MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY STATE UNIVERSITY
ACADEMIC AND RESEARCH MEDICAL INSTITUTE

Eastern Ukrainian Medical Journal

116, Kharkivska st., Sumy 40007, Ukraine eumj.med.sumdu.edu.ua
e-mail: eumj@med.sumdu.edu.ua ISSN: 2663-5909 (print)/2664-4231 (online)

How to cite: Atta SE, Ghannawi LA, Gharab K, Shakir OY, Khleef AH, Ismael SB. Analysis of mecA Gene in Methicillin-Resistant Staphylococcus aureus Isolated from a Diverse of Clinical Sources Specimens. *East Ukr Med J.* 2025;13(3):834-847

DOI: https://doi.org/10.21272/eumj.2025;13(3):834-847

ABSTRACT

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ANALYSIS OF *MECA* GENE IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM A DIVERSE OF CLINICAL SOURCES SPECIMENS

***Background*:** Conventional method was the best method for identifying the Methicillin-resistant gene in *Staphylococcus aureus*(*mecA*) due to various species of *staphylococci* that were expressed for various resistance levels.

***Objective*:** This study aimed to get an accurate detection of the *mecA* gene in *S. aureus* isolates, which mediates methicillin resistance in bacteria using the primers (*mecA*) to detect the mutations that occur in the *mecA* gene encoding for penicillin-binding protein (PBP2a) that is responsible for the intrinsic resistance to all β-lactams.

***Methods*:** Fifty clinical isolates were determined as *S. aureus* according to molecular and bacteriological ways. The susceptibility tests were performed on all bacterial isolates by disc diffusion and MIC methods using methicillin and six fluoroquinolones antibiotics.

***Results*:** From fifty isolates, 12 isolates were resistant to methicillin and all six antibiotics; 12 were resistant, three were intermediate, and 38 were sensitive to three or more tested antibiotics, in addition to confirming the resistance of *S. aureus* isolates by minimum inhibitory concentration test. The primary sources of *S. aureus* isolates were burns (10%), nose (16%), wounds (8%), operation room (10%), ear (20%), urine (8%), skin (6%), and throat (22%). Twelve resistant isolates were used to examine the mutations in *the mecA* gene. A direct sequence analysis found no mutations detected in *mecA*. The methicillin resistance was due to the *mecA* gene responsible for methicillin resistance.

***Conclusion*:** The absence of mutations in the *mecA* gene implies that resistance may be ascribed to alternative mechanisms, potentially including enhanced expression of penicillin-binding proteins or efflux pumps.

***Keywords*:** Mutations, *Staphylococcus aureus*, Antibiotics, MRSA, *mecA*.

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ABBREVIATIONS

AAMRSA – Animal-Associated Methicillin Resistance *Staphylococcus Aureus*
BP – Base pair
CAMRSA – Community-Associated Methicillin Resistance *Staphylococcus Aureus*
CIP – Ciprofloxacin
CLSI – Clinical & Laboratory Standards Institute
D.W. – Distilled water
FOX – Cefoxitin
HAMRSA – Healthcare-Acquired Methicillin Resistance *Staphylococcus Aureus*
LAMRSA – Livestock-Associated Methicillin Resistance *Staphylococcus Aureus*
LEV – Levofloxacin
LOM – Lomefloxacin
Me – Methicillin
MHA – Muller-Hinton Agar
MIC – Minimum Inhibitory Concentration
MRSA – Methicillin Resistance *Staphylococcus Aureus*
MSA – Mannitol Salt Agar
MSSA – Methicillin-Sensitive *Staphylococcus Aureus*
NA – Nalidixic Acid
NCBI – National Center for Biotechnology Information
NOR – Norfloxacin
OFX – Ofloxacin
PBP2a – Penicillin-Binding Protein 2a
PCR – Polymerase Chain Reaction
PVL – Panton-Valentine Leukocidin
SCC – Staphylococcal Chromosomal Cassettes
WBC – White Blood Cells

INTRODUCTION

Methicillin resistance *staphylococcus aureus* (MRSA) is a health problem worldwide; 95% of *S. aureus* strains resist methicillin due to *the mecA* gene that is expressed to penicillin-binding protein 2a (PBP2a protein), which has no affinity to binding with the penicillin group [1]. Three significant forms of (MRSA) isolates have been discovered, including healthcare-acquired MRSA, community-associated MRSA, and animal-associated MRSA [2]. CAMRSA occurs in healthy individuals. But healthcare-acquired MRSA occurs significantly in immune-compromised individuals or patients with a disease risk factor [3, 4]. While the *nuc* gene (*S. aureus*-specific chromosomal gene encoding thermo-nuclease) is used for the rapid detection of *overall S. aureus* (both MSSA (methicillin-sensitive *S. aureus*) and MRSA) from clinical samples [5]. As the title indicates, HAMRSA was generally founded in hospitals or other healthcare facilities for hospitalized patients or those who recently visited any form of healthcare. HAMRSA incidence is much greater in the Americas and East Asia than in Europe [6].

