Molecular Detection of the mecA and some Virulence Determinants in Methicillin-Resistant Staphylococcus aureus

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Abstract:

**Background:** Methicillin Resistant *Staphylococcus aureus* (MRSA) is globally acknowledged as a prominent contributor to both hospital-acquired and community infections. Understanding key virulence factors including coagulase production, hemolysis ability and biofilm formation, is crucial.

**Objective:** The study aimed to establish a molecular characterization of mecA gene and virulence factors genes (hla, icaA, and coa) in clinical isolates of MRSA obtained from two hospitals in Baghdad.

**Materials and Methods:** A hundred and five isolates were obtained from clinical sources from November 2022 to March 2023 and their antibiotic sensitivity was assessed using the agar diffusion test against seven different antibiotics (Azithromycin, Ciprofloxacin, Nitrofurantoin, Rifampin, Trimethoprim, Ofloxacin and Oxacillin), through Conventional Polymerase Chain Reaction, the presence of virulence factor genes including mecA, hla, icaA, and coa, was determined in MRSA isolates.

**Results:** All MRSA isolates (100%) harbored the mecA, hla, and icaA genes while the coa gene was recognized in 50% of the isolates. Regarding antibiotic susceptibility, all MRSA isolates (100%) demonstrated sensitivity to Nitrofurantoin. Additionally, 96.8% of the isolates were sensitive to Oxacillin.

**Conclusion:** Molecular detection of methicillin resistance genes and virulence genes can be used to diagnose MRSA isolates in hospitals. The presence of these genes may affect their pattern of sensitivity to antibiotics.

**Keywords:** Methicillin-resistant *Staphylococcus aureus*, mecA gene, hla gene. icaA gene, coa gene.

**Introduction**

*Staphylococcus aureus* is one of the most developing worldwide public health issues resulting from the emerging resistance to antimicrobial agents and leading to ineffective treatment (1). *S. aureus* is considered one of the most widely spreading bacterial infection in community and hospital settings as it possesses multiple mechanisms of antibiotic resistance, therefore, leading to severe infections (2). The problem is MRSA strains which previously spread in hospital settings but is currently occurring progressively in community settings. MRSA exhibits high infection and virulence (3). Understanding the prevalence of MRSA is of utmost significance for infection control, the prevention of severe infections and gaining insights into the mechanisms of resistance (4). These bacteria are responsible for widespread diseases spanning from acute skin abscesses to chronic endocarditis and osteomyelitis, affecting both hospital and community settings (5). In recent years, MRSA has become a global concern, with variations in its distribution from one region to another (6). For instance, in Zakho city MRSA frequency is 85% as reported by Hami & Ibrahim (7), while its reported 90% by Hameed et al. in Karbala city (8).

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MRSA strains exhibit resistance to β-lactam antibiotics through two mechanisms: first, they hydrolyze penicillin β lactams by production of penicillinase, and reduce binding affinity for β lactams through altered Penicillin-Binding Protein (PBP).

This alteration results in fighting almost all available β-lactam antibacterials except for the latest cephalosporins such as ceftaroline (9). The resistance mediated by penicillinase is determined by the *blaZ* gene while the resistance associated with PBP is determined by the mecA gene sited in the SCCmec gene (10, 11).

Locally, a study conducted at the Maternity and Children teaching hospital and Al Diwaniya teaching hospital in Iraq, *S. aureus* isolates from different cases were found to be highly resistant to the usually used antibiotics (12). Another Iraqi study, revealed that MRSA strains were resistant to various antibiotics such as azithromycin, methicillin, and ciprofloxacin, but not to ceftaroline (13).

The pathogenicity of MRSA strains relies on several virulence factors; for example, hemolysins which lead to development of diseases and are categorized into three types: alpha(α), beta(β) and gamma(γ). The alpha hemolysin is a toxin produced by the *Hla* gene of *S. aureus* acting as a virulence factor that
forms pores in cell membranes, disrupting epithelial barriers and leading to cell lysis and death (14). Biofilms which are multi-cellular bacterial communities embedded in an extracellular matrix pose a major challenge in the setting of non-communicable diseases. These biofilms provide protection to bacteria and increasing their resistance to antibiotics. Antimicrobial resistance is a prominent feature of biofilm-associated bacteria and facilitates their adhesion to infected areas (15). MRSA strains are known to produce biofilms by expressing polysaccharide intracellular adhesion (PIA), which is generated by transcription of the icaA gene operon products on chromosomes (16).

