Research Article

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) Typing of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from Community- and Hospital-Acquired Infections

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for the growing number of hospital- and community-acquired infections.

Objectives: In this study, we aimed to determine the prevalence of MRSA, its antimicrobial resistance profile, and molecular typing of strains isolated from different infections in Iran.

Methods: A total of 100 *S. aureus* strains were isolated from various clinical specimens from Al-Zahra Hospital of Isfahan, Iran during January-June, 2015. Antimicrobial susceptibility test was performed, using the disk diffusion method. For identifying the MRSA phenotype, oxacillin agar screening was performed. Detection of *mecA* gene among the isolates was performed via polymerase chain reaction (PCR) using specific primers, followed by multiplex PCR for SCC*mec* typing of MRSA isolates.

Results: In the present study, 54 (54%) isolates were identified as MRSA. Overall, 12 (22.23%) and 42 (77.7%) isolates were obtained from community- and hospital-acquired infections, respectively. SCC*mec* typing among MRSA isolates showed that 19 (35.18%), 13 (24.07%), 6 (11.11%), 5 (9.25%), and 3 (5.55%) isolates contained SCC*mec* type III, type I, type IV, type II, and type V, respectively; however, 8 (14.81%) isolates were nontypable.

Conclusions: In the current study, SCC*mec* type III isolates were the most common among 54 MRSA isolates in a teaching hospital in the center of Iran. This finding might be attributed to antibiotic pressure, facilitating clonal selection.

Keywords: *Staphylococcus aureus*, SCC*mec* type, CA-MRSA, HA-MRSA

1. Background

Staphylococcus aureus (*S. aureus*) is a major human pathogen, responsible for both community- and hospital-acquired infections worldwide. Methicillin resistance is induced by *mecA* gene, which is located on the *staphylococcal* cassette chromosome mec (SCC*mec*), a large heterologous genetic element, encoding a low-affinity penicillin-binding protein 2a (PBP2a), which inhibits the activity of β-lactam antibiotics (1-3).

The increasing prevalence of methicillin-resistant *S. aureus* (MRSA) complicates the treatment and management of *staphylococcal* infections (4). Therefore, MRSA screening is extremely important for epidemiological surveillance in order to prevent the spread of MRSA infections. To classify MRSAs, it is necessary to understand their genetic structure and detect all SCC*mec* types (2, 5). Although conventional MRSA typing techniques are not adequate for epidemiological surveys, numerous molecular techniques are available for this purpose (6, 7).

SCC*mec* typing is a polymerase chain reaction (PCR)-based method, which is essential for characterization of MRSA clones in epidemiological studies and provides important information about the mobile genetic elements of methicillin resistance (8, 9). SCC*mec* is an element involved in the horizontal transfer of resistant genes among *Staphylococcus* strains. It is also a marker for differentiation between hospital-acquired methicillin-resistant (HA-MRSA) and community-acquired methicillin-resistant (CA-MRSA) strains (10-12).

MRSA SCC*mec* elements have been classified into 5 different allotypes and sometimes show poor discriminatory

power ([12](#), [13](#)). SCC*mec* types I, II, and III are usually found in HA-MRSA and multidrug-resistant (MDR) strains, whereas CA-MRSA strains are mostly associated with SCC*mec* type IV or V ([14](#), [15](#)).

2. Objectives

The main objective of this study was to determine the prevalence of methicillin resistance among *S. aureus* strains, isolated from a teaching hospital in Isfahan and to identify molecular typing of MRSA isolates through SCC*mec* typing method. Such findings could promote effective infection control and moderate antibiotic use in hospitals and reduce the prevalence of MRSA in hospitals.

3. Methods

3.1. Bacterial Strains and Phenotypic Test

A total of 100 *S. aureus* strains were isolated from inpatients and outpatients, admitted to Al-Zahra Hospital of Isfahan, Iran during January-June, 2015. The isolates were obtained from different clinical specimens, including wounds, blood samples, urine samples, and abscesses. *S. aureus* isolates were identified through culture studies, as well as morphological and conventional biochemical tests, including Gram staining, catalase test, mannitol fermentation, slide coagulase test, tube coagulase test, and DNase test. Then, the isolates were classified as CA-MRSA or HA-MRSA, based on the recorded data of the patients. Hospital-acquired infection was defined as bacterial colonization after more than 48 hours of hospitalization.

3.2. Antimicrobial Susceptibility Test

The susceptibility of *S. aureus* isolates to antimicrobial agents was determined by disk diffusion method on Mueller-Hinton agar medium, according to the clinical and laboratory standards institute (CLSI) guidelines ([16](#)). The used antibiotics were as follows: vancomycin (30 μg), tetracycline (30 μg), gentamicin (10 μg), clindamycin (2 μg), ciprofloxacin (5 μg), rifampin (30 μg), cefoxitin (30 μg), levofloxacin (5 μg), and cotrimoxazole (25 μg) (HiMedia, India). Inducible macrolide-lincosamide-streptogramin B resistance was identified as the flat zone of clindamycin growth inhibition near the erythromycin disk (D-shaped). *S. aureus* strain ATCC25923 was used for the quality control of antibiotic susceptibility test.

