EJONS International Journal on Mathematic, Engineering and Natural Sciences | ISSN 2602 - 4136

| Article Arrival Date | Article Type | Article Published Date |
|---|---|---|
| 28.06.2023 | Research Article | 20.09.2023 |


# DETECTION AND GENETIC VARIATION OF SPA AND TST-1 GENE IN CLINICAL *Staphylococcus aureus* SAMPLES IN THI-QAR

THI-QAR İLİNDE KLİNİK *STAPHYLOCOCCUS AUREUS* ÖRNEKLERİNDE SPA VE TST-1 GENİN VARLIĞI VE GENETİK VARYASYONUNU

**[1]Ahmed Sabah Mohan**

Kırşehir Ahi Evran University Institute of Science and Technology, Kırşehir, Türkiye

ORCİD:0000-0002-1883-2134

**[2]Dr. Öğr. Üyesi Esin KIRAY**

Kırşehir Ahi Evran University Vocational School of Health Services, Kırşehir, Turkey.

ORCİD: 0000-0002-6908-5909

**[3] Dr. Öğr. Üyesi Amran Mezher Lawas**

Al Aayen Üniversitesi, Tıp Fakültesi, Anestezi Bölümü Thı-Qar, Iraq

ORCİD: 0000-0001-5258-9724



## ABSTRACT

Urinary tract infection (UTI) is one of the most common infectious diseases in humans. *Staphylococcus aureus*, a gram-positive bacterium, is one of the most common causes of UTI. In this study, it was aimed to investigate the presence and genetic variation of Staphylococcal protein A (spa) and toxic shock syndrome toxin-1 (Tsst-1) gene regions by determining the distribution of *S. aureus* in urine samples taken from UTI patients in Thi-qar province. Various biochemical methods and API-20 and Vitek2 compact systems were used for the identification of S. aureus. Fourteen different antibiotics, which are frequently used in the clinic, were used to determine the antibiotic susceptibility of the strains. PCR technique was used to determine the presence of spa and tsst-1 genes of *S. aureus* strains isolated from UTI patients. Genetic variation of strains was determined by DNA sequencing analysis method. In the study, *S. aureus* was found to be positive in 133 (66.5%) of the urine samples taken from 200 patients with UTI. The highest resistance rates in antibiotic susceptibility of the strains were determined as Ampicillin (100%), Cefoxitin (97.7%), Tetracycline (76.6%), Clindamycin (71.4%) and Trimethoprim-Sulphamethoxazole (54.1%), while Erythromycin (43.6%) and Doxycycline (4%). 42.8) was determined. On the other hand, the strains tested showed a high degree of susceptibility to Vancomycin and Novobiocin. All *S. aureus* isolates (n=55) were amplified with a 100% positive reaction against the spa gene, while the presence of the tsst-1 gene was determined at 20%. In DNA sequencing analysis, the percentage of genetic variation in the spa gene in native *S. aureus* isolates was found to be between 0.84-1.05%, while the genetic variation in the tsst-1 gene was found to be 0.23%. As a result of the study, it was seen that there was a significant increase in the prevalence of *S. aureus* in Iraq, and the genetic variation in the spa and tsst-1 gene was low.

**Key Words:** UTI, *Staphylococcus aureus*, Spa, mTsst-1

## ÖZET

İdrar yolu enfeksiyonu (İYE), insanlarda görülen en yaygın bulaşıcı hastalıklardan biridir. Gram pozitif bir bakteri olan *Staphylococcus aureus* İYE' nın en sık görülen etkenlerindendir. Bu çalışmada, Thı-qar ilinde İYE hastalarından alınan idrar örneklerinde *S. aureus*'un dağılımını belirleyerek Stafilokokal protein A (spa) ve toksik şok sendromu toksin-1 (Tsst-1) gen bölgelerinin varlığı ve genetik varyasyonu araştırılması amaçlanmıştır. *S. aureus*'un idendifikasyonunda çeşitli biyokimyasal yöntemler ile API-20 ve Vitek2 kompakt sistemleri kullanılmıştır. Suşların antibiyotik duyarlılıklarının belirlenmesinde klinikte sık olarak kullanılan on dört farklı antibiyotik kullanılmıştır. İYE hastalarından izole edilen S. aureus suşlarına ait spa ve tsst-1 genlerinin varlığını belirlemek amacı ile PCR tekniği kullanılmıştır. Suşlara ait genetik varyasyon DNA dizileme analizi yöntemi ile belirlenmiştir. Çalışmada İYE şikayeti olan 200 hastadan alınan idrar örneklerinin 133'ünde (%66.5) *S. aureus* pozitif olarak bulunmuştur. Suşların antibiyotik duyarlılıklarında en yüksek direnç oranları Ampisilin (%100), Sefoksitin (%97.7), Tetrasiklin (%76.6), Klindamisin (7%1.4) ve Trimetoprim-Sülfametoksazole (%54.1) olarak belirlenirken Eritromisin (%43.6) ve Doksisiklin (%42.8) olarak tespit edilmiştir. Diğer yandan, test edilen suşlar, Vankomisin ve Novobiyosine karşı yüksek derecede duyarlılık göstermiştir. Tüm *S. aureus* izolatları (n=55), spa genine karşı %100 oran ile pozitif reaksiyonla amplifiye edilirken, tsst-1 gen varlığı %20 oranında belirlenmiştir. DNA dizileme analizinde, yerli *S. aureus* izolatlarında spa genindeki genetik varyasyon yüzdesi (%0.84-1.05) arasında iken tsst-1 genindeki genetik varyasyon %0.23 olarak bulunmuştur. Çalışma sonucunda Irak'ta *S. aureus* prevalansında anlamlı bir artış olduğunu spa ve tsst-1 genindeki genetik varyasyonun düşük olduğu görülmüştür.

