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Assessment of Gene Expression of some Biofilm- Related Genes in *Staphylococcus aureus* under the Effect of *Lactobacillus acidophilus*

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Abstract

A wide range of infections result from the Gram-positive pathogen *Staphylococcus aureus*. One key virulence factor of this bacterium is the capacity to develop biofilm. Antibiotics are ineffective in treating a majority of infections linked with biofilm formation in this opportunistic bacterium. The current study was conducted to estimate the influence of cell-free supernatant (CFS) of *lactobacillus acidophilus* on the expression level of some biofilm-association genes in clinical strains of *S. aureus.* The antibiotic susceptibility pattern against twenty antibiotics and quantitative assay for biofilm development were examined for all bacterial isolates. Moreover, the agar well diffusion method was followed to evaluate the impact of CFS on the growth of *S.aureus*. In addition, real-time PCR was utilized to determine the impact of CFS extract on the transcription level of the genes involved in forming *S. aureus* biofilms (*fib , eno,sdrC*). In isolates constituting biofilm, a high percentage of antibiotic resistance was detected. The CFS exhibited an antimicrobial effect toward strong biofilm-forming isolates. In addition, the results of real-time PCR for the treatment group showed a drop in the expression degree of *fib and sdrC* genes compared to the control group; meanwhile, the *eno* gene displayed upregulation after treatment with CFS. In conclusion, biofilm construction is a key strategy that may participate in preventing antimicrobial agents from killing *S. aureus,* especially since all isolates were multidrug resistant with a high percentage of strong biofilm former. In addition, CFS of *lactobacillus acidophilus* affects the growth of *S. aureus* and biofilm formation by displaying downregulation for some mediating biofilm formation genes.

Keyword: Biofilm, *S. aureus, lactobacillus*, Antibiotic resistance

تقييم التعبير الجيني لبعض الجينات المرتبطة بالغشاء الحيوي في المكورات العنقودية الذهبية تحت تاثير العصيات اللبنية الحمضية

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الخلاصة

هنالك مدى واسع من الاصابات الناتجة عن البكتريا الممرضة الايجابية لصبغة غرام المكورات العنقودية الذهبية.احد عوامل الفوعة الرئيسية لهذة البكتريا هو القدرة على تطوير الاغشية الحيوية .المضادات الحيوية غير فعالة في علاج غالبية حالات العدوى المرتبطة بتوليد الأغشية الحيوية في هذه البكتيريا الانتهازية. أجريت الدراسة الحالية لتقدير تأثير الراشح الخالي من الخلايا لبكتريا العصيات اللبنية الحمضية على مستوى

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التعبير الجيني لبعض الجينات المرتبطة بالاغشية الحيوية في السلالات السريرية لبكتريا المكورات العنقودية الذهبية.تم اجراء اختيار الحساسية ضد عشرين مضاد حيوي والقياس الكمي لتكوين الاغشية الحيوية لكل العزلات البكترية. علاوة على ذلك، تم اتباع طريقة الانتشار في الآجار لتقييم تأثير الراشح الخالي من الخلايا على نمو المكورات العنقودية الذهبية بالإضافة إلى ذلك، تم استخدام real time PCR , لتحديد تاثير الراشح على مستوى التعبير الجيني لبعض الجينات المشاركة في تكوين الغشاء الحيوي (*fib , eno,sdrC*) للمكورات العنقودية الذهبية. في العزلات التي تشكل أغشية حيوية تم الكشف عن نسبة عالية من مقاومة المضادات الحيوية. أظهر الراشح الخالي من الخلايا تأثيرًا مضادًا للميكروبات تجاه العزلات المكونة للأغشية الحيوية من النوع القوي. بالإضافة إلى ذلك، أظهرت نتائج تفاعل real time PCR في المجموعة المعاملة انخفاضا في مستوى التعبير الجيني لجينات *fib, sdrC* بالمقارنة مع المجموعة الغير معاملة بينما اظهر جين *eno* ارتفاعا في مستوى التعبير الجيني بعد المعاملة بالراشح .وفي الختام فان بناء الغشاء الحيوي يعد استراتيجية تساهم في منع العوامل المضادة للمايكروبات من قتل بكتريا المكورات العنقودية الذهبية خاصة ان جميع العزلات اظهرت مقاومة متعددة للمضادات الحيوية ونسبة عالية من الغشاء الحيوي من النوع القوي. بالاضافة الى ان راشح العصيات اللبنية الحمضية اظهر تاثير على نمو وتكوين الغشاء الحيوي لبكتريا المكورات العنقودية الذهبية من خلال خفض مستوى التعبير الجيني لبعض الجينات المتوسطة للغشاء الحيوي.

