Emerge of multiple SCCmec elements in clinical isolates of Community associated methicillin-resistant Staphylococcus aureus in Iraq.

Ali N Alsharefi¹, Khairallah A S Mohammed¹* and Yahya A Abbas ²

¹ Department of Medical Lab Technology, College of Health and Medical Technology, Southern Technical University, Basrah, Iraq
² University of Thi-Qar, Thi-Qar, Iraq

*Correspondence author: dr.kmohammed@stu.edu.iq (Mohammed)

Abstract

Background: Methicillin Resistance Staphylococcus aureus has gained worldwide disrepute as a hospital superbug and it has appeared as a problematic pathogen in the community setting as well. According to their SCCmec types and associated epidemiological and virulence factors, they can be classified into hospital-acquired MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA). Purpose: The objectives of the present was to study the diversity and distribution of SCCmec elements among MRSA isolates from outpatients and patients on admission into hospitals in at time of admission into hospitals. Methodology: The S. aureus clinical isolates were initially identified phenotypically using various biochemical tests and then this identification was confirmed by PCR using species-specific 16S rRNA primer pairs. Methicillin Resistant was determined using the disk diffusion method. All the identified isolates were subjected to mecA detection. Simplex PCR was optimized for all the major SCCmec types (I to V) and PCR products were sequenced to confirm our results. Results: Out of the 349 isolates, 75 (21.48%) were S. aureus. The antibiogram analysis of the isolated S. aureus strains indicated that these strains showed high resistance to oxacillin (93.33%), methicillin (90.66%) and 54 (72%) isolates possessed the mecA gene. The results showed that the predominate
SCCmec type was type 1V 45% (34/75) followed by type I 6.6% (5/75) and 48% (36/75) carried multiple SCCmec types (type I and type IV). Types II, III and V were not detected. **Conclusion:** The existence of multiple SCCmec types in individual MRSA isolates rose difficulties in using SCCmec typing criterion.

**Key Words:** PCR, MRSA-CA, multiple SCCmec types, Iraq.

**How to cite this article:** Alsharefi AN, Mohammed KAS, Abbas YA (2020): Emerge of multiple SCCmec elements in clinical isolates of community associated methicillin-resistant Staphylococcus aureus in Iraq, Ann Trop Med & Public Health; 23(S16): SP231616. DOI: [http://doi.org/10.36295/ASRO.2020.231615](http://doi.org/10.36295/ASRO.2020.231615)

**Introduction:**

*S. aureus* is one of the main nosocomial microbes, and it is one of most five pathogen exist as normal habitat of the skin nasal origin at a minimum 25-30% of healthy persons. It can causes a variety of infections continue from mild infections to severe life threatening infections such as endovascular diseases, toxic shock syndrome (tss) and staphylococcal scalded skin syndrome (SSSS).¹ Treatment of these infections has become more difficult because of the emergence of multidrug resistance strains (Lowy, 1998; Seifert, and Wisplinghoff 2008). CA-MRSA is different from other healthcare-associated MRSA (HA-MRSA) strains; as it affects various groups of patients, exhibits differing antimicrobial susceptibility patterns, and rapidly spreads among healthy individuals within a community.¹² According to the Centers for Disease Control and Prevention (CDC), CA-MRSA refers to isolates obtained from outside hospital or from a patient within 48 hours of admission to hospital.¹ In addition, we can distinguish HA-MRSA from CA-MRSA SCCmec typing approach. CA-MRSA isolates have a smaller SCCmec element, usually SCCmec type IV or type V. These smaller elements contain the mecA gene and are perhaps more moveable, they are resistant to fewer non-β-lactam antibiotics and frequently carry the pvl gene.³ Yet, comparatively in our region, not enough information is available...
regarding the characterization of CA-MRSA. Our study aimed to characterize \textit{S. aureus} isolates collected from outpatients or patients upon admission into hospitals. The objectives were to study the diversity and distribution of \textit{SCCmec} elements in these isolates.

