ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2026, 11 (3): 66-78 doi: 10.28978/nesciences.263006

Antifungal and Antibiofilm Effects of LL-37 Against Candida albicans Isolated from Pregnant Women with Vulvovaginal Candidiasis: Impact on FKS1 Gene Expression

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Abstract

Vulvovaginal Candidiasis (VVC) is a frequently observed condition during pregnancy caused by the organism known as *Candida albicans*. The ability of *Candida* to form a biofilm and its capability of being resistant to antifungal medication makes treating this condition complicated. Therefore, the purpose of this study was to evaluate the antifungal and antibiofilm properties of the human antimicrobial peptide, LL-37, against *Candida* isolated from pregnant women with VVC; also, to investigate whether LL-37 affects the expression of the *FKS1* gene. Vaginal swabs were cultured from 150 symptomatic pregnant women in Baghdad. *Candida* species were identified using the chromogenic medium, the VITEK-2 system, and PCR for *18S rRNA*. The susceptibility of each isolate to antifungal agents was determined using the disk diffusion method. Biofilm formation was measured using the microtiter plate method. The MIC of LL-37 was determined using the broth microdilution method according to CLSI M27-A3 guidelines. Briefly, LL-37 was serially diluted two-fold in RPMI-1640 medium (pH 7.0) in 96-well plates at concentrations ranging from 7.8 to 500 µg/mL. A standardized inoculum of 0.5–2.5 × 10^3 CFU/mL was added to each well and plates were incubated at 35°C for 48 hours. The MIC was defined as the lowest concentration that inhibited visible fungal growth. The expression of the *FKS1* gene was evaluated using RT-qPCR. A total of 90 *Candida* isolates represented by 33.3% were identified as *C. albicans*. Of the 30 *C. albicans* isolates that were identified, 53.3% produced a thick biofilm and 60% were resistant to fluconazole. LL-37 demonstrated a minimum inhibitory concentration (MIC) against *Candida albicans* ranging from 62.5 to 250 µg/ml. Further testing established that the three separate isolates tested (each represented a different strain of *Candida albicans*) had their FKS1 expression significantly downregulated following LL-37 treatment by 48%, 66%, and 95%, respectively ($p < 0.05$). These results demonstrate that LL-37 is a novel agent that may be used to treat vulvovaginal candidiasis in pregnant women by exhibiting potent antifungal effects and antibiofilm capabilities against C. albicans. Additionally, down-regulation of the *FKS1* gene is one of the mechanisms through which LL-37 may exhibit its antifungal effects.

Keywords

LL-37, Candida albicans, vulvovaginal candidiasis, pregnancy, biofilm, FKS1 gene, antifungal resistance, antimicrobial peptide.

Available online: 01/09/2026

Introduction

Vulvovaginal candidiasis (VVC) is one of the most common vaginal infections worldwide, affecting approximately 75% of women at least once in their lifetime (Sobel & Vempati, 2024). During

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pregnancy, women's susceptibility to VVC increases significantly due to elevated estrogen and progesterone levels. These hormones increase glycogen levels in the vagina, promoting Candida adhesion to the vaginal epithelium (Arastehfar *et al*., 2021). While VVC is usually not life-threatening for the mother, untreated VVC can lead to complications such as chorioamnionitis and premature rupture of membranes, and increase the risk of oral thrush or systemic infection in the newborn (Ponomarova *et al*., 2023). *Candida albicans* is the most common causative agent of VVC, accounting for the majority of clinical isolates. A key factor in the persistence and pathogenicity of vulvovaginal candidiasis (VVC) is its ability to form biofilms complex, structured clumps of cells enclosed in the extracellular matrix (Ma et al., 2024). Biofilms form a physical barrier, blocking attacks from host immune cells and binding to antifungal drugs, leading to drug resistance up to 1000 times higher than that of free planktonic cells (Rodriguez-Castano et al., 2023). This protective environment results in high recurrence rates of fungal vaginitis (VVC) and decreased efficacy of traditional triazole drugs. The increasing resistance to antifungal drugs has prompted research to focus on antimicrobial peptides (AMPs) as alternative therapies. LL-37, the only human member of the cathelicidin family, is a cationic peptide primarily secreted by epithelial cells and neutrophils (Rodriguez-Castano *et al*., 2023). In addition to its role in the innate immune response, LL-37 exhibits potent antifungal and antibiofilm activity by disrupting fungal cell membranes and interfering with cellular signaling pathways essential for hyphal growth (Ma *et al.*, 2024).

At the molecular level, the fungal cell wall is an important target for the immune system and drug intervention. The *FKS1* gene is particularly important because it encodes the catalytic subunit of β-D-glucan synthase, an enzyme responsible for synthesizing core structural components of the Candida cell wall (Yadav *et al*., 2024). Mutations or altered expression of the *FKS1* gene are directly associated with reduced susceptibility to echinocandins and play a role in the structural integrity of the biofilm matrix (Ben-Ami & Kontoyiannis, 2012). Many studies have shown that antimicrobial peptides (AMPs) may weaken fungal structure and inhibit biofilm formation by regulating the expression of genes related to cell wall synthesis (Yadav *et al*., 2024). Numerous local studies have also indicated that antimicrobial peptides possess antimicrobial and antibiofilm activities (Aziz and Ghaima, 2025; Khalaf and Ghaima, 2026). Although the properties of LL-37 are well-known, its specific efficacy against

Notably, the specific efficacy of LL-37 against clinical isolates from pregnant women, who present unique hormonal and immunological conditions, remains insufficiently explored. This research gap provides the novelty and justification for the current study. The objective of this research was to explore the antifungal properties of LL-37 against *Candida albicans* associated with VVC when isolated from pregnant women, as well as to analyze its efficacy at inhibiting biofilm formation by this organism. The goal of this study was to determine whether further studies evaluating the ability of LL-37 to act as a potential new topical treatment for VVC are warranted.