Introduction

Staphylococcus aureus is a well-known opportunistic bacterium that is extensively present in different hosts, such as humans and animals. *S. aureus*. Constantly colonized approximately 20–30% of humans, with the residual 30% only being temporary carriers [1].

The colonization of this bacterium works as a reservoir from which bacteria can be introduced to the host body when the immune response weakens, increasing the risk of infection. *S. aureus* can adapt to different hosts and environmental conditions and produce various types of diseases. *S. aureus* produces multiple virulence factors that contribute to its ability to cause severe illnesses. These factors are categorized as secreted exotoxins and cell-surface-associated virulence determinants [2].

In addition, the construction of biofilm by *S. aureus* represented a significant virulence factor, which is facilitated mainly by the genes involved in intercellular adhesion and acts as one of the most efficient protective strategies of this opportunistic bacteria [3]. *S aureus* is one of the most effective pathogens in biofilm production, so this property enables it to attach and stay for a long period on the tissues of the host and medical devices[4]. *S. aureus* can express different genes that play crucial roles in biofilm constriction, such *fib , eno,* and *sdrC*. *Eno* gene encoding to laminin-binding protein and *fib* gene encoding to fibrinogen binding protein has a role in attachment of the cells on the solid surface, while *sdrC* gene encoding to serine-aspartate repeat protein has a role in cell-to-cell attachment and cells to solid surface attachment. The biofilm prevents antibiotics from reaching *S. aureus* and can evade the host immune system's destruction and develop into persistent cells [5].

The biofilm can also encourage *S. aureus* to spontaneously mutate, speed up the acceleration of heritable antimicrobial resistance, and greatly aid the capacity of bacteria to obtain or transfer the determinants of antimicrobial resistance carried by the plasmid via horizontal gene transfer. Globally, the distribution of multidrug-resistant bacteria has increased, posing a threat to public health as a result of their genes being shared with commensal microorganisms found in humans, animals, and the environment [6]it is, therefore, essential to discover and create novel antimicrobial agent and potent medication to treat illness caused by multidrug resistance (MDR) bacteria like *S. aureu*s [7].

Probiotics such as probiotic *Lactobacillus acidophilus* strains have been suggested to eliminate *S. aureus* infections associated with MDR or biofilm-producing. There are

different ways in which probiotics can appear therapeutic. For instance, the acidity of culture media through lactic acid, formation of H_2O_2, and bacteriocin have been associated with the decreasing growth of *S. aureus* by lactobacilli [8]. Due to little research regarding the impact of CFS on *S. aureus* growth, this study aimed to estimate the influence of CFS on *S. aureus* growth and its ability to decrease the capacity to produce strong biofilm in *S. aureus* isolates and also to look for the impact of CFS on *S. aureus* on gene expression for some genes enrolled with biofilm formation in this case impact of CFS on *S. aureus* growth will be investigated phenotypically as well as genetically.

Methodology

S. aureus Isolation and Growth Conditions

Thirty-three *S. aureus* isolates were obtained from patients suffering from urinary tract infections in different local hospitals in Baghdad city. The isolation and identification of *S. aureus* isolates are based on traditional morphological characteristics of isolates and results of biochemical tests; for morphological identification, the samples were grown on different types of medium, such as mannitol salts agar and blood agar, and kept at 37◦C for 24 hrs. The biochemical tests were achieved for further identification of isolates by using catalase, oxidase, and coagulase tests; after that, the identification of *S. aureus* in urine was confirmed using the VITEK 2 Compact System [9]. The specimens were taken from participants under their consent following the guidance of Helsinki instructions according to reference number CSEC/1023/0093 permitted by the College of Science.

