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Genetic estimation of the toxic shock syndrome genes for burn patients in Al-Qadisiyah Province

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Abstract:

Staphylococcus aureus is a commensal and opportunistic bacterium and the leading cause of human bacterial infections worldwide. *S. aureus* exhibits significant resistance to antibiotics, particularly methicillin-resistant Staphylococcus aureus (MRSA) strains. This resistance is a growing global concern, as *S. aureus* can rapidly adapt to various antibiotic treatments, making these bacteria difficult to treat. The ability to form biofilms enhances this resistance, allowing *S. aureus* to persist in infections and become resistant to antibiotics. Therefore, alternative treatment options to antibiotics are needed to control antibiotic resistance and biofilm formation, and cold plasma may be an alternative. The aim was to study genetic estimation of the toxic shock syndrome genes for burn patients in Al-Qadisiyah Province. 150 clinical samples were collected from various sources, including burns from patients aged 3–58 years at Al-Diwaniyah Teaching Hospital, Afak General Hospital, and the Specialized Burns Center during the period from February 2025 to May 2025. The results demonstrated the clear effectiveness estimation of the toxic shock syndrome genes for burn patients in Al-Qadisiyah Province.

Keywords: *S. aureus*, antibiotic resistance, clinical isolates, toxic shock syndrome

Introduction:

Toxic shock syndrome (TSS) is a rare but life-threatening condition caused by the release of bacterial toxins. It is a medical concern due to its severe and rapidly progressive symptoms. One of the most prominent culprits is the bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*, which produce toxins known as "superantigens." These toxins trigger an excessive and uncontrolled immune response (Smith et al., 2024), leading to a variety of serious systemic symptoms. The main symptoms of TSS include a sudden high fever and a severe loss of blood pressure, leading to shock, as well as failure of multiple vital organs such as the kidneys, liver, and respiratory system. TSS is a significant challenge in clinical medicine due to its rapid

progression and high mortality rate if not treated promptly and effectively (Subrahmanyam et al., 2024).

For burn patients, the risk of developing (TSS) is significantly higher. Because burns cause severe skin damage, the skin, which serves as a protective barrier against bacteria, becomes more susceptible to infection (Vivekanandan et al., 2025; Sabah and Kadhim, 2025). The deeper and more extensive the wounds, the greater the chance of bacteria entering the bloodstream. Consequently, there is a greater likelihood of the release of toxins that cause the syndrome, further exacerbating the patient's health condition. In addition, the physiological stress resulting from burn injury and medical treatment, which may include the use of antibiotics, can weaken the immune response and make the body more susceptible to microbial infections, including those that cause TSS (Nam et al., 2021). Furthermore, TSST-1 (toxic shock syndrome toxin 1) is one of the main toxins released by Staphylococcus aureus and contributes to the development of toxic shock syndrome (TSS) (Mumtaz et al., 2023).

This toxin bypasses the immune system's natural mechanisms, interacting directly with the body's T cells, triggering an excessive immune response known as a "cytokine storm." This storm is one of the main causes of the severe symptoms associated with toxic shock syndrome, such as circulatory shock and organ failure (Ding et al., 2016). In burn patients, the risk of developing TSS is particularly high. Burn wounds compromise the skin barrier, which normally acts as a defense against bacterial infection. When the skin is damaged, bacteria such as *Staphylococcus aureus* can easily colonize the affected areas and may spread systemically. These bacteria, which are often found on the skin and mucous membranes, can release toxins, including toxic shock syndrome toxin-1, which has been strongly linked to the onset of toxic shock syndrome (Guo et al., 2021). TSST-1 is particularly problematic because it bypasses normal immune processes, directly activating large numbers of T cells, leading to a massive release of inflammatory cytokines—a phenomenon known as a "cytokine storm." This storm is responsible for the rapid and severe symptoms seen in toxic shock syndrome, such as shock and acute organ failure (Abbood and Hateet, 2025).

