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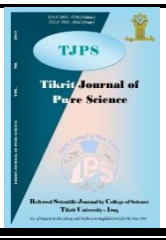

Evaluation of Geh gene expression in *Staphylococcus aureus* isolated from acne patients

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ABSTRACT

Acne vulgaris is a chronic skin disease that affects the hair follicle and sebaceous glands. *Staphylococcus aureus* colonizes human skin and causes infections ranging from local skin infections to systemic infections that may threaten human life due to its ability to produce many virulence factors and antibiotic resistance. The objective of this study is to measure the genetic expression of Geh gene responsible for producing the lipase enzyme from *S. aureus* isolated from acne patients. This study was conducted by collecting 130 swabs from acne patients at Tikrit Teaching Hospital. Using routine bacterial isolation and identification methods, 50 samples (38.4%) of the total samples were identified as *S. aureus* through its ability to grow in mannitol salt agar medium, the fermentation of mannitol sugar, and the change of the medium color to yellow. The *Geh* gene expression of ten *S. aureus* isolates from acne patients was examined using RT-PCR. The results showed that *Geh* gene was expressed in all isolates, with an expression ranging from 1.5 to 7.2, and that the mean folding of the *Geh* gene for ten samples was 4.2. This indicates the presence of high gene expression of the *Geh* gene in *S. aureus* isolates. Therefore, it is concluded that Lipase enzyme plays an important role in the pathogenesis of *S. aureus* in acne patients.

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تقييم التعبير الجيني لجين *Geh* في بكتيريا المكورات العنقودية الذهبية المعزولة من مرضى حب الشباب

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قسم علوم حياة، كلية العلوم، جامعة تكريت، تكريت، العراق

الملخص

حب الشباب الشائع هو مرض جلدي مزمن يؤثر على بصيلات الشعر والغدد الدهنية. تستعمر المكورات العنقودية الذهبية جلد الإنسان وتسبب التهابات تتراوح من الالتهابات الجلدية الموضعية إلى الالتهابات الجهازية التي قد تهدد حياة الإنسان، بسبب قدرته على إنتاج العديد من عوامل الضراوة ومقاومة المضادات الحياتية. هدفت الدراسة الى قياس التعبير الجيني لجين *Geh* المسؤول عن انتاج انزيم اللايبيز في بكتريا المكورات العنقودية الذهبية المعزولة من مرضى حب الشباب. جمعت 130 مسحة من مرضى حب الشباب في مستشفى تكريت التعليمي. باستخدام الطرق الروتينية تم تشخيص 50 عينة وبنسبة 38.4% كبكتريا مكورات عنقودية ذهبية من مجموع العزلات. من خلال قدرتها على النمو في وسط mannitol salt agar، وتخمر سكر المانيتول، وتم تأكيد التشخيص بالفحوصات الكيموحيوية. تم فحص التعبير الجيني لجين Geh لعشر عزلات من بكتريا المكورات العنقودية الذهبية المعزولة من مرضى حب الشباب باستخدام تقنية RT-PCR. أظهرت النتائج ان جين *Geh* كانت معبرة في جميع العزلات وبمدى تعبير من 1.5 الى 7.2 وان mean folding لجين *Geh* بالنسبة لعشر عينات كانت 4.2, وهذا يدل على وجود تعبير جيني عالي لجين Geh في عزلات المكورات العنقودية الذهبية . لذلك نستنتج ان انزيم اللابيز يلعب دور مهم في امراضية بكتريا المكورات العنقودية الذهبية لدى مرضى حب الشباب.

Introduction

Acne is a common chronic inflammatory skin disease of the pilosebaceous unit and involves internal and environmental factors such as androgen-induced sebum overproduction, ductal hyperkeratosis, and changes in the skin microbial environment [1]. *S. aureus* and *Propionibacterium acne* are the main causes of infections [2]. *S. aureus* are characterized by being Gram-positive bacteria with a spherical shape arranged in the shape of grape clusters, and are considered one of the most widespread microbes in the human body [3]. Cross-sectional research indicates that about 43% of people with acne carry *S. aureus* [4]. *S. aureus* produces multiple enzymes during infection, These enzymes enable it to invade and destroy host tissues as well as spread to other sites within the host. Lipase is one of these enzymes and is related to the virulence of *S. aureus* [5]. About 80% of clinical isolates of *S. aureus* isolated from pathological conditions such as impetigo, furunculosis, osteomyelitis, bacteremia, and peritonitis have lipolytic activity, and isolates from cases of widespread or deep infection have more lipolytic activity than those found in cases of local or superficial infections [6]. Most strains of *S. aureus* contain two types of lipase enzyme, Sal1 and *Geh* (Sal2), in addition to a putative esterase SAUSA300-0641. The enzymatic activity was clearly described for both Geh and