Antibiotic Sensitivity Profile

The Gram-positive sensitivity card (AST) was utilized using the Vitek 2 Compact System (BioMerieux/ France) to assess and evaluate the Antibiotic sensitivity patterns of all isolates under the study toward twenty different antibiotics. The isolates under the study were classified into (S) sensitive isolates, (I) susceptible dose-dependent and (R) resistant isolates. Moreover, the following formula was applied to determine the MDR index [10]

Index of MDR = The no of antibiotics that isolates show resistance / Total no of antibiotics examined

Quantitative Assay for Biofilm Formation

A sterile 96-flat-bottom polystyrene microtiter plate was utilized to examine quantitatively the capability of 33 *S. aureus* isolates to develop biofilms. One colony from the pure culture of each isolate was grown in 5ml of tryptic soy broth and put in an incubator for 24h at 37 °C. The density of bacterial growth for all isolates was adjusted to be equal to 0.5 McFarland density. Then, the bacterial growth was diluted by supplementing (10µl) of bacterial growth with (490 µl) of TSB -1% glucose. The wells were filled in triplicate with 150 µl of *S. aureus* growth. The wells containing sterile TSB-1% glucose without *S. aureus* growth acted as a negative control. The microtiter plate was put at 37 °C for 24hrs without shaking. After that, the bacterial growth was gently removed, and 200 µl of phosphate-buffered saline (PBS) was used to wash the well. Attachment bacteria were fixed by adding methanol, and wells were stained with 150 µL of crystal violet (0.1%) for fifteen minutes. The residual crystal violate was removed gently by washing the wells three times with PBS, and the microtiter plate was left for 60 minutes for air drying. The solubilization of the fixed stain was achieved by loading 150 µL ethanol (96%) for 30min.[11]. Next, the microtiter plate reader was used to read the optical density at 590 for each isolate (ODI) and control (ODC). The biofilm production capacity of *S. aureus* isolates was classified into 4 groups as follows: non-producers isolate (ODI < ODC), weak producers isolate (ODC < ODI < 2ODI), moderate producers isolate (2ODC < ODI < 4ODC), and strong producers isolate (ODI> 4ODC) [12, 13].

The Extraction of Cell-Free Supernatant (CFS) From lactobacillus acidophilus

The extraction of CFS from *lactobacillus acidophilus* was achieved by inoculating 10 ml of sterile MRS broth with two to three colonies of the fresh growth *Lactobacillus* strains and incubated in anaerobic conditions at 37°C for 48 hrs. The culture of bacteria was subjected to centrifuge at 3,000 rpm for 20 min at 4°C . The supernatant was collected in a fresh tube and passed through a sterile 0.45 milipore filter to remove all *lactobacillus acidophilus cells*. Then 0.1ml of CFS was cultured on MRS agar for 24 hrs at 37°C in anaerobic conditions to ensure the absence of *lactobacillus* cells. Finally, the supernatant was stored in the refrigerator until use[8].

The Impact of CFS on S. aureus Growth

The antimicrobial effect of CFS against *S. aureus* isolates was carried out following the agar well diffusion technique. Briefly, a sterile loop was used to pick up one colony from the pure culture of the *S. aureus* isolates and activated by culturing it in 5 ml of nutrient broth. The broth was left in an incubator for 18hrs at 37°C. Then the bacterial growth was diluted by nutrient broth to achieve a density equal to 0.5 density of McFarland suspension. The nutrient agar plates were used to culture it, and several wells of 5-millimeter diameter were made on each plate. A volume of 150 µl of extracted CFS was loaded into the wells. The well load with 150 µl of PBS represented negative control. The plates were put in an incubator for 24hrs at 37°C. Finally, the plate inhibition zones were measured in millimeters and compared with standard data [14].

