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# Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Gene Typing in Detection of Methicillin-Resistant *Staphylococcus aureus*: Toward Precise Detection in Health Care Facility

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## Abstract

**Background:** Blood stream infections (BSI) are considered key issues in critical care units. Methicillin resistant *Staphylococcus aureus*, MRSA-related infections, are considered a major health problem. This is attributed to the emerging new society dangerous strains with continuous antibiotics pressure and fluctuations in resistance patterns. **Aim:** We aimed to study epidemiology of methicillin resistance *S. aureus* (MRSA) infections by using conventional phenotypic methods [cefoxitin disk diffusion (CDD) and oxacillin screening agar] and molecular typing of the mec-gene (SCCmec) using multiplex PCR in Suez Canal University Hospital. **Methods:** 100 non-repetitive *staphylococcus aureus* were collected and identified morphologically and biochemically by standard laboratory procedures. The strains were considered MRSA if the MIC of oxacillin ≥ 4 μg/ml, and the inhibition zone of cefoxitin was ≤21 mm (CDD). Characterization of SCC*mec* elements in isolated MRSA strains was done via multiplex-PCR. **Results:** From total of 100 isolates, eighty were detected as MRSA by using CDD (sensitivity and specificity were 83.6% and 24.4% respectively) and only 65 by using oxacillin screening agar (sensitivity and specificity were 85.5% and 60% respectively). *MecA* gene was identified in 55 samples; the majority of isolates were SCC*mec* type IVa (63.7%). Both type I and III of SCC*mec* couldn't be detected. Antimicrobial sensitivity rates among SCC*mec*-V isolates were expectedly higher than those among Type-II isolates. SCC*mec* type II was characterized by 100% resistant to ciprofloxacin, erythromycin, oxacillin and cefepime as well as greater resistance to clindamycin (70%) with the same pattern between all typing strains (7 strains).

**Conclusion**: SCC*mec* types IVa and V are generally dominant in our community with no detection of SCC*mec* types I or III. PCR is the optimum method for MRSA detection.

## Keywords

MRSA, Blood Stream Infections, MecA

## 1. Introduction

The use of venous catheters would cause infections with significant morbidity and mortality with a tremendously high economic burden [1]. About 80,000 catheter-related bloodstream infections (CRBSIs) were reported annually among patients in critical care units, counting for up to 24,000 deaths with annual cost of 414 million dollars [2]. Central line-associated blood stream infections (CLABSI) are laboratory-confirmed bloodstream infections (LCBI) where a medical catheter was in place for more than two days on the day of event. The calendar date of catheter insertion is counted day one supported by the fact that the line secured in place at the time of the event or the day before [3].

According to (CDC 2016) recommendations, LCBI was identified if a pathogen was detected in one or more blood specimens and the detected organism is not linked to infections at other places (LCBI-1), or if the patient had one at least of these clinical features; fever (>38.0˚C), hypotension, or chills, with the same commensal identified from at least two blood specimens collected on separate times (LCBI-2) [2] [3].

Still *Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, and *Candida spp*. are the most frequently described causative pathogens [4]. Whereas (19% - 21%) of CLABSIs are attributed to Gram-negative bacilli [5]. Antimicrobial resistance is the common problem for all CLABSIs causing microorganisms [6].

Worldwide, the doubled prevalence rate of MRSA-related infections from 1996-2004 had raised a major public health problem. These strains have a common mobile genetic component (21-to-67 kb) included in their genome, known as the staphylococcal cassette chromosome *mec* (SCC*mec*), carrying the methicillin resistance (*mecA*) gene and other antibiotic resistance determinants [7] [8] [9]. In addition to the two vital genetic elements (the *mec* gene complex and the *ccr* gene complex), a terminal inverted and direct repeats and the junkyard (J) regions are included within SCC*mec gene* [10] [11] [12]. *mec* and *ccr* complexes have been classified into three classes (A, B, and C) and four allotypes (1, 2, 3, and 5) respectively. SCC*mec* types are usually composed of diverse grouping of these complex allotypes and classes.

