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Evaluation of the Antifungal Efficacy of *Lactobacillus* Strains Isolated from Human and Camel Urine Against Fluconazole-Resistant *Candida* spp.

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A B S T R A C T

Objective: The increasing prevalence of *Candida* infections and the emergence of resistance to chemical antifungal medicines, such as fluconazole, has prompted increased research into natural compounds with anti-*Candida* activity. Probiotics, in particular, are being investigated as promising alternatives due to their lower costs and reduced side effects compared to synthetic drugs. This study aimed to isolate *Lactobacillus* strains from human and camel urine and evaluate their antifungal activity against three Candida strains isolated from human vaginal samples.

Materials and Methods: In this research, *Lactobacillus* strains were isolated from human and camel urine. Then, the anti-*Candida* effect of isolated *Lactobacillus* spp. and two commercial probiotic formulations, Lactofem and Femi capsules containing *Lactobacillus* spp. 10^9 and 1.65×10^9 CFU/mL, respectively, was assessed using the microtiter plate method for determination of minimum inhibitory concentration (MIC). Finally, the interaction of *Lactobacillus* and fluconazole against *Candida* was investigated using the checkerboard method.

Results: *Lactobacillus brevis* and *Lactobacillus crispatus* were isolated from human urine samples, and *Lactobacillus brevis* was isolated from camel urine. The MIC of *Lactobacillus* spp. against *Candida albicans*, *Candida glabrata,* and *Candida krusei* was 1.87×10^7 CFU/mL. Antifungal effects of Lactofem and Femi against three *Candida* spp. were 6.25×10^6 CFU/mL and 2.062×10^7 CFU/mL, respectively. Among the probiotics, Lactofem had the highest, and Femi had the lowest anti-*Candida* effect. Fractional Inhibitory Concentration results show the synergistic effect of the combination of 9.37×10^6 CFU/mL *Lactobacillus* and 4 µg/mL fluconazole.

Conclusions: The finding of this study indicate that *Lactobacillus* spp. have potent antifungal activity against *Candida* infections and show synergistic effect when combined with fluconazole, suggesting their potential as an alternative or complementary therapeutic approach in antifungal therapy. Future research is recommended to focus on developing clinical formulations and validating efficacy through in vivo studies.

may be recommended for their applications as an alternative or complementary therapeutic approach in pharmaceutical formulations.

Keywords: Probiotic, Alternative medicine, Microtiter plate, Candidiasis, Fluconazole

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Candidiasis is a growing global health concern due to the increasing prevalence of antifungal resistance and immunosuppression. To solve this problem, there are only a limited number of medications to treat Candida infections [1]. Therefore, new therapeutic approaches are needed [2]. Certain antifungal drugs, including polyenes, azoles, and echinocandins, are utilized to treat Candida infections. Data reported from healthcare centers have shown widespread resistance to fluconazole among clinical isolates of Candida strains. Fluconazole is the most effective antifungal medication for treating Candida infections [3]. However, several side effects have been reported for these drugs, including GI disturbances, hepatotoxicity, and neurotoxicity [4]. Therefore, alternative treatments for opportunistic fungal infections have been proposed, along with the use of natural products. Thus, the need for biological control agents such as probiotic microorganisms and their bioactive metabolits has been predicted as an alternative strategy for treating fungal infections [5]. Probiotics are living microorganisms that, when consumed at suitable doses, cause beneficial effects on health by affecting microflora. Most probiotic strains belong to a large group of the main bacteria of the microbial flora of the human intestine [6]. Microbial, viability and health improvement are three key features of probiotics [3]. Lactic acid bacteria (LAB) have recently been recognized as generally recognized as safe (GRAS) due to their probiotic properties. Lactobacilli fight pathogenic bacteria by releasing antimicrobial metabolites [7]. Studies have reported that Lactobacillus spp. isolated from different sources have anti-Candida activities that can be used as protective agents [7-10].

The vaginal microbiota of healthy women is naturally overwhelmed by Lactobacillus spp., which play an important role in maintaining low pH and inhibiting the growth of pathogens [11]. Investigation has demonstrated that Lactobacillus spp. break down carbohydrates and create an acidic vaginal environment through the production of lactic acid and carbon dioxide, thereby inhibiting colonization by pathogenic microbes [12].

