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Antimicrobial and Antibiofilm Effects of GF-17 Peptide Against Methicillin-Resistant *Staphylococcus aureus*Isolated from Different Clinical Samples

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Abstract : Background: A major human pathogen that causes a variety of diseases is the gram-positive bacteria *Staphylococcus aureus*. Biofilm production increases its virulence and leads to antimicrobial resistance, especially in methicillin-resistant *S. aureus* (MRSA) strains. Antimicrobial peptides, including GF-17, have shown promise in the fight against *S. aureus* that is resistant to many drugs. Methods: Using biochemical tests, selective media, VITEK2-compact system, and molecular confirmation, 190 clinical samples were gathered from hospitals in Baghdad and screened for Methicillin-resistant *S. aureus* (MRSA). Twelve antibiotics were used in the disk diffusion method to assess antibiotic susceptibility. A microdilution technique was used to determine GF-17's minimum inhibitory concentration (MIC). GF-17's anti-biofilm activity was assessed with an ELISA reader and crystal violet staining. Furthermore, RT-qPCR was used to assess the expression of biofilm-associated genes (*icaA* and *rbf*). The purpose of this study is to assess how well GF-17 inhibits Methicillin-resistant *S. aureus* isolates that were taken from clinical samples. Results: Of the isolates, 44 were identified as MRSA and exhibited high levels of resistance to Vancomycin (61.3%) and Methicillin (100%). Strong antibacterial action was demonstrated by GF-17, with MIC values ranging from 7.8 to 15.6 µg/ml. 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 GF-17. In treated isolates, biofilm formation was considerably decreased, and RT-qPCR analysis showed that *icaA* expression was markedly downregulated but *rbf* expression was unaffected. Conclusion: When it came to Methicillin-resistant *S. aureus* (MRSA), GF-17 showed notable inhibitory and anti-biofilm efficacy. These results imply that it may be used as a therapeutic substitute to treat MRSA infections. Its clinical uses and mechanisms of action need to be investigated further.

Keywords: *Staphylococcus aureus*,MRSA, GF-17 peptide, biofilm genes, antibiotic resistance.

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Introduction

Staphylococcus aureus is a commensal gram-positive bacterium found in the normal skin flora of humans. *S. aureus* is included in the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species). These bacteria are involved in many clinical diseases such as skin and soft tissue infections, toxic shock syndrome, food poisoning, pneumonia, medical device-associated bloodstream infections, osteomyelitis, and infective endocarditis (1).Methicillin-resistant *Staphylococcus aureus* (MRSA) caused problems and difficult-to-treat infections when it first appeared in 1960 and developed resistance to all beta-lactam antibiotics that were widely used at the time (2).Hospital-acquired MRSA strains with multidrug resistance spread worldwide. In the 1980s, community-acquired MRSA strains became widespread and became known for causing skin and soft tissue infections in healthy individuals (1).

One of the most important virulence factors of Methicillin-resistant *S. aureus* (MRSA)is the formation of biofilms, which are organized collections of surface-associated microbial cells surrounded by an extracellular matrix of polymeric substances. Bacteria living in biofilms are more resistant to antimicrobial agents through a variety of mechanisms, including the inhibition of antimicrobial penetration into the biofilm, the diminished effectiveness of growth-dependent agents due to decreased metabolic activity, and the exchange of resistance genes between cells. As a result, infections caused by biofilms on prosthetic devices can act as persistent reservoirs and cause metastatic complications, such as endocarditis, deep-seated abscesses, septic arthritis, and osteomyelitis(3)

As a result of their co-evolution with bacteria, antimicrobial peptides are ancient chemicals that were perfected during an early period of mammalian evolution. AMPs constitute the majority of broad-spectrum antibacterial activity against bacteria, viruses, and fungi and are produced by almost all organisms. Their multifaceted roles in microbial destruction, inflammation, angiogenesis, and wound healing make them a vital component of the human innate immune system (4).The cationic antimicrobial peptide GF-17, a 17-mer-derived peptide from human cathelicidin LL-37, has a significant strength in the killing of the methicillin-resistant *Staphylococcus aureus* and Escherichia coli strains. (5)GF-17 peptide has perfect amphipathicity and greater hydrophobicity, resulting in increased haemolytic activity. It is also effective in removing biofilms, especially against Staphylococcus aureus. (6).

