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Investigation of Biofilm-Related Genes (*icaA* and *rbf*) in *Staphylococcus aureus* and Evaluation of LL-37 Peptide as an Antibacterial and Antibiofilm Agent

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ABSTRACT

Background: The main human pathogen that causes a variety of diseases is Staphylococcus aureus. The increasing prevalence of antibiotic-resistant bacteria may cause complications in addressing bacterial infections. Due to its extensive array of antibacterial and antibiofilm properties, the human peptide LL-37 is being considered as a potential substitute for conventional antibiotics. The objective of this research was to identify genes involved in the development of biofilms, and to assess the effectiveness of LL-37 in combating the resistant Staphylococcus aureus that are found in clinical samples, the purpose of this research was to assess the effectiveness of the compound as a biofilm inhibitor. **Methods:** Through biochemical analyses, selective media, VITEK-2, and molecular verification, 190 clinical samples were gathered from hospitals in Baghdad and analyzed for S. aureus. Identifying the increase of biofilms using the microtiter plate method. The PCR was employed to identify genes associated with biofilm formation (icaA and rbf). The lowest concentration of LL-37 that inhibited the growth of the bacterium was obtained by a microdilution method. The efficacy of LL-37 against biofilms was evaluated with a reader that employs ELISA and a stain that is composed of crystal violet. **Result:** 44 of the aislates were identified as S. aureus. All of the aislates developed biofilms, 17 of which were successful and 7 of which were MDR. Biofilm genes were employed to identify the icaA gene in 6 different isolates, while the rbf gene was encountered in all of them. The antibacterial activity of LL-37 was considered strong, with the MIC value of 62.5-250 µg/ml being demonstrated. Our results demonstrated a significant alteration to their capacity to form biofilms, as the aislates became less effective at forming biofilms, following the treatment of the antimicrobial peptide LL-37. In the treated strains, the formation of biofilms was markedly reduced. **Conclusion:** When compared to multiple-drug resistant S. aureus, LL-37 demonstrated a significant inhibitory effect and an anti-inflammatory capacity. These findings indicate that it could be a viable alternative to treat infections caused by MSAs. Its practical applications and mechanisms of action should be studied in greater detail.

Keywords: *Staphylococcus aureus*, LL-37 peptide, biofilm genes, antibiotic resistance.

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium that lives on the skin (as part of the normal human flora) and in the mucous membranes of humans and animals. Once the bacterium breaches the skin barrier, it can cause a variety of systemic diseases, including fever, acute and chronic infections, and various syndromes. The pathogenicity of the bacterium is influenced by several properties, such as its ability to form biofilms and antibiotic resistance (1). Several local studies have shown that local isolates of S. aureus are highly resistant to first-choice drugs, especially methicillin and vancomycin (2,3). Septic

arthritis, endocarditis, osteomyelitis, and infections associated with implanted medical devices are examples of various S. aureus infections that result in biofilm formation.

Biofilms are communities of bacteria that are attached to surfaces and surrounded by an extracellular matrix. Biofilms are believed to enhance human defenses and tolerance to antimicrobial therapy and to promote bacterial colonization on surfaces (4). S. aureus biofilm formation begins with surface adhesion and is regulated by a variety of microbial surface components. Once these molecules are recognized, polymeric intercellular adhesion proteins are synthesized. The ica operon (icaABCD) encodes this protein, which controls cell-cell binding. Studies have shown that the presence of MRSA can lead to the failure of antimicrobial therapy. These organisms can transform acute infections into chronic infections by invading host cells and spreading to other parts of the body (5).

Antimicrobial peptides are ancient chemicals that were perfected early in mammalian evolution due to their co-evolution with bacteria. AMPs represent the majority of broad-spectrum antimicrobial activity against bacteria, viruses, and fungi and are produced by nearly all organisms. They play a variety of roles in microbial destruction, inflammation, angiogenesis, and wound healing, making them an important component of the human innate immune system (6). The antimicrobial peptide (AMP) LL-37 is a promising antimicrobial agent. Over the years, methods have been discovered to rapidly and efficiently produce recombinant LL-37, which has improved the clinical and functional properties of this previously expensive peptide. Previous studies on the efficacy of the peptide LL-37 against existing biofilms have yielded inconsistent results (7).

The aim of this study was to evaluate the ability of the antimicrobial peptide LL-37 to inhibit multidrug-resistant Staphylococcus aureus isolated from various clinical samples. Furthermore, the antibiofilm activity of the peptide and genetic detection of biofilm-related genes (icaA and rbf) were investigated.

