Association between SCCmec types and antimicrobial resistance in clinical MRSA isolates

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Abstract

Background

MRSA is the causative agent of serious infections. MRSA isolates carry mecA gene which confers resistance to all β-lactams, markedly limiting the therapeutic options. SCCmec typing enables strain-based MRSA identification. This study aimed to identify the prevalent SCCmec types among clinical MRSA isolates in Alexandria, Egypt, and their association with antibiotic resistance. One hundred MRSA clinical isolates were identified and tested for susceptibility to antibiotics. SCCmec typing was done by PCR using previously published primers.

Results

Typeability was 75 %. SCCmec type V was the most predominant (45.3%), with a significant association with pyogenic lesions (53%, MCp <0.001). SCCmec type IV was significantly associated with nasal colonizers (50%, MCp 0.049). SCCmec type II was the most prominent in bloodstream infection (33 %). Various antibiotic resistance patterns were detected. SCCmec types II and III displayed the highest resistance, while SCCmec type IV showed the least resistance. There was a significant association between SCCmec types and antibiotic resistance (p = 0.02-0.001).

Conclusions

The only SCCmec types detected by PCR were SCCmec II-VI, with high resistance to gentamicin among all types. SCCmec type V was the most prevalent and was of relatively low resistance to antibiotics. SCCmec type IV was the least prevalent and showed the least resistance to antibiotics. There was a significant association between SCCmec types II and III and resistance to fluoroquinolones. Macrolides resistance was significantly associated with SCCmec type II. Tetracyclines resistance was significantly associated with SCCmec type III.

1. Background

MRSA has been recognized as a causative agent of a diversity of serious hospital and community-acquired infections, particularly pyogenic infections of the skin. It can also cause infections associated with medical instruments such as central-line-associated bloodstream infection. The rising threat of antibiotic resistance in Methicillin-resistant Staphylococcus aureus (MRSA) has been an impetus for research to unveil any associations that might have prognostic implications and may partially guide empirical therapeutic decisions. [1].

Clinically, resistance against many antibiotic classes is considered one of the characteristic features of MRSA infection, as it carries an altered form of penicillin-binding protein; PBP2a, which renders it less sensitive to most semisynthetic penicillin drugs. This protein is expressed via an acquired gene named mecA, which is carried within a highly conserved mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec). [2] [3].

MRSA has been known as a healthcare-associated (HA) infectious agent with high predominance all over the world since its emergence in 1960 [4]. It was highly implicated in multidrug-resistant healthcare-associated infections [5], unlike community-acquired MRSA (CA-MRSA) which first emerged in the 2000s. CA-MRSA occurred in either healthy individuals or any individual within two days of admission to the hospital with no history of any hospitalizations, surgeries, or long-term care facility stays in the previous year, as per the definition published by the CDC in 2005 [6].

Nowadays, CA-MRSA healthcare-associated outbreaks have been recorded in several countries worldwide, causing remarkable changes in the epidemiological distribution of MRSA worldwide and implying an increasingly difficult
distinction between CA-MRSA and HA-MRSA based on the aforementioned description [7]. Hence, the true prevalence of this community-dwelling organism may be underestimated or exaggerated [8]. Accordingly, it is now preferred to establish a strain-based definition for CA-MRSA because of its distinct epidemiology, genetic profile, antibiotic resistance pattern, and clinical presentation [6].

Bacterial typing is an indispensable epidemiologic tool that enables the identification of bacteria at the strain level, elaborating clonal relationships between them. It may be done phenotypically by methods such as antibiogram typing or serotyping. Alternatively, bacteria may be typed more precisely by genotypic methods, based on analyzing variations in the genetic elements [9].

Staphylococcal Chromosomal Cassette mec (SCCmec) typing is one of the well-recognized MRSA genotyping methods. It is based on the identification of the SCCmec element, which is carried on a genomic island that can easily transfer horizontally between strains by the site-specific action of two recombinases. SCC consists of 3 components; (i) mec gene complex, (ii)Ccr (cassette chromosome recombinase) gene complex, and (iii) J regions [10].

mec gene complex encompasses the mec gene, insertion sequences (IS), and the regulatory components mecR1 (signal transducer protein) and mecI (repressor protein). There are five known classes of the mec gene complex (A – E) based on variations in the assortments of insertion sequences and regulatory regions [10].

Ccr gene complex contains 8 open reading frames in addition to ccr gene(s). Three ccr allotypes have been detected in staphylococci (A-C). There are nine different forms of ccr gene complexes based on different combinations of ccr allotypes: 1 (A1B1), 2 (A2B2), 3 (A3B3), 4 (A4B4), 5 (C), 6 (A5B3), 7 (A1B6), 8 (A1B3), and 9 (C2).[10]

J regions are joining or junk regions that represent the third component of SCC. Despite being considered unessential components of the complex, they may contain determinants for additional antimicrobial resistance. SCCmec subtypes are defined by differences in the J-region DNA segment [6].

A unified nomenclature scheme for the cassette types has been established. SCCmec is the outcome of integrating the mec gene complex classes with the ccr gene complex types to categorize SCCmec components into types. There are thirteen different forms of SCCmec (I-XIII) found in MRSA strains so far [6].