Materials and methods

Isolation and Identification of Candida species

Between September 2024 and March 2025, researchers collected vaginal swab samples from 150 patients presenting with symptoms of vulvovaginal candidiasis (VVC) at three hospitals in Baghdad, Iraq, and isolated *Candida* from them. Samples were collected by gynecologists. Infection criteria included vulvar and vaginal itching, edema, erythema, and clumps of vaginal discharge adhering to the vaginal wall. Vaginal swabs were inoculated onto Sabouraud dextrose agar and Hi Crome™ Candida identification agar (Himedia, India) and incubated at 37°C for 48 hours. *Candida* species were identified using the VITEK-2 system based on colony color and characteristics, microscopic examination, and biochemical tests.

Testing for Antifungal susceptibility

Inoculate *Candida albicans* colonies into 5 ml of sterile saline and turbidity visually adjusted to 0.5 McFarland standard. A sterile cotton swab was dipped into the adjusted suspension and streaked evenly onto Mueller-Hinton Agar (MHA) to form a uniform lawn. Assess antifungal susceptibility using the disk diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (Brandolt *et al*., 2017). Recommended drugs include fluconazole (10 µg), nystatin (50 IU), and amphotericin B (100 IU). Incubate the disks at room temperature (35 °C) for 24 hours before evaluation. Measure the diameter of the inhibition zone on each antifungal drug disk in

millimeters using a ruler. CLSI standards are used to interpret all antifungal drug susceptibility (susceptible S, dose-dependent susceptibility SDD, and resistance R) (Table 1).

Table 1. Interpretation of antifungal zone sizes for disk diffusion method.

Anti-fungals discs	Disk Concentration	zone diameter (mm)		
		S	SDD	R
Amphotericin B	100 U	≤10	10-14	≥15
Fluconazol	10 µg	≥17	14-16	≤10
Nystatin	50 U	≤14	18-15	≥13

SDD stands for susceptible dose dependent, S sensitive, and R stands for resistant Biofilm formation detection.

The *Candida albican*'s colonies were cultured in Sabouraud Dextrose Agar at 35 C° for a total of 18 hours. The *Candida albican*'s suspension was then prepared to a concentration of 10^6 cells/mL in a Muller-Hinton Medium. Each well was washed with phosphate buffered saline (PBS) and then allowed to dry for 45minutes before being stained with 0.4% crystal violet. After washing each well 4times with sterile distilled water, the wells were treated with 95% ethanol and incubated for an additional 45minutes. Then, 100µL of solution from each well was transferred to a new well and the absorbance was measured at 595nm using a micro-plate reader. The absorbance values of the control samples were then subtracted from the respective absorbance values of the test wells (Jin *et al*., 2003). The calculated optical limit is obtained by adding three standard deviations of the negative control with the mean of the negative control-OD to obtain the cumulative value of the optical limit.

OD at a certain time point (t) was calculated using the following formula:

OD_c = average OD_{nc} + (3 x SD_{nc}).

There are four categories of biofilm density: 1.) No biofilm - ODC or greater; 2.) Slightly positive for biofilm - between 2 & ODC; 3.) Moderate to positively identified for biofilm between ODC & 2 x ODC; and 4.) Intensely positive for biofilm - greater than 4 x ODC (Stepanovic *et al*., 2000).

Molecular Study for the Isolated Candida albicans

Candida DNA Extraction

DNA was extracted from Candida albicans isolates using the Promega DNA Extraction Kit (Promega, USA) according to the manufacturer's instructions.

Candida albicans Identification

The extracted DNA was amplified using *Candida albicans*-specific primers. Table 1 lists the primers used for the 18S rRNA gene. The amplification procedure was identical to the previously described method (Zhang et al., 2020).

Detection *FKS1* gene by PCR

All amplification protocols for the research genes were strictly performed according to the previously described methods. All reagents used for amplification were purchased from Promega, with primers used in quantities of 2.5 µl and extracted DNA in quantities of 5 µl (Zhang *et al*., 2020). Separation was performed using the 100-base DNA molecular weight standard (Promega, USA) and electrophoresis on a 1.5% agarose gel at 80 V for 1.5 hours.

Table 2. Sequences of the primers use to amplify *FKS1* and *18S rRNA* Genes fragments

Primer Name	Sequence 5`-3`	Annealing Temp. (˚C)
FKS1-F1719	CATTGCTGTGGCCACTTTAG	57
FKS1-R2212	GATTTCCATTTCCGTGGTAGC	
18S Rrna-F	TCGACCCCGGAGAAGGA	60
18S Rrna-R	GCCTGCTGCCTACCTTGGA	

Revelation of PCR products

The amplification products were separated by electrophoresis on a 1.5% agarose gel with 1×Tris-alkali-boric acid-EDTA buffer at 80 V for 1 hour and 30 minutes. DNA molecular weight standards (100 bp)

were stained under UV light. The PCR products were then developed under UV light with ethidium bromide (BET) (0.5 µg/ml).