Scientific evidence suggests that camel urine exhibits effective inhibitory effects against various microbial pathogens. This includes antibacterial activity against pathogens like S. aureus and E. coli, and antifungal action against Aspergillus niger, Aspergillus flavus, and C. albicans [13]. Considering that the research showed the antimicrobial effect of camel urine, searching for Lactobacilli with antimicrobial properties in camel urine can strengthen the results of these previous findings.

Introduction

The aim of this study was to isolate Lactobacillus strains from human and camel urine samples and investigate the efficiency in growth inhibition of three Candida spp. isolated from the human vaginal specimens. Additionally, the synergistic interaction between Lactobacillus spp. and fluconazole against Candida strains was investigated using the checkerboard method.

Materials and methods

Probiotic drugs used

Two commercial probiotic formulations containing Lactobacillus spp. were included in this study for comparative analysis with isolated Lactobacillus strains. Lactofem capsule (Zist Takhmir LactoFem) containing a multi-strain formulation of bacteria named L. acidophilus, L. plantarum, L. fermentum, and L. gaseri, which enhances its potential broad-spectrum antimicrobial activity due to strain synergies. It also includes fructooligosaccharide as prebiotic, lactose, magnesium stearate and talc. Femi (Davo, Italy) was selected due to its diverse microbial composition containing six strains of beneficial bacteria, namely Bifidobacterium bifidum, Lactobacillus acidophilus, Lactobacillus Helveticus, L. brvis, L. plantarum and Salivarius, as well as cellulose microcrystals, magnesium stearate and lactoferrin. By evaluating these formulations, we aimed to assess whether naturally occurring urinary Lactobacilli show comparable or higher than antifungal activity to commercially available probiotics.

The manufacturer-reported CFU count was 109 CFU/mL per capsule for Lactofem and 1.65×108 CFU/mL for Femi. Capsules were stored at 4°C until use, strictly within the expiration period. Immediately before the experiments, the capsule contents were suspended in sterile MRS broth to achieve the desired concentration. Viability was confirmed using the plate count method, typically involving plate counting on MRS agar.

Yeast strain

In this study, three clinically resistant strains of C. albicans, C. glabrata, and C. krusei were obtained from the Department of Mycology, Faculty of Medicine, Isfahan University of Medical Sciences. These strains were originally isolated from human vaginal samples, though detailed donor information (e.g., age, health status) was not available for this study. According to the CLSI standard, the resistance of the Candida species to the antifungal fluconazole drug

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was confirmed by the microtiter plate method. MIC values were determined after 48-h incubation at 35°C, with visual turbidity assessed at 630 nm [14]. Isolates were categorized as susceptible (S), dose-dependent susceptible (DD-S), or resistant (R) based on the following MIC50 breakpoints:

Susceptible (S): MIC50 ≤ 8 µg/mL

Dose-Dependent Susceptible (DD-S): MIC50: 16 -32 µg/mL

Resistant (R): MIC50 ≥ 67 µg/mL [15].

Isolation of Lactobacilli from the human urine

Urine samples were collected from female volunteers aged 20–50 years. Midstream clean-catch urine was collected in sterile containers. Within 2 hours of collection, samples were cultured on blood agar (BA, Quelab, Canada) and incubated aerobically at 37°C for 24 h for bacterial enrichment. After 24 hours, the small, white, and opaque colonies formed on the surface of the blood agar medium were cultivated on the MRS agar medium (Biolife, Italy) and placed in an anaerobic condition with 4% CO2 in an incubator at 37°C for 24 hours [16].

Isolation of Lactobacillus from camel urine

Fresh urine samples were collected from healthy, adult camels of the Kalkouhi ecotype. Camel urine was selected due to its traditional use as an antimicrobial. Specific health metrics (e.g., diet, exact age) were not recorded; however, all camels were clinically healthy at the time of sampling.

