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Comparing the Effect of Probiotic and Fluconazole on Treatment and Recurrence of Vulvovaginal Candidiasis: a Triple-Blinded Randomized Controlled Trial

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Abstract

Vaginitis is a common problem in women. *Candida albicans* is responsible for more than 85% of vaginal fungal infections. The aim of this study was to compare the effects of probiotic and fluconazole on the treatment and recurrence of vulvovaginal candidiasis (VVC). This triple-blinded randomized controlled trial was conducted on 80 married women, aged 18–49 years, with VVC, as confirmed by clinical and laboratory diagnosis. The participants were allocated into two groups using blocked randomization method. The fluconazole-treated group received a single dose of fluconazole (150 mg) supplemented with 30 placebo capsules of probiotic, and the probiotic-treated group got 30 probiotic capsules containing 1×10^9 CFU/g *LA-5* with 1 fluconazole placebo capsule. The samples were taken from patients to evaluate the vaginal pH and microbiological tests before, 30–35 days, and 60–65 days after starting the treatment. The signs and symptoms were assessed before the intervention and the first and second follow-ups. Chi-square, Fisher's exact, independent *t*, and ANCOVA tests were then used for data analysis. There was no statistically significant difference between the two groups ($p = 0.127$) in the frequency of negative culture 30–35 days after starting the treatment, but the frequency of negative culture 60–65 days after starting treatment in the fluconazole group was significantly higher than that of the probiotic group ($p = 0.016$). The abnormal discharge and vulvovaginal erythema in the first and second follow-ups and also pruritus in the second follow-up in the fluconazole group were significantly lower than those in the probiotic group ($p < 0.05$). There was, however, no statistically significant difference in burning, frequent urination, dysuria, and dyspareunia between the groups ($p > 0.05$). *Lactobacillus acidophilus* supplementation had an effect similar to that of fluconazole in treating most symptoms of VVC, but it was less effective than the latter in preventing recurrence.

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Keywords Fluconazole · Probiotic · Vulvovaginal candidiasis

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Abbreviations

VVC Vulvovaginal candidiasis
IRCT Iranian Registry of Clinical Trials
LHLP Larger heat-labile proteins
MAP Membrane-active peptides
SHSL Small heat-stable antibiotics

Background

Vaginitis is a common problem in women. It is one of the most common reasons for women to visit health centers around the world [1]; therefore, it receives about 10 million visits per year [2]. Vaginitis is a general term that refers to the inflammation of the vaginal wall [3], leading to complaints such as itching, burning, and vaginal discharge [4]. The most common infections leading to vaginitis are bacterial vaginosis, vulvovaginal candidiasis, and trichomonas vaginitis [4].

Vulvovaginal candidiasis (VVC) is a fungal infection that affects the vaginal mucosa [5]; it is, in fact, the second most common vaginal infection after bacterial vaginosis, as it is diagnosed in more than 40% of women with vaginal complaints [6]. It affects about 75% of women at least once in their reproductive ages, and half of women get it more than once in their lifetimes [7]. *Candida albicans* is responsible for more than 85% of vaginal fungal infections. The other non-albicans species contributing to vaginal fungal infections include *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* [8]. The prevalence of *Candida* spp. in Tabriz has been reported to be 25.2% [9].

Affected women often complain of symptoms such as vaginal discharge, burning, itching, vulvovaginal erythema, dyspareunia [10], and urinary symptoms such as frequent urination and dysuria [11]. *Candida* vaginitis can cause complications such as pain and discomfort, anxiety, low self-esteem, dysfunction, and sexual problems [12].

There are several antifungal drugs for VVC that can be used orally or intravaginally [13]. Azoles are considered the first line of treatment [14]. Antifungal agents have a wide range of side effects, such as chills, dizziness, neutropenia, thrombocytopenia [15], hepatotoxicity, and gastrointestinal symptoms, such as nausea, vomiting, and neurological symptoms [16, 17]. Extensive and long-term use of oral and topical medications of azoles and other antifungal agents can increase the incidence of VVC caused by non-*albicans Candida* species that are more resistant to treatment [18]. Due to the resistance of some *Candida* spp. to common treatments, as well as the side effects of these drugs, disease recurrence and the high cost of medical treatments [19–21], the use of effective products with fewer side effects has been considered [22].

