Iranian Journal of Microbiology

Volume 14 Number 1 (February 2022) 56-66

ORIGINAL ARTICLE

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

Christiana Rezk Bottros Youssef*, Ashraf Ahmed Kadry, Amira Mohammed El-Ganiny

Department of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

Received: October 2021**, Accepted:** January 2022

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 SCC*mec* 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 SCC*mec* was typed by two methods.

Results: *S. aureus* rate was high in wounds, sputum, blood, and urine isolates. Antimicrobial resistance rates against cefotaxime, tetracycline, gentamicin, ciprofloxacin, erythromycin, azithromycin, clindamycin, chloramphenicol, 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 SCC*mec* element among 57% of isolates classified as SCC*mec* II (n=40), III (n=21), IVa (n=3), IVd (n=2), V(n=1), and four isolates contain both SCC*mec* II and SCC*mec* IV. Traditional typing by PCR for *mec* and *ccr* gene complexes was almost concordant with M-PCR. Furthermore, it was able to identify SCC*mec* 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 SCC*mec* cassettes.

Conclusion: Most MRSA isolates were MDR reflecting antimicrobials misuse. Detection of various SCC*mec* types among MRSA isolates indictae 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

*Corresponding author: Christiana Rezk Bottros Youssef, Ph.D, Department of Microbiology and Immunology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. Tel: +2-011-40922088 Fax: +2-055-2303266 Email: christianarezk@gmail.com

*mec*A gene, that is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) island (3).

SCC*mec* 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 SCC*mec* cassette by its excision or insertion into several integration sites. Three types of *ccr* have been reported (*ccr*A, *ccr*B, and *ccr*C) with multiple allotypes for each gene forming eight complexes (6).

Five *mec* gene and eight *ccr* gene complexes combination are essential in SCC*mec* nomenclature and assignment of new types. Availability of complete sequences from different MRSA isolates facilitated the assignment of thirteen (I-XIII) SCC*mec* types (7). SCC*mec* I, SCC*mec* II, and SCC*mec* III carry *mec*B and *ccr*1, *mec*A and *ccr*2, and *mec*A and *ccr*3 gene complexes, respectively. SCC*mec* IV is the smallest element that has a unique combination of *mec*B and *ccr*2 gene complexes. SCC*mec* V, SCC*mec* VI, SCC*mec* VII, and SCC*mec* VIII carry *mec*C2 and *ccr*5, *mec*B and *ccr*4, *mec*C1 and *ccr*5, and *mec*A and *ccr*4 gene complexes, respectively. SCC*mec* IX, SCC*mec* X, SCC*mec* XI, SCC*mec* XII, and SCC*mec* XIII carries *mec*C2 and *ccr*1, *mec*C1 and *ccr*7, *mec*E and *ccr*8, *mec*C2 and *ccr*5(C2) and *mec*A and *ccr*5(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 SCC*mec* elements. Thus, traditional method, in which *mec* and *ccr* were typed individually in a separate PCR, is more professional in typing novel and unidentified SCC*mec* 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 SCC*mec* 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 SCC*mec* typing with subsequent possible discovering of novel SCC*mec* in MRSA isolates. Finally, the relationship between SCC*mec* 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 mannitol 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 *mec*A 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 *mec*A 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 SCC*mec* elements was conducted by M-PCR reactions in a total volume of 50 µL/ reaction using specific primers illustrated in Table 1. SCC*mec* I, SCC*mec* III, SCC*mec* IVc, and SCC*mec* IVd were identified in one reaction while SCC*mec* II, SCC*mec* IVa, SCC*mec* IVb, and SCC*mec* V were