1 mL of camel urine sample was added to 100 cc of MRS-broth (containing 0.05% cysteine) with pH 6.5 (Optimal for initial broad bacterial growth) and incubated at 37 °C for 24 h in aerobic conditions to enrich all bacteria. Then, it was subcultured in MRS-broth with pH 4.5 (Selective for Lactobacillus spp., which dominate low-pH niches e.g., vagina at 37 °C and 4% CO2. Then, after culture on MRS agar, biochemical tests were performed for the final confirmation of Lactobacillus, such as gram staining, catalase, and sugar fermentation.

Molecular identification

Bacterial DNA extraction was done using the Iraizol kit (RNA BIOTECH, Iran). The universal bacterial primers DG47F (5′-AGGAGGTGATCCACCGCA-3′) and RW01R (5′-AACTGGAGGAAGGTGGGGAT-3′) [17] were used to amplify the 16S rRNA gene.

PCR amplification was carried out in a volume of 25 µl consisting of 2.5 µl PCR buffer 10X, 0.5 µl of each forward and reverse primer (final concentration: 0.2 µM each), 1 µl DNA template (~50–100 ng/µL), 0.5 µl Taq DNA Polymerase, 1 µl MgCl2 1mM. Amplification conditions were 10 minutes of preheating at 94°C, 1 minute of denaturation at 95°C, 45 seconds of primer annealing at 57°C, 1-minute extension step at 72°C and a post-cycling extension cycle of 10 minutes at 72°C for 30 cycles. Analysis of PCR production was performed by gel electrophoresis on a 1% agarose TAE gel. The PCR products were sequenced by the Rena Biotechnology Company (Isfahan, Iran). Then the homology of nucleotide sequences was evaluated by comparing them with the sequences in the NCBI database and by the BLASTn algorithm.

Determination of the MIC by microtiter plate method

Turbidity of equal to 0.5 Mc Farland was obtained from fresh cultures of yeasts and Lactobacilli in YPD and MRS broth media, respectively. A serial dilution of Lactobacillus was prepared 1:1 in MRS-broth. Then, 100 microliters of the Candida strains were poured into each well of the 96-well polystyrene plate. Wells considered containing 200 microliters of broth medium (100 microliters of YPD + 100 microliters of MRS) as a negative control, wells containing 100 microliters of yeast suspension + 100 microliters of MRS and also wells containing 100 µL Lactobacillus suspension + 100 µL MRS broth as a positive control. Three repetitions were considered for each yeast. Then, the surface of the plates was covered and incubated for 24 h at 37 °C, and the turbidity was read at a wavelength of 630 nm by ELISA reader [18].

In the case of Lactofem and Femi capsules, the bacterial count in each capsule was equal to 109 CFU/mL and 1.65 × 109 CFU/mL, respectively. The contents of each capsule were dissolved in one milliliter of MRS-broth medium and then diluted 1:10 in MRS medium to reach a concentration of 108 and 1.65 × 108 CFU/mL, respectively. The next steps were done as described for the Lactobacilli.

The Determining the amount of FIC and the interaction of two antimicrobial substances

FIC is an index that determines the type of interaction of two antimicrobial substances in combination with each other against the target microorganism. One of the methods for

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determining FIC is the checkerboard method. The term checkerboard refers to a pattern that consists of multiple dilutions of two antimicrobial substances tested at equal, higher, and lower MIC concentrations against the tested yeasts. In the columns of this table, each well contains the same amount of drug A that is diluted along the X axis, and in its rows, each well contains the same amount of drug B that is diluted along the Y axis. The result is that each square in the checkerboard contains a unique combination of two tested drugs.

In this method, the FIC for each drug was obtained by dividing the concentration of the required drug to inhibit the growth in a column or row by the MIC of the drug alone on the tested yeasts, and the FIC index was obtained according to the following formula from the sum of the FICs of the two available drugs.

$$\text{FICindex} = \text{FICA} + \text{FICB} = \frac{\text{MIC (A in presence of B)}}{\text{MIC (A alone)}} + \frac{\text{MIC (B in presence of A)}}{\text{MIC (B alone)}}$$

Here, A and B represent the concentrations of fluconazole and B Lactobacillus supernatant in combination that inhibit microbial growth, while MIC A and MIC B are their respective MICs when used alone.