Quantitative Analysis of FKS1 Gene Expression (RT-qPCR)
This is the core method for measuring the effect of LL-37 on FKS1 transcriptional levels.

1. Experimental Setup:
Sample Groups: Three representative Candida albicans isolates were selected for FKS1 expression analysis based on predefined inclusion criteria: (1) strong biofilm-forming ability (OD ≥ 4× ODc), (2) confirmed fluconazole resistance, and (3) successful FKS1 gene amplification by PCR. The three selected isolates (237G, 237R, 356) collectively represented the range of MIC values observed across all C. albicans isolates, ensuring diversity in antifungal susceptibility profiles. Two treatment groups were set up for each isolate:
Control Group (Untreated): Labeled as C237G, C237R, C356.
Treatment Group: Labeled as 237G, 237R, 356 (treated with LL-37, at the determined MIC concentration for each isolate).
Biological Replications: All qRT-PCR reactions were performed in biological triplicates (n = 3) to ensure statistical reliability. Results are presented as mean ± standard deviation.

2. RNA Extraction and cDNA Synthesis:
RNA Extraction and cDNA Synthesis: Total RNA was extracted from Candida albicans isolates (treated and untreated control groups) using TRIzol Reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA quality and quantity were assessed using a NanoDrop spectrophotometer (A260/A280 ratio ≥1.8). DNase I treatment (Promega, USA) was performed to eliminate genomic DNA contamination. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the GoScript Reverse Transcription System (Promega, USA) with random primers, following the manufacturer's protocol. The cDNA was stored at −20°C until further use.

3. Quantitative Real-Time PCR (qRT-PCR):
Target Gene: FKS1 (using the same primers as in Part 1 or a similar set of qPCR primers – not explicitly stated). Internal Reference Gene (Normalized Gene): 18S rRNA gene. Used to normalize expression data.
Method: Comparative CT method (2-ΔΔCT).
qRT-PCR Protocol: Quantitative real-time PCR was performed using the GoTaq qPCR Master Mix (Promega, USA) in a total reaction volume of 20 µL, containing 10 µL of 2× master mix, 1 µL of each primer (10 µM), 2 µL of cDNA template, and 6 µL of nuclease-free water. The cycling conditions were as follows: initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. All reactions were run in triplicate on a Bio-Rad CFX96 Real-Time PCR System. The 18S rRNA gene was used as the endogenous reference (housekeeping gene) to normalize FKS1 expression data.

4. Data Analysis (Fold Change Calculation):
Analysis is performed using the ΔΔCT method. Refer to Table 4 in the article for the steps: Step 1 (Calculating ΔCT): For each sample, subtract the CT value of 18S rRNA from the CT value of FKS1.
ΔCT = CT(FKS1) - CT(18S rRNA)
Control group C237G Example: 30.73 - 21.35 = 9.38
Step 2 (Calculate ΔΔCT): Subtract the control group's ΔCT value from the treatment group's ΔCT value.
ΔΔCT = ΔCT(treatment group) - ΔCT(control group)
Step 3 (Calculate fold change - expression ratio): Use Formula 2 - ΔΔCT.

5. Interpretation of Results (from Table 4):
Sample | Change (2 - ΔΔCT) | Percentage Reduction | Interpretation
A change value of 1.00 (all controls) indicates no change compared to baseline.
A change value less than 1.00 indicates downregulated gene expression (decreased gene expression) (Livak and Schmittgen, 2001).

Statistical Analysis

All experiments were performed in triplicate (n=3) and data are expressed as mean ± standard deviation (SD). Statistical comparisons between treated and control groups were performed using Student's t-test. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for multiple group comparisons. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, USA). Gene expression fold-change data were analyzed using the $2^{-\Delta\Delta CT}$ method and statistical significance of differences in ΔCT values between treated and untreated groups was assessed by independent samples t-test.

Results and discussion

Isolation and Identification of Candida

The isolation and identification of *Candida* species were based on their morphological characteristics (direct microscopic observation, Gram staining), culture properties, and biochemical characteristics. Swabs were inoculated onto HiChrom Candida identification agar and SDA (continuous identification agar) for culture. Since different Candida species exhibit different colors on nutrient media, chromogenic media are commonly used for the direct and rapid identification of yeasts, which confirmed the effectiveness of HiChrom Candida agar. Strains were identified using germ tube assays, cornstarch agar morphology, and HiChrom agar. All *Candida* isolates showed good growth after 48 hours of culture see Figure 1.

Figure 1. *Candida albicans* (green) colonies on HIChrom Candida identification agar after 48 hours of incubation at 37°C.

Hichrome Candida agar is a practical and rapid method for identifying Candida species, even in resource-constrained situations (Rajesh *et al*., 2016). Chromagar Candida medium has proven particularly useful in clinical mycology for detecting mixed yeast cultures and directly identifying *Candida albicans* (Faraj *et al*., 2020). Sabouraud dextrose agar (SDA) is a nutrient medium for fungi (yeasts, molds). In clinical samples, the pH needs to be raised to 5.6 to promote fungal growth while slightly inhibiting bacterial growth. Antimicrobial agents can be added to enhance antimicrobial efficacy. Chloramphenicol has been used as a screening agent to prevent the proliferation of competing microorganisms while successfully isolating Candida species. Incubation is performed at 37°C for 24 hours (Kaur *et al.*, 2024).

