Egyptian Journal of Medical Microbiology Volume 32 / No.3 / July 2023 41-49 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Effect of *Lactobacillus acidophilus* and *Lactobacillus johnsonii* on Growth, Phenotypic and Genotypic Expression of Virulence Factors of *Candida albicans* Causing Vulvovaginal Candidiasis

[1]Aliaa A. Abd El-Aala*, [2]Mohammed A.I. El-Kholy, [2]Alaa R. Awad, [3]Mohammed A. El Sharkawy

[1]Assistant lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt
[2]Professor of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Professor of Gynecology and Obstetrics, Faculty of Medicine, Cairo University, Cairo, Egypt

Key words:
L. acidophilus LA-5, L. johnsonii B-2178, virulence factors, C. albicans, vulvovaginal candidiasis

****Corresponding Author:***
Aliaa Ashraf Abd El-Aala, Assistant lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Al-Saray Street, El-Manial, Cairo, Egypt
Tel.: +201012530560
Aliaa_ashraf13@yahoo.com

ABSTRACT

Background: *Vulvovaginal candidiasis (VVC) causes significant morbidity among women of reproductive age, as well as a considerable financial burden on the health-care system. In the age of antibiotic resistance, using probiotic lactobacilli to treat VVC has become an appealing treatment approach.* ***Objective:*** *The goal of this research was to determine how L. acidophilus LA-5 and L. johnsonii B-2178 affect C. albicans growth, phenotypic and genotypic expression of virulence factors (biofilm and hyphal production) in females with VVC.* ***Methodology:*** *This study was conducted on 30 isolates of C. albicans recovered from 118 patients in the reproductive period, complaining of cheesy curd-like vaginal discharge and/or itching. The effect of L. acidophilus LA-5 and L. johnsonii B-2178 on growth of C. albicans isolates was determined. The effect of L. acidophilus LA-5 and L. johnsonii B-2178 cell-free supernatant (CFS) on* ***phenotypic expression of virulence factors (biofilm and hyphae formation) among C. albicans isolates*** *was determined. By using Quantitative Real-Time Reverse Transcription PCR (qRT-PCR), the effect of CFS of L. acidophilus LA-5 and L. johnsonii B-2178 on the transcription of C. albicans genes involved in the transition from yeast to hyphae and biofilm formation was determined.* ***Results:*** *Both L. acidophilus LA-5 and L. johnsonii B-2178 were effective in inhibiting C. albicans growth. The CFS of L. acidophilus LA-5 and L. johnsonii B-2178 significantly inhibited biofilm formation. The CFS of L. acidophilus LA-5 and L. johnsonii B-2178 reduced hyphae formation, but this reduction was not significant. The CFS of L. acidophilus LA-5 and L. johnsonii B-2178 significantly inhibited expression levels of virulence-related genes.* ***Conclusions:*** *L. acidophilus LA-5 and L. johnsonii B-2178 had growth inhibition effect, anti-biofilm effect, anti-hyphal effect and gene expression level inhibition on C. albicans isolates causing VVC.*

INTRODUCTION

C. albicans is a common microbe that can cause infections in both healthy and immunocompromised people. It is a member of the commensal flora of the mouth, vagina, urethra, stomach, and upper respiratory tracts[1,2]. Around 75% of all women of childbearing age are infected with VVC at least once in their lives, with 40–50% enduring at least one subsequent episode of infection, and 5–8% of women enduring at least four recurrent VVC episodes per year[3,4,5].

The first phase of *C. albicans* infection is attachment to host cells or medical devices, which is followed by hyphal development and biofilm formation[6]. The hyphal wall protein 1 gene (*HWP1*), agglutinin-like protein gene 3 (*ALS3*), and extent of cell elongation gene 1 (*ECE1*) are three genes in *C. albicans* that are critical for hyphal development and host cell attachment[7,8].

Due to the fact that *C. albicans* becomes less susceptible or insensitive to antifungal drugs in the biofilm, development of new therapeutic alternatives that prevent *C. albicans* adhesion, yeast-hyphal transition, and biofilm formation would be critical[9,10]. Drug resistance and side effects have limited the use of currently available antifungals as long-term preventive treatments for candida infections, so the use of probiotic lactobacilli to treat VVC has emerged as a promising treatment approach[11]. Lactobacilli are often used as probiotics to favor vaginal eubiosis and to counteract fungal and bacterial infections[12].

The aim of this study was to determine how one of the commercially available *Lactobacillus* species (*L. acidophilus* LA-5) and one of the healthy vaginal

Lactobacillus species (*L. johnsonii* B-2178) affect the growth, phenotypic and genotypic expression of the virulence factors (biofilm and hyphal formation) in *C. albicans* that causes VVC.

METHODOLOGY

This study is an observational descriptive study conducted to determine how one of the commercially available *Lactobacillus* species (*L. acidophilus* LA-5) and one of the healthy vaginal *Lactobacillus* species (*L. johnsonii* B-2178) affected growth, virulence factor phenotypic expression (biofilm and hyphal formation), and genotypic expression of virulence-related genes in *C. albicans* that causes VVC.

The study population included 118 female patients complaining of itching and/or cheesy curd-like vaginal discharge collected from patients attending the Gynecology and Obstetrics Out-patient Clinics of Kasr El-Aini University Hospitals at Cairo University, Faculty of Medicine during the months of January 2018 to July of the same year. The Ethical Committee of Cairo University, Faculty of Medicine authorized this work (has received the approval on 19/12/2017). All subjects gave their informed consent, and a complete clinical history was collected.