MATERIALS AND METHODS

Isolation and identification of bacteria

A total of 190 clinical samples from different sources (blood, urine, burn swabs, and wound swabs) were collected from three hospitals in Baghdad. The samples were transferred to brain heart infusion (BHI) broth and incubated overnight at 37°C. They were then spread on blood agar, mannitol salt, and HiCrome agar and incubated aerobically at 37°C for 24 hours. Single colonies were tested for coagulase, catalase, and Gram staining using standard microbiological methods. Biochemical identification was confirmed using the VITEK-2 Compact System and molecular testing.

Assessment of biofilm formation

The amount of biofilm produced by S. aureus was determined by the microtiter plate method; all isolates were cultured in brain heart infusion overnight at 37°C. Each isolate was added to tryptic soy broth (TSB) containing 1% glucose using a pipette and mixed thoroughly. The turbidity of the bacterial isolate suspension was measured using a McFarland turbidimeter No. 0.5. The culture of each isolate was inoculated in triplicate in a volume of 200 µl into sterile 96-well U-bottom microtiter plates. The plates were covered and incubated aerobically at 37°C for 24 h. To remove non-adherent bacteria, planktonic cells were rinsed twice with distilled water after the incubation period. Adherent bacterial cells in each well were fixed with 200 µl of 100% methanol for 20 min at room temperature. To stain adherent cells, 200 µl of 0.1% crystal violet was added to each well and allowed to stand for 15 min. After the staining process is complete, wash the plates two to three times with distilled water

to remove any additional dye. Allow the plates to cure at room temperature for about half an hour to ensure that they are completely dry. The dye is then removed by adding 33% acetic acid. The optical density (OD) is measured at a wavelength of 630 nm using an ELISA autoreader. The mean of the OD values of the sterile culture medium is subtracted from all test results. A cutoff value (ODc) is calculated to classify the isolate as a biofilm former or not (8).

DNA extraction

DNA was extracted using a bacterial DNA preparation kit (Geneaid Biotech Ltd., Taiwan). Following the protocol, the tubes were inverted for one minute to mix the samples and then centrifuged at 15,000 g for one minute. The supernatant was discarded and the contents of the tube were drained onto clean absorbent paper. The DNA pellet was then washed with 500 µl of buffer and the tube was spun several times. The tube was then centrifuged again at 15,000 g for one minute. The ethanol was carefully discarded and the DNA was air-dried at room temperature for 10-15 minutes. Subsequently, 50–100 µl of hydration solution was added to the dried DNA pellet. After incubation at 65°C for another hour, the DNA was stored at -20°C.

PCR Primers and Amplification

As indicated in Table 1, primers (Macrogen, South Korea) were used to identify and amplify the biofilm genes.

No.	Gene	Primer name	Sequence (3'-5')	Product size	Reference
1	*rbf*	*rbf* (F)	ACGCGTTGCCAAGATGGCATAGTCTT	190 bp	(9)
		rbf (R)	AGCCTAATTCCGCAAACCAATCGCTA		
2	*icaA*	*icaA*(F)	CTGGCGCAGTCAATACTATTTCGGGTGTCT	195 bp	(9)
		icaA (R)	GACCTCCCAATGTTTCTGGAACCAACATCC		
3	*16S rRNA*	*16S* (F)	GTAGGTGGCAAGCGTTATCC	229 bp	(10)
		16S (R)	CGCACATCAGCGTCAG		

Detection of biofilm-producing genes

S. aureus isolates that produced biofilm were identified by PCR amplification of the icaA, rbf, and 16s rRNA genes. The PCR results were examined using a UV transilluminator after being conducted on a 1% agarose gel that had been stained with ethidium bromide.

Table 2: Detection of biofilm-producing genes by using PCR

Stage	Temperature (°C)	Time	Cycle
Initial denaturation	92	5 min	1
Denaturation	92	30 sec	30
Annealing	58	30 sec	
Extension	72	1 min	
Final extension	72	5 min	1

Minimum inhibitory concentrations (MIC) of LL-37 peptide

MICs were determined using the microdilution method (microtiter plate assay with resazurin dye) according to the recommendations of the Clinical and Laboratory Standards

Institute. A 1:2 dilution series of the peptide LL-37 was used in Müller-Hinton broth (MHB) ranging from 1000 to 1.9 µg/ml. Subcultures of S. aureus were grown in brain heart broth at 37°C for 18 to 24 hours to prepare the bacterial inoculum for testing. To achieve a McFarland turbidity of 0.5, the bacterial solution was diluted to 1 × 108 CFU/ml. A final concentration of 5 × 105 CFU/ml was then achieved by diluting the material in MHB in a 1:2 ratio. The peptides were serially diluted and then added to the 96-well plate containing the diluted bacterial solution. A total of 200 µl was added to each well, including 100 µl of the diluted peptide solution and 100 µl of the bacterial solution. For positive and negative growth controls, one series of wells contained MHB alone and another series of wells contained MHB and S. aureus. After 24 hours at 37°C, resazurin (6.75 mg/ml) was added to each well. A color change was observed after four hours of incubation. Wells in which the blue color of resazurin remained unchanged after incubation reached values above the MIC (11).

