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# Molecular Study of the Toxic Shock Syndrome Gene Tsst-1 in Staphylococcus Aureus Isolated from Different Female Cases

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## ABSTRACT

This study aimed at molecular investigation of the toxicity gene for toxic shock syndromeTsst-1, which is encoded by the T gene, produced by Staphylococcus aureus and isolated from multiple female cases. Accordingly, approximately 180 samples were collected from different female cases from inpatients and patients attending Imam Al-Sadiq Hospital, Al-Qasim General Hospital, and Al-Hashimiyya Hospital in Babylon Governorate.

Biochemical tests were carried out for the samples taken, where they were cultured on blood agar medium, and it was noted that there were significant differences at a level of significance of 5%, and the culture results were 120 (66.7%) positive bacterial isolates for Gram stain, compared to no recording of 60 (33.3%) samples of bacterial growth on blood agar media. Staphylococcus aureus 45 isolates (37.5%) were cultured on mannitol-saline medium and the diagnosis was confirmed byAPI staph. and the phaetek system (Vitek 2), where the results showed that there were no significant differences at the level of significance of 5% for the isolates grown on manthol saline medium. The drug sensitivity of the Staphylococcus aureus isolates towards antibiotics was tested by using the disc spread method on Acar Mueller-Hinton medium and according to the Karby-Bauer method. There are significant differences at the level of significance of 5%, and some virulence factors were investigated outwardly, as significant differences were found at the level of significance of 5%. Among the virulence factors were the formation of the biofilm, the percentage of which was 89%, the production of hemolytic enzyme by 100%, and the production of the enzyme coagulase (plasma coagulase). ) by 100%

**Keywords:** *Molecular, Isolated, Toxic, Syndrome*

## INTRODUCTION

The bacteria of Staphylococcus aureus are among the most important common pathogens associated with serious infections in the community and hospitals. They have been considered for a long time a major problem for public health. They are coexisting organisms on the human body in a natural way, as they inhabit the nasal mucosa at a rate of 25-30%. They are also present on the skin and in the female genital tract. in women(Senok, 2009) It is considered an opportunistic pathogen when it causes a group of diseases such as supportive infections, whether superficial or deep, as well as because it causes a wide range of infections, including systemic infections,

which are life-threatening infections such as bone marrow infection, endocarditic, destructive abscess, pneumonia, meningitis, bacteremia, and septicemia. joints, calluses of scalded skin, and toxic shock syndrome (Zainulabdeen and Dakl, 2021) I have conditions suitable for them, for example, I have a decrease in the body's immune defense mechanisms and the presence of some chronic diseases such as cancers and diabetes, as well as I have skin burns and wounds and when I have other pathological organisms such as viruses, Mahfouz et al.2010)) Staphylococcus aureus has been called super antigens due to its production of toxins. When toxins enter the bloodstream, they will bind to class II his to compatibility complex proteins (MHc-) and affect the immune system by activating T-cell lymphocytes, leading to Massive release of many cytokines by systemic toxicity, which suppresses the immune response and the onset of symptoms (Krakauar et al, 2019).

One of the important characteristics of Staphylococcus aureus is the ability to secrete toxins that cause serious diseases.(ETB, ETA) implicated in scalded skin syndrome (Plata et al, 2009), toxic shock syndrome toxin Tsst-1 implicated in toxic shock syndrome (Hakimi et al, 2018), and enterotoxins implicated in staphylococcal food poisoning (Lu et al, 2022) It is known that each of these toxins has strong effects on the cells of the immune system and is encoded by genes. For example, the tst gene encodes an extracellular toxin called toxic shock syndrom toxin-1 that causes toxic shock syndrome Tsst-1 (Sapugahawatte et al, 2020). This syndrome is associated with the presence of aureus-producing Staphylococcus aureus. Tsst-1 in the vagina during the menstrual cycle, as well as for pregnant women at the end of the eighth month until childbirth and postpartum, or in other locations as a result of complications from infection with Staphylococcus aureus components, especially the skin or respiratory tract, and complications from surgeries (Senok,

### *Work methods*

#### *Sample collection*

180 samples were collected from different female cases from inpatients and patients attending Al-Qasim General Hospital and Al-Hashemiah Hospital in Babylon Governorate.

