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Molecular Detection of Virulence Factors in Clinical Isolates of *Staphylococcus aureus*

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Abstract

The 16S rRNA gene demonstrated a high degree of diagnostic accuracy (100%) , which reinforces the reliability of the results . High prevalence rates were recorded for PVL (90%) and clfA (80%), indicating the presence of highly virulent strains and their impact on infection . The differences in results compared to other studies are attributed to several factors, including environmental factors , sample type, and geographic location . *S.aureus* is an opportunistic pathogen responsible for a wide range of infections, ranging from mild skin infections to serious systemic diseases. This study aims to identify virulence factors in *S.aureus* bacteria isolated from various clinical cases. A total of 140 clinical samples were collected from patients admitted to Diwaniya Teaching Hospital between November 2025 and January 2026. The samples were diagnosed by microscopic examination and cultured on blood agar and menthol salt agar , followed by testing using the 16S rRNA gene and screening for virulence factors, including PVL and CLF-A, using PCR molecular methods. The results showed that 16S rRNA gene amplification was successful in 100 % of all samples. The PVL gene was detected in 18 out of 20 samples (90 %) , while the clfA gene was detected in 16 samples (80 %). These results indicate a widespread prevalence of virulence factors in the isolates included in the study. The study confirms the effectiveness of the 16S rRNA gene in molecular diagnosis, and indicates a widespread prevalence of the virulence genes PVL and clfA in S.aureus isolates , reflecting the virulence of these isolates and their ability to cause severe infections , with the distribution of these genes influenced by environmental factors , sample type, and geographic location.

Keywords: *Staphylococcus aureus*, PCR, 16S rRNA, PVL, clfA, virulence factors, molecular diagnosis

الكشف الجزيئي عن عوامل الضراوة في العزلات السريرية للمكورات العنقودية الذهبية

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الخلاصة

أظهر جين 16 S rRNAدقة تشخيصية عالية (100%)، مما يعزز موثوقية النتائج .وسُجلت معدلات انتشار عالية لـ PVL (90%)وclfA (80%)، مما يشير إلى وجود سلالات شديدة الضراوة وتأثيرها على العدوى .تُعزى الاختلافات في النتائج مقارنةً بالدراسات الأخرى إلى عدة عوامل، منها العوامل البيئية ونوع العينة والموقع الجغرافي .تُعد بكتيريا المكورات العنقودية الذهبية (*S. aureus*) من مسببات الأمراض الانتهازية المسؤولة عن طيف واسع من العدوى، بدءًا من التهابات الجلد الخفيفة وصولًا إلى الأمراض الجهازية الخطيرة .تهدف هذه الدراسة إلى تحديد عوامل الضراوة في بكتيريا المكورات العنقودية الذهبية المعزولة من حالات سريرية مختلفة .جُمعت 140 عينة سريرية من مرضى أُدخلوا إلى مستشفى الديوانية التعليمي بين نوفمبر 2025 ويناير 2026. شُخِّصت العينات بالفحص المجهري، وزُرعت على أجار الدم وأجار ملح المنثول، ثم خضعت لاختبار جين S rRNA16، وفُحصت عوامل الضراوة، بما في ذلك PVL وCLF-A، باستخدام تقنيات تفاعل البوليميراز المتسلسل (PCR). أظهرت النتائج نجاح تضخيم جين 16 S rRNAفي جميع العينات (100%). وتم الكشف عن جين PVL في 18 عينة من أصل 20 (90%)، بينما تم الكشف عن جين clfAفي 16 عينة (80%) . تشير هذه النتائج إلى انتشار واسع لعوامل الضراوة في العزلات المشمولة في الدراسة . تؤكد الدراسة فعالية جين 16 S rRNAفي التشخيص الجزيئي، وتشير إلى انتشار واسع النطاق لجينات الضراوة PVL و clfA في عزلات *S.aureus* ، مما يعكس ضراوة هذه العزلات وقدرتها على التسبب في عدوى شديدة، مع تأثر توزيع هذه الجينات بالعوامل البيئية ونوع العينة والموقع الجغرافي.

الكلمات المفتاحية: *Staphylococcus aureus*, PCR, 16S rRNA, PVL, clfA ، عوامل الضراوة، التشخيص الجزيئي

1. Introduction

S.aureus belongs to the genus Staphylococcus. It is spherical in shape and typically arranges in clusters resembling grape clusters when viewed under a microscope. It is Gram-positive, non-motile, and non-spore-forming. It ferments mannitol on Mannitol Salt Agar, turning the medium yellow, and is positive for catalase and coagulase. It is an opportunistic pathogen and a common colonizer of human skin; however, once it breaches the skin barrier, it can cause a variety of infections [1]. *S. aureus* is characterized by its high capacity to acquire multiple resistance mechanisms against antibiotics [2]. Staphylococcal virulence factors are characterized by their often overlapping roles in various pathogenic processes leading to adhesion to and invasion of host structures, followed by intracellular colonization, persistence within cells, and immune evasion [3].

