Investigating the relation between resistance pattern and type of Staphylococcal cassette chromosome mec (SCCmec) in methicillin-resistant Staphylococcus aureus

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ABSTRACT

Background and Objectives: MRSA became a widely recognized cause of mortality worldwide with necessity of its epidemiological pattern study. Typing of MRSA isolates was performed molecularly based on SCCmec type and relation to resistance pattern was investigated.

Materials and Methods: Out of 200 clinical specimens, S. aureus was detected phenotypically and confirmed as MRSA by PCR in 124 isolates obtained from associated laboratories of different hospitals of Zagazig, during 2018-2019. Antimicrobial resistance pattern was detected and MRSA SCCmec was typed by two methods.

Results: S. aureus rate was high in wounds, sputum, blood, and urine isolates. Antimicrobial resistance rates against cepotaxime, tetracyclines, gentamicins, ciprofloxacin, erythromycin, azithromycin, clindamycin, cloramphenicol, sulfamethoxazole-trimethoprim, linezolid and vancomycin were 82.3%, 65.3%, 56.4%, 45.1%, 37.1%, 32.3%, 32.3%, 25%, 7.3%, 2.4% and 0%, respectively. Multiplex-PCR(M-PCR) was able to detect SCCmec element among 57% of isolates classified as SCCmec II (n=40), III (n=21), IVa (n=3), IVd (n=2), V(n=1), and four isolates contain both SCCmec II and SCCmec IV. Traditional typing by PCR for mec and ccr gene complexes was almost concordant with M-PCR. Furthermore, it was able to identify SCCmec types VI, IX, and XIV among 1, 3 and 18 isolates, respectively. No Statistical correlation was established between type of cassette and rate of antimicrobial resistance. Phylogenetic analysis reveals that all ccr types were related to each other and no significant variation in the same ccr type of different SCCmec cassettes.

Conclusion: Most MRSA isolates were MDR reflecting antimicrobials misuse. Detection of various SCCmec types among MRSA isolates indicate the complexity of MRSA epidemiology and increase chance for gene sharing creating new types. The presented investigation was important in understanding MRSA epidemiology and diversity in Egypt providing advice for improving therapeutic protocols.

Keywords: Methicillin resistant Staphylococcus aureus; Antimicrobial; Resistance; Molecular typing; Cassette chromosome recombinase

INTRODUCTION

Staphylococcus aureus (S. aureus) is a widespread pathogen causing skin infections, pneumonia, osteomyelitis, endocarditis, bacteremia, and toxic shock syndrome (1). Methicillin-resistant S. aureus (MRSA) is a serious cause of hospital and community-acquired infections (2). Methicillin resistance is usually acquired through the expression of a mutated penicillin-binding protein (PBP2a) encoded by
mecA gene, that is located on the staphylococcal cassette chromosome mec (SCCmec) island (3).

SCCmec is a mobile genetic island characterized by the presence of mec gene complex, and cassette chromosome recombinase (ccr) gene complex (4). Five classes of mec gene complex (A - E) have been identified with many subclasses in various types of MRSA isolates. Class A mec, B mec and C mec are the most common types (5). The ccr gene complex encodes DNA recombinase enzymes that catalyze the mobility of the SCCmec cassette by its excision or insertion into several integration sites. Three types of ccr have been reported (ccrA, ccrB, and ccrC) with multiple allotypes for each gene forming eight complexes (6).

Five mec gene and eight ccr gene complexes combination are essential in SCCmec nomenclature and assignment of new types. Availability of complete sequences from different MRSA isolates facilitated the assignment of thirteen (I-XIII) SCCmec types (7). SCCmec I, SCCmec II, and SCCmec III carry mecB and ccr1, mecA and ccr2, and mecA and ccr3 gene complexes, respectively. SCCmec IV is the smallest element that has a unique combination of mecB and ccr2 gene complexes. SCCmec V, SCCmec VI, SCCmec VII, and SCCmec VIII carry mecC2 and ccr5, mecB and ccr4, mecC1 and ccr5, and mecA and ccr4 gene complexes, respectively. SCCmec IX, SCCmec X, SCCmec XI, SCCmec XII, and SCCmec XIII carries mecC2 and ccr1, mecC1 and ccr7, mecE and ccr8, mecC2 and ccr5(C2) and mecA and ccr5(C2) gene complexes, respectively (8).