The aim of this study is to evaluate the role of the antimicrobial peptideGF-17 as inhibitory agent against Methicillin-resistant *S. aureus* (MRSA)isolates of *Staphylococcus aureus* isolated from different clinicalsamples. Also, investigate the antibiofilm activity of this peptide and its effect on the gene expression of the biofilm genes (*icaA* and *rbf*).

Materials and Methods

Isolation and identification of bacteria

A total of 190clinical samples were collected from different sources (blood, urine, burn swabs, and wound swabs) from 3 hospitals in Baghdad.Specimens were transferred to Brain Heart Infusion (BHI) broth and incubated overnight at 37 °C then streaking on Blood, Mannitol salt, and HiCrome agar and incubated for 24 hours at 37 °C under aerobic conditions. Single colonies were subjected to Gram staining, catalase, and coagulase tests according to standard microbiological methods.VITEK-2 compact system and molecular detection were employed to confirm the biochemical identification.

Antibiotic Susceptibility Testing

Antibiotic resistance was assessed using the disk diffusion technique on Mueller Hinton agar. The isolates of *S. aureus* were evaluated using the Kirby-Bauer disk diffusion method with 12 different antibiotics. Methicillin(MET), Gentamicin (GEN), Ciprofloxacin(CIP), Cefotaxime(CTX), Azithromycin (AZM), Clindamycin(CD), Cefazolin(CZ), Tetracycline (TE), Vancomycin(VA), Levofloxacin(LEV), Tigecycline(TGC), and Trimethoprim-sulfamethoxazole (COT) were the antibiotic discs utilized in this study. After the agar plates were incubated at 37 °C for 24 hours, the percentage of susceptible, intermediate, or resistant isolates was used to evaluate and interpret the inhibition zone using CLSI breakpoint interpretation guidelines (7).

Minimum inhibitory concentrations (MIC) of GF-17 peptide

As recommended by the Clinical and Laboratory Standards Institute, the MIC was calculated using the microdilution technique (Microtiter Plate Assay with Resazurin Dye). A 1:2 serial dilution of GF-17 peptide was used in Mueller Hinton Broth (MHB) at dosages ranging from 3.9 to 500 µg/ml. A subculture of *S. aureus* was cultivated in brain heart broth for 18 to 24 hours at 37°C in order to create the bacterial inoculums for the test. In order to achieve a turbidity of 0.5 on the McFarland scale, the bacterial solution was diluted to $1x10^8$ CFU/mL.A final concentration of 5x105 CFU/mL was then achieved by diluting the materials in MHB 1:2. Before being added to a 96-well plate containing a diluted bacterial solution, the peptide was serially diluted. A total of 200 µl was added to each well, comprising 100 µl of peptide solution and 100 µl of diluted bacterial solution. In order to conduct both positive and negative growth controls, MHB alone was added to one line of wells and S. aureus with MHB to another. After 24 hours at 37°C, resazurin (6.75 mg/ml) was added to each well, and color changes were observed after 4 hours of incubation. Wells that showed no color change (blue Resazurin color stayed consistent) after incubation scored higher than the MIC (8).

Antibiofilm activity of GF-17 peptide

This test was performed on four strains that showed strong biofilm formation ability in the biofilm production assay. The effect of different concentrations of GF-17 peptideat sub-MIC peptide, to inhibit the ability of *S. aureus*cells to form a biofilm was assessed using the TCP method adopted by Zhang*et al*. (2021) (9).In 96-well polystyrene microtiter plates, about 100 µl of 0.5 McFarland bacterial cultures were added to each well along with 100 µl of the antibiofilm agent at appropriate doses. The plates were then incubated for 24 hours at 37°C. Positive controls for the growth of the biofilm were wells free of antimicrobial agents. Following incubation, the medium and non-adherent cells were taken out, and sterile PBS was used to wash the wells three times. After the plates were allowed to air dry, 100% ethanol was used to resolubilize the color. Using an ELISA reader (BioTek, Korea), the optical density (OD) of every well was measured at 570 nm. Every assay was carried out three times.

