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A Molecular Study of Toxic Shock Syndrome Toxin gene (*tsst-1*) in β-lactam Resistant *Staphylococcus aureus* Clinical Isolates

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Abstract
Three hundred and sixty different samples were collected from different sources, including wound, burn, nasal, and oral swabs from several hospitals in Baghdad. A number of 150 (53%) *Staphylococcus aureus* samples were isolated and identified among a total of 283 samples. Then, the spread of the Toxic Shock Syndrome Toxin-1 gene (*tsst-1*) was investigated in β-lactamase resistant *S. aureus*. According to the source of samples, the distribution of *S. aureus* isolates was found to be significantly higher ($p < 0.01$) in wound samples as compared to other sources. According to the age, a highly significant distribution ($p < 0.01$) was recorded in the age group of 15-30 years, whereas gender comparison showed no statistically significant differences. All the isolates were subjected to susceptibility test against eight β-Lactam antibiotics by using the disc diffusion method. The antimicrobial susceptibility test showed that *S. aureus* had maximum resistance percentage to Carbenicillin (97.3%), while the lowest resistance rate was against Meropenem, with a sensitivity rate of up to 82%. In addition, 144 (96%) out of the 150 *S. aureus* isolates have multiple drug resistance (MDR). All the isolates were subjected to polymerase chain reaction to amplify *tsst-1* toxin gene. A number of 70 isolates (46.7%) were found to be positive for *tsst-1* gene. The results showed no significant correlation between *tsst-1* gene with the individual antibiotic resistance and the multi-drug resistance patterns of the isolates ($p = 0.226$).

Keywords: *tsst-1* gene, *S. aureus*, β-lactam resistance, Multiple Drug Resistance, Clinical isolates.

دراسة جزيئية لجين الصدمة السمية (*tsst-1*) في العزلات السريرية للمكورات العنقودية الذهبية المقاومة لمجموعة مضادات بيتا لاكتام

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الخلاصه
جمعت ثلاثمائة وستين عينة مختلفة من مصادر مختلفة شملت مسحات الجروح والحروق والأنف والفم من العديد من المستشفيات في بغداد. عزلت 150 (53٪) من المكورات العنقودية الذهبية والتعرف عليها من بين 283 عينة، ثم تم فحص انتشار جين الصدمة السامة (*tsst-1*) في المكورات العنقودية الذهبية المقاومة لل β-lactamase. توزع عزلات المكورات العنقودية الذهبية (150) طبقا لمصدر العينات، وجد دلالة إحصائية بدرجة عالية (p <0.01) في عينة الجروح مقارنة ببقية العينات. بينما حسب العمر، وجد دلالة إحصائية بدرجة عالية (p <0.01) الموزعة حسب الفئة العمرية 15-30 عام، لكن حسب الجنس لم يظهر

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أي قيمة ذات دلالة إحصائية. خضعت جميع العزلات لاختبار الحساسية ضد ثمانية من المضادات الحيوية β-lactamase باستخدام طريقة انتشار القرص. أظهر اختبار الحساسية لمضادات الميكروبات أن نسبة Carbenicillin كانت لها أقصى نسبة مقاومة (97.3٪) ، بينما أظهرت Meropenem أدنى معدل مقاومة مع معدلات حساسية تصل إلى 82٪. بالإضافة إلى ذلك، فإن 144 (96٪) من أصل 150 عزلة من المكورات العنقودية الذهبية لها مقاومة متعددة للأدوية (MDR). خضعت جميع العزلات لتفاعل البلمرة المتسلسل لتضخيم جين السمية *tsst-1*. وجدت 70 عزلة (46.7٪) إيجابية لجين السمية *tsst-1*. لم يظهر الارتباط بين جين *tsst-1* والحساسية للمضادات الحيوية أي قيمة ذات دلالة إحصائية مع كل نمط مقاومة للمضادات الحيوية للعزلات ونمط المقاومة للأدوية المتعددة (p = 0.226).

Introduction

Staphylococcus aurous is a major cause of multiple infections that range from the superficial skin to deeper infections, such as hair follicle abscesses, deep tissue infection, and systemic infections that involve the lungs, blood, and bones [1]. *S. aureus* is both a commensal organism and an important opportunistic human pathogen, causing a variety of community and hospital associated infections, such as bacteremia, sepsis, endocarditis, pneumonia, osteomyelitis, arthritis, and skin diseases [2].

Antimicrobial resistance is one of the most serious health threats. Infections from resistant bacteria are very common and some pathogens have become resistant to multiple types or classes of antibiotics. *S. aureus* became a major public health concern as a result of the steadily increasing incidence of antimicrobial resistance, particularly the Methicillin resistant *S. aureus* (MRSA) [3].