Among the most prominent of these factors is Panton-Valentine leukocidin (pvl), a potent cytotoxin considered an important virulence factor for some strains of Staphylococcus aureus [4]. A associated with severe infections [5]. The molecular weight of the gene is estimated at 34 kDa [6]. It is a two-component toxin that causes the degradation of complement receptors on the membranes of white blood cells. These toxins are encoded by two genes, LukS-PV and LukF-PV, and act together as subunits that assemble on the membrane of host defense cells (white blood cells, monocytes, and macrophages) [7, 8]. Clumping factor A (clfA) is responsible for the adhesion of bacterial cells to fibrinogen and fibrin in the presence of *S. aureus* [9]. *S.aureus* contains two types of clumping factors, CIFA and ClfB, which act as adhesives; specifically, CIFA, when mixed with plasma, differs from a coagulation factor [10], as the former triggers a strong immune response in the host [11]. Therefore, the aim of study was to detect of virulence factors in clinical isolates of *S. aureus.*

2. Materials and Methods

A total of 140 clinical samples were collected from inpatients and outpatients at Diwaniya Teaching Hospital between November 2025 and January 2026 from various sources, including urine, wounds, and the middle ear (Table 1) , with the aim of studying the isolation and diagnosis of *Staphylococcus aureus*. The initial diagnosis was performed using microscopic examination after staining with Gram stain, where the bacteria appeared as Gram-positive cocci clustered in groups resembling clusters. The diagnosis was then confirmed by culturing the samples on differential media, such as mannitol salt medium, where the salt ferments and the medium turns yellow , The samples were also inoculated onto blood agar, where the bacterial colonies exhibited growth accompanied by beta-hemolysis, characterized by a clear zone around the colonies resulting from the breakdown of red blood cells .

To improve diagnostic accuracy, a molecular method was used that relies on the amplification of the 16S rRNA gene via PCR , which provided species-level confirmation of the isolates. Virulence genes (clfA, pvl) were then detected using PCR , given their role in increasing the bacteria's ability to adhere to tissues , evade the immune system , and cause inflammation , DNA was extracted from bacterial isolates using a ready-to-use extraction kit from Geneaid, following the manufacturer's instructions and procedures. The primer sequences used to amplify the PVL, ClfA, and 16S rRNA genes are listed in (Table 2). The primers were resuspended by dissolving the freeze-dried primer product, then preparing the primer master mix by adding PCR water (nuclease-free water) according to the manufacturer's instructions, as shown in Table 3 , The PCR tubes were placed in the thermal cycler, and the thermal cycling parameters were adjusted according to each starting material,

as shown in (Table 4). The PCR products were analyzed according to the manufacturer's instructions by agarose gel electrophoresis.

2.1 Statistical Analysis

Statistical analysis was performed using the chi-square (X^2) test to measure the degree of independence among the variables. This was conducted using IBM SPSS Statistics, Version 27. A p-value of 0.05 or less is considered statistically significant [12].

3. Results and Discussion

The results showed that the 140 samples, 32 (22.85%) tested negative in the culture test , while 108 (77.13 %) tested positive. Among the 108 positive samples, there were 20 isolates (14.8%) suspected to be of the *S.aureus* species (Table 1). Microscopic examination following Gram staining revealed that the bacteria appeared as Gram-positive cocci clustered in grape-like clusters Figure 1, In addition, cultures of the samples on blood agar showed growth accompanied by beta-hemolysis, which is characterized by the presence of a clear zone around the colonies resulting from the breakdown of red blood cells (Figure 2).

Table 1- Bacterial isolation by sample source

Sample Source	**Total Number Tested**	**Number of Positive**	**Results Percentage**
Urine Samples	68	16	23.52
Middle ear samples	26	1	3.84
Wounds	46	3	6.52
Total	140	20	14.28
Chi-square value		9.32	
Calculated significance value		0.009*	