The quick characterization of mec element types by multiplex PCR (M-PCR) technique was developed by Zahng et al. (9). Although the M-PCR guarantee rapid identification of variant mec element, unfortunately, it can only detect five SCCmec elements. Thus, traditional method, in which mec and ccr were typed individually in a separate PCR, is more professional in typing novel and unidentified SCCmec types. However, it requires multiple primer sets, which is relatively expensive and time-consuming (10).

The last decade includes a lot of changes in MRSA epidemiological pattern in Egypt, with changes in its spread and resistance to antimicrobial agents. The resistance was dramatically increased against both old and recent groups of antimicrobials with an increasing rate of multidrug resistance (MDR) that associated with accumulation of resistance genes in SCCmec elements (11).

The current study aims to investigate MRSA epidemiology including incidence and change in antimicrobial resistance patterns in Egyptian local hospitals. Comparing two common methods for SCCmec typing with subsequent possible discovering of novel SCCmec in MRSA isolates. Finally, the relationship between SCCmec types and resistance patterns was investigated.

MATERIALS AND METHODS

Isolation and identification of S. aureus. Two hundred clinical specimens of inpatients were obtained from associated laboratories of different hospitals of Zagazig, Sharkia province in Egypt during 2018-2019. Clinical specimens were from: wounds (n=55), sputum (n=35), blood (n=30), ascites fluids (n=25), urine (n=20), ear swabs (n=20) and diabetic foot swabs (n=15).

Isolates were primary cultured and purified on nutrient agar medium (non-selective medium) and manitol salt agar medium (selective medium). S. aureus was identified through morphological characters on different media, Gram staining, and biochemical tests (12). MRSA was identified phenotypically by disk diffusion method and genotypically by amplification of mecA gene using PCR. MRSA isolates were kept in glycerol stocks at -20°C till further use, isolates were recovered from glycerol stocks by plating on nutrient agar and incubating at 37°C for 24 h.

Antimicrobial resistance profile. Antibiogram of S. aureus isolates was performed by the disk diffusion method. All isolates were tested against 12 antimicrobials from 10 classes. The disks were purchased from Oxoid (Hampshire, England) and include: methicillin (ME, 5 µg), cefotaxime (CTX, 30 µg), vancomycin (VA, 30 µg), azithromycin (AZM, 15 µg), erythromycin (E, 15 µg), gentamicin (CN, 10 µg), tetracycline (TE, 10 µg), ciprofloxacin (CIP, 5 µg), clindamycin (DA, 2 µg), sulfamethoxazole-trimethoprim (SXT, 25 µg), chloramphenicol (C, 30 µg) and linezolid (Lzd, 3 µg). The inoculum was optimized to 0.5 McFarland turbidity standard and inoculated on Muller Hinton Agar (Oxoid, Hampshire, England) as recommended by the Clinical and Laboratory Standards Institute (CLSI). The plates were incubated at 37°C for 18 h after placing antimicrobial disks. Zones of inhibition were measured, and the results were interpreted ac-
cording to the criteria of CLSI (13).

**Molecular identification and classification of MRSA.** *S. aureus* isolates were primary treated with 19.5 μL 10 mM Tris-HCl plus 0.5 μL lysostaphin (1 mg/mL) in 37°C for 30 min before extraction of genomic DNA (gDNA) by gDNA purification kit (Thermo scientific, Germany) according to the recommended instructions (14).