Probiotics are live microorganisms that, when prescribed in an adequate amount, have health effects on the host [23]. Recently, probiotics such as *Lactobacillus* spp. and *Bifidobacterium* spp. have been considered for the prevention and treatment of VVC [24, 25]. Some studies have reported that VVC is caused by the loss or decrease of some vaginal flora, and lactobacilli could be useful in the prevention of vaginal infections [26, 27]. *Lactobacillus* spp. are the natural microbiota of the human vulva and vagina, playing a key role in suppressing potential pathogens [28]. Probiotics can be administered orally, intravaginally, or in combination [29]. In most studies, probiotics have been used as an adjunctive therapy after treatment with an antifungal drug to increase the effectiveness of the antifungal drug [26, 30, 31]. It is proposed that the metabolites of the gut microbiota and probiotics could travel through the bloodstream to affect the distant sites of such organisms as the vagina, which may change its flora [32]. Free phenol and para-cresol are the bacterial metabolites produced by *Clostridium difficile* after antibiotic treatment [33]. These metabolites can enter the circulation and reduce keratin 10 expression in keratinocytes, as well as disrupting the integrity of the epidermal barrier [34]. Miyazaki et al. [34] demonstrated that skin hydration could be improved by reducing serum total levels of phenol, which could be reduced by the daily administration of prebiotic *galactooligosaccharide* and *Bifidobacterium breve*. In addition, the consumption of lactobacilli could be used to prevent genital infections and improve genital flora [28]. Probiotics used in the prevention and treatment of *Candida* infections include *Limosilactobacillus fermentum* RC-14, *L. fermentum* B-54, *Lacticaseibacillus rhamnosus* GR-1, *L. rhamnosus* GG, and *Lactobacillus acidophilus* [18].

Given the numerous reports on the effect of probiotics against VVC [35, 36], a more effective and newer antifungal drug with probiotic origin is needed. Therefore, this study aimed to evaluate the effect of probiotic capsules with fluconazole capsules on the treatment and improvement of symptoms in women, aged 15–49 years, with VVC. The present research is one of the few studies comparing the effect of probiotics with the antifungal drug on the treatment and recurrence of VVC.

Methods

Type of Study and Participants

Eighteen women with VVC, who had been referred to Al-Zahra and Taleghani teaching hospitals and health centers in Tabriz, Iran, from May 2021 to January 2022, were enrolled in this randomized, triple-blinded (participants, outcome evaluators, and statistical analysts were blind) controlled clinical trial. The study was approved by the

ethics committee of Tabriz University of Medical Sciences (ethical code: IR.TBZMED.REC.1399.848) and registered in the Iranian Registry of Clinical Trials (IRCT code: IRCT20110826007418N5).

Regarding the inclusion criteria, married women, aged 15–49, with positive *Candida* spp. culture result and willingness participated in the study. On the other hand, the exclusion criteria included pregnancy; lactation; menopause; use of antibiotics; immunosuppressive drugs; vaginal medications during the last 2 weeks; autoimmune diseases; chronic diseases such as diabetes, menstrual bleeding, or any abnormal uterine bleeding during the visit of the participants; recurrent vulvovaginal candidiasis (four or more cases during the year); symptoms of drug allergy; and consumption of any probiotic products.

Sampling

The researcher explained the study aims and procedures to the patients; we assessed the participants based on the eligibility criteria and asked them to sign a written informed consent form. To diagnose VVC, two sterile swabs were taken from the posterior fornix of the vagina from eligible individuals. We measured vaginal pH using pH meter paper. The first sample was stretched on a slide. We conducted the Whiff test by adding 1–2 drops of 10% potassium hydroxide to it. The second sample was placed inside the tube containing sterile physiological serum and sent back to the laboratory and cultured in Sabouraud Dextrose Agar (SDA; Merck, Germany) containing 0.05% chloramphenicol (Sigma-Aldrich, USA). The cultured medium was incubated at 37 °C for 18–24 h. The growth colonies were transferred to Chromo agar; after 24 h, the type of colonies was identified. In addition, a stained slide with methylene blue was prepared by a microbiologist for the direct observation of fungal cells, clue cells, and other abnormalities. The patients with positive Wiff test, *Trichomonas vaginitis*, and other non-*Candida* vaginitis were excluded.

Randomization and Intervention

Allocation of individuals in groups was done randomly using a computerized table of random numbers through random blocks of 4 and 6 with an allocation ratio of 1:1. Probiotic supplementations or fluconazoles were prepared in sequentially numbered bottles based on the allocation sequence and delivered to the participants in the order in which they entered the study. The sequence generation and preparation of the bottles were done by a person not involved in the sampling or data collection.