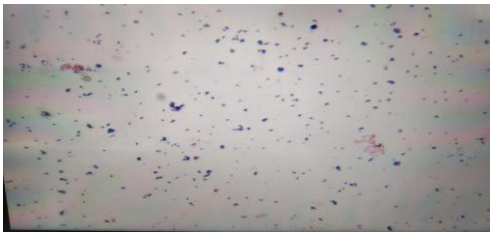


Figure -1 A microscopic image of *S.aureus* bacteria

PCR results using 16S rRNA (1.500 bp) primers showed distinct bands at the specific site in all bacterial isolates (20/20), with a 100 % positivity rate and no negative samples recorded. The presence of bands of approximately 1.500 bp in all lanes indicates that the amplification process was successful, confirms the integrity of the DNA extraction, and demonstrates the effectiveness of the PCR reaction in this study. The 16S rRNA gene is a conserved gene widely used in the field of molecular bacterial diagnosis, as it is present in all bacterial species and is characterized by conserved and variable regions that facilitate species identification (Figure 3). Polymerase chain reaction (PCR) results obtained using the PVL gene primer (433 bp) revealed the presence of bands at the target site in 18 out of 20 *S.aureus*

samples, representing 90% of the isolates, while no amplification was observed in the first and fifth samples, representing 10% of the isolates (Figure 4).

Table 2- polymerase chain reaction (PCR) Primers

Sources	Prepositions	(3 -'5 ') Sequence	Bp
(Marín et al., 2012)	*16srDNA-F*	AGAGTTTGATCCTGGCTCAG	1500
	16srDNA-R	CTACGGCTACCTTGTTACGA	
(Tristan et al., 2003)	*CLF-F*	ATTGGCGTGGCTTCAGTGCT	292
	CLF-R	CGTTTCTTCCGTAGTTGCATTTG	
(McClure et al., 2006)	*PVL-F*	ATCATTAGGTAAAATGTCTGGACAT GATCCA	433
	PVL-R	GCATCAAGTGTATTGGATAGCAAA AGC	

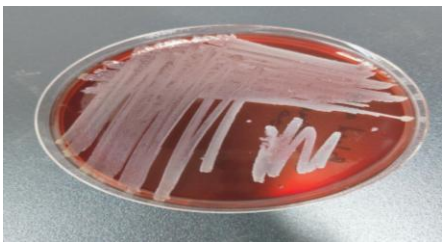


Figure -2 Growth of S.aureus on blood agar

The presence of bands at a size of 433 bp indicates successful gene amplification via PCR, confirming the presence of the PVL gene in most of the isolates in this study. The absence of bands in some samples confirms that these isolates do not possess the gene, or it may be due to genetic differences in the primer binding process. ClfA is one of the most important virulence factors in S.aureus, as it contributes to the destruction of white blood cells and leads to tissue necrosis, thereby increasing the severity and danger of the infection [13]. Similarly, PCR results using primers specific to the clfA gene (292 bp) revealed bands at the expected location in 16 out of 20 samples of *S.aureus* isolates, representing 80 % of the total, while no amplification was observed in 4 samples (20 %), namely samples 4, 12, 13, and 18 (Figure 5).

Table 3- Components of the polymerase chain reaction (PCR) mixture

Components	Volume
Green Master Mix 2X	µL 12.5
Nuclease-free water	µL 5.5
Primers forward, 10µM	µL 1.5
Primers reverse, 10µM	µL 1.5
DNA template	microliters 4
Final sample volume	microliters 25

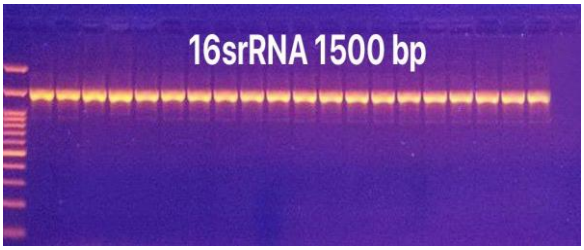
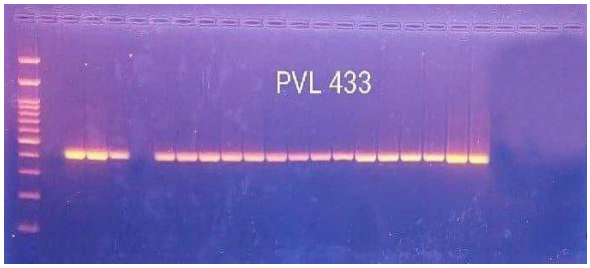


Figure -3 Electrophoresis of the 16SrRNA gene on agarose gel

The appearance of bands of size (292 bp) indicates successful amplification of the gene by PCR and confirms the presence of the clfA gene in most of the isolates used in this study. Conversely, the absence of bands in some isolates indicates that these isolates either do not contain the gene or possess mutations that affect primer binding. The clfA gene (coagulation factor A) is one of the virulence factors in *S.aureus*, it plays a key role in the bacteria's adhesion to host cells by binding to fibrinogen , thereby contributing to bacterial colonization and the spread of infection.