SCCmec typing has recently become part of the well-recognized nomenclature of MRSA, which enables getting information about SCCmec-typed MRSA isolates. SCCmec typing can be performed by Whole-genome sequencing and subsequent data analysis using bioinformatics tools such as SCCmecFinder. However, the conventional method of SCCmec typing using conventional PCR remains to be more widely applied, particularly in low-income countries. [11]

This study aimed to identify the SCCmec types of MRSA strains causing clinical infection and/or nasal colonization state and their associated antibiotic resistance. The study was performed on 100 non-repetitive MRSA strains randomly collected from different types of clinical specimens submitted for routine culture and antimicrobial susceptibility testing to the Microbiology laboratory of the Medical Research Institute, Alexandria University. MRSA strains were collected over the period from September 2019 to March 2021, disregarding the source of infection being healthcare-associated or community-acquired.

2. Results
The 100 MRSA isolates collected during the study period included 60 isolates from pyogenic skin infection including abscess aspirates and wound swabs, 14 from bloodstream infection, 9 from lower respiratory tract infection, and 5 from urinary tract infection, in addition to 12 colonizing isolates collected from nasal swabs.

2.1. Antimicrobial susceptibility testing results:

Antimicrobial resistance patterns to the 14 tested antibiotics, other than cefoxitin, varied among the 100 MRSA isolates. The highest resistance among all isolates was to gentamicin (71%), followed by Tetracycline (44%), while the highest sensitivity was to vancomycin (100%), linezolid (97%), and rifampicin (93%). Resistance to fluoroquinolones was detected in 23–24% of the isolates, while 10% were resistant to trimethoprim/sulfamethoxazole. As for macrolides, resistance was detected in 23–25%. Regarding clindamycin, out of the 100 MRSA isolates under study; 85% were sensitive, while 8% were constitutively resistant to clindamycin and 4% showed induced resistance by positive D-test (Table 1).

<table>
<thead>
<tr>
<th>Antibiotics (OXOID™, Thermo Scientific™)</th>
<th>No. of samples</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin (FOX, 30 µg)</td>
<td>100</td>
<td>100</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin (CN, 10 µg)</td>
<td>100</td>
<td>71</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Azithromycin (AZM, 15 µg)</td>
<td>95*</td>
<td>24</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>Clarithromycin (CLR, 15 µg)</td>
<td>95*</td>
<td>23</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>Erythromycin (E, 15 µg)</td>
<td>95*</td>
<td>25</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>Clindamycin (DA, 2 µg)</td>
<td>100</td>
<td>12</td>
<td>3</td>
<td>85</td>
</tr>
<tr>
<td>Tetracycline (TE, 30 µg)</td>
<td>100</td>
<td>44</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>Doxycycline (DO, 30 µg)</td>
<td>100</td>
<td>29</td>
<td>2</td>
<td>69</td>
</tr>
<tr>
<td>Minocycline (MH, 30 µg)</td>
<td>100</td>
<td>9</td>
<td>15</td>
<td>76</td>
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<tr>
<td>Ciprofloxacin (CIP, 5 µg)</td>
<td>100</td>
<td>23</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>Levofloxacin (LEV, 5 µg)</td>
<td>100</td>
<td>24</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole (SXT, 1.25/23.75 µg)</td>
<td>100</td>
<td>10</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>Rifampicin (RD, 5 µg)</td>
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<td>5</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>Linezolid (LZD, 30 µg)</td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>Vancomycin (6 µg/ml) **</td>
<td>100%</td>
<td>100</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

* Macrolides (Azithromycin, Erythromycin and Clarithromycin) were not tested with the 5 isolates from urine.

** Testing was done by Vancomycin screening agar according to CLSI guidelines [13].
The 100 MRSA isolates showed different antimicrobial resistance patterns, the two most prominent resistance patterns were resistance to gentamicin, doxycycline, and Tetracycline, as well as resistance to gentamicin only (17%) each. This was followed by resistance to gentamicin and Tetracycline (12%). On the other hand, 14% of the isolates were sensitive to all tested antibiotics other than cefoxitin (Table 2).
<table>
<thead>
<tr>
<th>Antibiotic resistance patterns</th>
<th>All isolates (No.)</th>
<th>Untyped isolates (No.)</th>
<th>SCCmec types</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DO, MH, DA, RIF, TE</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DO, MH, TE, DA</td>
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<td>3</td>
<td>1</td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DA, RIF, TE, SXT</td>
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<td>1</td>
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</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DA, SXT, RIF</td>
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<td>1</td>
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</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, RIF, SXT</td>
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<td>1</td>
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<tr>
<td>CN, AZM, CLR, E, CIP, LEV, TE</td>
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<tr>
<td>CN, AZM, CLR, E, DA</td>
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</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, SXT</td>
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<td>1</td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV</td>
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<tr>
<td>AZM, CLR, E, DO, TE, DA</td>
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<tr>
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<td>3</td>
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<tr>
<td>AZM, CLR, E, CIP, LEV</td>
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<td>1</td>
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<td>CN, E, CIP, LEV, DA</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>CIP, LEV, DO, MIN, TE</td>
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<td>1</td>
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<tr>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>CN, DO, TE, RD</td>
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<td></td>
</tr>
<tr>
<td>CN, DO, TE</td>
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<td>1 5 7</td>
</tr>
<tr>
<td>CN, TE</td>
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<td>CN, DA</td>
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<td>1</td>
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</tr>
<tr>
<td>CN, E</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CIP, LEV</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TE</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SXT</td>
<td>4</td>
<td>1</td>
<td>2 1</td>
</tr>
<tr>
<td>CN</td>
<td>17</td>
<td>6</td>
<td>3 1 7</td>
</tr>
<tr>
<td>No resistance</td>
<td>14</td>
<td>3</td>
<td>1 4 5 1</td>
</tr>
</tbody>
</table>
2.2. Molecular identification and typing of MRSA strains:

$mecA$ gene was successfully amplified in the 100 MRSA isolates included in this study by conventional PCR. SCC$mec$ typing of MRSA isolates was done by observing bands specific to each SCC$mec$ type by conventional PCR and only the typed isolates were confirmed by SYBR- Green real-time PCR to ensure the specificity of amplification by melting curve analysis. Out of the 100 MRSA isolates, only 75 (%) were successfully SCC$mec$-typed using previously published primers specific to each of SCC$mec$-types I-XII (Table 3).
### Table 3
Sequence of primers used in this study

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotide sequences</th>
<th>Amplicon size (bp)</th>
<th>Annealing temp. (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>mec A F</td>
<td>mecA CCTAGTAAAGCTCCGGAA</td>
<td>331</td>
<td>53</td>
<td>[16]</td>
</tr>
<tr>
<td>mec A R</td>
<td>CTAGTCCATTCCGCTCCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I-F</td>
<td>SCC mec I GCTTTAAAGAGTGCTCGTTACAGG</td>
<td>613</td>
<td>50</td>
<td>[17] and [18]</td>
</tr>
<tr>
<td>Type I-R</td>
<td>GTTCTCTCATAGTATGACGTCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II-F</td>
<td>SCC mec II CGTTGAAGATGATGAAGCG</td>
<td>398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II-R</td>
<td>CGAAATCAATGGTTAATGGACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III-F</td>
<td>SCC mec III CCATATTGTGTACGATGC</td>
<td>280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III-R</td>
<td>CCTTAGTTGTCGTAACAGATCG</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Type IV-F</td>
<td>SCC mec IV GCCTTATTGGAAGAACCG</td>
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<td>53</td>
<td></td>
</tr>
<tr>
<td>Type IV-R</td>
<td>CTACTCTTCTGAAAAGCGTCC</td>
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<td></td>
<td></td>
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<tr>
<td>Type V-F</td>
<td>SCC mec V GAACATTGTTACTTAAATGAGCG</td>
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<td>50</td>
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<td>Type V-R</td>
<td>TGAAAGTTGTACCTTGACACC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>mec F</td>
<td>SCC mec VI CGTTATAAGTGTAACGATGGTTTTTTG</td>
<td>126</td>
<td>54</td>
<td>[19]</td>
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<tr>
<td>mec I R</td>
<td>TCATCTGCAGAATGGGAAGTT</td>
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<td></td>
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<tr>
<td>ccrB4 F</td>
<td>CGAAGTATAGACACTGGAGCGATA</td>
<td>134</td>
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<tr>
<td>ccrB4 R</td>
<td>GCGACTCTCTTGGCGTTTA</td>
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<td></td>
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</tr>
<tr>
<td>IS1272J-F</td>
<td>GAAGCTTTGGGCCGATAAAGA</td>
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<td></td>
<td></td>
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<tr>
<td>IS1272J-R</td>
<td>GCACGTCTCGTTTAGACCAATC</td>
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<tr>
<td>Type VII-F</td>
<td>SCC mec VII GTGACGTGGATATTGCAGTGG</td>
<td>473</td>
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<td>[20]</td>
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<tr>
<td>Type VII-R</td>
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<td></td>
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<tr>
<td>Type VIII-F</td>
<td>SCC mec VIII AGCGACGTGAACAAACACCGCTACTTACTCAA</td>
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<tr>
<td>Type VIII-R</td>
<td>TGGTTGGAATGAGAACAGTGTTAAGATC</td>
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<tr>
<td>Type IX-F</td>
<td>SCC mec IX TGGCATGGTTGATAGAACAGTG</td>
<td>642</td>
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<td>Primers</td>
<td>Nucleotide sequences</td>
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<td>Annealing temp. (℃)</td>
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<td>----------------------</td>
<td>--------------------</td>
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<td>------------</td>
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<tr>
<td>Type IX R</td>
<td>TCACTAATTTTGCTCCTACGTCT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Type X F SCCmec X</td>
<td>ATTTACGCGGATGCGTTGAC</td>
<td>708</td>
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<tr>
<td>Type X R</td>
<td>TATGCGATTGCGCAGGTGAT</td>
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<td></td>
<td></td>
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<tr>
<td>Type XI F SCCmec XI</td>
<td>GGCGATAACAACGACACATCC</td>
<td>255</td>
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<tr>
<td>Type XI R</td>
<td>TGTTAGTGCTTTGACGCTCTT</td>
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<tr>
<td>Type XII F SCCmec XII</td>
<td>AGAAGACGGAGGACATCGACA</td>
<td>371</td>
<td></td>
<td></td>
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<tr>
<td>Type XII R</td>
<td>TCGCTTCTTCACGCCATCTT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Among the 75 typed MRSA isolates: SCCmec type V (45.3%) was the most frequently encountered, followed by SCCmec type VI (16%), SCCmec types II and III which were found each in 13.3% of the isolates, and SCCmec type IV in 12% of the isolates. None of the isolates gave amplicons specific to SCCmec types I, VII, VIII, IX, X, XI, and XII by conventional PCR.

The typed isolates included only 45/60 isolates from pyogenic skin lesions, 12/14 isolates from bloodstream infection, 7/9 from lower respiratory tract infection, and all 5 isolates from urinary tract infection. As for the 12 nasal colonizers, only 6 isolates were typeable.