Specimen collection:

Vaginal swabs were obtained from the vaginal walls and fornix of all patients using a sterile disposable speculum.

Isolation and identification of *C. albicans*:

- Vaginal swabs were cultured on Sabouraud Dextrose Agar (SDA) and incubated for 24- 48 hours at 37ºC.
- Gram stain, germ tube test, subculture on Rice Tween-80 agar, and HiCrom *Candida* Differential agar (Himedia, India) were used to identify colonies suspected of being *C. albicans.*

Culturing of lactobacilli strains:

Department of Dairy Science, Faculty of Agriculture, Cairo University provided *L. acidophilus* (LA-5®) and *L. johnsonii* (B-2178). *L. acidophilus* LA-5 and *L. johnsonii* B-2178 were cultured on De Man, Rogosa and Sharpe (MRS) agar plates (Himedia, India) anaerobically using Anaerogas Pack (Himedia, India) at 37°C for 48 hours to obtain separate colonies.

Determination of the effect of lactobacilli on:

a) Growth of *C. albicans* using radial streak technique:

- Half McFarland of *L. acidophilus* LA-5 **and** *L. johnsonii* B-2178 prepared in MRS broth (Himedia, India) was inoculated on MRS agar plates by filling a circle in the plate's centre and were incubated anaerobically at 37°C for 48 hours.
- After 48 hours of anaerobic incubation, the plates were seeded with *Candida* strains (0.5 McFarland) by radial lines of inoculum from the plate's perimeter to the centre, and aerobically incubated.
- The inhibition zone size was measured after 24 hours of incubation at 37°C to determine the microbial interactions.
- By subtracting the circle diameter (CD, cm) of the *Lactobacillus* spreading zone from the inhibition zone diameter detected (IZD, cm), the growth inhibitory activity (GI) was estimated. GI = (IZD-CD)/ 2[13].

b) Phenotypic expression of virulence factors (biofilm and hyphae formation) among *C. albicans* isolates:

i. Biofilm formation using the co-culture method

- One hundred µl **of each** *Candida* strain (0.5 McFarland) **suspended in** brain heart infusion broth (BHIB) medium enriched with 0.25 percent glucose **plus** either 100 µl **of CFS of** *L. acidophilus* LA-5 **or** 100 µl **of CFS of** *L. johnsonii* B-2178 **were pipetted onto a** polystyrene flat-bottomed 96-well plates **and each isolate was tested in duplicate[14,15].**
- A positive control of 100 µl **of each** *Candida* strain (0.5 McFarland) **suspended** in BHIB supplemented with 0.25% glucose **plus** 100 µl **of MRS broth was used**. Sterile plane BHIB was used as a negative control **and** biofilm formation **was** determine**d (fig. 1) as described by ElFeky and Gohar[16]**. The optical density (OD) of each well was measured using an ELISA reader set at 540 nm**[14].**

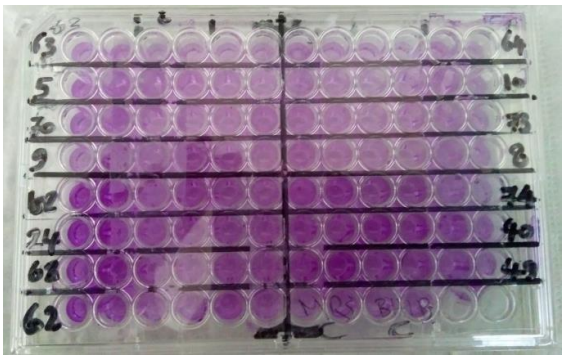

Fig. 1: The inhibitory effect of *L. acidophilus* LA-5 and *L. johnsonii* B-2178 on biofilm formation by *C. albicans* isolates.

ii. Hyphae formation using the hyphae-formation liquid medium assay

- Hyphal growth assay was performed by preparing *C. albicans* suspensions (0.5 McFarland) in Roswell Park Memorial Institute 1640 medium (RPMI 1640 medium) (Lonza, USA) containing 10% human serum (hyphae-inducing condition).
- A mixture of 900 µl of *C. albicans* suspensions (0.5 McFarland) and either 100 µl **of CFS of** *L. acidophilus* LA-5 **or** 100 µl **of CFS of** *L. johnsonii* B-2178 was placed in each well of a 24-well microplate and incubated at 37°C for 4 hours under aerobic conditions. A One hundred µl MRS broth instead of CFS plus 900 µl of *C. albicans*

suspensions (0.5 McFarland) was used as a positive control (fig. 2).

- Under a light microscope, the number of individual yeast cells vs the number of hyphae in the population was counted to determine the amount of inhibition of the yeast-to-hyphae transition. More than 100 cells were counted for each well. (Hyphae percent in Control – Hyphae percent in CFS)/Hyphae percent in Control × 100 was used to calculate the hyphae inhibition rate (%)[17].

