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Prevalence of Multidrug Resistant *Staphylococcus aureus* and their Pathogenic Toxins Genes in Iraqi Patients, 2022-2023

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ABSTRACT

Background and Aim: Methicillin Resistance *Staphylococcus aureus* (MRSA) causes staph infections, produces numerous toxins and virulence factors, and displays antibiotic resistance. Therefore, this study aimed to detect MRSA and its antibiotic-resistant patterns and evaluate the toxins genes in *S. aureus* isolates from Baghdad, Iraq.

Materials and Methods: Two hundred twenty bacterial samples were collected from different clinical sources in Iraq, 2022-2023. The diagnosis was made using traditional culture, microscopic examinations, and molecular diagnosis using the *16srRNA* gene and *mecA* gene used for Methicillin Resistance detection. In addition, Antibiotic resistance patterns were detected using VITEK-2. Also, the toxins genes were determined by sequencing.

Results: Fifty isolates were identified as *S. aureus*, and the strains showed high resistance to Benzylpenicillin, Erythromycin, Oxacillin, and Clindamycin. PCR showed a prevalence of the *mecA* gene in methicillin resistance *S. aureus* isolates by 100%, while toxin genes that were present in *S. aureus* were *LukD/E* gene 50(100%), *eta* gene 50(100%), *etd* gene 47(94%), *LukS/F* gene 34(68%) and *tst* gene 21(42%). All isolates tested negative for the *etb* gene. The results of the sequencing analysis of the studied genes showed that there were no genetic mutations. They were 100% identical except for *the eta gene,* and the results indicated three genetic mutations.

Conclusion: All *S. aureus* isolates had the *mecA* gene for methicillin resistance, and *S. aureus* possessed toxin genes. The sequencing analysis of the *eta* gene indicated the presence of various mutations, including the silent mutations.

Keywords: Antibiotic resistance, DNA Sequencing, methicillin resistance, *Staphylococcus aureus,* Toxin genes

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1. Introduction

Staphylococcus aureus, a Gram positive, coagulase-positive pathogen belonging to the family *Staphylococcaceae,* and it is the most prevalent in terms of its pathogenicity to humans **(1)**. Toxins are one of the most important substances that prevent *S. aureus* from invading host cells and disease occurrence. They have high molecular weight and play an important role in disrupting the physiological functions and developing the infection in the host. Toxins in *S. aureus* are divided into three groups, including the group of superantigens (Sags) that cause toxic shock syndrome (TSST), enterotoxins (SEs), exfoliative toxins (ETs) that cause Staphylococcal scalded skin syndrome (SSSS) and a group of cytotoxins that cause decomposes host cells by creating holes in cell membranes, include leukotoxins, α-hemolysin, β-hemolysin and γ-hemolysin **(2)**.

In hospitals, the *S. aureus* bacteria is one of the most significant contributors to wound and burn infections. It can also cause minor skin infections, severe tissue infections, and sepsis, and this bacterium can infect the body's organs and cause diseases such as acute endocarditis. It can sometimes lead to fatalities and

skin conditions characterized by abscesses, inflammation of hair follicles, and the development of pimples caused by these bacteria. Moreover, Kawasaki disease occurs in children, especially in developing countries, leading to vasculitis, which can have a significant impact on the coronary arteries. It can also affect older people, particularly those with AIDS **(3, 4)**.

Staphylococcus aureus enhances pathogenicity by producing some enzymes and toxins. These agents include exfoliative toxins secreted by certain strains of *S. aureus* bacteria. Exfoliative toxins consist of two serotypes: exfoliative toxin A, encoded by the *eta* gene carried on the phage genome and thermally stable, and exfoliative toxin B, encoded by plasmid-borne gene *etb,* which is destroyed by heat. Some studies indicate the existence of the tertiary serotype (toxin D). Exfoliative toxin D pattern encoded by the etd gene that separates the epidermis layers in places of infection because of its ability to lyses protein and dissolve polysaccharides in the intercellular of the skin, causing exfoliate and then death and also inflammation in the skin cell membranes **(5)**.