Table 1. Primers used for classification of SCC*mec* type

Target	Primer	Nucleotide sequence 5′ to 3′	Amplicon size (bp)	Ref.
MRSA identification	*mec*A-F	GTAGAA ATGACTGAACGTCCGATAA	310	(15)
	*mec*A-R	CCA ATTCCACATTGTTCGGTCTAA		
SCC*mec* I	Type I- F	GCTTTAAAGAGTGTCGTTACAGG	613	
	Type I- R	GTTCTCTCATAGTATGACGTCC		
SCC*mec* II	Type II- F	CGTTGAAGATGATGAAGCG	398	
	Type II- R	CGAAATCAATGGTTAATGGCAA		
SCC*mec* III	Type III- F	CCATATTGTGTACGATGCG	280	(9)
	Type III- R	CCTTAGTTGTCGTAACAGATCG		
SCC*mec* IVa	Type IVa- F	GCCTTATTCGAAGAAACCG	776	
	Type IVa- R	CTACTCTTCTGAAAAGCGTCG		
SCC*mec* IVb	Type IVb- F	TCTGGAATTACTTCAGCTGC	493	
	Type IVb- R	AAACAATATTGCTCTCCCTC		
SCC*mec* IVc	Type IVc- F	ACAATATTTGTATTATCGGAGAGC	200	
	Type IVc- R	TTGGTATGAGGTATTGCTGG		
SCC*mec* IVd	Type IVd- F	CTCAAAATACGGACCCCAATACA	881	
	Type IVd- R	TGCTCCAGTAATTGCTAAAG		
SCC*mec* V	Type V- F	GAACATTGTTACTTAAATGAGCG	325	
	Type V- R	TGAAAGTTGTACCCTTGACACC		
Class A *mec* (MecI-*mec*R1)	mI6mA7[b]	CATAACTTCCCATTCTGCAGATGATATACCA	1965 (type II)	(16)
		AACCCGACAACTACA	1797 (type III)	
Class B *mec* (IS1272)	IS5	AACGCCACTCATAACATATGGAA	1996	(10)
	mA6	TATACCAAACCCGACAAC		
Class C *mec* (IS431-*mec*A)	IS2	TGAGGTTATTCAGATATTT CGATGT	2072	(17)
	mA2	AACGTTGTAACCACCCCAAGA		
*ccr*1 (*ccr*A1)	*α 1*	AACCTATATCATCAATCAGTACGT	695	(18)
	Bc	ATTGCCTTGATAATAGCCITCT		
*ccr*2 (*ccr*A2)	*α 2*	TAAAGGCATCAATGCACAAACACT	937	
	Bc	ATTGCCTTGATAATAGCCITCT		
*ccr*3 (*ccr*A3)	*α 3*	AGCTCAAAAGCAAGCA ATAGAAT	1791	(19)
	Bc	ATTGCCTTGATAATAGCCITCT		
*ccr*4 (*ccr*A4)	A4.2	GTATCAATGCACCAGAACTT	1287	(20)
	B4.2	TTGCGACTCTCTTGGCGTTT		
*ccr*5 (*ccr*A5)	ɤ-F	CGTCTATTACAAGATGTTAAGGATAAT	518	
	ɤ- R	CCTTTATAGACTGGATTATTCAAAATAT		

identified in another reaction. Each reaction includes 1.5 µL (50 pmol) of each specific primer, 25 µL of EconoTaq 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 SCC*mec* was also performed by amplification of *mec* and *ccr* complexes. PCRs were performed for *mec*A, *mec*B and *mec*C 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 *mec*A, *mec*B, and *mec*C, 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 SCC*mec* 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 *ccr*1 gene of SCC*mec* IX (3 strains). MZ291466, MZ291467, and MZ291468 for *ccr*2 gene of SCC*mec* II, SCC*mec* IV, and non-typeable SCC*mec*, respectively. MZ291469 for *ccr*3 gene of SCC*mec* III and MZ291470 for *ccr*2 gene of SCC*mec* VI. MZ291471, MZ291472 and MZ291473 for *ccr*2 gene of SCC*mec*V, and SCC*mec*XIV (2 strains). MZ291474, MZ291475, MZ291476, and MZ291477 for *Mec*I, *Mec*R1, IS1272, and IS431.1 of *mec*A, *mec*B and *mec*C cassette chromosomes, respectively.

Statistical analysis. The relationships between frequencies of antimicrobial resistance, MDR, and SCC*mec* 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 *mec*A 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-

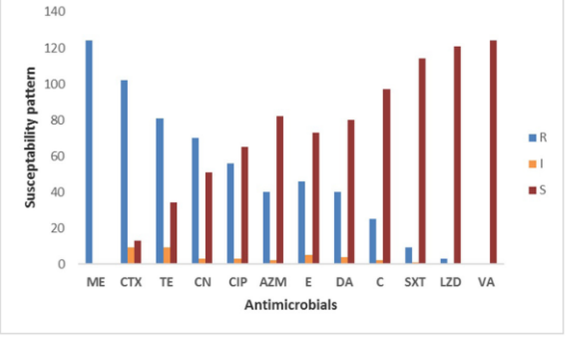

Fig. 1. Susceptibility patterns of MRSA clinical isolates to different antimicrobials.