Studying the prevalence of antibodies in patient populations at risk for developing toxic shock syndrome, such as burn patients, could significantly contribute to identifying individuals most susceptible to this syndrome. These studies could provide the basis for accurate assessments of an individual's immune status, enabling more precise and effective treatment and prevention. Furthermore, understanding the role of antibodies in counteracting these toxins may help develop new methods to stimulate or strengthen the immune system, thereby reducing the risk of developing the syndrome (Abdulrazzaq and Ali, 2025). Therefore, the aim was to study genetic estimation of the toxic shock syndrome genes for burn patients in Al-Qadisiyah Province.

Materials and Methods:

Bacterial Isolates:

150 clinical samples were collected from various sources, including burns from patients aged 3–58 years at Al-Diwaniyah Teaching Hospital, Afak General Hospital, and the Specialized Burns Center during the period from February 2025 to May 2025.

Diagnosis:

All bacterial isolates were initially identified using Gram staining and biochemical tests (oxidase, catalase, citrate uptake test, and growth at 4 and 42°C). Diagnosis was confirmed using the VITEK2 device and PCR by detecting the presence of the 16S rRNA gene. Primers for this gene were designed using the NCBI-GenBank Database and the Primer3plus primer design software. These primers were prepared by the Korean company Macrogene, as shown in Table (1).

Antibiotic susceptibility test (AST):

Susceptibility testing was performed for the antibiotics Piperacillin, Amoxicillin/clavulanate, Ceftriaxone, Cefotaxime, Imipenem, Gentamicin, Amikacin, Ciprofloxacin, and Levofloxacin using the Kirby-Bauer disk diffusion method according to the recommendations of the Clinical Laboratory Standards Institute (CLSI). The bla OXA-10, aac(6')-Ib, and gyrA genes were also screened for resistance to antibiotics, β-lactams, aminoglycosides, and fluoroquinolones, respectively, using PCR. Primers for each gene were designed using the NCBI-GenBank Database and the Primer3plus primer design software. These primers were prepared by the Korean company Macrogen (Brun et a., 2018) (Table 1).

Detection of *S. aureus:*

The ability of *S. aureus* to form biofilms was detected phenotypically by crystal violet staining using a 96-well microtiter plate, and molecularly using PCR to detect the presence of the lasR gene, a diagnostic marker for biofilm production by this bacterium. The primers for this gene were designed using the NCBI-GenBank Database and the Primer3plus primer design software. These primers were prepared by the Korean company Macrogen (Table 1).

Table (1): Shows the names of the primers, their nitrogenous base sequences, and amplification product size

primers		**nitrogenous base sequences(5'→3')**	**amplification product size**
***S. aureus* detection**	**F**	ACGGTCTTGCTGTCACTTATAG	**519 bp**
	R	CACTGGTGTTCCTCCATATCTC	
mec A	**F**	AAGTGGAAAATTGGAGTGGAG	**130 bp**
	R	GTAGTTGCCGACGATGAAG	
MRSA	**F**	TCCTGCGCTACCAATGACTT	**760 bp**
	R	TGCGACACCAGGATTTGACT	

Exposure:

Bacterial suspensions were prepared at a concentration of 0.5 McFarland, with three replicates for each isolate and a control group. Using the disk diffusion method, the bacteria were cultured on Muller-Hinton agar medium, and the plates were incubated for 24 hours at 37°C. After bacterial growth appeared, Each plate was exposed to cold plasma using an argon-powered Plasma Jet device at 25°C, with an 11 kV power source, a frequency of 12 kHz, and a gas flow rate of 4 liters per minute. The nozzle of the device was directed directly at the center of the plate, maintaining a distance of 1 cm between the nozzle and the plate surface (Alkan et al., 2023). Different time periods (3, 6, and 9) were applied to each isolate.

Statistical analysis:

The study results were statistically analyzed to determine significant differences. The Chi-square test and the LSD test were used, and significant differences were determined at the 5% level using the Statistical Package for Social Sciences (SPSS) version.

Results:

The phenotypic and biochemical results showed that 15 (15%) isolates were *S. aureus*, distributed among different clinical cases, 60% (6 isolates) from the skin, 40% (4 isolates) from the urine, 20% (2 isolates) from the nose, 20% (2 isolates) from the sputum, and 10% (1 isolate) from the eyes. Five isolates were molecularly identified using PCR, as the results of the primer amplification targeting the 16S rRNA gene showed that all bacterial isolates contained 100% of this gene with a product size of 519 bp (Figure 1).

