infection due to non-C. albicans species is not far from expectation [19-22]. According to the theory that any disruption in vaginal microbiota could lead to VVC, different studies were carried out to define a therapeutic and protective role for probiotic lactobacillus [9, 13, 23-25]. The ability of probiotics in maintaining and recovering the normal vaginal microbiota, and their potential ability to resist Candida gave rise to the concept of using probiotics for the treatment of VVC [26].

During the recent decades, probiotics, known as a part of normal human bacterial flora, have been defined as a new strategy for challenging vaginal candidiasis by different routes, including the reduction of intravaginal pH [27], improvement of mucosal defense barrier against yeast cells, improvement of vaginal normal flora, and production of specific molecules, such as bacteriocins, extracellular proteins or hydrogen peroxide [28, 29]. However, the role of probiotics in the treatment of vaginal candidiasis has been controversial up to now. Results of different studies have indicated the beneficial role of probiotics in the treatment of VVC [30, 31].

Numerous studies have been performed to determine the therapeutic role of probiotics; nevertheless, most of them had some limitations which could have influenced their outcomes. These limitations included poor design, unverified VVC, and randomization regardless of uncomplicated and complicated VVC caused by non-albicans Candida species, particularly C. glabrata which are intrinsically resistant to fluconazole. Moreover, some studies did not mention how the episodes of recurrent VVC were identified before their inclusion in the trials. Perhaps they were only diagnosed just through self-diagnosis and vaginal fluid cultures were not obtained [13, 23, 32].

This study was conducted to compare the clinical and microbiological efficacy of treatment with fluconazole and treatment with fluconazole plus oral and vaginal probiotics capsules. Moreover, it aimed to identify the Candida species and their susceptibility to fluconazole for further justification of the efficacy of probiotics in the treatment of vaginal candidiasis.

Materials and Methods

Trial design and participant

This study was a randomized, double-blind, placebo-controlled clinical trial registered in the Iranian registry of the clinical trial (http://www.irct.ir: IRCT2016090529710N1) which was conducted at Naghavi and Imam Reza gynecology clinics affiliated to Kashan University of Medical Sciences (KAUMS), Kashan, Central Iran, from July 2017 to March 2020.

The inclusion criteria were VVC based on 2015 Sexually transmitted Diseases Treatment Guidelines and age range of 18–48 years old [14]. Exclusion criteria were consumption of antifungal drugs
(systemic or intravaginal) within 4 weeks before the intervention, diabetes, allergic reactions to fluconazole and probiotics to both administration routes (vaginal or oral), pregnancy, affliction by other vaginal infections, and usage of antibiotics during the intervention.

**Study design**

All women who were admitted to Naghavi and Imam Reza Gynecology clinics with signs and symptoms of VVC, such as vulvar burning, vulvar itching, abnormal vaginal discharge, and dyspareunia visited by a gynecologist as well as the patients with approved VVC based on laboratory exams were included in this study. The subjects were randomly divided into two groups. One of the groups received fluconazole plus 150-mg vaginal probiotic (Lactovag) supplements, including Lactobacillus acidophilus \( \times 10^9 \), Lactobacillus plantarum \( \times 10^9 \), Lactobacillus rhamnosus \( \times 10^9 \) Lactobacillus gasseri \( \times 10^9 \) colony-forming unit/day for 14 nights and oral probiotic (Lactofem) supplements, including Lactobacillus acidophilus \( \times 10^9 \), Lactobacillus plantarum \( \times 10^9 \), Lactobacillus fermentum \( \times 10^9 \), Lactobacillus gasseri \( \times 10^9 \) colony-forming unit/day for 30 days (n=38). The other one received fluconazole150-mg plus vaginal and oral placebo for 14 nights and 30 days (n=38).

Placebos and probiotic capsules were identical as they had similar colors, shapes, sizes, and packages made by Zist-Takmir Pharmaceutical Company (Tehran, Iran). Patients with recurrent VVC took a 150-mg oral dose of fluconazole every 72 h (three doses in total). All patients were recruited after 30-35 days and were reassessed (clinical and mycological) if their treatment had been completed. Randomization was managed using computer-generated random numbers and randomized allocation was conducted by a trained midwife at a gynecology clinic.