Nevertheless, CAMRSA was generally seen in the community and not nosocomial. Panton-valentine leukocidin (PVL gene) has been detected in young people; the strains containing this gene are coded for β-pore-forming exotoxins, leading to damage in white blood cells (WBC) and, initially, necrosis tissues [7]. In addition, livestock-associated MRSA (LAMRSA) is a new type of MRSA identified in animals such as pigs and cattle. LAMRSA can be seen in animals and transmission to people who come into contact with animals [8]. The *S. aureus* bacterium grown to achieve successful survival of β-lactam antibiotics through

multiple molecular and mechanical approaches; the *S. aureus* methicillin-resistant *mecA* gene has translated into alternative 78-KD penicillin-binding protein 2a (PBP2a) as the new variants were not recognized by β-lactam antibiotics (penicillin) The bacterial cell wall was synthesized by peptidoglycan layers without interruption, *mecA* gene is present on the chromosomes of MRSA strains. Staphylococcal chromosomal cassettes (SCCmec) included five groups with molecular weights of 20 to 68Kb [9, 10]. The PBP2a is encoded by the *mecA* gene, which is carried by a mobile genetic element [11].

The phylogenetic tree serves as a valuable tool for identifying genetic markers associated with specific traits or behaviors of *S. aureus*, including antibiotic resistance or virulence. The identification of these markers can contribute to predicting strain behavior and the development of diagnostic tools or therapeutic interventions. MRSA clinical isolates become resistant to several antibiotic types, e.g., (β-lactam antibiotics, macrolides, aminoglycosides, clindamycin, and fluoroquinolones) [12]. Plasmid-mediated resistance in *S. aureus* remains an essential route of multidrug resistance. However, this is dwarfed by chromosomally mediated resistance as the principal mechanism of resistance in multidrug resistance *S. aureus* [13, 14]. Bacteria may utilize additional mechanisms, such as efflux pumps or altered target sites, which can contribute to developing resistance. The efflux of antibiotics has been identified as an effective resistance mechanism among antibiotic-resistant *Staphylococcus aureus*. Additionally, regulatory mechanisms that control the expression of *mecA* or other genes associated with resistance [15, 16]. These mechanisms may involve increased production of β-lactamase, methicillinase production, acquisition of structurally modified normal PBPs, or the emergence of slight colony variants of *S. aureus.* MRSA strains often exhibit multidrug resistance, and the *mec* region may contain multiple resistance determinants, leading to the concentration of resistance genes in this region [17].

METHODS

Isolation and Identification of Staphylococcus aureus

A total of 185 bacterial specimens from burns, throat, wounds, urine, skin infections, operation room noise, and ears were collected aseptically from various clinical sources. The swap specimens were then transferred to the laboratory under cooling conditions. Specimens were cultured in mannitol salt agar (MSA) and chrome agar and incubated at 37°C for 24 hours under aerobic conditions. All the *S. aureus* isolates are subjected to different biochemical and morphological tests, in addition to the VITEK2 system, to ensure their identity [18].

Antibiotic Susceptibility of Staphylococcus aureus

Kirby-Bauer disc diffusion was the basic method for bacterial investigation and susceptibility testing. It detected the most effective bacterial therapy technique on Muller-Hinton agar media. In this study, disc diffusion was used for fifty *S. aureus* isolates against methicillin and fluoroquinolone antibiotics (Nalidixic Acid, Ciprofloxacin, Norfloxacin, Ofloxacin, Lomefloxacin, and Levofloxacin) [19–21]. Fifty *Staphylococcus aureus* isolates identified previously were cultured in nutrient broth to get turbidity equal to McFarland and incubated for 24 hours at 37°C. Then, a sterile cotton swab was placed in a nutrient broth tube by Shaking the cotton swab on Muller-Hinton agar media in several directions from the plates [22]. Place the plates at room temperature and wait 10 minutes for complete absorption. Then, via antibiotic forceps, press discs of antibiotics on agar. Only four discs were placed on the plate. After fifteen minutes of discs being applied, incubate the plates for 24 hours at 37 °C. The inhibition zone diameters of isolates were compared with CLSI 2021[23].

Determination of Minimum Inhibitory Concentration (MIC) for Antibiotics

Based on the CLSI, 2021 [23] The agar dilution method was used to find the minimum inhibitory concentration (MIC) for resistant *S. aureus* isolates. Nine-fold dilutions (0.25 to 64 µg/ml) were prepared for Ciprofloxacin, Ofloxacin, and Levofloxacin; nine-fold dilutions (0.5 to 128 µg/ml) were prepared for Cefoxitin and Lomefloxacin; nine-fold dilutions from 1 to 256 µg/ml were prepared for Norfloxacin; and nine-fold dilutions from 2 to 512 µg/ml were prepared for Nalidixic acid using D.W. Prepare Muller-Hinton agar (MHA) and sterilize in an autoclave, then cool to 45°C. After mixing the media well, pour it into Petri dishes. Transmit three colonies using a sanitary loop into a Brain Heart infusion broth tube. Incubate at 37°C for 24 hours; it should be equal to the 0.5 McFarland standard. Add 1 ml of broth and dilute with normal saline (1:10), then place 100 microliters from each inoculum on the agar surface via a micropipette. Leave plates to dry for 10 minutes and make wells on each agar plate using Cork Borer agar. Label the tubes with the concentration of diluted antibiotics on each agar plate to identify their activity and concentration in every well by placing 75 µl of each dilution. The plates were incubated neatly overnight. Incubate for 24 hours at 37 °C and use one plate of MHA without antibiotics as a control [24].