The *mecA* gene of MRSA isolates was investigated by PCR using specific primers (Table 1). PCR was performed in Biometra thermocycler (Göttingen, Germany) that adjusted at 95°C for 5 min as initial denaturation step followed by 30 cycles of 94°C for 30 sec, 52°C for 30 sec and 72°C for 30 sec, followed by a final elongation step at 72°C for 10 min. Identification of five types of SCCmec elements was conducted by M-PCR reactions in a total volume of 50 μL reaction using specific primers illustrated in Table 1. SCCmec I, SCCmec III, SCCmec IVc, and SCCmec IVd were identified in one reaction while SCCmec II, SCCmec IVa, SCCmec IVb, and SCCmec V were

### Table 1. Primers used for classification of SCCmec type

| Target                  | Primer        | Nucleotide sequence 5’ to 3’ | Amplicon size (bp) | Ref. |
|-------------------------|---------------|-----------------------------|--------------------|------|
| MRSA identification     | *mecA*-F      | GTAGAAATGACTGAAAGTGCGATCAA  | 310                | (15) |
|                         | *mecA*-R      | CCAATCCACATTTGCTGCGATCTAA  |                    |      |
| SCCmec I                | Type I- F     | GCTTTAAAGAGTGCTGTACAGGG    | 613                |      |
|                         | Type I- R     | GTTCTCTCATATGACTGCTCCC     |                    |      |
| SCCmec II               | Type II- F    | CGTCTGAAGATGTAAGCGG        | 398                |      |
|                         | Type II- R    | CGAAATCATGGTTAAATGGCAA     |                    |      |
| SCCmec III              | Type III- F   | CCAATTTGGTGATCGATGCG       | 280                | (9)  |
|                         | Type III- R   | CTTACTCTTGGTAAAGCCTGC      |                    |      |
| SCCmec IVa              | Type IVa- F   | GCCCTATTGCGAAGAAACCG       | 776                |      |
|                         | Type IVa- R   | CTAATCGTTCGATGATCGATTGC    |                    |      |
| SCCmec IVb              | Type IVb- F   | TTCTGGAATTACTTCCAGTCG      | 493                |      |
|                         | Type IVb- R   | AAACAAATATTGCTCCCTACTC    |                    |      |
| SCCmec IVc              | Type IVc- F   | ACAATTTGGTATATCGGAGCG      | 200                |      |
|                         | Type IVc- R   | TTGATGATGAAATTTGCTGG       |                    |      |
| SCCmec IVd              | Type IVd- F   | CTCAAAATACGCAGCCACATACCA  | 881                |      |
|                         | Type IVd- R   | TGCTCAAGATATTGCTAAAG       |                    |      |
| SCCmec V                | Type V- F     | GAACATTTGGTACGATGAGCC      | 325                |      |
|                         | Type V- R     | TGGAAGGTTGTAACTTCCAGCACCC |                    |      |
| Class A mec (MecI-mecR1)| ml6mA7b       | CATAGGCTCCCTTCTTCAGATGATACCAA | 1965 (type II) | (16) |
|                         |              | AACCGCAACAAATCA            | 1797 (type III)    |      |
| Class B mec (IS1272)    | IS5          | AACGCGCATCTATAAGATGGAA     | 1996               | (10) |
|                         | mA6          | TATCCAAACCCGACAA           |                    |      |
| Class C mec (IS431-mecA)| IS2          | TGGAGGTATCTAGATTTTGCATGT   | 2072               | (17) |
|                         | mA2          | AACGTGGTAAACCCCAAGA        |                    |      |
| ccr1 (ccrA1)            | α1           | AACGCTATCATACTACGACTAATGC | 695                | (18) |
|                         | Bc           | ATGCGGTCTGATAGACCTCTCT    |                    |      |
| ccr2 (ccrA2)            | α2           | TAAAGGGCATCAGACACACACT    | 937                |      |
|                         | Bc           | ATGCCTGATGATACCTCTCT      |                    |      |
| ccr3 (ccrA3)            | α3           | AGCTCAAAGAGCAATAGAAT      | 1791               | (19) |
|                         | Bc           | ATGGCCTGATATAGCCCTCT      |                    |      |
| ccr4 (ccrA4)            | A4;2         | GTATCAATGCGACAGAATTTT     | 1287               | (20) |
|                         | B4;2         | TTGCACACTCCTCTTGCAGTTT    |                    |      |
| ccr5 (ccrA5)            | y-F          | CGTCTATACAGATGTTAGAGATAAT | 518                |      |
|                         | y- R         | CTTTATAGACTGGGTAATCCAAAAT |                    |      |
identified in another reaction. Each reaction includes 1.5 μL (50 pmol) of each specific primer, 25 μL of EcoRedaq plus Green 2× Master Mix (Lucigen, USA), 5 μL of DNA template. Cycling conditions were, 94°C for 10 min, followed by 30 cycles of 94°C for 45 sec, 52°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 10 min. The presence of bands at target size on 1.2% stained gel was documented.