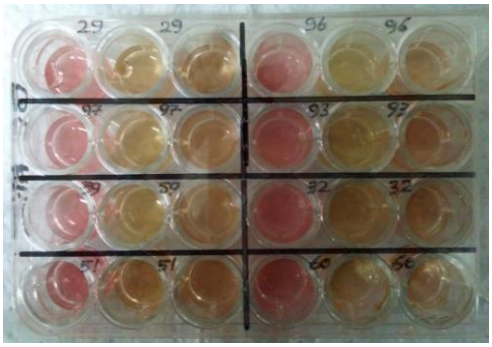

Fig. 2: Testing of hyphae formation among *C. albicans* isolates and the inhibitory effect of CFS *L. acidophilus* LA-5 and *L. johnsonii* B-2178 on it.

b) Genotypic expression of *C. albicans* isolates virulence factors (biofilm and hyphae formation):

- One hundred µl **of each** *Candida* strain suspension (0.5 McFarland) prepared in **RPMI 1640** with 10% human serum plus 100 µl **MRS broth were cultured at** 37°C for 24 hours to measure the levels of transcription of *C. albicans* genes related to yeast-to-hyphae transition and biofilm formation (*HWP1*, *ALS3* and *ECE1*).
- While for determining the effect of CFS of *L. acidophilus* LA-5 **and** *L. johnsonii* B-2178 on the transcription of these genes, the above was used but the 100 µl **MRS broth were replaced** by either 100 µl **of CFS of** *L. acidophilus* LA-5 **or** 100 µl **of CFS of** *L. johnsonii* B-2178[17].
- Gene expression levels of *HWP1*, *ALS3*, *ECE1* and *GSP1* (GTP-binding protein) (as a reference gene) were quantified by qRT-PCR using primer sequences and cycling conditions as described by **Sun et al.**[10] and **Wang et al.**[17] in StepOne Real Time PCR System (Applied Biosystems) at the department of Medical Biochemistry, Faculty of medicine, Cairo university as shown in table (1).

Table 1: List of primers used for qRT-PCR

Gene	Function	Primer sequence (5´–3´)	Reference
GSP1	a protein that binds to GTP (Reference gene or housekeeping gene)	F: TGAAGTCCATCCATTAGGAT R: ATCTCTATGCCAGTTTGGAA	**Sun et al.**[10] and **Wang et al.**[17]
HWP1	Hyphal cell wall protein	F: TGGTGCTATTACTATTCCGG R: CAATAATAGCAGCACCGAAG	
ALS3	Agglutinin-like protein	F: CTAATGCTGCTACGTATAATT R: CCTGAAATTGACATGTAGCA	
ECE1	Extent of cell elongation protein	F: GCTGGTATCATTGCTGATAT R: TTCGATGGATTGTTGAACAC	

The formula $R = 2^{-\Delta\Delta Ct}$ was used to calculate the normalized value of the expression level in comparison to the calibrator as described by **Wang et al.**[17].

ΔΔCt = sample ΔCt - average control group ΔCt

ΔCt = target gene Ct - housekeeping gene Ct

Statistical analysis:

The data were statistically analyzed using version 26 of the Statistical Package for Social Science (SPSS) software program. For quantitative variables, mean and standard deviation were used, whereas for qualitative variables, frequency and percentage were used. The Student's t-test was used to compare two groups statistically. One-way ANOVA was used to compare several groups, followed by the least significant difference test. P-values of less than 0.05 were deemed statistically significant, while those of less than 0.01 were deemed highly significant.

RESULTS

The present study included 118 patients in the reproductive period, complaining of cheesy curd-like vaginal discharge and/or itching. History taking (containing name, age, parity, and symptoms suggestive of vaginitis), gynecological examination (for evidence

of vulvovaginitis), and vaginal swabbing were taken from all patients.

Prevalence of VVC caused by *C. albicans* among the studied group:

Out of the 118 patients that were included in the study, 40 (34%) were positive for VVC, while 78 (66%) patients were negative. Out of the 40 *Candida* isolates, 30 (75%) isolates were identified as *C. albicans*, while 10 (25%) isolates were classified as non-albicans Candida.

Effect of lactobacilli on growth of *C. albicans* using radial streak method:

Effect of L. acidophilus LA-5

- **Out of the 30 *C. albicans* isolates, 23 (76.7%) were inhibited by** *L. acidophilus* LA-5 (fig. 3), while 7 (23.3%) were not inhibited.
- **The GI by** *L. acidophilus* LA-5 ranged from 0-13 mm (fig. 4), with a mean of 4 ± 4 mm.

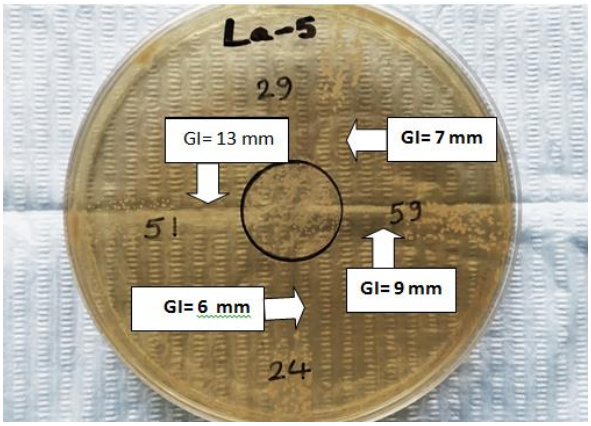

Fig. 3: Growth inhibition of 4 *C. albicans* isolates by *L. acidophilus* LA-5 by radial streak method.

Effect of L. johnsonii B-2178

- **Out of 30 *C. albicans* isolates, 16 (53.3%) were inhibited by** *L. johnsonii* B-2178, while 14 (46.7%) were not inhibited.
- **The GI by** *L. johnsonii* B-2178 ranged from 0-10 mm (fig. 4), with a mean of 3 ± 4 mm.

