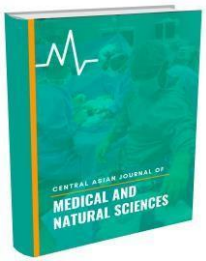

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*Article*

# Distribution of the SSCmec Types among Staphylococcus Aureus that Methicillin Resistant (MRSA) Isolates in Iraq

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**Abstract:** One of the primary causes of nosocomial infections is "Staphylococcus aureus", particularly "methicillin-resistant" strains. This drug resistance has become an international public health concern. Due to the lack of studies, especially on the presence of genotypes in MRSA species in the Diwaniyah city in Iraq. The objective of the present investigation was to determine extent of the distribution and diversity of these patterns s.aureus bacteria that had been eliminated from various medical and environmental resources within specific hospitals and health centers in the governorate, using phenotypic and molecular methods. Total of 565 samples were collected for this purpose, including 330 clinical samples (Urine 200 samples, Sputum 70 samples, and Burns 60 samples), 235 environmental samples for the period from September to November 2020. Based on the results of culture results , traditional biochemical and microscopic examinations, the virulence of 80 S. aureus isolates was determined, while methicillin-resistant MRSA isolates were phenotypically diagnosed using the disc diffusion method of cefoxetine and molecularly based on the methicillin resistance gene (mecA) and monoplex PCR technique , 72 (90%) isolates have been identified as MRSA, and by comparing the molecular and phenotypic methods, it was found that the cefoxitin disc diffusion test is highly accurate and effective in detecting MRSA strains. Staphylococcal chromosome cassette methicillin-resistance (SCCmec) for four main types (I, II, III, and V) and four subtypes (IVa, IVb, IVc, and IVd) was also carried out using (Multiplex PCR). The results showed that 7 isolates (9.72%) contain the SCCmec V subtype, 7 (9.72%) contain the SCCmec IVa subtype, 6 (8.33%) contain the SCCmec IVb subtype, and 5 isolates (6.94%) contained the subtype SCCmec IVc, while 48 isolates were not identified.

**Keywords:** Methicillin-Resistant Staphylococcus Aureus (MRSA), Nosocomial Infections, SCCmec Typing, Phenotypic and Molecular Detection, Antibiotic Resistance in Iraq

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## 1. Introduction

Even though Staphylococcus aureus is a common human commensal, it can also cause infections in people, some of which can be dangerous if they are brought on by strains of the bacteria that are resistant to antibiotics [1]. Hospitals and the outside world are both equally embracing antibiotic-resistant S. aureus, particularly MRSA, which is becoming a major public health risk [2].

Since its introduction to medicine in 1960, methicillin, a semisynthetic penicillin, has been used to treat staphylococci that produce beta-lactamases. Methicillin-resistant Staphylococcus aureus (MRSA) was found, however, in 1960 [3]. Over the course of the next sixty years, MRSA became recognized as the most common and well-known antimicrobial-resistant strain. Following the introduction of methicillin, MRSA was found in healthcare settings more frequently, although it was rare to isolate the bacteria in clinical

settings prior to this. This was noted in the first report on MRSA. In the United Kingdom, for instance, the percentage of methicillin-resistant S. aureus isolates from blood cultures elevated during the 1990s, peaking at 40% around the year 2000 before declining. In the 2010s, it dropped below 10%. A relationship between unsuitable use of antimicrobials and high rates of antimicrobial resistance, not only to methicillin but also to other antimicrobials, has been theorized. Therefore, the prevalence of MRSA remains a useful indicator of proper antimicrobial usage and good infection control and prevention practices [4][5]. Penicillin-binding protein 2a (PBP2a) is a protein that has low affinity for beta-lactam antibiotics and is responsible for the methicillin resistance of MRSA strains. The mecA gene, which is found on the chromosome via a large gene cassette called the staphylococcal cassette chromosome mec (Sccmec), encodes PBP2a [6][7].

SCCmec are "mobile genetic elements" (MGEs), that are crucial in the development of virulence factors and antibiotic resistance techniques since these are primarily found in hospital-associated MRSA strains and are spread by horizontal gene transfer in three different ways: conjugation, transmission, and transformation. It is characteristically grouped into types I, II, III, IV, and V, rendering to ccr and mec alleles . The SCCmec type IV is further grouped into a, b, c, and d types [8][9].