The formation of germ tubes

Atypical germ tube tests can produce germ tubes, which often indicate the presence of diagnostic Candida albicans (Figure 2). It is recommended to examine at least five clear germ tubes before confirming a positive isolate. This is because there are many similarities between *Candida dublin* and the closely related *Candida albicans*. Microscopic morphology, the ability to form germ tubes in serum,

and the formation of budding spores with pseudohyphae, fungal hyphae, and chlamydospores are all routine methods for identifying Candida albicans (Jan *et al*., 2017).

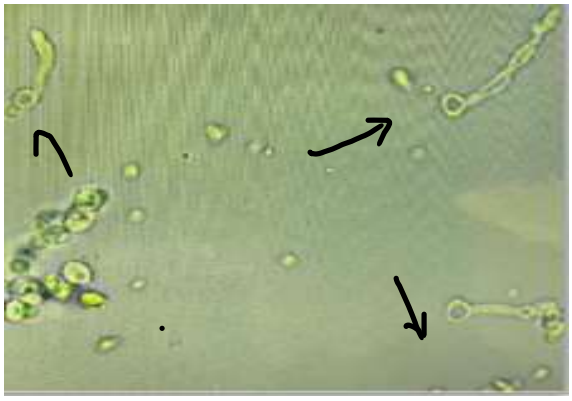

Figure 2. *Candida albicans* positive test in germ tube (direct microscopic examination at40x).

Biochemical Assays

Identification of isolates was performed using standard growth-based methods and the API 20 kit. *Candida albicans* was identified using API 20C Aux (bioMérieux, France). The API 20C Aux Candida identification system utilizes a carbohydrate assimilation pattern to distinguish different species within the Candida genus. Results showed positive growth in vaginal swab cultures on the API 20C Aux chromatogram, identifying 90 *Candida* isolates (60%) (Table 3), including 30 *Candida albicans*, 14 *Candida krusei*, 22 *Candida glabrata*, and 24 *Candida tropicalis*, all isolated from clinical vaginal swab samples.

Table 3. The prevalence of *Candida* species among 150 vaginal swabs from females' patients.

Candida Spp.	**No. of Isolates**	**%**
C. albicans	30	33.3%
C. glabrata	22	24.4%
C. Krusei	14	15.5%
C. tropicalis	24	26.6%
Total	90	100%

Various yeasts can act as opportunistic pathogens in people with weakened or suppressed immune systems (22-23). A local study tested vaginal swab samples from 97 women suspected of having vaginitis, and the results showed that 67 samples were positive and 30 were negative. The *Candida* species isolated included: *Candida albicans* (46 strains, 68.65%), *Candida tropicalis* (11 strains, 16.41%), *Candida paracanthosis* (7 strains, 10.44%), and *Candida kefir* (3 strains, 4.47%) (Hasan, 2017).

Test for Antifungals susceptibility

The resistance of 30 *Candida albicans* isolates to commonly used antifungal agents was determined using the disk diffusion method. Antifungal susceptibility testing results showed that *Candida albicans* was highly susceptible to amphotericin B (70%), while its resistance to fluconazole was high (60%) and nystatin was moderate (40%) according to CLSI standards. The antifungal susceptibility test results of 30 *Candida albicans* isolates are summarized in Table 4.

Table 4. Candida albicans antifungal susceptibility patterns

Antibiotic	**Concentration (microgram\disc)**	**Resistant**	**Intermediate**	**Sensitive**
Fluconazol	10	18 (60%)	3 (10%)	9 (30%)
Nystatin	50 U	12 (40%)	2 (6.6%)	16 (53.3%)
Amphotericin B	100 U	8 (26.6%)	1 (3.3%)	21 (70%)

Antifungal resistance is a serious problem and is growing at an alarming rate in the treatment of *Candida* infections (Hashemi *et al*., 2019). Azole antibiotics are commonly used for treatment, but due to their antifungal properties, Candida has developed resistance not only to azoles but also to polyenes and echinocandins (Bhattacharya and Sae-Tia, 2020). Candida glabrata is considered the most resistant strain and was classified as a multidrug-resistant infection in a study of Saudi Arabian women. On the other hand, *Candida albicans* and *Candida tropicalis* are highly susceptible to terbinafine. *Candida albicans* is resistant to fluconazole, chlorothiazide, and nystatin, while Candida tropicalis, as the most susceptible strain, responded to all tested antifungal agents except nystatin (Yassin *et al*., 2020).

Biofilm Formation

Microtiter plate method was conducted for detection of biofilm formation among all *C. albicans* isolates as showed in figure 3.

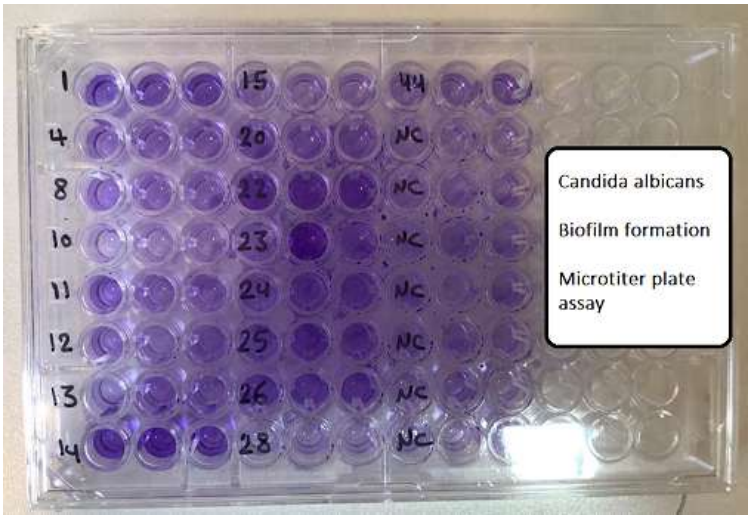

Figure 3. Biofilm formation detection of *Candida albicans* isolates by microtiter plate assay.