Each bottle contained 30 white capsules and one red capsule; they were divided into two types: the first type contained a red capsule of fluconazole (150 mg) and 30 white capsules of probiotic placebo; the second one had 30 white capsules of probiotic and a red capsule of fluconazole placebo. The probiotic capsules contained 1×10^9 CFU/g *L. acidophilus* LA-5 that had been ordered from Christian Hansen (Hoersholm, Denmark) and potato starch (Shahdine, Iran). The placebo capsules only contained potato starch and were similar in appearance to probiotic supplementation. Fluconazole oral capsules were bought from Amin Pharmaceutical Co (Iran). For the placebo of fluconazole, the capsules were filled with pure potato starch; they were similar to fluconazole capsules in terms of appearance.

The women were asked to use a red capsule on the first day; then, other capsules were used once a day. The participants were advised to keep the drugs in a refrigerator (below 25 °C), take them after lunch, drink a full glass of water before taking the capsules, and not to get any other medications at least 2 h before and after taking them. In addition, they were asked to observe personal hygiene during the treatment period and not use vaginal showers, vaginal creams, herbal medicines, and antibiotics, as well as other medicines.

Outcomes and Data Collection

Follow-up

During the study (days 12 to 15), a telephone call reminded the regular use of medications to investigate any possible side effects. The first and second follow-ups were performed 30–35 days and 60–65 days after starting the treatment. In addition, we took the samples from patients to evaluate the vaginal pH and microbiological tests. We assessed the signs and symptoms for vaginal discharge, itching, burning, inflammation, and vulvovaginal erythema, dysuria, and dyspareunia. The level of patients' satisfaction and side effects was determined. All untreated patients were treated with the routine treatment (clotrimazole) after the second follow-up (60–65 days after starting treatment).

Statistical Analysis

SPSS software, version 20.0 (Chicago, IL, USA), was used for statistical analysis. Pearson chi-square, Mann–Whitney *U*, and Fisher's exact test were then used to compare the qualitative data obtained for the probiotic-treated and fluconazole-treated groups. The independent *t*-test and ANCOVA were also used for the comparison of the groups in terms of vaginal pH before and after intervention, respectively. Also, we conducted repeated measures ANOVA for the vaginal pH and reported results of effects of group, time, and group × time. All analyses were done based on intention-to-treat. Significance was set at p-value < 0.05.

Results

Totally, we randomly assigned 80 patients with vulvovaginal candidiasis who had inclusion criteria to the probiotic or fluconazole groups. Thirty-five to 40 days after starting the treatment, four patients in the probiotic group (two patients due to nausea, one due to stomach problems, and one other due to the lack of cooperation) and seven patients in the fluconazole group (one patient due to nausea and six due to the lack of cooperation) were excluded from the study. However, in the first follow-up, out of seven patients excluded from the fluconazole group, the symptoms of four of them were checked by phone (Fig. 1). In the fluconazole group, seven participants did not attend for examination. One participant refused to take the medicine due to a nausea, one was hospitalized, one was infected with coronaviruses, one participant was not present in Tabriz, and three participants did not answer their phones. In the probiotic group, four participants did not attend for examination. Three participants did this due to a nausea and stomach problem, and one did not answer the phone calls.

The mean age, weight, and BMI of the patients were 35.59 ± 4.36 years old, 73.12 ± 11.64 kg, and 27.23 ± 4.16 kg/m^2 in both groups, respectively. Most of the participants were housewives (95%), and 40% of them had undergraduate education and used withdrawal as a contraception method. There was no statistically significant difference between the groups in terms of sociodemographic characteristics ($p > 0.05$) (Table 1).

The frequency of negative culture days 35–40 after starting treatment was 27.8% ($n = 10$) in the probiotic group and 45.5% ($n = 15$) in the fluconazole group; there was no statistically significant difference between the two groups ($p = 0.127$). Meanwhile, the frequency of negative culture 60–65 days after starting treatment in the fluconazole group (46.9%) was significantly higher than that in the probiotic group (19.4%) ($p = 0.021$) (Table 2).