**Clinical assessment**

All women with one or several signs and symptoms, such as abnormal vaginal discharge, vaginal itching, vaginal burning, and dyspareunia which are considered major complaints of vaginitis, and those who were admitted to the Naghavi and Imam Reza gynecology clinics were visited by a gynecologist. All suspected patients that met the inclusion criteria were introduced for registration of demographic and medical information and vaginal sampling. A vaginal sample was taken by two sterile swabs from vaginal secretions for mycological and vaginal pH assessment.

**Sample size determination**

The sample size for this trial was calculated based on a prior study (9). The probiotic-treated group showed significantly less vaginal discharge associated with any of the above-mentioned symptoms of candidiasis and lower positive culture, compared to the placebo group (10.3% vs. 35.6%; \( P = 0.03 \)) (10.3% vs. 38.5%; \( P = 0.014 \)). The sample size was calculated for both symptoms of candidiasis and positive culture but a greater sample size was considered. The type one (\( \alpha \)) and type two errors (\( \beta \)) were determined to be 0.05 and 0.20, respectively. Moreover, 10% for loss to follow-up was added; hence, finally, the sample size was determined 40 subjects per group.

\[
\begin{align*}
\text{Sample size } n &= \left( z_{1-\alpha/2} + z_{1-\beta} \right)^2 \left( p_1(1-p_1) + p_2(1-p_2) \right) \\
&= \frac{1}{2}\left( z_{1-\alpha/2} + z_{1-\beta} \right)^2 \left( p_1 + p_2 \right) \\
&= 100 \left( z_{1-\alpha/2} + z_{1-\beta} \right)^2 \left( p_1 + p_2 \right)
\end{align*}
\]

**Randomization**

Randomization was performed by a simple method and using random numbers generated by computer software (Stat Trek software). In this method, the computer selects random numbers, and randomization and allocation were concealed from the investigators and participants until the final analyses were completed. Randomized allocation was conducted by a trained midwife at a gynecology clinic.

**Mycological assessment**

Vaginal pH was assessed using an impregnated swab obtained from the lateral vaginal wall by a pH indicator strip (Macherey-Nagel, Duren, Germany). Further detections were conducted by direct examination (Gram staining and wet mount preparation) and culture. Bacterial vaginitis was ruled out by Amsel criteria, namely clue cells in the Gram staining smear, pH ≥ 4.5, and positive whiff test. In addition, wet mount preparation ruled out trichomoniasis. The culture was performed by inoculation of the impregnated vaginal swab with vaginal discharge on Sabouraud dextrose agar (Biolfie, Italy) supplemented with chloramphenicol (0.5μg/ml) based on a previous study [33]. *Candida* species were identified using morphological and physiological properties and produced color in CHROM agar *Candida* (bioMérieux, Marcy l’Etoile, France). Subsequently, genomic DNA was extracted from all the test isolates and all the strains were identified by the previously-described polymerase chain reaction-restriction fragment length polymorphism method [34, 35].

**Antifungal assessment**

The antifungal susceptibility testing of fluconazole was assessed by the microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI, documentM27-A3) [36].

**Outcomes**

Clinical symptoms, including vaginal discharge, vaginal burning, vaginal itching, and dyspareunia, were considered the primary outcomes, and fungal culture was considered the secondary outcomes.

**Ethical statement**

The study protocol was approved by Research Ethics Committee, KAUMS, Iran. Written, informed consent for vaginal sampling and subsequent treatments were taken from all participants before
Results
In total, 70 patients (probiotic [n=32] and placebo [n=38]) completed the trial. In this study, the compliance was noticeable, although 10 patients (intervention: 8, control: 2) did not complete the trial. No adverse effects were observed in patients with VVC following probiotic supplementation. There were no significant differences between the two groups in terms of social demographic information, sexual activity, and type of contraception methods (Opicon One-Step, intrauterine device, male condom, and natural methods) at the baseline of the trial (Table 1).

Vaginal symptoms, such as abnormal vaginal discharge, vaginal itching, vaginal burning, dyspareunia, and pH were not statistically different between the two groups at the baseline of the study. Therefore, the signs and complaints between the two groups were similar. Rates of infection with vaginal candidiasis due to non-albicans Candida species among the probiotic supplementation and the placebo group were 21.1% and 21.8%, respectively, which had no significant difference at the baseline of the study. Moreover, the rate of resistance and dose-dependent susceptible strains between the two groups had no significant difference (P=0.181) (Table 1).