## 2. Materials and Methods

**Collection of Samples:**

Between September 2020 and November 2020, 565 samples were collected from various sources in a random manner and included clinical and environmental samples. The clinical samples included 330 samples (urine 200 samples, sputum 70 samples, burns and wounds 60 samples), while hospital environmental samples included 235. The samples were taken from inpatients and outpatients at the teaching hospital and the Specialized Center for Burns, children's and women's hospital, in Diwaniya Governorate. For different age groups and both genders. Cotton wipes containing transport media are used to keep skin pus, burn and ecological specimens. Using sterile plastic containers to obtain specimens of sputum and urine. The specimens were then all brought to the laboratory after stored in ice-filled plastic cabinet to prevent microbiological development.

**Aureus separation and identifying :**

Blood agar and Manitol salt agar were the culture mediums utilized for the streaking technique of culturing medical and ecological specimens, all plates were cultured aerobically for 24 hours at 37 °C, the procedure was then repeated to obtain pure colonies[10]. The phenotype of isolates cultivated in culture medium and biochemical testing were used to recognize the isolates.

**Phenotypic Detection of MRSA**

MRSA is detected by the Cefoxitin Disc Diffusion Test using Cefoxitin Disc (30 μg). Staphylococcus aureus strains with MRSA that showed a zone of inhibition of less than 21 mm to the cefoxitin disc were recognized.

**Purification of bacterial genetic DNA**

Using (The genetic extracted DNA reagent) manufactured by the United States organization Genaid, DNA was extracted from the s. aureus bacteria according to the company's procedures.

**Molecular detection of MRSA**

PCR method testing has been employed to verify the authenticity of MRSA strains. by detecting the mecA gene using the technique of monoplex PCR. The reaction mixture consists of 12.5 μl of GoTaq Green Master mix (Promega, USA), 0.5 μl of each primer (10 pmol/μl), and 5 μl of template DNA. The final measurement of PCR was 25 μl, and it concluded by adding 15.2 μl of nuclease-free water before putting it in the thermal cycler. The amplification conditions that were applied were as follows: A 5-minute initial

denaturation phase was followed by 35 cycles of denaturation (15 seconds at 94°C), annealing (15 seconds at 55°C), and extension (20 seconds at 72°C), ending in a 5-minute final extension at 72°C.

**Molecular detection of staphylococcal cassette chromosome mec (SCCmec)**

Multiple polymerase chain reaction technology was used to detect the genes of staphylococcal types, where several primers that were similar in temperature were added in different sizes, as shown in the table (2). The thermal cycles are also shown in a table (3).

**Table 1.** Primers of staphylococcal cassette chromosome mec (SCCmec) detection.

| Genes | | Primer | Sequence (5'→3') | Product Size | Reference |
|---|---|---|---|---|---|
| SCC*mec* genes | Type I | F | GCTTTAAAGAGTGTCGTTATAGG | 613 | [11] |
| | | R | GTTCTCTCATAGTATGACGTCC | | |
| | Type II | F | CGTTGAAGATGATGAAGCG | 398 | |
| | | R | CGAAATCAATGGTTAATGGACC | | |
| | Type III | F | CCATATTGTGTACGATGCG | 280 | |
| | | R | CCTTAGTTGTCGTAACAGATCG | | |
| | Type Iva | F | GCCTTATTCGAAGAAACCG | 776 | |
| | | R | CTACTCTTCTGAAAAGCGTCG | | |
| | Type IVb | F | TCTGGAATTACTTCAGCTGC | 493 | |
| | | R | AAACAATATTGCTCTCCCTC | | |
| | Type IVc | F | ACAATATTTGTATTATCGGAGAGC | 200 | |
| | | R | TTGGTATGAGGTATTGCTGG | | |
| | Type IVd | F | CTCAAAATACGGACCCCAATACA | 881 | |
| | | R | TGCTCCAGTAATTGCTAAAG | | |
| | Type V | F | GAACATTGTTACTTAAATGAGCG | 325 | |
| | | R | TGAAAGTTGTACCCTTGACACC | | |