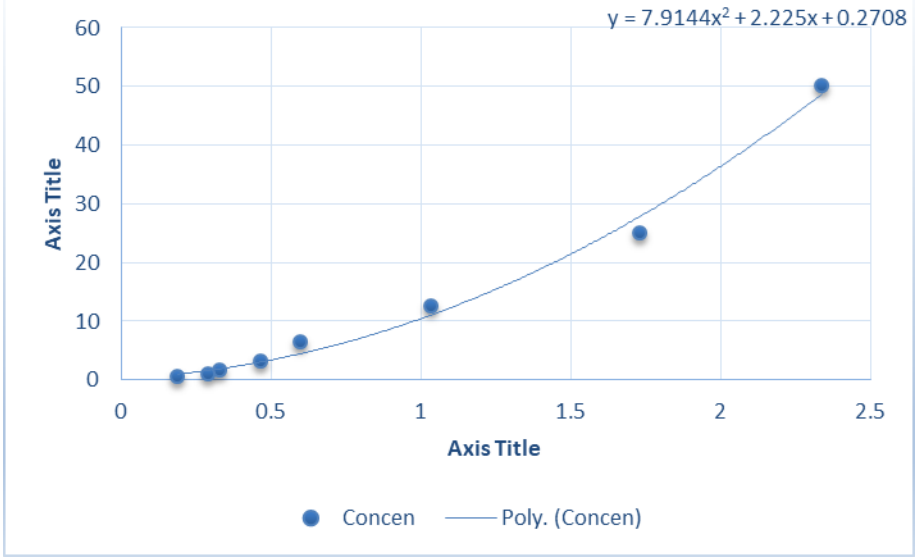

Figure (1) IL-1 α amplification

Antibiotic susceptibility testing:

The results of the disk diffusion antibiotic susceptibility testing showed that the isolates varied in their resistance to the tested antibiotics. The highest resistance rate was 67% to the

antibiotic. PCR results also showed that the isolates possessed the RNA 16 gene, which is responsible for resistance to β-lactam antibiotics, at 80% (Figure 2).

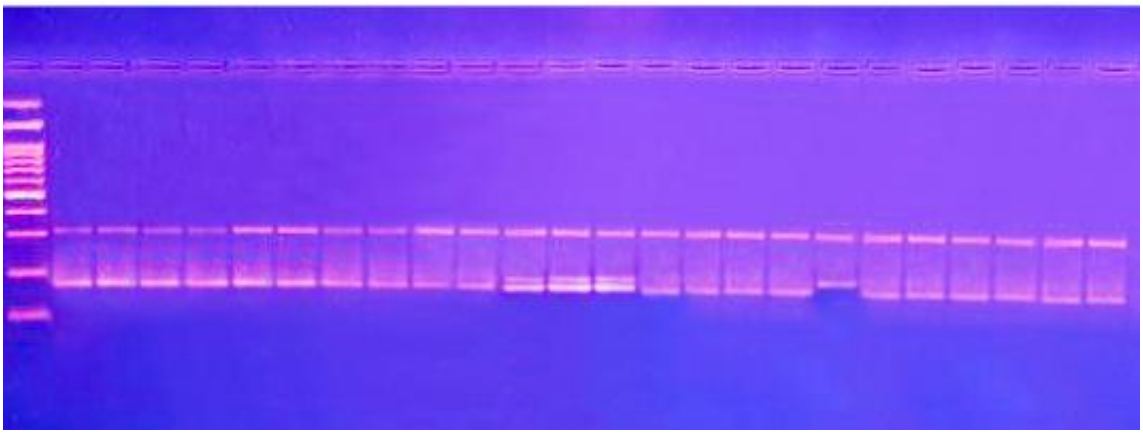

Figure (2): Agarose gel electrophoresis showing the PCR analysis of the RNA 16 gene in *S. aureus*. M represents the marker ladder with a size of 100-1500 bp, holes (1, 2, 3, 4) show the isolates positive for the RNA 16 gene with a PCR result of 576 bp, while hole (5) shows the isolate negative for this gene. Electrophoresis conditions: agarose gel (1.5%), potential difference (100 V), current (80 A), time (1 h).