The Effect of CFS on Gene Expression of Some Biofilm-Mediated Genes

The impact of CFS on the expression level of *fib , eno,* and *sdrC* genes was evaluated for three isolates (multidrug resistance and strong biofilm former) by utilizing real-time PCR, and the experiment was divided into two groups; the control group included untreated tubes (containing 8ml of sterileTSB-1% glucose with 0.8 ml of 0.5 McFarland culture of *S. aureus*) and treated group include treated tubes (containing 8ml of sterile TSB-1% glucose with 0.8 ml of McFarland culture of *S. aureus* plus the sub-inhibitory concentration of CFS). The suspension of each group was loaded onto a 6-well microplate, and the plate was kept for 24 hrs at 37°C. Then, the bacterial growth was removed, and cells were collected. The bacterial RNA was collected according to the guidelines of TRIzol™ Reagent (Thermo Scientific, USA). A Quantus Fluorometer was adopted to evaluate the concentration of extracted RNA. A real-time PCR procedure (One-step Promega, USA) was adopted to detect the difference in the *fib, eno*, and *sdrc* gene expression levels in treated and untreated groups with CFS. The quantitative RT-PCR component was prepared by adding 5 µl q PCR Master Mix(50X), RT mix (0.25 µl), $MgCl_2$ (0.25 µl), 0.5 µl of forward primer (10µM), 0.5 µl reverse primer (10µM), nuclease-free water (2.5 µl) and 1µl of templet RNA (1 ng/µL). The specific primers used in the current study and all steps of the RT-PCR program and their details, are illustrated in Table 1. The normalization of gene expression was achieved by using housekeeping gene. Finally, the results of RT-PCR were analyzed according to the ΔΔCT technique[15].

Table 1: Primers Utilized in RT-PCR and RT-PCR Condition

Name of Gene	sequence of Primers	Annealing temperature°C	Size of product	Reference
fib	**Forward** CGTCAACAGCAGATGCGAGCG **Reverse** TGCATCAGTTTTCGCTGCTGGTTT	60	239	[16]
eno	**Forward** TGCCGTAGGTGACGAAGGTGGTT **Reverse** GCACCGTGTTCGCCTTCGAACT	60	195	[16]
sdrC	**Forward** AAAAGGCATGATACCAAATCGA **Reverse** AATTCTCCATTCGTATGTTCTG	53	144	[17]

Program of RT-PCR			
Steps	Temperature °C	m: s	Cycles
Activation of reverse transcriptase Enzyme	37	15:00	1
Initial Denaturation	95	05:00	1
Denaturation	95	00:20	40
Annealing	54 or 60 or 63	00:20	40
Extension	72	00:20	40

Statistical Analysis

The P-value was calculated relying on the determination of the proportion of the data under the study using the Chi-squared test, and a P value that is less or equal to 0.05 was considered a significant result [18].

Results

Antibiotic Sensitivity Patterns

All *S. aureus* isolates were found to be resistant to at least one of the 20 antibiotics according to the results of antibiotic sensitivity patterns. A high percentage of resistance was found toward Benzylpenicillin and oxacillin 97%, followed by Erythromycin 78%, Piperacillin/ Tazobactam75%, and Tetracycline 51%. Conversely, a low percentage of resistance was indicated against Fusidic acid 30%, vancomycin 24%, Levofloxacin 12%, Tobramycin 6%and Moxifloxacin 3%. Additionally, 100% of isolates were sensitive to Gentamicin, linezolid, and Tigecycline, and 97% of isolates exhibited sensitivity to Nitrofurantoin, Rifampicin, and sulfamethoxazole, as shown in Figure 1. Moreover, all isolates (100%) under the study were multidrug resistant, exhibiting resistance to a minimum of 3 or more distinct classes of antibiotics with values of MDR index around 0.15 - 0.45, as mentioned in Table 2. The highest rate of multidrug resistance was demonstrated in three isolates, which showed resistance to 9 antibiotics with a MDR index of 0.45; meanwhile, the lowest MDR index was observed in seven isolates that resisted 3 antibiotics. The MDR score greater than 0.2 suggests an increased risk of antibiotic exposure to the bacterium.