F: Forward primer , R: Reverse primer

**Table 2.** Components and sizes of the reaction mixture for Multiplex PCR.

| A combination of PCR reactions | The volume (µL) |
|---|---|
| (Master mix 2X) | 25µl |
| (Primer forward) (10 µM) | 0.5–5.0µl |
| (Primer reverse) (10 µM) | 0.5–5.0µl |
| DNA template | 1–5µl |

| | |
|---|---|
| (Nuclease-Free Water) | to 50µl |
| **Total volume** | 50 **µl** |

**Table 3.** Thermal cycle conditions for Multiplex PCR technology.

| Multiplex gene | Thermal ˚C / Period | | | | | Cycle no. |
|---|---|---|---|---|---|---|
| | First denaturing | State of cycling | | | Late extension | |
| | | Denaturing | Annealing | Extension | | |
| The genes for SCC*mec* types | 95C°/4min | 95C°/45sec | 62C°/50sec | 72C°/2min | 72C°/10min | 35* |
| | 95C°/4min | 95C°/45sec | 55C°/50sec | 72C°/2min | 72C°/10min | 35* |

**Analytical statistics**

The Statistical Package for Social Science (SPSS) was used to statistically examine the present research's information. The statistics program was utilized in the 23 version. The Chi-square was utilized to examine the research's data, and significant differences were found to be less than or equal to 0.05.

## 3. Results

In the present investigation, (80) isolates of S. aureus bacteria which identified according to phenotypic cultural characteristics, microscopy characteristics, and biochemical tests. [10]

Whereas MRSA strains were by phenotype diagnosed by cefoxitin disc diffusion(FOXDD) at dosage of 30µg, in accordance with the CLSI 2020 standards [8], utilizing the altered Kerby-Bawer disc diffusion procedure.

MRSA strains were identified from S. aureus strains that involve inhibiting zone 21 millimeters in diameter or less per disc. Cefoxiten was classified MRSA , as the findings indicated that 70 isolates, (87.5%), were MRSA, and only 10 isolates, (12.5%), were Methicillin-sensitive Staphylococcus aureus (MSSA)table (4).

As for molecular detection, by investigating the mecA gene using pcr technology, the results showed it is shown in Figure (1) that 72 isolates, representing 90%, were MRSA, and only 8, representing 10%, were MSSA. This means that the cefoxitin discs method gave only two isolates, with a false negative rate of 2.5%.

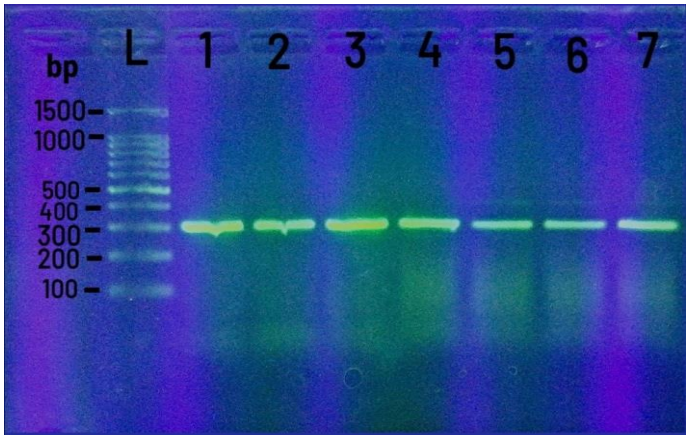


**Figure 1.** Agarose gel electrophoresis of a multiplexed PCR product for (mecA) gene was detected. L: DNA Ladder 1500-100bp, amplified products of mecA gene; 304bp band, lanes 1-7 positive results.

**Table 4.** Prevalence of MRSA isolates based on collection type.

| Specimens Source | | Number Of MRSA Isolates N=72 | P Value |
|---|---|---|---|
| Clinical Samples | Urine | 29 | 0.01* |
| | Sputum | 14 | |
| | Burn | 10 | |
| Environmental Samples | | 19 | |

In the current study, staphylococcal cassette chromosome mec (SCCmec) were investigated in 72 MRSA isolates using (Multiplex PCR) technology to detect the primers shown in Table (1). The results of the molecular examination showed a wide spread of these gens among the isolates, as 24 isolates showed a percentage of (33.33%) it contains at least one type, as shown in Figure (2), while the remaining 48 isolates with a percentage of (66.66) did not give any positive result for any type table (5).