This method determines synergism by FIC index ≤ 0.5, non-reactivity between two drugs by FIC index = 1, and antagonism by FIC index = 2 [19].

100 microliters of Candida was added to the well to investigate the interaction between Lactobacillus and fluconazole at a concentration of 1-5 × 10^3 [14].

Aggregation assay

Overnight cultures of two *Lactobacillus isolates from urine* were diluted in phosphate buffer saline (PBS) to an optical density (OD600 nm) of 0.5. 4 mL aliquots of cell suspensions were vortexed and then incubated for 4 h at 37°C. After incubation, the OD600 nm values of the cell suspensions were measured using a spectrophotometer. The autoaggregation percentages (%) L was calculated using the following formula:

100 × [1-(ODA/ODB)]

ODA = Absorbance after 4 h of incubation

ODB = Initial absorbance (before incubation).

The coaggregation levels between the Lactobacilli spp. and the Candida spp. were determined by a spectrophotometric coaggregation method. The Lactobacilli cell suspension was prepared the same procedure described for the autoaggregation assay, except OD600 nm Candida cultures were adjusted to 1.0 in PBS solution. A 2 mL volume of *Lactobacillus* cell suspension was mixed with 2 mL of each Candida cell suspension and incubated for 4 h at 37°C. Then OD600 nm was read using the spectrophotometer. The coaggregation percentages were reported as:

Percentage of coaggregation (%)=100×[(ODL+ODC)−2 (ODM)](ODL+ODC)

ODL = Absorbance of Lactobacillus

ODC= Absorbance of Candida

ODM = Absorbance after 4 h of co-incubation [20].

Statistical analysis

The experimental data were analyzed using IBM SPSS Statistics 23 (IBM Corp., USA. For comparisons among multiple groups, Use One-Way ANOVA followed by Tukey's post hoc test for normally distributed data or the Kruskal-Wallis test for non-parametric data. A p-value < 0.05 was considered statistically significant. Graphs were generated using Microsoft Excel 2010.

Result

Assessment of Candida strains resistance to fluconazole

According to the results obtained in Table 1 and Figure 1, all resistant strains (C. albicans, C. glabrata, C. krusei) showed high-level fluconazole resistance (MIC50 ≥64 µg/mL), while susceptible C. albicans (MIC50 ≤8 µg/mL) retained sensitivity.

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Table1: Susceptibility test results of fluconazole against the Candida species

Candida spp.	MIC50 based on fluconazole concentration in (µg/mL)
Resistant C. albicans	≤ 64
Resistant C. glabrata	≤ 64
Resistant C. krusei	≤ 64
Susceptible C.albicans	≥ 8

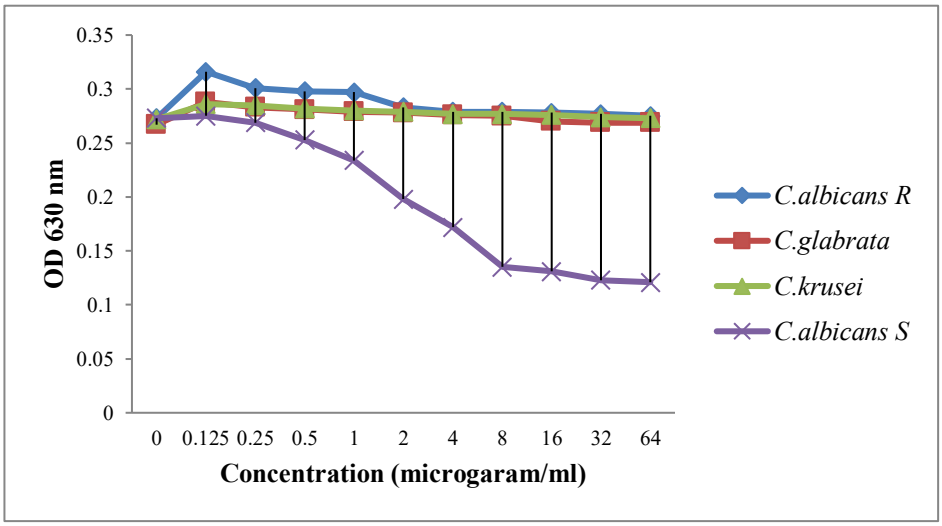

Figure 1: The inhibitory effect of fluconazole against Candida strains growth

Isolation and Identification of Lactobacilli

Two Lactobacillus (HL246 and HL182) were isolated from 15 sample of human urine samples, and one (CL) was isolated from 5 camel urine samples. The bacteria were identified according to biochemical tests, as can see in Table 2.