One of the extracellular toxins that *S. aureus* secretes is Pantone Valentine leukocidin (PVL). It was first discovered in 1894 by the scientist Van de Velde. In 1932. One of the most important factors of virulence in the S. aureus resistant to methicillin MRSA acquired in the community by its effect on multinucleated blood cells, which is a multicomponent protein toxins that works to break down the membranes, causing holes in the membrane of granulocyte and phagocytic membrane and thus causes the killing of white blood cells for humans and rabbits, and that the mechanism of this poison includes a change in the permeability of potassium ion, which leads to the entry of substances into the cell and works to form holes in the plasma membrane, which leads to the decomposition of cells and the exit of cytoplasm granules **(6)**. Most strains of *Staphylococcus aureus* isolated from patients produce toxic shock syndrome toxin, a single polypeptide chain; the coding gene test has a molecular weight of approximately 22 kDa. In 1980, it was discovered in the United States that it is the leading cause of toxic shock syndrome and causes multiple infections. This toxin works in association with MHC Class II and with T cell receptors and thus leads to the activation of T cells to secrete vast amounts of cytokines such as (IL-8, IL-2, and TNF), and this is called indirect effect because it possesses superantigen qualities. The direct effect as a result of the interaction of the poison with T cells and endothelial cells thus leads to a disease known as toxic shock syndrome. Its symptoms include high temperature, low blood pressure, rash, peeling and muscle peeling, circulatory failure, diarrhea, vomiting, peeling of the skin, and hypoalbuminemia and causes many complications such as inflammation of the lungs, lung abscesses, and urinary tract infections UTI. If treatment is not done after a period of the onset of symptoms, toxic shock may occur. Kill him after 24 hours **(7)**. Hence, the study aims to detect some toxin genes and analyze nucleotide sequences in some *S. aureus* isolates.

2. Materials and Methods

2.1. Collection, Isolation and Detection of Bacteria

In this cross-sectional study, 220 samples were randomly collected: 30 samples of urinary tract infection, 50 samples of wounds, 40 samples of burns, 30 samples of sputum, and 70 samples of cervix. The samples were collected from several hospitals in Baghdad: educational laboratories, Baghdad Teaching Hospital, Shaheed Ghazi Al-Hariri Hospital for Specialized Surgery, and Burns and Wounds Hospital /Medical City from October 1st, 2022, to January 1st, 2023. The samples were cultured in blood agar media and mannitol salt agar and then were incubated at 37°C for 24 hours. The VITEK system and 16srRNA PCR did the final detection. The bacteria that had *the mecA gene were considered* resistant to Methicillin. The PCR temperature cycling for *mecA* gene detection and toxin genes is shown in Table 1.

2.2. Antibiotic Susceptibility Test

The VITEK-2 (bioMérieux, USA) was used to determine the sensitivity of *S. aureus* isolates to 15 antibiotics. These include: Benzylpenicillin, Oxacillin, and Fusidic acid, Linezolid, Gentamicin, Ciprofloxacin, Moxifloxacin, Erythromycin, Teicoplanin, Clindamycin, Rifampicin, Vancomycin, Tetracycline, Tigecycline and Trimethoprim / Sulfamethoxazole.

2.3. DNA Isolation

DNA was extracted from the bacterial isolates under study using a Zymo (USA) Genomic DNA Extraction kit (Zymo Research, USA, R2014(50prep) following the manufacturer's instructions **(8)**.

2.4. Molecular Detection of Toxin Genes for *S. aureus*

This study used PCR to detect the toxins' genes, including Luk S/F, Luk E/D, tst, eta, etb, etd, as shown in Table 1. According to the manufacturer (Integrated DNA Technologies Company, Canada), the reaction mixture for diagnosing the genes consisted of 25 µL for each reaction, which included 1.5 µL of DNA template, 1µL of primer Forward, 1 µL of primer Reverse, 100 µL Concentration and 16 µL of ion-free distilled water and 5 µL GO Tag green master mix. The following

program was used to determine the reaction conditions, with some modifications, as shown in Table 2 **(8)**.