Identification of SCCmec was also performed by amplification of mec and ccr complexes. PCRs were performed for mecA, mecB and mecC using specific primer pairs (Table 1). PCR was conducted in a 25 μL reaction mixture. The thermocycling conditions were 94°C for 10 min, 30 cycles of denaturation at 1 min at 94°C, 1.5 min for annealing, and 1 min at 72°C for elongation and then final elongation step at 72°C for 10 min. The annealing temperature was adjusted at 51°C, 45°C, 47°C for mecA, mecB, and mecC, respectively. ccr genes were detected by two M-PCR. The first and second M-PCR were optimized for the identification of ccr 1-3 and ccr 4-5, respectively. The cycling parameters were 94°C for 10 min, 30 cycles of 94°C for 1 min, 50°C in first M-PCR and 55°C in second M-PCR for 1 min, and 72°C for 45 sec and then final extension step at 72°C for 10 min. The presence of bands at target size on 1.2% stained gel was documented.

Sequencing and phylogenetic analysis. Representative fifteen amplicons that represent different types of mec and ccr genes were selected, purified, and sequenced to investigate the relation of the same type of ccr in different SCCmec elements. Amplicons were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. The purified products were submitted to the University of Minnesota Genomic Center (UMGC), Saint Paul, MN, USA for sequencing. The data was assembled using Sequencher 5.4.6 software then blasted on NCBI.

Molecular Evolutionary Genetic Analysis (MEGA X) was used for sequences alignment, and phylogenetic tree construction (21). A phylogenetic tree was constructed by selecting Tamura 3-parameter +G as a maximum likelihood model in MEGA X based on the lowest BIC (Bayesian Information Criterion) score. The obtained sequences were submitted to GenBank with the following accession numbers, MZ291463, MZ291464, and MZ291465 for ccr1 gene of SCCmec IIX (3 strains), MZ291466, MZ291467, and MZ291468 for ccr2 gene of SCCmec II, SCCmec IV, and non-typeable SCCmec, respectively. MZ291469 for ccr3 gene of SCCmec III and MZ291470 for ccr2 gene of SCCmec VI, MZ291471, MZ291472 and MZ291473 for ccr2 gene of SCCmecV, and SCCmecXIV (2 strains), MZ291474, MZ291475, MZ291476, and MZ291477 for MecI, MecR1, IS1272, and IS431.1 of mecA, mecB and mecC cassette chromosomes, respectively.

Statistical analysis. The relationships between frequencies of antimicrobial resistance, MDR, and SCCmec element type were analyzed using Chi-square test in IBM SPSS statistics software (version 25). P>0.05 was considered to be statistically non significant.

RESULTS

Identification of MRSA. S. aureus was confirmed among 124 isolates out of 200 clinical specimens. All isolates were identified morphologically through golden yellow colored colonies, yellow fermentation, and β- haemolysis on nutrient agar medium, mannitol salt agar medium, and blood agar medium, respectively. All S. aureus isolates were confirmed to be MRSA (100%) by PCR amplification of mecA gene.

Antibiogram. Antibiogram for 124 MRSA isolates is shown in Fig. 1. The highest resistance was observed in methicillin (100%), cefotaxime (82.3%), tetracycline (65.3%), and gentamicin (56.4%). Moderate resistance was detected for ciprofloxacin (45.1%), erythromycin (37.1%), azithromycin (32.3%), clinda-

![Fig. 1. Susceptibility patterns of MRSA clinical isolates to different antimicrobials.](http://ijm.tums.ac.ir)
mycin (32.3%), and chloramphenicol (25%). The lowest resistance was against sulfamethoxazole-trimethoprim (7.3%) and linezolid (2.4%). None of the isolates were resistant to vancomycin. Resistance to β-Lactam and tetracyclin classes were the highest.