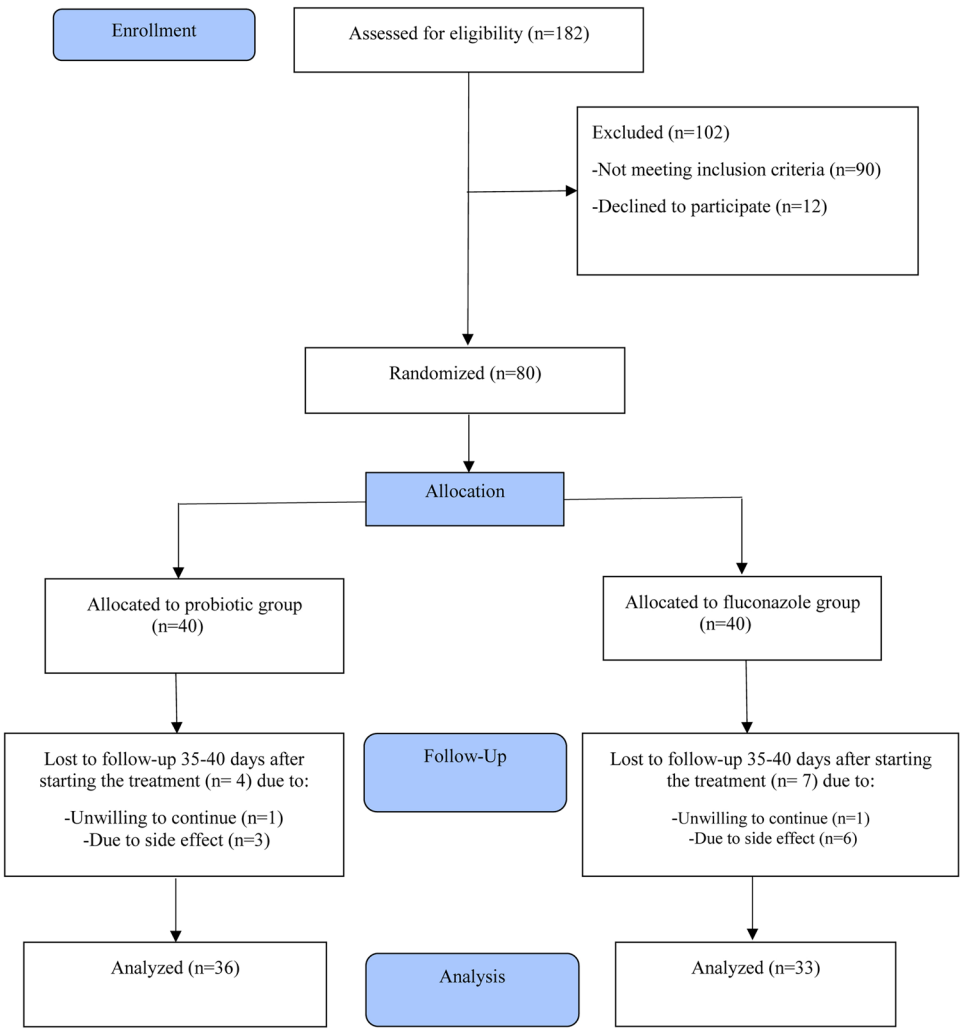

Fig. 1 Flow chart of the study

Table 1 Sociodemographic characteristics of the participants by the study groups

Characteristic	Probiotic ($n=40$) Mean (*SD*)	Fluconazole ($n=40$) Mean (*SD*)	*p*-value
Age	35.10 (4.41)	36.07 (4.30)	0.320*
BMI	27.54 (4.07)	26.93 (4.27)	0.705*
Age of marriage	19.97 (3.57)	21.12 (4.67)	0.220*
	Number (percent)	Number (percent)	
Education level			0.345[a]
Illiterate	1 (25)	1 (2.5)	
Under diploma	24 (60)	16 (40)	
Diploma	10 (25)	20 (50)	
University	5 (12.5)	3 (7.5)	
Job			1.000[b]
Housewife	38 (95)	38 (95)	
Self-employment	1 (2.5)	0	
Employee	1 (2.5)	2 (5)	
Husband's education			0.271[a]
Illiterate	3 (7.5)	0	
Under diploma	20 (50)	20 (50)	
Diploma	14 (35)	16 (40)	
Academic	3 (7.5)	4 (10)	
Husband's job			0.309[b]
Self-employment	30 (75)	29 (72.5)	
Employee	5 (12.5)	9 (22.5)	
Worker	5 (12.5)	2 (5)	
Number of pregnancies			0.508[b]
None	1 (2.5)	2 (5)	
One	7 (17.5)	5 (12.5)	
Two	19 (47.5)	18 (45)	
Three	10 (25)	10 (25)	
Four	3 (7.5)	3 (7.5)	
Five	0	2 (5)	
Number of deliveries			1.000[b]
None	1 (2.5)	2 (5)	
One	10 (25)	10 (25)	
Two	26 (65)	23 (57.5)	
Three	3 (7.5)	5 (12.5)	
Type of the last delivery			0.860[b]
None	1 (2.5)	2 (5)	
NVD	14 (35.0)	15 (37.5)	
CS	25 (62.5)	23 (57.5)	
Number of living children			0.874[b]
None	2 (5)	2 (5)	
One	11 (27.5)	10 (25)	
Two	23 (57.5)	24 (60)	
Three	4 (10)	4 (10)	
Number of abortions			0.315[b]
None	28 (70)	26 (65)	
One	9 (22.5)	9 (22.5)	
Two	3 (7.5)	2 (5)	
Three	0	3 (7.5)	
Contraception			0.922[b]