**Materials and Methods**

**Bacterial isolates**

A total of 349 specimens were collected from patients at hospitals. The isolates were collected from urine samples (159), followed by tonsil swabs (90), nasal swabs (28), wound swabs (21), burn swabs (39), blood samples (3), vagina swabs (6), and sputum (3). Specimens were collected from outpatients or patients upon admission into hospital and were obtained with sterile swabs that were then cultivated in Petri dishes containing blood agar. These samples were incubated for 24 h at 37°C before inoculation onto mannitol salt agar (Oxoid) for isolation and identification of \textit{S. aureus}. Gram stains and other biochemical tests (Catalase, coagulase and API Staph System) were performed to confirm the identification of \textit{S. aureus}. The following patients were excluded: recent admission to hospitals, patients on haemodialysis, recent surgical operation, or presence of an intravenous cannula at the time of swab taking. Only patients who agreed to participate and provided informed consent were included in the study.

**Antibiotic susceptibility test**

Antibiotic susceptibility testing to Methicillin (10 µg) and Oxacillin (1 µg) was carried out on Mueller-Hinton agar (Oxoid Limited, Hampshire, England) using the Kirby-Bauer disk diffusion method, according to the recommendations by the Clinical and Laboratory standard Institute.
DNA isolation and PCR conditions

DNA extraction was carried out with a mercantile DNA isolation kit (Promega, USA) according to the manufacturer’s instructions.

**Simplex PCR:** Simplex PCR was used to amplify a 228 bp region of the 16S rRNA gene fragment of *S. aureus*, which is highly conserved in the species, and to detect *mecA* with specific primers. Also, it used to detect staphylococcal cassette chromosome *mec* Type IV (SCCmec Type IV), all primers used in this study and the size of PCR products shown in table (1)

The reaction mix had a final volume of 25 µl consisting of 2 µl (50-100 ng) DNA, 1 µl (20 pmol) of each primer, 12.5 µl of master mix (Taq DNA polymerase, dNTPs, MgCl₂ and reaction buffers; Promega) and 8.5 µl of nuclease free water.

The PCR amplification program was as follows: an initial denaturation step at 94°C for 4 min; followed by 35 cycles of denaturation at 94°C for 1 min; an annealing temperature for (*SCC*, *ccrC* and *dcs*) at 53°C for 30 second min, and for (*kdp, Cif, Ccrb2* and *MecA*) at 56°C for (30) second and an extension step at 72°C for 1 min, followed by a final extension at 72°C for 4 min.

**DNA Sequencing of PCR products**

All PCR products (20 µl) from All PCR product were sent to Macrogen Company (South Korea) for DNA sequencing. The alignment of DNA sequences was performed using BLAST (basic local alignment search tool) https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch., and Bioedit Sequence Alignment Editor program.
Results:

Out of the 349 clinical samples, 75 (21.48%) isolates were phenotypically identified as *S. aureus*, and this identification was confirmed by PCR using species-specific primers (Table 1). All 75 *S. aureus* (100%) samples exhibited positive results for the 16S rRNA gene and 54 (72%) samples possessed the *mecA* gene. The *S. aureus* isolates showed the most resistance to Oxacillin (70 samples, 93.33%), followed by Methicillin (68 samples, 90.66%).

All MRSA isolates were subjected to SCCmec typing using simples PCR. Out of the 75 sample 34 (45%) were type IV, 5 (6.6%) were type 1 and 36 (48%) were multiple types (I and IV). Types II, III and V were not detected.

Out of the five isolates type I, two isolates showed only CIF2 products, while three isolates showed *cif* and dcs products, but no amplification for other regions. CIF2 region is specific for type I whereas dcs is specific for type I, II, IV and VI. However, as these isolates did not show products for the types (*ccrB2*, *kdp*, SCCmec) which are specific for type IV and II respectively, they consider as type I. Furthermore, alignment of DNA sequences of PCR products using BLAST (basic local alignment search tool) program and BioEdit Sequence Alignment Editor showed 97% similarity *Staphylococcus aureus* DNA, type-I staphylococcal cassette chromosome mec - ID: AB033763.2:18468-18892.