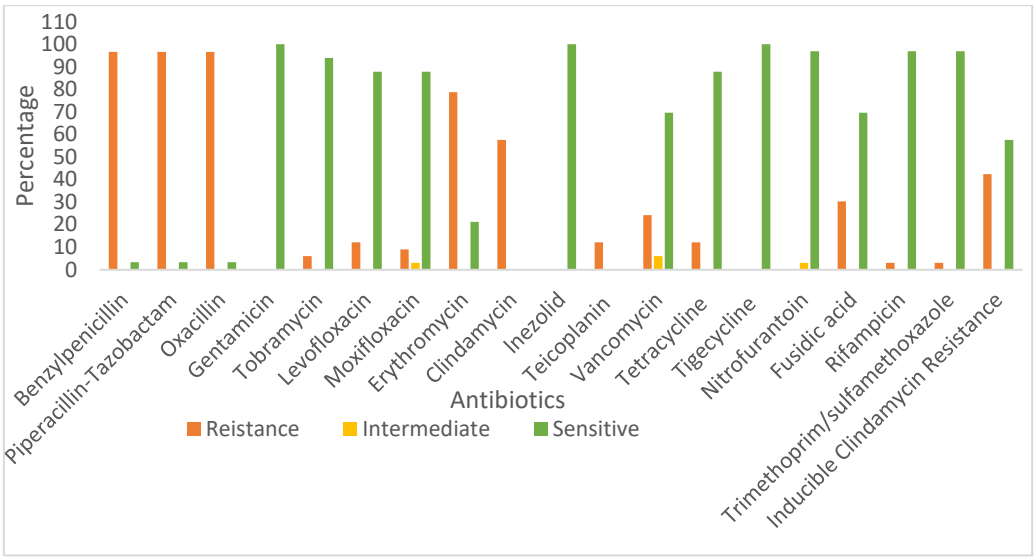


Figure 1 : Results of Antibiotic Sensitivity Pattern of *S. aureus*

Table 2 : Resistance Frequency in *S. aureus* isolates and MDR index values.

Number of isolates	Number of antibiotic resistances	Values of MDR index
7	3	0.15
2	4	0.2
6	5	0.25
4	6	0.3
7	7	0.35
4	8	0.4
3	9	0.45

Quantitative Assay for Biofilm Formation

The result of the biofilm constriction assay indicated that 96.96 % (32/33) of *S. aureus* isolates gave a positive result for biofilm constriction compared to 3.03% (1/33) of isolates were non-biofilm producers with significantly different (p = 0.0001). The capacity of biofilm development for positive isolates under the study shows various levels of biofilm production. Only two isolates (6.06%) were defined as weak biofilm producers, while (48.48%) of isolates were indicated as moderate biofilm producers, followed by (42.42%) of isolates that were noticed to be strong -biofilm development. The comparison between weak and strong biofilm producers was checked, and it was not statistically significant, with a P value of 0.3, as illustrated in Table 3.

Table 3: Results of Biofilm Development Assay in *S. aureus*

Biofilm development	Number of isolates	Percentage	P value
Non-producer isolates	1	3.03%	
Biofilm producer isolates	32	96.96%	**P = 0.0001**
Total	33	100%	

Groups of biofilm in biofilm development isolates

Biofilm Degree	Number of isolates	Percentage	P value
Weak producer	2	6.06%	p =0.3
Moderate producer	16	48.48%	
Strong producer	14	42.42%	
Total	32	96.96%	

The Impact of CFS on Growth of S. aureus

The inhibitory influence of CFS on *S. aureus* was examined using the agar well diffusion technique. The results indicated that CFS possesses an antimicrobial impact that results in the appearance of clearing zones on the surface of the plate, as shown in Figure 2. The clearing zone diameter ranged from 20 to 25 mm. The formation of organic acids, production of H_2O_2, and bacteriocin are the main factors involved in the antimicrobial activity of CFS.