Table 1 (continued)

Characteristic	Probiotic ($n=40$) Mean (*SD*)	Fluconazole ($n=40$) Mean (*SD*)	*p*-value
Condom	5 (12.5)	5 (12.5)	
IUD	7 (17.5)	10 (25)	
OCP	4 (10)	2 (5)	
Tubectomy	2 (5)	1 (2.5)	
Vasectomy	2 (5)	2 (5)	
Withdrawal	20 (50)	20 (50)	
Medical history			0.615[b]
None	39 (97.5)	36 (90)	
Cardiac problems	1 (2.5)	1 (2.5)	
Asthma	0	1 (2.5)	
Kidney stone	0	1 (2.5)	
Deaf	0	1 (2.5)	
Medication history			1.000[b]
None	39 (97.5)	39 (95)	
Propranolol	1 (2.5)	0	
Hydrochlorothiazide	0	1 (2.5)	

[*]Independent-samples *t*-test

[a]Linear-by-linear association

[b]Fisher's exact test

The mean pH before the intervention was higher in the probiotic group than in the fluconazole one (4.16 ± 0.26 vs. 4.02 ± 0.25, $p=0.019$). Meanwhile, the mean pH was not statistically different based on the ANCOVA test and the control of the baseline value, 30–35 ($p=0.921$) and 60–65 days ($p=0.879$) after starting the treatment (Table 3). According to repeated measure ANOVA, the effects of group ($p=0.943$), time ($p=0.123$), and group × time (0.602) were not statistically significant.

The most common complaint of the patients before the intervention was abnormal vaginal discharge (95%), 23 patients (57.5%) in the probiotic group, and 20 (50%) in the fluconazole group suffered from severe discharge. Thirty-five to 40 days after starting treatment, the amount of discharge in both groups was decreased; this was such that in the probiotic group, only six persons (16.7%) and, in the fluconazole group, only one person (2.8%) complained of severe discharge ($p=0.035$). In addition, 60–65 days after

Table 2 Comparison of study groups in terms of the vaginal culture results in different time points

Culture	Day 0			Days 35–40			Days 35–40		
	Probiotic ($n=40$) *n* (%)	Fluconazole ($n=40$) *n* (%)	*p*-value	Probiotic ($n=36$) *n* (%)	Fluconazole ($n=33$) *n* (%)	*p*-value	Probiotic ($n=36$) *n* (%)	Fluconazole ($n=33$) *n* (%)	*p*-value
Negative	0	0		10 (27.8)	15 (45.5)	0.127*	7 (19.4)	15 (45.5)	0.021*
Positive	40	40		26 (72.2)	18 (54.5)		29 (80.6)	18 (54.5)	
Isolated *Candida* spp.			0.806[a]			0.074[a]			0.034[a]
C. albicans	25 (62.5)	22 (55)		15 (41.7)	6 (18.2)		16 (44.4)	6 (18.2)	
C. glabrata	7 (17.5)	7 (17.5)		5 (13.9)	5 (15.2)		6 (16.7)	5 (15.5)	
C. parapsilosis	4 (10)	5 (12.5)		4 (11.1)	1 (3)		4 (11.1)	1 (3)	
C. krusei	3 (7.5)	6 (15)		2 (5.6)	6 (18.2)		3 (8.3)	6 (18.2)	
C. tropicalis	1 (2.5)	0		0	0		0	0	

[*]Pearson chi-square

[a]Fisher's exact test

Table 3 Comparison of study groups in terms of the vaginal pH in different time points

Vaginal pH	Probiotic Mean (*SD*[b])	Fluconazole Mean (*SD*[b])	Mean difference (95%CI)	*p*-value
Day 0	4.16 (0.26)	4.02 (0.25)	0.137 (0.02 to 0.25)	0.019*
Days 35–40	4.05 (0.16)	4.01 (0.15)	−0.003 (−0.07 to 0.06)	0.921[a]
Days 60–65	4.07 (0.17)	4.01 (0.15)	0.005 (−0.06 to 0.07)	0.879[a]

*Independent-samples *t* test

[a]ANCOVA with adjusting the baseline value

[b]Standard deviation

starting treatment, this number remained unchanged in the probiotic group; in the fluconazole group, no person complained of severe discharge, which was still statistically significant ($p = 0.035$).