### 2.3. Statistical correlation between SCCmec types and clinical condition

A statistically significant association was found between SCCmec types and pyogenic skin infection ($MC_p < 0.001$), as SCCmec type V MRSA was the most prominent among all isolates from pyogenic skin lesions, isolated from 24/45 (53%) of the lesions. Type V was also the most prominent among isolates from lower respiratory tract infection 3/7 (43%), as well as urinary tract infection 3/5 (60%). As for bloodstream infection, type II was the most prominent 4/12 (33%), followed by type V 3/12 (25%). No statistically significant association was found between SCCmec types and different types of clinical infection other than pyogenic skin lesions. In nasal colonization, however, type IV was the most prominent 3/6 (50%), with a statistically significant association, $MC_p = 0.049$ (Table 4).
Table 4
Correlation between SCCmec types and their source clinical condition

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of typed isolates</th>
<th>Type II (n = 10)</th>
<th>Type III (n = 10)</th>
<th>Type IV (n = 9)</th>
<th>Type V (n = 34)</th>
<th>Type VI (n = 12)</th>
<th>MCP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Pyogenic lesions</td>
<td>45</td>
<td>3 13.6</td>
<td>7</td>
<td>15.5</td>
<td>3</td>
<td>6.7</td>
<td>24</td>
</tr>
<tr>
<td>Blood stream infection</td>
<td>12</td>
<td>4 33.3</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
<td>16.6</td>
<td>3</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>6</td>
<td>1 16.6</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
<td>50.0</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory tract infection</td>
<td>7</td>
<td>1 14.2</td>
<td>2</td>
<td>28.5</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td>Urinary tract infection</td>
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<td>1 20.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>20.0</td>
<td>3</td>
</tr>
</tbody>
</table>

*p: p value for Chi square test (Monte Carlo) association between different categories

*: Statistically significant at p ≤ 0.05

2.4. Statistical correlation between SCCmec types and antibiotic resistance

Concerning Antimicrobial resistance, SCCmec types II and III had the highest resistance. SCCmec type II was resistant mainly to gentamicin, macrolides (p = 0.002 - 0.001) followed by fluoroquinolones (p < 0.001). SCCmec type III showed high resistance to fluoroquinolones (p < 0.001) followed by gentamicin and Tetracyclines (p < 0.001). On the other hand, SCCmec type IV showed the least resistance to antibiotics followed by SCCmec type V and VI (Table 5). Intermediate susceptibility to Linezolid was detected in 3 isolates, that were of SCCmec types III, V, and VI.
Table 5
Correlation between SCCmec types and antibiotic resistance

<table>
<thead>
<tr>
<th>SCCmec types (n = 75)</th>
<th>No.</th>
<th>Type II (n = 10)</th>
<th>Type III (n = 10)</th>
<th>Type IV (n = 9)</th>
<th>Type V (n = 34)</th>
<th>Type VI (n = 12)</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Gentamicin (CN)</td>
<td>54</td>
<td>9 90.0</td>
<td>8 80.0</td>
<td>3 33.3</td>
<td>24 70.6</td>
<td>10 83.3</td>
<td>0.075</td>
</tr>
<tr>
<td>Azithromycin (AZM)</td>
<td>19</td>
<td>7 70.0</td>
<td>5 50.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>Clarithromycin (CLR)</td>
<td>18</td>
<td>7 70.0</td>
<td>4 40.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.002*</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>19</td>
<td>7 70.0</td>
<td>5 50.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>Clindamycin (DA)</td>
<td>11</td>
<td>0 0.0</td>
<td>4 40.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.133</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>35</td>
<td>0 0.0</td>
<td>6 60.0</td>
<td>2 22.2</td>
<td>17 50.0</td>
<td>10 83.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Doxycycline (DO)</td>
<td>24</td>
<td>0 0.0</td>
<td>6 60.0</td>
<td>1 11.1</td>
<td>8 23.5</td>
<td>9 75.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Minocycline (MH)</td>
<td>9</td>
<td>0 0.0</td>
<td>6 60.0</td>
<td>0 0.0</td>
<td>2 5.9</td>
<td>1 8.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>22</td>
<td>6 60.0</td>
<td>9 90.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Levofloxacin (LEV)</td>
<td>23</td>
<td>6 60.0</td>
<td>9 90.0</td>
<td>1 11.1</td>
<td>5 14.7</td>
<td>2 16.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Trimethoprim/ Sulfamethoxazole (SXT)</td>
<td>9</td>
<td>3 30.0</td>
<td>1 10.0</td>
<td>3 33.3</td>
<td>1 2.9</td>
<td>1 8.3</td>
<td>0.020*</td>
</tr>
<tr>
<td>Rifampicin (RD)</td>
<td>5</td>
<td>1 10.0</td>
<td>0 0.0</td>
<td>1 11.1</td>
<td>0 0.0</td>
<td>3 25.0</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

\( \chi^2 \): p value for Chi square test (Monte Carlo) association between different categories

*: Statistically significant at \( p \leq 0.05 \)

Most of the isolates with the same SCCmec type displayed the same pattern of resistance to antibiotics. For instance, simultaneous resistance to gentamicin and tetracycline was displayed by 8 isolates typed as SCCmec type V, also resistance to gentamicin, doxycycline, and tetracycline was displayed by 5 isolates of SCCmec type V and 7 isolates of SCCmec type VI (Table 2).