**Anahtar Kelimeler:** İYE, *Staphylococcus aureus,* Spa, Tsst-1

## 1. INTRODUCTION

*Staphylococcus aureus* strains can cause a wide spectrum of clinical manifestations ranging from superficial infections to life-threatening serious infections. *S. aureus* is the most common cause of community-acquired infections, while it is the second most common cause of nosocomial bacteremia in hospitals (Kourtis et al., 2019). Pyomyositis infective skin and soft tissue infections, endocarditis, pleuropulmonary infections, sepsis and osteoarticular infections are the most common clinical *S. aureus* infections. Other clinical infections originating from *S. aureus* that pose a significant public health threat are necrotizing pneumonia, urinary tract infections (UTIs), meningitis, osteomyelitis, epidural abscess, and toxic shock syndrome (Tong et al., 2015).



Various virulence factors such as surface protein, biofilm, exoenzymes, exotoxins and exfoliative toxins formed by *S. aureus* bacteria are the factors that increase the severity of the disease. In addition, the ability to acquire resistance to more than one antibiotic class and to have antibiotic resistance genes such as mecA, VanA, and staphylococcal exotoxins make *S. aureus* a difficult pathogen to treat (Costa et al., 2013).

Staphylococcal protein A (spa) is one of the best virulence factors that is fixed to the cell wall, binds to the host immunoglobulin and impairs the host immune response. Spa protein can inhibit host immune responses by being released from the bacterial surface during growth in order to suppress host immunity (Shi et al., 2021). In addition, it has been reported in various studies that the spa secreted from the bacterial surface affects the behavior of a widespread microbial community, affecting colonization and coinfection with other microbial community (Armbruster et al., 2016).

Toxic shock syndrome (TSS) is a rare but severe disease characterized by fever, hypotension, skin rash and multiple organ dysfunction. The syndrome is characterized by high fever, hypotension, diarrhea, erythroderma, confusion and renal failure. It is usually seen in healthy menstruating women who use intravaginal protection such as tampons and are colonized by *S. aureus*, which produces toxic shock syndrome toxin 1 (tsst-1). TSS due to intravaginal protection is associated with the potential to proliferate and use the buffered menstrual products of the colonizing *S. aureus* strain as a growth medium (Ross and Shoff, 2022).

Recent studies show a significant spread of methicillin-resistant *S. aureus* in Iraq (Kareem et al., 2015; Kareem et al., 2020). It is thought that the determination of the epidemiological distributions of these microorganisms will be of great importance in order to control their spread in community and hospital environments. Therefore, our study aimed to determine the antibiotic resistance profile of *S. aureus*, the presence and genetic variation of the spa and tst-1 gene, by detecting the incidence of *S. aureus* in patients with UTI in Iraq.

## 2. MATERIAL AND METHOD

### 2.1. Collection of Patient Samples

Urine samples were collected from patients who applied to XXXX Training Hospital with the complaint of UTI infection between February and June 2021. Consent form was signed by the patients included in the study, and information about the age, gender, place of residence of the patients and whether they were outpatients or inpatients were obtained.

### 2.2. Identification of Bacteria

All urine specimens collected were collected on blood agar, Mannitol Saline Agar (MSA), MacConkey agar, and *Staphylococcus* Medium No. It was inoculated on 110 agar media. After 24 hours of incubation at 37°C, naive isolated colonies were passaged into brain heart infusion agar to confirm identification. At the end of the incubation, *Staphylococci* were identified according to the size, transparency, color, shape, marginal structure, hemolysis status and height of the colonies in the culture medium (MacFaddin, 2000).

### 2.3. Biochemical Tests

All biochemical tests used in this study were prepared according to Atlas, (2010) and Markey et al., (2014). After applying catalase, oxidase and coagulase tests from biochemical tests, other tests were carried out.



### 2.4. Deoksiribonükleaz (DNaz) Testi

The Deoxyribonuclease Test was used to measure the ability of microorganisms to synthesize the heat-stable DNase enzyme. The enzyme is also used to depolymerize and decompose deoxyribonucleic acid (DNA), to measure the thermonuclease stability of DNases of *S. aureus* against heat, and to determine the pathogenicity of *S. aureus* with coagulase negative reaction. Microorganism cultures to be examined were incubated at 37 °C for 18-24 days after sowing on DNase Test Agar. At the end of the period, a few drops of HCl (1N) were added to the culture and the hydrolysis of DNA by the bacterial DNase enzyme was monitored for 15-30 minutes. An opaque color is formed around the colony in negative cultures, while an open area appears around the colony in positive samples that perform DNase synthesis (Markey, 2014).

### 2.5. Novobiocin Testi

The novobiocin test was used to differentiate coagulase-negative staphylococci and to identify resistant (R) *S. saprophyticus*. Disk diffusion method was used in the realization of the test. After the diagnostic susceptibility test agar plate was dried, the sterile swab was dipped in a 24-hour nutrient broth culture and used to inoculate evenly across the surface of the medium. The plate was then allowed to dry. The Novobiocin "5µg" disc was placed on the mid-surface with sterile forceps and gently pressed, and kept at room temperature for 15 minutes. It was then incubated at 37°C for 24 hours and the zone diameter in the inhibition zone was measured in millimeters, and it was determined that the isolates were susceptible or resistant (Atlas, 2010).

### 2.6. Urease Test

In the urease test, the change of color seen in the medium from yellow to pink after incubation of pure bacterial colonies on urea agar for 24-48 hours at 37°C was evaluated as positive (Markey, 2014).

### 2.7. Hemolysis Test

In order to determine the hemolysis test of bacterial isolates, pure colonies inoculated on blood agar plates were incubated at 37°C for 18-24 hours. The development of transparent areas around the bacterial colonies indicates the susceptibility of the bacteria to the secretion of hemolysis.-Staph için Analitik Profil İndeksi (Api - 20 Staph). API Staph is a standardized *Staphylococcus* and *Micrococcus* genus identification system for *Staphylococcus* spp. used to identify species (Atlas, 2010).