Of the 30 *Candida albicans* isolates, 16 (53.3%) formed strong biofilms, 10 (33.3%) formed medium biofilms, and only 4 (13.3%) formed weak biofilms. Furthermore, all isolates were found to form biofilms (Table 5).

Table 5. Distribution of biofilm formation ability among *Candida albicans* Isolates.

Candida Albicans	Biofilm formation		
	Strong	Moderate	Weak
Total no. of Isolates =30	16	10	4
%	53.3%	33.3%	13.3%

A biofilm has cells that are grouped together in a matrix made up of their own material. Biofilms can also consist of other living things, such as bacteria and protozoa. The cells of a biofilm can accomplish many important tasks that benefit each member, including obtaining food, responding to and eliminating waste products, growing, communicating with each other and protecting themselves. Fungi grow into large aggregates made up of many interconnected hyphae held together by a matrix. Depending on the type of fungi, this matrix may be thick or thin, and the shape of the matrix is often dependent on the chemical compounds produced by the fungi, which provide a means for cell-to-cell communication by allowing them to use quorum sensing. The importance of biofilms in facilitating the virulence of mixed infections has been recognized as a major virulence factor in the pathogenicity of the host (Biswas *et al*, 2024). Based on clinical case reports and unpublished studies, one or more bacteria can be isolated from different sites of infection in fungal diseases. Although mixed biofilms can form between fungi such as Fusarium, further research is needed. While this is not yet certain, it is particularly likely to occur in immunocompromised individuals because both Candida species can form biofilms on both biological and non-biological surfaces (Bautista *et al*., 2019). Candida biofilms can lead to the persistence or exacerbation of a variety of chronic inflammatory diseases, as well as acute, deep, systemic *Candida* infections. *Candida albicans* remains the most common causative agent of fungal biofilm infections (Oliveira *et al*., 2020). Most commercially available antifungal drugs are

either ineffective against *Candida* biofilms or only effective at high concentrations, with significant side effects (Gulati and Nobile, 2016).

Detection of Candida albicans using the 18S rRNA gene

The 16 *Candida albicans* isolates used in this study, especially those with strong biofilm-forming ability and resistance to fluconazole, were all detected by PCR using the *18S rRNA* gene. All clinical *Candida albicans* isolates tested contained the *18S rRNA* gene (150 bp) (Figure 4).

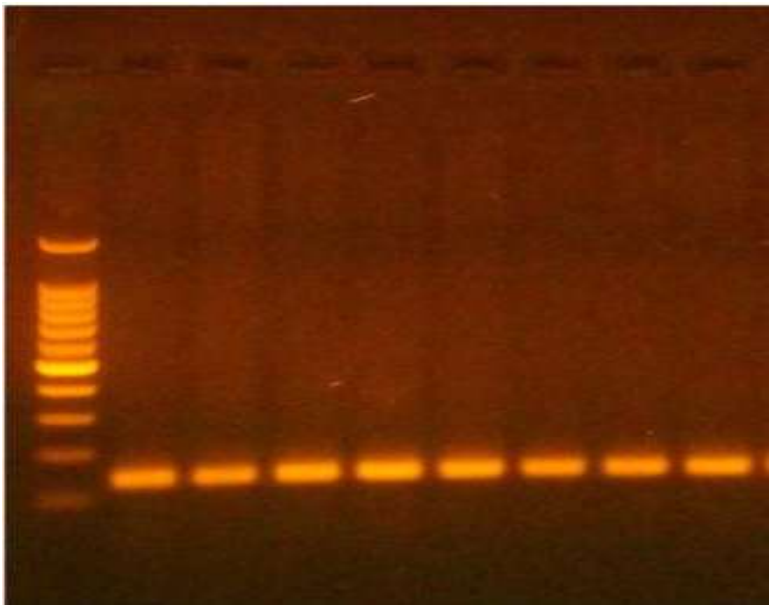

Figure 4. Agarose gel electrophoresis for the amplification of *18S rRNA* gene of *Candida albicans* had been fractionated on 1.5% electrophoresis gel agarose stained in Eth.Br. M: 100base per ladder marker. Lanes 1-10 resemble 150bp (1.5hours, 80 volts).