Furthermore, a main virulence factor of S. aureus is coagulase, which contributes to pathogenic infections such as endocarditis, abscess formation and staphylococcal bacteremia (17). Coagulase, an enzyme-like protein, converts fibrinogen to fibrin and leads to the formation of a plasma clot. Consequently, it enhances the pathogenesis of S. aureus, promotes persistent infection, plays a role in immune evasion and facilitates the spread of the bacterium through host tissues (18). Therefore, the current study aims to find the molecular characterization of mecA gene and some virulence factors genes (hla, icaA, and coa) in clinical isolates of MRSA obtained from patients.

**Materials and Methods**

**Samples collection:** Between November 2022 and March 2023, 105 specimens (nostril, throat, urine, sputum and wound samples) were collected in sterilized transport tube media from Abu Ghraib General Hospital and Al Yarmouk Teaching Hospital laboratories.

**Isolation and Identification of S. aureus and detection of MRSA isolates:** All specimens were inoculated on Mannitol salt agar (MSA) (Accumix, England). The cell culture plates were incubated overnight at 37°C. Biochemical tests for isolated bacteria were identified by oxidase, coagulase, urease and catalase tests (10). Phenotypic detection of MRSA isolates was done by utilizing a Methicillin disc (10 μg) and measurement of inhibition bacterial zone of growth after cultured on Muller Hinton medium according to the CLSI guidelines (19). Next, the confirmation of identified isolates was carried out via the VITEK 2 identification device.

**Antibiotics Susceptibility Test (AST):** Antibiotic susceptibility test using seven different antibiotics [Azithromycin (15 μg), Rifampin (5 μg), Trimethoprim (10 μg), Nitrofurantoin (100 μg), Ciprofloxacin (10 μg), Ofloxacin (5 μg) and Oxacillin (5 μg)] was performed for MRSA isolates by KB test according to Bauer et al. in 1966 (20) and CLSI (19).

**Molecular Detection of mecA, icaA, coa, and hla genes**

**DNA Extraction:** Norgen’s Blood DNA Isolation Mini Kit (Norgen, Canada), OneTaq DNA Polymerase Kit (NEB, England) and the Qubit Double-stranded DNA high sensitivity Kit (ThermoFisher, USA) were used for extracting pure DNA from Methicillin-Resistant S. aureus isolates depending on their manufacturer's guidelines. After that, NanoDrop spectrophotometer was used to accurately determine sample concentration between 10 pg/μl and 100 ng/μl (21).

**Polymerase Chain Reaction assay:** All Methicillin-Resistant S. aureus isolates were checked molecularly for hla, icaA, coa and mecA genes using conventional PCR. The amplification procedure for these genes included the initial denaturation phase heated to 94°C for 5 minutes then, at 94°C, 38 cycles of denaturation for 30 seconds, annealing for 45 seconds at 57°C, extension for 45 seconds at 72°C, and final extension for 7 minutes at 72°C. Electrophoresis of the conventional PCR product in a 2% Tris-acetate-EDTA (TAE) agarose gel electrophoresis with 1x TAE buffer for 80 minutes at 80 volts and dyed with RedSafe dye (22). The primer sequences used in conventional PCR for the detection of mecA, icaA, coa, and hla genes in MRSA isolates, and the primer sequences are shown in Table 1.