### 2.8. Identifying Isolates with the VITEK®2 Compact System

The automated VITEK2 Compact system (Biomerieux, France) was used to validate the manual species identification. It was carried out in accordance with the VITEK2 compact system manufacturer's instructions.

| | **spa gen bölgesi** | **tst-1 gen bölgesi** |
|---|---|---|
| **Forward primer (F)** | TATAGTTCGCGACGACGTCC | CCCTTTGTTGCTTGCGACAA |
| **Reverse primer (R)** | AGCACCAAAAGCTGACAACA | ACCACCCGTTTTATCGCTTG |
| **PCR product length** | 705 bç | 538 bç |
| **Protocol type** | Touchdown PCR protokolü | Simple 3-adımlı PCR protokolü |

### 2.9. Antibiotic Susceptibility Test

Antibiotic susceptibility testing of *S. aureus* isolates was performed by the disc diffusion method (Somerville, 2022). The Clinical and Laboratory Standards Institute (CLSI) was used as a criterion for selecting antibiotic discs (CLSI, 2018). Brain-heart water was used to activate bacteria. Cultures adjusted to 0.5 McFarland standard after growth were seeded on Muller Hinton Agar plates with the aid of sterile swaps. After antibiotic discs were placed on the plates, they were incubated at 37°C for 18-24 hours. After incubation, the inhibition zone diameters (including the disc diameter) were measured in millimeters (mm). The diameter of the inhibition zone for each antibiotic was compared with the standards and classified as susceptible (S), moderate (I), or resistant (R).



### 2.10. Detection of Spa and Tst-1 Gene Regions of *S. aureus* Strains

PCR test was used to detect spa and tst-1 genes in *S. aureus* isolates isolated from urine samples taken from patients with UTI infection. All PCR primers used in this technique were designed in this study. Bacterial genomic DNA (Presto™ Mini gDNA Bacteria Kit) kit was used for DNA extraction from *S. aureus* isolates. The kit was used according to the manufacturer's instructions. Nanodrop was used to measure the total DNA isolated.

### 2.11. PCR Karışımının Hazırlanması

(GoTaq Green PCR master kit) was used for PCR testing. PCR was performed on T100 PCR Thermocycler (BioRad-USA). The PCR thermocycler conditions methodology for each gene was calculated using the Optimase ProtocolWriterTM web program. The PCR conditions applied for the spa and tst-1 gene regions are given in Table 1.

**Table 1.** Primer sequences for the spa and tst-1 gene regions

### 2.12. DNA Dizileme Yöntemi

DNA sequencing method was used to identify *S. aureus* isolates, analyze their phylogenetic trees and determine homology sequence similarities. Applied Biosystems DNA sequencing analyzes were performed by purchasing services from Macrogen company in South Korea. Results were read against the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology

Information (NCBI). A genetic tree was drawn using the Molecular EvolİYEonary Genetics Analysis Version 6.0 (MEGA version 6.0) program to show the number and type of genetic mutations in the study genes and then the genetic changes between *S. aureus* isolates and standard isolates. Afterwards, the isolates were registered on the gene bank site (NCBI Genbank Submission) and their gene bank numbers were obtained.

### 2.13.Statistical analysis

Chi-square statistics were used for statistical analysis in all study descriptive statistics groups. The significance of (P-0.01) was determined. Social Science Statistical Package for Windows (SPSS) was used to perform all analyzes (version23.0, SPSSInc, Chicago, III).

## 3. RESULTS

### 3.1. UTI Patient Data

133 (66.5%) of 200 fresh urine samples taken from male and female patients who applied to the hospital with the complaint of UTI between February-June 2021 were *Staphylococcus* spp. was found to be positive.

No significant growth was observed in the remaining 67 patients (33.5%). The demographic information of the patients who applied to XXXXX Training and Research Hospital with UTI symptoms is summarized in Table 2.

The highest rates of UTI were seen between 55 patients (27.5%) and the 51-70 age group, while the lowest infection rate was found in the 11-20 age group with 25 patients (12.5%). There is no statistically significant difference in age group distribution (P≤0.01).

Of the 200 patients included in this study, 136 (68%) were female and 64 (32%) were male. While reproduction was observed in 73% of the female patient population, the reproduction in the male patient population was 51% lower. Growth was observed in 76% of the 124 patients included in the study from urban areas, while reproduction was observed in 51% of 76 patients from rural areas.



As indicated in the sampled population (Table 2), the majority of UTI patients were 118 (59%) outpatients, while 82 (41%) were inpatients. While bacterial growth was observed at the rate of 73% in the outpatient group, this rate increased to 78% in the inpatient group. As shown in Table 2, there was no significant change in the patient distribution (P≥ 0.01).

**Table 2.** Characteristics of the UTI patient groups included in the study

| Parameter | | Number of patients | Percentage (%) |
|---|---|---|---|
| **Size of sample** | | **200** | **100** |
| **Bacterial Growth** | Growth | 133 | 66.5 |
| | No growth | 67 | 33.5 |
| **Gender** | Female | 136 | 68 |
| | Male | 64 | 32 |
| **Resident** | Urban | 124 | 62 |
| | Rural | 76 | 38 |
| **Hospital status** | Outpatient | 118 | 59 |
| | Inpatient | 82 | 41 |

### 3.2. *Staphylococcus* identification

*Staphylococcus* colonies on Mannitol Salt Agar Medium were observed to have different morphological shapes and colors compared to bacteriological culture. *S. aureus* isolates ermented mannitol and turned red agar into yellow. Colonies appear round and smooth with yellow zones in

yellow (Fig. 1). On blood agar, *S. aureus* isolates appear as large, round, smooth, white to yellow, shiny, opaque, and clear hemolysis zones as shown in Figure 1.