MDR was detected in 79 isolates (63.7%). Among the MDR isolates, 17 (21.5%), 20 (25.3%) and 42 (53.2%) isolates were resistant to three, four and five groups of antimicrobials, respectively. Interestingly, resistance was higher against all antimicrobial agents in the mid-age group (21-40 years) of both genders more than younger and older patients. Male gender was the predominant in the resistance profile for all antimicrobial agents in all age groups (Table 2).

**SCCmec classification among MRSA isolates.** Out of the 124 isolates, SCCmec element was detected in 71 isolates (57%) by M-PCR. Among the 71 typeable isolates, 40 (56%), 21 (29.5%), 3 (4.2%), 2 (2.8%), and 1 (1.4%) isolate belonged to SCCmec types II, III, IVa, IVd, and V, respectively. Four isolates were found positive for both SCCmec II and SCCmec IV, SCCmec type I and subtypes, IVc, or IVb were not found among tested isolates. However, 53 (43%) isolates were non-typeable using M-PCR.

Furthermore, all isolates were characterized using traditional PCR typing against mec (A, B and C) and ccr (1-5) gene complexes. The result was almost concordant with M-PCR, with ability to characterize more SCCmec types. SCCmec VI, SCCmec IX (C1), SCCmec XIV (A5) were detected among 1, 3 and 18 isolates. The newly 18 SCCmec XIV elements were classified as 5 (SCCmec XIV& II), 7 (SCCmec XIV& III), and 6 SCCmec XIV. One sample was considered as a novel type in combination between mecC and ccr2. Nineteen and four non-typeable isolates had only ccr or mec gene complex, respectively. 23 out of 124 (18.5%) isolates were only amplified against the mecA gene, potentially consider as variant sub-types or new types (Table 3).

**Relations between antimicrobial resistance and SCCmec cassette type.** Statistically, no significance and no correlation were established between the type of antimicrobial resistance and type of detected cassettes (P>0.05). However, strains carrying SCCmec II, III, IV, and XIV were resistant to most of the used antimicrobials while strains with SCCmec V, VI, and IX were not resistant against most antimicrobials. No association between the presence of multiple SCCmec cassettes and increasing resistance to antimicrobials was established (Fig. 2 and Table 4).

Statistically, no significance between the type of SCCmec cassettes and MDR was established (P>0.05). However, overall SCCmec cassettes, most of SCCmec II (68.6%) and SCCmec III (82%) showed MDR. Strains with SCCmec II were unique to show MDR against 3-8 groups of antimicrobials with a maxi-

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**Table 2.** Age and sex classification for patients tested against different antimicrobial agents.

| Antimicrobial agents | Age 0-20 | | | Age 21-40 | | | Age 41-60 |
|----------------------|---------|---|---|---------|---|---|---------|
|                      | M       | F | T | M       | F | T | M       | F | T |
| ME                   | 26      | 11| 37| 34      | 18| 52| 22      | 13| 35|
| CTX                  | 22      | 7 | 29| 28      | 15| 43| 19      | 11| 30|
| VA                   | 13      | 7 | 20| 19      | 11| 30| 14      | 6 | 20|
| CN                   | 8       | 5 | 13| 15      | 6 | 21| 4       | 2 | 6 |
| AZM                  | 9       | 5 | 14| 16      | 7 | 23| 6       | 3 | 9 |
| E                    | 20      | 10| 30| 21      | 12| 33| 13      | 5 | 18|
| CIP                  | 10      | 6 | 16| 17      | 8 | 25| 12      | 3 | 15|
| DA                   | 9       | 4 | 13| 12      | 7 | 19| 5       | 3 | 8 |
| SXT                  | 2       | 2 | 4 | 2       | 4 | 6 | -       | 1 | 1 |
| C                    | 8       | 4 | 12| 7       | 5 | 12| 1       | 1 | - |
| LZD                  | -       | - | - | 2       | 2 | 1 | 1       |    | 1 |