Table 1. The PCR optimum temperature condition for detection *is 16srRNA, mecA gene, and toxin genes*.

No.	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	5 min	1
2	Denaturation	94°C	45 Sec	35
3	Annealing		45 sec	
	16 srRNA	57°C		
	MecA	56°C		
	LukS/F	55°C		
	LukE/D	55°C		
	Tst	55°C		
	eta	57°C		
	etb	57°C		
	etd	57°C		
4	Extension	72°C	1 min	
5	Extension -2	72°C	7 min	1

Table2. Size amplicon and the sequence of oligonucleotide primers.

Target gene	Initial Sequence From 5′ to 3′	Product Size (bp)	Reference
16srRNA	F-5′- AACTCTGTTATTAGGGAAGAACA -3′	756	(8)
	R-5′- CCACCTTCCTCCGGTTTGTCACC -3′		
mecA	F-5′- GATGAAATGACTGAACGTCCGATAA-3′	310	(9)
	R-5′- CCAATTCCACATTGTTTCGGTCTAA-3′		
LukS/F	F-5′- ATCATTAGGTAAAATGTCTGGACATGATCCA-3	433	(9)
	R-5′- GCATCAAGTGTATTGGATAGCAAAGC-3		
LukE/D	F-5′- TGAAAAAGGTTCAAAGTTGATACGAG -3′	269	(9)
	R-5′- TGTATTCGATAGCAAAAGCAGTGCA -3′		
tst	F-5′- ACCCCTGTTCCCTTATCATC-3′	326	(10)
	R-5′- TTTTCAGTATTTGTAACGCC -3′		
eta	F- 5′- TTTGCTTTCTTGATTTGGATTC-3′	494	(11)
	R-5′- GATGTGTTCGGTTTGATTGAC -3′		
etb	F-5′- ACAAGCAAAAGAATACAGCG -3′	226	(10)
	R-5′- GTTTTTGGCTGCTTCTCTTG -3′		
etd	F-5′- CGGAAAGTCTGCAGGTGATT -3′	193	(12)
	R-5′- TCCAGAATTTCCCGACTCAG-3′		

2.5. Gel Electrophoresis

Bio Basic INC (Canada) was used at a concentration of 2% in 5 µL of Red Safe Nucleic Acid Staining Solution. Then, DNA ladders containing between 100 and 1500 base pairs and a voltage difference of 5 volts for an hour. UV light from Optima (Japan) was used for imaging.

2.6. DNA Sequencing

PCR Product reaction of *LukS/F-PV, LukD/E*, *eta,* and *etd*, with forward primer and reverts primer genes, was sent to Macrogen Inc (South Korea Geumchen, Seoul). The results were read and analyzed using the BioEdit sequence Alignment Editor Software DNASTER (Madison, WI, USA), which can be found on the

website of the National Center for Biotechnology Information (NCBI) at the following link (http://www.ncbi.nlm.nih.gov). Protein translation was performed using the Cluster Omega program to identify the number and type of mutations and their impact on protein translation, available on the https://www.ebi.ac.uk/Tools/msa/clustalo website **(13)**.

3. Results

3.1. Clinical Samples Source and Detection

Two hundred twenty samples were collected from various clinical cases, including genders, different age groups, and several hospitals in Baghdad. The frequency of sample by type and distribution of *S. aureus* by age and gender are shown in Table 3. Finally, 50 samples were determined by 16srRNA PCR as *S. aureus*.

Table 3. Source and distribution by age groups of *S. aureus* isolates**.**

Source of isolate	Number of isolates	Percentage%
Urine	10	20%
1-10 year	0	0.0%
10-20 year	1	2%
20-30 year	1	2%
More 30 year	8	16%
Wounds	12	24%
1-10 year	0	0.0%
10-20 year	0	0.0%
20-30 year	0	0.0%
More 30 year	12	24%
Cervicitis	8	16%
1-10 year	2	4%
10-20 year	1	2%
20-30 year	0	0.0%
More 30 year	2	4%
Burns	15	30
1-10 year	0	0.0%
10-20 year	0	0.0%
20-30 year	0	0.0%
More 30 year	15	30%
Sputum	5	10%
1-10 year	0	0.0%
10-20 year	0	0.0%
20-30 year	2	4%
More 30 year	6	12%
Total number	50	100%

3.2 Molecular Detection and Confirmation of *S. aureus* Isolates

To confirm the results of the cultural diagnosis, microscopic, Vitek-2 compact system, the molecular diagnosis of *S. aureus* isolates was performed by PCR technique using specific primers for the16SrRNA gene. The results showed that all *S. aureus* isolates **(50)** had a positive gene result (Table 4).