Antibiofilm activity of LL-37 peptide

In this test, four strains that showed high biofilm formation ability in the biofilm production test were used. The inhibitory effect of LL-37 peptide at different concentrations below the MIC concentration on the biofilm formation ability of S. aureus cells was studied using the method described by Zhang et al. (2021) (12) The developed TCP method was tested. About 100 µl of bacterial culture at 0.5 McFarland density was added to each well of a 96-well polystyrene microtiter plate along with 100 µl of an appropriate dose of antibiofilm substance. The culture plates were then incubated at 37°C for 24 h. The positive control for biofilm growth was a well without antimicrobial agent. After incubation, non-adherent planktonic cells and culture medium were removed, and the wells were washed three times with sterile PBS. After the plates were air-dried, the ink was re-dissolved using 100% ethanol. The optical density (OD) of each well was measured at 570 nm using an ELISA reader (BioTek, South Korea). Each test was performed three times.

RESULTS AND DISCUSSION

Isolation and identification of *S. aureus*

From 190 isolates (from various clinical sources between November 2024 to march 2025) 44 isolates (23.2%) regrouped characteristics (culture, biochemistry and molecular) as *Staphylococcus aureus*. Also these isolates were identified by VITEK2 system.

Microtiter plate method for detection of biofilm formation

The microtiter plate technique was employed to find out if *Staphylococcus aureus* could produce biofilms. 44 isolates were tested and the results showed that 17 (38.6%) isolates were strong in forming biofilms, 21 (47.7%) were moderate and 6 (13.6%) were weak in forming. (Figure 1).

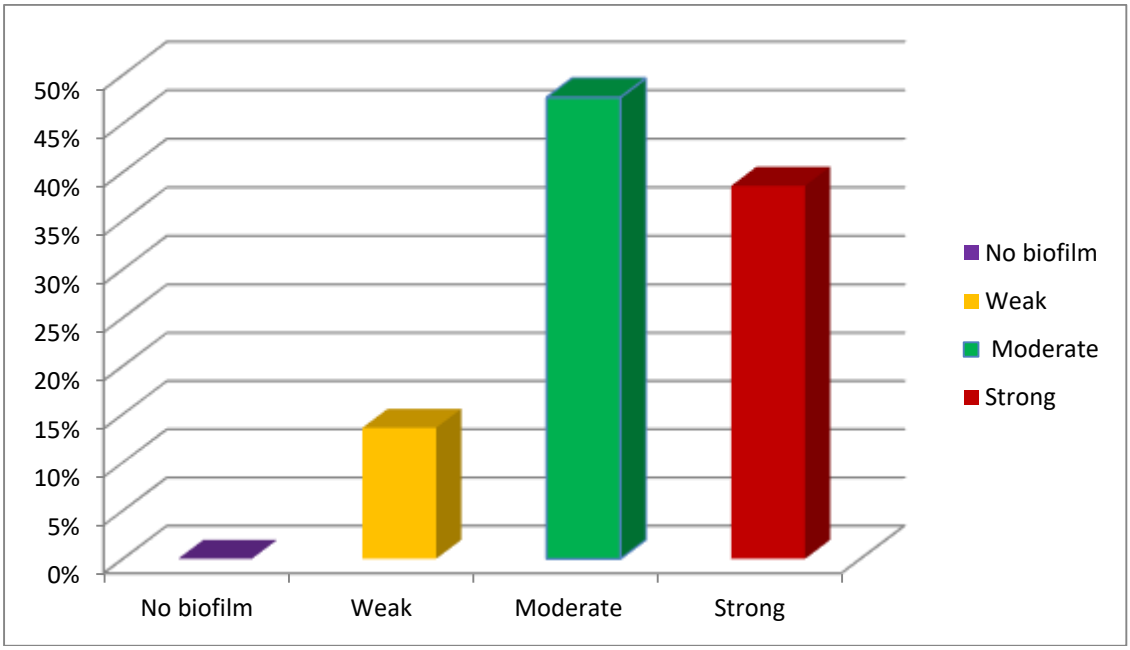

Figure (1). The biofilm formation by *S. aureus* isolated from clinical samples.

All isolates were biofilm-producing and no non-biofilm-producing isolates were found.