ME=Methicillin, CTX=Cefotaxime, VA=Vancomycin, CN=Gentamicin, AZM=Azithromycin, E=Erythromycin, TE=Tetracycline, CIP=Ciprofloxacin, DA=Clindamycin, SXT=Sulfamethoxazole-Trimethoprim, C=Chloramphenicol, LZD=Linezolid.
Table 3. SCCmec complex typing using Traditional PCR.

| SCCmec type | Mec complex type | Ccr complex type | No. of isolates |
|-------------|------------------|------------------|-----------------|
| II          | A                | 2                | 31 (25%)        |
| III         | A                | 3                | 14 (11.3%)      |
| IVa         | B                | 2                | 3 (2.4%)        |
| IVd         |                  |                  | 2 (1.6%)        |
| V           | C                | 5                | 1 (0.8%)        |
| Scmec II + Sccmec IV | A, B | 2 | 4 (3.2%) |
| SCCmec VI   | B                | 4                | 1 (0.8%)        |
| SCCmec IX   | C                | 1                | 3 (2.4%)        |
| SCCmec XIV  | A                | 5                | 6 (4.8%)        |
| SCCmec XIV, III | A | 5, 3 | 7 (5.6%) |
| SCCmec XIV, II | A | 5, 2 | 5 (4.03%) |
| Novel SCCmec | C                | 2                | 1 (0.8%)        |
| Only ccr complex | - | - | 19 (15.3%) |
| Only mec complex | - | - | 4 (3.22%) |
| Only mecA gene | - | - | 23 (18.5%) |

Fig. 2. Count of antimicrobial resistant strains in different SCCmec cassettes.

mum number of resistant strains (14 strains) against 6 groups of antimicrobials (Table 5).

mec and ccr sequences analysis. Alignment of 361 nucleotide (nt) in all ccr sequences reveal that similarity between all types is 35-39%. All ccr1 samples were obtained from SCCmec IX type. Nucleotide (nt) comparison (355 nt) revealed 100% similarity within sequences and 99% with HF569112 and 98% with AB930126, AB033763 and KF527883 reference strains with 5-7 nt substitution. Amino acids (aa) similarity with reference strains was 100%. Three ccr2 representative samples from SCCmec II, IV and putative novel type (2C) were sequenced. Nucleotide and aa comparison (355 nt and 118 aa, respectively) revealed 100% identity within sequences and with
Table 4. Antimicrobial resistant strains in different SCCmeC cassettes classified by traditional PCR.

| AB | Total SCCmeC II | Total SCCmeC III | Total SCCmeC IV | SCCmeC V (n=1) | SCCmeC VI (n=1) | SCCmeC IX (n=3) | Total SCCmeC XIV |
|----|----------------|-----------------|---------------|--------------|---------------|----------------|----------------|
| ME | 40             | 21              | 9             | 1            | 1             | 3              | 18             |
| CTX| 33             | 21              | 7             | -            | -             | 2              | 18             |
| VA | -              | -               | 0             | -            | -             | -              | -              |
| AZM| 17             | -               | 6             | -            | -             | 1              | 7              |
| E  | 22             | 9               | 7             | -            | -             | 1              | 7              |
| TE | 28             | 9               | 7             | -            | 1             | 1              | 14             |
| CIP| 23             | 14              | 5             | -            | -             | 1              | 12             |
| C  | 8              | 15              | 4             | -            | -             | 1              | 5              |
| DA | 19             | 3               | 5             | -            | -             | 1              | 7              |
| SXT| 2              | 8               | 1             | -            | -             | -              | 1              |
| LZD| -              | 1               | 1             | -            | -             | -              | 1              |
| CN | 24             | 1               | 6             | -            | -             | 1              | 14             |

Table 5. Distribution of multi drug resistant strains on different SCCmeC cassettes classified by traditional PCR.