3.3. Phenotypic Detection of MRSA Isolates

All *S. aureus* isolates were screened for oxacillin resistance, using agar screening method. Resistance to methicillin was defined as the growth capacity in the agar screening medium, including 4% NaCl and 8 μg/mL of oxacillin (Sigma, USA). Also, *S. aureus* ATCC 25923 was used as the control.

3.4. Genotypic Detection of mecA Gene

For this purpose, DNA extraction was performed for all the isolates, which were grown overnight on nutrient agar at 37°C, using the K0512 DNA kit (Fermentas, Germany) in accordance with the manufacture's instructions. Using specific primers listed in [Table 1](#), amplification of the 583-bp fragment of *mec*A gene was performed through PCR.

The amplification reaction mixture (25 μL) contained 4 μL of DNA template, 2.5 μL of PCR buffer (10X), 0.75 μL of MgCl2 (50 mM), 0.5 μL of deoxynucleotide triphosphates (dNTPs, 10 mM), 1 μL of each primer (2 μL in total), 0.25 μL of Ex-Taq DNA polymerase (5 u/μL), and 15 μL of distilled water. PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 7 minutes.

3.5. Multiplex PCR for SCCmec Typing

SCC*mec* typing for 54 MRSA isolates was performed via multiplex PCR method. Primers shown in [Table 1](#) were used for this purpose. Each PCR round was performed in a final volume of 25 μL, consisting of 4 μL of DNA template, 2.5 μL of PCR buffer (10X), 0.75 μL of MgCl2 (50 mM), 0.5 μL of dNTPs (10 mM), 0.5 μM of βF1, α3R1, ccrR, and ccrF primers, 0.3 μM of 1272F1, 1272R1, 5RmecA, and 5R431 primers (3.2 μL in total), 0.25 μL of Ex-Taq DNA polymerase (5 u/μL), and 13.8 μL of distilled water.

DNA was amplified with a thermocycler (Eppendorf, Germany), and multiplex PCR conditions were as follows: initial denaturation at 94°C for 4 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, and final extension at 72°C for 10 minutes.

4. Results

During 6 months, 100 *S. aureus* isolates from various clinical specimens were collected from Al-Zahra Hospital of Isfahan. The isolates were obtained from urinary tract infections (UTIs) (n = 23, 23%), blood samples (n = 21, 21%), wounds (n = 20, 20%), abscesses (n = 14, 14%), and other samples (n = 22, 22%). The rate of isolation in females and males

Table 1. Primers Used in the Present Study

Target	Primer	Sequence	Product Size (bp)	Reference
mecA	F	AAAATCGATGGTAAAGGTTGGC	583	(17)
	R	AGTTCTGCAGTACCGGATTTG		
SCCmec	βF	ATTGCCTTGATAATAGCCYTCT	937	(18)
	α3R	TAAAGGCATCAATGCACAAACACT		
	ccrCF	CGTCTATTACAAGATGTTAAGGATAATCCTTTA	518	
	ccrCR	TAGACTGGATTATTCAAAATAT		
	1272F1	GCCACTCATAACATATGGAA	1415	
	1272R1	CATCCGAGTGAAACCCAAA		
	5RmecA	TATACCAAACCCGACAACTAC	359	
	5R431	CGGCTACAGTGATAACATCC		

was 62 (62%) and 38 (38%), respectively. The patients' average age was 47 years (range, < 1 - 89 years).

Antimicrobial susceptibility test was performed via disk diffusion method. The results showed that *S. aureus* resistance to cefoxitin (54%) was the highest resistance rate, followed by tetracycline (52%), gentamicin (30%), clindamycin (29%), ciprofloxacin (20%), rifampin (19%), cotrimoxazole (10%), and levofloxacin (10%). The results indicated that all the isolates were sensitive to vancomycin. Also, inducible resistance to clindamycin was found in 2 isolates.

In the present study, presence of *mecA* gene in all the isolates was evaluated, using the oxacillin agar screening method and confirmed by molecular methods. The results showed that 54 (54%) isolates were identified as MRSA, using the agar screening method (phenotypic detection). Overall, 13 (24.07%) out of 54 isolates were obtained from wounds, 10 (18.51%) from blood, 8 (14.81%) from UTI samples, 6 (11.11%) from abscesses, 5 (9.25%) from trachea, and 12 (22.22%) from other samples.