Molecular identification of *S. aureus* isolates

The isolates *S. aureus* that were MDR and strong in forming biofilms used in this study and were identified by PCR using the *16S rRNA* gene for diagnosing of *S. aureus*. All the tested *S. aureus* clinical contained *16S rRNA* gene (229 bp) (Figure 2).

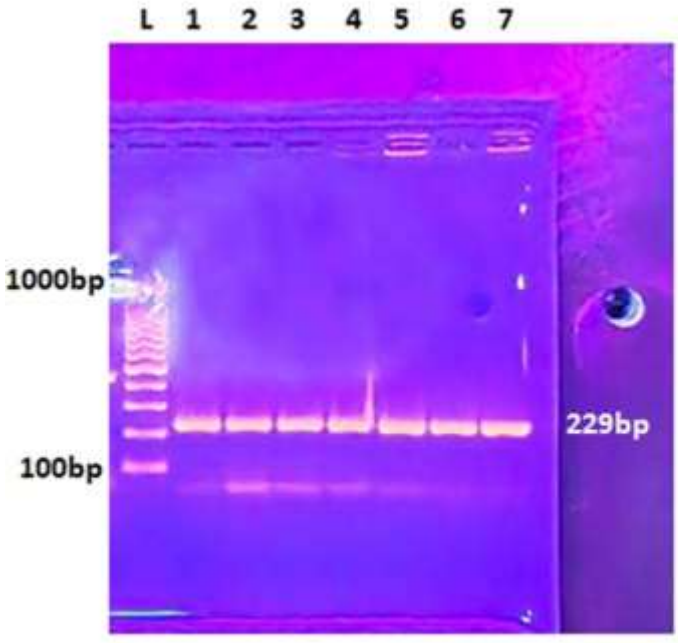

Figure (2): *S. aureus* isolates' 16s rRNA gene amplification was separated using gel electrophoresis in 1% agarose gel (229 bp band) .

The amplification of DNAs from phylogenetically different bacteria by targeting conserved sections of the *16S rRNA* gene has proven an effective method for bacterial detection and identification. The present data revealed that PCR with the *16S rRNA* gene was an excellent approach for detecting *S.aureus* spp. isolates. These findings align with previous research indicating that detecting and sequencing this gene can effectively identify clinical isolates of *S. aureus* (13).

Detection of biofilm genes

Biofilm formation by *S. aureus* increases resistance to antimicrobial agents, posing a significant clinical challenge. Numerous studies have shown the involvement of various genes in the formation of biofilms. Essential proteins that produce polysaccharide intercellular adhesion (PIA), which improves cell-to-cell contact and encourages the formation of biofilms in Staphylococcus species, are encoded by the intracellular adhesion (ica) cluster (icaADBC). *icaA* is one of these genes that play critical roles in biofilm formation. In order to help direct the choice of suitable treatments, it is crucial to identify the presence of the ica locus in S. aureus isolates in addition to phenotypic biofilm detection (14). Seven multidrug-resistant *S. aureus* isolates that were potent biofilm-forming strains were selected for *icaA* gene detection. It was found in six isolates, while only one was missing. This suggests that the icaADBC gene alone is not responsible for biofilm formation.

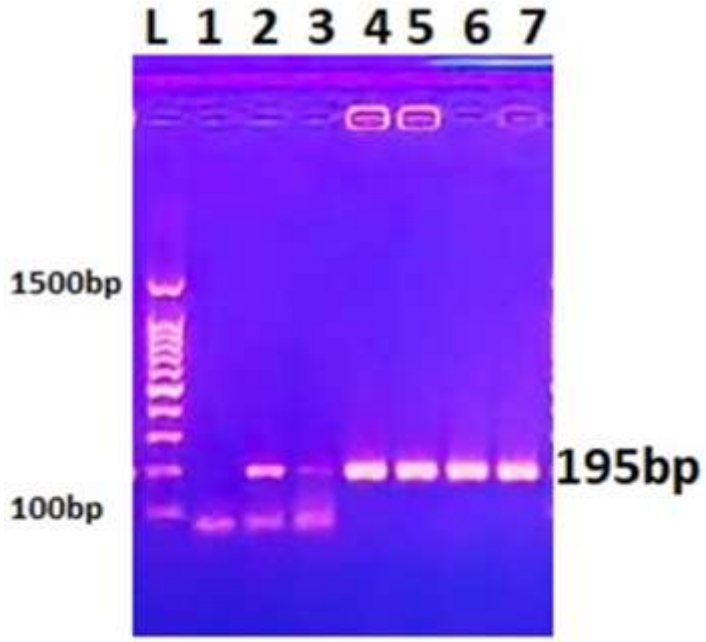

Figure (3): *S. aureus* isolates' *icaA* gene amplification was separated using gel electrophoresis in 1% agarose gel (195 bp band) .