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Sal1. *Geh* analyzes long and short-chain triglycerides, and the optimum pH for this enzyme to work is 8.0 [7]. Sal1 analyzes short-chain triglycerides, and the optimum pH for the work of this enzyme is 6.0 [8]. Both *Geh* and Sal1 show high degree of similarity, especially in the mature lipase portion, and are organized as pre-pro-enzymes [9]. A study was conducted to find out how *S. aureus* lipase interacts with the host immune system and weakens the process of innate immune recognition of the microbe. It was found that the *Geh* type of lipase enzyme prevents the process of activation of innate cells in culture. It was also found that a mutation in *Geh* gene leads to stimulating the production of proinflammatory cytokines during infection, an increase in innate immune activity, as well as an improvement in the process of removing bacteria from infected tissues [10]. In addition, recent studies have revealed the ability of *Geh* gene to hydrolyze host lipids and liberate free fatty acids, then use them in the synthesis of phospholipids for bacterial membranes [11]. Lipases produced by *S. aureus* have been shown to impair host granulocyte function, and enhance bacterial survival during host defense by inactivating bactericidal lipids [5].

Materials and Methods

Isolation and Identification

One hundred and thirty swab samples were collected from acne patients from July 2023 to December 2023 at Tikrit Teaching Hospital in the city of Tikrit, using sterile transport swabs. Bacterial isolates were grown on blood agar medium using the streaking method and incubated at 37°C for 24 hours. After that, lysis was observed in the dish, where the appearance of transparent areas around the colonies indicates the positive result and the ability of the bacteria to analyze the blood. Depending on the type of lysis, the appearance of a transparent, colorless area around the colonies is known as beta hemolysis, or the appearance of a green belt around the colonies, known as alpha hemolysis, either In the case of a negative result, no transparent area appears around the colonies and is known as gama hemolysis [13]. Bacterial isolates were grown on Mannitol salt agar medium. The color change of the medium from red to yellow indicates a positive result and fermentation of the mannitol sugar. MSA is considered a diagnostic and differential medium for isolating Staph. Aureus [14].

Microscopic and Morphological Features

Gram stain was exploited to identify *S. aureus* isolates that appeared as Gram-positive cocci with an arrangement resembling grape-like clusters [15].

Biochemical Test

Catalase Test

This test was performed by transferring a pure colony, 18-24 hours old, grown on nutrient agar medium using a sterilized wooden stick to a clean glass slide, then adding a drop of hydrogen peroxide reagent at a concentration of 3%. The appearance of air bubbles is evidence of the bacteria's ability to produce the catalase enzyme and is evidence of a positive result.

Coagulase Test

This test was performed by adding 0.5 milliliters of liquid bacterial culture that had been previously grown at 37°C for 24 hours to clean

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and sterile test tubes containing 0.5 milliliters of blood plasma. The tubes were incubated at 37 degrees for (2-4) hours. Plasma coagulation was observed in the tubes, and the formation of a clot or clot is evidence of a positive result.

Gene Expression

Gene expression was measured using the Two Step PCR method. Initially, RNA was converted to cDNA using RT PCR Master Mix. The polymerase chain reaction was then performed using SYBR qPCR master mix in a separate tube. The relative quantitative method was used to measure the amount of gene expression, where the gene expression of the sample was measured based on a reference sample known as the control.

The gene expression of the *Geh* gene, which is responsible for producing lipase enzyme, was measured for 10 samples isolated from acne patients representing target samples and 3 samples isolated from the skin of healthy people representing control samples.

The expression value in both samples was measured by comparing the CT results for each isolate with the CT results for the reference gene. The 16sRNA gene was used as a reference gene for the *Geh* gene, as shown in Table (1).

Table 1: Primers used for real time PCR.

Primers	Primer sequence 5′→3′	Size bp	reference
Geh F	CCACGCTGCAGATGAATTAG	113	designed for this study
Geh R	CCCATTGAGACAAACCGAAG		
16S rRNA F	GGGACCCGCACAAGCGGTGG	191	21
16S rRNA R	GGGTTGCGCTCGTTGCGGGA		

RNA Extraction

To extract RNA from *S. aurues* isolates, pure bacterial isolates growing on nutrient broth medium were transferred to 1.5 ml centrifuge tubes (Eppendorf tubes). Then, the tubes were centrifuged at 13,000 rpm for two minutes. The supernatant were discarded, and RNA remains in a sediment at the bottom [16]. RNA were then extracted following manufacturer instructions. (TransZol Up Plus RNA Kit, TransGen Biotech/China)

Complementary DNA (cDNA) Synthesis

cDNA is manufactured from RNA using RT master mix, where 10 µl of the extracted RNA from each sample is added in PCR Eppendorf tubes. The tubes are incubated in a thermal cycler for 25 minutes, after which the temperature in the device is set to 85°c for 5 seconds in order to Reverse Transcriptase inactivation. The manufactured cDNA is either used directly or stored at -20 degrees for later use.