β-lactam antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems target transpeptidase enzymes that synthesize the bacterial cell wall and act cytostatically on bacteria by irreversibly inactivating peptidoglycan transpeptidases [4]. The resistance to β-lactam antibiotics can be due to the expression of a single mechanism of resistance or to the additive effects of several mechanisms. The resistance to β-lactam antibiotics in bacteria could be due to four mechanisms [5, 6]; (I) resistance by increased encoding of efflux pump genes, (II) resistance by decreased antibiotics uptake before β-lactam reaches bacterial penicillin-binding protein (PBP) targets, (III) resistance by alteration of the target site resistance caused by alterations in PBPs, and (IV) resistance by enzymatic inactivation (antibiotic-inactivation enzymes), such as β-lactamases.

S. aureus resistance to β-lactam antibiotics is conferred through the acquisition of the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins, and carbapenems). This allows for resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections. As such, the glycopeptide vancomycin is often deployed against MRSA [7, 8].

S. aureus is a dangerous pathogen which has proved versatile in developing resistance to antimicrobials and acquiring virulence factors. Significant efforts have been undertaken to clarify the importance that specific molecular determinants have in defining *S. aureus* virulence and regulatory systems governing the expression of the virulence genes. This is considered now as an active field of research aiming at the development of new methods for therapy against infectious diseases [9].

The entire genome of *S. aureus* was sequenced in 2001 and the ongoing molecular and genetic dissection of *S. aureus* revealed a large number of surface adhesions, secreted enzymes, and toxins that make invasion possible [10].

Staphylococcus aureus harbor a number of mobilizable exogenous DNA stretches, including insertion sequences, transposons, bacteriophages, and pathogenicity islands (also referred to as genomic islands) [11], which contain specific determinants responsible for disease and antibiotic resistance. These exogenous elements explain the high capacity of *S. aureus* to undergo horizontal gene transfer and to exchange genetic elements with other organisms, including both staphylococcal and non-staphylococcal genera [12].

Staphylococcal superantigens (SAgs) include staphylococcal enterotoxins (SEs) and toxic shock syndrome toxine-1 (TSST1) [13]. These toxins were classified as members of the pyrogenic toxin superantigen family because of their biological activities and structural relatedness. Superantigen bypass normal antigen presentation and have strong T-cell mitogenic activity as a result of direct binding to specific regions on T-cells and the major histocompatibility complex class II molecules of antigen - presenting cells [14].

More than twenty distinct superantigenic toxins are known to be produced by *S. aureus*. Both MRSA and MSSA can harbor one or more superantigenic toxin genes. The pathogenic mechanism and virulence factors are assumed to be different between MRSA and MSSA [15].

The aims of this study include the isolation of *S. aureus* and the investigation of the spread of *tsst-1* gene in β-lactam resistant *S. aureus* clinical isolates.

Materials and Methods

Specimens Collection

From October 2019 until February 2020, wound, burn, nasal, and oral swab samples were collected from patients and outpatients of Al-Yarmouk Teaching Hospital, Baghdad Hospital Laboratories, and Teaching Laboratories Institute in the Medical City, as well as several private laboratories in Baghdad/ Iraq. The samples were cultured immediately after collection for the purposes of diagnosis.

S. aureus isolation and identification

All samples were cultured on brain heart infusion (BHI) agar and/ or broth and incubated aerobically for 24 hrs at 37°C to test bacterial growth. Then, the growth was subcultured in mannitol salt agar and blood agar and incubated overnight at 37 ˚C. *S. aureus* isolates were identified by using conventional and molecular methods. The molecular method included the detection of *16R rRNA* gene by using the polymerase chain reaction (PCR) technique at the microbiology research laboratory of the Department of Biotechnology/ University of Baghdad. Microscopic examination was performed for Gram stained samples to confirm their Gram positivity.

Antibiotic susceptibility tests

The susceptibility of *S. aureus* isolates against antibiotics was detected by the disk diffusion (Kirby-Bauer) method using Muller-Hinton (MH) agar [16]. Eight antibiotics (Bioanalyses, Turkey) were used in this study, namely Amoxicillin-Clavulanic acid, Carbenicillin, Cefotaxime, Cefoxitin, Cephalexin, Meropenem, Methicillin, and Oxacillin. The diameter of inhibition zone was measured and compared to the chart provided by the National Committee for Clinical Laboratory Standard institute (CLSI, 2018) [17]. The isolates were considered as MDR when they showed resistance to 3 or more types of antibiotics.

Molecular Assay

The genome of *S. aureus* was extracted by using Geneaid™ DNA isolation kit. The extracted DNA was used as a template for the PCR analysis, which was performed for the detection of the gene associated with the identification of *S. aureus* (*16S rRNA*) and *tsst-1* gene. The sequence of primers and size of amplification are described in Table-1.