There was no statistically significant difference between **the GI of** *L. acidophilus* LA-5 and **of** *L. johnsonii* B-2178 on the 30 *C. albicans* isolates (P-value = 0.349)

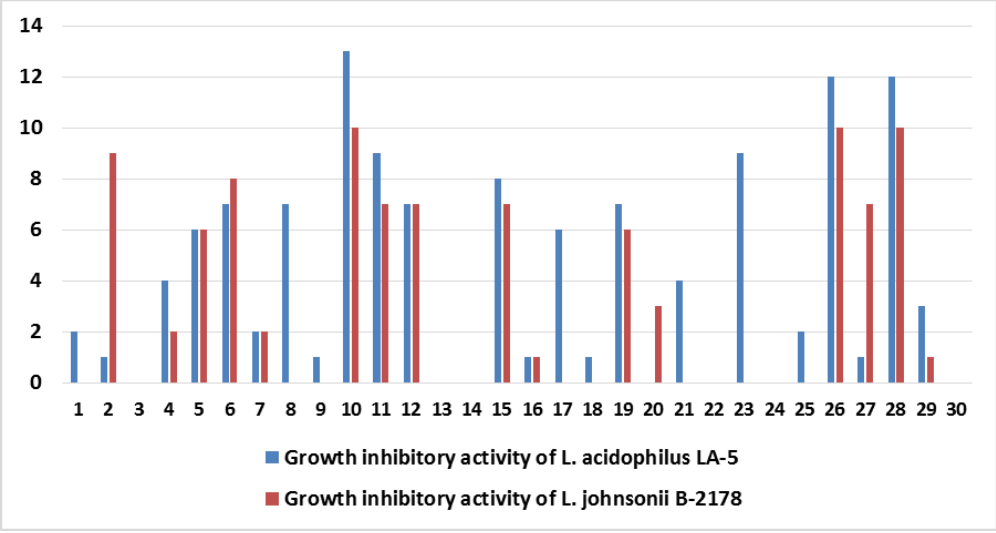

Fig. 4: GI **of** *L. acidophilus* LA-5 and *L. johnsonii* B-2178 on the 30 *C. albicans* isolates in (mm).

Effect of CFS of lactobacilli on expression of virulence factors of *C. albicans* isolates phenotypically:

a. Biofilm formation

Among the 30 *C. albicans* isolates, 27 (90%) were biofilm producers.

Effect of CFS of L. acidophilus LA-5:

- The OD levels in untreated *C. albicans* (control) ranged from 0.709 to 3.575, with a mean of 1.079 ± 0.523, while the OD levels in *C. albicans* treated with CFS of *L. acidophilus* LA-5 ranged from 0.6 to 0.958, with a mean of 0.716 ± 0.094.
- There was a statistically significant inhibition in OD levels between untreated *C. albicans* (control) and treated *C. albicans* with CFS of *L. acidophilus* LA-5 (P-value < 0.001) (fig. 5).

Effect of CFS of L. johnsonii B-2178:

- The OD levels in untreated *C. albicans* (control) ranged from 0.709 to 3.575, with a mean 1.079 ± 0.523, while the OD levels in *C. albicans* treated with CFS of *L. johnsonii* B-2178 ranged from 0.609 to 0.834, with a mean 0.711 ± 0.066.
- There was a statistically significant inhibition in OD levels between untreated *C. albicans* (control) and treated *C. albicans* with CFS of *L. johnsonii* B-2178 (P-value < 0.001) (fig. 5).

There was no statistically significant difference in OD inhibition between the effect of CFS of *L. acidophilus* LA-5 and that of *L. johnsonii* B-2178 on treated *C. albicans* (P-value = 1) (fig. 5).

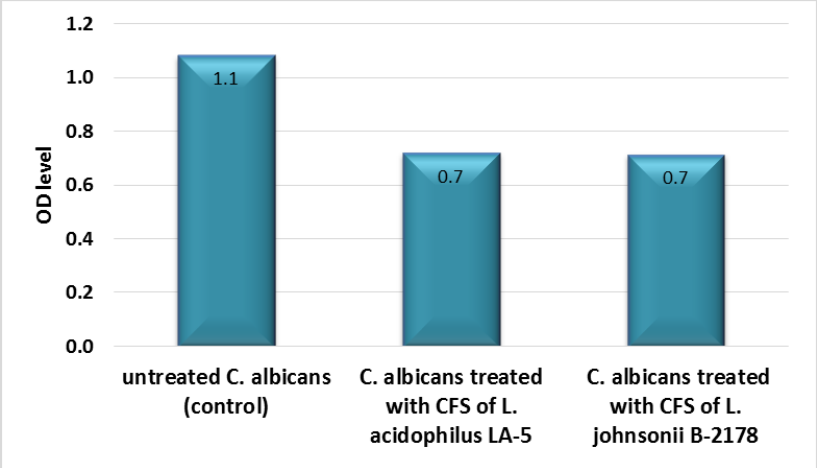

Fig. 5: Mean of OD levels in untreated *C. albicans* (control), in *C. albicans* treated with CFS of *L. acidophilus* LA-5 and in *C. albicans* treated with CFS of *L. johnsonii* B-2178.

b. Hyphae formation

Effect of CFS of L. acidophilus LA-5

- In the 30 *C. albicans* isolates, there was a reduction in hyphae percentage in treated *C. albicans* with CFS of *L. acidophilus* LA-5 compared to untreated *C. albicans*, however this reduction was not statistically significant (fig. 6 and 7).
- The hyphae inhibition rate (%) by CFS of *L. acidophilus* LA-5 ranged from 0.45 to 68, with a mean of 21.374 ± 18.36.