Polymerase Chain Reaction (PCR)

The Promega extraction kit extracted bacterial DNA (template) for (50) *S. aureus* isolates. In PCR amplification, 25 µl was perpetrated by using 3µl of DNA template, 9.1 µl Nuclease-free water, 12.5 µl of PCR 2X Master mix kits, and 0.2 µl of each poly-nucleotides primers for *mecA* gene with sequence:

- *mecA* F: TGCAGTACCGGATTTGCC
- *mecA* R: TCGATGGTAAAGGTTGGC
- *nuc* F: GCGATTGATGGTGATACGGTT
- *nuc* R: GAGGCGAAGTCTTGGGTAAAAAC**.**

Thermo-cycler device (Eppendorf, Germany) was used with the appropriate conditions (Table 1). After that, 5 µl of PCR product for the sample was analyzed using gel electrophoresis on 1% (w/v) Tris-acetate buffer agarose gel containing 0.1 µl/mL of ethidium bromide.

TABLE 1. The optimal conditions of PCR amplification

Step	No. of Cycle	Time– Temperature
Initial Denaturation	1-Cycle	5 minutes – 94º C
Denaturation	30-Cycles	30 seconds – 94º C
Annealing		30 seconds – 54º and 60º C
Extension		30 seconds – 72º C
Final-Extension	1-Cycle	10 minutes – 72º C

Note: The annealing temperature for the fluoroquinolone resistance gene (mecA) was 60ºC and 54°C for the (nuc) gene

DNA Sequencing

A 20 µl of purified PCR yields that screened *the nuc* and *mecA* gene and primers were sent to Macro Gene Company in Korea for DNA Sequencing. The consequent sequences *of S. aureus* isolates were aligned by Bio-edit and Mega7 software and compared with NCBI databases to test the presence of mutations in the *mecA* gene.

Statistical analysis

All the data underwent a Pearson Chi-Square test to detect any significant variations ($p < 0.05$) in isolate source and antibiotic susceptibility for each antibiotic. Additionally, Pearson and Spearman's correlations were employed to assess the relationship between mutation and the isolates associated with methicillin and fluoroquinolone resistance.

TABLE 2. Sources and percentages of Staphylococcus aureus samples and isolates

Source of Samples	No. of Samples	No. of Isolates
Burn	23	5 (10%)
Ear	25	10 (20%)
Nose	21	8 (16%)
Operation room	20	5 (10%)
Skin	28	3 (6%)
Throat	23	11 (22%)
Urine	18	4 (8%)
Wound	27	4 (8%)
Total	185	50

RESULTS

Isolation and Identification of Staphylococcus aureus

All *S. aureus* isolates were subjected to primary identification tests using different biological methods (gram staining, cultural characteristics, biochemical tests, and vitek2 system). All bacteria Isolates were subjected to the VITEK system to identify suspected bacteria at the species level. The fifty isolates have 93% *Staphylococcus aureus* properties (as shown in Table 2).

Antibiotic Susceptibility and Minimal Inhibitory Concentration (MIC) of Staphylococcus Aureus

In this study, antibiotics disc diffusion was used in fifty *S. aureus* isolates against methicillin and multiple types of antibiotics. The tested *S. aureus* isolates indicated resistance to methicillin and multiple types of fluoroquinolone antibiotics. The diameter of isolate inhibition zones was compared with (CLSI 2021) [23]. These isolates were subjected to the MIC tests under antibiotics disc diffusion. Through an account of the diameters inhibition zones of antibiotics by corresponding them to the standard diameters of CLSI, 2021 (as shown in Table 3) [23].

The 12 resistant isolates in the Disc diffusion procedure were subjected to MIC tests. The results presented that *S. aureus* isolates were resistant to nalidixic acid and lomefloxacin at MIC ranged (32, 64, 128, 512, and 1024µg/ml), and the highest MIC was registered in wound and throat (1024 µg/ml). However, the lower MIC range can be seen in Ciprofloxacin antibiotic (0.5, 1, and 2 µg/ml) specific in burn with (0.5 µg/ml). While *S. aureus* isolates were resistant to another kind of fluoroquinolone antibiotics, cefoxitin ranged from (8-512 µg/ml). The results of MIC are illustrated in Table 4.

Molecular identification of S. aureus

The fifty *S. aureus* isolates were selected and identified previously by biochemical tests, morphological, and VITEK-2 System as *S. aureus* and determined the antibiotic susceptibility, were further characterized by amplifying a conserved region of the thermo stable nuclease that encodes for *nuc* gene by using specific primers (*nuc* primers) for confirming

Staphylococci species [25]. Only *S. aureus* could be recognized by *nuc* primer from other *staphylococci*. PCR products of all *S. aureus* isolates were expressed for specific gene sequences, which means these isolates were *S. aureus* (see Figure 1). The PCR product of bacteria isolates appears in the form of a single band of DNA; the molecular size is about (276 bp) compared with the DNA ladder size as a marker (100bp)[26]. The results of diagnoses of gene detection were close to those (of Naorem *et al*. 2020) [27] results, which detected DNA fragments (276 bp) in all *S. aureus* specimens. These results were confirmed by the accuracy of morphological and biochemical tests used to identify the selected isolates at the species level [28].