Table 1- Primer sequences used in this study

Gene	Primer	Nucleotide sequence (5´ to 3´)	PCR product (bp)	Reference
16S rRNA	F:	ACGGTCTTGCTGTCACTTATA	257	[18]
	R:	TACACATATGTTCTTCCCTAATAA		
tsst-1	F:	CTGGTATAGTAGTGGGTCTG	271	[19]
	R:	AGGTAGTTCTATTGGAGTAGG		

F: Forward primer, R: Reverse primer, bp: Base pair.

Components for monoplex PCR reaction were prepared in a total volume of 20µl, including 10µl of Go Taq®Green Master mix (2X), 1µl for each primer (10 pmol), 3µl of DNA template, and 5µl of nuclease-free water. The amplification reaction was carried out using PCR thermal cycler (MultiGene, Labnet, USA). Initial denaturation was achieved in 5 minutes at 94ºC, followed by 35 cycles of denaturation at 94ºC for 1 minute, extension at 72ºC for 1 minute, and a final extension for 8 min. at 72ºC. The annealing conditions for *16S rRNA* were 30 seconds at 52ºC and for *tsst-1* were 2 minutes at 54ºC. The amplified PCR products were detected by electrophoresis through 1.5% agarose gels to determine the size of amplified fragments, stained with a safe stain, and visualized by UV-transilluminator.

Statistical Analysis

Data are presented as frequency and percentage, while Chi-square (χ^2) was applied to test the data in this study. p value ≤ 0.05 was considered statistically significant and p value ≤ 0.01 was considered

highly statistically significant, by using IBM Statistical Package for the Social Sciences (SPSS) version 25 (2017) [20].

Results and Discussion

Isolation of Bacteria

The clinical samples (n= 360) included wound, burn, nasal, and oral swabs. According to gender, the samples were distributed into 204 (56.7%) samples from males and 156 (43.3%) samples from females. The age of the participants ranged from 5 to 80 years, with a mean age of 36.5±0.93 years.

Distribution of clinical samples according to the source of samples is summarized in Table-2. Among the total samples, only 77 (21.4%) showed no growth, whereas 283 (78.6%) samples showed growth. The microscopic examination was applied to all samples that exhibited growth, after applying Gram stain to detect their response to staining. The results showed that 185 (51.4%) isolates were Gram-positive and 98 (27.2%) isolates were Gram-negative.

Table 2- Distribution of clinical samples.

Samples	Growth samples No. (%)	Non- growth samples No. (%)	Total No. (%)	*P-value* [a]
Wound	85 (23.6%)	5 (1.4%)	90 (25%)	<0.0001**
Burn	83 (23.1%)	7 (1.9%)	90 (25%)	<0.0001**
Nasal	65 (18.1%)	25 (6.9%)	90 (25%)	<0.0001**
Oral	50 (13.8%)	40 (11.2%)	90 (25%)	0.292 [NS]
Total	283 (78.6%)	77 (21.4%)	360 (100%)	<0.0001**
P-value [a]	0.009**	<0.0001**	-	

Data presented as [a] Chi-square goodness of fit. NS=Non-significant, ** the correlation is significant at the $p < 0.01$ level (Highly Significant).

Identification and Prevalence of *S. aureus* isolates

According to the rotation laboratory test identification results (culture, biochemical and API Staph. and VITEK 2 systems), 150 isolates (53%) of *S. aureus* were identified amongst the 283 isolates that exerted bacterial growth. The prevalence of *S. aureus* according to the samples source revealed that wound samples recorded the highest number of isolates, reaching 59 isolates (39.3%). Nonetheless, the lowest number of isolates (n= 18) was isolated from oral swabs, as shown in Table-3. The distribution of the 150 *S. aureus* isolates according to the source of samples revealed a highly significant ($p < 0.01$) higher number in wound samples as compared to the other sources. While according to the age, a highly significant ($p < 0.01$) distribution was found in the age group of 15-30 years, while gender distribution showed no statistically significant differences.

Table 3- Distribution of *S. aureus* (150) isolates according to the source of samples, gender, and age.

	Status	No. of *S. aureus* isolates	Percent %	*P-value* [a]
Source of Sample	Wound	59	39.3	<0.0001**
	Burn	43	28.7	
	Nasal	30	20	
	Oral	18	12	
Gender	Male	85	56.7	0.102 [NS]
	Female	65	43.3	
Age (years)	<15	16	10.7	<0.0001**
	15-30	49	32.7	
	31-45	42	28	
	46-60	28	18.6	
	>60	15	10	

Data presented as [a] Chi-square goodness of fit. NS=Non-significant, ** the correlation is significant at the $p < 0.01$ level (Highly Significant).

Recent local studies reported the isolation of *S. aureus* from different clinical samples. Jaddoa (2018) [21] recorded 70% from total isolates as *S. aureus*. Mohammed and Flayyih (2018) [22] recorded that 38.83% isolates were identified as *S. aureus*. A study by Fayyad *et al*. (2019) [23] recorded that 24.7% isolates were identified as *S. aureus* in Baghdad.