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Table2: Biochemical characteristics of three isolated *Lactobacillus strains*

Isolated	Test											
	Catalase	Growth at 15 °C	Growth at 45 °C	Growth at pH 4.5	Growth at pH 6.5	Arabinose	Galactose	Lactose	Maltose	Mannitol	Sucrose	Raffinose
HL246	-	+	-	+	+	+	-	+	+	-	+	+
HL182	-	-	+	+	+	+	+	+	+	-	+	-
CL	-	+	-	+	+	+	-	+	+	-	+	+

Nucleotide sequences were submitted to the NCBI database. PCR amplification revealed that every three isolates were Lactobacilli. Their accession numbers are shown in Table 3.

Table3: Sequencing results of strains isolated from urine

Code	Species	Accession number
HL246	Lactobacillus brevis	PP496779
HL182	Lactobacillus crispatus	PP500771
CL	Lactobacillus brevis	PP500980

MIC results

MIC determination by microtiter plate using isolated Lactobacilli and probiotic capsules indicates the same MIC. The result for Lactobacilli isolated from human urine against C. albicans, C. glabrata, and C. krusei equals 1.87 × 107 CFU/mL. According to the results presented in Table 4, Lactofem has the most antifungal effect compared to others, followed by isolated Lactobacilli from human and camel urine, and finally, Femi.

Table4: MIC results of lactobacilli isolated against Candida strain (CFU/mL)

Candida strain	Lactobacillus brevis	Lactobacillus crispatus	Lactobacillus brevis	Lactofem	Femi
C. albicans	1.87×10^{7}	1.87×10^{7}	1.87×10^{7}	6.25×10^{6}	2.062×10^{7}
C. glabrata	1.87×10^{7}	1.87×10^{7}	1.87×10^{7}	6.25×10^{6}	2.062×10^{7}
C. krusei	1.87×10^{7}	1.87×10^{7}	1.87×10^{7}	6.25×10^{6}	2.062×10^{7}

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Microscopic analysis of cell morphology

Structural and morphological changes of C. krusei were observed after 24h exposure to Lactobacillus compared to control (without Lactobacillus) (Figure 2). Incubation of C. krusei with Lactobacillus after 24 hours shows the multinucleation of Candida cells due to inhibition of their germination by Lactobacillus (white arrows). (Figure 2D). According to the results obtained from the SEM images, the size of Lactobacillus in co-culture with Candida (Figure 2F) has increased compared to the culture alone (Figure 2E).