TABLE 3. Antibiotics susceptibility of S. aureus isolates to Fluoroquinolones antibiotics and ratio of each antibiotic

Antibiotic	S	%	I	%	R	%
Ciprofloxacin	38	76	0	0	12	24
Levofloxacin	38	76	0	0	12	24
Lomefloxacin	34	68	4	8	12	24
Nalidixic acid	20	40	2	4	28	56
Norfloxacin	38	76	0	0	12	24
Ofloxacin	38	76	0	0	12	24

Note: S = Sensitive, I = Intermediate, R = Resistance

TABLE 4. Actual MIC ranges for S. aureus resistance isolates

Resistance Isolates		Actual MIC range (µg/ml)						
Source	No.	NA	CIP	NOR	OFX	LOM	LEV	FOX
Burn	1	64	0.5	16	32	512	16	128
	4	512	2	32	64	512	32	128
Ear	9	512	1	32	128	256	32	32
Nose	16	512	1	32	32	256	32	128
	17	512	2	32	32	512	32	64
Operation Room	24	512	1	32	64	512	32	64
Skin	29	512	2	32	64	512	32	128
Throat	32	1024	1	64	32	64	16	64
	42	512	2	32	32	512	32	256
Urine	43	32	2	64	64	128	32	64
Wound	47	128	1	16	32	32	32	128
	50	1024	1	8	32	1024	16	64

Note: No = Number of resistance isolates, NA= Nalidixic Acid, CIP= Ciprofloxacin, NOR= Norfloxacin, OFX= Ofloxacin, LOM= Lomefloxacin, LEV= Levofloxacin and FOX= Cefoxitin

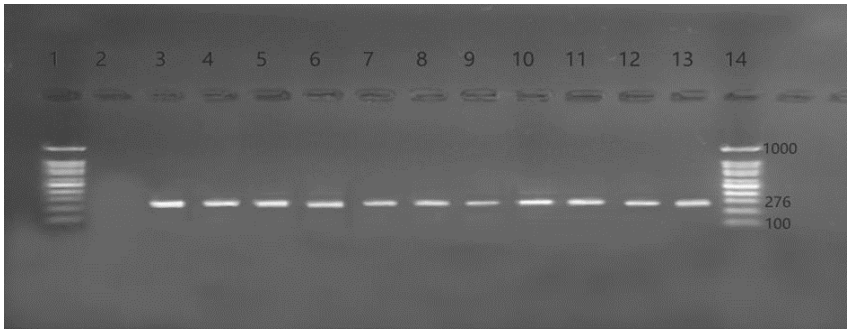

FIGURE 1. Agarose gel stained with Ethidium Bromide showing PCR products with nuc gene (276 bp) primers for S. aureus extracted DNA. The electrophoresis resulted in 70 volts for 70 min. Lines (1 and 14) for DNA marker (100bp), Lines 3-13 represented positive results of S. aureus isolates that gave the amplified product (276 bp), Line 2: Negative control

Phylogenetic tree analysis

The construction of a phylogenetic tree is essential for advancing research into the genetics and epidemiology of *S. aureus*. Phylogenetic tree analysis resulted from the alignment using the BLASTn program for diagnostic gene *(nuc)* sequences from the direct sequencing *of S. aureus* isolates. MEGA7 program software was used for phylogenetic tree analysis, as shown in Figure 2, showing the relation between the diagnostic gene sequences of current isolates with known *S. aureus* strains [29].