3. Discussion

MRSA infection is of global concern worldwide. Epidemiologic studies about MRSA rely on the use of standard nomenclature that identifies the prevailing strains at the chromosomal level [11]. SCCmec typing is one of the internationally recognized MRSA typing methods. [12, 13].
Pyogenic skin infection is the most common clinical presentation of MRSA infection. Sixty percent of the isolates in this study were collected from pyogenic skin lesions, followed by bloodstream infection (14%), lower respiratory tract infection (9%), and urinary tract infection (5%). Another study about MRSA in Egyptian hospital laboratories also reported a similar proportion of isolates from pyogenic lesions (64.3%) and bloodstream infection (9.5%) [14]. Similarly, it was reported in Kuwait that the majority of MRSA isolates were from wounds and pus, followed by blood [15]. Also, in the United Arab Emirates, pyogenic lesions and bloodstream infection were the sources of 73.4% and 15.2% of MRSA isolates, respectively [16].

Seventy-five percent of our isolates were SCC\textit{mec} typeable by PCR. Several studies worldwide employed SCC\textit{mec} typing by PCR for identification of the prevailing SCC\textit{mec} types in their regions and reported varying degrees of typeability that were all less than 100%. For instance, a study in Denmark reported 98% typeability by multiplex PCR [17]. Another study in Portugal reported 97.4% typeability [18]. A more recent study in Palestine reported a typeability of 96.4%[19]. Also in Alexandria, Mansoura, and Cairo, Egypt, the reported typeability was 90%, 94% and 88.8%, respectively. [20]. [21]. [22]. A lower percentage of typeability (77%) was reported by a study in Rwanda [23], which was close to the findings of the current study.

The high percentage of isolation of SCC\textit{mec} type V (45.3%) followed by SCC\textit{mec} type IV (16%) and types II and III (13.3% each) among the 75 typeable MRSA isolates in our study was in accordance with the findings of several studies, worldwide. A recent study in a tertiary hospital in Cairo, Egypt, reported that half of their MRSA isolates were SCC\textit{mec} type V (50%) followed by SCC\textit{mec} type VI (17%) [22]. Also, a study carried out in four University Teaching Hospitals in Iran, reported that SCC\textit{mec} type V was the most prevalent (66.7%) among their clinical MRSA isolates [24]. Moreover, other studies conducted in Armenia [25], and in Iran [26] stated that, SCC\textit{mec} types V and VI were the most identified among MRSA isolated from hospitals.

Consistently, a study in Saudi Arabia reported the detection of SCC\textit{mec} type IV in 77.3% of their isolates, followed by SCC\textit{mec} type V (13.2%), and type III (9.4%) [12]. Similarly, a study in Kuwait reported that the majority of their isolates belonged to SCC\textit{mec} type IV (39.5%) followed by SCC\textit{mec} type III (34.4%) [15]. In Africa, a study assessed the SCC\textit{mec} types in correlation with spa types and reported that isolates of the common spa types harbored SCC\textit{mec} types IV followed by type V, with a minority harboring SCC\textit{mec} type I.[27]

Conversely, a study in Alexandria conducted on 72 MRSA isolates collected over 4 months in 2015, reported that 57% of their MRSA isolates harbored SCC\textit{mec} type III and only 11% were of SCC\textit{mec} type V [20]. The discrepancy between their most prevalent SCC\textit{mec} type (type III) and our results (type V) may be attributed to the fact that the study was conducted 4 years earlier, and it focused mainly on typing of MRSA isolates collected from healthcare-associated infections which represented 80% of their typed isolates. On the other hand, our study intentionally disregarded the source of infection, and typing was performed on randomly selected isolates including nasal colonizers, to allow for a better representation of the SCC\textit{mec} types prevalent in Alexandria, Egypt.

SCC\textit{mec} type I was not detected in any of our isolates. Despite being undetected in Egypt and nearby regions, a study on a small scale in Rwanda, reported the detection of SCC\textit{mec} type I in 56% of the 39 MRSA isolates included in their study. They also reported that SCC\textit{mec} type IV was the second most common type among their isolates (17.9%), while SCC\textit{mec} types II and V were undetectable [23].

Apart from that, a study in Hungary stated that SCC\textit{mec} type IV accounted for the vast majority of their MRSA isolates (66.7%), followed by SCC\textit{mec} type II (23.5%), and SCC\textit{mec} type I (9.2%). They reported that SCC\textit{mec} type V was detected in only one isolate, while SCC\textit{mec} types III and VI were not found [28].
The discrepancy in the distribution of SCCmec types reported from different geographic regions, and even from the same region at different points of time, can be attributed to the high plasticity of this region, and the limited capabilities of the conventional PCR detection method, in addition to the differences in the sensitivity and specificity of the primers used, which may eventually result in missed identification of some SCCmec types.

In the present study, SCCmec type V isolates were the most predominantly isolated (53%) from pyogenic skin lesions, with a statistically significant correlation ($p < 0.001$). This was in accordance with the findings reported by a study in Mansoura University Hospital which stated that SCCmec type V is significantly associated with burns and abscesses, and of a moderate association with wound sources[21].

SCCmec IV showed the least resistance to antibiotics, while SCCmec types II and III displayed the highest resistance to antibiotics and were significantly associated with resistance to fluoroquinolones ($p < 0.001$). The association between SCCmec type III and fluoroquinolones resistance was in accordance with the findings of previous studies in Egypt and Iran [20, 29].

Similarly, in Hungary, it was reported that SCCmec type II is associated with the highest level of resistance to antibiotics while SCCmec type IV is associated with low resistance [28]. Also, a Russian study reported that Isolates carrying SCCmec type III demonstrated higher antibiotic resistance than SCCmec type IV [30].