This may be due to the fact that *S. aureus* is one of the main causes of hospital (Nosocomial) and community-acquired infections, which can lead to serious consequences [24]. *S. aureus* is considered as one of the important pathogens with the potential to cause opportunistic infections, being also a member of the normal flora in the body [25].

However, *S. aureus* is an opportunistic pathogen that can be isolated from different sources of infections and cause human infections. *S. aureus* infections have been reported to increase, with important clinical manifestations that include bacteraemia, infective endocarditis, skin and soft tissue infections, osteoarticular infections, and pleuropulmonary infections. Other clinical infections include epidural abscess, meningitis, toxic shock syndrome, and urinary tract infections [25].

The skin is the largest organ of the body and, along with the underlying soft tissue which includes the fat layers, fascia, and muscle; it represents the majority of the tissues in the body. Skin and soft tissue infections (SSTIs) are commonly caused by *S. aureus*, specifically MRSA. They cause mild infections on the skin, such as sores or boils, but also more serious skin infections, such as furuncles, impetigo, or infected wounds and burns. *S. aureus* also causes pneumonia, urinary tract infection, blood infections (if bacteria enter blood stream they cause bacteremia in adults and sepsis in infants), osteomyelitis, toxic shock syndrome, and scalded skin syndrome [26].

The proportion of *S. aureus* isolated from the oral source in this study was lower than that from the other source. This is due to the fact that the oral microbiota stability may be changed or influenced by internal factors of the host, such as tooth loss [27], immunological conditions, and ageing, as well as external factors, such as smoking and oral hygiene [28].

Antibiotics Susceptibility of *S. aureus*

In past and present years, *S. aureus* showed resistance to both new and traditional antibiotics and, thus, the treatment of antibiotic resistant bacteria represents a therapeutic problem. For this reason, investigating the susceptibility pattern is useful to determine the future challenges of effective therapy.

One hundred and fifty isolates were subjected to the susceptibility test for eleven types of β-lactam antibiotics, as previously mentioned. The antibiotics susceptibility of *S. aureus* demonstrated an obvious level of resistance against the used antibiotics, as shown in Figure-1.

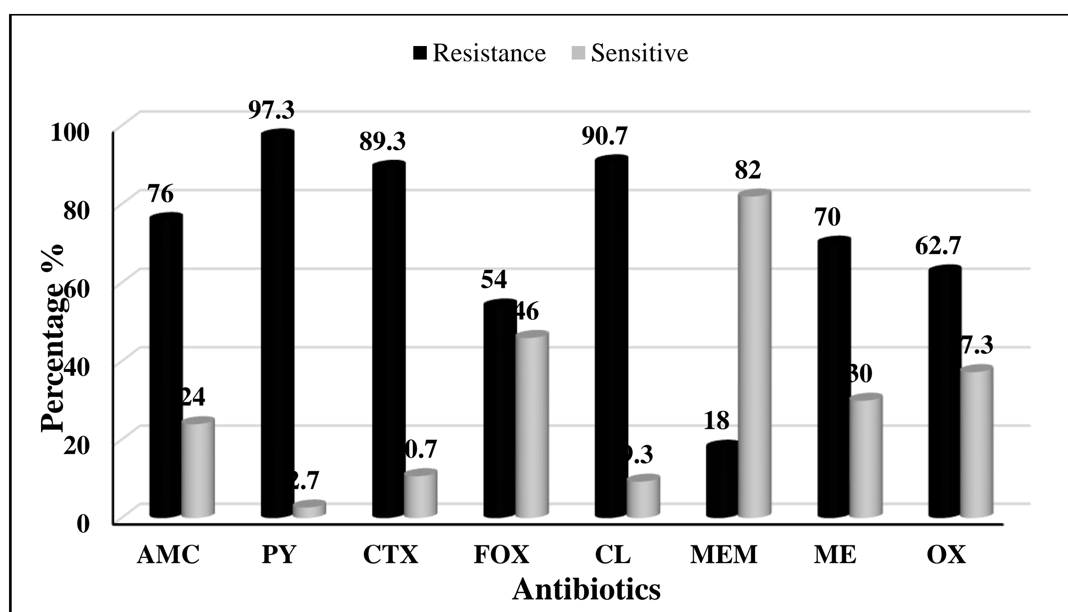

Figure 1- Percentage results of antibiotics susceptibility test against *S. aureus* isolates.

*AMC: Amoxicillin/ Clavulanic acid, PY: Carbenicillin, CTX: Cefotaxim, FOX: Cefoxitin, CL: Cephalexin, MEM: Meropenem, ME: Methicillin, OX: Oxacillin.

The antimicrobial susceptibility of *S. aureus* isolates showed that 114 isolates (76%) were resistant to Amoxicillin/ Clavulanic acid. This result is in agreement with that reported by Alsaadi (2017) who found a susceptibility of 76.1% [29].