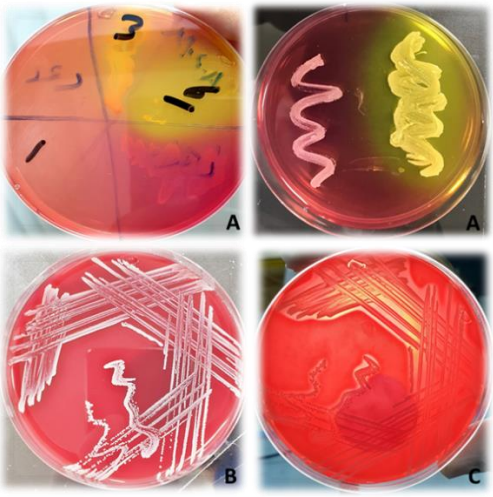


**Figure 1.** *S. aureus* on blood agar show clear zone surrounding the isolates

### 3.3. Biochemical Tests

*Staphylococcus* spp. According to the biochemical test results performed on the cultures, catalase, gelatinase, DNase, coagulase and hemolysis tests were positive, while the oxidase test was negative. S. aureus cultures were sensitive to Novobiocin and positive for DNAse activity (Figure 2).

*Staphylococcus* spp. According to the API 20 Staph test result used to confirm the diagnosis of isolates, S. aureus isolates showed a high percentage varying between 86% and 97%, different from the kit index. The study continued with biochemical results and 55 S. aureus strains, which were identified with the automatic VITEK2 Compact system (Biomerieux, France) device.



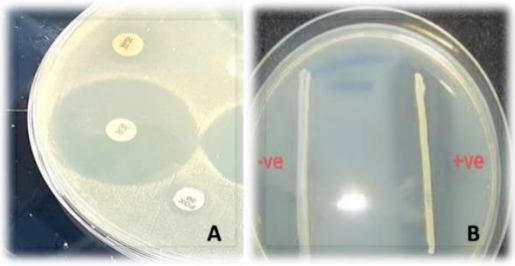


**Figure 2.** Novobiocin susceptibility (A) and positive DNAse activity (B) images of *S. aureus* isolates

### 3.4. Antibiotic Susceptibility Test

It was observed that there were significant differences in the resistance rates of the bacteria tested in the antibiotic susceptibility test of all *S. aureus* samples isolated from patients with UTI. The highest resistance rate (100%) of *S. aureus* isolates with species identification (n=55) was for Ampicillin and (97.7%) Cefoxitin, and resistance rates against Tetracycline, Clindamycin and Trimethoprim-Sulphamethoxazole and Erythromycin, respectively (76.6%, 71.4%) , 54.1% and 43.6%. The susceptibility profile of the isolates to antibiotics is given in Table 3.

**Table 3.** Antibiogram results of *Staphylococcus aureus* isolates

| No | Antibiotic | Symbol | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|---|
| **1** | Ampisilin | AMP | 0 | 0 | 133(%100) |
| **2** | Sefoksitin | FOX | 2(%1.5) | 1(%1.0) | 130(%97.7) |
| **3** | Tetrasiklin | TE | 25(%18.7) | 6(%4.5) | 102(%76.6) |

| | | | | | |
|---|---|---|---|---|---|
| **4** | Klindamisin | CD | 30(%22.5) | 8(%6.0) | 95(%71.4) |
| **5** | Trimetoprim | TMP | 40(%30) | 21(%15.8) | 72(%54.1) |
| **6** | Eritromisin | E | 56(%42.1) | 19(%14.2) | 58(%43.6) |
| **7** | Kotrimoksasin | COT | 47(%35.2) | 29(%21.7) | 57(%42.8) |
| **8** | Doksisiklin | DO | 49(%36.8) | 30(22.5%) | 54(%40.6) |
| **9** | Siprofloksasin | CIP | 45 (%33.7) | 35(%26.2) | 53(%39.8) |
| **10** | Netilimisin | NET | 66 (%49.5) | 17(%12.0) | 50(%37.6) |
| **11** | Nitrofuransiyon | NIT | 95(%71.4) | 8(%6.0) | 30(%22.5) |
| **12** | Vankomisin | VA | 111(%83.4) | 10(%7.5) | 12(%9.1) |
| **13** | Novobiyosin | NV | 118(%88.7) | 3(%2.2) | 12(%9.0) |

### 3.5. Detection of spa Gene in *S. aureus* Isolates

In our study, the presence of the spa gene in *S. aureus* isolates from UTI patients was determined using PCR technique. The spa gene was amplified with a positive reaction (1400-260 bp) in all *S. aureus* isolates (100%). Spa genotyping results are given in Table 4 and agarose gel electrophoresis images showing the PCR product analysis of the spa gene are given in Figure 3.

**Table 4**. Spa genotyping results

| ***S. aureus* UTI isolate No.** | **Number of repeat polymorphism site** | **~band size (bp)** | **Genotype** |
|---|---|---|---|
| **UTI.1** | 2 | 705bp, 590bp | Type B1 |
| **UTI.2** | 2 | 705bp, 590bp | Type B1 |
| **UTI.3** | 1 | 530bp | Type A1 |
| **UTI.4** | 2 | 705bp, 590bp | Type B1 |
| **UTI.5** | 2 | 705bp, 590bp | Type B1 |
| **UTI.6** | 1 | 705bp | Type A2 |
| **UTI.7** | 1 | 705bp | Type A2 |
| **UTI.8** | 1 | 705bp | Type A2 |
| **UTI.9** | 1 | 705bp | Type A2 |
| **UTI.10** | 1 | 705bp | Type A2 |
| **UTI.11** | 1 | 705bp | Type A2 |
| **UTI.12** | 2 | 705bp, 590bp | Type B1 |
| **UTI.13** | 2 | 705bp, 590bp | Type B1 |
| **UTI.14** | 1 | 705bp | Type A2 |
| **UTI.15** | 2 | 705bp, 590bp | Type B1 |
| **UTI.16** | 2 | 705bp, 590bp | Type B1 |
| **UTI.17** | 4 | 1400bp, 750bp, 705bp, 590bp | Type D1 |
| **UTI.18** | 1 | 705bp | Type A2 |
| **UTI.19** | 1 | 705bp | Type A2 |
| **UTI.20** | 3 | 590bp, 530bp, 260bp | Type C1 |
| **UTI.21** | 2 | 705bp, 590bp | Type B1 |
| **UTI.22** | 1 | 530bp | Type A1 |
| **UTI.23** | 2 | 705bp, 590bp | Type B1 |
| **UTI.24** | 2 | 705bp, 590bp | Type B1 |