Table 4. Source and number of genes possessed by *S. aureus*

No	Source	Diagnostic gene	Virulence genes					
		16srRNA No. (%)	*mecA* No. (%)	*LukS* No. (%)	*lukE* No. (%)	*Tst* No. (%)	*eta* No. (%)	*etb* No. (%)	*etd* No. (%)
1	UTI	10(20)	10(20)	7(14)	10(20)	0	10(20)	0	9(18)
2	Wounds	12(24)	12(24)	11(22)	12(24)	0	12(24)	0	10(20)
3	Cervicitis	8(16)	8(16)	5(10)	8(16)	8(16)	8(16)	0	8(16)
4	Burns	15(30)	15(30)	6 (14)	15(30)	9(18)	15(30)	0	15(30)
5	Sputum	5(10)	5(10)	5(10)	5(10)	4 (8)	5(10)	0	5(10)

3.3. Methicillin Resistance Prevalence

The PCR result to detect the mecA gene showed that all 50 (100%) S. aureus strains harbored the mecA gene and were considered MRSA (Table 5).

3.4. Antibiotics Susceptibility Prevalence

The current study demonstrated that *S. aureus* isolates exhibited the highest resistance to Benzylpenicillin at 100%, followed by Erythromycin at 78%, Oxacillin at 76%, and Clindamycin at 74%. In contrast, the remaining antibiotics showed resistance rates of 42% for Ciprofloxacin, 40% for Moxifloxacin, 20% for Gentamycin, 40% for Tetracycline, 14% for Fusidic Acid, 6% for Rifampicin, and 14% for Trimethoprim/Sulfamethoxazole. The isolates showed less resistance to Vancomycin, Linezolid, and Teicoplanin at 5%, 2%, and 2%, respectively. Tigecycline displayed a sensitivity rate of 100%, as shown in Figure 1.

3.5 Multidrug Antibiotics Resistant (MDR) Patterns

The results showed that there are 10 different patterns of S. aureus resistance against the fifteen antibiotics, as shown in Table 5. The current study demonstrated that 80% of *S. aureus* isolates exhibited multiple drug resistance (MDR).

Table 5. The Profile of multiple antibiotic resistances among *S. aureus* isolates.

Antibiotic type	Types of resistances	MDR	Number of isolates
Antibiotype1 Resistant for 11 antibiotics	Ben, Oxa, Genta, Moxi, Cip, Eryth, Clind, Fusid, Tet, Rifam, Trime	2	2(4%)
Antibiotype2 Resistant for 10 antibiotics	Ben, Oxa, Cip, Moxi, Eryth, Clind, Tet, Genta, Linez, Teico	1	1(2%)
Antibiotype3 Resistant for 9 antibiotics	Ben, Oxa, Genta, Moxi, Cip, Eryth, Clind, Tet, Fusid	1	1(2%)
Antibiotype4 Resistant for 8 antibiotics	Ben, Oxa, Cip, Moxi, Eryth, Clind, Tet, Genta	4	4(8%)
Antibiotype5 Resistant for 7 antibiotics	Ben, Oxa, Cip, Moxi, Eryth, Clind, Tet	7	7(14%)
Antibiotype6 Resistant for 6 antibiotics	Ben, Oxa, Cip, Moxi, Eryth, Clind	4	4(8%)
Antibiotype7 Resistant for 5 antibiotics	Ben, Oxa, Eryth, Clind, Tet	5	5(10%)
Antibiotype8 Resistant for 4 antibiotics	Ben, Oxa, Eryth, Clind	1	1(2%)
Antibiotype9 Resistant for 3 antibiotics	Ben, Eryth, Clind	15	15(30%)
Antibiotype10 Resistant for 2 antibiotics	Ben, Oxa	0	7(14%)
Total number		40 (80 %)	