The maximum percentage of resistance (97.3%, 146 isolates) was observed s against Carbenicillin. An earlier study also reported a high resistance to Carbenicillin (100%) [30].

The isolates expressed high resistance against the third generation of cephalosporins, as represented by a value of 89.3% (134 isolates) for Cefotaxime. A previous study by Hasan and Ismael (2018) reported that all the isolates (100%) were resistant to Cefotaxime [31].

The susceptibility rate of the isolates toward Cefoxitin was 54% (81 isolates). A previous study published by Fayyad *et al*. (2019) showed approximately similar resistance results at 59.1% [23].

The results also showed that 90.7% (136) of *S. aureus* isolates were resistant to Cephalexin. A previous study by Salih *et al*. (2017) reported a lower percentage of 53.9% [32].

In addition, the results showed that, among 150 *S. aureus* isolates, 105 isolates (70%) were resistant to Methicillin (MRSA) and 94 isolates (62.7%) were resistant to Oxacillin. A previously study by Salih *et al*. (2017) showed high resistance of *S. aureus* against methicillin (93.4%) and Oxacillin (100%) [32].

Finally, *S. aureus* isolates showed the lowest rates of resistance toward Meropenem with a sensitivity rate that reached to 82%. A previous study by Abd-Alamer and Al-Khozai (2016) also showed low resistance (20%) [33]. However, Hasan and Ismael (2018) reported that all isolates were not resistant to Meropenem (0%) [31].

S. aureus is known for its ability to become resistant to antibiotics. Infections caused by antibiotic resistant strains often occur in epidemic waves that are initiated by one or a few successful clones [34].

Resistance development in *S. aureus* to beta lactams occurs through the acquisition of a genomic island called staphylococcus cassette chromosome (SCCmec) carrying the methicillin resistance determinant mecA. This in turn codes for an alternative penicillin binding protein with less susceptibility to methicillin. In addition, resistance to penicillin is acquired via acquisition of plasmids coding for beta lactam resistance [35]. Penicillin resistance is mediated by blaZ gene which codes for beta lactamase enzymes. Two differently transcribed genes, known as *blaI* and *blaRI*, regulate these genes [36]. Beta lactamase is an extracellular enzyme synthesized upon exposure to the beta lactams class of antibiotics. It hydrolyzes the beta lactam ring, thereby reducing the therapeutic effect of penicillin [37].

The increasing resistance among local bacterial isolates results from the random and excessive consumption of antibiotics, physical connection to animals, and consumption of contaminated meat. Resistance spread in opportunistic and pathogenic bacteria is an evolutionary process that results from challenging the selective pressure provided by antibiotics through evolutionary forces represented by the consequence of mutations and variants that are selected in response to environmental factors [6].

Multiple Drug Resistance of *S. aureus*

Grouping of isolates in order to obtain a pattern of resistance is important for clearing the view of their infectivity behavior. Accordingly, the results shown in Figure-2 indicate that 144 (96%) of *S. aureus* isolates show multiple resistance to various types of antibiotics used in this study.

The obtained results showed that eight isolates have the highest multi-resistance to antibiotics, which represent 5.3% of the isolates; these isolates show resistance to eight antibiotics. Also, eight isolates have the lowest multi-resistance (5.3%); they are resistant to three antibiotics only. Moreover, the highest percentage was observed in the group which exhibit resistance to seven antibiotics, represented in 40 (26.7%) isolates.