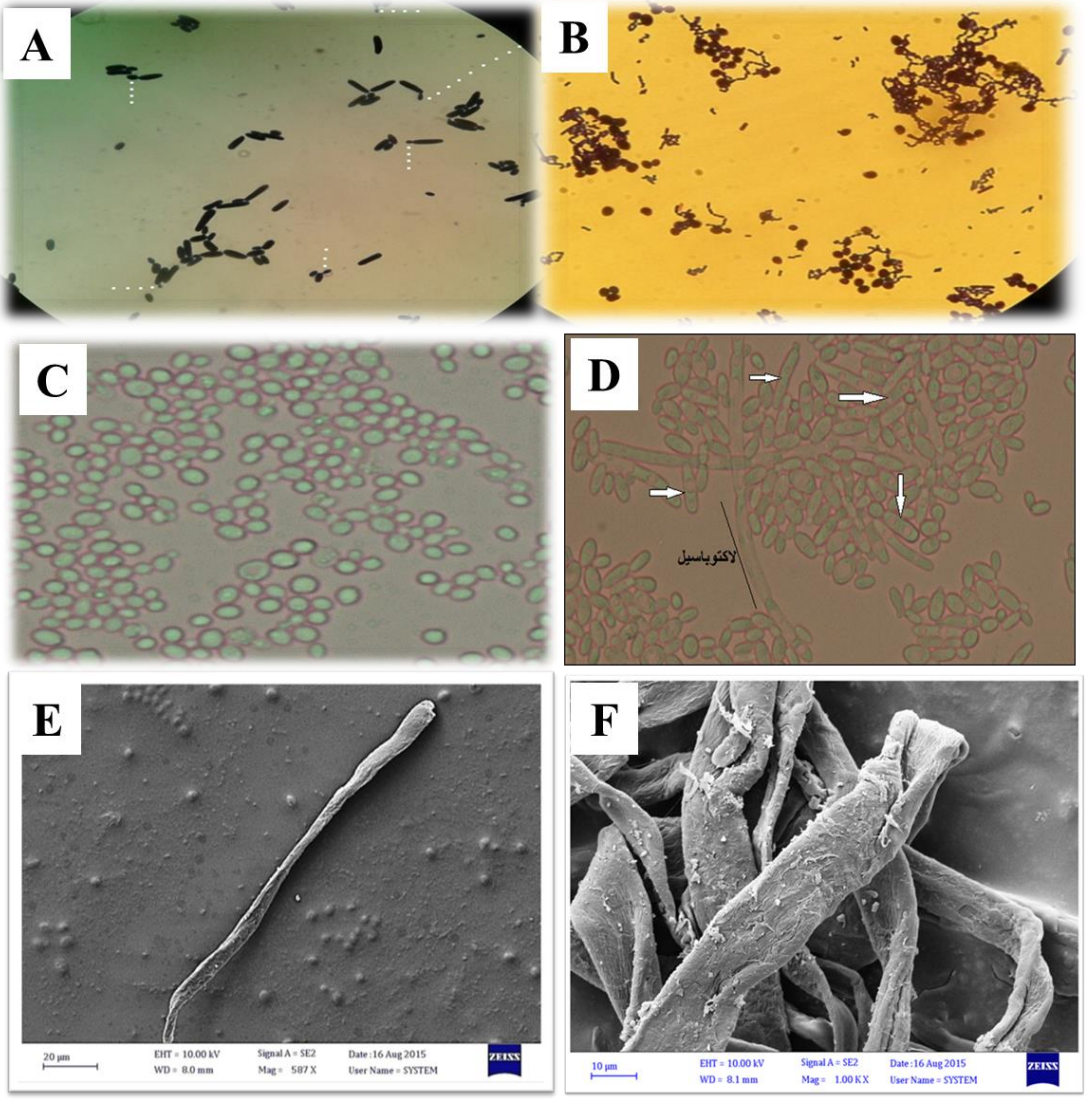

Figure 2: Microscopic image of Candida cells treated with Lactobacillus after 24 h. A) light microscope ×100 Candida B) light microscope ×100 Candida and Lactobacillus C) Fluorescent microscope from Candida D) fluorescence microscope Candida and Lactobacillus E) SEM microscope Lactobacillus F) SEM microscope Candida and Lactobacillus

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The results of autoaggregation and coaggregation

The FIC calculation for each well indicates the synergistic effect of fluconazole and Lactobacillus at a concentration of 5 µ g/mL fluconazole and 4×10^7 CFU/mL Lactobacillus.

Significant synergy (FIC index ≤0.5) was observed between fluconazole and L. brevis strain HL246 against C. albicans as well as L. crispatus strain HL182 against C. albicans.

To calculate the percentage of autoaggregation, 2 Lactobacillus spp. were selected. The absorption of 2 isolates was measured at time zero and after 4 hours. The absorbance of the isolates before incubation was 0.432 and after 4 h incubation was 0.438.