Before the intervention, 16 patients (40%) in the probiotic group and 15 (37.5%) in the fluconazole group complained of vulvovaginal erythema. After the intervention, the frequency of erythema was decreased in both groups. Upon 35–40 and 60–65 days, after starting treatment, none of the patients in the study groups complained of severe erythema and edema, but this difference in the fluconazole group was significantly less than that in the probiotic one ($p < 0.05$). There was, however, no statistically significant difference between the two study groups in the frequency of other symptoms such as itching, burning, frequent urination, dysuria, and dyspareunia 35–40 and 60–65 days after starting treatment ($p > 0.05$) (Table 4).

In the vaginal examination, there was no statistically significant difference in terms of cervical appearance, vaginal inflammation, amount, and color of secretions between the two groups before the intervention ($p > 0.05$). There was also no statistically significant difference in the signs in the first and second follow-up between the two groups, except for vaginal inflammation, which was significantly lower in the first ($p = 0.006$) and second ($p = 0.015$) post-intervention follow-up (Table 5).

The level of satisfaction with treatment in the fluconazole group was significantly higher than that in the probiotic group ($p = 0.020$) 35–40 days after starting the treatment. The side effects of treatment with probiotics were observed in two people with the symptoms of nausea ($n = 1$) and flatulence ($n = 1$). In addition, the side effects of treatment with fluconazole were observed in two people with the symptoms of nausea ($n = 1$) and stomach problem ($n = 1$).

Discussion

In this study, probiotics were less effective than fluconazole in improving and preventing the recurrence of discharge and vulvovaginal erythema. However, there was no statistically significant difference between probiotics and fluconazole in improving other clinical signs and symptoms. The frequency of negative culture 35–40 days after starting the treatment in the study groups was not statistically significant, thus indicating the effect of probiotics in the treatment of VVC, but the frequency of negative culture 60–65 days after starting treatment in the fluconazole group was significantly higher, which could indicate the greater effect of fluconazole in preventing the recurrence of the disease.

In this study, the most common complaints of patients in both groups were abnormal vaginal discharge (95%) and pruritus (78.7%), which was similar to other studies [15, 37]. In addition, the vaginal pH of 93.8% of the patients was 4–4.5, which was similar to that found by Hainer and Gibson [4], thus demonstrating that the vaginal pH in VVC is usually in the normal range (4 to 4.5).

There are very few studies that have used probiotics alone to treat or prevent the recurrence of VVC [38, 39], while most works have used probiotics as an adjunctive therapy after treatment with an antifungal drug [26, 31, 40]. However, the antifungal effect of probiotics has been reported in many studies [41, 42]. Several studies have demonstrated that some probiotics including *L. rhamnosus*, *L. casei*, and *L. acidophilus* could reduce the pathogenicity of *Candida* spp. by inhibiting biofilm formation. Among these lactobacilli, there are differences in the number of probiotics required to produce this inhibitory effect, thus indicating that the effect of probiotics is species-dependent [43].

Martinez et al. [26] prescribed one dose of fluconazole plus probiotics supplement (containing 1×10^9 CFU *L. rhamnosus* GR-1 and *L. reuteri* RC-14) twice a day for the first group; the second group received fluconazole plus placebo of probiotic for 28 days. In the fourth week, the probiotic group had significantly lower vaginal discharge and other symptoms of vaginitis like itching, burning, dyspareunia, and dysuria. In addition, the presence of yeast detected by culture was significantly lower, but there was no statistically significant difference between the two groups in the presence of pH > 4, which was similar to the results of the present study.

Kovachev and Vatcheva-Dobrevska [40] also demonstrated the improvement of the clinical symptoms of VVC in the patients receiving vaginal probiotics including *L.*

Table 4 Comparison of study groups in terms of the frequency of symptoms in different time points