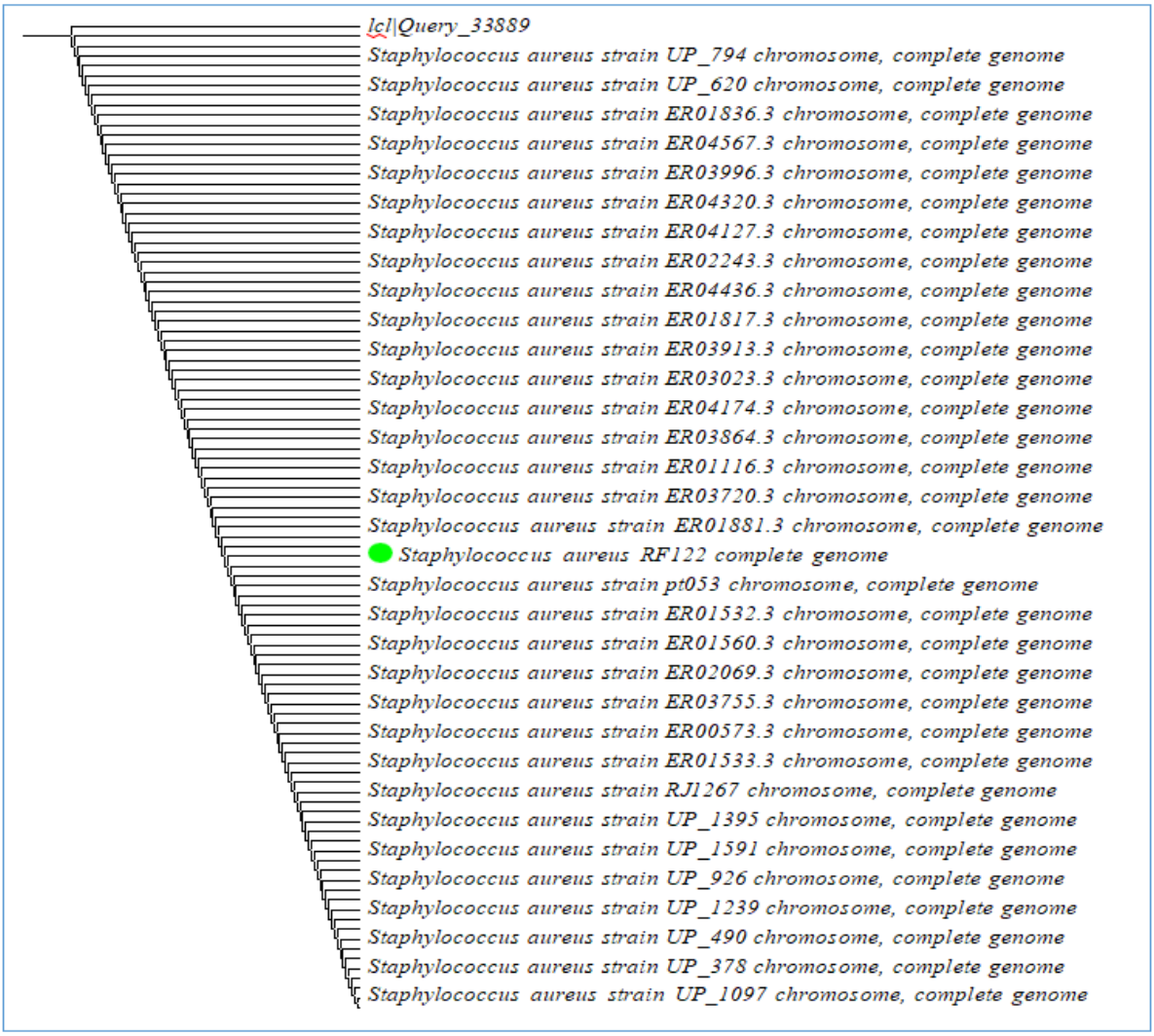

FIGURE 2. Phylogenetic tree by MEGA7 using Neighbor-Joining method showing the classification of the current isolates and nearest strains of S. aureus. Note: RF122 was the primary target strain (shown in green color)

Detection of a methicillin-resistant gene (mecA)

The conventional approach was the best procedure for identifying the Methicillin-resistant gene in *S. aureus* (*mecA*) due to various species of *staphylococci* that were expressed for various resistance levels[30]. PCR identification was used in this study to accurately detect the *mecA* gene in *S. aureus* isolates, which mediates methicillin resistance in bacteria using the primers (*mecA*) defined in the methodology. All the positive isolates for *nuc* that are characterized as MRSA by the (methicillin Disc Diffusion and MIC test) are subjected to detect (*mecA*) gene in PCR procedure (as shown in Figure 1) [31]. The Molecular size of *mecA* is about (525 bp) compared with DNA ladder size as a marker (100bp). The positive *mecA* isolates were compared with the methicillin sensitivity test and cefoxitin MIC [32]. The relation between genotypic and phenotypic was 100% detected in methicillin and cefoxitin resistance [33]. All *S. aureus* isolates give a positive result, and the negative *mecA* isolates are not detected by PCR or the methicillin disc diffusion (as shown in Figure 3) [34].

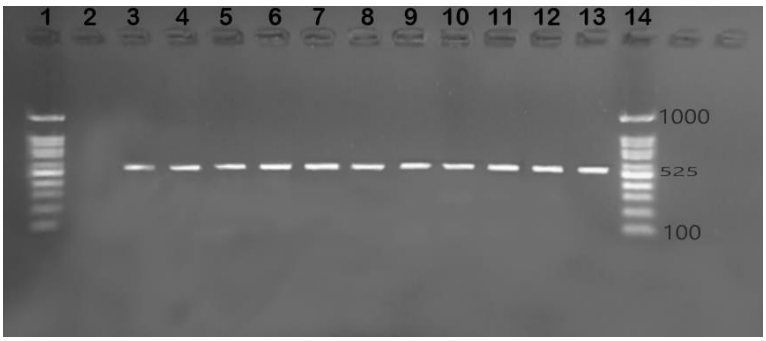

FIGURE 3. An agarose gel stained with Ethidium Bromide dye showing PCR products with mecA gene 525bp primers for extracted DNA. The electrophoresis process resulted at 70 volts for 70 min. Lanes 1 and 14 represent DNA markers (100-1000bp), Line 2 is for negative control, and Lanes 3-13 represent a band of positive results of amplified PCR products (525bp)

The present study gave amplified *nuc* primers a fragment of the size of 276 bp. The sequence alignment (accessory application) of the *nuc* gene was then resulted by Bio-Edit program software when comparing *S. aureus* isolates and reference strain sequences that are available in the Gene Bank database (Figure 4) [35]. The similarity of current nucleotides resulted from NCBI (BLASTn program). However, showing 99% to 100% of the main similarity of current sequences to *S. aureus* known strains [36]. The *nuc* sequences were subjected to MEGA7, and the alignment of the starting sequences was used to remove all trimmings from the start and end [37].

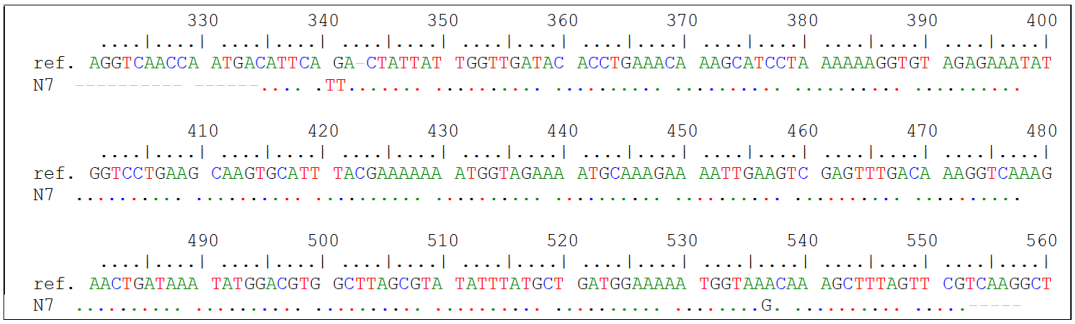

FIGURE 4. DNA sequence alignment of S. aureus isolates with its corresponding reference sequence of the nuc gene by Bio-Edit software, with alterations in each isolate (Table 3-7). Ref = Reference sequence of nuc gene of S. aureus strain RF122 (Wild type). The symbol "n" indicates to S. aureus resistant isolates