Gene expressionof *(icaA* and *rbf*) genes using RT- PCR assay

Four isolates of Methicillin-resistant *S. aureus* (MRSA)each with two biofilm genes (*icaA* and *rbf*) and varying MIC values, were used in the design of this experiment. Before and after the treatments, the gene expression of the four resistant isolates' genes was assessed. These agents were used in the treatment at concentrations below the minimum inhibitory concentration (MIC) to promote bacterial growth and resistance induction. After 24 hours, the measurement was made. Every well was duplicated, and every plate was copied. A study on gene expression made use of the copy.

Total RNA extraction

TRIzolTM Reagent (Promega, USA) protocol was followed in order to isolate RNA from the sample. A QuantusFluorometer was used to measure the extracted RNA concentration in order to evaluate the quality of samples for use in subsequent applications.

Preparation of primers

The primers were lyophilized and supplied by the Macrogen Company. Lyophilized primers were dissolved in nuclease-free water to a final concentration of 100 pmol/µl in order to create a stock solution. Ten microliters of primer from the stock solution which was kept at -20 ºC, were combined with ninety microliters of nuclease-free water to create a functional primer solution with a concentration of ten pmol/µl. Previous studies have indicated that specific primers were obtained (Table 1) for the purpose of detecting gene expression.

Table (1): Sequences of primers that used to gene expression of biofilm genes.

Gene	Primer name	Sequence (3'-5')	Product size	Reference
rbf	*rbf* (F)	ACGCGTTGCCAAGATGGCATAGTCTT	190 bp	(22)
	rbf (R)	AGCCTAATTCCGCAAACCAATCGCTA		
icaA	*icaA*(F)	CTGGCGCAGTCAATACTATTTCGGGTGTCT	195 bp	(22)
	icaA (R)	GACCTCCCAATGTTTCTGGAACCAACATCC		
16S rRNA	*16S* (F)	GTAGGTGGCAAGCGTTATCC	229 bp	(23)
	16S (R)	CGCACATCAGCGTCAG		

One step Quantitative Real-time PCR Assay (QRT-PCR)

The extracted RNA, primers and RT-qPCR master mix were thawed at 4 ºC, and mixed well by vortex, RT-qPCR reaction tubes placed into the thermocycler q- PCR. The reaction mix of one-Step quantitative RT-PCR including 5 µl of qPCRMasterMix, 0.25 µl of T mix and $MgCl_2$, 0.5 µl of Forwardand reverse primers, 2.5 of NucleaseFreeAquatic, 1 µl of RNA, and the total volume was 10 µl. Table 2, presented the reaction conditions of one-Step quantitative RT-qPCR.The gene expression was measured by using the ΔΔCt method. (10)

Table (2): Thermocycler program for One-Step quantitative RT-qPCR .

Step	Temperature	Time	Cycles
Reverse Transcription	37 °C	15 minutes	1
a.intial Denaturation	95	10:00	40
b.Denaturation	95 °C	00:20	
c. Annealing	60,66 or 60°C	00:20	
d. Extention	72 °C	00:20	

***16S rRNA*: 60°C; *icaA*: 66°C; *rbf.*: 60°C.**

Statistical Analysis

The SAS (2018) software was utilized to ascertain the impact of distinct groups on the study parameters. In this study, the least significant difference, or LSD, was employed to compare means in a meaningful way.

Results and discussion

Isolation and identification of Methicillin-resistant *S. aureus* (MRSA)

The biochemical tests, VITEK-2 compact system, CHROMagar, and mannitol salt agar medium identified 44 Methicillin-resistant *S. aureus*

(MRSA)isolates from all bacterial cultures analyzed.