Effect of CFS of L. johnsonii B-2178

- In the 30 *C. albicans* isolates, there was a reduction in hyphae percentage in treated *C. albicans* with CFS of *L. johnsonii* B-2178 compared to untreated *C. albicans*, however this reduction was not statistically significant (fig. 6 and 7).
- The hyphae inhibition rate (%) by CFS of *L. johnsonii* B-2178 ranged from 0.6 to 66.74, with a mean of 22.735 ± 21.64.

There was no statistically significant difference in hyphae percentage reduction and hyphae inhibition rate between the effect of CFS of *L. acidophilus* LA-5 and that of *L. johnsonii* B-2178 on treated *C. albicans* (p-value = 0.545 and 0.794, respectively).

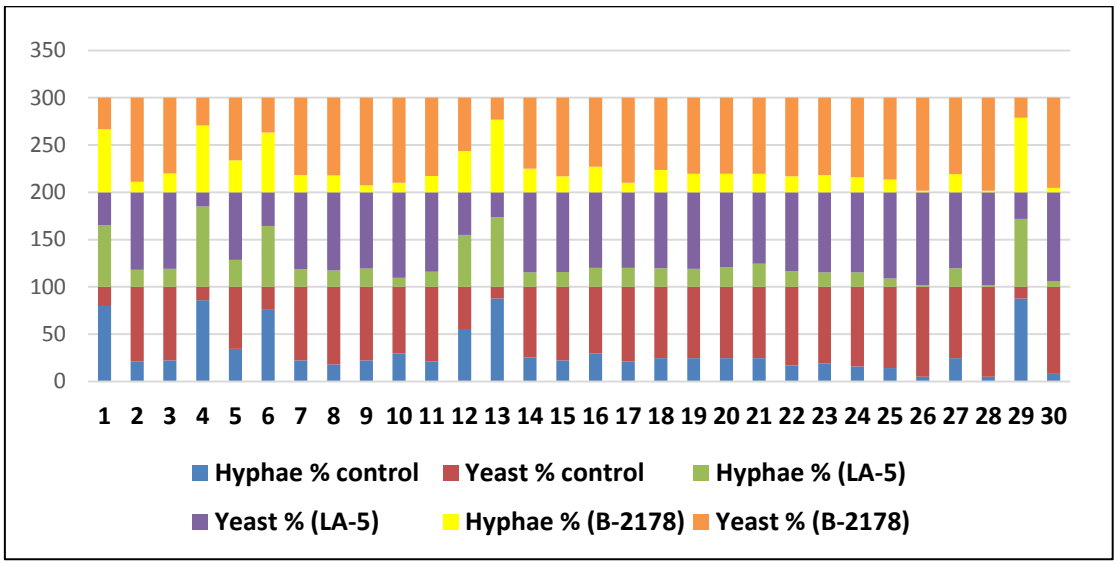

Fig. 6: Hyphae % and yeast % in untreated *C. albicans*, treated *C. albicans* with CFS of *L. acidophilus* LA-5 and treated *C. albicans* with CFS of *L. johnsonii* B-2178 of the 30 tested isolates.

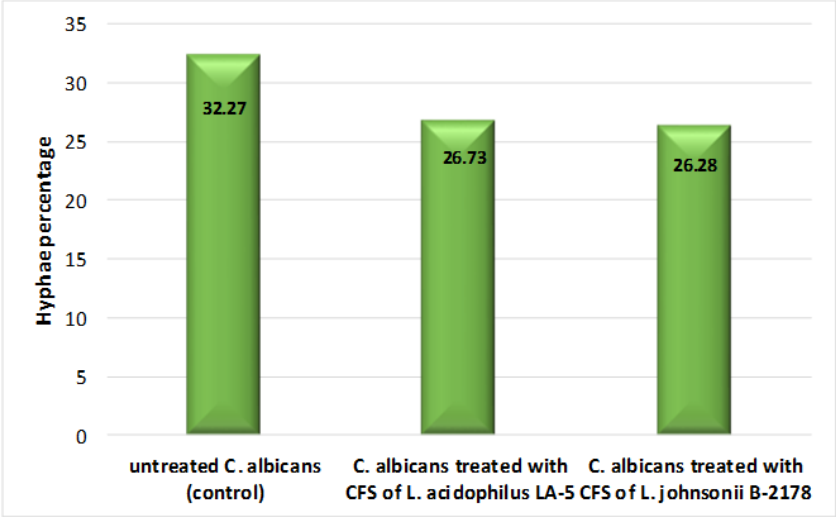

Fig. 7: Mean of hyphae percentage in untreated *C. albicans* (control), in *C. albicans* treated with CFS of *L. acidophilus* LA-5 and in *C. albicans* treated with CFS of *L. johnsonii* B-2178.

Effect of CFS of *L. acidophilus* LA-5 and *L. johnsonii* B-2178 on expression of virulence factors genes among *C. albicans* isolates:

Effect of CFS of L. acidophilus LA-5 on:

a. *ALS3* gene expression

- The expression levels in untreated *C. albicans* ranged from 0.627 to 2.361, with a mean of 1.0451 ± 0.348, while in treated *C. albicans* with CFS of *L. acidophilus* LA-5 ranged from 0.062 to 1.331, with a mean of 0.399 ± 0.267.

b. *ECE1* gene expression

- The expression levels in untreated *C. albicans* ranged from 0.62 to 1.44, with a mean of 1.032 ± 0.255, while in treated *C. albicans* with CFS of *L. acidophilus* LA-5 ranged from 0.091 to 1.954, with a mean of 0.436 ± 0.392.

c. *HWP1* gene expression

- The expression levels in untreated *C. albicans* ranged from 0.665 to 1.424, with a mean of 1.024 ± 0.227, while in treated *C. albicans* with CFS of *L. acidophilus* LA-5 ranged from 0.047 to 1.182, with a mean of 0.458 ± 0.32.