In this study, we collected 42 (77.7%) MRSA isolates from inpatients (HA-MRSA) and 12 (22.23) isolates from outpatients (CA-MRSA). The patients with CA-MRSA were younger than those with HA-MRSA. Among 100 strains tested with PCR (genotypic detection), 54% were positive for *mecA* gene. Finally, to determine the SCC*mec* type of MRSA isolates, SCC*mec* typing was evaluated among *S. aureus* isolates. As the findings revealed, 19 (35.18%), 13 (24.7%), 6 (11.11%), 5 (9.25%), and 3 (5.55%) MRSA isolates contained SCC*mec* type III, type I, type IV, type II, and type V, respectively; also, 8 isolates (14.81%) were nontypable.

5. Discussion

Today, MRSA is one of the most frequent nosocomial pathogens worldwide (19). The rate of antibiotic resistance, including methicillin resistance, is high in developing countries such as Iran, which might be due to the uncontrolled high rate of antibiotic prescription. The mean incidence of MRSA infections in Iranian hospitals has been reported to be 52.7% $\pm$ 4.7 (20). Overall, CA-MRSA infections occurred in healthy individuals outside healthcare settings without any risk factors and could act as a source of transfer to community.

On the other hand, recent studies have demonstrated that the epidemiology of MRSA has changed, and CA-MRSA strains are now being introduced into the hospital setting. Detection of the epidemiology, clinical syndrome, and antibiotic resistance of possible CA-MRSA allows early initiation of treatment and control measures (21, 22). The prevalence of HA-MRSA in the present study was 77.7% (n = 42), which is high. In this regard, Stranden et al. and Moghadami et al. (14, 23) have reported similar findings.

As presented in Table 2, based on the antimicrobial susceptibility profile in the current study, the highest resistance of *S. aureus* isolates was reported against cefoxitin (54%), oxacillin (54%), and tetracycline (52%), followed by gentamicin (30%), clindamycin (29%), ciprofloxacin (20%), rifampicin (19%), cotrimoxazole (10%), and levofloxacin (10%). Incidentally, all the isolates were susceptible to vancomycin in the present study. These rates were lower than those reported by Moghadam et al. and Ghasemian et al. (24, 25).

In the present study, 16% of MRSA isolates were MDR, which is the main cause of failure in antibiotic therapy and increasing cost of treatment for patients and healthcare systems throughout the world (20). The phenotypic

Table 2. Antimicrobial Susceptibility Test

Antibiotics	Susceptibility, Number of Isolates (%)					
	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Ciprofloxacin	66	66	14	14	20	20
Vancomycin	100	100	0	0	0	0
Gentamicin	60	60	10	10	30	30
Clindamycin	66	66	5	5	29	29
Tetracycline	43	43	5	5	52	52
Levofloxacin	82	82	8	8	10	10
Rifampin	76	76	5	5	19	19
Cefoxitin	42	42	4	4	54	54
Cotrimoxazole	87	87	3	3	10	10

methods for MRSA detection are simple and relatively cost-effective, although they are subjected to variations in environmental conditions, such as incubation time, medium pH, and inoculum size. The associated drawbacks are among several reasons for replacing these methods with molecular ones, which are accurate and have good reproducibility (12).

Detection of *mecA* gene in the present study by PCR method revealed that phenotypic and genotypic methods produce similar results regarding the susceptibility of *S. aureus* to methicillin (MRSA). The present study showed that the prevalence of resistant strains to methicillin has steadily increased in comparison with a previous study performed in the same hospital (20% vs. 54%) (5, 26). Furthermore, a similar prevalence rate (54%) was reported by Moghadami et al. in Shiraz, Iran (21). In contrast, Ghasemian et al. reported a prevalence rate of 25% in Tehran in 2015, and Orrett et al. showed a prevalence rate of 20.8% from nosocomial sources in Trinidad (3).

Based on SCC*mec* typing, MRSA strains are classified into 5 different allotypes, 2 of which have been identified in Asian countries. The clonotype II is distributed in Japan and Korea, whereas clonotype III is predominant in some other Asian countries, such as Saudi Arabia, India, China, and Thailand (8, 27, 28). We used SCC*mec* typing to monitor the epidemiology of MRSA isolates, collected from hospital- and community-acquired infections. Distribution of SCC*mec* types among *S. aureus* isolates demonstrated that 19 (35.18%), 13 (24.07%), 6 (11.11%), 5 (9.25%), and 3 (5.55%) strains harbored SCC*mec* type III, type I, type IV, type II, and type V, respectively; however, 8 (14.81%) strains were nontypable (Figure 3).
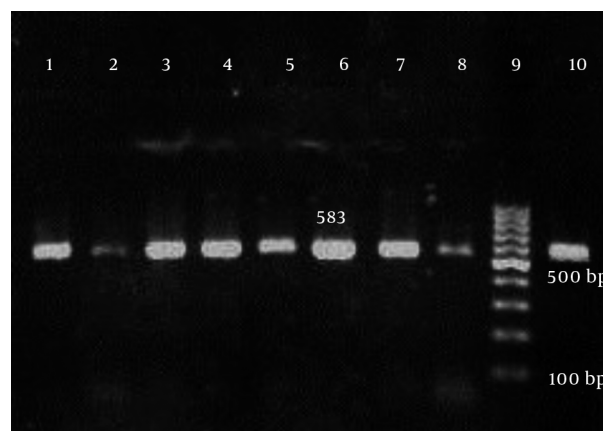
Similar findings in agreement with the present study