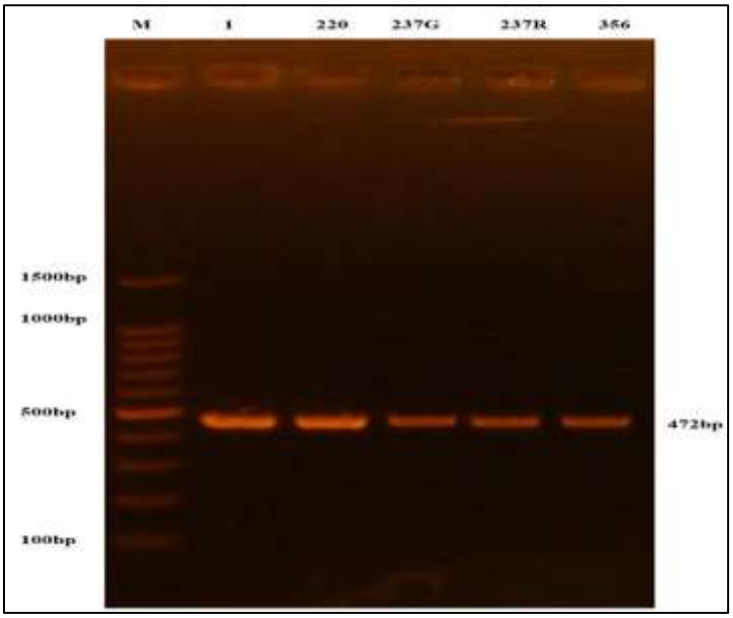

Figure 5. Fractionated on a 1.5% Agarose Gel Electrophoresis (Ethidium Bromide Stained). 100bp ladder was used in Marker's lane, 1-356 were 472bp PCR products. (1.5% Agarose Gel Electrophoresis; 100 volts and 60 minutes)

The figure 6 displays clear, distinct bands in lanes 1 through 356, matching the position of the 514 bp marker. The presence of these bands confirms that the *FKS1* gene was successfully and specifically amplified from the genomic DNA of all tested *Candida albicans* isolates. The absence of non-specific bands or smearing indicates that the PCR reaction and gel electrophoresis were performed with high specificity and accuracy.

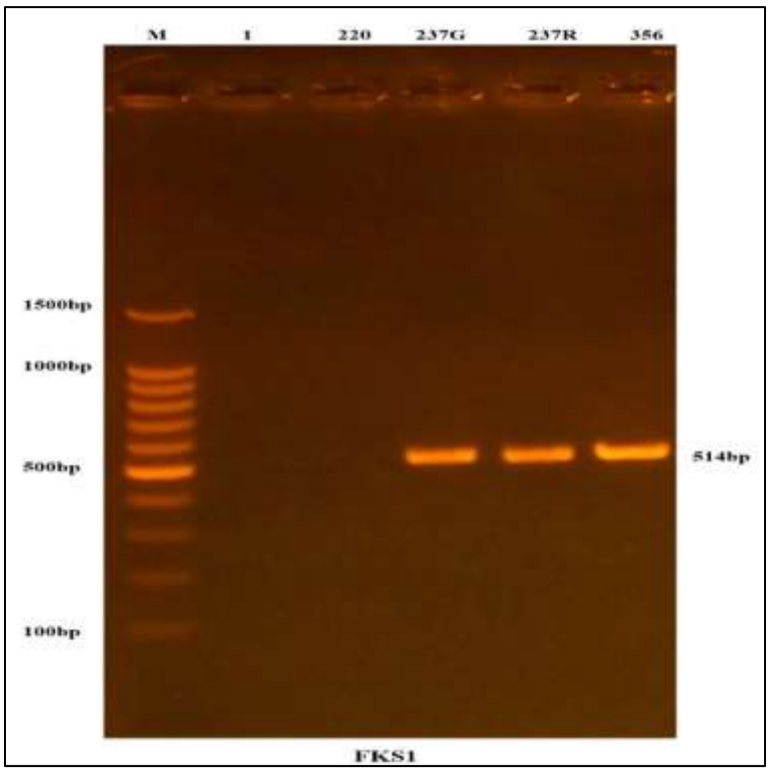

Figure 6. The amplification of the *FKS1* gene of *Candida albicans* species with samples run on a 1.5% agarose gel and stained with Ethidium Bromide after PCR (M=100bp ladder marker). All 1-3 samples have a PCR product size of 514 bp. (Electrophoresis on 1.5% agarose gel, using 100 volts for 60 minutes.).

FKS1 encodes the catalytic subunit of β-1,3-glucan synthase, an enzyme essential for synthesizing the primary structural component of the fungal cell wall. The downregulation of *FKS1* is particularly significant because β-1,3-glucan is a core component of the biofilm matrix (Czajka *et al*., 2023).
LL-37 is an antimicrobial peptide derived from human cathelicidin, known for its broad-spectrum activity against bacteria, fungi, and viruses. The varying MIC values observed in this study indicate differences in the sensitivity of different Candida albicans isolates to this peptide. Mechanism of action: LL-37 typically disrupts fungal cell membranes through pore formation or detergent-like dissociation. It can also penetrate cells and interact with intracellular target structures such as RNA or DNA. Strain variability: The differences in MIC values (between 62.5 and 250 µg/ml) may be due to variations in cell wall composition or the presence of fungal proteases that degrade the peptide. Higher MIC values (e.g., 250 µg/ml) suggest that these specific strains may have stronger resistance to the innate immune response, potentially leading to persistent infection.