All of the samples were cultured by streaking on Blood, Mannitol salt, and HiCrome agar and incubated for 24 hours at 37 °C. The findings showed that the colony on the blood agar is composed of bacteria with Large, creamy white color, and the ability to hemolyze blood and the type of hemolysis. *S. aureus* on blood agar often exhibits beta hemolysis. On Mannitol salt agar, the colonies exhibited Mannitol fermenting with yellow colonies as shown in Figure (1).

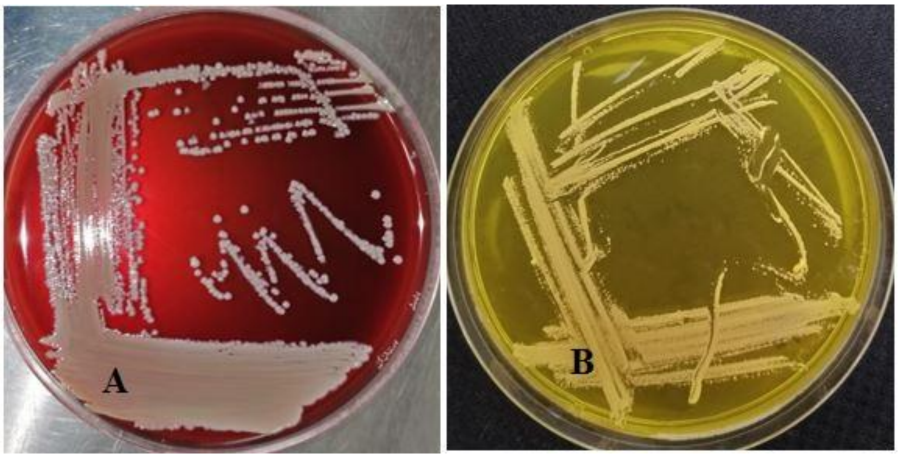


Figure (1) : *Staphylococcus aureus*colonies on :(a)Blood agar. (b)Mannitol salt agar.

Peptone special in the medium supplies the essential nitrogeneous, carbonaceous required for the growth. Phenol red is pH indicator. Mannitol in the medium is fermented by *Staphylococcus aureus* and the chromogenic mixture incorporated in the medium is specifically cleaved by *Staphylococcus aureus* to give greenish coloured colonies which are easily distinguishable,HiCrome™ Staph Selective Agar is a selective chromogenic medium recommended for the isolation and enumeration of coagulase positive staphylococci from clinical and non-clinical specimens. HiCrome is the selective agent and inhibits most bacteria by contain High concentration of sodium chloride that also helps in inhibiting the accompanying microflora. The positive results of *S. aureus* are the colonies with green color as shown in Figure (2).

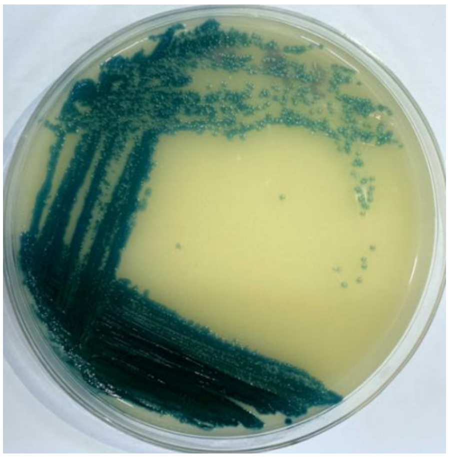


Figure (2): *Staphylococcus aureus*colonies on HiCrome™ Staph Selective Agar.

Antibiotic Susceptibility of *S. aureus*

Using the disk diffusion method, the resistance and sensitivity of 44 *Staphylococcus aureus* isolates to 12 antibiotics were tested.All isolates in this study were resistant to Methicillin (100%), i.e. all isolates were (MRSA). The isolates also showed the highest resistance to Vancomycin (61.3%), azithromycin (36.3%), and cefotaxime (25%). They showed intermediate resistance to gentamicin and cefazolin (11.3%), ciprofloxacin and trimethoprim-sulfamethazole(9%). The lowest resistance rate was to clindamycin (6.8%), tetracycline and tigecycline (2.2%).