A Lactobacillus strain and a Candida strain were selected to calculate the coaggregation percentage. The absorption of Lactobacillus and Candida was measured at time zero and after 4 hours. Absorption of Lactobacillus and C. albicans before incubation was 0.514 and 0.8, respectively, and after 4 h of incubation was 0.540. The percentage of autoaggregation and coaggregation was calculated according to the obtained values.

$$\text{autoaggregation \%} = \{1 - (\frac{ODA}{ODB})\} \times 100 = \{1 - (\frac{0/438}{0/432})\} \times 100 = \%\ 1.38$$

$$\text{coaggregation \%} = \frac{\{(ODL+ODC)-2(ODM)\}}{(ODL+ODC)} \times 100 = \frac{(0.514+0.8)-2(0.54)}{0.514+0.8} \times 100 = \%\ 17.8$$

According to the obtained results, the percentage of spontaneous accumulation is 1.38%, and the percentage of combined accumulation is 17.8%.

Table5: Plate microtiter results in the investigation of the interaction of fluconazole + lactobacillus Candida

Fluconazole	02 0.7	00 0.9	009 0	00 0.9	00 0.4	01 0
	02 0.6	00 0.3	008 0	01 0.2	00 0.1	00 0.8
	02 0.5	01 0.2	006 0	00 0.6	01 0.3	01 0.2
	02 0.4	00 0.6	019 0	00 0.7	00 0.4	01 0.1
	02 0.2	01 0.1	012 0	00 0.8	01 0.4	01 0.5
	02 0.7	00 0.1	001 0.4	00 0.2	02 0.6	02 0.8
	0	1	2	3	4	5
OD	Lactobacilli					

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Discussion

Lactobacillus strains can be isolated from diverse sources, including fermented dairy products and various human-associated places such as the gastrointestinal tract, vaginal mucosa, oral cavity, breast milk, and feces [21]. In the present study, human and camel urine samples were used to obtain Lactobacilli with anti-Candida effects. L. brevis and L. crispatus were isolated from human urine samples. The reported sources of Lactobacillus brevis are milk, cheese, mouth, digestive tracts of humans and rats, cow manure, sauerkraut, sourdough, and fodder [22]. Lactobacillus brevis was isolated from the women's vagina [23]. There are few reports on isolating other Lactobacillus species from human urine. A study announced the isolation of Lactobacillus cholehominis from a human source [24]. In 2009, Darbro et al. isolated Lactobacillus delbrueckii from women's urine [25]. In another study, Lactobacillus equicursoris was isolated from human urine [26]. In this study, L. brevis was isolated from urine of the camel. There are few studies on the isolation of Lactobacillus from camel urine and evaluation of its antibacterial potential [27]. This may be because the Lactobacillus population in camel urine is very low, requiring repeated enrichment and purification for isolation.

In a study, 30 Lactobacillus strains were isolated from caries-free samples, and the inhibitory effects were directly evaluated on two clinical strains of C. albicans and one reference strain. The isolated Lactobacillus strains were assessed for their antimicrobial activity against C. albicans biofilms in laboratory conditions. They showed that Lactobacillus isolates can inhibit *C. albicans* biofilms [9].

In this research, Lactobacilli isolated from the urine of human and camels showed their anti-Candida effect on the studied strains in the microtiter plate methods. Also, two probiotic drugs, Lactofem and Femi, were investigated for anti-candidal effects. Lactofem drug against C. albicans, C. glabrata, and C. krusei had the MIC equal to 6.25×10^6 CFU/mL. In addition, the Fami drug against C. albicans, C. glabrata, and C. krusei had the same and equal MIC with 2.062×10^7 CFU/mL; the difference in MICs is significant ($P \leq 0.05$). The greater anti-Candida effect of Lactofem compared to clinical isolates can be because Lactofem is a mixture of 4 probiotic species while we used only one species. The difference in the MIC of Lactofam and Femi may be also related to their microbial composition. Lactofam includes 4 types of beneficial bacteria, namely Lactobacillus acidophilus, Plantarum, Fermentum, and Gaseri, as well as the prebiotic fructooligosaccharide lactose, magnesium stearate, and talc. Femi contains 6 types of beneficial bacteria, namely Bifidobacterium bifidum, Lactobacillus acidophilus, Heloticus, Bruis, Plantarum, and Salivarius, as well as microcrystals of cellulose, magnesium stearate, and lactoferrin.