Thirty-four I isolates showed PCR products either for *ccrB2* only (25) or together with dsc. The region *ccrB2* is specific to type II and IV, whereas dsc region is specific to I, II, IV, and VI. However, these isolates did not showed amplification to any of other regions. So they considered as type IV. Furthermore, alignment of DNA sequences of PCR products using BLAST program and BioEdit Sequence Alignment Editor showed 99% similarity with *Staphylococcus aureus* DNA, type-I staphylococcal cassette chromosome mec - ID: AB033763.2:18468-18892.
DNA, type-IV.1 (IVa) staphylococcal cassette chromosome mec - ID: AB063172.2:11696-11959.

Thirty-six isolates showed PCR product for both cif and ccrB2 regions in the same isolate, 11 from 36 these isolates positive for CIF2 and ccrB2, while other 25 isolates were positive for CIF2, dsc and ccrB2, but negative for other regions. The alignment of DNA sequences of PCR products showed 99% similarity Staphylococcus aureus DNA, type-I staphylococcal cassette chromosome mec - ID: AB033763.2:18468-18892 and type-IV.1 (IVa) staphylococcal cassette chromosome mec - ID: AB063172.2:11696-11959.

The association between SCCmec types the presence of mecA gene shown in table 2. The results suggest that the isolates with SCCmec types IV and multiple type (I +IV) are significantly resistant to methicillin and oxacillin.

![Simplex PCR](image)

**Figure 1.** Simplex PCR for SCCmec types on, lanes 1, 4, 7, 13 bands CIF2 gene (495bp), lanes 5, 11, 14 dcs gene (342bp), lanes 9, 12, 15, ccrB2 gene (311bp).
Discussion:

In the present study, out of 349 clinical samples, 75 (21.48%) isolates were identified as multidrug-resistant *S. aureus*. These results are comparable to the incidence found in northern Iraq (22.3%)\(^{11}\) and Palestine (24%),\(^{12}\) but this is lower than the results reported by Huang *et al.*, in the USA (42%).\(^{13}\) Only 72% of the isolates identified as MRSA were positive for the *mecA* gene. This could indicate that these MRSA isolates have a different mechanism for methicillin resistance than through the *mecA* gene.

The results of the antibiotic sensitivity tests revealed high resistance to oxacillin (93.33%) and methicillin (90.66%), which is adjacent to the results (81%) stated by Fey *et al.* in USA,\(^{14}\) but differs from the findings of other studies in South Africa (73.3%) and Kenya (29%) reported by Akanbi *et al.*\(^{15}\) and Gitau, *et al.*,\(^{16}\) respectively.

The incidence of the *SCCmec* types found in this study suggest that *SCCmec* type IV is the abundant type (52%) among MRSA in our community, while the *SCCmec* element of type IV was only documented in 10.5% of the isolates studied by Nagasundaram and Sistla.\(^{17}\) on other hand, *SCCmec* type IV was found in 78.6 and 43.5% of the MRSA isolates from the Philippines and the Republic of Korea.\(^{18}\) Our result indicate the absence of type II which is similar to those reported by Lawung *et al.* from Thailand, in which *SCCmec* type II was not found.\(^{19}\) On the other hand, this was in complete contrast to a study by Buntaran *et al.* from Indonesia, which reported that *SCCmec* type II is the predominate type.\(^{20}\) In contrast to our, studies reported that the predominant *SCCmec* type in most Asian countries except Japan and the Republic of Korea is type III.\(^{21}\) In addition, our results showed the existence of the *SCCmec* multiple type (48%) in our region, which is comparable with those obtained by Nagasundaram and Sistla.\(^{17}\) Few isolate
(6.6%) possess the elements of SCCmec type I. The frequency of the SCCmec types found in this study compared with data in the literature shown in table 3.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

No conflicts of interest to declare

**Funding Statement**

No funding is associated with this study.

**Ethical Approval**

The proposal on which the paper is based was approved by the Health Research and Ethical Committee in Thi-Qar province. Written consent was obtained from all participants.