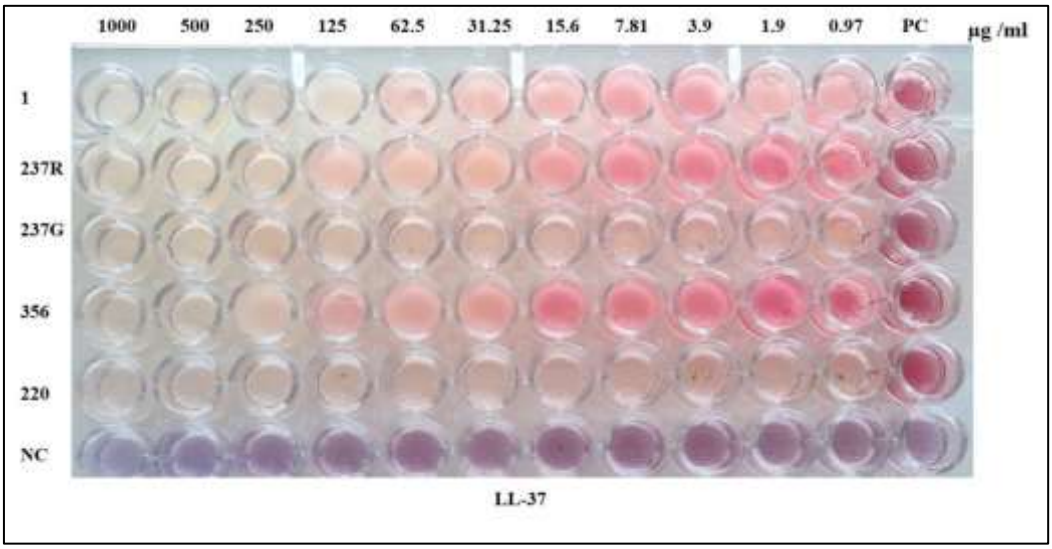

Figure 7. The Minimum Inhibitory Concentration (MIC) of LL-37 peptide on candida albicans.

These results are consistent with the broad-spectrum antifungal properties of LL-37, particularly highlighting the variability in its activity within the same species.
The observed differences in MIC values are consistent with the complex multimodal mechanism of action of LL-37. Unlike traditional antifungal drugs that target a single specific metabolic pathway (e.g., ergosterol synthesis), LL-37 is a cationic host defense peptide that primarily exerts its effects through physical disruption of microbial membranes. A previous review by Neshani *et al*. (2025) emphasizes that LL-37 is effective against more than 16 fungi, with mechanisms of action including membrane rupture, cell wall disruption, and induced oxidative stress. LL-37's amphiphilic α-helical structure allows it to insert into negatively charged *Candida* membranes, leading to membrane disintegration or pore formation, similar to the action of a detergent.
The varying sensitivities (62.5 to 250 μg/ml) suggest that some strains possess intrinsic resistance mechanisms that mitigate these effects. Strains requiring higher MIC values (≥ 250 μg/ml) may exhibit alterations in cell wall composition, such as increased chitin or mannose protein content, which can create physical barriers and reduce peptide permeability. Furthermore, the secretion of fungal proteases is a well-established virulence factor in *Candida albicans*. These proteases can degrade LL-37, thereby neutralizing its antifungal activity and leading to higher MIC values. This proteolytic degradation remains a significant challenge for the clinical application of peptide-based therapies (Voronko *et al*., 2025).
Looking ahead, while LL-37 provides a promising foundation for the development of novel antifungal drugs, our data also highlight the need for structural optimization. Current strategies focus on developing LL-37 analogs (e.g., truncated peptide KR-12), which retain antifungal activity but exhibit lower cytotoxicity and greater resistance to protease degradation. In summary, LL-37 is an effective effector molecule against innate immunity against *Candida albicans*, but its efficacy varies by strain. Future therapeutic applications may require the development of stable LL-37 analogs or combination therapies to overcome resistance mechanisms such as proteolytic degradation (Zhang *et al*., 2025).

The *FKS1* gene (typically involved in Candida cell wall synthesis) was present and expressed under all test conditions. Due to the high overlap of curves (Figure 8), there was no significant difference in FKS1 DNA (or cDNA) levels among samples. In a gene expression study (RT-qPCR), similar Ct values would indicate identical FKS1 transcription levels in the control and treatment groups. Since there was no internal reference gene (standard gene), expression levels could not be normalized; however, the tight clustering of curves indicates good experimental technique. The results indicate that *FKS1* gene amplification was successful, stable, and reproducible, with no detectable differences between samples.