#### *Isolation and identification of bacteria*

The female samples were planted on blood agar media, then the dishes were incubated at 37 °C for 18 to 24 hours, then transferred to manifold saline medium, and then incubated at 37 °C for 48 hours. Then the growing bacterial isolates were initially diagnosed based on their agricultural characteristics in terms of colonies size, texture and color. As well as its ability to analyze red blood cells on the center of blood acres and then conduct a confirmatory examination (ApI staph)

#### *testing the sensitivity of bacteria to antibiotics*

The drug sensitivity of Staphylococcus aureus isolates towards antibiotics was tested using the disc spread method on Acar Mueller-Hinton medium and according to the method.

Kirby and Bauer Method

#### *Phenotypic detection of some virulence factors*

#### *Biofilm formation test*

This test was conducted by placing the growing bacterial colonies in glass test tubes containing tryptic soy broth, to which 1% glucose was added, and they were observed at a temperature of 37 degrees Celsius for a period of 24 hours.(Hassan et al, 2011)

#### *Hemolytic enzyme production test*

I followed the method described before Chart. And his group 1998 to investigate the ability of the isolated bacteria to produce the enzyme hemolytic. A sample of human blood drawn immediately and containing anticoagulant was used for this, then the plasma was separated and the precipitate of red blood cells was obtained, which were then washed with physiological salt solutions, and then the cells were sediment by centrifugation, then 5% of the precipitate was added to the base. Sterile blood aquifers to note the ability of bacterial isolates to analyze blood through the formation of transparent halos around the growing colonies

#### *Plasma coagulant enzyme production test*

This test was performed using the test tube method Test tube by adding 0.5 ml of undiluted blood plasma to test tubes containing 0.5 cryptic

soy broth inoculated with the target bacterial isolates, then the tubes were incubated in a water bath at a temperature of 37 degrees Celsius for four hours, during which a monitoring process was observed, as the clot formed as evidence of a positive The Test (Macfaddin, 2000)

***Molecular examinations***

***Firstly Genomic DNA extraction***

Nucleic acid extraction from Staphylococcus aureus was carried out using the prepared kit G-spin Genomic DNA E extraction kit, according to the instructions of the supplying company, Promega/USA, and the extraction was carried out according to the company's instructions.

***Second - PCR reaction***

Primers for the PCR were designed PCR of the Tsst-1 gene, primers were prepared by Promega/USA, and a PCR master mix was prepared. Using the GO Tag G2Green Master Mix kit, according to the manufacturer's instructions and as shown in the table

***Preparation of PCR solutions***

| Components | Concentration | Volume (50µl) |
|---|---|---|
| 2X PCR Taq Master Mix | 1X | 25µl |
| forward primer | 10µM/µl | 4µl |
| reverse primer | 10µM/µl | 4µl |
| ddH2O | - | 13µl |
| DNA | 40 ng | 4µl |

After completing the preparation of the polymerase chain reaction mixture, the tubes were closed with careful mixing with the rotary mixer for five seconds, then the tubes were transferred to the thermo cycler for the polymerase chain reaction to perform the DNA amplification process according to the optimal conditions for the thermal cycles of bacteria, represented by the processes of separating the DNA denaturation strip and the association of primers with the strip. Separate annealing and DNA extension

***Third Thermal cycles program for DNA amplification***

The polymerase chain reaction was carried out using a the rmocouple Thermo cycler PCR

The device has been programmed as shown in the table

***Conventional PCR conditions***

| Phase | Tm(°C) | Time | Cycles |
|---|---|---|---|
| **Initial denaturation** | 94°C | **5 min** | **1X** |
| **Denaturation** | 94°C | **30 sec.** | **35X** |
| **Annealing** | 56°C | **30 sec.** | |
| **Extension** | 72°C | **1 min** | |
| **Final extension** | 72°C | **5 min** | **1X** |

***Fourthly Electrophoresis in an agarose gel***

Agarose gel electrophoresis was performed for the purpose of detecting L. bundles The extracted DNA and the amplified DNA according to the method of (Sambrook et al, 1989), and after the migration process was completed, the gel containing product was examined. PCR and amplified DNA using the ultraviolet light source to investigate the presence of DNA and to determine the bands and measure their molecular weights when compared to the standard values of the DNA marker

## RESULTS AND DISCUSSION

The results of the statistical analysis of the primary isolation on the medium of blood agar showed that 120 samples out of the total number

of samples gave bacterial growth by 66.7%. As for those that did not show growth, they reached 60 samples and their percentage was 33.3%, where significant differences were found among them at the level of significance of 5%, due to the reason for this. To the presence of other pathogens such as fungi, Chlamydia and parasites, or to the efficacy of antibiotics used in the treatment of diseases caused by bacteria.