Regarding sensitivity, the highest sensitivity rates were recorded for tigecycline, trimethoprim-sulfamethazoleand clindamycin .

Table (3):Percentages of antimicrobial susceptibility rate of 44 *S. aureus*isolates against 12 antimicrobial agents

Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
MET	(0) 0%	(0) 0%	(44) 100%
VA	(17) 38.6%	(0) 0%	(27) 61.3%
COT	(40) 90.9%	(0) 0%	(4) 9%
CZ	(39) 88.6%	(0) 0%	(5) 11.3%
CD	(40) 90.9%	(1) 2.2%	(3) 6.8%
CIP	(38) 86.3%	(2) 4.5%	(4) 9%
CTX	(33) 75%	(0) 0%	(11) 25%
LE	(39) 88.6%	(4) 9%	(1) 2.2%
AZM	(28) 63.6%	(0) 0%	(16) 36.3%
GEN	(38) 86.3%	(1) 2.2%	(5) 11.3%
TE	(39)88.6%	(4) 9%	(1) 2.2%
TGC	(42) 95.4%	(1) 2.2%	(1) 2.2%

Methicillin (MET), Gentamicin (GEN), Ciprofloxacin(CIP), Cefotaxime(CTX), Azithromycin (AZM), Clindamycin(CD), Cefazolin(CZ), Tetracycline (TE), Vancomycin(VA), Levofloxacin(LEV), Tigecycline(TGC), and Trimethoprim-sulfamethoxazole (COT)

The findings of the local study in Baghdad hospitals demonstrated for the susceptibility of 68 *S. aureus* isolates to 22 antimicrobial agents showed that all isolates were 100% resistant to cefotaxime and that the percentages of isolates that were resistant to ceftriaxone, cefoxitin, and erythromycin were 99, 87, and 65 %, respectively. On HiCromeMeReSa Agar Base medium, all 68 isolates were found to be MRSA; however, in an antibiotic sensitivity test, 66 of the 68 isolates were methicillin resistant (11).

According to AbdulRazzaq*et al*. local study (2022) (12), the presence of the *van A* gene indicated that all isolates were vancomycin resistant, however the presence of the *van B* gene showed that the isolates were (66.6%). Furthermore, our findings are contradicted by the substantial resistance to azithromycin and cefoxitin, as well as the 52.8% and 14.2% resistance to tetracycline and vancomycin, respectively.

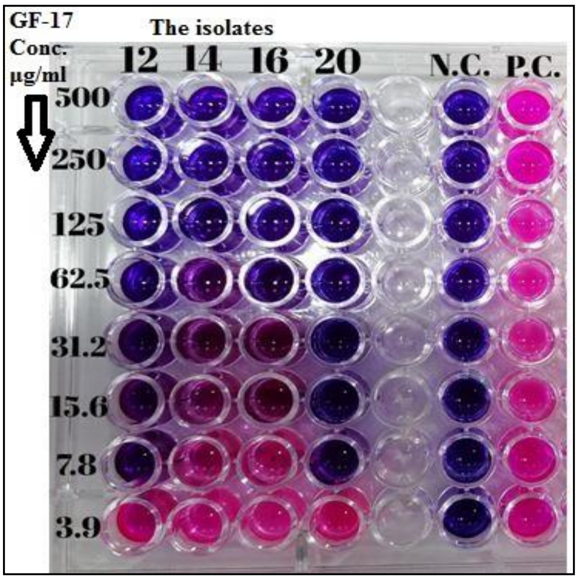
Globally, the treatment of infections caused by methicillin-resistant *S. aureus* (MRSA) strains is a serious and persistent challenge. A nonnative gene that encodes a penicillin-binding protein (PBP2a) with a much reduced affinity for β-lactam antibiotics is typically acquired to confer resistance. Because of this resistance, β-lactams' target, cell-wall production, can proceed even when antibiotic doses are normally inhibitory. A proteolytic signal transduction system consisting of a sensor protein (MecR1) and a repressor (MecI) regulates the expression of the mecA gene, which encodes PBP2a and is carried on a unique mobile genetic element (SCCmec)(13).

