

ORIGINAL ARTICLE

Staphylococcal Cassette Chromosome *mec* Typing of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Isolates in Sohag University Hospital, Egypt

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ABSTRACT

Key words:

CA-MRSA, SSTIs, PVL, SCCmec

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Background: community-acquired MRSA is an emerging pathogen increasingly reported worldwide. It is the most commonly reported microorganism to cause skin and soft tissue infections. **Objectives:** This study aimed to detect CA-MRSA in skin and soft-tissue infections (SSTIs), their pattern of antibiotic resistance, with molecular characterization of the isolated strains by detection of PVL gene and SCCmec typing. **Patients & Methods:** A total of 180 pus samples were collected from patients attending at outpatient clinics of General Surgery and Dermatology departments, Sohag University Hospital. *Staph aureus* was isolated and MRSA detected phenotypically by using cefoxitin discs, subculturing on ORSAB medium and detection of PBP by PBP2' Latex Agglutination Test. Antibiotic susceptibility testing was done by disc diffusion method and confirmed by Vitek 2 compact system. Conventional PCR was used to detect *mecA* gene and PVL gene, while SCCmec typing was done by multiplex PCR. **Results:** Eighty five (47.2 %) *S. aureus* strains were isolated; 36 (42.4%) out of them were diagnosed phenotypically as MRSA and confirmed by *mecA* gene testing. Twenty three (63.6%) of proved CA-MRSA were PVL positive. Typing of SCC showed 16 strains (44.4%) SCCmec type V, 10 (27.7%) SCCmec type IVd and 10 (27.7%) were untypable. **Conclusion:** CA-MRSA is an important cause of SSTIs in our community. PVL gene is a good diagnostic marker for CA-MRSA identification. SCCmec types V, IVd are the most prevalent CA-MRSA types in our community.

INTRODUCTION

Staphylococcus aureus is an ubiquitous organism widely distributed in nature and it can be found on the skin or in anterior nares in about 30% of normal individuals¹. It is the most commonly isolated organism from human infections and it causes variety of infections ranging from localized skin and soft-tissue infections (SSTIs) to serious systemic infections including; pneumonia, endocarditis, septic arthritis, osteomyelitis and sepsis².

Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other β -lactam antimicrobial drugs. They emerged by the late 1960s and were confined largely to hospitals (HA-MRSA)³. At the mid-1990s, a new MRSA clones known as community-associated MRSA (CA-MRSA) were reported in healthy people in the community lacking exposure to the health care system⁴. They are commonly reported to cause skin and soft tissue infections, rarely

implicated in severe infections as necrotizing fasciitis, pyomyositis and septic arthritis².

HA-MRSA strains carry a large staphylococcal chromosomal cassette *mec* (SCCmec) type I, II, or III. These cassettes contain *mecA* gene, in addition they carry resistant genes to many classes of non- β -lactam antimicrobials. HA-MRSA strains rarely carry the genes for the Pantone-Valentine leukocidin (PVL)⁵. In contrast, CA-MRSA isolates commonly carry smaller SCCmec elements, SCCmec type IV or type V. These smaller elements also carry a smaller *mecA* gene and are presumably more mobile, allowing to spread more efficiently in community settings. They are resistant to fewer non- β -lactam classes of antimicrobials and frequently carry PVL genes⁶.

The global epidemiology of CA-MRSA varies widely, with geographical differences in the predominant clones and their antibiogram⁵. The prevalence of HA-MRSA in Egypt is high reaching 50%⁷. The data on CA-MRSA in Egypt are limited particularly regarding the antibiotic susceptibility and molecular

characterization. The study aims to detect CA-MRSA in Sohag University Hospital, their antibiotic susceptibility pattern and their molecular characterization.