It should be mentioned that 35 days after the intervention, vaginal complaints and negative culture (clinical and mycological improvement) increased in both groups. Furthermore, there was a significant difference between the groups before and after the treatment. Moreover, only the patients in the intervention group had a significant difference (P=0.021) in terms of the decrease in pH (<4.5) (Table 2).

The logistic regression test indicated a significant difference (P<0.05) in burning, discharge, and itching in the probiotic supplementation group, and the odds ratios for these complaints were 6.21, 7.38, and 13.82, respectively. However, dyspareunia and pH < 4.5 were not significantly different between these two groups (P>0.05) (Table 2).

Mycological cure (negative culture) between the two groups did not have a significant difference (P=0.184). However, the mycological cure (68.4%) in the probiotic supplementation group was higher than in the placebo group. The difference between the ratios of albicans/non-albicans and VVC/RVVC in the two groups was not significant in terms of mycological response, which has not been evaluated in other studies (Table 2).

Table 1. Baseline demographic characteristics of study groups

| Variables         | Fluconazole and probiotic (n=38) | Fluconazole and placebo (n=32) | P-value |
|-------------------|----------------------------------|--------------------------------|---------|
| Age (year)        | 34.6±4.15                        | 33.7±4.13                      | 0.389   |
| Relapse (year)    | 3.42±1.88                        | 3.44±2.09                      | 0.928   |
| Sexual activity (week) | 1.84±0.86                     | 2.31±1.35                      | 0.214   |
| Occupation Employed (%) | 2 (5.3)                      | 4 (11.5)                       | 0.402   |
| Unemployed (%)    | 36 (94.7)                        | 28 (87.5)                      |         |
| Contraceptive method |                                  |                                |         |
| OCP (%)           | 3 (7.9)                          | 0 (0)                          |         |
| IUD (%)           | 15 (39.5)                        | 20 (62.5)                      |         |
| TL (%)            | 2 (5.3)                          | 0 (0)                          | 0.280   |
| Male Condom (%)   | 12 (31.6)                        | 7 (21.9)                       |         |
| Natural Methods (%) | 4 (10.5)                    | 2 (6.3)                        |         |
| Not at risk (%)   | 1 (2.6)                          | 2 (6.3)                        |         |
| Antifungal susceptibility | susceptible≤8µg/ml | Dose-dependent susceptible 16-32 8µg/ml Resistance≥64 |
| Susceptible (%)   | 30 (78.9)                        | 29 (90.6)                      | 0.181   |

Table 2. Clinical and mycological outcomes of study groups seven days after treatment.

| Complaints          | Fluconazole and probiotic (n=38) | Fluconazole and placebo (n=32) | P-value** | OR |
|---------------------|----------------------------------|--------------------------------|-----------|----|
|                     | Before treatment | After treatment | Before treatment | After treatment |                     |                     |
| Vulvar burning      | No | %     | No | %     | No | %     | No | %     | P=0.001* | P=0.001* | (0.011) | 6.21 |
| Vaginal discharge   | 36 | 94.7 | 7 | 18.4 | 3 | 96.1 | 20 | 62.5 | P=0.002* | P=0.001* | (0.000) | 7.38 |
| Vulvar itching      | 31 | 81.6 | 2 | 5.3 | 28 | 87.5 | 14 | 43.8 | P=0.001* | P=0.001* | (0.001) | 13.82 |
| Dyspareunia         | 19 | 75.0 | 4 | 10.5 | 18 | 56.5 | 6 | 18.8 | P=0.001* | P=0.001* | (0.394) | 6.21 |
| pH<4.5             | 10 | 26.3 | 20 | 52.8 | 12 | 40 | 14 | 45.2 | P=0.021* | P=0.015* | (0.527) | 1.36 |
| Culture             | 38 | 100 | 13 | 31.6 | 32 | 100 | 16 | 53.1 | P=0.001* | P=0.001* | (0.184) | 1.92 |
| C. albicans        | 30 | 78.9 | 6 | 46.2 | 25 | 78.1 | 9 | 56.2 | P=0.001* | P=0.001* | (0.181) | 3.658 |
| C. non-albicans    | 8 | 21.1 | 7 | 53.8 | 7 | 21.8 | 7 | 43.8 | P=0.062* | P=1.00* | (0.814) | 1.92 |

* McNemar's test  ** Logistic regression
The minimum inhibitory concentration50 (MIC50) of fluconazole for Candida isolates of the two groups was similar (1 μg/ml). The Logistic regression test did not show a significant difference between the two groups in terms of the frequency of fluconazole susceptible strains in comparison to resistance and dose-dependent susceptible strains (P=0.181).