In a study, Lactobacillus crispatus was isolated from the vagina and after investigating its anti-Candida effect by microtiter plate method, the researchers concluded that this Lactobacillus has fungicidal properties [28], contrary to the results obtained in the present study, none of the isolates or probiotic drugs had a fungicidal effect (MFC), and this could be related to resistance of the used the Candida species to fluconazole.

One study showed the anti-Candida effect of Lactobacillus fermentum isolated from human samples against C. albicans and C. glabrata species by agar overlay method and microtiter plate with filtered supernatant and suggested that this bacterium can be used to treat or prevent Vaginal candidiasis [29]. The anti-Candida effect of FCS of Lactobacillus plantarum strain LR/14 was investigated by Sharma and Srivastava in 2014, and it was announced that disruption of the integrity of the membrane could lead to the leakage of cell contents and cause the death of 90% of the Candida [18].

In the clinical research, three times a week for 30 days, Lactobacillus casei and L. fermentum probiotics with concentrations of 2×10^7-10^9 and 5×10^7-10^9 were consumed by volunteers, and their saliva samples were taken before and after 30 days, then cultured to identify and count the number of Candida, the number of Candida significantly decreased from 92.9% to 85.7%, and anti-Candida IgA significantly decreased after treatment with probiotics [8].

In the present study, the percentage of co-aggregation and autoaggregation is calculated, and the co-aggregation percentage is shown to be higher than the autoaggregation percentage. It was shown that the co-accumulation of Lactobacillus and Candida can be the one mechanism of Lactobacilli that inhibits the growth of this yeast. A study stated that the autoaggregation percentage of two samples of Lactobacillus is 23.75%, and the co-aggregation percentage of Lactobacillus and Candida together is 62-66.1% [20].

In the present research, in the SEM images prepared shown with the incubation of Lactobacillus with Candida, Lactobacillus cells have formed biofilm, so the size of Lactobacillus and the diameter of their wall have increased.
In a study, it has been stated that aggregation is the first step for forming biofilm by Lactobacillus strains, which prevents

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the growth and proliferation of pathogenic microorganisms [30]. As can be seen in the images prepared in this study with a fluorescent microscope without staining, the germination power of yeast cells decreases after 24 hours of exposure to Lactobacillus. As a result, yeast cells are prepared for germination but cannot germinate, leading to the formation of multinucleated cells. This may be showing that Lactobacillus metabolites or cell-surface interactions may disrupt cytokinesis or nuclear division and trapp Candida in an abortive growth situation. Furthermore, in the SEM images in this research revealed that Candida cells incubated alone exhibited an average diameter of 3.24 µm, whereas those co-cultured with Lactobacillus spp. showed a significant increase in size, reaching 4.76 µm. This morphological alteration, characterized by enlarged cell dimensions, may indicate that Lactobacillus spp. inhibits germination of Candida. Multinucleation and cell enlargement are signs of viable but non-germinative states, which could control Candida virulence and biofilm formation. If Lactobacillus spp. can support this effect in vivo, it may serve as a probiotic supplement to reduce Candida pathogenicity. Further research should evaluate whether these cells are eventually lysed or regain normal function once Lactobacillus is removed. Microorganisms, microbial agents, and natural plant-based antioxidants play a significant role in the prevention and treatment of various infectious, fungal, and microbial diseases [31-33].

Conclusion

The rising prevalence of fluconazole-resistant Candida strains, coupled with the adverse effects of conventional antifungal therapies and the growing population vulnerable to candidiasis, underscores the potential utility of natural alternatives for prevention and treatment. Such options may offer reduced side effects, lower costs, and greater accessibility. Findings from this study demonstrate that probiotic Lactobacillus strains, both independently and in combination with fluconazole, exhibit significant inhibitory effects against a broad spectrum of Candida strains. Notably, these results were observed in fluconazole-resistant clinical isolates, suggesting that the therapeutic efficacy of this approach may be even greater against fluconazole-susceptible Candida species.

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Data Availability

All relevent data generated or analyzed during this study are available within the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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