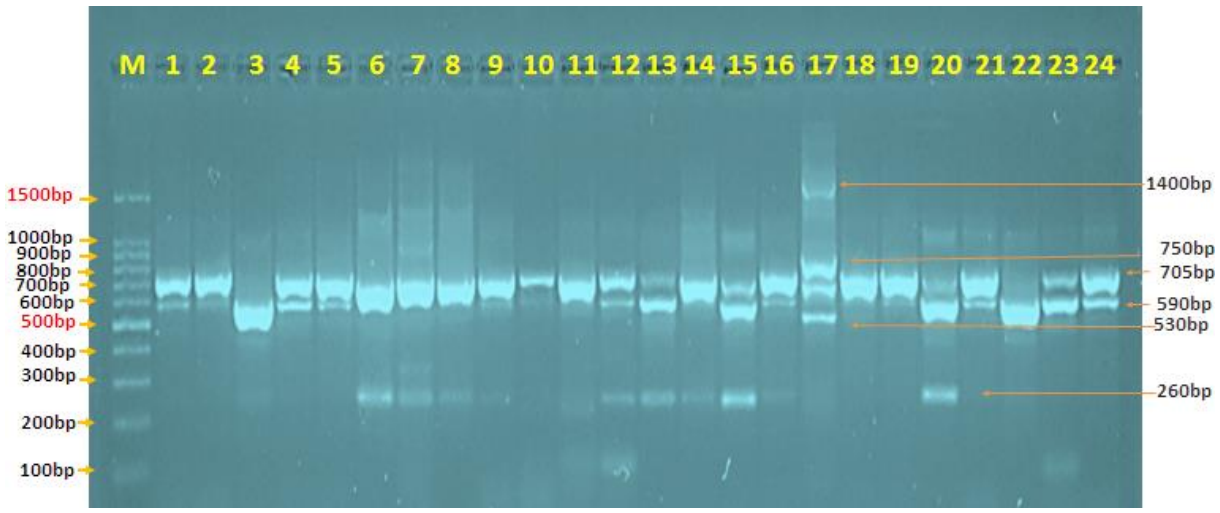


**Figure 3.** Agarose gel electrophoresis image that showed the PCR product analysis of *S. aureus* protein A (spa) gene in urinary tract infection *S. aureus* isolates. Where Marker ladder (1500-100bp), lane (1-24): showed some positive spa gene at (1400-260bp) PCR product size.

### 3.6. Detection of the tst-1 Gene in S. aureus isolates

In this study, the presence of tst-1 gene in *S. aureus* samples isolated from UTI patients was determined using PCR technique. In all S. aureus isolates (n=55), it was amplified to the tst-1 gene with 11 (20%) positive reactions (538bç) (Figure 4).


**Figure 4.** Agarose gel electrophoresis image that showed the PCR product analysis of toxic shock syndrome toxin-1 (*tst-1*) gene in urinary tract infection *S. aureus* isolates. Where Marker ladder (1500-100bp), lane (1-20): showed some positive *tst-1* gene at (538bp) PCR product size.

### 3.7. Spa Gene DNA Sequence Analysis Results

DNA sequencing method was performed to determine the analysis of genetic variation (substitution Mutations) in the spa gene of *S. aureus* isolates (IQ-UTI.1-IQ-UTI.2) and related NCBI-Blast. Phylogenetic tree genetic association analysis showed that native *S. aureus* isolates (IQ-UTI.1 - IQ-UTI.2) were associated with NCBI BLAST *S. aureus* genetic cluster variant 2 isolate in total genetic variation (0.060) (0.020%). As shown in Figure 5, the homology sequence identity between the *S. aureus* isolates isolated in the study (IQ-UTI.1 - IQ-UTI.2) and the related *S. aureus* India isolate with NCBI BLAST was determined as 98.95% - 99.16%. Analysis of genetic variation (substitution Mutations) in the spa gene was found between native *S. aureus* isolates (IQ-UTI.1-IQ-UTI.2) and the NCBI-Blast-related *S. aureus* isolate. The percentage of total genetic variation (0.84-1.05%) varies between 4 and 5 substitution mutations (Table 5). Finally, native *S. aureus* IQ-UTI.1-IQ-UTI.2 isolates were sent to NCBI Genbank as IQ-UTI-1 and IQ-UTI-2 with accession numbers OL411975 and OL411976.

**Table 5.** NCBI-BLAST analysis of genetic variation among *S. aureus* isolates.

| Local isolate | Homology sequence analysis |
|---|---|

| | Accession number | Number Mutations | Type of Mutation | Mutation (%) | Identity (%) |
|---|---|---|---|---|---|
| **UTI.No.1** | OL411975 | 5 | A/C, A/C, A/T, A/T, A/T | %1.05 | %98.95 |
| **UTI.No.2** | OL411976 | 4 | A/T, C/A, A/T, A/T | %0.84 | %99.16 |

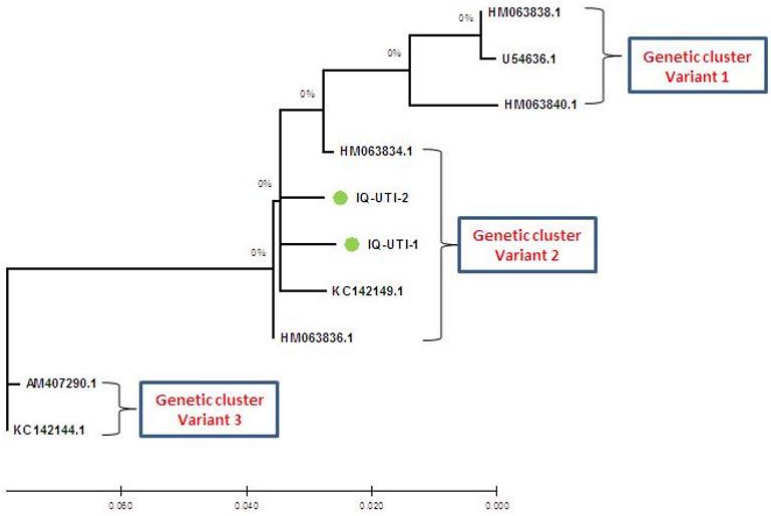




**Figure 5.** Phylogenetic tree analysis based immunoglobulin G binding protein A (*spa*) gene partial sequence in local *S. aureus* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *S. aureus* isolates (UTI-IQ.1 and UTI-IQ.2) were showed closed related to NCBI BLAST *S. aureus* genetic cluster variant 2 isolate at total genetic change (0.060-0.020%).