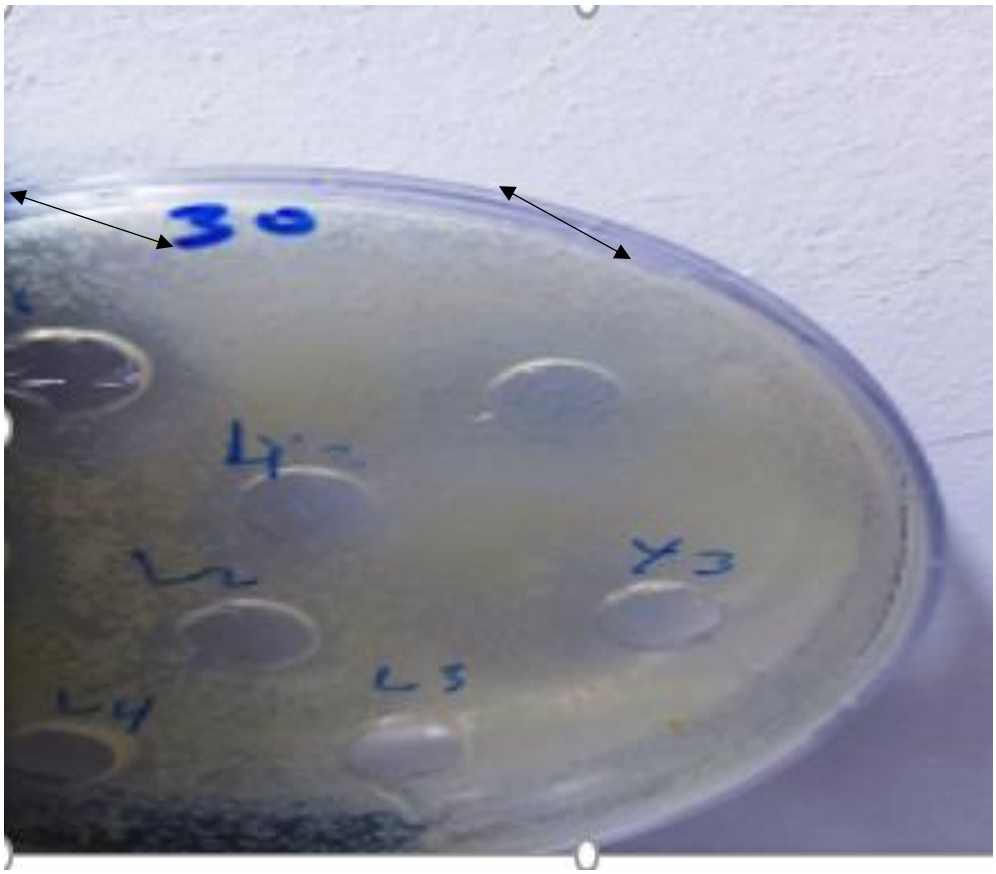


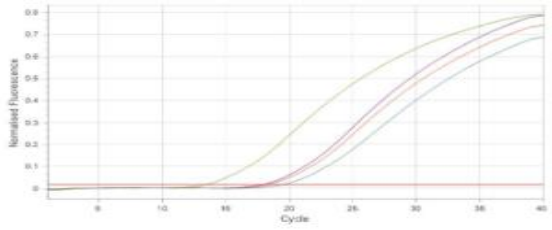
Figure 2 : Antimicrobial Effect of CFS on *S. aureus* Growth

Impact of CFS on Gene Expression of Some Biofilm-Mediated Genes

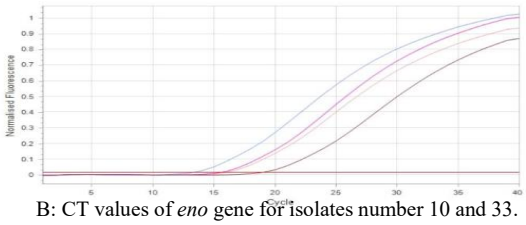
The Analysis and interpretation of gene expression results according to the Livak method indicated that CFS(Sub–MIC) caused a drop in the expression level of *fib* and *SdrC* genes in two strong biofilm development isolates. However, the CFS resulted in an increase in the expression level for the *eno* gene in two strong biofilm producer isolates, as illustrated in Table 4 and Figure 3.