Our results are consistent with a study conducted by Mahmood and Hussein (2022) that focused on genes encoding adhesion proteins and the ability of S. aureus isolates to form biofilms. The results showed that the amplicon of the intercellular adhesion gene icaA was present in 7 (77.8%) of the biofilm-producing S. aureus isolates (15). Another study showed that 75% of biofilm-producing S. aureus isolates contained the ica operon (16). However,

another study found that all S. aureus isolates possessed both the icaA and icaD genes (17). A previous study of S. aureus isolates isolated from burns also found a weak association between biofilm formation on Congo red agar plates and the presence of the biofilm gene (icaA) detected by PCR. Eleven of the 16 isolates tested with CRA (68.7%) formed biofilms. PCR results showed that 13 (81.3%) isolates contained the icaA gene, of which 2 isolates did not form biofilms on CRA plates but were positive for the icaA gene. The data from this study indicated that the presence of the icaA gene was required for the ability of the isolates to form biofilms on Congo red plates, however, this was affected by the genotype of the icaA gene (18).

A study discovered that Rbf acts as a positive biofilm regulator. It enhances PIA-dependent biofilm production in S. aureus by attaching to the sarX promoter, it increases sarX transcription and decreasing icaR expression indirectly (19). Polymerase chain reaction (PCR) was utilized to identify the regulatory gene for the icaADBC operon (rbf). All seven isolates with strong biofilm production contained the rbf gene (100%). These findings show that *S. aureus* uses the rbf gene to generate biofilms. The results were consistent with a study by Shin et al., who tested two strains of S. aureus, one knocked out of the rbf gene (CYL1135) and the other complemented with the rbf gene (CYL1106). Using crystal violet staining, the capacity of CYL1135 and CYL1106 to produce biofilms was investigated. Compared to CYL1135, CYL1106 had a four-fold greater capacity to produce biofilms. (20).

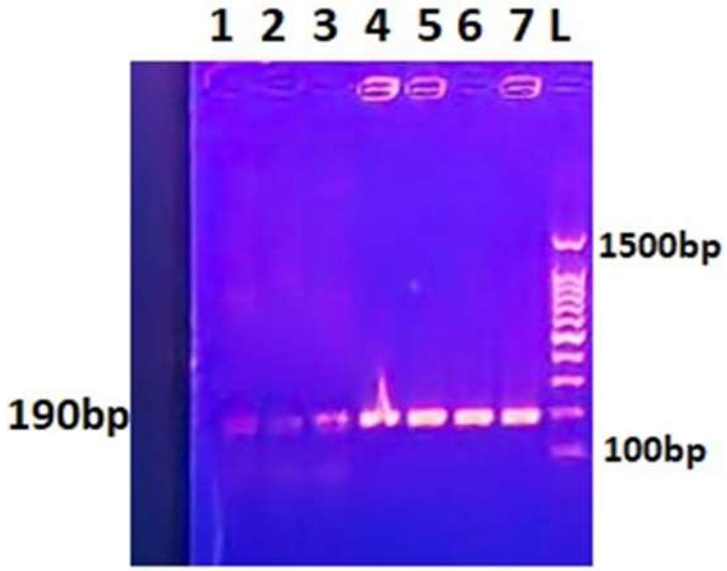

Figure (4): *S. aureus* isolates' *rbf* gene amplification was separated using gel electrophoresis in 1% agarose gel (190 bp band) .

Minimum Inhibitory Concentrations (MICs) of LL-37 against *S. aureus*

Using the microtiter method with resazurin stain to test 4 isolates of multidrug-resistant *Staphylococcus aureus* that are strong in forming biofilms, The antimicrobial peptide LL-37's minimum inhibitory concentrations against the four isolates revealed that the inhibitory activity range was (62.5 - 250 µg/ml). Isolates S.16 was affected by the lowest concentration

(62.5 µg/ml), while isolates S.12, S.14 and S.20 were inhibited by a concentration (250 µg/ml) as shown in the figure 5 and table 3.

Table (3). The minimum inhibitory concentrations of LL-37 Peptide against *Staphylococcus aureus* isolates at concentrations (1000-1.9 µg/ml).