METHODOLOGY

Study design and patients

This is a descriptive cross-sectional study which was conducted during the period from January 2016 to September 2016 at the Medical Microbiology & Immunology department and Central Research Laboratory, Sohag Faculty of Medicine. A total of 180 pus samples were collected from patients with skin and soft tissue infections attending at outpatient clinics of General Surgery and Dermatology departments, Sohag University Hospital. Patients were selected according to the criteria of the Centers for Disease Control and Prevention (CDC), which defined CA-MRSA infection as: identification of MRSA in a patient with signs and symptoms of infection, either in the outpatient setting or within 48 hours after admission to a hospital, with no history of MRSA infection or colonization; no history of admission to a hospital during the previous year; and no history of dialysis, surgery, permanent indwelling catheters, or medical devices⁸.

Data collection

The following data were collected from the included patients: age, name, sex, onset of the lesion, size, site and number of abscesses, previous antibiotic intake and its type, hospitalization within the last year and associated medical conditions; diabetes, hypertension, cancer or renal failure. Informed consents were taken from all the participants. The study was approved by the Research Ethics Committee of Sohag Faculty of Medicine, Sohag University.

Identification of staphylococcal isolates

Pus samples were collected by sterile cotton swabs from the base of abscess, immediately labeled and sent to the lab. Swabs plated out on different culture media; mannitol salt agar, blood agar and nutrient agar. Morphologically suspected staphylococcal colonies were confirmed by gram staining and biochemically by catalase and coagulase tests.

Screening of MRSA among the identified *Staph aureus* isolates was done phenotypically by using cefoxitin discs, subculturing on Oxacillin Resistance Screening Agar Base (ORSAB) (Oxoid, UK) and detection of PBP by PBP2' Latex Agglutination Test (Oxoid, UK) following the manufacturers' protocol. Phenotypically identified MRSA colonies were preserved in 15% glycerol and tryptone soya broth at -70 °c for subsequent PCR testing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion method and E-test according to Clinical and Laboratory Standards Institute (CLSI,

2014) recommendations⁹. The used antimicrobial disks and E-test strips (Oxoid, UK) were: Pencillin 10u, Oxacillin 1u, Cefoxitin 30u, Teicoplanin 30u, Linezolid 30u, Quinpristin- dalfopristin 15u, Gentamicin 1u, Kanamycin 30u, Clindamycin, Tetracycline 30u, Doxycycline 30u, Trimethoprim sulphamethoxazole 25u, Rifampicin 5u, Ciprofloxacin 5u, Erythromycin 15u and Vancomycin MIC strips for E-test.

The antibiotic susceptibility pattern was confirmed by Vitek2 compact system (bio Mérieux, France).

Molecular analysis

DNA extraction

Extraction was done by the boiling method; few *Staph aureus* colonies from overnight growth were diluted in 50 ul distilled water then heated to 100°C for 10 minutes then centrifuged at 3000 rpm for 10 minutes. The clear supernatant is collected into a new tube and used.

Detection of *mecA* and *Panton-Valentine* genes by Polymerase chain reaction (PCR)

PCR amplification of *mecA* and PVL genes were carried out in a thermocycler (Biometra, Germany). The used primers were listed in table (1). The reaction volume was 25ul containing 1.25 uL of each the forward and reverse primers (Invitrogen, USA), 2 ul of the extracted DNA, 0.125 uL dreamTaq DNA polymerase (Thermo Scientific, USA), 2.5 dreamTaq green buffer (Thermo Scientific, USA), 0.5ul nucleotides (komabiotech, Korea), 0.75ul Mgcl (komabiotech, Korea) and 17.5 uL nuclease free water.

Thermal cycling program for *mecA* gene included the following: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 1min (denaturation), 58.5°C for 30 seconds (annealing) and 72°C for 1 min (extension), then final elongation at 72°C for 10 minutes followed by a hold at 4°C.

Thermal cycling program for PVL gene included the following: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 sec (denaturation), 55°C for 30 seconds (annealing) and 72°C for 1 min (extension), then final extension at 72°C for 10 minutes followed by a hold at 4°C. The amplified products (*mecA*; 851 bp and PVL; 433 bp) were separated by gel electrophoresis on 2% agarose gel and stained with 0.5 µg/mL ethidium bromide. The gel was photographed by gel documentation system (*Ingenius, Syngene, USA*).