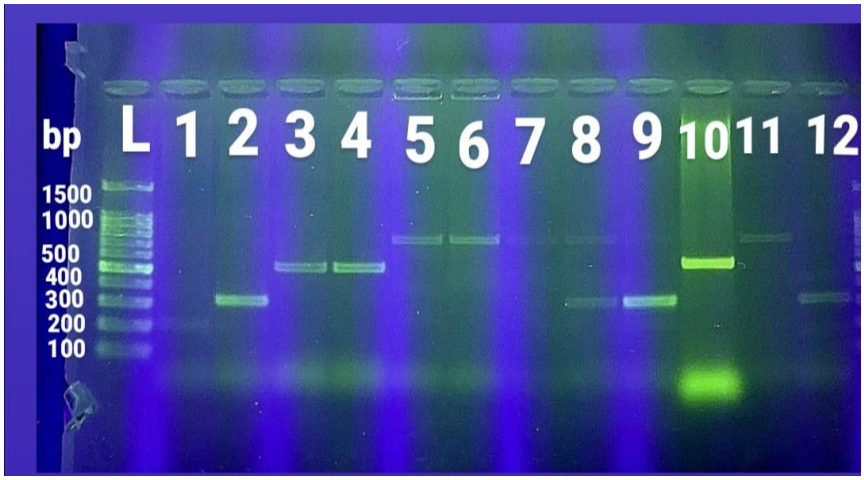


**Figure 2.** Agarose gel electrophoresis of a multiplexed PCR product for (SCCmec) genes were detected. L: DNA Ladder 1500-100bp, amplified products of SCCmec genes; 1: SCCmec IVc- 200 bp /2,9,12: SCCmec V- 325 bp /3,4,10: SCCmec IVb- 493 bp/5,6,7,11: SCCmec Iva- 776 bp /8: Isolation contains two genes: SCCmec V- 325 and SCCmec Iva- bp 776.

**Table 5.** Prevalence of SCCmec types in MRSA isolates according to isolation sources.

| Specimens source | | Total no of MRSA n=72 | SCC*mec* IVa n=7 | SCC*mec* IVbn=6 | SCC*mec* IVcn=5 | SCC*mec* Vn=7 | Untypable SCC*mec* n= 48 | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Clinical samples | Urine**(%)** | 29 | 1 (14.28%) | 6(100%) | 2(40%) | 4(57.14% | 18(37.5%) | 0.001* |
| | Sputum**(%)** | 14 | 4(57.14%) | 0 | 0 | 1 (14.28%) | 9(18.75%) | 0.03* |
| | Burn**(%)** | 10 | 0 | 0 | 0 | 0 | 10(20.83) | |
| Environmental samples**(%)** | | 19 | 2(28.57%) | 0 | 3(60%) | 2(28.57%) | 12(25%) | 0.002* |

## 4. Discussion

Most MRSA strains have acquired chromosomal genetic elements called Staphylococcal Cassette Chromosome mec (SCCmec) that have been incorporated into their genome, including antibiotic resistance factors , the mecA gene and genes responsible for virulance factors [12].The technique of identifying SCCmec types is molecularly

important to distinguish between types of MRSA and form a clear vision of its clonal and epidemiological classification and to control the spread of resistance between strains and study its epidemiology [12].

Present research revealed that the (FOXDD) technique indicated susceptibility (100%), as well as the current findings is in agreement with a number of earlier investigations. [13] [14]. This high sensitivity of cefoxitin can be explained by Through increased the manifestation of the protein PBP2a encoded by the mecA genes, cefoxitin is an stimulant of the mecA gene, which results in enhanced MRSA detection [15].

This approach is regarded as the best since numerous researchers have found that the "cefoxitin disc diffusion (FOXDD)" assay more clearly reveals the presence of the mecA gene than the disc diffusion assays that use "oxacillin disc diffusion (OXDD)".[16].

Out of the 80 isolates of staphylococcus aureus, 72 isolates (90%) were categorized as MRSA based on the findings of the PCR technique that identified the mecA gene. Table (4) summarizes the data, which indicates that there were substantial differences at the probability levels ($P<0.05$) and 40.2% of these isolates were found to be from urine samples, suggesting that the prevalence of MRSA strains varies throughout clinical specimens.