The results of Fisher's exact test showed a significant difference between the two groups in terms of itching, discharge, and pH ≥ 4.5. Accordingly, the improvement of vaginal symptoms and pH in the probiotic group was more considerable than that in the control group (Table 3). Finally, the adjusted logistic regression model indicated that the infection rate (positive culture) in the control group was higher (odd ratio=2.99) but not significant (P=0.059). When patients with resistance and dose-dependent strains were excluded, positive culture in the control group was higher (OR=4.7) than the probiotic group with a significant difference (P=0.025) (Table 4).

**Discussion**

Results of this study clarified that supplementary treatment with probiotics led to a significant improvement in clinical symptoms of major VVC, such as burning, discharge, and itching. Moreover, mycological cure that was considered negative culture in the probiotic supplementation group was more prevalent in the probiotics group, compared to the control group although the difference between these two groups was insignificant (P=0.184).

In total, 16 patients (41%) with fewer than four episodes during the previous year, who were treated with a single dose of 150 mg fluconazole, had positive culture (44% vs. 56%, P=0.291) in probiotic and placebo groups. However, in a study performed by Martinez et al., the rate of positive culture in patients treated with a single dose of fluconazole was 10.3%, while that of the probiotic and placebo group was 38.5% [9].

The rate of mycological cure for all patients was 58.5%, which was close to the results of a study performed by Sobel et al. (1995) that reported 63% mycological cure after 35 days of treatment with an oral single dose of fluconazole. However, based on their findings, the corresponding rates for the patients with four or more episodes during the past year were 43% and %69 for fewer episodes [37]. Nevertheless, in the present study, positive culture was equal in patients with fewer and more than four episodes during the previous year. This consistency could be justified by the fact that the patients with four or more episodes were considered cases of recurrent VVC; therefore, they were treated with three doses of fluconazole every 72 h.

In a study performed in Iran, Sekhavat et al. reported a remarkable mycological and clinical response (98.6%) to an oral single dose of fluconazole in women with acute VVC [38]. Fluconazole is one of the most commonly prescribed drugs for Candida infections but some Candida species, such as C. glabrata, exhibited a low intrinsic susceptibility to azole derivatives. Moreover, several studies demonstrated the ability of Candida species for developing resistance during exposure to azoles [39, 40]. More importantly, each isolate has a unique susceptibility pattern based on the infection site, and this issue was confirmed by several studies that indicated Candida isolates from oropharyngeal candidiasis were more resistant than candidemia isolates [41, 42]. Badiee et al. (2011) performed a study in the south of Iran and found that C. glabrata strains were more resistant than C. albicans strains (45.5% vs. 10.5%) [43].

According to CLSI document M27-A3 guidelines, Candida species with MICs ≥ 64 μg/ml and MICs 16-32 μg/ml are defined as Candida resistant and dose-dependent, respectively, which could play the role of a confounding factor, while these issues have been ignored in more studies. Therefore, the elimination of possible limitations was conducted by Candida identification and antifungal susceptibility to fluconazole. Moreover, for better colonization, probiotics were administrated both orally and intravaginally, and only patients with positive culture were included in the present study.

Soble et al. (2003) in their study demonstrated that clinical and mycological responses were more prevalent in patients with complicated vaginal candidiasis due to isolates with MICs ≤ 1 μg/ml in the baseline, compared to those of isolates with
MICs > 1µg/ml at 35-day follow-up. Despite higher rates of clinical and mycological cures in patients with MICs \( \leq 1 \mu g/ml \), this difference was not statistically significant [44]. In the present study, MIC50 (1 µg/ml) was similar among the two groups. However, fluconazole resistance and dose-dependent isolates in the placebo group were more than the intervention group (21.1% vs. 9.4%) but there was no significant difference between the two groups in this regard.

The results of this study indicated that clinical response to mycological response significantly improved in both groups. This was not unexpected since both groups underwent fluconazole therapy which is defined as a choice drug with the least toxicity and optimal compliance in patients who have experienced more than one episode of VVC [45, 46].