Staphylococcal cassette chromosome mec typing by multiplex PCR

Multiplex-PCR typing assay contained 8 pairs of primers including the unique and specific primers for *SCCmec* types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V. These primers and their respective concentrations used in the PCR are listed in Table 1. The reaction volume was 25µL containing 2µL of the extracted DNA and 23µL PCR reaction mixture (as mentioned above). The amplification was performed in Thermal Cycler (Biometra, Germany) beginning with an

initial denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 min and another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min, ending with a final extension step at 72°C for 10 min

and followed by a hold at 4°C. The amplified products were separated by gel electrophoresis on 2% agarose gel and stained with 0.5 µg/mL ethidium bromide. The gel was photographed by gel documentation system (Ingenius, Syngene, USA).

Table 1 : List of primers

Gene	Oligonucleotide sequence (5-3)	Conc. (µM)	Product size (bp)	Reference
mecA gene			851	11
mecA-QF1	GTGGAATTGGCCAATACAGGAAC			
mecA-QR1	ACGTTGTAACCACCCCAAGA			
PVL gene				
luk-PV-1	ATCATTAGGTA AAAATGTCTGGACATGATCCA		433	12
luk-PV-2	GCATCAASTGTATTGGATAGCAAAAAGC			
SCCmec gene				10
Type I-F	GCTTTAAAGAGTGTCTGTTACAGG	0.048	613	
Type I-R	GTTCTCTCATAGTATGACGTCC			
Type II-F	CGTTGAAGATGATGAAGCG	0.032	398	
Type II -R	CGAAATCAATGGTTAATGGACC			
Type III-F	CCATATTGTGTACGATGCG	0.04	280	
Type III-R	CCTTAGTTGTCGTAACAGATCG			
Type IVa-F	GCCTTATTCGAAGAAACCG	0.104	776	
Type IVa-R	CTACTCTTCTGAAAAGCGTCG			
Type IVb-F	TCTGGAATTACTTCAGCTGC	0.092	493	
Type IVb-R	AAACAATATTGCTCTCCCTC			
Type IVc-F	ACAATATTTGTATTATCGGAGAGC	0.078	200	
Type IVc-R	TTGGTATGAGGTATTGCTGG			
Type IVd-F5	CTCAAATACGGACCCCAATACA	0.28	881	
Type IVd-R6	TGCTCCAGTAATTGCTAAAG			
Type V-F	GAACATTGTTACTTAAATGAG	0.06	325	
Type V-R	TGAAAGTTGTACCCTTGACACC			

RESULTS

A total of 180 pus samples were collected (160 from general surgery and 20 from dermatology outpatient clinics). One hundred strains (55.5%) were identified as *Staphylococcus spp.* Eighty five (47.2 %) of them were diagnosed as *Staph aureus*. Out of the 85 *Staph aureus* isolates; 36 (42.4%) strains were confirmed phenotypically as MRSA.

According to the antibiotic susceptibility testing by disc diffusion and Vitek2 compact system, the isolated CA-MRSA strains were sensitive to most of the used antibiotics. However, they showed resistance to tetracycline and gentamicin (90% and 25% respectively) (Table 2).

According to the molecular analysis; *mec A* gene was examined in all *Staph aureus* strains (85) and it was only detected in the phenotypically identified MRSA strains (36; 42.4%) (Figure1). Panton-Valentine gene was evaluated in 23 (63.9%) of genetically confirmed MRSA isolates (36)(Figure 2). *SCCmec* typing of the isolated CA-MRSA strains revealed 16 strains (44.4%)

SCCmec type V and 10 strains (27.7%) *SCCmec* type IVd while 10 strains (27.7%) were untypable (Figure 3).