Table 4 : Calculation of ΔCT,ΔΔCT and fold change for genes under study (C: control T: treated)

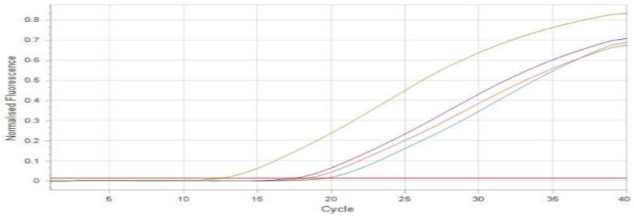
Isolates code	*16SrRNA*	*fib*	*ΔCT*	*ΔΔCT*	*Fold change*	*Result*
C10	11.22	12.54	1.32	0.00	1.00	Decrease
T10	13.51	17.50	3.98	2.66	0.16	
C18	11.95	11.51	-0.44	0.00	1.00	Decrease
T18	11.32	10.99	-0.33	0.11	0.92	
C33	13.67	19.63	5.96	0.00	1.00	Increase
T33	12.55	18.35	5.80	-0.16	1.12	
Isolates code	***16SrRNA***	***eno***	**ΔCT**	**ΔΔCT**	**Fold change**	**Result**
C10	11.22	13.33	2.11	000	1.00	Decrease
T10	13.51	16.01	2.50	0.39	0.76	
C18	11.95	11.37	-0.58	0.00	1.00	Increase
T18	11.32	8.15	-3.17	-2.59	6.02	
C33	13.67	19.03	5.37	0.00	1.00	Increase
T33	12.55	15.61	3.06	-2.31	4.94	
Isolates code	***16SrRNA***	***sdrC***	**ΔCT**	**ΔΔCT**	**Fold change**	**Result**
C10	11.22	13.41	2.18	0.00	1.00	Decrease
T10	13.51	17.99	4.47	2.29	0.20	
C18	11.95	11.35	-0.6	0.00	1.00	Decrease
T18	11.32	12.09	0.77	1.37	0.38	
C33	13.67	19.44	5.77	0.00	1.00	Increase
T33	12.55	18.25	5.71	-0.07	1.05	



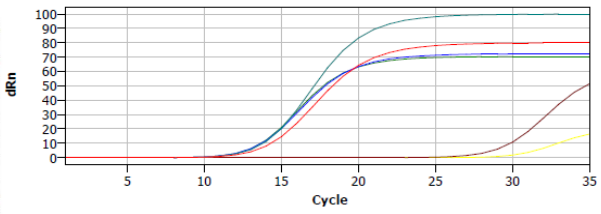
A:CT values of *sdrC* gene for isolates number 10 and 33.Green line: indicates control of isolate 10, blue line: control of isolate33, purple line: test of isolate 10, orange line: test of isolate 33



B: CT values of *eno* gene for isolates number 10 and 33. pink line: indicates control of isolate 10, brown line: control of isolate33, blue line: test of isolate 10, purple line: test of isolate 33



C: CT values of *fib* gene for isolates numbers 10 and 33. Green line: indicates control of isolate 10, blue line: control of isolate33, purple line: test of isolate 10, orange line: test of isolate 33



D: CT values for *eno, fib* and *sdrC* of control for isolate 18. Blue line: *sdrC*, green line*: eno*, dark green: *fib*

Figure (3): Illustrate the values of CT for the genes under study for the isolates number 10, 18, and 33.

Discussion

A pattern of antibiotic susceptibility tests performed on isolates under study revealed a high resistance rate ranging from 97% to 51%, mostly for Penicillin, Oxacillin, and Erythromycin, and this was agreed with Adhikari *et al.,* [19] by which high resistance was detected mostly against Penicillin and Erythromycin. On the other hand, resistance to antibiotics in *S. aureus* isolates was reported in Azithromycin, Erythromycin, and Clindamycin with around 82 % [20]. Moderate resistance to antibiotics was detected to vancomycin at 24% while it dropped down to 2%, as reported by Adhikari *et al*., [19]. All isolates exhibited sensitivity to linezolid and gentamicin, which was compatible with [21]. All isolates were multidrug-resistant, with 100% as announced in our study, compared to 94% reported by An *et al.,* [20]. The resistance of antibiotics in bacteria arises due to different mechanisms, and the reason why resistance for a particular antibiotic was high in isolates in the current study in comparison with other isolates in other studies is that the isolate under investigation possesses this mechanism while it is absence in other due to different gene sequences among isolates example mutation may occur on target site for an antibiotic so the antibiotic cannot bind with a specific target; therefore, resistance is raised in isolates under investigation[22].