In the three genes, there was a statistically significant inhibition in expression levels between treated *C. albicans* with CFS of *L. acidophilus* LA-5 and untreated *C. albicans* (P-value < 0.001).

Effect of CFS of L. johnsonii B-2178 on:

a. *ALS3* gene expression
- The expression levels in untreated *C. albicans* ranged from 0.627 to 2.361, with a mean of 1.045 ± 0.348, while in treated *C. albicans* with CFS of *L. johnsonii* **B-2178** ranged from 0.08 to 0.853, with a mean of 0.4 ± 0.217.

b. *ECE1* gene expression
- The expression levels in untreated *C. albicans* ranged from 0.62 to 1.44, with a mean of 1.032 ± 0.255, while in treated *C. albicans* with CFS of *L. johnsonii* **B-2178** ranged from 0.074 to 0.944, with a mean of 0.315 ± 0.237.

c. *HWP1* gene expression
- The expression levels in untreated *C. albicans* ranged from 0.665 to 1.424, with a mean of 1.024 ± 0.227, while in treated *C. albicans* with CFS of *L. johnsonii* **B-2178** ranged from 0.03 to 1.634, with a mean of 0.411 ± 0.42.

In the three genes, there was a statistically significant inhibition in expression levels between treated *C. albicans* with CFS of *L. johnsonii* B-2178 and untreated *C. albicans* (P-value < 0.001). However, no statistically significant difference was detected in the expression levels inhibition between treated *C. albicans* with CFS of *L. acidophilus* LA-5 and CFS of *L. johnsonii* B-2178 (P-value = 1).

DISCUSSION

VVC causes significant morbidity among women of reproductive age, as well as a considerable financial burden on the health-care system due to increased vaginitis-related medical costs. It is one of the most common manifestations of *Candida* infection, estimated to affect approximately 75% of women at some point in their lifetime[3,4,5].

In the current study, 40 (34%) of the 118 individuals tested were positive for VVC SDA culture, while 78 (66%) tested negative. Thirty out of the 40 (75%) *Candida* isolates were identified as *C. albicans*, while the remaining 10 (25%) were classified as non-albicans Candida. In the study conducted by Kiasat et al.[18], the prevalence rate of VVC was (39.76%). *C. albicans* was the predominant species (71.1%), while (28.9%) were non-albicans Candida. Whereas, lower rate (23%) of VVC was detected in a study conducted by Farahyar et al.[19]. However, *C. albicans* was the predominant species (97%), while (3%) were non-albicans Candida.

In the present study, **out of the 30 *C. albicans* isolates, 23 (76.7%) were inhibited by** *L. acidophilus* LA-5, while 7 (23.3%) were not inhibited using the radial streak method, whereas **16 (53.3%) were inhibited by** *L. johnsonii* B-2178 and 14 (46.7%) were not inhibited. There was no statistically significant difference between **the growth inhibitory activity of** *L. acidophilus* LA-5 and **of** *L. johnsonii* B-2178 on the 30 *C. albicans* isolates (P-value = 0.349). Coman et al.[13] used the radial streak method to investigate the inhibitory activity of two probiotic strains, *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502, and their 1 : 1 combination, called SYNBIO, against eight *Candida* strains. The probiotics utilized in this investigation suppressed the growth of all *Candida* strains, with SYNBIO having the strongest inhibitory efficacy against *C. krusei* ISS4 when compared to the single strains.

In the present study, among the 30 *C. albicans* isolates, 27 (90%) were biofilm producers. Lower rates (40%), (20.6%) and (21.6%) were reported in studies conducted by ElFeky and Gohar[16], Tulasidas et al.[20] and Haggag[21], respectively.

This variation in biofilm production could be due to the complexity and diversity of the transcriptional control over processes as biofilm formation within pathogenic yeasts of the *Candida* genus, resulting in variation between species and strains within the same species[22].

In the current study, there was a statistically significant inhibition in OD levels between untreated *C. albicans* (control) and treated *C. albicans* with CFS of *L. acidophilus* LA-5 (P-value < 0.001) and CFS of *L. johnsonii* B-2178 (P-value < 0.001). **Orsi et al.[14] studied the impact of** crude filtrate supernatant fluids (CFSF) of **several *Lactobacillus* species (*L. rhamnosus, L. acidophilus, L. plantarum*, and *L. reuteri*) on two *C. albicans* strains biofilm and hyphal production.** *Lactobacillus* affected *Candida* biofilm production in a dilution- and time-dependent manner. They found consistent inhibitory effects in all the cases, as shown by the reductions in the OD values, strictly related to the CFSF dilutions. Three out of 4 CFSF (i.e., those from *L. acidophilus*, *L. plantarum* and *L. reuteri*) caused significant OD reductions ($p < 0.05$), always detectable at the 1:4 dilution, against both *Candida* strains, at 24 and 48 h. Also Rossoni et al.[23] investigated the influence of *L. rhamnosus* 5.2, *L. fermentum* 20.4, and *L. paracasei* 28.4 supernatants on *C. albicans* ATCC 18804 and *C. albicans* CA230S biofilm. In comparison to the control group, the biofilms generated by *C. albicans* ATCC 18804 in the presence of their supernatants showed a considerable reduction (P-value = 0.0153, 0.0303 and 0.0121, respectively). While in case of *C. albicans* CA230S, the biofilm generated in the presence of *L. paracasei* strain 28.4 supernatant showed a considerable reduction in comparison to the control group (P-value = 0.0049).