Table 2: Antibiotic resistance pattern of the isolated CA-MRSA according to Vitek2 Compact system

Antibiotic	MIC	%
cefoxitin screening		37
benzyl penicillin	≥ 0.5	100
oxacillin	≥ 4	100
gentamicin	≤ 0.5	25
ciprofloxacin	≤ 0.5	0
levofloxacin	0.25	0
moxifloxacin	≤ 0.25	0
erythromycin	≤ 0.25	0
clindamycin	≤ 0.25	0
Quinopristin /dalfopristin	≤ 0.25	0
linezolid	2	0
vancomycin	< 0.5	0
tetracycline	≥ 16	90
Tigecycline	≤ 0.12	0
nitrofurantoin	≤ 16	0
rifampicin	≤ 0.5	0
trimethoprim /Sulpha-methoxazole	≤ 10	0
inducible clindamycin resistance		0

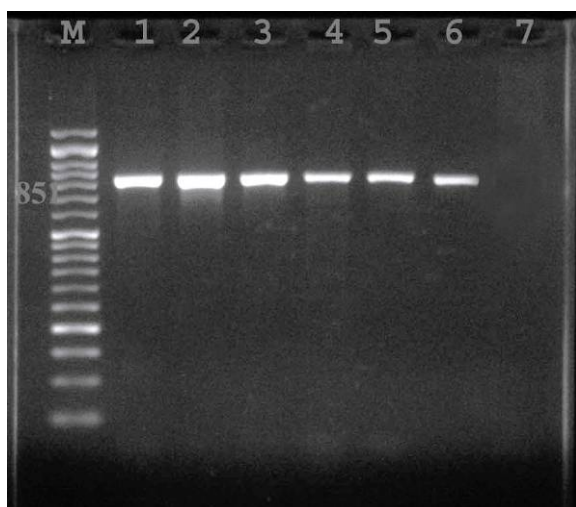


Fig. 1: Agarose gel electrophoresis of PCR-amplified products of the *mecA* gene. Lane M; DNA ladder (50 bp DNA Marker). Lanes 1-6; MRSA (851 bp). Lane 7; negative control

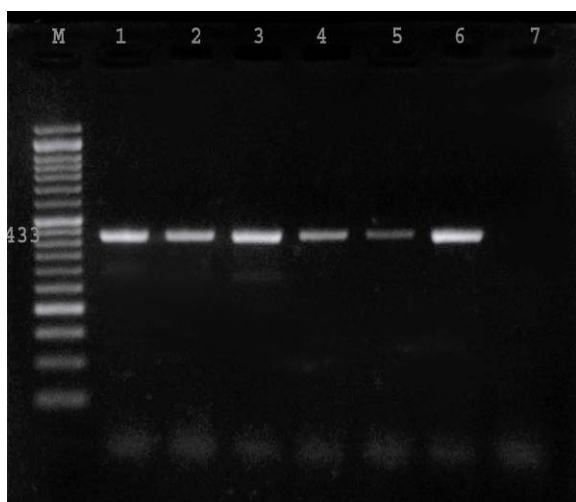


Fig. 2 :Agarose gel electrophoresis of PCR-amplified products of the Panton-valentine gene. Lane M; DNA ladder (50 bp DNA Marker). Lanes 1-6; PVL positive MRSA strains (433 bp). lane 7; negative control

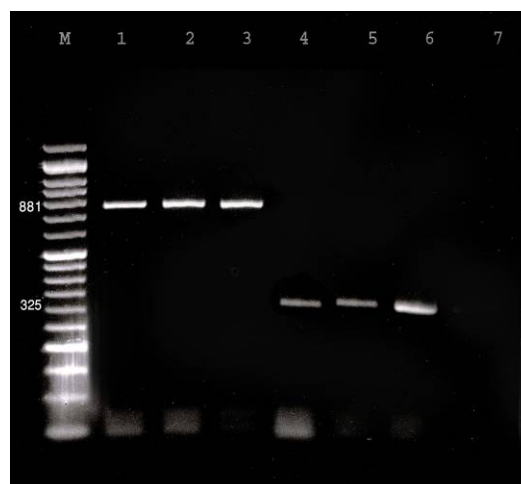


Fig. 3: Agarose gel electrophoresis of multiplex PCR-amplified products of *SCCmec* typing of MRSA. Lane M; DNA ladder (50 bp DNA Marker). Lanes 1-3; *SCC* type IVd (881 bp), Lanes 4-6; *SCC* type V (325 bp), lane 7; negative control.