### 3.8. Tsst-1 Gene DNA Sequence Analysis Results

DNA sequencing method was applied for the analysis of genetic variation (transformer Mutations) in the NCBI-Blast tsst-1 gene related to IQ-UTI.3 of the native *S. aureus* isolate. Phylogenetic tree analysis showed that the most similarity was associated with the NCBI BLAST *S. aureus* tst-1 gene with the accession number MH920611.1 of the native *S. aureus* isolate (IQ-UTI.3). As shown in Figure 6, the genetic variation is between (0.00050-0.00350%). Homology sequence similarity between native *S. aureus* isolate IQ-UTI.3 and NCBI BLAST related *S. aureus* isolate was found to be 99.77%. After analysis of genetic variation (substitution Mutations) in the tst-1 gene between native *S. aureus* isolates (IQ-UTI.3) and NCBI-Blast-related *S. aureus* isolate, the genetic variation was found to be 0.23%. Finally, the native *S. aureus* IQ-UTI-3 isolate was submitted to NCBI Genbank as IQ-UTI-3, accession numbers OL411977.

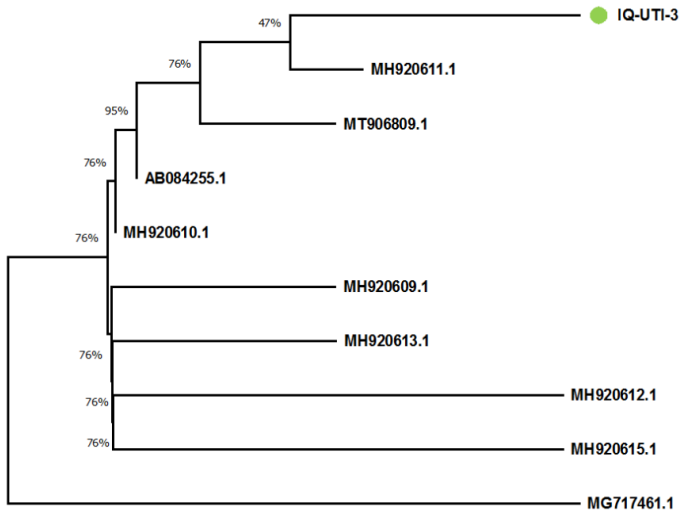


**Figure 6.** Phylogenetic tree analysis based toxic shock syndrome toxin-1 (tsst-1) gene partial sequence in local *S. aureus* isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *S. aureus* isolates (UTI-IQ.3) were showed closed related to NCBI BLAST *S. aureus* isolate (MH920611.1) at total genetic change (0.00350-0.00050%).

## 4. DISCUSSION

UTI is a common disease and is a common cause of morbidity, usually in outpatients and hospitalized patients. It affects every person from all age groups and different geographical regions (Wagenlehner and Naber, 2006). In UTI, patients may have symptomatic or asymptomatic manifestations. The present study was conducted for the morphological and molecular detection of *S. aureus* isolated from UTI patients in AL-Nasiriyah city. The findings of this study showed a very high level of positivity, 66.5%. In a similar study conducted in Iraq, the positivity rate of *S. aureus* in cultures isolated from urine culture was 57.9%. These rates are similar to our study (Hammoudi, 2013). On the other hand, in another study, the rate of positive culture was found to be 41.6% (Alsamarai et al., 2016). This rate is quite low compared to our positive rate. These differences in the distribution of pathogens can be explained by different fields of studies, as well as the drugs used in the treatment and have an important role in the distribution and spread of the pathogen.



In our study, most of the patients with UTI symptoms were women with a rate of 68%. The high prevalence of infection in women is due to anatomical and pathogenic factors such as the shortness of the urethra, the easy access of pathogens to the urethra, and therefore the shorter distance of bacteria to the urinary tract. For example, as a woman's estrogen levels decrease with menopause, the risk of UTI increases further due to the loss of the protective vaginal flora (Ismail et al., 2018). Apart from these, contraceptive use, childbirth and menopause can be counted as other factors (Dielubanza and Schaeffer, 2010). As in our study, it is an expected result that UTI is seen more frequently in women than in men (Fadhel et al., 2013).

In our study, the incidence of infections was found to be higher in urban areas than in rural areas. In a similar study, the results were similar, with a ratio of (62.9%) and (37.1%) for urban and rural areas, respectively (Seifu and Gebissa, 2018). One of the reasons why these rates are different is that the population density and environmental pollution are higher in urban areas compared to rural areas.

In the patient groups included in our study, the incidence of UTI was found to be higher in the outpatient groups than in the hospitalized group. The results of our study were found to be compatible with other studies (Harsha et al., 2013; Iregbu and Nwajiobi-Princewill, 2013). The reason for the higher infection rates in outpatients can be attributed to predisposing factors such as dirty toilet facilities and inadequate drainage (Hannah et al., 2011).