In 1990s, adult and pediatric patients with no risk factors for acquiring Healthcare-Associated MRSA strains (HA-MRSA) were diagnosed with MRSA

infections for the first time. This was defined as Community-Associated MRSA (CA-MRSA) [13]. It included mobile, little SCC*mec* type IV or V (containing the *mecA* gene) which can be simply relocated to other *S. aureus* strains than bigger SCC*mec* elements (types I, II, and III), converting them to a major cause of significant threat to the public health [14] [15] [16].

To identify the molecular epidemiology of MRSA, SCC*mec* typing protocols using single multiplex PCR reaction have been established to detect types I, II, III, IV, and V based on the nature of the *mec* and *ccr* gene complexes, and are additionally classified into IVa and IVd subtypes according to differences in their J region DNA [17] [18]. In 2007, Milheiric and his colleagues divided the SCC*mec* typing system into three stages: ccrB sequencing, SCC*mec* multiplex PCR and SCC*mec* IV multiplex PCR for subtyping of SCC*mec* type IV strains [19]. In 2007, Kondo and his team published another protocol based on five multiplex PCR reactions that couldn't be realistic for regular practice [20].

The study aimed to evaluate different detection methods for identifying MRSA. Additionally, we aimed to highlight the molecular epidemiology of MRSA infections in Suez Canal University Hospital.

## 2. Subjects and Methods

### 2.1. Samples Collection

A cross-sectional descriptive study was carried out during the period from May 2018 to May 2019 on patients with clinical presentations suggestive of CLABSI in Intensive Care Units (ICUs), Suez Canal University Hospital, Ismailia, Egypt. All age groups were included. Informed consent was taken from each patient to use their data in the current research work. A total of 100 non repetitive *staphylococcus aureus* were isolated from routine blood cultures, requested for patients admitted to ICUs, in Microbiology Laboratory in Suez Canal University Hospital, Ismailia, Egypt, for different laboratory work.

### 2.2. Isolation and Classification of Staphylococcal Isolates

Standard laboratory procedures according to morphologic and biochemical reactions were used to identify staphylococci from different isolates. The *S. aureus* ATCC 25923 was our reference strain. The isolates were preserved in glycerol 15% (v/v) in brain heart infusion broth (BHIB, Oxoid, Basingstoke, UK) at −80˚C and then recovered at the Microbiology Laboratory by subculturing in BHIB at 37˚C for 24 h followed by two further subcultures on brain heart infusion agar [21].

The sensitivity to antimicrobials was performed using the disk diffusion method on Mueller-Hinton Agar (Oxoid, UK) [22]. The following antibiotics were used to determine the antibiotic susceptibility patterns; penicillin (10 mg), cefoxitin (30 mg), ceftaroline (30 mg), erythromycin (15 mg), amikacin (30 mg), linezolid (30 mg), trimethoprim-sulfamethoxazole (25 mg), minocycline (30 mg), levofloxacin (5 mg), clindamycin (2 mg), tetracycline (10 mg), kanamycin

(30 mg), mupirocin (200 mg), gentamicin (10 mg), chloramphenicol (30 mg), Rifampicin (5 mg), and Ciprofloxacin (5 mg) (Oxoid, England).

## 2.3. Identification of Methicillin Resistance

Methicillin resistance was confirmed for all isolates by the following.

### 2.3.1. Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) of oxacillin (Sigma St. Louis, Mo, USA) was determined using Cation-Adjusted Muller Hinton Broth (CAMHB) according to CLSI guidelines. An MIC of oxacillin ≥ 4 μg/ml was considered MRSA and ≤2 μg/mL was considered methicillin sensitive *S. aureus* (MSSA) [22].

### 2.3.2. Cefoxitin Disk Diffusion (CDD) Method

The antibiotic susceptibility was performed using cefoxitin (30 μg) disks (surrogate test for oxacillin) by Kirby-Bauer disk diffusion method as recommended by CLSI 2017. Resistance was reported if the inhibition zone of cefoxitin was ≤21 mm and sensitive if ≥22 mm [22].

### 2.3.3. Oxacillin Agar Screening

Culture on mannitol salt agar containing oxacillin was performed. Media was prepared by adding 11.1 gm of mannitol salt agar base into 100 ml of distilled water and autoclaved. When the autoclaved medium temperature reaches around 50˚C, we added oxacillin as a solution with a final concentration of 6 μg/ml of medium. Any growth in the cultured media was considered as MRSA [22].