Out of the 80 isolates of Staphylococcus aureus, 72 isolates (90%) were categorized as MRSA based on the findings of the PCR technique that identified the mecA gene. Table (4) summarizes the data, which indicates that there were substantial differences at the probability levels. ($p < 0.05$), and had been shown the 40.2% of the isolates were obtained from urine samples, followed by sputum samples., which was 19.4%, and finally burns, which was 13.8%, and among environmental samples, which was 26.3%, that indicating a difference the variation in MRSA strain occurrence between clinical specimens.

The prevalence of MRSA strains has raised lately and is currently a serious danger, particularly in hospitals and other healthcare facilities. Our findings are consistent with numerous studies carried out in various parts of Iraq, where rates of MRSA strains were found to be similar to these reported here as follow: 80% in the study [17] in Baghdad, 88% in a study [18] in Karbala, 91.6% in a study [19] in Babylon, 65.3% [20]. Furthermore, the diagnostic techniques utilized in various investigations varied, which has an impact on the strain s' percentage frequency. Furthermore, distinct study designs could be the cause of the disparity in results amongst the various governorate of Iraq.

There is a discernible increase in rates of infections worldwide, even if the prevalence of MRSA differs between hospitals and nations. As an illustration, Iran has a comparatively high MRSA prevalence of 70.8% , higher than that of certain other Asian and African countries, [21] and at 40.8%. % and 57.1% in Tabriz and Kerman, respectively [22], Kuwait (39.8%) [23], and Syria (29.5%)[24] , while in Egypt it was The percentage is 95.8% [25].

These findings demonstrate that MRSA has evolved genes that increase its resistance to antibiotics, which has made it more deadly. This finding was supported by [26] , in addition to the high ability to rapidly replicate MRSA, It renders possible for bacteria to propagate more quickly and become more resistant to medicines.[27] [28] .

The statistical results showed that there were significant differences at the probability level of $P < 0.05$ between the prevalence of types and sources of isolation, as shown in Table (5). The results showed that the most common types among the 72 MRSA isolates were the main type, SCCmec V, and the secondary type, SCCmec IVa, with a percentage of (%) 9.72) and 7 isolates for each, as the prevalence of the SCCmec V type was higher in clinical samples than in environmental samples, as it gave 4 isolates (57.14%) isolated from urine, and only one isolate (14.28%) isolated from sputum, while only two isolates showed (57.14%) isolated from urine (14.28%). 28.57% of environmental samples showed positive results for this type. As for the SCCmec IVa type also, its presence rate in clinical samples was higher than environmental samples, as 4 isolates showed (14.28%) isolated from sputum and one isolate (14.28%) showed only two isolates (28.57%) of the environmental samples tested positive for this type

Our findings converged with the results of the study [12] in that the two types, SCCmec V and SCCmec IVa, are the most widespread among MRSA isolates. Our results also converged with the results of the study [29], as the prevalence of the two types was SCCmec V and SCCmec IVa are (31%) and (29.3%) respectively.

The secondary type SCCmec IVb showed the second highest prevalence rate after the previous two types, as 6 isolates (8.33%) gave positive results for it, as all of these isolates were isolated from urine, with a percentage of (100%), while the prevalence rate of the secondary type SCCmec IVc was (6.94%) and 5 isolates, as only 2 isolates (40%) isolated from urine and 3 isolates (60%) isolated from environmental samples showed positivity for this type. These results were higher than what the researchers reached in the study [29] , as the prevalence of SCCmec IVb and SCCmec IVc types was (1.7%) and (3.4%), respectively.

As for the remaining types, none of the isolates under study showed positive results for them, and this is consistent with the study [30] in that the main type SCCmec III did not appear in any of the isolates studied, while our results contradicted the study [29] , the isolates studied showed prevalence rates of the categories SCCmec I, SCCmec II, SCCmec III, and SCCmec IVd, which are (8.6%), (19%), (34.5%), and (19%), respectively. Our results also contradicted the study of [31] , as resistance rates for types SCCmec III, SCCmec I, and SCCmec II were recorded as (44.44%), (27.77%), and (16.66%), respectively.

## 5. Conclusion

a. The current study is only a primary investigation of the spread of resistant strains of S. aureus bacteria in hospital and health center environments.
b. The study also demonstrated the possibility of identifying resistance types in these bacteria, which helps in knowing the most widespread types and thus the possibility of finding solutions to limit this spread.
c. Therefore, we recommend conducting more in-depth studies on these types to keep hospital environments free of these deadly strains.

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