Symptoms	Day 0			Day 35			Day 60		
	Probiotic ($n=40$) *n (%)*	Fluconazole ($n=40$) *n (%)*	*p*-value*	Probiotic ($n=36$) *n (%)*	Fluconazole ($n=36$) *n (%)*	*p*-value*	Probiotic ($n=36$) *n (%)*	Fluconazole ($n=33$) *n (%)*	*p*-value*
Discharge			0.302			0.035			0.035
None	0	4 (10)		11 (30.6)	17 (47.2)		14 (38.9)	19 (57.6)	
Mild	7 (17.5)	7 (17.5)		11 (30.6)	13 (36.1)		10 (27.8)	10 (30.3)	
Moderate	10 (25)	9 (22.5)		8 (22.2)	5 (13.9)		6 (16.7)	4 (12.1)	
Severe	23 57.5)	20 (50)		6 (16.7)	1 (2.8)		6 (16.7)	0	
Itching			0.025			0.096			0.019
None	3 (7.5)	14 (35)		22 (61.1)	28 (77.8)		20 (55.6)	27 (81.8)	
Mild	8 (20)	5 (12.5)		7 (19.4)	6 (16.7)		12 (33.3)	5 (12.5)	
Moderate	11 (27.5)	9 (22.5)		2 (5.6)	0		0	0	
Severe	18 (45)	12 (30)		5 (13.9)	2 (5.6)		4 (11.1)	1 (3)	
Burning			0.342			0.155			0.069
None	14 (35)	19 (47.5)		28 (77.8)	32 (88.9)		25 (69.4)	29 (87.9)	
Mild	7 (17.5)	5 (12.5)		2 (5.6)	3 (8.3)		6 (16.7)	1 (3)	
Moderate	8 (20)	7 (17.5)		2 (5.6)	1 (2.8)		1 (2.8)	3 (9.1)	
Severe	11 (27.5)	9 (22.5)		4 (11.1)	0		4 (11.1)	0	
Erythema			0.525			0.006			0.015
None	24 (60)	25 (62.5)		27 (75)	35 (97.2)		30 (83.3)	33 (100)	
Mild	6 (15)	10 (25)		6 (16.7)	1 (2.8)		3 (8.3)	0	
Moderate	9 (22.5)	5 (12.5)		3 (8.3)	0		3 (8.3)	0	
Severe	1 (2.5)	0		0	0		0	0	
Frequent urination			0.724			0.317			0.338
None	37 (92.5)	36 (90)		35 (97.2)	36 (100)		35 (97.2)	33 (100)	
Mild	1 (2.5)	2 (5)		0	0		0	0	
Moderate	1 (2.5)	2 (5)		0	0		0	0	
Severe	1 (2.5)	0		1 (2.8)	0		1 (2.8)	0	
Dysuria			0.128			0.680			0.783
None	27 (67.5)	33 (82.5)		31 (86.1)	32 (88.9)		32 (88.9)	29 (90.6)	
Mild	5 (12.5)	3 (7.5)		1 (2.8)	1 (2.8)		1 (2.8)	0	
Moderate	3 (7.5)	1 (2.5)		1 (2.8)	2 (5.6)		1 (2.8)	2 (6.3)	
Severe	5 (12.5)	3 (7.5)		3 (8.3)	1 (2.8)		2 (5.6)	1 (3.1)	
Dyspareunia			0.726			0.091			0.129
None	22 (55)	22 (55)		28 (77.8)	33 (91.7)		28 (77.8)	30 (90.9)	
Mild	6 (15)	8 (20)		2 (5.6)	2 (5.6)		3 (8.3)	2 (6.3)	
Moderate	3 (7.5)	5 (12.5)		2 (5.6)	0		2 (5.6)	0	
Severe	9 (22.5)	5 (12.5)		4 (11.1)	1 (2.8)		3 (8.3)	1 (3.1)	

*Mann–Whitney *U*

acidophilus, *L. rhamnosus*, *Streptococcus thermophiles*, and *L. delbrueckii*, with an antifungal drug being more in comparison to the group receiving an antifungal drug alone. In addition, Carriero et al. [44] came up with a similar result, showing that the proportion of symptoms improvement in the group receiving *Lactiplantibacillus plantarum* after fluconazole treatment was higher than that in the group getting fluconazole alone. Kohler et al. [45] also demonstrated that probiotics reduced the expression of the genes involved in the biosynthesis of ergosterol and a drug efflux pump involved in fluconazole's resistance in *C. albicans*. In addition, several mechanisms could be considered for the inhibition of VVC by different species of lactobacilli, such as the production of biosurfactants like surlactin; reduction in luminal pH; production of various antimicrobial substances including acetic acid, lactic acid, and hydrogen peroxide; bacteriocins such as larger heat-labile proteins (LHLP), non-lanthionine-containing membrane-active peptides (MAP),

Table 5 Comparison of the study groups in terms of the frequency of signs in different time points