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).

SCC*mec* classification among MRSA isolates. Out of the 124 isolates, SCC*mec* 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 SCC*mec* types II, III, IVa, IVd, and V, respectively. Four isolates were found positive for both SCC*mec* II and SCC*mec* IV. SCC*mec* 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 SCC*mec* types. SCC*mec* VI, SCC*mec* IX (C1), SCC*mec* XIV (A5) were detected among 1, 3 and 18 isolates. The newly 18 SCC*mec* XIV elements were classified as 5 (SCC*mec* XIV& II), 7 (SCC*mec* XIV& III), and 6 SCC*mec* XIV. One sample was considered as a novel type in combination between *mec*C and *ccr*2. 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 *mec*A gene, potentially consider as variant sub-types or new types (Table 3).

Relations between antimicrobial resistance and SCC*mec* 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 SCC*mec* II, III, IV, and XIV were resistant to most of the used antimicrobials while strains with SCC*mec* V, VI, and IX were not resistant against most antimicrobials. No association between the presence of multiple SCC*mec* cassettes and increasing resistance to antimicrobials was established (Fig. 2 and Table 4).

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

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

Antimicrobial agents	Age0-20			Age21-40			Age41-60		
	M	F	T	M	F	T	M	F	T
	Noofresisrantisolats			Noofresisrantisolats			Noofresisrantisolats		
ME	26	11	37	34	18	52	22	13	35
CTX	22	7	29	28	15	43	19	11	30
VA	-	-	-	-	-	-	-	-	-
CN	13	7	20	19	11	30	14	6	20
AZM	8	5	13	15	6	21	4	2	6
E	9	5	14	16	7	23	6	3	9
TE	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	2	4	6	-	1	1
C	8	4	12	7	5	12	1	-	1
LZD	-	-	-	-	2	2	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. SCC*mec* complex typing using Traditional PCR.

SCC*mec* 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%)
Scc*mec* II + Scc*mec* IV	A, B	2	4 (3.2%)
SCC*mec* VI	B	4	1 (0.8%)
SCC*mec* IX	C	1	3 (2.4%)
SCC*mec* XIV	A	5	6 (4.8%)
SCC*mec* XIV, III	A	5, 3	7 (5.6%)
SCC*mec* XIV, II	A	5, 2	5 (4.03%)
Novel SCC*mec*	C	2	1 (0.8%)
		Non typeable	
Only *ccr* complex	-	-	19 (15.3%)
Only *mec* complex	-	-	4 (3.22%)
Only *mec*A gene	-	-	23 (18.5%)