Amino acid alignment of mecA gene

The *mecA* nucleotide sequences were analyzed by operating program software (Bio-Edit) using the toggle translation option from the alignment menu. The sequence alignment was used to detect the alterations or changes in the amino acids of *S. aureus* isolates (see Figure 5) [38]. Nevertheless, in *the mecA* gene, the mutations did not occur in amino acids or nucleotides due to alterations or substitution, and the resistance to methicillin antibiotics occurred due to the *mecA* gene responsible for methicillin-resistant [39]. All *S. aureus* isolates were non-mutant in this study because no mutations were recorded in the Data Analysis of DNA Sequencing [40] .

Mutant isolates associated with antibiotics

To evaluate the relation between *S. aureus* isolates and mutations associated with methicillin and fluoroquinolone resistance by comparing antibiotic sensitivity and MIC tests with the molecular analysis of the target genes using SPSS software. Statistical analysis results are based on methicillin and fluoroquinolones antibiotics with mutations and isolation sources. The tables and figures below summarize the relationship in this study between antibiotics, mutations, and isolation sources [41].

Non-mutant isolates associated with methicillin susceptibility

In this study, no mutation was observed in sensitive and resistant *S. aureus* isolates, as mentioned in Table 5. It was reported that about 100% of *S. aureus* strains were resistant to methicillin (Reygaert, 2013), and considering this aspect, these fifty MRSA isolates may not have any mutation in the *mecA* gene [40].

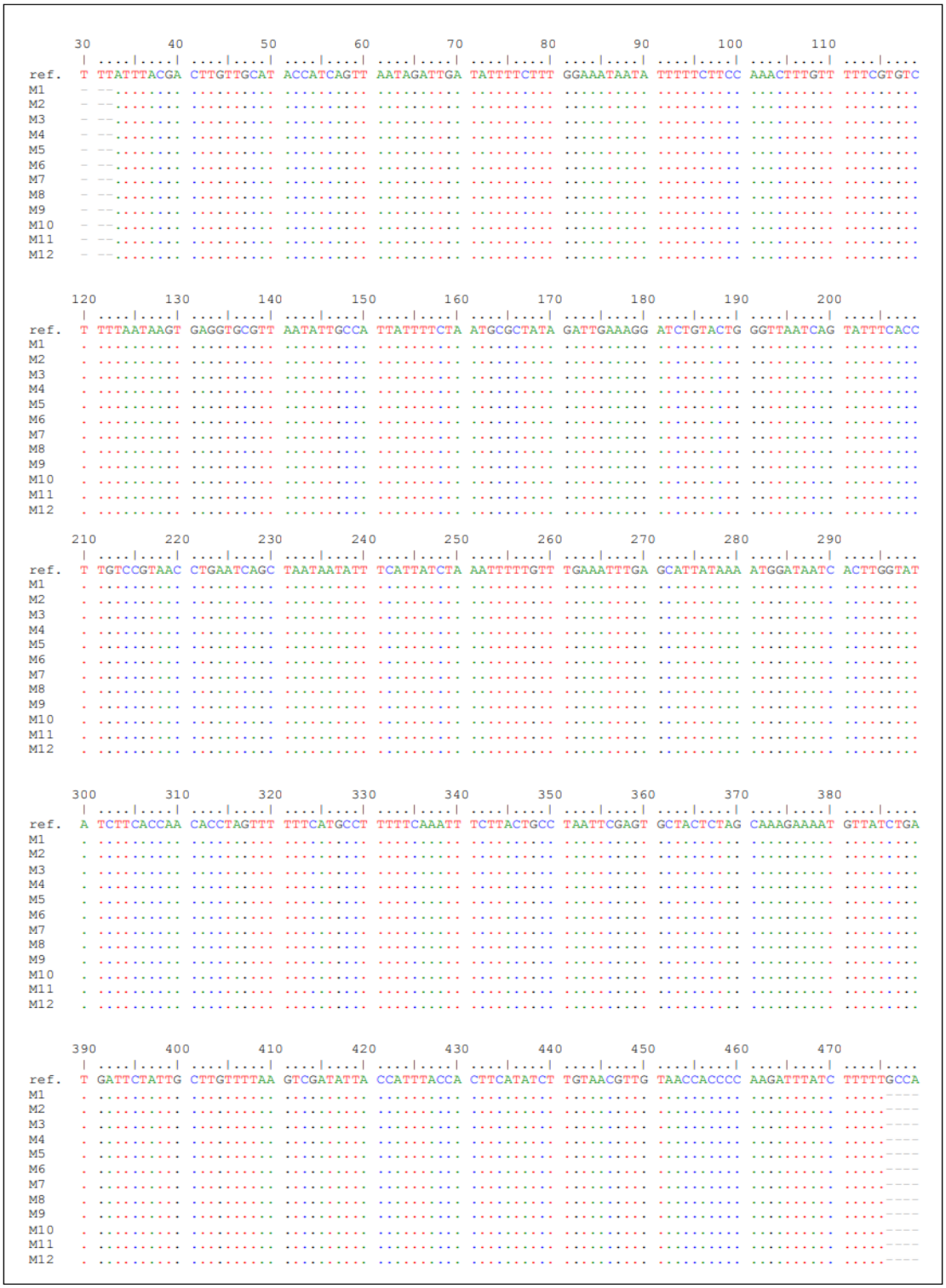

FIGURE 5. DNA sequence alignment of S. aureus isolates with its corresponding reference sequence of the mecA gene by Bio-Edit software, with no alterations in each isolate Ref=Reference sequence of mecA gene of RF122 (Wild type). The symbol "M" in black color indicates resistant isolates