Oxa: Oxacillin, Genta: Gentamicin, Cip: Ciprofloxacin, Moxi: Moxifloxacin, Eryth: Erythromycin, Ben: Benzylpenicillin, Clind: Clindamycin, Linez: Linezolid, Teico: Teicoplanin, Vanco: Vancomycin, Tet: Tetracyclin, Tige: Tigecycline, Fusid: Fusidic acid, Rifam: Rifampicin, Trime: Trimethoprim/ Sulfamethoxazole

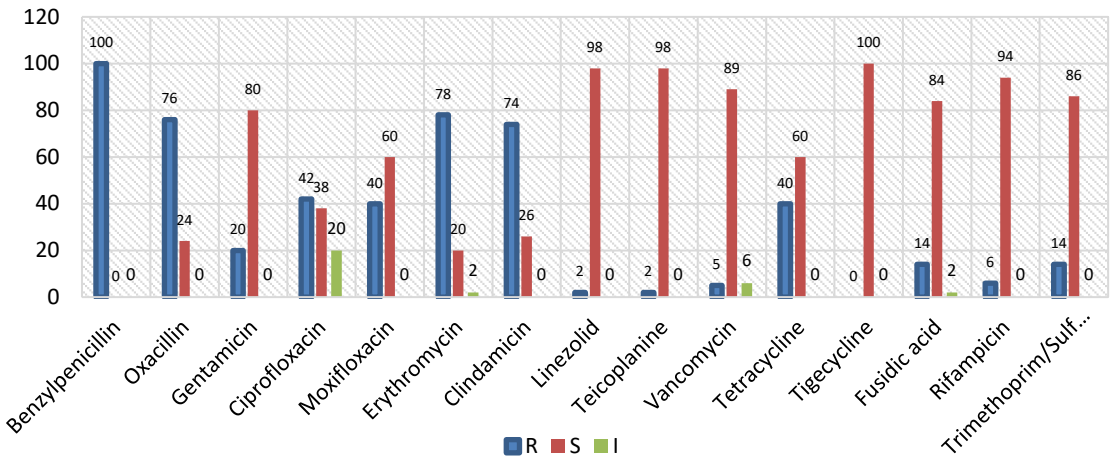

Figure 1. Antibiotic resistant percentage of *S. aureus* isolates

3.6 Detection of Leucocidin and Toxic Shock Syndrome Toxin

The genes of toxins possessed by *S. aureus* bacteria were detected: *Luks/F-PV, LukE/D* that 34 (68%) isolated belong to *S. aureus* bacteria possesses the gene Luks/F-PV. The investigation results of the *LukE/D-PV* gene indicate that all 50 (100%) isolated samples belonging to S. aureus isolates possess the gene LukE/D-PV, and 21 (42%) isolates harbored the test gene (Table 4).

3.7 Detection of Exfoliative Toxins

The current study revealed that all *50(100%) S. aureus* isolates possessed the *eta* gene and tested negative for the *etb* gene. Moreover, 47 (94%) *S. aureus* isolates were found to carry the *etd* gene (Table 4).

3.8 DNA Sequencing

The analysis of the four toxins genes (Tst, LukE/D, LukS/F, etc.) showed *no genetic mutations, and the match rate was 100% except for the eta* gene, as shown in Figure 2.

3.7. GeneBank accession numbers

The DNA sequences of the partial *eta* gene from the representative isolates have been deposited in the GenBank database under accession numbers OQ557480 at the following link: https://www.ncbi.nlm.nih.gov/nuccore/OQ557480

Table 6. Changes in nitrogen bases and their effect on the translation of the eta gene's amino acid for isolated *S. aureus*.