| MDR | SCCmeC elements |
|-----|-----------------|
|     | II (N=40) | III (N=21) | IV (N=9) | V (N=1) | VI (N=1) | IX (N=3) | xIV (N=18) |
| 3   | 2               | 1           | 1        | -       | -        | -        | -         |
| 4   | 7               | 8           | -        | -       | -        | -        | 7         |
| 5   | 1               | 2           | 1        | -       | -        | -        | -         |
| 6   | 10              | 4           | 2        | -       | -        | -        | 4         |
| 7   | 5               | 2           | 3        | -       | -        | 1        | 3         |
| 8   | 1               | -           | -        | -       | -        | -        | -         |
| Total (%) | 26 (65%) | 17 (81%) | 7 (77.8%) | - | - | 1 (33.3%) | 14 (77.8%) |

N = number of individual and combined cassette

AJ810121 and AB096217 reference strains. One ccr3 representative sample from SCCmeC III type was sequenced. Comparison of 326 nt and 108 amino acid (aa) revealed 100% similarity with AB047088, AB014436 and MG874130 reference strains. ccr4 is the only sample of SCCmeC VI was sequenced. Comparison of 399 nt and 177 aa revealed 100% identity with AF411935 and reference strain. ccr4 nt and aa identity were 92% and 98% with EF126185 reference strain due to 27 nt substitutions and 4 aa mutation. Three ccr5 represent SCCmeC V and SCCmeC XIV was sequenced. Comparison of 407 nt and 135 aa revealed 100% identity within sequences. The nt similarity was 99%, 94%, 93% and 63% while aa similarity was 99%, 99%, 99%, 98% and 67% with EF190468 (ccrC1-7 of S. epidermidis), HE980450 (ccrC1-10), EF190467 (ccrC 1-6 of S. haemolyticus) and KR187111 (ccrC2) reference strains (Fig. 3).

**DISCUSSION**

MRSA is a major worldwide cause of healthcare and community-acquired infections. Our study revealed the predominance of mecA gene among all S. aureus isolates in agreement with previous Egyptian studies (22, 23). Generally, there is no definite explanation for increasing MRSA infection in males than females. Previously, male gender and increased age were linked to acquisition of MRSA (24). Furthermore, a high rate of S. aureus infections in males may attributed to occupational risk or due to decrease expression of virulence factors by female hormones (25). Interestingly; the mid-age group in
our study was more exposed to MRSA infection that may support the hypothesis of correlation between increase activity with direct and indirect exposure to infection sources.

In this study, resistance was commonly detected for CTX (82.3%), TE (65.3%), CN (56.4%) and CIP (45.1%). Previous MRSA antibiogram in Egypt performed by Elshimy et al. (26) confirmed resistance against TE (39.1%), CN (37.9%), and CIP (23.5%). The ascending increase of resistance against several antibiotic groups over time is alarming and proof failure in MRSA combating. Increasing in MDR rate (63.7%) reflects the hazardous use of antimicrobial agents and the possible encoding of novel resistance genes (27).

Decreased resistance to chloramphenicol (20.2%) may be attributed to banning its use in food producing animals and awareness about its dangerous side effects (28). Low resistance to sulfonamide is related to the common thought that it causes renal problems, but modern sulfonamide has mild risk of crystallization if patient drink enough water. Hence, its prescription should be encouraged due to its reported low resistance and the strategy of old drugs revival (29). Although no isolates were resistant to vancomycin in this survey, resistance begins to appear against it all over the world (30). Vancomycin, linezolid and other antimicrobials that show moderate rate of resistance should be kept away from misuse prescription by physicians.

Understanding MRSA cassette composition may ultimately improve strategies to combat MRSA, identify strains origins, and select suitable antimicrobial therapy. The detection of new MRSA SCCmec became a continuous process (7). The M-PCR was preferred in detection as it avoids the disadvantage of difficult interpretation but it only detect five SCCmec types (31). Due to emerging of new SCCmec types and subtypes, new M-PCR is in need to reduce typing effort and time of SCCmec. Using real-time PCR followed by High resolution melting (HRM) analysis was approved as a appropriate procedure with higher specificity, sensitivity and reproducibility for SCCmec typing. It is the most suitable, rapid and cheaper technique for evaluating the chromosomal cassette of MRSA with higher accuracy compared to conventional M-PCR (32).