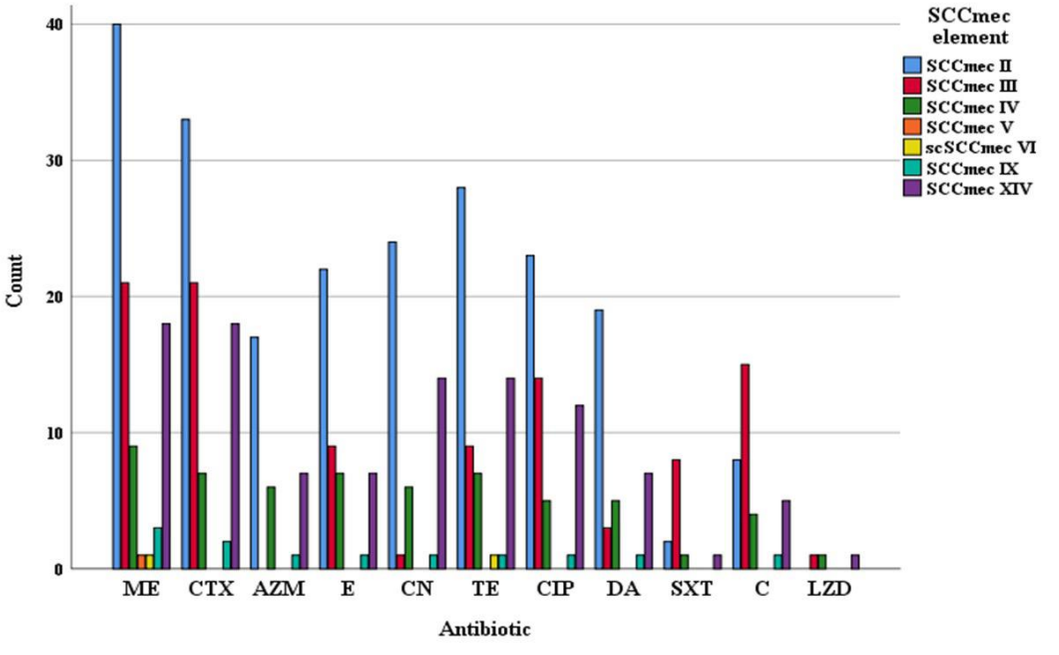

Fig. 2. Count of antimicrobial resistant strains in differernt SCC*mec* cassettes.

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 *ccr*1 samples were obtained from SCC*mec* 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 *ccr*2 representative samples from SCC*mec* 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 SCC*mec* cassettes classified by traditional PCR.

AB	Total Scc*mec* II	Total Scc*mec* III	Total Scc*mec* IV	Scc*mec* V (n=1)	SCC*mec* VI (n=1)	SCC*mec* IX (n=3)	Total SCC*mec* 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. Distrubtion of multi drug resistant strains on different SCC*mec* cassettes classified by traditional PCR.

MDR	SCC*mec* 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 *ccr*3 representative sample from SCC*mec* III type was sequenced. Comparison of 326 nt and 108 amino acid (aa) revealed 100% similarity with AB047088, AB014436 and MG874130 reference strains. *ccr*4 is the only sample of SCC*mec* VI was sequenced. Comparison of 399 nt and 177 aa revealed 100% identity with AF411935 and reference strain. *ccr*4 nt and aa identity were 92% and 98% with EF126185 reference strain due to 27 nt substitutions and 4 aa mutation. Three *ccr*5 represent SCC*mec* V and SCC*mec* 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 (*ccr*C1-7 of *S. epidermidis*), HE980450 (*ccr*C1-10), EF190467 (*ccr*C 1-6 of *S. haemolyticus*) and KR187111 (*ccr*C2) reference strains (Fig. 3).

DISCUSSION

MRSA is a major worldwide cause of healthcare and community-acquired infections. Our study revealed the predominance of *mec*A 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

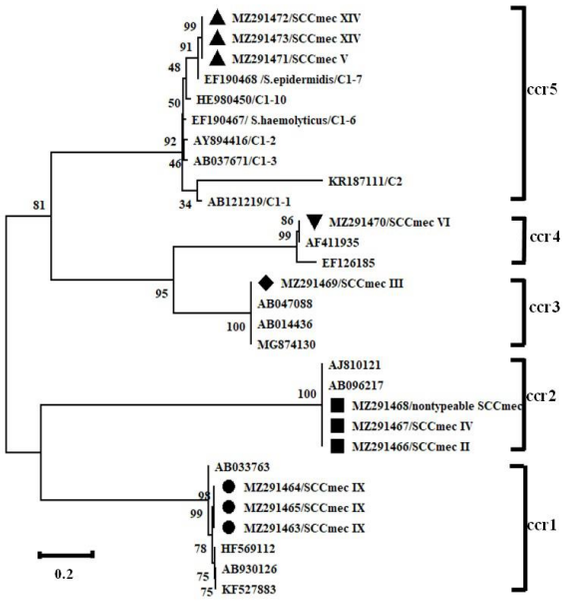

Fig. 3. Phylogenetic tree showing relationship between different *ccr* of different SCC*mec* elements. The tree was constructed by selecting Tamura 3-parameter +G as maximum likelihood model in MEGA X based on the lowest BIC score and 1000 bootstraps.

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 alerting 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 prescrption should be encourged due to its reported low resistance and the stratgey 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 SCC*mec* 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 SCC*mec* types (31). Due to emerging of new SCC*mec* types and subtypes, new M-PCR is in need to reduce typing effort and time of SCC*mec*. Using real-time PCR followed by High resolution melting (HRM) analysis was approved as a appropriate procedure with higher specificity, sensitivity and reproducibility for SCC*mec* 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 SCC*mec* II (35.5%) revealed its predominance. In agreement with Abd El-Hamid and Bendary (33) who classified 33.33% of their isolates as SCC*mec* II. Rates of SCC*mec* V and IV were very low, contrary to Sobhy et al. (34) who reported SCC*mec* V as the most predominant (50%).