Signs	Day 0			Day 35			Day 60		
	Probiotic ($n=40$)	Fluconazole ($n=40$) n (%)	p-value	Probiotic ($n=36$) n (%)	Fluconazole ($n=33$) n (%)	p-value	Probiotic ($n=36$) n (%)	Fluconazole ($n=33$) n (%)	p-value
Appearance of cervix			0.769*			0.867*			0.867*
Normal	31 (77.5)	29 (72.5)		29 (80.6)	28 (84.8)		29 (80.6)	28 (84.8)	
Inflamed	8 (20)	8 (20)		6 (16.7)	4 (12.1)		6 (16.7)	4 (12.1)	
Lesion	1 (2.5)	3 (7.5)		1 (2.8)	1 (3)		1 (2.8)	1 (3)	
Vaginal inflammation			0.369†			0.017†			0.017[a]
Present	24 (60)	20 (50)		20 (55.6)	9 (27.3)		20 (55.6)	9 (28.1)	
Absent	16 (40)	20 (50)		16 (44.4)	24 (72.7)		16 (44.4)	24 (27.7)	
Amount of discharge			1.000*			0.125†			0.075[a]
Normal	4 (10)	4 (10)		9 (25)	14 (42.4)		9 (25)	15 (45.5)	
Abnormal	36 (90)	36 (90)		27 (75)	19 (57.6)		27 (75)	18 (54.5)	
Color of discharge			0.217*			0.946*			0.946*
Gray	2 (5)	0		1 (2.8)	1 (3)		1 (2.8)	1 (3)	
White	29 (72.5)	27 (67.5)		23 (63.9)	20 (60.6)		23 (63.9)	20 (60.6)	
Colorless	8 (20)	13 (32.5)		11 (30.6)	12 (36.4)		11 (30.6)	12 (36.4)	
Yellow	1 (2.5)	0		1 (2.8)	0		1 (2.8)	0	

*Fisher's exact test

[a]Pearson chi-square test

and small heat-stable lantibiotics (SHSL); and stimulation of adaptive and innate immune responses, such as the synthesis of inflammatory cytokines [46, 47].

The study done by Vladareanu et al. [39] showed that the oral administration of *L. plantarum* P17630 to patients with a history of recurrent vulvovaginal candidiasis (RVVC), in comparison to placebo, improved the colonization of lactic acid bacteria in the vagina and reduced some clinical symptoms including discharge and vaginal inflammation and redness. In addition, no side effects were reported during the study, thus recommending the use of this oral product for the successful prevention of vulvovaginal candidiasis episodes. Ang et al. [38] also reported that the use of probiotics in pregnant women with VVC for 8 weeks reduced abnormal discharge, itching, and burning, in comparison to the placebo group, but there was no statistically significant difference between the two groups in the quality of sexual activity.

In a review conducted by Hempel et al. [48], the probiotics were well tolerated in 235 studies, and 387 studies accurately reported the presence or absence of side effects. In some case studies, bacteremia was potentially associated with the use of probiotics, but randomized controlled trials showed no statistically significant increased relative risk (RR) of the overall number of experienced side effects, such as gastrointestinal problems and infection, in the probiotic group, as compared to the placebo ones.

The use of lactobacilli, in comparison with oral or topical antifungal therapies, could reduce the risk of pathogenic resistance to treatment and the risk of side effects, as well as decreasing the financial costs of production and purchase. In addition, such a treatment is more acceptable for women looking for more natural ways to maintain their health [36].

The use of probiotics alone for the treatment of VVC was one of the strengths of the present study. The use of a single species (LA-5) can also determine its specific effect on VVC. One of the limitations of the study was, however, the loss of the follow-up of the first and second examinations in 10% ($n=4$) of the probiotic group and 17.5% ($n=7$) of the fluconazole one; this could be the reason for non-cooperation in both groups, which were almost the same. In addition, different types of probiotics with different doses should be used to evaluate their specific effect on the treatment of VVC. The effect of oral and vaginal use of lactobacilli should also be assessed.

Conclusion

L. acidophilus can reduce most symptoms of *Candida* vaginitis, like fluconazole, although it was less effective than the latter in improving some of the symptoms. The culture results also showed that *L. acidophilus* could be used to treat *Candida*, but fluconazole was more effective in preventing the recurrence. Considering the obtained findings, the laboratory effect on the inhibition of *Candida* spp., the side effects of chemical drugs, and treatment failure due to the

emergence of resistant species, further studies are needed to evaluate the anti-*Candida* properties of *L. acidophilus* by performing more clinical trials and identifying exact mechanisms.

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Author Contribution All of the authors contributed to the conception and design of the study and revised the manuscript. ZM drafted the manuscript under direct supervision of PY. MM and SMAC conducted statistical analysis. AHR helped to prepare medicine. HSK and PG conducted the microbiological evaluations. All authors read and approved the final manuscript.

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Data Availability The datasets generated and/or analyzed during the current study are not publicly available due to the limitations of ethical approval involving the patient data and anonymity, but are available from the corresponding author upon reasonable requests.

Declarations

Ethics Approval and Consent of Participants This study was approved by the ethics committee of the research and technology deputy of Tabriz University of Medical Sciences (IR.TBZMED.REC.1399.848). The informed written consent was obtained from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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