In the current study, high rate of SCCmec II (35.5%) revealed its predominance. In agreement with Abd El-Hamid and Bendary (33) who classified 33.3% of their isolates as SCCmec II. Rates of SCCmec V and IV were very low, contrary to Sobhy et al. (34) who reported SCCmec V as the most predominant (50%). In the current study, 43% of isolates were non-typeable using M-PCR. Traditional methods can identify more SCCmec types, however non-typeable strains still present with a lower rate (37%). Five out of 617 Asian MRSA isolates, 11 out of 375 Spanish MRSA isolates, four out of 113 American MRSA isolates and 13 out of 453 Canadian MRSA isolates were classified as non-typeable strains by Ito et al. (31), Perez-Roth et al. (35), Chung et al. (36) and Zahng et al. (9), respectively. Samples with only ccr or mec may be exposed to SCCmec element recombination, insertion of a new structure, or structural re-arrangements (35).

Absence of SCCmec element in some strains may refer to extreme polymorphism (4). Absence of ccr gave an indication that mecA gene probably, was not carried by a SCCmec element and previously reported by Berglund and Soderquist, (37) and Ruppe et al. (38). Presence of multiple SCCmec types in clinical MRSA isolates is common. Increasing the rate of multi SCCmec types in a single isolate may attribute to infection with many types of staphylococcus isolates. In India, 54.71% and 59% of MRSA isolates...
were confirmed to carry multiple SCCmec types by Bhutia et al. (39) and Nagasundaram et al. (40) respectively. From an epidemiological point of view, detection of various SCCmec elements in the tested strains, suggests that numerous MRSA clones are spread in Egyptian hospitals.

In agreement with Bimanand et al. (41), statistical analysis revealed absence of a significant correlation between types of SCCmec cassette in MRSA isolates and drug resistance. In this study no relation was detected between MDR and any type of detected cassette, Although strong relation was previously detected between MDR and SCCmec types I and III (42). In agreement with previous literature, most of SCCmec type III (82%) contain a large number of resistance genes in coagulase negative staphylococci (43).

The main objective of ccr sequencing was to detect differences between same ccr type in different SCCmec elements. The results showed that different ccr genes are diverse with less than 50% nt similarity as in Ito et al. study (44). All ccr genes of one type was in the same allotype as nt identity was >85% (45).

No significant variation among same ccr type of different SCCmec types indicating possible frequent recombination between different types of SCCmec elements. The minor phylogenetic variation between ccr2 in different SCCmec II and IV was not detected in phylogenetic tree as in tree constructed by Ito et al. (44). The classification reveals that only a single sample was SCCmec VI with ccr4 decreasing the chance for comparison with other SCCmec types with ccr4. Complete similarity of ccr2 in SCCmec II, IV harboring isolates may reveal to a common ancestor. ccrC of the sequenced MRSA samples were close to ccrC of S. epidermidis and S. haemolyticus. The high rate of ccrC1 in S. epidermidis and S. haemolyticus isolates increase the probability that SCCmecV was preferentially associated with this species and increases the thought that ccrC may invade S. aureus through the interspecies genes transmission process (46).

Further investigation including other staphylococci species should apply for a better understanding of relationship between SCCmec element compositions in different species.

CONCLUSION

In conclusion, S. aureus is a common pathogen among all types of infections in Egyptian hospitals with a predominant MRSA isolates. Most MRSA isolates were MDR reflecting misuse of antimicrobials especially in mid-age group of patients. Detection of various SCCmec types and subtypes including II, III, IVa, IVd, V, VI, IX and XIV plus the ability to identify novel SCCmec type (mecc and ccr2) among MRSA isolates by PCR against mec and ccr gene complexes indicate the complexity of MRSA epidemiology and increase chance for gene sharing creating new types. The presented data was important to understand MRSA epidemiology in Egyptian hospitals, alarming its dangerous diversity and provide advice for improving combating protocols.

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