Table 4- Cycling conditions for polymerase chain reaction (PCR)

Gene	**temperature**					**Cycle No**
	Initial denaturation	**Cycling condition**			**Final extension**	
		Denaturation	**Annealing**	**Extension**		
16srDNA	95	95	55	72	72	35
clfA	95	95	58	72	72	
pvl	95	95	55	72	72	



Figurer - 4 Electrophoresis of the PVL gene on agarose gel

The results of this study are consistent with numerous studies that have demonstrated the use of the 16S rRNA gene as a standard marker in the molecular analysis of bacteria [14] noted that this gene is one of the most widely used genes in the identification and classification of bacteria due to its conservative nature, Subsequent studies have also shown that the success rate of 16S rRNA gene amplification is typically close to 100 % in the presence of pure DNA and under appropriate conditions for PCR , which supports these findings [15]. The achievement of a 100 % positive rate in this study indicates the quality of the laboratory work and confirms that all samples were suitable for molecular testing,

consequently, the results for other genes such as PVL and clfA can be relied upon with a high degree of accuracy.

Table 5- Microbial isolation based on morphological characteristics,, and molecular diagnostics

result	Issue	percentage
S.aureus	20	14.28
Other bacteria	88	62.85
No bacterial growth	32	22.85
Total number	140	100
Chi-square value	84.68	
Calculated significance value	0.0001*>	

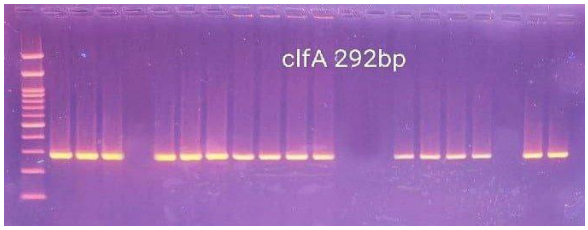


Figure -5 Electrophoresis of the ClfA gene on agarose gel

In this context, the results of this study show a very high prevalence of the PVL gene (90%), a rate higher than that reported in many previous studies. One such study found that the prevalence of the gene was relatively low, ranging from 10% to 30% in some clinical isolates, particularly those associated with hospitals [13]. Other studies have also indicated that the prevalence of the PVL gene is higher in community-associated (CA-MRSA) isolates compared to hospital-associated isolates, with rates reaching 50% or higher depending on the type of sample and geographic location [16], while a recent study reported varying rates of gene presence ranging from 20% to 80%, indicating significant variation in the distribution of this gene among different isolates [17]. The high prevalence recorded in this study (90%) may reflect the prevalence of highly virulent strains in the samples studied, or it may be related to the nature of the clinical samples used, suggesting the virulence of these isolates and their ability to cause severe infection.

Turning to the clfA gene, the current results indicate that it is highly prevalent (80%) among the *Staphylococcus aureus* isolates included in the study, suggesting its important role in enhancing the bacteria's ability to adhere and cause infection. In addition, the variation in prevalence rates compared to previous studies reflects the influence of environmental factors, sample type, and geographic location on the prevalence of virulence factors. The findings of this study are consistent with several previous studies that reported a high prevalence of the clfA gene among *S.aureus* isolates, One study showed that the gene prevalence rate was 36.3 % among isolates, which is lower than the rate found in this study (80 %), indicating differences in gene prevalence based on geographic location and sample type [18]. Other studies have also shown that the prevalence of the clfA gene ranges from 63.7 % to 100 % in many isolates, which supports the high prevalence recorded in this study [19]. In a molecular-level study conducted in Iran , very high prevalence rates of the gene were

recorded in 93.7 % of the isolated samples , which is slightly higher than the results of the current study, indicating that the prevalence of this gene is a major factor in the level of toxicity. On the other hand, another study reported a prevalence rate of this gene as low as 22 %, this significant discrepancy may be attributed to differences in the samples used or in the methodology employed in the study [19]. Some studies have reported that this gene may be present in up to 100 % of certain isolates, confirming its role in the pathogenicity of the bacterium [20].

4. Conclusion

Based on the results of the current study and the relevant discussion, we conclude that the 16S rRNA gene demonstrated high efficacy as a molecular detection tool , with a 100 % positive rate, which confirms the accuracy of the laboratory procedures and the integrity of all samples tested , and supports the reliability of the results regarding the other genes included in the study. The results also revealed a high prevalence of the PVL gene at 90 %, a rate higher than that recorded in many previous studies, suggesting the potential presence of highly virulent strains in the studied samples and their ability to cause severe infection. The study also demonstrated a high prevalence of the clfA gene at 80 %, indicating its importance in promoting bacterial adhesion to host cells. The variation in gene prevalence rates compared to other studies reflects the influence of environmental factors , sample type , and geographic location , indicating the importance of these factors in determining the genetic characteristics and virulence factors of S.aureus isolates.

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