In the present study, all *C. albicans* isolates, showed a reduction in hyphae percentage when treated with CFS of *L. acidophilus* LA-5 and CFS of *L. johnsonii* B-2178 compared to untreated *C. albicans*, however this reduction was not statistically significant. The mean of hyphae inhibition rate (%) by CFS of *L. acidophilus* LA-5 was 21.374 ± 18.36%, whereas for CFS of *L.*

johnsonii B-2178 it was 22.735 ± 21.64%. There was no statistically significant difference in hyphae percentage reduction and hyphae inhibition rate between the effect of the CFS of the two *Lactobacillus* species (p-value = 0.545 and 0.794, respectively). Orsi et al.[14] investigated the effect of lactobacilli CFSF on yeast-to-hyphal form transition. Quantification of the yeast cells revealed that only about 10% of *C. albicans* still retained the yeast-like structure (90% hyphae) in the control groups, while in parallel studied groups exposed to the CFSF yeast-cell pecentage increased and ranged between 50% and 80% (between 50% and 20% hyphae, respectively). They reported that the CFSF from *L. acidophilus* was more effective in preventing dimorphic transition (as indicated by the highest percent of yeast-like cells) with respect to *L. rhamnosus* CFSF. Also, Wang et al.[17] found that the yeast-to-hyphae transition *of C. albicans* treated with CFS from *L. crispatus*, *L. gasseri*, and *L. jensenii* was significantly reduced and had hyphae inhibition rates of 88.3 ± 3.02%, 84.9 ± 6.0%, and 81.9 ± 6.2%, respectively. Allonsius et al.[24] found that hyphae inhibition rates varied greatly among the studied strains, ranging from 91 % (*L. casei* AMBR2) to 14 % (*L. plantarum* WCFS1). Higher reduction in hyphae percentage in these studies compared to the present study may be due to the fact that these studies investigated the effect of different *Lactobacillus* species other than those used in the present study on few *C. albicans* strains.

In the present study, in the three genes (*ALS3*, *ECE1* and *HWP1*) there was a statistically significant inhibition in expression levels between untreated *C. albicans* and treated *C. albicans* with CFS of *L. acidophilus* LA-5 (P-value < 0.001) and CFS of *L. johnsonii* B-2178 (P-value < 0.001). However, no statistically significant difference was detected in the expression levels inhibition between treated *C. albicans* with CFS of *L. acidophilus* LA-5 and CFS of *L. johnsonii* B-2178 (P-value = 1). James et al.[25] determined the effects of probiotic CFS on the transcription of *C. albicans* genes related to adhesion, biofilm formation and virulence. The combined supernatants of *L. plantarum* SD5870 and *L. helveticus* CBS N116411 considerably lowered the expression of *C. albicans* genes involved in the yeast–hyphae transition, such as *ALS3* by 70% (P < 0.0001) and *HWP1* by > 99% (P < 0.0001), according to qRT-PCR results. In the study conducted by Ribeiro et al.[26], the expression levels of the adhesion genes (*ALS3* and *HWP1*) in interaction with *L. rhamnosus* supernatant were considerably downregulated (P = 0.0001) compared to the control, with 0.09-fold decrease in *ALS3* and 0.14-fold drop in *HWP1*. Wang et al.[17] showed that *L. crispatus* CFS suppressed the expression of hyphae-specific genes *ALS3* (0.140-fold), *HWP1* (0.075-fold), and *ECE1* (0.045-fold). The results of the qPCR assay revealed that the isolates *L. rhamnosus* 5.2, *L. fermentum* 20.4, and *L. paracasei* 28.4 influenced *C. albicans* biofilm formation by dramatically downregulating the expression of the *ALS3* and *HWP1* genes, according to Rossoni et al.[23].

CONCLUSION

It may be deduced from this research that *C. albicans* was the most common cause of VVC. Both *L. acidophilus* LA-5 and *L. johnsonii* B-2178 were effective in inhibiting *C. albicans* growth. Most of *C. albicans* isolates in this study exhibited biofilm formation, while all the isolates exhibited hyphal formation. The CFS of *L. acidophilus* LA-5 and *L. johnsonii* B-2178 significantly inhibited biofilm formation by *C. albicans* isolates. The CFS of *L. acidophilus* LA-5 and *L. johnsonii* B-2178 reduced hyphae formation, but this reduction was not significant. The CFS of *L. acidophilus* LA-5 and *L. johnsonii* B-2178 significantly inhibited expression levels of virulence-related genes; *ALS3*, *ECE1* and *HWP1*.
To the best of my knowledge, no conflict of interest, financial or others exist.