No.	Nitrogen bases	Change in the nitrogen base	Site	Amino acid	Change in amino acids
1	Thymine	Cytosine	1145213	Lysin	lysin
2	Cytosine	Thymine	1145192	Methionine	Methionine
3	Guanine	Cytosine	1145025	Cysteine	Serine

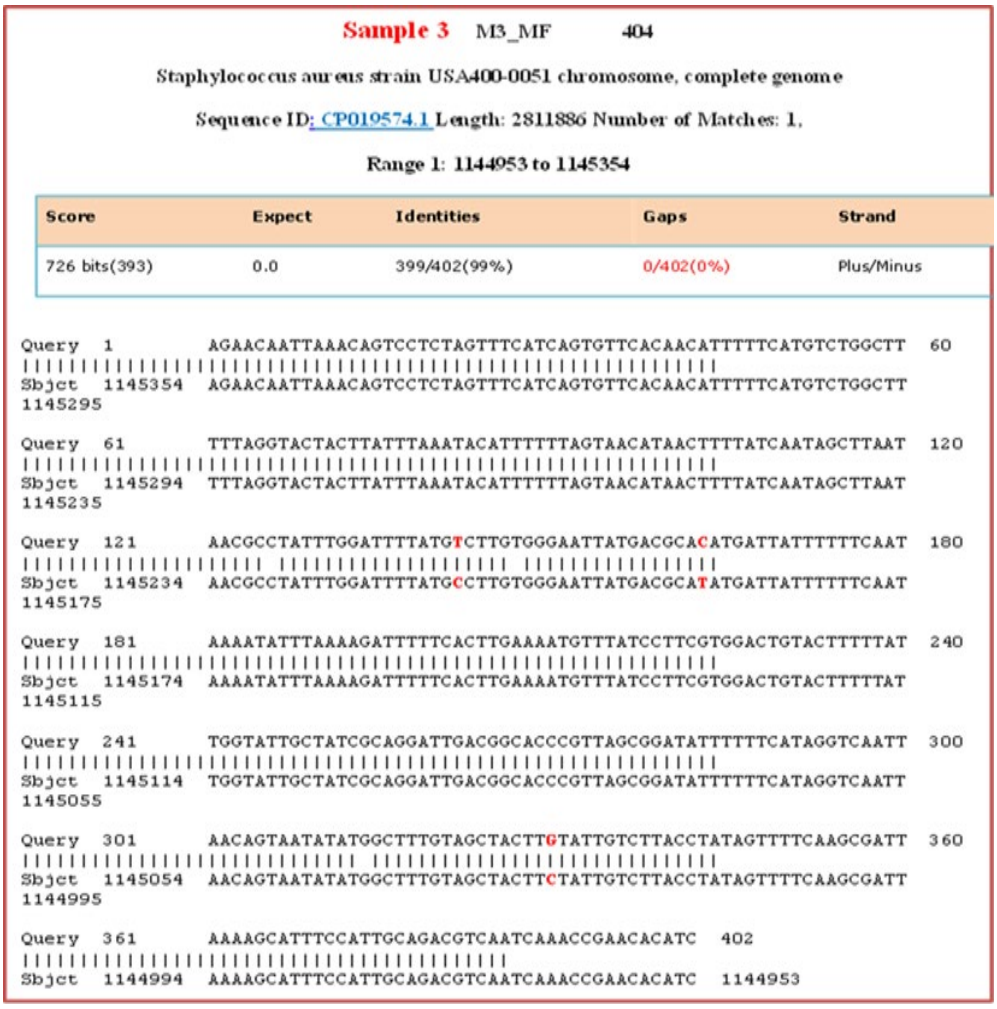

Figure 2. Analysis of multiple sequences of the reference *eta* gene with isolates of *S. aureus* using *BioEdit* Sequence Alignment Editor Software

4. Discussion

S. aureus is one of the most prevalent bacterial pathogens. It can infect both healthy individuals and those with underlying health conditions, leading to numerous severe infections worldwide each year (14).