### 2.3.4. Molecular Typing Using Multiplex-PCR

Fresh overnight plate cultures of MRSA strains were obtained. Extraction of DNA from bacterial colonies was done using Qiagen DNA Mini kit 51304. mecA gene and SCC*mec* genotyping were determined for all isolates via a single multiplex PCR as illustrated by Zhang *et al.* [16]. Multiplex PCR was carried out in a 25 μl total volume using 2 μl volume of DNA template added to 23 μl of PCR buffer containing (50 mM KCl, 20 mM TRis-HCl pH 8.4, 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, various concentrations of each primer were used [16], and 1.0 unit of *Taq* polymerase (**Table 1**).

The optimal cycling conditions using Eppendorf Mastercycler® nexus PCR thermal cycler were: first denaturation step at 94˚C for 5 min, then 10 cycles of 94˚C for 45 sec, 65˚C for 45 sec, and 72˚C for 90 sec. A further 25 cycles of 94˚C for 45 sec, 55˚C for 45 sec, and 72˚C for 2 min terminated by a final extension step at 72˚C for 10 min and followed by a final hold at 4˚C. Inclusion of NTC (Non template control) in each experiment was done.

PCR products were analyzed using 2.5% agarose gel (Promega, Madison, USA). Syngene G.Box (Syngene, UK) was used to take photos for the stained gel. The SCC*mec* genotypes were determined according to the amplicon size. DNA ladder (100 bp) was used as a marker.

Table 1. Primers used in the SCC*mec* IV multiplex PCR [16].

| Primer | Oligonucleotide sequence (5'-3') | Conc (μM) | Amplicon size (bp) | Specificity |
|---|---|---|---|---|
| **Type I-F** | GCTTTAAAGAGTGTCGTTACAGG | 0.048 | 613 | SCC*mec* I |
| **Type I-R** | GTTCTCTCATAGTATGACGTCC | | | |
| **Type II-F** | CGTTGAAGATGATGAAGCG | 0.032 | 389 | SCC*mec* II |
| **Type II-R** | CGAAATCAATGGTTAATGGACC | | | |
| **Type III-F** | CCATATTGTGTACGATGCG | 0.04 | 280 | SCC*mec* III |
| **Type III-R** | CCTTAGTTGTCGTAACAGATCG | | | |
| **Type IVa-F** | GCCTTATTCGAAGAAACCG | 0.104 | 776 | SCC*mec* IVa |
| **Type IVa-R** | CTACTCTTCTGAAAAGCGTCG | | | |
| **Type IVb-F** | TCTGGAATTACTTCAGCTGC | 0.092 | 493 | SCC*mec* IVb |
| **Type IVb-R** | AAACAATATTGCTCTCCCTC | | | |
| **Type IVc-F** | ACAATATTTGTATTATCGGAGAGC | 0.078 | 200 | SCC*mec* IVc |
| **Type IVc-R** | TTGGTATGAGGTATTGCTGG | | | |
| **Type IVd-F** | CTCAAAATACGGACCCCAATACA | 0.28 | 881 | SCC*mec* IVd |
| **Type IVd-R** | TGCTCCAGTAATTGCTAAAG | | | |
| **Type V-F** | GAACATTGTTACTTAAATGAGCG | 0.6 | 325 | SCC*mec* V |
| **Type V-R** | TGAAAGTTGTACCCTTGACACC | | | |
| **MecA147-F** | GTG AAG ATA TAC CAA GTG ATT | 0.046 | 147 | mecA |
| **MecA147-R** | ATG CGC TAT AGA TTG AAA GGA T | | | |

## 3. Results

The conventional microbiological methods, culture and sensitivity using CDD detected 80 isolates as MRSA (80%). Oxacillin screening agar method (mannitol salt agar with oxacillin) detected 65 isolates as MRSA (65%).

Multiplex PCR was performed to screen the existence of *mecA* genes in the entire isolates. *mecA* gene was identified in 55 isolates out of 100 isolates. Surprisingly, 9 isolates (9%) of PCR positive samples were detected as MSSA by CDD conventional methods and 8 isolates (8%) were detected as MSSA by oxacillin agar screening method.

Regarding mecA gene PCR negative samples (45 samples), 34 samples (34%) were detected as MRSA by CDD method and 18 isolates (18%) were identified as MRSA using oxacillin agar screening technique.