**Table 1: The Primers sequences used for detection of icaA, mecA, coa, and hla genes**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>F: GTTGTAGAAAATGTCATTATGGGG</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>R: TAGAACCTTGAGCCTCTT</td>
<td></td>
</tr>
</tbody>
</table>

| Hla    | F: TTTTCTTTTTCAGGAAAGGCAG       | 400       |
|        | R: CTTCGATTATATCTGTCGCT         |           |

| Coa    | F: CTGGAGGGAAAAATTGGAAGAAGC    | 179       |
|        | R: CAGTGATGCTTCTCTCTAA        |           |

| icaA   | F: GTATAAGGGCAAGTGCAAC         | 554       |
|        | R: CACGTCTACAAAATGAGTC        |           |

**Statistical analysis:** The R software package was used to analyze the data to determine the sensitivity of the bacteria under study to different antibiotics. The percentages and numbers of resistant and sensitive ones for each type of antibiotic used were determined at a significance level of (p<0.05) (23).

**Results**

**Isolation of Staphylococcus aureus and MRSA**

Only 84 (80%) samples grew on MSA, and based on the primary diagnosis, 47 isolates of these were S. aureus (Table 2) and were examined for antibiotic sensitivity tests by the Kirby-Bauer method to identify methicillin sensitivity of S. aureus. Out of these 47 isolates, 31 (66.0%) were resistant, 8 (17.0%) were intermediate, and 8 (17.0%)
We were sensitive (Table 3). The confirmation of identification was performed by the VITEK2 system. All 31 bacterial isolates were recognized as MRSA based on the primary diagnosis and the VITEK2 system results.

### Table 3: Susceptibility of 47 MRSA isolates to Methicillin

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>31 (66%)</td>
<td>8 (17%)</td>
<td>8 (17%)</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Chi Square test (X²)

**Antibiotics susceptibility test:** A sensitivity test was conducted using seven different antibiotics (Azithromycin, Ciprofloxacin, Nitrofurantoin, Rifampin, Trimethoprim, Ofloxacin and Oxacillin) to determine the sensitivity of MRSA isolates by agar diffusion test.

The results showed that all 31 MRSA isolates (Table 4) were sensitive to Nitrofurantoin at a rate of 100%. However, they exhibited no sensitivity to Ciprofloxacin. Azithromycin, on the other hand, showed a sensitivity rate of 35.5% for MRSA and a resistance rate of 54.8%. Additionally, 29.0% of MRSA isolates were resistant to Trimethoprim, while 9.7% showed resistance to Rifampin and Ofloxacin. Moreover, 96.8% of the isolates were sensitive to Oxacillin, 83.9% were sensitive to Rifampin and 71% were sensitive to Ofloxacin.

### Table 4: Antibiotics susceptibility test of MRSA isolates against seven different antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>% of isolates</th>
<th>sensitive</th>
<th>intermediate</th>
<th>resistant</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>11 (35.5)</td>
<td>2 (6.5)</td>
<td>18 (58.1)</td>
<td>0.01**</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0</td>
<td>14 (45.2)</td>
<td>17 (54.8)</td>
<td>0.354</td>
<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>31 (100)</td>
<td>0</td>
<td>0</td>
<td>0.00***</td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>26 (83.9)</td>
<td>2 (6.5)</td>
<td>3 (9.7)</td>
<td>0.0216*</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1 (3.2)</td>
<td>21 (67.7)</td>
<td>9 (29.0)</td>
<td>0.865</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>22 (71.0)</td>
<td>6 (19.4)</td>
<td>3 (9.7)</td>
<td>0.0453*</td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>30 (96.8)</td>
<td>0</td>
<td>1 (3.2)</td>
<td>0.004**</td>
<td></td>
</tr>
</tbody>
</table>

Chi square test (X²)

**Identification of mecA gene and virulence factor genes:** The conventional PCR test was used to identify the main virulence factor genes including mecA, icaA, coa, and hla genes that are responsible for methicillin resistance, polysaccharide production, coagulase production, and blood lysis, respectively. Ten MRSA isolates were used for determining these genes. The results detected that all samples contained mecA, hla, and icaA genes (100%), the coa gene was identified in 50% of the isolates, as shown in Figure 1.

![Picture](image1.png)

**Figure 1:** Multiplex Conventional PCR of amplified PCR products for mecA (226bp), hla (400bp), icaA (554bp), and coa (179bp) from MRSA isolates

**Discussion**

*S. aureus* is a main contributor to the hospital-acquired and community contagions globally. The transformation of *S. aureus* from methicillin-sensitive to methicillin-resistant is primarily due to the acquirement of the mecA gene (24, 25).