LL-37 conc. (µg/ml)	The isolate code				Positive control	Negative control
	S12	S14	S16	S20		
1000	–	–	–	–	+	–
500	–	–	–	–	+	–
250	–	–	–	–	+	–
125	+	+	–	+	+	–
62.5	+	+	–	+	+	–
31.25	+	+	+	+	+	–
15.6	+	+	+	+	+	–
7.8	+	+	+	+	+	–
3.9	+	+	+	+	+	–
1.9	+	+	+	+	+	–

P.C.: Positive control, N.C.: Negative control

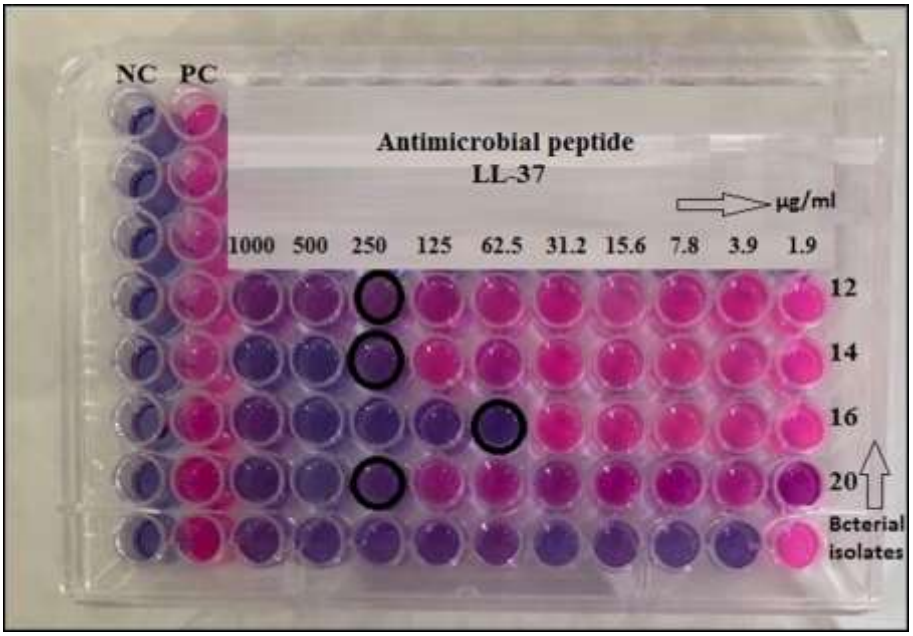

Figure (5). The minimum inhibitory concentrations of LL-37 Peptide against *Staphylococcus aureus* isolates at concentrations (1000-1.9 µg/ml). (In 10 mM potassium phosphate buffer at pH 7.5)

The anti-biofilm effect of LL-37 peptide was tested using sub-MIC by microtiter plate method, adding crystal violet dye and reading the results using ELISA reader. The experiment was conducted on four isolates of multidrug-resistant *Staphylococcus aureus* that are strong in forming biofilms, and the minimum inhibitory concentration (MIC) was tested for them. Our results showed a clear effect on their ability to form biofilms, as the isolates became weak in forming biofilms, after having been strong before treatment with the antimicrobial peptide LL-37.