Minimum Inhibitory Concentrations (MICs) of GF-17 against Methicillin-resistant *S. aureus* (MRSA)

Using the microtiter method with resazurin stain to test 4 isolates of Methicillin-resistant *S. aureus* (MRSA)that are strong in forming biofilms, the results of the minimum inhibitory concentrations of the antimicrobial peptide GF-17 against the four isolates showed that the range of inhibitory activity was (15.6 - 7.8 µg/ml). Because the peptide GF-17 is short and has a high activity, the concentration was started at 500 µg/ml and reached a concentration of 3.9µg/ml. Isolates S.12 and S.20 were affected by the lowest concentration (7.8µg/ml), while isolates S.14 and S.16 were inhibited by a concentration (15.6µg/ml) as shown in the figure(3) and table (4).

Table (4): The minimum inhibitory concentrations of against Methicillin-resistant *S. aureus* (MRSA)isolates at concentrations (500-3.9 µg/ml)

GF-17 conc. (µg/ml)	The isolate code				Negative control	Positive control
	S12	S14	S16	S20		
500	_	_	_	_	_	+
250	_	_	_	_	_	+
125	_	_	_	_	_	+
62.5	_	_	_	_	_	+
31.25	_	_	_	_	_	+
15.6	_	_	_	_	_	+
7.8	_	+	+	_	_	+
3.9	+	+	+	+	_	+



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

Figure (3) : The minimum inhibitory concentrations of against *Staphylococcus aureus* isolates at concentrations (500-3.9 µg/ml).

Examine GF-17's capacity to alter the model membranes of gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. According to the molecular dynamics studies, the peptide is stabilized on the membrane surface and quickly forms hydrogen bonds and electrostatic interactions with the phosphate headgroups of the model membranes. Additionally, the binding with the membrane surface is energetically promoted by both polar and nonpolar interactions. The study also shown that the key residues Arg23 and Lys25 were essential for GF-17's binding to both gram-positive and gram-negative model membranes(5).

The previous study was evaluated the antibacterial properties and effectiveness of synthetic Scolopendin A2 peptides and LL-37 fragment GF-17D3 against resistant clinical strains using both in vitro and in vivo models. With MICs of 64 µg/ml, 8 µg/ml, and 16 µg/ml, respectively, Scolopendin A2 demonstrated antibacterial efficacy against *P. aeruginosa*, *S. aureus*, and *A. baumannii*, while LL37 demonstrated antibacterial efficacy against *P. aeruginosa*, *S. aureus*, and A. *baumannii* with MICs of 128 µg/ml, 32 µg/ml, and 32 µg/ml, respectively. At 1 x MIC, both AMPs reduced biofilms by > 96%. The results showed that Scolopendin A2 had anti-biofilm activity at 1/4 × MIC and 1/2 × MIC concentrations of 47.9 to 63.8%, while LL37 showed anti-biofilm activity at 1/4 × MIC and 1/2 × MIC concentrations of 21.3 to 49.6% against three pathogens (14).

Antibiofilm activity of GF-17

The anti-biofilm effect of GF-17 peptide was tested using sub-MIC bymicrotiter plate method, adding crystal violet dye and reading the results using ELISA reader. The experiment was conducted on four isolates of Methicillin-resistant *S. aureus* (MRSA)that are strong in forming biofilms, and the minimum inhibitory concentration (MIC) was tested for them. The current 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 GF-17. It was found that the biofilm inhibition percentage was 91.22% for the isolate S14, while the minimum inhibition was 31.89% for the isolate S12, by using the sub-MIC of the peptide GF-17.