RT-qPCR

The RT-PCR reaction was performed using 2x UltraSYBR Mixtur (WithLow ROX) manufactured by MEBEP TECH BIOSCIENCE company. The reaction mixture for RT-PCR was prepared as shown in the table (2).

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Table 2: Components of the RT-PCR reaction used in gene expression for each gene

Component	Volume
UltraSYBR Mixture (WithLow ROX)	12µl
Forward Primer	0.5µl
Reverse Primer	o.5µl
cDNA Template	2µl
Nuclease-free water	up to 25µl

The thermal cycling steps were then programmed as shown in the table (3).

Table 3: RT-PCR Cycling Program

Step	Temperature °C	Duration or time	Cycles
Pre-denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing	60°C	30 sec	
extension	72°C	30 sec	

Analysis of Gene Expression

The Livak method was used to measure the gene expression value by using the following equation:

$\Delta Ct(control) = Ct_{(control\ gene)} - Ct_{(refrence\ gene)}$

$\Delta ct(target) = Ct_{(target\ gene)} - Ct_{(refrence\ gene)}$

$\Delta\Delta Ct = \Delta Ct(targ) - \Delta Ct(con.)$

$Fold = 2^{-\Delta\Delta Ct}$.

Result and Discussion

Samples Collection and Isolation

A number of 130 swabs were obtained from acne cases at Tikrit Teaching Hospital in the city of Tikrit. *S. aureus* isolates were identified phenotypically through their growth on MSA medium, their ability to ferment mannitol sugar and turn the color of the medium to yellow. A number of 50 samples were identified, and 38% of the isolates belonged to *S. aureus*. Also, 50 swabs were collected from healthy people who did not suffer from acne, 10 samples (20%) of the total samples were identified as *S. aureus* through its ability to grow in MSA medium, the fermentation of mannitol sugar, and the change of the medium color to yellow.

Identification of Culture Characteristics

The results of culture on blood agar medium showed that the S. aureus were beta hemolysis through their ability to completely decompose red blood cells and the appearance of a transparent halo around the colonies, as shown in figure1B.

The results of cultivation on mannitol medium also showed a change in the color of the medium from red to yellow due to the ability of bacteria to ferment mannitol sugar as shown in the figure1A.

Microscopic Features

The results of microscopic examination showed the presence of bacterial cells in the form of gram-positive cocci with an arrangement mostly grape-like clusters, as shown in the figure 1C.

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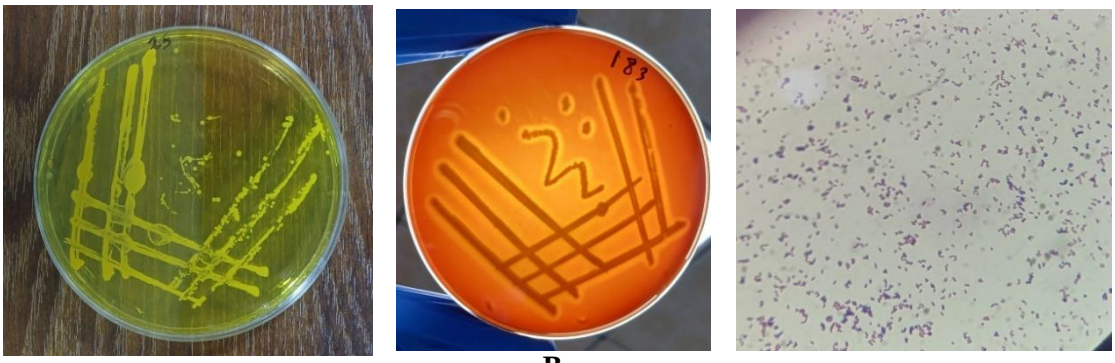
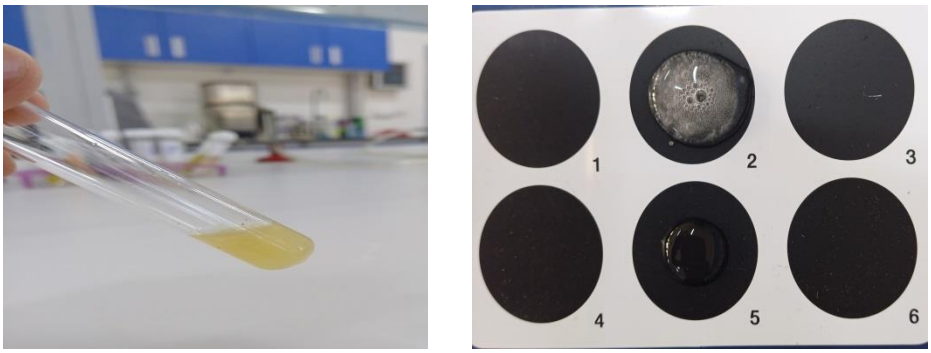