In this study, Table 3, the prevalence of S. aureus in burn infection was found to be 30%, similar to other studies **(15)**, which found that the highest rate of *S. aureus* in wound infections was 59%, and in other studies **(16, 17)**. it was similar to the current research. The study found that the highest percentage of *S. aureus* isolates was found in males (54%) and females (46%), respectively; **(18, 19)** stated that the highest prevalence rate of S. aureus in females 44% and 22.6%, respectively compared to males 20% and 56% respectively. The first result was much lower than in this study, while the second was similar. The patients were classified into different age groups from one year to 30 years, and as shown in Table 3**,** the highest prevalence of *S. aureus* in the age group between (30 years and over). The result of the current study differed from the study **(20)** in that the highest incidence rate in the age groups between 1-20 years was (45.1%) followed by the age groups 41-60 years (29.4%). The reason for the different percentage of isolation of *S. aureus* isolates and infection between age groups may be due to several factors, including the time of collecting samples, geographical location, source of isolation, the number of samples, the duration of their stay in the hospital, the health status of patients, their chronic diseases and other diseases, and the treatment used.

In this study, S. aureus isolates showed less resistance to Vancomycin, Teicoplanin, Linezolid Rifampicin, and Fusidic Acid, as shown in Figure 1, and all isolates were sensitive to Tigecycline, which supports the findings of several studies **(18, 21-24)**. *S. aureus* susceptibility to these antibiotics can be linked due to their low use, high costs, low market availability, and toxic side effects **(25)**. The pattern of sensitivity to other antibiotics to *S. aureus* isolates was similar to other studies where the most commonly used antibiotics, such as Benzylpenicillin, Oxacillin, and Gentamycin, were included. The isolates showed high antibiotic resistance, identical to many studies

(20, 22, 26, 27). The reason may be due to the bacteria having several mechanisms to resist these antibiotics, including the generation of intrinsic resistance and the modification of the target site Penicillin-linked proteins (PBps) as well as the production of β-Lactamase enzymes, the degradation of beta-lactam (β-lactam) antagonists, possessing the three modified enzymes aminoglycoside acetyltransferase (AACs), aminoglycoside-Nucleotidyltransferases (ANTs) and aminoglycoside phosphotransferase (APHs) and encoding genetic elements resulting in bacterial resistance to aminoglycoside antagonists **(28, 29)**.

In contrast, other studies, including **(30, 31)**, showed that S. aureus isolates appeared less resistant to clindamycin and gentamicin, possibly due to the low frequency of antibiotics used in the population studied and the source of isolates.

In this study, *S. aureus* isolates showed moderate resistance to ciprofloxacin and moxifloxacin antibiotics, which was compatible with several studies **(20, 26, 32)**. The reason for this resistance may be due to several mechanisms, including the possession of flush pump bacteria such as NorA encoded for the *NorA* gene, a change in the permeability of the antibiotic, or mutations in the DNA gyrase encoded for the *gryA* and *gry B* genes and mutations in topoisomerase encoded for the *ParC* and *ParE* genes that confer resistance to Fluoroquinolone antibiotic **(33)**.

Accordingly, the results shown in Table 5 indicate that 40 (80%) of *S. aureus* isolates show multiple resistance to various types of antibiotics used in this study. The results showed that 40 isolates have the highest multi-resistance to antibiotics, representing 4(8%) of the isolates showing resistance to eight antibiotics. Also, 1(2%) isolate has the lowest multi-resistance, which is resistant to four antibiotics only. Moreover, the highest percentage was observed in the isolates that exhibit resistance to three antibiotics, represented in 15(30%). The result of the study differed from **(34)**, which reported that *S. aureus* exhibited six distinct antibiotic resistance patterns. Our MDR result agrees with previous studies conducted in **(23)**, which found that S. aureus is resistant to multiple antibiotics by 71.8%. MDR patterns vary between studies and can be linked to many factors, including the source of isolation, their ability to avoid the effects of antibiotics, and differences in antibiotic concentration. Several studies have identified bacterial sources as an essential determinant of MDR **(35)**.