The prevalence for MRSA in the present study differs from other studies which may be due to various factors, including poor hygiene, antibiotic excessive use, regional conflicts and close contact, such as through religious visits.

The majority of MRSA isolates showed Azithromycin and Ciprofloxacin resistance which agrees with other studies (26), such as Bastidas et al. at 61.8% (27) and Ponveli et al. at 16% (28). Additionally, studies in Jordan showed a 15% resistance for Azithromycin and a 28.6% resistance to Ciprofloxacin in Pakistan (29). In this study, Nitrofurantoin demonstrated sensitivity among MRSA isolates aligning with previous findings (30). Consequently, Nitrofurantoin is considered an effective antibiotic for treating MRSA infections, as no resistance was observed among the tested isolates.

Resistance rates for Rifampin (9.7%) and Trimethoprim (29.0%) were seen in this study, showing some agreement with findings by Bai et al. for Rifampin (5.9%) (31), but showing a higher rate (11.8%) for Trimethoprim than in the study by Ibrahim et al. (32).

The frequency of MRSA isolates resistance to Oxacillin was 3.2%, in contradiction to the results of Khasawneh et al. (33), who found a resistance rate of 42.1% for MRSA isolates in Jordan. The 9.7% resistance to Ofloxacin disagrees with Sohail et al. (34), who found a resistance rate of 98%.

In the current study, Ofloxacin exhibited a sensitivity rate of 71% for MRSA isolates, which is close to the results of Nedly et al. (35), who reported a sensitivity of 50% for Ofloxacin. Hense, the most effective antibiotics for treating MRSA as found in the current study were Ofloxacin, Rifampin.
and Oxacillin since only a small number of MRSA isolates exhibited resistance to these drugs. The molecular detection method of the icaA, mecA, coa, and hla genes was achieved using multiplex PCR. Vieira et al. found that 100% of MRSA isolates from clinical sources were mecA producers (36). MRSA is a primary human pathogen causing significant morbidity worldwide and is closely monitored by the World Health Organization. This pathogen contributes to biofilm formation, acute human infections and the development of antibiotic resistance (37).

A study by Bayirli et al. reported that 92.6% of isolates exhibited alpha-hemolysis, while 1.6% displayed beta-hemolysis (38). Additionally, the current study reveals that MRSA isolates which possess the mecA gene were phenotypically susceptible to Nitrofurantoin. The expression of the icaA gene through the study revealed all isolates had positive results and expressing the gene, which is similar to the findings of Azmi et al. (39), who reported a prevalence of 100% for the icaA gene in MRSA.

Regarding the coa gene, the current study reported that it was found in 50% of MRSA isolates, which was lower than that reported by Hazem (86.6%) (40), which may be due to differences in sample treatment, geographical locations, and the dynamic nature of bacterial evolution.

The study revealed that Oxacillin and Nitrofurantoin as effective treatments for MRSA infections but with controlled use to prevent antibiotic resistance.

Conclusion
Molecular detection of methicillin resistance genes and virulence genes can be used to diagnose MRSA isolates in hospitals. The presence of these genes may affect their pattern of sensitivity to antibiotics, especially with the increasing antibiotic resistance due to antibiotic misuse, and person-to-person transmission.

Author contributions:
The manuscript should mention the contribution of each author to the research done; Study conception & design: (Huda S.A. Al-Hayanni). Literature search: (Wed L. Khalil). Data acquisition: (Wed L. Khalil). Data analysis & interpretation: (Wed L. Khalil & Huda S.A. Al-Hayanni). Manuscript preparation: (Wed L. Khalil). Manuscript editing & review: (Wed L. Khalil & Huda S.A. Al-Hayanni).

Authors' declaration:
Conflicts of Interest: The authors declare no conflict of interest.

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for republication attached to the manuscript. Authors sign on ethical consideration’s approval-Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad according to the code number (5296/22) on (18/ 10/ 2022).

Limitations: The study was based on only two hospitals (Abu Ghraib General Hospital and Al Yarmouk Teaching Hospital) hence, the findings don’t represent the whole population.

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