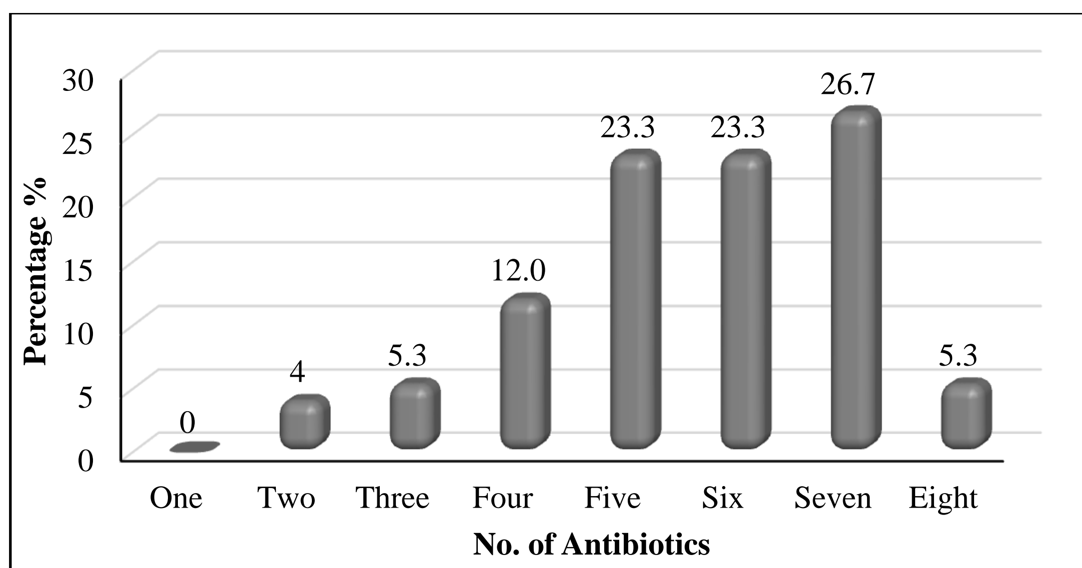

Figure 2- Multiple Antibiotic Resistance of *S. aureus*.
*the numbers from one to eight means the number of antibiotics resisted.

Our result of MDR is in agreement with previous studies conducted in Iraq by Abdul-Wahab (2015) [38] who found that all *S. aureus* isolates were MDR, as well as by Abu-Raghif *et al*. (2016) [39] and Saber *et al*. (2019) [40]. In addition, another study conducted in Nigeria by Ismail *et al*. (2015) [41] reported that 93% of *S. aureus* isolates were MDR.

The factors affecting the multi–drug resistance among bacterial strains are as follows; first, including the community acquired bacterial isolates along with hospital isolates would have provided a much better picture of resistance patterns of strains in a certain geographical area [42]. Second, molecular typing and plasmid profile of the bacterial isolates, which have become a major cause of nosocomial infections with MDR strains, should be analyzed. The rapid spread of bacteria expressing MDR has necessitated the discovery of new antibacterial and resistance modifying agents [43].

The MDR index shows that the majority of the isolates were resistant to three or more antibiotics. This indicates that the isolates involved do not respond to the effects of all the antibiotics. This could be attributed to possession of multiple resistance genes in the bacterial genome that enable them to resist all the antibiotics. The MDR shown by *S. aureus* is usually associated with increased expression of multiple antibiotic resistance genes, including those coding for β-Lactam, aminoglycoside, and other type of resistance [6, 41]. The MDR patterns are different, which could be linked to many factors that include the source of the isolate, its ability to evade antibiotic effects, and the variation in antibiotic concentration. Many studies have identified bacterial source as an important determinant of MDR, especially due to *S. aureus* when it occurs in nosocomial infections [44].

However, the MDR phenomenon is linked with the overexpression of efflux pumps that recognize a broad range of unrelated chemicals compounds. Resistance to any antibiotic is almost entirely achieved through efflux pump networks. Hence, evolution has provided bacteria with enormous abilities to survive even in toxic conditions [45].

Molecular diagnosis of *S. aureus* isolates

In order to confirm the results of the biochemical test, the molecular diagnosis of *S. aureus* isolates was performed by PCR technique using specific primers for *16S rRNA* gene. The results showed that all *S. aureus* isolates (150) had a positive result as a single DNA band of PCR product with a length of 257 bp (Figure-3).

For confirmation, the primers did not anneal with another type of infection bacteria. The PCR reaction for the amplification of DNA extracted from different bacterial isolates (*Staphylococcus epidermidis* and *Streptococcus pneumoniae*) was conducted at same reaction conditions with no appearance of any band. This is an evidence of the efficiency of this method for the diagnosis of *S. aureus* isolates.

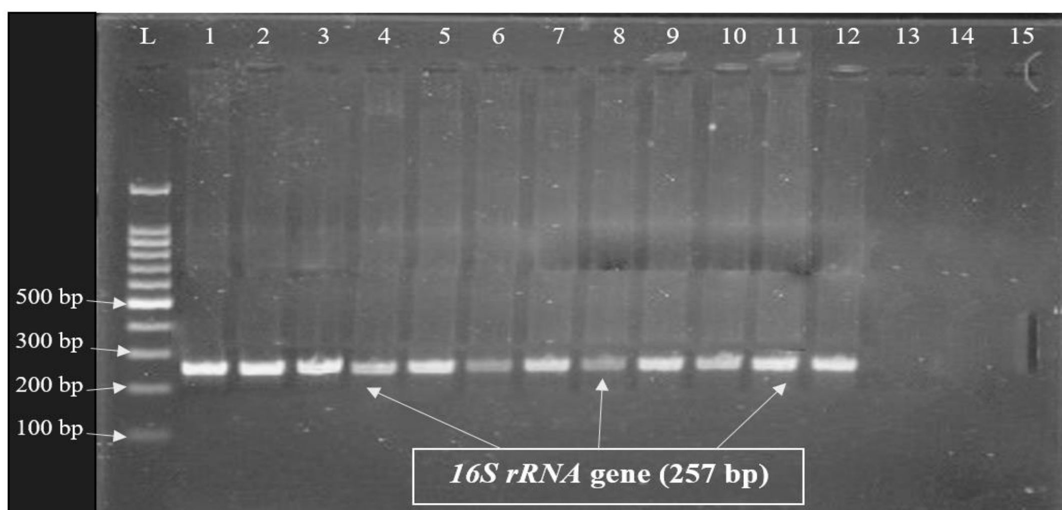

Figure 3- Gel electrophoresis of amplified *16S rRNA* (257bp) gene for *S. aureus* identification using conventional PCR. Agarose 1.5%, 70 V for 90 min, stained with safe gel stain and visualized on a UV transilluminator.