Many researchers corroborated this by using the *16SrRNA* gene to diagnose *S. aureus* species and distinguish it from other species (**10**). They explained using the same gene to confirm that the *16SrRNA* gene is one of the conversed genes carried on the chromosome, which has a length of 720 base pairs and consists of 239 amino acids. It is used in genetic diagnosis and finding an evolutionary relationship between organisms and bacteria due to evolution and variation at low rates in the genetic region, which is the diagnosis of *S. aureus* **(36)**.

mecA is an emerging gene responsible for Staphylococcal methicillin resistance. In the current study, Figure 2, the prevalence rate of the mecA gene among S. aureus isolates was 100% in line with many studies **(37, 38)**, showing that all isolates gave a positive result for the gene. Our results sharply contradicted the evidence published in local research in Iraq, which found that 10 isolates (11.11%) possess the gene. This may be because all isolates in the current study have a gene that is carried on a mobile cassette Chromosome (SSCmec), enters the S. aureus chromosome, combines with it, and then mediates resistance to methylene antibodies and several beta-lactam antagonists **(39)**. Studies have indicated that the absence of *the mecA gene among S. aureus isolates may be due to their possession of other diverse cassette genes encoded to other genes,* such as *mecD, mecB,* and *mecC* genes **(40)**.

For the potential to produce leucocidin toxin, we measured the prevalence of lukE/D in S. aureus isolates, and we found that the rate of prevalence was significantly higher by 100% compared to the report **(41)** who found that 63(82.8%) out of a total of 79 isolates possessed *LukD/E* gene. However, it differed from the results of **(42)**, which indicated that 17 (51.5%).

The superantigen TSST-1 is an extracellular protein that causes toxic shock syndrome and is encoded by the *tst* gene **(7)**. *tst* encoded by a highly mobile pathogenicity island (SaPI) (43). Our results showed *tst* prevalence rates of 21(42%) in *S. aureus isolates*. These rates agreed with (35, 44), who recorded (44 % and 46.7%) in Iraq.

Exfoliative toxins, encoded by eta and *etb* genes, *etd* are responsible for skin and cutaneous tissue infections and scalded skin syndrome **(45)**. In this study, neither *etb* were found in *S. aureus* isolates. This is similar to *S. aureus* strains in Iran **(11)**. They reported that *eta*, *etb*, and *etd* genes were present in 115(76.7%), 25(16.7%), and 81(54%) of S. aureus isolates, respectively. While 92% of *S. aureus* isolates carried the *etb* gene, eta was not detected **(46)**. The result of the current study differed from these findings.

The results of the sequencing analysis of the eta gene, as shown in Figure 2**,** Table 6**,** the presence of silent mutations showed that this type of mutation occurs when the substitution of a single nitrogen base in the DNA results in a new genetic code that encodes the same original amino acid that did not affect the

protein sequence and the change of amino acids, in addition to the presence of mutations of another type called missense mutations, this type of mutation occurs either one change of the genetic code of the resulting protein or an acid change One amino for the resulting protein **(47)**.

The results were inconsistent with the findings of **(48)** when analyzing the DNA sequence of the *eta* gene in *S. aureus*, as it was found that there were no genetic mutations.

5. Conclusion

All *S. aureus* isolates had the *mecA* gene for methicillin resistance and showed high resistance to Benzylpenicillin and less resistance to Vancomycin, Linezolid, and Teicoplanin antibiotics. The results of the detection of the toxin genes showed that *S. aureus* possessed each of the toxin genes *Luks/F-PV, LukD/E-PV, tst, eta,* and *etd*, while all isolates under study did not have the etb gene. The sequencing analysis results of the *eta* gene indicated the presence of silent mutations that did not impact protein translation, alongside other mutations that resulted in alterations to the arrangement of amino acids and protein translation.

Acknowledgment

Further molecular techniques are needed to study S. aureus's other virulence and antibiotic resistance genes, the effect of mutations on these genes, and their role in bacterial pathogenesis.

Ethics Approval

The samples were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health No. 47737 on 13/11/2022.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

R.Z: Experimental studies, data collection, Manuscript preparation, editing.

R.M.: Experimental Design, Manuscript review, and final decision of the manuscript

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