Phytochemical detection of biofilm production was performed using the microtiter plate method. Results showed that these isolates were 100% biofilm-forming. Molecular detection revealed the mec A gene, responsible for biofilm formation in *S. aureus*. Results showed that all isolates were biofilm-producing and possessed this gene at 100% expression.

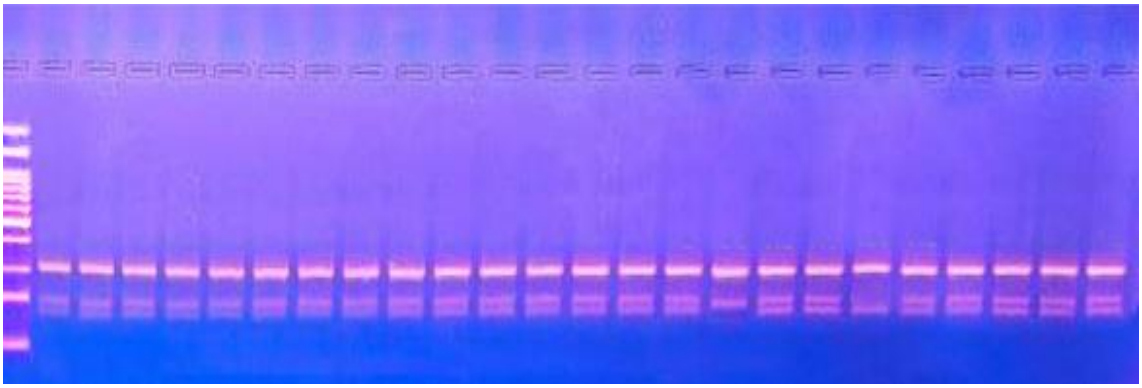

Figure (3) Agarose gel electrophoresis showing the PCR analysis of mec A gene in *S. aureus*. M represents the marker ladder with a size of 100-1500 bp, and the wells (S1, S2, S3, S4, S5) show the isolates positive for mec A gene with a PCR product of 235 bp. Electrophoresis conditions: agarose gel (1.5%), voltage (100 V), current (80 A), time (1 h).

The effect of cold plasma on antibiotic resistance:

The results showed that the resistance of bacterial isolates to antibiotics decreased as the exposure duration increased. Consequently, there was a significant increase in the effectiveness

of antibiotics after exposure to cold plasma, as an increase in the diameters of inhibition was observed for most antibiotics, as shown in Table (2) and Figure (2).

Table (2): Diameters of inhibition of antibiotics for *S. aureus* after exposure

antibiotics	burn infections			
	0	3	6	9
Piperacillin	15Aa	16Ab	16Ab	16Ab
Augmentin	18Ba	18Ba	21Bb	21Bb
Ceftriaxone	12Ca	19Bb	18Cb	19Cb
Cefotaxime	18Ba	19Ba	22Bb	22Bb
Imipenem	40Da	40Ca	46Db	47Dc
Gentamicin	20Ea	20Da	20Ba	20Ca
Amikacin	22Fa	24Eb	25bE	26bE
Ciprofloxacin	13Ga	12Fb	13Fc	13Fc
Levofloxacin	16Ha	18Bb	19Cb	19Cb
LSD	0.91	1.12	1.21	1.2

Effect of biofilm formation in *S. aureus*:

The results of exposing bacterial isolates to cold plasma showed a significant decrease in the optical density associated with biofilm formation, indicating that cold plasma inhibits biofilm formation in direct proportion to the increase in exposure time. Significant differences ($P < 0.05$) were observed between the control group (0 minutes) and the treated isolates (3, 6, and 9 minutes). The control group (without exposure) had the highest value in biofilm formation, while the isolates treated with plasma for 9 minutes showed a loss of their ability to form biofilms. Biofilm formation was classified as strong (> 0.24), moderate (0.12-0.24), and non-forming (< 0.12) based on (Akpor et al., 2024). The optical density (OD) values of the isolates treated with cold plasma for different periods (3, 6, 9) minutes were compared with a control group (without exposure (Table 2).