In the current study, 43% of isolates were non-typeable using M-PCR. Traditional methods can identify more SCC*mec* 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 SCC*mec* element recombination, insertion of a new structure, or structural re-arrangements (35).

Absence of SCC*mec* element in some strains may refer to extreme polymorphism (4). Absence of *ccr* gave an indication that *mec*A gene probably, was not carried by a SCC*mec* element and previously reported by Berglund and Soderquist, (37) and Ruppe et al. (38). Presence of multiple SCC*mec* types in clinical MRSA isolates is common. Increasing the rate of multi SCC*mec* 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 SCC*mec* types by Bhutia et al. (39) and Nagasundaram et al. (40) respectively. From an epidemiological point of view, detection of various SCC*mec* 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 SCC*mec* cassette in MRSA isolates and drug resistance. In this study no relation was detected between MDR and any type of detected casstette, Although strong relation was previously detected between MDR and SCC*mec* types I and III (42). In agreement with previous literature, most of SCC*mec* 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 SCC*mec* 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 SCC*mec* types indicating possible frequent recombination between different types of SCC*mec* elements. The minor phylogenetic variation between *ccr2* in different SCC*mec* 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 SCC*mec* VI with *ccr*4 decreasing the chance for comparison with other SCC*mec* types with *ccr*4. Complete similarity of *ccr*2 in SCC*mec* II, IV harboring isolates may reveal to a common ancestor. *ccr*C of the sequenced MRSA samples were close to *ccr*C of *S. epidermidis* and *S. haemolyticus.* The high rate of *ccr*C1 in *S. epidermidis* and *S. haemolyticus* isolates increase the probability that SCC*mec*V was preferentially associated with this species and increases the thought that *ccr*C 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 SCC*mec* 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 SCC*mec* types and subtypes including II, III, IVa, IVd, V, VI, IX and XIV plus the ability to identify novel SCC*mec* type (*mecc* and *ccr*2) among MRSA isolates by PCR against *mec* and *ccr* gene complexes indictae 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.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the medical staff at clinical laboratories in Zagazig University hospitals and El-Ahrar educational hospital for providing clinical specimens.

REFERENCES

1. Götz F, Bannerman T, Schleifer KH (2006). The genera *Staphylococcus* and *Micrococcus.* In: *The Prokaryotes.* Archaea.Bacteria: Firmicutes, Actinomycetes. Eds, M Dworkin, S Falkow, E Rosenberg, KH Schleifer, E Stackebrandt. Springer, 3rd ed, New York, NY, pp. 5-75.
2. Raygada JL, Levine DP. Methicillin-resistant *Staphylococcus aureus*: a growing risk in the hospital and in the community. *Am Health Drug Benefits* 2009; 2: 86-95.
3. Miragaia M. Factors contributing to the evolution of Meca-mediated β-lactam resistance in Staphylococci: update and new insights from whole genome sequencing (WGS). *Front Microbiol* 2018; 9: 2723.
4. Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, et al. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. *Antimicrob Agents Chemother* 2006; 50: 1001-1012.
5. Yamaguchi T, Ono D, Sato A. Staphylococcal Cassette Chromosome *mec* (SCC*mec*) analysis of MRSA. *Methods Mol Biol* 2020; 2069: 59-78.
6. Turlej A, Hryniewicz W, Empel J. Staphylococcal cassette chromosome *mec* (SCC*mec*) classification and