The CDD and oxacillin agar screening method results were compared using two-way table analysis. Both methods were evaluated versus PCR. The sensitivity and specificity for CDD method were (83.6% and 24.4%) respectively, with 57% accuracy, 57.5% PPV and 55% NPV. On the other hand, the sensitivity and specificity for oxacillin agar screening method were (85.5% and 60%) respectively, with 74% accuracy, 72.3% PPV and 77.1% NPV.

Three different SCC*mec* classes were identified, most of isolates 35/55 (63.7%)

were type IVa (amplicon size 776 bp) followed by 13/55 isolates (23.6%) were type V (amplicon size 325 bp), and 7/55 isolates (12.7%) type II (amplicon size 398 bp) as shown in (**Figure 1**). Surprisingly, SCC*mec* types I and III were completely absent.

### Relation between Antibiotic Resistance and Multiplex PCR

SCC*mec* type II was 100% resistant to erythromycin, ciprofloxacin, oxacillin and cefepime as well as to clindamycin (70%) with the same pattern between all typing strains. SCC*mec* type IVa isolates illustrated clindamycin resistance (60%) and erythromycin (35%) with 30% of them showed the same antibiotic resistance. Finally, MRSA strains SCC*mec* type V showed recurrent resistance to aminoglycosides.

## 4. Discussion

The emergence of continuous antibiotics pressure and fluctuations in resistance patterns in the novel community acquired MRSA virulent strains resulted in serious blood stream infections in the last two decades [23]. Methicillin resistance in staphylococci is due to the expression of a modified penicillin-binding protein (PBP), PBP 2a encoded by the *mecA* gene that is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) [10].

Our study utilized two recommended standard tests to screen for MRSA, Cefoxitin disk diffusion (CDD) and oxacillin agar screening. The sensitivities of these 2 tests to detect MRSA as compared to Polymerase Chain Reaction in detecting of mecA gene were 83.6% and 85.5% respectively. The sensitivity of 83.6% of CDD means that the test can detect only 83 cases out of 100 as true positive and 17 cases will be misdiagnosed. This may affect the treatment decision, strategy, cost and hospital stay.

The specificity of oxacillin agar screening methods was higher than of CDD (60% and 24.4% respectively) but both were below 90%, which can't be accepted as a standard method for diagnosis MRSA cases especially with low accuracy of both tests (57% for CDD and 47% for oxacillin agar screening). Our findings were similar to Pillai *et al.* [24] which reported that the sensitivity and specificity of oxacillin disk diffusion (ODD) test were (93.5%, 83.5%) respectively, whereas that of oxacillin agar screening was found to be (87.1%, 89.3%) respectively. Cauwelier *et al.*, mentioned that the sensitivities of both oxacillin disk diffusion method and agar screening method are 83.5% and 91.7% respectively, and both were 100% specific compared with PCR for mecA detection [25].

In our study, only 55 isolates were confirmed MRSA strains by means of PCR detecting mecA gene. Surprisingly, out of mecA PCR positive isolates, only 9 isolates were diagnosed as MSSA by CDD and 8 isolates were diagnosed as MSSA by oxacillin agar screening phenotypically. This may attributed to the fact that conventional tests are subjective tests depend on many factors as incubation time, PH and salt concentration of the culture medium and finally the inoculum

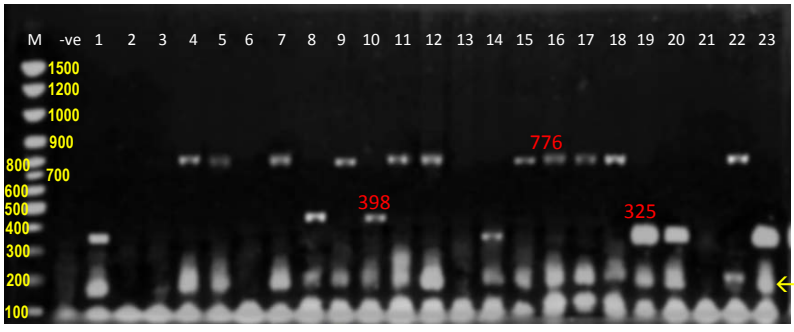


**Figure 1.** SCC*mec* Typing PCR results; M: 1500 bp marker. –ve: negative control. Lanes 2, 3, 6, 13, and 21 were *mecA* negative. Lanes 4, 5, 7, 9, 11, 12, 15 - 18, and 22 were type IVa positive. Lanes 1, 14, 19, 20, and 23 were positive for type V. Lanes 8, and 10 were type II positive.