Methicillin susceptibility relies heavily on the source of isolation against methicillin; the skin isolate showed a significant variability ($p<0.05$) compared to *S. aureus*' other resistance isolates. This refers to these isolates having 100% resistance against methicillin compared with 33.3% skin isolate within the source of isolation. However, it was the same picture observed in sensitive isolates, where skin isolates showed a significant variability ($p<0.05$) in comparison to *S. aureus* other sensitive isolates, which means these isolates have 0% resistance against methicillin compared with 66.6% skin isolate within the source of isolation (Table 6 and

Figure 6), this may be skin normal flora which is sensitive against methicillin. These results were disagreed by Wróbel *et al.,* (2018), who found 4% methicillin-resistant *S. aureus* isolate in 1% of a patient's skin, whereas methicillin-sensitive *S. aureus* was not detected [42].

TABLE 5. Non-mutant isolates against methicillin susceptibility

Methicillin	**Mutation**	**Total**
	Non-mutant isolates	
Count	0	0
Expected Count	0	0
% within Mutation	0%	0%
Count	50	50
Expected Count	50.0	50.0
% within Mutation	100.0%	100.0%
Count	50	50
Expected Count	50.0	50.0
% within ME	100.0%	100.0%
% within Mutation	100.0%	100.0%

TABLE 6. Methicillin susceptibility depending on the source of isolation

Methicillin		**Source of isolation**								**Total**
		Burn	Ear	Nose	Operation room	Skin	Throat	Urine	Wound	
S	Count	0a	0a	0a	0a	0b	0a	0a, b	0a, b	2
	Expected Count	.2	.4	.3	.2	.2	.4	.2	.2	2.0
	within ME	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%	100.0%
	within the source of isolates	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0%
R	Count	5a	10a	8a	5a	3b	11a	4a, b	4a, b	50
	Expected Count	4.8	9.6	7.7	4.8	2.9	10.6	3.8	3.8	50.0
	within ME	10.4%	20.8%	16.7%	10.4%	6.2%	22.9%	8.3%	8.3%	100.0%
	within the source of isolates	100.0%	100.0%	100.0%	100.0%	100%	100.0%	100.0%	100.0%	96.0%
Total	Count	5	10	8	5	3	11	4	4	50
	Expected Count	5.0	10.0	8.0	5.0	3.0	11.0	4.0	4.0	50.0
	within ME	10.0%	20.0%	16.0%	10.0%	6.0%	22.0%	8.0%	8.0%	100.0%
	within the source of isolates	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Note: S = Sensitive, I = Intermediate, R = Resistance

DISCUSSION

A total of 185 bacterial samples were collected from different clinical sources, and only 50 samples (27% of total isolates) were given the typical biochemical tests and characteristics of morphology specific to *Staphylococcus aureus* strains [43]. The fifty Isolates formed yellow (golden-color) colonies due to fermenting the mannitol salt by changing the phenol from red to golden and tolerating the high amount of salts in an MSA selective medium (Mannitol salt agar) [44]. These biochemical reactions give the typical characteristics of *S. aureus* strain morphology [45]. The primary biochemical tests for specimens showed a positive reaction for catalase, coagulase, MR-VP, and Voges Proskauer tests. Nevertheless, it was given a negative reaction for oxidase tests [46]. The VITEK2 system was used to identify *Staphylococcus aureus* [47]. Catalase is a catalysis enzyme that decomposes (hydrogen peroxide) H2O2 to water and oxygen. Then, it prevents the toxic metabolites from accumulating [48]. The *S. aureus* isolates were differentiated from other *Streptococcus* genera by giving a positive catalase

reaction. However, *S. aureus* gave negative reactions for the oxidase enzyme, which differentiated from other *Micrococcus* genera [49]. They were subjected to coagulase reactions, which differentiate between *S. aureus* species (positive coagulase) and other *Staphylococci* species (negative coagulase) due to reaction coagulase enzymes of bacteria with prothrombin of human blood and form staphylothrombin (clot of blood) led to converting the fibrinogen into fibrin [50]. *Staphylococcus aureus* isolates gave positive results in methyl red reaction and Voges Proskauer reaction. Mutations that occur in the 16S rRNA gene might change the structure of the ribosome, which can confer resistance to antibiotics that target the ribosome. However, Mutations that affect protein synthesis could impact the overall fitness of the bacterium, affecting its ability to survive and compete in various environments. This indicates that the genetic dimension between Iraq and the isolates of the world is extremely relative, and 16S rRNA analysis is considered a good discrimination approach for distinguishing unrelated isolates. The mutation in the 16S rRNA region may be associated with fluoroquinolone antibiotic-resistant phenotype [51]. The utilization of a phylogenetic tree provides valuable assistance in the classification and identification of strains based on their genetic profiles. This method is particularly beneficial for discerning between strains that exhibit similar phenotypes yet possess different genetic backgrounds [52].