In recent years, *S. aureus* has become resistant to both new and conventional antibiotics, which poses a therapeutic problem in the treatment of antibiotic-resistant bacteria. Therefore, investigation of the susceptibility pattern is of great importance in determining the future challenges of effective treatment. World wide studies have also shown high rates of resistance to ampicillin (100%), cefoxitin (97.7%), followed by tetracycline (76.6%) (Onanuga and Awhowho, 2012). Ampicillin resistance may be due to structural modification of the enzymatic action (β-lactam effect) or to blocking access to the target by altering the outer membrane permeability, and may be due to alteration of the antibiotic target site (Chakraborty et al., 2011). In our study, it was observed that the isolates tested against vancomycin and novobiocin antibiotics were sensitive (83.4%) and (88.7%). In a study conducted in Iraq in 2021, it was aimed to identify the sources of methicillin-resistant *S. aureus*, which was seen to spread significantly in Iraq, and to control their spread in community and hospital settings. In the study, 65 (22.4%) of 290 clinical specimens were *S. aureus*, and 62 (95.4%) of them were found to be resistant to oxacillin and methicillin (Kareem et al., 2015; Kareem et al., 2020).

Various techniques have been developed for the identification of *S. aureus*, including phenotypic and genotypic analysis such as spa and tst-1gene detection, which is considered a genetic marker used for rapid and direct identification of *S. aureus* (Ostojić and Hukić, 2015). Spa typing is based on the polymorphism site of the gene encoding the protein A spa. The spa typing method is based on the number of tandem repeats and the sequence variation in the X region of the protein A gene. Numerous studies have shown different spa gene patterns among *S. aureus* strains isolated from patients in different geographic regions of the World (Kareem et al., 2020; Furuya et al., 2010). In this study, the genotypic expression rate of the spa gene in UTI samples was 24 (100%) with a different number of repeat polymorphism sites, especially in UTI. The obtained study data seem to be in agreement with other similar studies (Goudarzi et al., 2018).

The biological toxicity of *S. aureus* superantigens (SAg) makes them critical contributors to causing life-threatening infections. Toxic shock syndrome toxin-1 (TSST-1), encoded by the tst-1 gene, is an important member of SAgs and can cause staphylococcal toxic shock syndrome (TSS) in a susceptible host (Spaulding et al., 2018). In this study, all *S. aureus* isolates were amplified to the tst1 gene (538 bp) with 4 (20%) positive reactions. These results were reported as 22% and 18% in other studies (Alni et al., 2013; Wang et al.,2017). In another study, the tst-1 gene positivity rate was found to be 26.31%, while in another study it was determined as 44.42% (Costa et al., 2018).



## 5. RESULTS

In conclusion, our study showed that there was a significant increase in the prevalence of *S. aureus* in Iraq. Genetic variation analysis in spa and tsst-1 gene was determined by DNA sequencing method and genetic variation in spa gene was found to be between 0.84-1.05%, while genetic variation in tsst-1 gene was found to be 0.23%. The antibiotic resistance profile of the tested isolates in our study was found to be compatible with other studies. Regular screening of UTI pathogens will contribute to reducing the prevalence of this disease. Immunological and histopathological studies, determination of virulence factors and polymorphism and fragment mapping studies are required to follow the effect of *S. aureus* in UTI diseases.

## ETHICS COMMITTEE APPROVAL

This study was carried out with the approval of the XXXX Medical Faculty Non-Invasive Clinical Research Ethics Committee (Decision No: 35 and Date: 19.11. 2020).

## CONFLICT OF INTEREST

The authors declared no conflict of interest regarding this article.

## REFERENCES

Alni, R. H., Mohammadzadeh, A., Mahmoodi, P., Alikhani, M. Y. (2018). Detection of toxic shock syndrome toxin (TSST) gene among *Staphylococcus aureus* isolated from patients and

healthy carriers. *Avicenna Journal of Clinical Microbiology and Infection*, 5(1), 14249-14249. https://doi.org/10.5812/ajcmi.14249

Alsamarai, A. G. M., Abdul-Rahman, Latif, I , Abdul-Aziz, M. M. (2016). Urinary tract infection in Iraq: Evaluation of early detection methods and etiology. *World Journal of Pharmacy and Pharmaceutical Sciences*, 5(7), 181-194. Retrieved from https://doi.org/20959/wjpps20167-7196

Armbruster, C. R., Wolter, D. J., Mishra, M , Hayden, H. S., Radey, M. C., Merrihew, G., et al. (2016). *Staphylococcus aureus* protein A mediates interspecies interactions at the cell surface of *Pseudomonas aeruginosa*. *ASM Journals - American Society for Microbiology*, 7:e00538-e1516. Retrieved from https://doi.org/10.1128/mBio.00538-16

Atlas, R. M. (2010). Handbook of microbiological media. CRC press. 4th ed. Boca Raton, United. Retrieved from https://doi.org/10.1201/EBK1439804063

Bauer, A. W., Kirby, W. M., Sherris, J. C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493-496.

Chakraborty, S. P., KarMahapatra, S., Bal, M., Roy, S. (2011). Isolation and identification of vancomycin resistant *Staphylococcus aureus* from post operative pus sample. *Al Ameen Journal of Medical Sciences*, 4(2),152-168.

Costa, A. R., Batistão, D. W., Ribas, R. M., Sousa, A. M., Pereira, M. O., Botelho, C. M. (2013). *Staphylococcus aureus* virulence factors and disease. Microbial pathogens and strategies for combating them. *Journal of Science Education and Technology*, 1, 702-710.

Costa, F. N., Belo, N. O., Costa, E. A., Andrade, G. I., Pereira, L. S., Carvalho, I. A., (2018). Frequency of enterotoxins, toxic shock syndrome toxin-1, and biofilm formation genes in *Staphylococcus aureus* isolates from cows with mastitis in the Northeast of Brazil. *Tropical Animal Health and Production*, 50(5), 1089-1097. Retrieved from https://doi.org/10.1007/s11250-018-1534-6.

Dielubanza, E. J., Schaeffer, A. J. (2011). Urinary tract infections in women. *Medical Clinics of North America*, 95(1), 27-41. Retrieved from https://doi.org/10.1016/j.mcna.2010.08.023

Fadhel, A. N., Abureesha, R. A., Al-azzawi, R. H. (2013). Prevalence of *Staphylococcus epidermidis* and *Staphylococcus aureus* and their biofilm ability among Iraqi patients suffering from urinary tract infection. Iraqi Journal of Science, 54(3): 547-552.