size [26]. Out of 45 isolates detected as MSSA by mecA PCR, only 11 isolates were detected as MSSA by CDD method and only 27 isolates were detected as MSSA by oxacillin agar screening. These false positive strains by conventional methods may be due to the heterogenous phenotypes of methicillin resistance in staphylococcus species "moderately resistant *S. aureus*" (MODSA). It might not be easy to differentiate MODSA from true MRSA strains carrying mecA gene owing to their overproduction of penicillinase (penicillinase hyper producers) [27] [28].

Despite that types I, II and III of the SCC*mec* are the highest prevalent HA-MRSA strains in western countries as Europe, Switzerland and USA [29] [30]. These strains were either undetected (type I and III) or sparsely detected (12.7%; type II) in our work.

We noticed the general dominance of MRSA strains carrying SCC*mec* types IVa and V (63.7% and 23.6% respectively). This data was matched with a study from Switzerland in 2010 [31] which reported the absence of type III and presence of types I and II in very low proportions (10%). In healthcare-associated infections, some studies reported 87% isolation rates of SCC*mec* types IV and V, others reported comparable distribution of SCC*mec*-IV and SCC*mec*-II/III types among the MRSA isolates [32] [33]. Fatholahzadeh and his colleagues reported 98% isolation rates of SCC*mec* type III or IIIA and only 2% for SCC*mec* type IV, but didn't isolate both types I and II [34]. These results are alike most Asian studies [35].

We couldn't identify MRSA isolates of the SCC*mec* types I or III, probably due to the limited number of isolates analysed. To our knowledge, we are the first study describing the SCC*mec* typing in our hospital with such high prevalence of SCC*mec* types IV and V.

Antimicrobial sensitivity rates among SCC*mec*-V isolates were expectedly higher than those among Type-II isolates. However, SCC*mec* type II was 100% resistant to ciprofloxacin, erythromycin, oxacillin and cefepime as well as greater resistance to clindamycin (70%). This was matched by **Davis** and his group who pointed the superior antibiotics sensitivity pattern among Type IV isolates com-

pared to Types-II/III isolates [32].

In this study, the majority of cases (63.7%) were carrying SCC*mec*-IV and all were of the subtype SCC*mec*-Iva. The resistance was mainly to clindamycin (60%) and erythromycin (35%). In 2008, Fatholahzadeh reported resistance most of SCC*mec* types III and IIIA to, erythromycin, azithromycin, kanamycin, ciprofloxacin, cotrimoxazole gentamicin, netilmicin, ofloxacin, and tetracycline [35].

This study has many limitations because MRSA surveillance cultures are not routinely performed, so it was difficult to track the source and starting time of MRSA acquirement. We also need more studies to recognize the risk factors for the acquisition of blood stream infections (BSI) as it is essential to follow up the performance of infection control preventive measures. Similarly, to our knowledge, no available data are published until now characterizing molecular, clinical and epidemiological basis for CA-MRSA colonization and infection. Additional prospective epidemiological work is required to assess the level of CA-MRSA strains in healthcare facilities.

## 5. Conclusion

In conclusion, SCC*mec* types IVa and V are generally dominant in our community with no detection of SCC*mec* types I or III. Antimicrobial sensitivity rates among SCC*mec*-V isolates were expectedly higher than those among Type-II isolates. PCR is the optimum method to be used in detecting these serious infections and preferred over the usual standard methods. PCR can pick up the wrong negative results in addition to the sensitivity, specificity, accuracy and the rapid diagnosis of MRSA strains as the detection of *mec*A gene can last for only 5 h from the bacterial isolation. Hence, the conventional methods are not reliable for detecting MRSA strains especially in seriously ill-patients.

## Ethical Considerations

The study was approved by the medical ethics committee of our institute in agreement with the 1964 Helsinki declaration and its later modification.

## Conflicts of Interest

Authors declare no conflicts of interests.

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