A B C

Fig. 1: (A) *S. aureus* on mannitol salt agar. (B): hemolysis by *S. aureus* on blood agar (C) *S. aureus* visualization under microscope

Biochemical Tests

The positive result of the Catalase test showed the formation of air bubbles on the slide, as shown in the figure2B, while the positive result of the Coagulase test showed the plasma coagulating in the tube and forming a clot as shown in the figure2A.



A B

Fig. 2: (A) Coagulase test (B) Catalase Test

Quantitative Gene Expression Results

The results showed that the Geh gene was expressed in all isolates, with an expression range between 1.5 and 7.2, and that the mean folding of the Geh gene for ten samples was 4.2, as shown in Table 4.

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Table 4: the values of Folding change for Geh gene

Samples	Ct(Geh gene)	Ct(16SRNA)	Δct	ΔΔct	Folding
S1	30.777	14.653	16.124	-0.867	1.824
S2	29.615	13.252	16.363	-0.628	1.545
S3	31.822	16.605	15.217	-1.774	3.420
S4	31.564	16.491	15.073	-1.918	3.779
S5	32.044	17.917	14.127	-2.864	7.280
S6	30.909	16.072	14.837	-2.154	4.451
S7	31.287	16.888	14.399	-2.592	6.029
S8	31.585	16.546	15.039	-1.952	3.869
S9	31.936	17.561	14.375	-2.616	6.130
S10	31.153	16.258	14.895	-2.096	4.275
Control 1	34.639	15.666	18.973	1.982	4.260
Control 2	34.269	18.324	15.945	-1.046	Mean Geh
Control 3	32.237	16.182	16.055	-0.936	
			16.991	0	1
			mean		control

This indicates an increase in *Geh* gene expression in *S. aureus* isolates that isolated from acne patients. This is due to the presence of bacteria in an area rich in fatty materials. *S. aureus* produce lipase enzyme to break down these materials to obtain their nutritional requirements. *S. aureus* may use them as a mechanism to evade the immune system, thus helping bacteria to colonize in this area and increasing their pathogenicity.

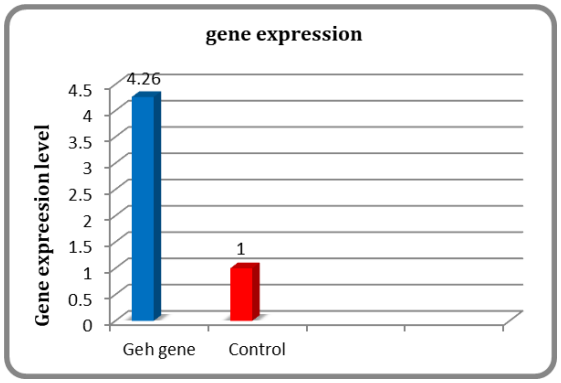

Fig. 3: Fold change of Geh gene in *s. aureus*

The increase in the gene expression of geh gene of *S. aureus* isolated from acne results from the skin containing Sebocytes, which are specialized in producing sebum in the hair follicles [17]. Therefore, *S. aureus* produces lipase which breaks down host fats into free fatty acids to gain nutrients, that consequently enhance the bacterial colonization process and the possibility of disease occurring [10]. *S. aureus* is an opportunistic pathogen that uses lipase for colonization and infection [18]. Clinical data indicate that lipase may contribute to spread and infection of *S. aureus* by circumventing host innate immunity via inactivation of bactericidal lipids and may interfere with the phagocytosis process as well as chemotaxis of granulocytes [19]. Lipase enzyme is one of the proteins that

contribute to the virulence of S. aureus bacteria [20].

Although the main role of lipase enzyme is to hydrolyze glycerol esters, lipase secreted by *S. aureus* and exposed to the surface has been described as an invasive factor for the host, thus facilitating the pathogenesis of the disease .

Conclusions

The results of the study showed that there is a high gene expression of the *Geh* gene responsible for producing the lipase enzyme in *S. aureus* isolated from acne patients due to the nature of the lipid-rich environment in the skin. The bacteria use this enzyme to obtain nutrients and inhibit the innate immune response.

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