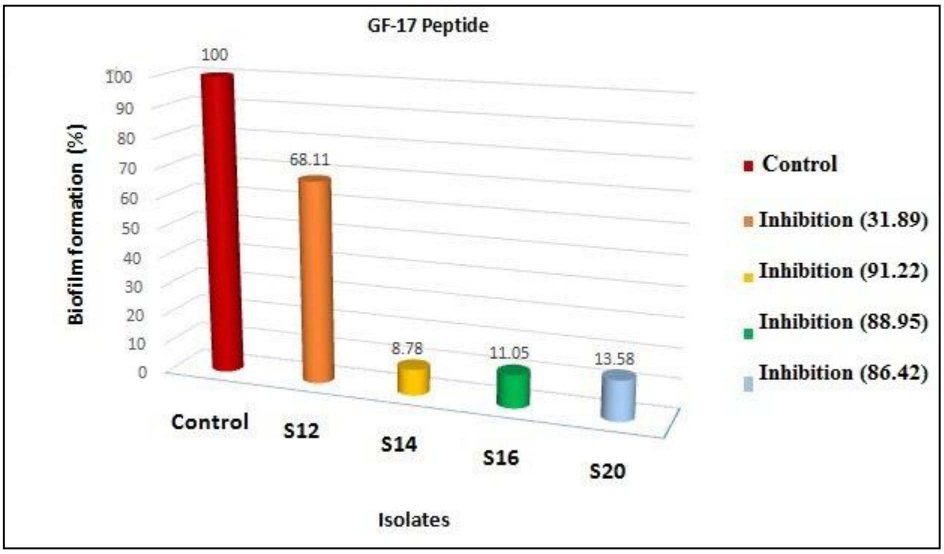


Figure (4): Biofilm formation inhibition of 4 Methicillin-resistant *S. aureus* (MRSA)isolates by sub-inhibitory concentration of antimicrobial peptides GF-17.

The local findings of Jabur and Kandala, (2022) (11) indicated that 56 of 68 isolates generated biofilm to varying degrees, according to the results of the microtiter plate method (MTPs) for biofilm detection, while 66 (97%) of the 68 isolates had the icaAD gene, as determined by PCR.After isolation, the sixty *S. aureus* isolates were identified. The isolates were separated into three biofilm types using the microtiter plate method: strong (25%) middling (66.6%) and weak (8.3%). 100% of isolates had ica operon, 100% had clumping factors genes, and 0% had (bap), according to the results of the PCR assay. And there was a correlation between *S. aureus* biofilm formation and the icaADBC operon (15).

According to previous study, the GF-17 antimicrobial peptide inhibits the attachment of bacteria to surfaces, prevents the growth and formation of biofilms, and also disrupts pre-formed biofilms. The study included the anti-biofilm effects of GF-17 against hospital- and community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which is consistent with the results of our study (16). Also, in a study by Decker *et al.*, the GF-17 peptide showed activity against methicillin-resistant *Staphylococcus aureus* (MRSA) in standard antimicrobial susceptibility, killing kinetics, and membrane permeability tests performed in vitro using planktonic bacteria. The peptide was also effective against both prior to biofilm formation and pre-formed biofilms (17).

Evaluate the gene expression of Biofilm genes

The minimum inhibitory concentration of each sample was determined and bacterial growth with the antimicrobial peptide (GF-17) was compared by this technique, where the treated and untreated samples were analyzed by RT-PCR in a quantitative manner. The analysis focused on determining mRNA expression of the genes for the biofilm formation (*icaA* and *rbf*). Quantitative RT-PCR software was used to detect the Ct values of amplified genes. Gene expression fold change was calculated by ΔΔCtvalue using relative quantification (RQ) method (10).Our results showed that the biofilm gene *icaA*was significantly down-regulated in the local isolateswith a fold change of 0.52 (figures 5). The results of this experiment were the average of gene expression of four isolates in comparison with the fold change of the control (the fold change is 1). The gene expression fold of the four isolates for the *rbf* gene was close to that of the control group, with a fold change of 1.26 .