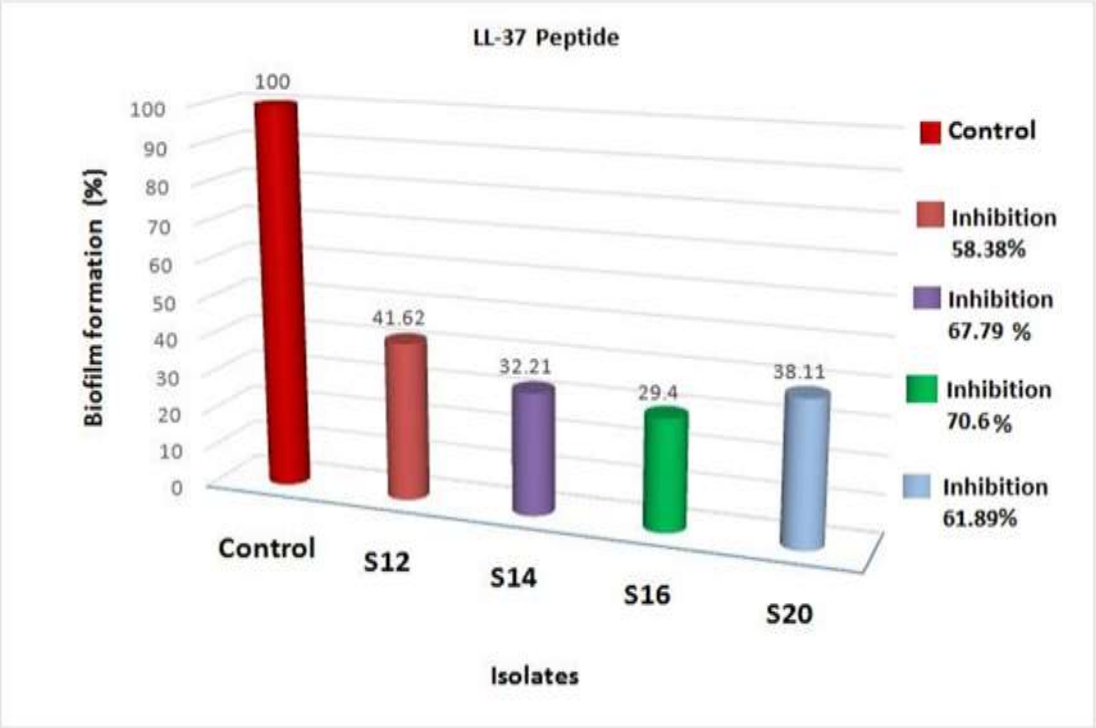

Figure (6). Biofilm formation inhibition of 4 *S. aureus* isolates by sub-inhibitory concentrations of antimicrobial peptides LL-37.

A previous study investigated the efficacy of silver nanoparticles (AgNPs) and the antimicrobial peptide LL-37 against Staphylococcus aureus. The authors found that LL-37 was the most effective antimicrobial agent, reducing the colony count by more than 4 logs. On the other hand, traditional antibiotics and silver nanoparticles did not show such high efficacy and only reduced the colony count by less than 1 log. Kinetic experiments also showed that LL-37 could rapidly kill S. aureus biofilms. Overall, the results of the study suggest that LL-37 is an effective antimicrobial agent against S. aureus biofilms and could be used in clinical settings to remove biofilms and treat joint prosthesis infections (7). The current findings are supported by a number of local studies showing that the antimicrobial peptide LL-37 affects the growth of pathogenic bacteria and the formation of biofilms (21, 22). In another study, the antimicrobial and antibiofilm activities of LL-37 were investigated against biofilm-positive, methicillin-resistant Staphylococcus aureus (MRSA) strains and biofilm-positive, methicillin-sensitive Staphylococcus aureus (MSSA) strains isolated from chronic wound infections. The peptide LL-37 showed inhibitory effects against the S. aureus strains (23). In another study, the antimicrobial peptide LL-37 and its reduced derivatives LL-13 and LL-17 were tested in vitro alone and in combination with vancomycin against a range of resistant S. aureus strains, including methicillin-resistant S. aureus (MRSA) and vancomycin-resistant S. aureus (VRSA). Both LL-13 and LL-17 showed significant ability

to prevent biofilm formation, almost completely blocking biofilm formation, while LL-37 reduced biofilm formation by half (24).

CONCLUSION

A substantial association between adhesion genes, particularly icaA and rbf, expressed by *Staphylococcus aureus* and multidrug-resistant (MDR) clinical isolates has been determined through this study, indicating the significance of genes linked to biofilms in the pathogenicity of *Staphylococcus aureus*. Results indicate that all isolates in the study are biofilm producers as all the isolates possesses the ability to create biofilms and the presence of *rbf* gene in all the isolate. The *icaA* gene was also positive in majority of the strong Biofilm producing strains. More importantly, the human peptide of antimicrobial peptide LL-37 was found to possess substantial antibacterial and antibiofilm activity.

REFERENCES

1-Mollaahmadi, C. M., Anzabi, Y., & Shayegh, J. (2021). Comparison of the Frequency of Biofilm-Forming Genes (icaABCD) in Methicillin-Resistant S. aureus Strains Isolated from Human and Livestock. 76(6), 1655–1663.

2- **AbdulRazzaq, A.B., Shami, A.M., Ghaima K.K., 2022**. Detection of vanA and vanB genes Among Vancomycin Resistant Staphylococcus aureus Isolated from Clinical Samples in Baghdad Hospitals. Iraqi Journal of Biotechnology, 20(1), 19-25.

3- **Jassim, S.A., and Kandala, N.J. 2021.** Molecular Detection of Enterotoxin Genes of Multiresistant *Staphylococcus aureus* Isolates from Different Sources of Food. (2021). *Iraqi Journal of Science*, 62(1), 61-74.

4- Ma, R., Qiu, S., Jiang, Q., Sun, H., Xue, T., Cai, G., & Sun, B. (2017). AI-2 quorum sensing negatively regulates rbf expression and biofilm formation in Staphylococcus aureus. International Journal of Medical Microbiology, 307(4-5), 257–267.

5- Mahmood, A. R., Hussein, N. M., & Hussein, N. M. (2022). Detection of biofilm-forming genes in Staphylococcus aureus clinical isolates by PCR assay. Journal of Natural Science, Biology and Medicine, 13(1).

6-Zeth, K., & Sancho-Vaello, E. (2017). The Human Antimicrobial Peptides Dermcidin and LL-37 Show Novel Distinct Pathways in Membrane Interactions. Frontiers in Chemistry, 5.