DISCUSSION

Staph aureus is traditionally the most common cause of skin and soft tissue infections (SSTIs), ranging from the benign (e.g., impetigo and uncomplicated cellulitis) to the life threatening infections. In the past decades community-associated skin and soft tissue infections were increasingly reported worldwide². In the present study 180 pus specimen were collected from patient's skin infections attending at dermatology and general surgery outpatient clinics. A total of 100 strains (55.5%) were identified as *Staphylococcus spp.*, and 85 (47.2 %) were identified as *Staphylococcus aureus*. A similar result was found by Maina et al.¹³ in Kenya but higher figures were reported by Sobhy et al.¹⁴ and Abdallah et al.¹⁵ in Egypt. Lui et al. also found that 71.6% of cases of SSTI were caused by community-associated *S. aureus* in a large multicentre study in China¹⁶.

Methicillin resistance, which indicates resistance to all β -lactam antibiotics, is mediated by the *mecA* gene encoding penicillin-binding protein2a (PBP2a) or penicillin binding protein2' (PBP2'), which unlike other penicillin-binding proteins its active site does not bind methicillin or other β -lactam antibiotics.

Many phenotypic methods have been developed to detect resistance to β -lactam antibiotics as cefoxitin discs screening, with a sensitivity of 100% as reported by many studies^{14,17} and detection of penicillin binding protein 2' (PBP2') by latex agglutination test¹⁸. In the present study 36 (37%) out of the 85 *S. aureus* strains; were resistant to cefoxitin discs (100% sensitivity) and were penicillin binding protein 2' (PBP2') producers

(100% sensitivity). The same results were reported by Velasco et al.¹⁹ and Sobhy et al.¹⁴.

Confirmation of methicillin resistance was achieved by detection of *mecA* gene. All of the 36 (42.4%) CA-MRSA strains were positive for *mecA* gene, this is comparable to that reported by a previous study conducted in Alexandria where 47.4% were positive for *mecA* gene¹⁴. A lower rate of MRSA (25%) was reported by Maina et al., in Kenya¹³, and a rate of 20.23% was reported by Ghebremedhin et al. in Nigeria²⁰. Although CA-MRSA accounted for only 2.6% in China by Liu et al.¹⁶. A higher rate of 61% was reported in USA by Forcade et al.²¹.

The Panton-Valentine Leukocidin (*PVL*) is a leukocidin. Which is a two-component pore-forming toxin with cytolytic activity on certain cells of the immune system (neutrophils, macrophages, and monocytes)²². It is encoded by the *lukS-PV* and *lukF-PV* genes. *PVL*-producing *S. aureus* was linked to severe and recurrent skin and soft tissue infections. *PVL*-positive MRSA also was suspected to be associated with severe community onset-pneumonia⁸.

PVL genes are mainly carried by community-associated MRSA (CA-MRSA), and it was defined as a stable marker for it in many studies. Approximately 60 to 100% of CA-MRSA strains have been shown to carry *PVL* genes in USA⁸. There are differences of *PVL* gene carriage from different geographical area and from different isolated clones¹.

In the current study 23 (63.6%) of isolated CA-MRSA were *PVL* positive which is higher than a previous two Egyptian studies where *PVL* positive MRSA was 19.04%¹¹ and (33.33%)¹⁴. It was also detected in 20.3% of MRSA isolates in a study in Kenya¹³. About 9.8% of the CA-MRSA strains were *PVL* positive in a study in china¹⁶. In a study in Russia only 6.26% of the isolated CA-MRSA carries *PVL* genes²³. Higher results were reported in a study in Tunisia where *PVL* positive MRSA was 79% from isolated CA-MRSA strains²⁴. According to our study, *PVL* is a good marker for CA-MRSA infections as it was detected in more than 60% of the isolates.