The most common resistance patterns among our isolates were; resistance to gentamicin only, and simultaneous resistance to gentamicin, doxycycline, and Tetracycline, each detected in 17% of the isolates. Contrary to our findings, a study conducted in a Hungarian tertiary care hospital reported that the most prevalent phenotype of resistance was to erythromycin, clindamycin, and ciprofloxacin [28]. On the other hand, a study in Kuwait reported that a high proportion of their isolates was resistant to tetracycline, erythromycin, ciprofloxacin, and trimethoprim/sulfamethoxazole [15].

Our isolates displayed very high resistance to gentamicin (71%), with no statistical difference between different SCCmec types. This was followed by resistance to tetracycline (44%). Resistance to fluoroquinolones and macrolides was less (23–25%), while resistance to trimethoprim/sulfamethoxazole (10%) and Rifampicin (5%) was low. All isolates were susceptible to vancomycin, however, 3 isolates displayed intermediate susceptibility to Linezolid. This could be probably due to the over-prescription of this drug by physicians in Egypt.

In Spain, it was reported that ciprofloxacin resistance was the highest (85%) in MRSA, followed by erythromycin resistance (65%), gentamicin resistance (35%), and tetracycline resistance (30%). All MRSA strains were susceptible to trimethoprim/sulfamethoxazole and rifampicin, which was not far from our susceptibility results for these 2 antibiotics [31]. Also, a study in Palestine reported that resistance to erythromycin in MRSA was 63.4%, and to ciprofloxacin was 39.3%, with 18.8% resistance to trimethoprim/sulfamethoxazole [19].

Constitutive clindamycin resistance was displayed by 8% of our isolates, while 4% showed inducible resistance with a positive D-test. The percentage of clindamycin resistance was slightly higher in a study conducted in Spain which reported that 11.7% of their MRSA isolates have inducible clindamycin resistance [31]. Even higher percentages were reported in Kuwait, where the authors reported that inducible and constitutive clindamycin resistance among their MRSA isolates were 14.4% and 37.8%, respectively [15].

4. Conclusions
The only SCC\textit{mec} types detected by PCR were SCC\textit{mec} II-VI, with high resistance to gentamicin among all types. SCC\textit{mec} type V was the most prevalent and was significantly associated with pyogenic lesions and of relatively low resistance to antibiotics. SCC\textit{mec} type IV was the least prevalent and showed the least resistance to antibiotics. There was a significant association between SCC\textit{mec} types II and III and resistance to fluoroquinolones. Macrolides resistance was significantly associated with SCC\textit{mec} type II. Tetracyclines resistance was significantly associated with SCC\textit{mec} type III.

5. Methods

5.1. Bacterial isolates

Primary isolation of 100 \textit{Staphylococcus aureus} strains from clinical specimens was done by culture on Blood agar plates. Identification was done by colony morphology, and the characteristic microscopic morphology of Gram-stained films. This was further confirmed by the positive reaction to biochemical tests; namely, catalase test, slide coagulase test, tube coagulase test and mannitol fermentation [32].

\textit{S. aureus} colonies were tested for methicillin resistance by the Kirby-Bauer method using cefoxitin disc (30 ug). Only cefoxitin-resistant isolates (≤ 21mm) after 16–18 hours were identified as MRSA and included in this study [33]. Subculture on ORSAB (Oxacillin Resistance Screening Agar Base) and observation of the characteristic blue colonies of MRSA was also performed as a further confirmatory step for phenotypic identification [34].

The Kirby-Bauer disc diffusion method was used for antimicrobial susceptibility testing of the isolates to 14 types of antibiotics routinely tested in the Microbiology laboratory of the Medical Research Institute. The sizes of the zones of inhibition were interpreted according to the CLSI M100 (31st edition) recommendations. Susceptibility of the isolates to vancomycin was screened by means of Vancomycin screening agar. Inducible clindamycin resistance was observed by D-test [33].

5.2. Molecular techniques

5.2.1. DNA extraction

DNA was extracted from MRSA isolates by boiling method. Briefly, colonies of fresh overnight MRSA culture on blood agar were washed and resuspended in 500 µl distilled water. The bacterial suspension was put in a boiling water bath for 15 minutes, immediately chilled on ice for 2–3 minutes, then centrifuged at 14000 rpm for 15 minutes. The supernatant was used as the stock DNA extract for PCR amplification and kept at -20°C till the time of subsequent testing by PCR. [35].

5.2.2 PCR detection of \textit{mecA} gene and SCC\textit{mec} typing of MRSA

Molecular detection of methicillin resistance by conventional PCR amplification of \textit{mecA} gene was done on all samples. Identification of staphylococcal chromosomal cassettes \textit{mec} types was done using previously published SCC\textit{mec} type-specific primers, and observation of the amplicon size corresponding to each type on agarose gel (Table 3) [17, 36–39].

A 10 µmolar working solution of each primer was prepared using DNase-free water. PCR reaction (25 µl) contained: 12.5 µl of MyTaq™ HS Red Mix (2x), 1µl of F primers (10 picomoles/µl), 1µl of R primers (10 picomoles/µl), 3 µl of DNA extract, and 7.5 µl of PCR grade water. Negative control was prepared by the addition of the same contents to
the tubes with water placed instead of the extract. Conventional PCR amplification was carried out on Veriti Thermal Cycler (Applied Biosystems), using gene-specific thermal cycling conditions.

All thermal profiles included one cycle of initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C, annealing at primer-specific temperatures, then extension at 72°C for 45 seconds, in addition to one cycle of final extension at 72°C for 1 minute (Table 3).

Detection of the amplified target genes was done using gel electrophoresis with 1.7% (w/v) agarose, carried out on Mupid-exU System gel electrophoresis equipment. The size of the amplicons was determined using a 100 bp DNA ladder (Thermoscientific GeneRuler, US).