The result of biofilm formation assay indicated that most of the isolates constituted biofilm, with 96.96 % mainly as strong and moderate, weak biofilm producers. In comparison, only 3.03% of the isolates were non-forming biofilm. The reason for producing strong biofilm in isolates under investigation was due to the fact that the isolates showed high virulence factors, especially all isolates collected from patients who attended hospitals, and biofilm formation is considered one of the virulence factors handled by the bacteria to defend against immune attack strategies. This result highlights the potential role of biofilm as a virulence factor in isolates under study and its contribution to antibiotic resistance, especially since the prevalence of multidrug resistance was 100%. In other words, biofilm development may encourage the isolates to persist in their host by introducing resistance to antimicrobial agents. The matrix's anti-penetration ability, the presence of polysaccharides, antibiotic-modifying enzymes, external DNA, and bacteriophages promote biofilm resistance and antibiotic tolerance[23].

Aniba *et al* [24] also showed 90% of isolates were biofilm producers, but only 50% were detected as multidrug-resistant. Pokhrel *et al.,* [21] declare that 80% of isolates were among moderate and strong biofilm development isolates. However, Tuon *et al.,* [5] revealed that around 20% of isolates did not produce biofilm. In addition, a high percentage of non-biofilm producers (59%), was reported by Leshmen *et al.,* [25]. The impact of CFS on *S. aureus* growth was investigated in the current study, and it showed a clear inhibition zone with a diameter ranging between 20-25 mm. This indicates CFS possesses a great inhibitory impact on *S. aureus.* The formation of organic acids, production of H_2O_2, and bacteriocin are the main factors involved in the antimicrobial activity of CFS. This result was compatible with Saidi *et al.,* [8], who showed the inhibition zone caused by CFS ranged between 37 and 50 mm. Reduction in the growth of *S. aureus* mainly due to the reduction in the PH caused by the production of organic acid by *lactobacillus acidophilus* as well as the production of lytic proteins such as LysM-containing peptidoglycan binding protein and peptidase (protein) [26] . The effect of CFS on expression degree for some genes mediating biofilm formation was reported in the current study. *Eno* gene encoding to laminin binding protein and *fib* gene encoding to fibrinogen binding protein have a role in attachment of the cells on a solid surface, while *sdrC* gene encoding to serine-aspartate repeat protein has a role in cell to cell attachment and cells to solid surface attachment [27]. The effect of CFS on gene expression for *eno*, *fib,* and *sdrC* was investigated in three isolates (strong biofilm producers and

multidrug resistance). For the *eno* gene, the result revealed that two isolates showed upregulation in expression level while one isolate appeared downregulation. However, expression in the *fib* and *sdrC* genes in two isolates showed downregulation, while one isolate appeared upregulation. This result may indicate that CFS decreases the *S. aureus* capacity to develop biofilm by decreasing cell to cell contact and contacting the cells with sold surface.Probiotics as anti-biofilm strategies several studies have demonstrated that certain probiotics such as lactic acid bacteria are capable of preventing cell adhesion and controlling biofilm development by several pathogens [28]. To the best of our knowledge, this is the first research to report the influence of CFS of *lactobacillus acidophilus* on gene expression of *eno*, *fib* and *sdrC*. However, Saidi *et al.,* [8] showed that the CFS of *lactobacillus spp* significantly decrease in gene expression for *sarA, icaA, sea* and *tst*. Other studies showed the effect of other substances on *eno*, *fib* and *sdrC,* Savirin and trans-Cinnamaldehyde and Manuka honey were significantly decrease in gene expression for *eno*, *fib* [29, 30] .

Conclusion

Biofilm formation is a powerful strategy that may help prevent antimicrobial agents from killing *S. aureus,* especially since all isolates were multidrug resistant with a high percentage of strong biofilm former. In addition, CFS of *lactobacillus acidophilus* affects the growth of *S. aureus* and biofilm formation by displaying downregulation for some genes mediating biofilm formation. Our study has some limitations due to the gene expression being carried out for all genes under study with only three isolates; however, we highly recommend increasing the number of isolates as a future aspect.

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