Discussion:

The 150 samples collected, 15 isolates (15%) were found to be *S. aureus*. Ding et al. (2016) explained that discrepancies in the prevalence of the bacteria between studies could be due to variations in clinical samples, hospitals, populations examined, geographic regions, and healthcare practices (Adnan et al., 2023). The isolates in the current study were distributed unequally across different clinical settings. This variation in the prevalence of *S. aureus* may be attributed to differences in sample size, study population, length of hospital stay, patient exposure to high-risk medical devices, and prescription of antibiotics without antibiotic susceptibility testing. The studied isolates demonstrated high resistance to β-lactam antibiotics, which is linked to their possession of the blaZ gene, which is responsible for resistance to β-lactam antibiotics (Jeong et al., 2024).

Antibiotic resistance in bacteria poses a serious global health challenge, driven by molecular mechanisms such as gene mutations, efflux pumps, enzymatic degradation of antibiotics, target site modifications, and biofilm formation. Horizontal gene transfer (HGT) accelerates the spread of resistance genes across bacterial populations. These mechanisms contribute to the emergence of multidrug-resistant (MDR) strains, rendering conventional antibiotics ineffective. Biofilm formation is one of the most important mechanisms of antibiotic resistance in bacteria. The isolates in the current study demonstrated a high capacity to form biofilms, which is linked to their high 100% presence of the icaD gene, thus explaining their multiple antibiotic resistances (Kemal et al., 2025).

After exposing the studied bacterial isolates to cold plasma, the results of the antibiotic susceptibility test for *S. aureus* showed a significant increase in the diameters of inhibition for most antibiotics. This confirms that cold plasma treatment improves the effectiveness of antibiotics against *S. aureus*, and in some cases, the bacteria regain their sensitivity to antibiotics. This is consistent with the findings of, which showed that cold plasma treatment enhances the effects of various antibiotics in reducing *S. aureus*, and bacteria can regain their sensitivity to antibiotics (Kang et al., 2021).

Gram-negative bacteria are generally more susceptible to cold plasma than Gram-positive bacteria. Gram-negative bacteria have a thinner outer membrane and peptidoglycan layer, and the proteins and LPS in the outer membrane are sensitive to ROS molecules. Therefore, cold plasma damages the outer membrane, leading to damage to the cell wall and cell membrane. In contrast, Gram-positive bacteria are less susceptible to oxidative damage because they lack an outer membrane and are covered by a thick layer of peptidoglycan. As a result, cold plasma cannot easily damage their cell wall, but the active chemicals they produce can enter the cell and cause oxidative damage to intracellular components (18). The results shown in Table (2) showed a significant decrease in the ability of *S. aureus* to form a biofilm. Prolonged exposure time rendered the bacteria unable to form a biofilm. The results of our study were consistent with those (19, 20). The results showed that longer treatment led to a decrease in the bacteria's ability to survive. It showed that increasing plasma exposure time and decreasing distance led to a reduction in the number of *S. aureus* cells in the biofilm (Khosravi et al., 2021).

Direct bacterial cell injury, disruption of the extracellular matrix, and altered gene expression associated with biofilm formation may be the underlying mechanisms for the anti-biofilm effect of cold plasma, ultimately leading to the elimination of the biofilm. Cold plasma affects the essential components of the biofilm. Through lipid oxidation, protein modification and degradation, and the disruption of carbohydrate chemical bonds, the EPS is destroyed (Mohammed and Al-Marjani, 2024). This biochemical change in the EPS results from oxidation processes mediated by ROS and RNS molecules. When the EPS is disrupted, the adhesion of the biofilm to the immobilized surface decreases, ultimately leading to disruption of the three-dimensional biofilm structure or even disintegration (Namini et al., 2023).

Conclusion:

The study found that cold plasma is effective in reducing *S. aureus* resistance to antibiotics and its ability to form biofilms. These results support the idea that PCR technology could be used to eliminate pathogenic bacteria instead of antibiotics, thereby treating some diseases associated with these bacteria.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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