5.2.3 Real-time time PCR confirmation of typing results

Further confirmation of PCR amplicon specificity was done for typed isolates by SYBR Green real-time PCR followed by melting curve analysis. Real-time PCR was carried out on Agilent Stratagene MX 3000P Quantitative PCR System using SensiFAST™ SYBR Lo-ROX® master mix, with gene-specific thermal cycling conditions. All thermal profiles started with an initial denaturation step (one cycle) at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 5 seconds, annealing at primer-specific temperatures, extension at 72°C for 20 seconds, and eventually, one cycle of melting curve analysis at temperatures specific to each primer pair (Table 3).

Abbreviations

\textit{SCCmec}: staphylococcal cassette chromosome \textit{mec}

\textit{MRSA}: Methicillin-Resistant \textit{Staphylococcus aureus}

\textit{CA-MRSA}: community-acquired MRSA

\textit{HA-MRSA}: healthcare-associated MRSA

\textit{Ccr}: (cassette chromosome recombinase)

Declarations

Ethics approval and consent to participate

The research design has been approved by the Ethics Committee of the Medical Research institute, Alexandria University. IORG#: IORG0008812. All experiments were performed in accordance with relevant guidelines and regulations. The study does not involve any humans or animals, it was performed on clinical samples that were submitted to the microbiology laboratory of the Medical Research Institute for routine analysis. The authors guarantee the privacy regarding patients’ data.

Consent for publication

Not applicable

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare.

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Author contributions

Aliaa Aboulela: planned and supervised the experiments, worked out almost all of the technical details, discussed the results, and wrote the final manuscript with input from all authors.

Mirette Roufaeil: carried out the experiments, performed the analysis, discussed the results and wrote the draft manuscript.

Ola Abdel Kader: conceived the original idea, supervised the findings of this work, discussed the results, and critically revised the manuscript.

Shahinda Rezk: planned and supervised the experiments, worked out almost all of the technical details, discussed the results and contributed to writing of the final manuscript.

Acknowledgement

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References


Tables

Table 1

Results of antibiotic susceptibility testing of the 100 MRSA isolates

<table>
<thead>
<tr>
<th>Antibiotics (Oxoid™, Thermo Scientific™)</th>
<th>No. of samples</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Cefoxitin (FOX, 30 µg)</td>
<td>100</td>
<td>100%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin (CN, 10 µg)</td>
<td>100</td>
<td>71%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Azithromycin (AZM, 15 µg)</td>
<td>95*</td>
<td>25.2%</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Clarithromycin (CLR, 15 µg)</td>
<td>95*</td>
<td>24.2%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Erythromycin (E, 15 µg)</td>
<td>95*</td>
<td>26.3%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Clindamycin (DA, 2 µg)</td>
<td>100</td>
<td>12%</td>
<td>3</td>
<td>3%</td>
</tr>
<tr>
<td>Tetracycline (TE, 30 µg)</td>
<td>100</td>
<td>44%</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Doxycycline (DO, 30 µg)</td>
<td>100</td>
<td>29%</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Minocycline (MH, 30 µg)</td>
<td>100</td>
<td>9%</td>
<td>15</td>
<td>15%</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP, 5 µg)</td>
<td>100</td>
<td>23%</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>Levofloxacin (LEV, 5 µg)</td>
<td>100</td>
<td>24%</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole (SXT, 1.25/23.75 µg)</td>
<td>100</td>
<td>10%</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Rifampicin (RD, 5 µg)</td>
<td>100</td>
<td>5%</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Linezolid (LZD, 30 µg)</td>
<td>100</td>
<td>0%</td>
<td>3</td>
<td>3%</td>
</tr>
<tr>
<td>Vancomycin (6 µg/ml) **</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>0%</td>
</tr>
</tbody>
</table>

* Macrolides (Azithromycin, Erythromycin and Clarithromycin) were not tested with the 5 isolates from urine.

** Testing was done by Vancomycin screening agar according to CLSI guidelines [13].

Table 2

Antibiotic resistance patterns and the corresponding SCCmec types
<table>
<thead>
<tr>
<th>Antibiotic resistance patterns</th>
<th>All isolates (No.)</th>
<th>Untyped isolates (No.)</th>
<th>SCCmec types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DO, MH, DA, RIF, TE</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DO, MH, TE, DA</td>
<td>4</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DA, RIF, TE, SXT</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, DA, SXT, RIF</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, RIF, SXT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, TE</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, DA</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV, SXT</td>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>CN, AZM, CLR, E, CIP, LEV</td>
<td>3</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>AZM, CLR, E, DO, TE, DA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CN, CIP, LEV, DO, MIN, TE</td>
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<td></td>
<td>3</td>
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<tr>
<td>AZM, CLR, E, CIP, LEV</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CN, E, CIP, LEV, DA</td>
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<td></td>
<td>1</td>
</tr>
<tr>
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<td>AZM, CLR, E</td>
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<td></td>
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</tr>
<tr>
<td>CN, CIP, LEV</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CN, DO, TE</td>
<td>17</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>CN, TE</td>
<td>12</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>CN, DA</td>
<td>1</td>
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</tr>
<tr>
<td>DO, TE</td>
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</tr>
<tr>
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<tr>
<td>CIP, LEV</td>
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<td>TE</td>
<td>1</td>
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</tr>
<tr>
<td>SXT</td>
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</tr>
<tr>
<td>CN</td>
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<tr>
<td>No resistance</td>
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Table 3