In our study 10 (43.5%) carried *SCCmec* of type V, 5 (21.7) % of them carried *SCCmec* type IV d element and 8(34.7%) untypable *SCCmec*, the results are similar to a previous Egyptian study, 50% carried *SCCmec* V, (16.7%) *SCCmec* IV, and (33.3%) were untypable¹⁴. Although Mariem et al. reported that 13.6% of *PVL* positive were untypable and the rest carry *SCCmec* IV²⁴.

The staphylococcal cassette chromosome *mec* (*SCCmec*), is a 21- to 67-kb mobile genetic element, which harbors the methicillin resistance (*mecA*) gene and other antibiotic resistance determinants²⁵. *SCCmec* typing is one of the most important molecular tools used for understanding the epidemiology and clonal strain relatedness of MRSA¹⁰.

To-date, ten *SCCmec* types have been identified²⁶. Some cassettes, *SCCmec* (types I, II, and III, are large

and possess mobile genetic elements (MGE), such as integrated plasmids and transposons, and are frequently associated with hospital-acquired MRSA. while the emerging community-acquired MRSA strains possess small, mobile *SCCmec* type IV or V genetic elements which contain the *mecA* gene with or without additional antibiotic resistance genes and are more easily transferred to other strains of *S. aureus* than larger *SCCmec* (types I, II, and III) elements³.

In this study Typing of *SCC* using Zhang et al. protocol¹⁰ shows: 16 (44.4%) out of the 36 CA-MRSA strains were *SCCmec* type V, and 10 (27.7%) was *SCCmec* type IVd. However, 10 (27.7%) were untypable. Similar results were reported in a study from Alexandria, at which *SCCmec* type V were 50% and 5.56% was classified as type IVc, while 44.44% were untypable. In a study in Russia all isolated CA-MRSA strains were *SCCmec* type IVc²³. In a study in India, the isolated CA-MRSA were *SCCmec* IVc (53.5%) and *SCCmec*V (46.5%)²⁷. In another study in Tunisia isolated CA-MRSA carried type IVc (89.2%) or nontypeable *SCCmec* (10.7%)²⁴.

Untypable strains that did not fit into the *SCCmec* typing can be designated as possibly new *SCCmec* types (<http://www.sccmec.org>)²⁸.

CA-MRSA isolates have typically known to be susceptible to most non-β-lactam antimicrobial drugs compared to HA-MRSA¹. These criteria were used as a marker of CA-MRSA but there is a recent emergence of multidrug-resistant CA-MRSA, thus confounding the current serious public health problem⁶.

In our study, the isolated CA-MRSA show sensitivity to most used non β -lactam antibiotics except for tetracycline (90% were resistant) and 25% show resistance to gentamicin. Both drugs have widely been used for the treatment of *S.aureus* infections in the community. Resistance to these drugs usually acquired by plasmids or transposons carrying resistance genes to these drugs⁶. A previous study conducted in Egypt also found that 27.78% of CA-MRSA were resistant to gentamicin, 11.11% were found to be resistant to cotrimoxazole and 100% resistant to fusidic acid, with no resistance to tetracycline¹⁴. In a study conducted in Tunisia the CA-MRSA isolated showed resistance to gentamicin (7%) and tetracycline (75%) in addition to resistance to other antibiotics²⁴. It is noteworthy that all isolates were susceptible to trimethoprim /Sulphamethoxazole, clindamycin, and linezolid which enable clinicians to have a number of options when choosing empiric treatment of CA-MRSA infections in our hospital.

Our study provided important data about the prevalence of CA-MRSA in SSTIs and their antibiotic susceptibility pattern. These data could guide the clinicians with the effective antibiotic therapy. Moreover, this study also provided important molecular data regarding the CA-MRSA *SCCmec* gene types and subtypes prevalent in our community.

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