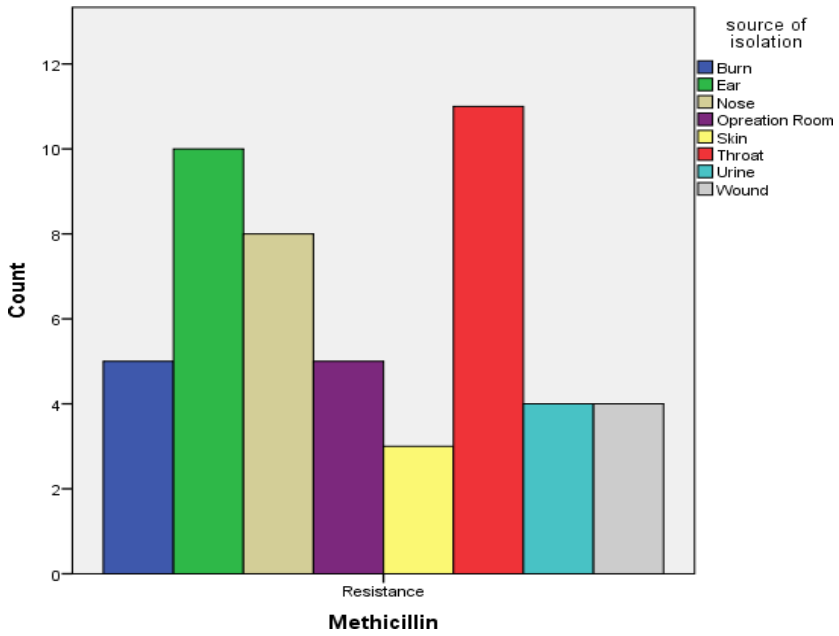

FIGURE 6. Distribution of methicillin susceptibility depending on the source of isolation

In this study, the percentage of resistance isolates to Ciprofloxacin, Norfloxacin, ofloxacin, and levofloxacin antibiotics was 12% of the total fifty *S. aureus* isolates. These results did not relate to the antibiotic sensitivity of AL-Marjani et al. [21], who showed 16% of bacterial isolates were resistant to Ciprofloxacin antibiotics and disagreed with Al-Jebouri and Mdish [53], who showed 40% bacterial isolates were resistance to Ciprofloxacin, Disagreed with Ikeagwu et al., [54] who showed 6.7% resistance to Norfloxacin, 35% resistance to ofloxacin and 40% to levofloxacin. In lomefloxacin antibiotics, 24% of *S. aureus* isolates were resistant; this case disagreed with Abd El Tawab [55], who found that 12% of *S. aureus* isolates were resistant to lomefloxacin. In nalidixic acid, the results showed 56% of *S. aureus* isolates resistance, 40% sensitive, and 4% intermediate. These results disagreed with Khaleel et al.[56], who showed that all *S. aureus* isolates (100%) were resistant to nalidixic acid. The 12 resistant isolates in the disc diffusion method were subjected to MIC tests. The results presented test that *S. aureus* isolates were resistant to nalidixic acid and Lomefloxacin at MIC ranged (32, 64, 128, 512, and 1024 µg/ml) and the highest MIC in wound and throat (1024 µg/ml). However, the lower MIC range can be seen in Ciprofloxacin antibiotic in the range (of 0.5, 1, and 2 µg/ml), specifically in burn with (0.5 µg/ml)[57]. In comparison, *S. aureus* isolates were resistant to another type of fluoroquinolone antibiotics that ranged from (8-512 µg/ml). This study completed the PCR method at 60 C annealing temperature. The 12 resistant isolates to the fluoroquinolone antibiotics group showed a clear band in agarose gel with the same molecular weight of *mecA* primer that compared with DNA ladder (100-1000 bp) at 70 volts for 70 min[58]. However, after DNA sequencing for 12 isolates resistant to methicillin and

six experiments with fluoroquinolones antibiotics, no mutations occurred in the *mecA* gene [59]. The *mecA* gene in *Staphylococcus aureus* is naturally associated with methicillin resistance. However, *mecA*-positive strains can resist cefoxitin and methicillin without mutations in the gene. This could be expected from other resistance mechanisms, such as raised countenance of penicillin-binding-proteins (PBPs) or efflux pumps. It is also wrong to believe in the possibility of other resistance genes or mechanisms in these strains. Methicillin resistance can be influenced by genetic variability within other related genes [60].

CONCLUSION

The resistant isolates to methicillin were selected to scan the mutation's occurrence by direct sequencing. This investigation identified no mutations in the *mecA* gene, and the methicillin resistance was due to the *mecA* gene being responsible. The absence of mutations in the *mecA* gene implies that resistance may be ascribed to alternative mechanisms, potentially including enhanced expression of penicillin-binding proteins or efflux pumps. The extensive utilization of F.Q.s in human and animal healthcare has led to many antibiotic-resistant pathogens.

AUTHOR CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: study conception and design: Lujain A. Ghannawi, Karam Gharab, and Safaa Ehssan Atta; data collection: Omar Yasir Shakir; analysis and interpretation of results: Safaa Ehssan Atta and Lujain A. Ghannawi; draft manuscript preparation: Shahad Basil Ismael, Asmaa Hamid Khleef, and Safaa Ehssan Atta. All authors reviewed the results and approved the final version of the manuscript.

FUNDING

The research did not receive financial support from any institution.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to sincerely thank everyone who helped with this study, especially those who contributed invaluable time and effort. We also thank Mustansiriyah University/ National Diabetes Center for their assistance and direction during this study.

ETHICS STATEMENTS

All subjects gave their informed consent for inclusion before they participated in the study, the study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Mustansiriyah University.

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Received 02.09.2024 ***Accepted 01.02.2025***