Sequence of primers used in this study
<table>
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<tr>
<th>Primers</th>
<th>Nucleotide sequences</th>
<th>Amplicon size (bp)</th>
<th>Annealing temp. (°C)</th>
<th>References</th>
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<tr>
<td><strong>mec A F</strong></td>
<td>CCTAGTAAAGCTCGGAAG</td>
<td>331</td>
<td>53</td>
<td>[16]</td>
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<td><strong>mec A R</strong></td>
<td>CTAGTCCATTCGGTCCA</td>
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<tr>
<td><strong>Type I-F</strong></td>
<td>SCCmec</td>
<td>GCTTTAAAGAGTGCTGTACAGGG</td>
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<td>CGAAATCAATGGTTAATGGAACC</td>
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<td>SCCmec III</td>
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<td><strong>IS1272J-F</strong></td>
<td>GAAGCCTTTGGGCGATAAAGA</td>
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<td>Type X F</td>
<td>SCC mec X</td>
<td>ATTTACGCCGATGCGTTGAC</td>
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<tr>
<td>Type X R</td>
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<td>TATGCGATTGCGAGGTGAT</td>
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<td>Type XI F</td>
<td>SCC mec XI</td>
<td>GGCATACAACGACACATCC</td>
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<td>Type XI R</td>
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<td>TGTTAGTGCTTGACGCTCTT</td>
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<tr>
<td>Type XII F</td>
<td>SCC mec XII</td>
<td>AGAAGACGGAGGACATGCA</td>
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<td>TCGCTTCTTCAACGCCATCTT</td>
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Table 4

**Correlation between SCC mec types and their source clinical condition**

<table>
<thead>
<tr>
<th>Source</th>
<th>SCC mec types (n = 75)</th>
<th>No. of typed isolates</th>
<th>Type II (n = 10)</th>
<th>Type III (n = 10)</th>
<th>Type IV (n = 9)</th>
<th>Type V (n = 34)</th>
<th>Type VI (n = 12)</th>
<th>MC p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<tr>
<td>Pyogenic lesions</td>
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<td>45</td>
<td>3</td>
<td>13.6</td>
<td>7</td>
<td>15.5</td>
<td>3</td>
<td>6.7</td>
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<tr>
<td>Blood stream infection</td>
<td></td>
<td>12</td>
<td>4</td>
<td>33.3</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
<td>16.6</td>
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<tr>
<td>Nasal swab</td>
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<td>6</td>
<td>1</td>
<td>16.6</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
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<tr>
<td>Respiratory tract infection</td>
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<td>7</td>
<td>1</td>
<td>14.2</td>
<td>2</td>
<td>28.5</td>
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<td>0.0</td>
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<tr>
<td>Urinary tract infection</td>
<td></td>
<td>5</td>
<td>1</td>
<td>20.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>20.0</td>
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</tbody>
</table>

p: p value for **Chi square test (Monte Carlo)** association between different categories

*: Statistically significant at p ≤ 0.05

Table 5

**Correlation between SCC mec types and antibiotic resistance**
<table>
<thead>
<tr>
<th>Resistant antibiotics</th>
<th>No.</th>
<th>Type II (n = 10)</th>
<th>Type III (n = 10)</th>
<th>Type IV (n = 9)</th>
<th>Type V (n = 34)</th>
<th>Type VI (n = 12)</th>
<th>$\text{Mc}p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin (CN)</td>
<td>54</td>
<td>9 90.0</td>
<td>8 80.0</td>
<td>3 33.3</td>
<td>24 70.6</td>
<td>10 83.3</td>
<td>0.075</td>
</tr>
<tr>
<td>Azithromycin (AZM)</td>
<td>19</td>
<td>7 70.0</td>
<td>5 50.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>Clarithromycin (CLR)</td>
<td>18</td>
<td>7 70.0</td>
<td>4 40.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.002*</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>19</td>
<td>7 70.0</td>
<td>5 50.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>Clindamycin (DA)</td>
<td>11</td>
<td>0 0.0</td>
<td>4 40.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>0.133</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>35</td>
<td>0 0.0</td>
<td>6 60.0</td>
<td>2 22.2</td>
<td>17 50.0</td>
<td>10 83.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Doxycycline (DO)</td>
<td>24</td>
<td>0 0.0</td>
<td>6 60.0</td>
<td>1 11.1</td>
<td>8 23.5</td>
<td>9 75.0</td>
<td>&lt;0.001*</td>
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<tr>
<td>Minocycline (MH)</td>
<td>9</td>
<td>0 0.0</td>
<td>6 60.0</td>
<td>0 0.0</td>
<td>2 5.9</td>
<td>1 8.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>22</td>
<td>6 60.0</td>
<td>9 90.0</td>
<td>1 11.1</td>
<td>4 11.8</td>
<td>2 16.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Levofloxacin (LEV)</td>
<td>23</td>
<td>6 60.0</td>
<td>9 90.0</td>
<td>1 11.1</td>
<td>5 14.7</td>
<td>2 16.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Trimethoprim/ Sulfamethoxazole (SXT)</td>
<td>9</td>
<td>3 30.0</td>
<td>1 10.0</td>
<td>3 33.3</td>
<td>1 2.9</td>
<td>1 8.3</td>
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<td>Rifampicin (RD)</td>
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<td>1 10.0</td>
<td>0 0.0</td>
<td>1 11.1</td>
<td>0 0.0</td>
<td>3 25.0</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

$p$: $p$ value for Chi square test (Monte Carlo) association between different categories

*: Statistically significant at $p \leq 0.05$