typing methods: an overview. *Pol J Microbiol* 2011; 60:95-103.
7. McClure JA, Conly JM, Obasuyi O, Ward L, Ugarte-Torres A, Louie T, et al. A novel assay for detection of methicillin-resistant *Staphylococcus aureus* directly from clinical samples. *Front Microbiol* 2020; 11: 1295.
8. Liu J, Chen D, Peters BM, Li L, Li B, Xu Z, Shirliff ME. Staphylococcal chromosomal cassettes *mec* (SCC*mec*): a mobile genetic element in methicillin-resistant *Staphylococcus aureus. Microb Pathog* 2016; 101: 56-67.
9. Zahng K, MCClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcus cassette cassette chromosome *mec* types I to v in methicillin-resistant *Staphylococcus aureus. J Clin Microbiol* 2005; 43: 5026-5033.
10. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002; 40: 4289-4294.
11. Elshabrawy WO, Zaki ME, Kamel MF. Genetic and phenotypic study of methicillin-resistant *Staphylococcus aureus* among patients and health care workers in Mansoura university hospital, Egypt. *Iran J Microbiol* 2017; 9: 82-88.
12. Collee JG, Mackie TJ, McCartney JE (1996). Mackie & McCartney practical Medical Microbiology: 14th ed. Churchillivingstone. New York.
13. Wayne PA (2016). Performance standards for antimicrobial disk susceptibility tests; approved standard—11th ed. CLSI document M02-A11. Clinical and Laboratory Standards Institute.
14. Chapaval L, Moon DH, Gomes JE, Duarte FR, Tsai SM. An alternative method for *Staphylococcus aureus* DNA isolation. *Arq Bras Med Vet Zootec* 2008; 60: 299-306.
15. Sun J, Yang M, Sreevatsan S, Davies PR. Prevalence and characterization of *Staphylococcus aureus* in growing pigs in the USA. *PLoS One* 2015; 10(11): e0143670.
16. Ji Y. Methicillin-resistant *Staphylococcus aureus* (MRSA) protocols. Preface. *Methods Mol Biol* 2007; 391:v. :10.1007/978-1-59745-468-1.
17. Svensson K, Hellmark B, Söderquist B. Characterization of SCC*mec* elements in methicillin-resistant *Staphylococcus epidermidis* isolated from blood cultures from neonates during three decades. *APMIS* 2011; 119: 885-893.
18. Ito T, Katayama Y, Asada K, Mori N , Tsutsumimoto K, Tiensasitorn C, et al. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus. Antimicrob Agents Chemother* 2001; 45:1323-1336.
19. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec, ccr,* and major differences in junkyard regions. *Antimicrob Agents Chemother* 2007; 51: 264-274.
20. Urushibara N, Paul SK, Hossain MA, Kawaguchiya M, Kobayashi1 N. Analysis of Staphylococcal cassette chromosome *mec* in *Staphylococcus haemolyticus* and *Staphylococcus sciuri*: identification of a novel *ccr* gene complex with a newly identified *ccr*A allotype (*ccr*A7). *Microb Drug Resist* 2011; 17: 291-297.
21. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018; 35: 1547-1549.
22. El-Baz AM, Yahya G, Mansour B, El-Sokkary MMA, Alshaman R, Alattar A, et al. The Link between occurrence of class I integron and acquired aminoglycoside resistance in clinical MRSA isolates. *Antibiotics (Basel)* 2021; 10:488.
23. Youssef CR, Kadry AA, Shaker GH, El-Ganiny AM. The alarming association between antibiotic resistance and reduced susceptibility to biocides in nosocomial MRSA isolates from two regional hospitals in Egypt. *Arch Microbiol* 2021; 203: 3295-3303.
24. Morgan M, Evans-Williams D, Salmon R, Hosein I, Looker DN, Howard A. The population impact of MRSA in a country: the national survey of MRSA in Wales, 1997. *J Hosp Infect* 2000; 44: 227-239.
25. Humphreys H, Fitzpatick F , Harvey BJ. Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant *Staphylococcus aureus*: are they real, do they matter and why? *Clin Infect Dis* 2015; 61: 1708-1714.
26. Elshimy R, Khattab RA, Zedan H, Hosny AEDS , Elmorsy TH. Study on prevalence and genetic discrimination of methicillin-resistant *Staphylococcus aureus* (MRSA) in Egyptian hospitals. *Afr J Microbiol Res* 2018; 12: 629-646.
27. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infect Drug Resist* 2020; 13: 3255-3265.
28. Lee MH, Lee HJ, Ryu PD. Public health risks: chemical and antibiotic residues-review. *Asian-Australas J Anim Sci* 2001; 14: 402-413.
29. Cassir N, Rolain JM, Brouqui P. A new strategy to fight antimicrobial resistance: the revival of old antibiotics. *Front Microbiol* 2014; 5: 551.
30. Jian Y, Lv H, Liu J, Huang Q, Liu Y, Liu Q , Li M. Dynamic changes of *Staphylococcus aureus* susceptibility

to vancomycin, teicoplanin, and linezolid in a central teaching hospital in Shanghai, China, 2008–2018. *Front Microbiol* 2020; 11: 908.

31. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccr*C. *Antimicrob Agents Chemother* 2004; 48: 2637-2651.
32. Sadeghi Y, Salami SA, Kananizadeh P, Mozhgani SH, Pourmand MR. Real-time PCR followed by high-resolution melting analysis–a new robust approach to evaluate SCC*mec* typing of methicillin-resistant *Staphylococcus aureus. Future Microbiol* 2019; 14:155-164.
33. Abd El-Hamid MI, Bendary MM. Comparative phenotypic and genotypic discrimination of methicillin resistant and susceptible *Staphylococcus aureus* in Egypt. *Cell Mol Biol (Noisy-le-grand)* 2015; 61: 101-112.
34. Sobhy N, Aly F, El Kader OA, Ghazal A, Elbaradei A. Community-acquired methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections (in a sample of Egyptian population): Analysis of *mec* gene and staphylococcal cassette chromosome. *Braz J Infect Dis* 2012; 16: 426-431.
35. Perez-Roth E, Lorenzo-Diaz F, Batista N, Moreno A, Mendez Alvarez S. Tracking methicillin-resistant *Staphylococcus aureus* clones during a five-year period (1998 to 2002) in a Spanish hospital. *J Clin Microbiol* 2004; 42: 4649-4656.
36. Chung M, Dickinson G, De Lencastre H, Tomasz A. International clones of methicillin-resistant *Staphylococcus aureus* in two hospitals in Miami, Florida. *J Clin Microbiol* 2004; 42: 542-547.
37. Berglund C, Soderquist B. The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden-possible horizontal transfer of a staphylococcal cassette chromosome *mec* between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus. Clin Microbiol Infect* 2008; 14: 1048-1056.
38. Ruppe E, Barbier F, Mesli Y, Maiga A, Cojocaru R, Benkhalfat M, et al. Diversity of staphylococcal cassette chromosome *mec* structures in methicillin-resistant *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* strains among outpatients from four countries. *Antimicrob Agents Chemother* 2009; 53: 442-449.
39. Bhutia KO, Singh T, Adhikari L, Biswas S. Molecular characterization of community- & hospital-acquired methicillin-resistant & methicillin-sensitive *Staphylococcus aureus* isolates in Sikkim. *Indian J Med Res* 2015; 142: 330-335.
40. Nagasundaram N , Sistla S. Existence of multiple SCC*mec* elements in clinical isolates of methicillin-resistant *Staphylococcus aureus. J Med Microbiol* 2019; 68: 720-727.
41. Bimanand L, Taherikalani M, Jalilian FA, Sadeghifard N, Ghafourian S, Mahdavi Z, et al. Association between biofilm production, adhesion genes and drugs resistance in different SCC*mec* types of methicillin resistant *Staphylococcus aureus* strains isolated from several major hospitals of Iran. *Iran J Basic Med Sci* 2018; 21: 400-403.
42. Murugesan S, Perumal N, Mahalingam SP, Dilliappan SK , Krishnan P. Analysis of antibiotic resistance genes and its associated SCC*mec* types among nasal carriage of methicillin resistant coagulase negative staphylococci from community settings, Chennai, Southern India. *J Clin Diagn Res* 2015; 9: DC01-5.
43. Bouchami O, Achour W, Mekni MA, Rolo J , Hassen AB. Antibiotic resistance and molecular characterization of clinical isolates of methicillin-resistant coagulase-negative staphylococci isolated from bacteremic patients in oncohematology. *Folia Microbiol (Praha)* 2011; 56:122-130.
44. Ito T, Kuwahara-Arai K, KatayamaY, Uehara Y, Han X, Kondo Y, et al. Staphylococcal cassette chromosome *mec* (SCC*mec*) analysis of MRSA. *Methods Mol Biol* 2014; 1085: 131-148.
45. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* 2009; 53: 4961-4967.
46. Bouchami O, Hassen AB, De Lencastre H , Miragaia M. High prevalence of *mec* complex C and *ccrC* is independent of SCC*mec* type V in *Staphylococcus haemolyticus. Eur J Clin Microbiol Infect Dis* 2012; 31: 605-614.