Lane (L): 100 bp DNA ladder.
Lane (1-12): Amplicons of the *16S rRNA* gene for *S. aureus* (isolates from S1-S12).
Lane (13-14): Control, DNA extracted from different bacterial isolates (*Staphylococcus epidermidis* and *Streptococcus pneumoniae*).
Lane (15): Negative control (had all components of PCR mixture with the substitution of water instead of DNA template).

Detection of *tsst-1* gene

The bacterial DNA of *S. aureus* was amplified to detect the superantigen of *tsst-1* gene using PCR technique in a monoplex pattern with the utilization of specific primers. The expression of *tsst-1* gene was confirmed by agarose gel electrophoresis and visualized on a UV transilluminator, as shown in Figure-4, were the amplification revealed a product of 271bp. From the total of 150 *S. aureus* isolates, only 70 isolates (46.7%) were found to be positive for *tsst-1* gene.

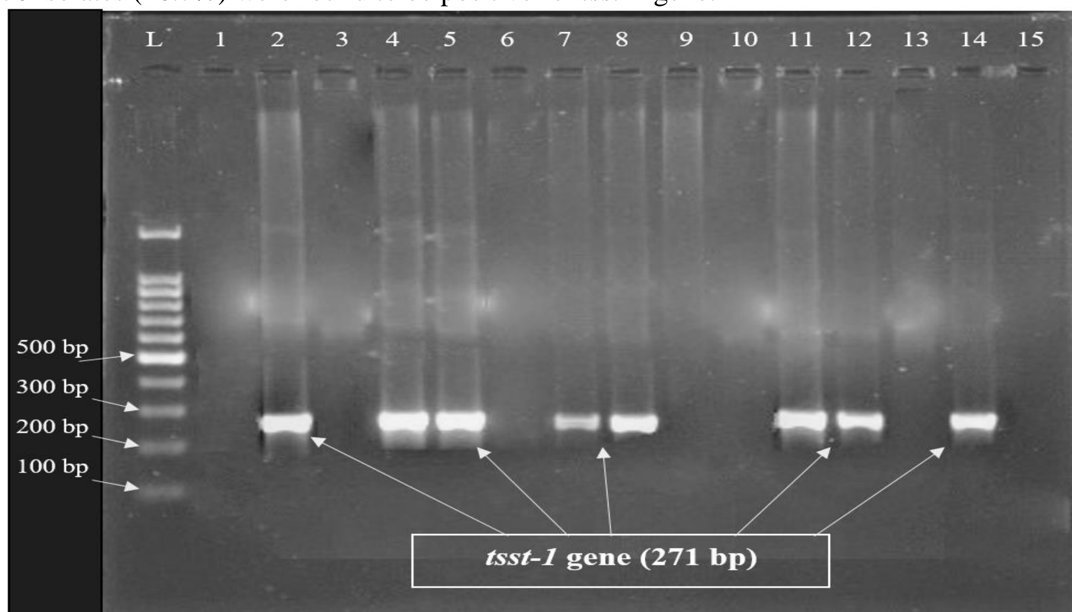

Figure 4- Gel electrophoresis of amplified *tsst-1* (271bp) gene from *S. aureus* using conventional PCR. Agarose 1.5%, 70 V for 90 min, stained with safe gel stain and visualized on a UV transilluminator.

Lane (L): 100 bp DNA ladder.
Lane (1-14): Amplicons of the *tsst-1* gene (isolates from S71-S84).
Lane (15): Negative control (had all the components of PCR mixture with the substitution of water for DNA template).

The above results agree with those of a local study by Hamad and Melconian (2016) [46] who found that the *tsst* gene is present in 43.2% of *S. aureus* isolates. Another study by Alajealy *et al*. (2017) [47] found that the *tsst* gene is present in all *S. aureus* isolates (100%), while Koosha *et al*. (2016) found it in 68% of the isolated [19]. The *tsst* gene expression reported by Ezeamagu *et al*. (2018) [48] and Ahrabi *et al*. (2019) [49] had lower percentages than that shown in the present study (14% and 1.5%, respectively.