Vaginal discharge and burning were the most common symptoms in both groups, which is similar to the results of other studies [13, 40]. In a previous study conducted in Kashan, itching was reported as the most common symptom and vaginal discharge was only reported in 34% of patients [33]. Major studies about probiotic supplement therapy have generally focused on bacterial vaginitis and regarded probiotics as a supportive treatment. Therefore, only a few studies have investigated the role of probiotics in the treatment of VVC. Findings of some of these studies have indicated that probiotics are effective for the treatment of vaginal candidiasis [9, 22, 25, 29, 47, 48].

In other studies, the role of probiotics, as a complementary treatment, is not promising and remains a controversial issue [13, 23, 32]. Moreover, the placebo-controlled trial studies about the assessment of fluconazole plus probiotics in vaginal candidiasis are limited [9, 48]. In a randomized double-blind clinical trial, Martinez et al. compared the effectiveness of the administration of a single dose of fluconazole plus probiotic (Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14) and fluconazole plus placebo for the treatment of vaginal candidiasis. According to their findings, concomitant use of probiotics with fluconazole played a significant role in the reduction of vaginal discharge associated with other symptoms, such as itching, burning, and dyspareunia, compared to the control group (10.3% vs. 34.6%; \( P=0.03 \)) and positive culture (10.3% vs. 38.5%; \( P=0.014 \)) [9].

The current study is similar to the one carried out by Martinez et al. as both groups were treated with fluconazole [9]. After identification of Candida species, they found that the prevalence rates of non-albicans species in the probiotic and placebo group were 6.7% and 15.4% respectively. While in the present study, these rates were 21.1% and 21.9% for the probiotic and placebo groups, respectively [9]. However, neither of these studies were influenced by non-albicans species nor had a similar finding in terms of the significant improvement of vaginal symptoms in the probiotic group, compared to the placebo group. The similarity between the two studies, in terms of fluconazole therapy, study populations, and duration of the intervention could be one of the main reasons for the similarity with the study conducted by Martinez et al. [9].

Nouraei et al. [48] in a double-blind clinical trial study showed that vaginal candidiasis treatment with fluconazole plus oral probiotics supplementation in comparison to the probiotic supplementation group had an equal effect on the reduction of complaints and symptoms. However, the probiotic supplementation had better therapeutic effects on elimination and recovery time in vaginal candidiasis. Afrakhte et al., in a double-blind clinical trial study, demonstrated that vaginal candidiasis treatment with clotrimazole and clotrimazole plus probiotics had equal outcomes and positive responses in the two groups (52.5% and 56.3%, respectively) (\( P=0.499 \)). Moreover, in the aforementioned study, among different signs, only improvement of inflammation and redness had a significant difference [13].

In another study, Wagner et al. declared that infections due to C. albicans are correlated with the induction of pro-inflammatory responses in vaginal epithelial cells. However, according to them, estrogen and lactobacilli suppress the expression of NF-κB-related inflammatory genes and modulate the morbidity of C. albicans through modification of cytokine production by vaginal epithelial cells [49]. In their study, Murina et al. noted that fluconazole plus probiotics have a synergistic effect through maintenance of homeostasis and balance in vaginal and intestinal flora [50].

In the current study, the mycological cure was improving among the probiotic supplementation group but this improvement was insignificant. However, when patients with susceptible dose-dependent and resistant strains were excluded, the rate of mycological cure in the probiotics group was higher and had a significant difference while this form of assessment had been neglected in other studies. This exclusion could be one of the major reasons why probiotic supplementation therapy had different outcomes whereas complementary therapy for VVC became controversial in more studies.

**Conclusion**

The results of the current study indicated that the administration of probiotics as a complementary treatment with fluconazole was effective. More specifically, it plays a principal role in the elimination of signs and symptoms of VVC as well as the assessment of antifungal susceptibility of Candida species isolated from vaginal candidiasis besides treatment with probiotics. This could lead to a bright future on the horizon of a complementary treatment with probiotics.

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**Authors’ contribution**
M.N. designed and managed the study. Z.V., P.T., Z.D., R.C.H., and T.F. performed the specimen collection. M.A. performed the antifungal susceptibility tests. M.S. contributed to statistical analysis. M.N. and I.H. prepared the manuscript.

**Conflicts of interest**
The authors declare that there was no conflict of interest in this study.

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