Consent for publication
Not applicable
Availability of data and material
Data are available upon request
Competing interests
The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article none.
Funding
Authors did not receive any grants from funding agencies

REFERENCES

1. Whaley S, Berkow E, Rybak J, et al. Azole antifungal resistance in *C. albicans* and emerging non-albicans *Candida* species. Front. Microbiol 2017; 7:2173.
2. Ciurea CN, Kosovski I, Mare AD, et al. *Candida* and Candidiasis-Opportunism Versus Pathogenicity: A Review of the Virulence Traits. Microorganisms 2020; 8: 857.
3. Peters BM, Yano J, Noverr MC, et al. *Candida* vaginitis: when opportunism knocks, the host responds. PLoS Pathog. 2014; 10:e1003965.
4. Cassone A. Vulvovaginal *Candida albicans* infections: pathogenesis, immunity and vaccine prospects. BJOG 2015; 122:785–94.
5. Nsenga L and Bongomin F. Recurrent *Candida* Vulvovaginitis. *Venereology* 2022; *1*:114-123.

6. Dos SAP, McArthur CP and Africa CW. Multi-drug resistant oral *Candida* species isolated from HIV positive patients in South Africa and Cameroon. Diagn. Microbiol. Infect. Dis. 2014; 79:222–27.
7. Fan Y, He H, Dong Y, et al. Hyphae-specific genes HGC1, ALS3, HWP1, and ECE1 and relevant signaling pathways in *Candida albicans*. Mycopathologia 2013; 176:329–35.
8. Vila T, Romo JA, Pierce CG, et al. Targeting *Candida albicans* filamentation for antifungal drug development. Virulence 2016; 7:1–9.
9. Inigo M, Peman J and Del PJ. Antifungal activity against *candida* biofilms. Int J Artif Organs 2012; 35: 780–91.
10. Sun L, Liao K and Wang D. Effects of Magnolol and Honokiol on Adhesion, Yeast-Hyphal Transition, and Formation of Biofilm by *Candida albicans*. PLoS ONE 2015; 10(2):e0117695.
11. Matsubara VH, Bandara HMHN, Mayer MPA, et al. Probiotics as Antifungals in Mucosal Candidiasis. Clinical Infectious Diseases® 2016; 62(9):1143–53.
12. Spaggiari L, Sala A, Ardizzoni A, et al. *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, and *L. reuteri* Cell-Free Supernatants Inhibit *Candida parapsilosis* Pathogenic Potential upon Infection of Vaginal Epithelial Cells Monolayer and in a Transwell Coculture System *In Vitro*. Microbiol Spectr 2022; 10(3):e0269621.
13. Coman MM, Verdenelli MC, Cecchini C, et al. In vitro evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501®, *Lactobacillus paracasei* IMC 502 and SYNBIO against pathogens. Journal of Applied Microbiology 2014; 117(2):518–27.
14. Orsi CF, Sabia C, Ardizzoni A, et al. Inhibitory effects of different lactobacilli on *Candida albicans* hyphal formation and biofilm development. J. Biol. Regul. Homeost. Agents 2014; 28:743–52.
15. Oliveira VM, Santos SS, Silva CR, et al. *Lactobacillus* is able to alter the virulence and the sensitivity profile of *Candida albicans.* J. Appl. Microbiol. 2016; 121:1737–44.
16. ElFeky D and Gohar N. Evaluation of Virulence Factors of *Candida*: Egypt. J. Med. Microbiol 2016; 25(1):27-36.
17. Wang S, Wang Q, Yang E, et al. Antimicrobial Compounds Produced by Vaginal *Lactobacillus crispatus* Are Able to Strongly Inhibit *Candida albicans* Growth, Hyphal Formation and Regulate Virulence-related Gene Expressions. Front. Microbiol 2017.; 8:564.
18. Kiasat N, Rezaei-Matehkolaei A, Mahmoudabadi AZ, et al. Prevalence of Vulvovaginal Candidiasis in Ahvaz, Southwest Iran: A Semi-Large Scale Study. Jundishapur J Microbiol. 2019; 12(3):e89815.
19. Farahyar S, Izadi S, Razmjou E, et al. Low prevalence of antifungal resistant *Candida africana*, in the *C. albicans* complex causing vulvovaginal candidiasis. Heliyon 2020; 6:e03619.
20. Tulasidas S, Rao P, Bhat S, et al. A study on biofilm production and antifungal drug resistance among *Candida* species from vulvovaginal and bloodstream infections. Infection and Drug Resistance 2018; 11:2443-48.
21. Haggag EMMH. Mannose Binding Lectin Gene Polymorphism versus Microbial Virulence in the Pathogenesis of Vulvovaginal Candidiasis. Thesis submitted for fulfillment of the MD degree in Medical Microbiology and Immunology 2020, Faculty of Medicine, Cairo University.
22. Cavalheiro M and Teixeira M. *Candida* Biofilms: Threats, Challenges, and Promising Strategies. Front. Med. 2018; 5:28.
23. Rossoni RD, de Barros PP, de Alvarenga JA, et al. Antifungal activity of clinical *Lactobacillus* strains against *Candida albicans* biofilms: identification of potential probiotic candidates to prevent oral candidiasis. Biofouling 2018; 34(2):212-25.
24. Allonsius CN, Vandenheuvel D, Oerlemans EFM, et al. Inhibition of *Candida albicans* morphogenesis by chitinase from *Lactobacillus rhamnosus* GG. Scientific Reports 2019; 9:2900.
25. James KM, MacDonald KW, Chanyi RM, et al. Inhibition of *Candida albicans* biofilm formation and modulation of gene expression by probiotic cells and supernatant. J. Med. Microbiol. 2016; 65: 328–36.
26. Ribeiro FC, de Barros PP, Rossoni RD, et al. *Lactobacillus rhamnosus* inhibits *Candida albicans* virulence factors *in vitro* and modulates immune system in *Galleria mellonella*. J Appl Microbiol. 2017; 122:201–11.