(AL-Douri, 2011) The categories of women were divided into four groups, and the percentage of bacterial isolates grown on menthol medium from pregnant women at the end of the eighth month was 32%. As for pregnant women at the end of the ninth and tenth months, the percentage was 33.3%, postpartum women 40%, and women using contraceptives, the metallic IUD, 43%. Where it was observed that there were no significant differences at a level of significance of 5%, and the diagnosis of the isolates of the Streptococcus aureus components was confirmed by the Vitek device and the use of the API staph system))

Biochemical tests were investigated, and the isolated strains showed a positive result for each of the catalane enzyme and the plasma coagulant enzyme, and a negative result for the oxidize enzyme, and they appeared as golden yellow colonies on manifold salt acres.

***Phenotypic detection of some virulence factors biofilm formation***

A test was conducted to investigate the ability of bacterial isolates to form a biofilm layer using the tube method, and the positive result was recorded when biofilms formed on the inner walls and bottom of the tubes in the form of a violet layer. Its composition and the result was consistent with the study Al-Omari and her group 2013, as they found that the percentage of biofilm-producing isolates amounted to 87.5%, and biofilm formation is one of the most prevalent virulence factors.

***Plasma coagulant enzyme production Coagulate***

The results of the current study showed that all 45 isolates of Streptococcus aureus recorded a positive result for the plasma coagulant enzyme, with a rate of 100%. The formation of vegetation is evidence of the ability of the bacteria to produce the enzyme. Fibrin of bacteria from phagocytosis works to isolate them from other defenses of the host, which makes the bacteria more virulent (Ratajczak et al, 2021).

***Hemolytic enzyme production Hemolytic***

Showed up All 45 isolates tested positive for the hemolytic enzyme, by observing the areas of decomposition surrounding the growing bacterial colony on the medium of blood plasma. it gaves The isolates analyzed type alpha blood, and this result was consistent with the results of Jaber 2022, as all werep Zlatha is a producer of this enzyme

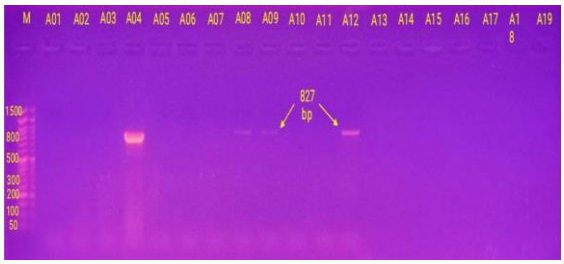
***Examination of sensitivity to antibiotics***

The drug sensitivity of Staphylococcus aureus isolates towards antibiotics was tested by using the disc spread method on Acar Mueller-Hinton medium and according to the Karby-Bauer method, where Staphylococcus aureus isolates showed resistance to antibiotics.Penicillin, Vancomycin, and Rifampcin increased by 95%, 80%, and 60%, respectively, while sensitivity to Gentamicin and linezolid antibiotics increased by 60% and 90%, respectively.

As for the levelcThus, the DNA of 45 isolates belonging to the components of Staphylococcus aureus was extracted, and the production of genotoxic shock syndrome toxins was investigated using a techniquePCR and specialized primer provided by Promega/USA. And designed according to the primers design program, the results showed the amplification of the primers of the Tsst-1 gene - encoding the production of toxic shock syndrome toxins, and after transferring them on an agarose gel 1.5 gm and staining with a dye

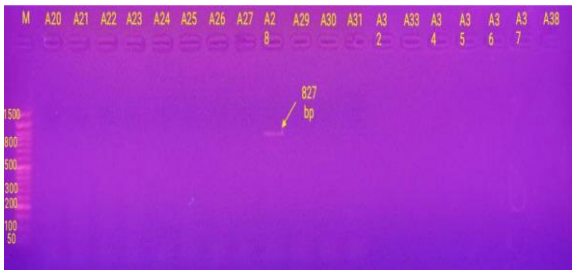
Red safe nucleic acid and examining it under ultraviolet light, we concluded that 7 isolates contained the Tsst-1 chromosomal gene at a rate of 15.5%. When referring to the sample sources, it was noted that the highest percentage of this gene was in the category of pregnant women in the ninth and tenth months, 20%, followed by the category of postpartum women, 16.6%. And then the women using the metallic IUD contraceptive, and the percentage of this gene presence in their isolates was 13.3%, while the lowest percentage was in the category of pregnant women at the end of the eighth month, 12.5%, where it was

observed that there were no significant differences at the level of significance of 5%.




***PCR products of gene TSST-1 of Staphylococcus aureus***

The size of the PCR product is 827 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). Electrophoresis conditions: V: 90, Time: 42 minutes. M: DNA ladder

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