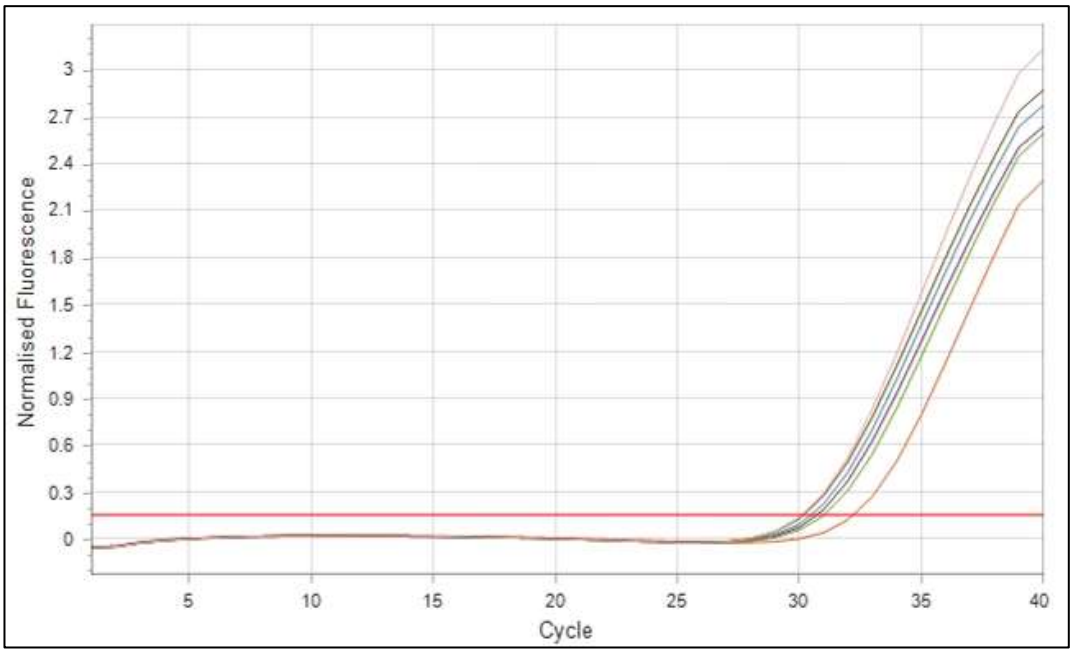

Figure 8. Amplification curves of *FKS1* gene

Quantitative real-time PCR (qRT-PCR) results showed that treatment with peptide LL-37 significantly downregulated FKS1 gene expression in all three tested Candida albicans isolates. Expression levels

were normalized to 18S rRNA housekeeping gene expression. In isolate 237G, FKS1 expression was reduced by 0.52-fold (48%) compared to the untreated control group (C237G). The reduction in FKS1 expression was more significant in isolate 237R, decreasing by 0.34-fold (66%) compared to the control group (C237R). The most significant downregulation of FKS1 expression was observed in isolate 356, which showed a reduction of only 0.05-fold (95%) compared to the untreated control group (C356).

Table 6. The expression level of *FKS1* gene before and after adding LL-37 peptide for three *C. albicans* isolates.

Sample	*18S rRNA*	FKS1	DCT	DDCT	Folding
C237G	21.35	30.73	9.38	0.00	1.00
237G	20.17	30.49	10.32	0.94	**0.52**
C237R	22.10	31.04	8.94	0.00	1.00
237R	19.70	30.19	10.49	1.55	**0.34**
C356	26.32	32.23	5.91	0.00	1.00
356	20.03	30.13	10.11	4.20	**0.05**

These data indicate that LL-37 can persistently inhibit FKS1 expression in different isolates, although the degree of inhibition varies (relative expression levels range from 0.05 to 0.52). The downregulation of FKS1 by LL-37 is an important finding with significant implications for antimicrobial therapy. FKS1 encodes the catalytic subunit of β-1,3-glucan synthase, an enzyme crucial for β-glucan synthesis, a key structural component of fungal cell walls (Garcia-Rubio et al., 2020). Reduced expression of this gene typically impairs cell wall integrity and makes fungi more susceptible to osmotic stress and immune recognition (Arendrup and Patterson, 2017). One of the local studies revealed the role of the antimicrobial peptide IDR-1018 in the gene expression of the antibiotic resistance genes among *P. aeruginosa* (Zedan and Ghaima, 2026).

These data suggest that the mechanism of action of LL-37 involves not only increasing microbial cell membrane permeability but also actively inhibiting cell wall synthesis at the transcriptional level. LL-37 reduces β-glucan production by decreasing FKS1 expression. This is particularly beneficial because β-glucan is a pathogen-associated molecular pattern (PAMP) that can be recognized by the host's immune system, particularly Dectin-1. Although the table shows downregulated β-glucan expression, some studies have suggested that sub-inhibitory levels of certain peptides may trigger compensatory stress responses. However, persistently reduced β-glucan expression was observed in all three isolates examined here, suggesting that LL-37 may disrupt cell wall stability pathways, such as the PKC or MAPK pathways (Ridyard and Overhage, 2021; Lee *et al*., 2023).

LL-37's ability to inhibit FKS1 has clinical significance. Echinocandins (e.g., caspofungin) are first-line antifungal agents that directly inhibit glucan synthase. These data suggest that LL-37 may have a synergistic effect with echinocandins. Furthermore, FKS1 gene mutations are a major mechanism of resistance to echinocandins. If LL-37 reduces the expression of this gene, it may reduce the selective pressure of resistance mutations or potentially resensitize resistant strains, although this requires further investigation (Román *et al*., 2022).

Conclusion

This study confirms that *Candida albicans* remains the predominant pathogen causing vulvovaginal candidiasis in pregnant women in Baghdad, with a significant proportion of strains exhibiting strong biofilm-forming ability and high resistance to fluconazole. The human antimicrobial peptide LL-37 demonstrated potent antifungal activity against these clinical isolates and significantly inhibited biofilm formation. At the molecular level, LL-37 downregulated FKS1 gene expression in a strain-specific manner, reducing FKS1 gene expression by up to 95% in one isolate, thereby impairing cell wall integrity and β-glucan synthesis. These results indicate that LL-37 has two mechanisms of action: direct disruption of the cell membrane and transcriptional repression of key cell wall synthase genes. The MIC values and gene expression responses of different isolates suggest the existence of intrinsic, strain-specific resistance mechanisms, including possible proteolytic degradation or alterations in cell wall composition. Overall, LL-37 shows promise as a candidate drug for topical treatment of azole-resistant and biofilm-associated *Candida albicans* infections in pregnancy-associated vulvovaginal candidiasis.

Further research is needed to optimize its stability and evaluate its synergistic effect with traditional antifungal drugs.

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