The variable distribution of *S. aureus* superantigenic toxin genes in different areas may be explained by the fact that they are mostly carried by mobile genetic elements (MGEs), resulting in a pronounced heterogeneity in the endowment with toxin genes of individual *S. aureus* strains [50]. Population genomic analysis has provided a framework for an improved understanding of the temporal and spatial scales of the motility of MGEs and their associated toxin genes [51].

The justification presence of *tsst* gene *S. aureus* strains isolated from patients characterized with toxic shock syndrome (TSS) because the *tsst* gene presents in up to 70% of the *S. aureus* strains isolated from patients with TSS [47].

On the other hand, the presence of *tsst-1* gene does not mean the expression of protein. Recent investigations with isogenic pairs of TSST- 1 secreting and TSST-1 -deficient *S. aureus* suggested that TSST- 1 is a virulence determinant, not only in TSS but also in other staphylococcal infections. It was inferred that the toxin was not expressed among the tested population [19].

This variation could be attributed to the geographical location, as well as different methodology encountered in these studies, as their detection of the *tsst-1* gene was carried out at the protein level only. The expression of *tsst-1* gene does not grant the production of the toxin. Therefore, despite the presence of the gene, the toxin might not have been expressed among the tested group, which could explain the differences in the reported *tsst-1* gene prevalence [52].

Distribution of the *tsst-1* gene with antibiotic susceptibility analysis

The isolates harboring the *tsst-1* gene were resistant to the described antibiotics and the antibiotic resistance pattern of strains without the *tsst-1* gene was approximately similar (i.e. no significant correlation between them), as shown in Table-4. There was no significant correlation between the distribution of *tsst-1* gene and the multi-drug resistance pattern (p = 0.649) (Table-5).

Table 4- The comparison of antibiotic resistance pattern with the presence or absence of *tsst-1* gene.

Antibiotic		***tsst*-1 gene**		
		Present	**Absent**	***p-value***
Amoxicillin/ Clavulanic acid	Count %	56 49.1%	58 50.9%	0.85 NS
Carbenicillin	Count %	69 47.3%	77 52.7%	0.51 NS
Cefotaxim	Count %	64 47.8%	70 52.2%	0.6 NS
Cefoxitin	Count %	39 48.1%	42 51.9%	0.74 NS
Cephalexin	Count %	65 47.8%	71 52.2%	0.61 NS
Meropenem	Count %	14 51.9%	13 48.1%	0.85 NS
Methicillin	Count %	52 49.5%	53 50.5%	0.9 NS
Oxacillin	Count %	45 47.9%	49 52.1%	0.68 NS

Data presented as [a] Chi-square goodness of fit. NS=Non-significant.

Table 5- Distribution of Multi-Drug Resistance pattern in all Clinical Isolates, with the presence or absence of *tsst-1* gene.

MDR		*tsst-1* gene		Total
		Present	Absent	
Three	Count	2	6	8
	%	1.3%	4%	5.3%
Four	Count	7	11	18
	%	4.7%	7.3%	12%
Five	Count	17	18	35
	%	11.3%	12%	23.3%
Six	Count	22	13	35
	%	14.7%	8.7%	23.3%
Seven	Count	17	23	40
	%	11.3%	15.3%	26.7%
Eight	Count	4	4	8
	%	2.7%	2.7%	5.3%
Total	Count	70	80	150
	%	46.7%	53.3%	100%
p-value		0.226[NS]		

Data presented as [a] Chi-square independence test. NS=Non-significant.

Some other researchers have also agreed with our study, where the presence of *tsst-1* gene was not significantly associated with either present or absent β-lactam resistance [53, 54, 55] .

However, several other studies disagreed with our results, which reported a significant association between β-lactam resistance and *tsst-1* gene [19, 48].

Earlier studies reported a high prevalence of *tsst-1* gene among MSSA and β-lactam sensitive strains, but MRSA and β-lactam resistant strains typically do not and/ or less produce these superantigens (i.e. TSST-1, SEB, and SEC exotoxins)[19, 54].

The discrepancy between our findings and other records may be due to a difference in geographic regions. It has been revealed that the virulence gene profiles of *S. aureus* strains isolated from various locations are different. Since records of the distribution of the mentioned genes in Iraq and its neighboring countries are limited, it is possible that the high frequency of strains harboring the *tsst-1* gene and the elevated drug resistance among them may be due to differences in geographic regions. Moreover, using a drug resistance pattern obtained from this work might be helpful for selecting an antibiotic of choice to be utilized in close areas.

Conclusions

In conclusion, *S. aureus* is the most isolated bacteria in our samples than the other bacterial isolates, especially in wound samples. An obvious level of resistance was exhibited against β-Lactam antibiotics. The highest resistance was against Carbenicillin, while the lowest was toward Meropenem, in addition to the presence of a high percentage of MDR. The *tsst-1* gene is present in less than half of the isolates. There was no significant correlation between *tsst-1* gene and each of the β-Lactam antibiotic resistance patterns as well as the MDR isolates.

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