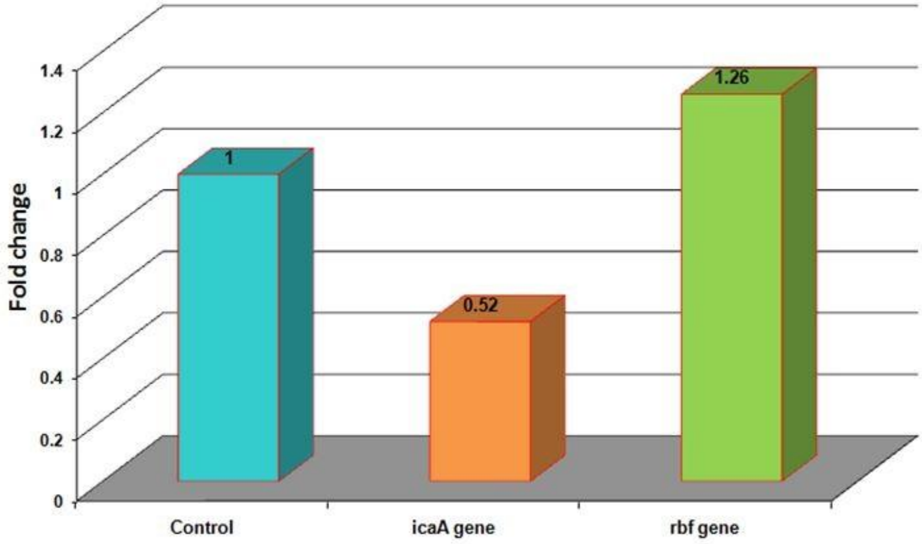


Figure (5): Fold of gene expression of biofilm genes for different *S. aureus* isolates at the sub-inhibitory concentrations of antimicrobial peptide GF-17 depending on ΔΔCt method.

The previous studies that use relative quantification usually compare the expression levels of a given gene across several samples. Reverse transcription quantitative PCR (RT-qPCR) differs from other gene expression methods because of this. The amplification was recorded using the cycle threshold, also known as the Ct value. The housekeeping gene used in this study was the *16S rRNA* gene. The gene's utility in molecular studies is predicated on its steady expression in the cells or tissues being examined under various circumstances (18).

One of the studies about the effect of antimicrobial peptide LL-37 on the gene expression of biofilm formation genes indicated that Following exposure to a suboptimal dose of LL-37, a statistically significant difference in the expression levels of the *atlA*, *RNAIII*, and *agrA*genes was seen between MRSA and MSSA bacteria. In the end, the necessary LL-37 antimicrobial concentration was fairly high; nonetheless, the LL-37 antibiofilm concentration might be suitable for human application against MRSA and MSSA strains that form biofilms (19).Antimicrobial peptides have several main anti-biofilm mechanisms, including: (1) breakdown or disruption of the membrane potential of cells embedded in biofilms; (2) disruption of bacterial cell signaling systems; (3) degradation of the biofilm matrix and polysaccharide; (4) inhibition of the alarm one system to prevent the bacterial stringent response; and (5) downregulation of genes involved in binding protein transport and biofilm formation (20).

Compared to AgNPs or antimicrobial peptide (AMP), the AMP, PDA, AgNPsnanocomposite shown better antibacterial action against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria. Crucially, the nanocomposites' effect reduced the mRNA expression of genes linked to biofilms..The outcome verified that the nanocomposite decreased the mRNA expression of genes relevant to biofilms. Furthermore, it was hypothesized that the AMP, PDA, AgNPs inhibited the production of biofilms by reducing the expression of the proteins las I and rh II, fim H. By suppressing the transcription level of genes linked to biofilm, the nanocomposite may prevent the production of biofilm, according to research on antibacterial mechanisms (21).

Conclusion

The GF-17 peptide demonstrated substantial inhibitory activity, effectively decreasing the growth of bacteria. Additionally, GF-17 significantly impaired biofilm formation. Additionally, gene expression study showed that the *icaA* gene, which is essential for biofilm formation, was significantly downregulated, whereas the rbf gene was unaffected.According to these results, GF-17 may be a viable substitute treatment for MRSA infections, especially those linked to biofilm formation, which frequently makes treatment more difficult and results in antibiotic resistance.

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