7- Kang, J., Dietz, M. J., & Li, B. (2019). Antimicrobial peptide LL-37 is bactericidal against Staphylococcus aureus biofilms. PLOS ONE, 14(6), e0216676.

8- Achek R, Hotzel H, Nabi I, Kechida S, Mami D, Didouh N, Tomaso H, Neubauer H, Ehricht R, Monecke S, El-Adawy H. Phenotypic and Molecular Detection of Biofilm Formation in *Staphylococcus aureus* Isolated from Different Sources in Algeria. Pathogens. 2020 Feb 24;9(2):153.

9- Cue, D., Lei, M. G., Luong, T. T., Kuechenmeister, L., Dunman, P. M., O'Donnell, S., Rowe, S., O'Gara, J. P., & Lee, C. Y. (2009). Rbf Promotes Biofilm Formation by Staphylococcus aureus via Repression of icaR, a Negative Regulator of icaADBC. Journal of Bacteriology, 191(20), 6363–6373.

10- AlMosawi, R., Hanadi Abdulqadar Jasim, & Haddad, A. (2024). Identification of S.aureus by specific 16S rRNA and detection of mec A gene from clinical samples in patients of Basrah governorate in Iraq.

11- Bajiya N, Kumar N, Raghava GPS. Prediction of inhibitory peptides against E.coli with desired MIC value. Sci Rep. 2025 Feb 8;15(1):4672.

12- Zhang Y, Lakshmaiah Narayana J, Wu Q, Dang X, Wang G. Structure and Activity of a Selective Antibiofilm Peptide SK-24 Derived from the NMR Structure of Human Cathelicidin LL-37. Pharmaceuticals (Basel). 2021 Nov 30;14(12):1245.

13- Ahmed, Z. F., & Al-Daraghi, W. A. H. (2022). Molecular Detection of medA Virulence Gene in Staphylococcus aureus Isolated from Iraqi Patients. Iraqi Journal of Biotechnology, 21(1)..

14-Cue, D., Lei, M. G., & Lee, C. Y. (2012). Genetic regulation of the intercellular adhesion locus in staphylococci. Frontiers in Cellular and Infection Microbiology, 2.

15- Mahmood, A. R., Hussein, N. M., & Hussein, N. M. (2022). Detection of biofilm-forming genes in Staphylococcus aureus clinical isolates by PCR assay. Journal of Natural Science, Biology and Medicine, 13(1).

16- Cue, D., Lei, M. G., & Lee, C. Y. (2013). Activation of sarX by Rbf Is Required for Biofilm Formation and icaADBC Expression in Staphylococcus aureus. Journal of Bacteriology, 195(7), 1515–1524.

17-Shin, H.-J., Yang, S., & Lim, Y. (2021). Antibiotic susceptibility of Staphylococcus aureus with different degrees of biofilm formation. Journal of Analytical Science and Technology, 12(1).

18- Ahmed, S. T., Abdallah, N. M., Al-shimmary, S. M., & Salih, M. (2021). The Role of Genetic Variation for icaA Gene Staphylococcus aureus in Producing Biofilm. International Journal of Drug Delivery Technology, 11(2), 390–395.

19- Eftekhar, F., & Dadaei, T. (2011). Biofilm Formation and IcaAB Gene Carriage in MRSA. Iranian Journal of Basic Medical Sciences, 14(2), 132–136.

20- Torlak, E., Korkut, E., Uncu, A. T., & Şener, Y. (2017). Biofilm formation by Staphylococcus aureus isolates from a dental clinic in Konya, Turkey. Journal of Infection and Public Health, 10(6), 809–813.

21- **Rifaat, R.M., Ghaima, K.K. 2023.** The Effect of Antimicrobial Peptide LL-37 on Biofilm Formation of Klebsiella pneumoniae Isolated from Catheter-Associated Urinary Tract Infection Patients. Iraqi Journal of Biotechnology, 22(1), 193-199.
22- **Al-Sabagh, F.S., Ghaima, K.K. 2022.** Synergistic Effect of Antimicrobial Peptide LL-37 and Ciprofloxacin against Multidrug Resistant Pseudomonas aeruginosa Isolated from Burn Infections. Iraqi Journal of Biotechnology, 21(1), 32-38.
23- Demirci, M., Yigin, A., & Demir, C. (2022). Efficacy of antimicrobial peptide LL-37 against biofilm forming Staphylococcus aureus strains obtained from chronic wound infections. Microbial Pathogenesis, 162, 105368.

24- Shurko, J. F., Galega, R. S., Li, C., & Lee, G. C. (2018). Evaluation of LL-37 antimicrobial peptide derivatives alone and in combination with vancomycin against S. aureus. The Journal of Antibiotics, 71(11), 971–974.