**Acknowledgment:** The authors thank Ms Zahraa H Abdulkareem for her excellent technical help.

**References**


4. Gaillot O, Wetsch M, Fortineau N, Berche P. Evaluation of CHROMagar Staph. aureus, a new chromogenic medium, for isolation and presumptive identification of


6. Clinical Laboratory and Standards Institute. M100-S29 Performance Standards for antimicrobial susceptibility testing; Twenty-ninth informational supplement; Wayne 2019.


13. Huang H, Flynn NM, King JH, Monchaud C, Morita M, Cohen SH. Comparisons of community-associated meticillin-resistant Staphylococcus aureus (MRSA) and hospital-


### Table 1. Primer used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequencing 5’to 3’</th>
<th>SCCmec type, region</th>
<th>Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIF2</td>
<td>F TTCGAGTTGCTGATGAAGAAGG</td>
<td>I, J1 region</td>
<td>495</td>
<td>Oliveira et al.⁷</td>
</tr>
<tr>
<td></td>
<td>R ATTTACCACAAGGACTACCAGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CcrC</td>
<td>GTACTCGTTACAATGTTTGG F</td>
<td>V, ccr complex</td>
<td>449</td>
<td>Milheirico et al.⁸</td>
</tr>
<tr>
<td></td>
<td>ATAATGGCTTCATGCTTACC R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dcs</td>
<td>F CATCCTATGATAGCTTGTC</td>
<td>I, II, IV, and VI, J3 region</td>
<td>342</td>
<td>Oliveira et al.⁷</td>
</tr>
<tr>
<td></td>
<td>R CTAATCATAGCCATGACCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CcrB2</td>
<td>F AGTTTCTCAGAAAATCGAAGC</td>
<td>II and IV, ccr complex</td>
<td>311</td>
<td>Milheirico et al.⁸</td>
</tr>
<tr>
<td></td>
<td>R CGATATAGAAAAGGTTAGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kdp</td>
<td>F AATCATCTCAGAAATCGAAGG</td>
<td>II, J1 region</td>
<td>284</td>
<td>Oliveira et al.⁷</td>
</tr>
<tr>
<td></td>
<td>R CGAATGAAATGAAAGATGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec</td>
<td>F CATTGGTGAAACACAGTACG</td>
<td>III, J1 region</td>
<td>243</td>
<td>Milheirico et al.⁸</td>
</tr>
<tr>
<td></td>
<td>R GATTGGAGACTCCTAAAGC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Association \textit{mecA} gene with \textit{SCCmec} types

<table>
<thead>
<tr>
<th>SCCmec type</th>
<th>mecA +</th>
<th>mecA -</th>
</tr>
</thead>
<tbody>
<tr>
<td>type I</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>type IV</td>
<td>20 (58.8%)</td>
<td>14 (41.2%)</td>
</tr>
<tr>
<td>Multiple types (I + IV)</td>
<td>31 (86.1%)</td>
<td>5 (13.8%)</td>
</tr>
</tbody>
</table>

Table 3. Incidence of the \textit{SCCmec} types found in this study compared with data in the literature

<table>
<thead>
<tr>
<th>SCCmec types</th>
<th>Incidence in this study</th>
<th>Incidence in other study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>6.67%</td>
<td>3%</td>
<td>Asghar \textsuperscript{22}</td>
</tr>
<tr>
<td>Type II</td>
<td>0%</td>
<td>0%</td>
<td>Lawung \textit{et al.}\textsuperscript{19}</td>
</tr>
<tr>
<td>Type III</td>
<td>0%</td>
<td>0.6%</td>
<td>Japoni \textit{et al.}\textsuperscript{24}</td>
</tr>
<tr>
<td>Type IV</td>
<td>45.33%</td>
<td>43.5%</td>
<td>Song \textit{et al.}\textsuperscript{18}</td>
</tr>
<tr>
<td>Type V</td>
<td>0%</td>
<td>1%</td>
<td>Rebic \textit{et al.}\textsuperscript{23}</td>
</tr>
<tr>
<td>Multiple types</td>
<td>48%</td>
<td>59%</td>
<td>Nagasundaram \textit{et al.}\textsuperscript{17}</td>
</tr>
</tbody>
</table>