Furuya, D., Tsuji, N., Kuribayashi, K., Tanaka, M., Hosono, Y., Uehara, N., Watanabe, N. (2010). Evaluation of spa typing for the classification of clinical methicillin-resistant *Staphylococcus aureus* isolates. *Japanese Journal of Infectious Diseases,* 63(5), 364-367.

Goudarzi, M., Abiri, P., Nasirian, S., Afshari, S. (2018). SCCmec and spa typing of *Staphylococcus aureus* strains isolated from patients with urinary tract infection: emergence of spa types t426 and t021 in Iran. *Jundishapur Journal of Microbiology*, 11(5):e62169. Retrieved from https://doi.org/10.5812/jjm.62169

Hammoudi, A. M. (2013). Urinary tract infection of adults in Baghdad City. *International Journal of Current Microbiology and Applied*, 2, 1-6.

Hannah, K. I., Gunturu, R., Joyce, M., Theresa, O. (2011). The incidence of nosocomial Urinary Tract Infections in Kenyatta National Hospital. *Baraton Interdisciplinary Research Journal*, 1(2), 12-21.

Harsha, T. R., Vijaya, D., Nagarathnamma, T. (2013). Bacteriological profile and comparison of Antibiotic susceptibility pattern of uropathogens by conventional disk diffusion and Rapid mini API Method. *Journal of Pharmacy and Bioallied Sciences*, 30(30), 7-12.

Iregbu, K. C., Nwajiobi-Princewill, P. I. (2013). Urinary tract infections in a tertiary hospital in Abuja, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 14(3), 169-173. Retrieved from http://dx.doi.org/10.4314/ajcem.v14i3.9

Ismail, M. D., Ali, I., Hatt, S., Cronenwett, A. W., Marrs, C. F., Foxman B. (2018). Association of *Escherichia coli* ST131 lineage with risk of urinary tract infection recurrence among young women. *Journal of Global Antimicrobial Resistance*, 13, 81-84. Retrieved from https://doi.org/10.1016/j.jgar.2017.12.006

Kareem, S. M., Aljubori, S. S., Ali, M. R. (2020). Novel determination of spa gene diversity and its molecular typing among *Staphylococcus aureus* Iraqi isolates obtained from different clinical samples. *New Microbes and New Infections*, 29, 34, 100653. Retrieved from https://doi.org/10.1016/j.nmni.2020.100653

Kareem, S. M., Al-Jubori, S. S, Ali, M. R. (2015). Prevalence of erm genes among methicillin resistant *Staphylococcus aureus* MRSA Iraqi isolates. *International Journal of Current Microbiology and Applied*, 4(5), 575–585.

Kourtis, A. P., Hatfield, K., Baggs, J., Mu, Y., See, I., Epson, E., et al. (2019). Vital Signs: Epidemiology and Recent Trends in Methicillin-Resistant and in Methicillin-Susceptible *Staphylococcus aureus* Bloodstream Infections - United States. MMWR Morb Mortal Wkly Rep, 68(9), 214-219. Retrieved from https://doi: 10.15585/mmwr.mm6809e1

MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria pp: 912, 3rd. Lippinocott Williams and Wilkins Philadelphia, USA.

Markey, B. K., Leonard, F. C., Archambault, M., Cullinane, A., Maguire, D. 2014. Clinical Veterinary Microbiology, 105-120. 2nded. Mosby Elsevier, Edinburgh.

Onanuga, A., Awhowho, G. O. (2012). Antimicrobial resistance of *Staphylococcus aureus* strains from patients with urinary tract infections in Yenagoa, *Nigerian Journal of Pharmaceutical and Applied Science* 4(3), 226. Retrieved from https://doi.org/10.4103/0975-7406.99058

Ostojić, M., Hukić, M. (2015). Genotypic and phenotypic characteristics of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, isolated on three different geography locations. *Bosnian Journal of Basic Medical Sciences* 15(3), 48. Retrieved from https://doi.org/10.17305/bjbms.2015.402

Ross, A., Shoff, H. W. (2022). Toxic Shock Syndrome. In: StatPearls Treasure Island: StatPearls Publishing.

Seifu, W. D., Gebissa, A. D. (2018). Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (İYE) in Shashemene referral hospital, Ethiopia. *BMC Infectious Diseases* 18(1), 30-37. Retrieved from https://doi.org/10.1186/s12879-017-2911-x

Shi, H., Tang, J., An, C., Yang, L., Zhou, X. (2021). Protein A of *Staphylococcus aureus* strain NCTC8325 interacted with heparin. *Archives of Microbiology*, 203, 2563–2573. Retrieved from https://doi.org/10.1007/s00203-021-02255-0

Somerville, G. A., Parrett, A. A., Reed, J. M., Gardner, S. G., Morton, M., Powers, R. (2022). Human Serum Alters the Metabolism and Antibiotic Susceptibility of *Staphylococcus aureus*. *Journal of Proteome Research*, 21(6), 1467-1474. Retrieved from doi: 10.1021/acs.jproteome.2c00073

Spaulding, A. R., Salgado-Pabon, W., Kohler, P. L., Horswill, A. R., Leung, D. Y., Schlievert, P. M. (2013). *Staphylococca*l and *streptococcal* superantigen exotoxins. Clinical Microbiology Reviews 26, 422–447. Retrieved from https://doi.org/10.1128/CMR.00104-12

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603- 661. Retrieved from https://doi.org/10.1128/CMR.00134-14

Wagenlehner, F. M. E., Naber, K. G. (2006). Current challenges in the treatment of complicated urinary tract infections and prostatitis. Clinical Microbiology and Infection, 12, 67-80. Retrieved from https://doi.org/10.1111/j.1469-0691.2006.01398.x

Wang, M., Zheng, Y., Mediavilla, J., Chen, L., Kreiswirth, B., Song ,Y., et al. (2017). Hospital dissemination of tst-1-positive clonal complex 5 (CC5) methicillinresistant *Staphylococcus aureus*. *Frontiers in Cellular and Infection Microbiology*, 7,101. Retrieved from https://doi.org/10.3389/fcimb.2017.00101