Figure 1. Agarose gel electrophoresis of *mecA* genes as PCR amplification products. Lane 1, positive control (*S. aureus* ATCC33591); lane 2 - 8, positive *mecA* sample (583 bp); and lane 9, 100-bp DNA ladder.

have been reported by Japoni et al., while contradictory results have been reported by Pe'rez-Va'zquez et al., Chongtrakool et al., and Chongtrakool et al. (29). While SCC*mec* type III (35.18%) showed the highest prevalence in the present study, frequency of SCC*mec* type V (5.55%) was low. These results were consistent with previous studies performed in Iran and other countries (24, 27, 30, 31).

As expected, SCC*mec* types V and IV have been overrepresented among MRSA strains, isolated from community-acquired infections. Only 2 isolates with SCC*mec* type IV belonged to HA-MRSA, while SCC*mec* types I, II, and III were associated with MRSA strains, isolated from hospital-acquired infections (HA-MRSA) (5, 14). In the present study, SCC*mec* typing did not show 100% typeability and had poor
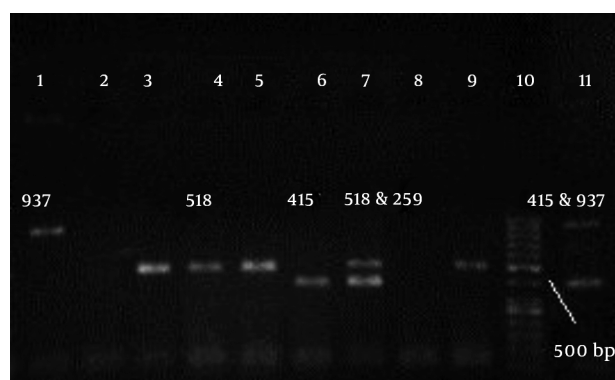
Figure 2. Agarose gel electrophoresis of SCC*mec* genes as the multiplex PCR amplification products in MRSA isolates. Lane 1, SCC*mec* type II (937 bp); lane 3, 4, 5 and 9, SCC*mec* type III (518 bp); lane 6, SCC*mec* type I (415 bp); lane 7, SCC*mec* type V (518 and 359 bp); lane 10, 50-bp DNA ladder; and lane 11, SCC*mec* type IV (415 and 937 bp).
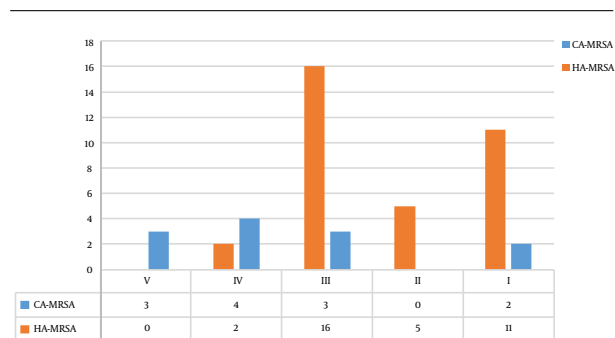

Figure 3. SCC*mec* Typing of MRSA Isolated Separately from Community and Hospital Setting

discriminatory power, as 8 (8%) MRSA isolates were nontypable (32).

Distribution of the isolates on the basis of the specimen source revealed that 75% of MRSA strains with SCC*mec* type III (as the predominant SCC*mec* type) were obtained from blood samples, which is consistent with several previous studies (27, 33). Overall, these findings propose that antibiotic pressure and lack of standard control measures might be responsible for the increased prevalence of MRSA in Al-Zahra hospital in comparison with the previous study in this hospital. Also, frequent screening of susceptibility patterns of MRSA can be useful in decreasing the incidence rates.

Finally, it appears that SCC*mec* typing provides useful information for determining the epidemiological relationship of a group of MRSA isolates, recovered from hospitals and/or community in Iran. To determine the exact antimicrobial susceptibility profiles and encoding resistance genes among MRSA isolates in the community and hospital settings of Iran, comprehensive research and large-scale studies are needed to provide further information on the phenotypic and genotypic characteristics of MRSA. Also, effective infection management for controlling the spread of CA-MRSA infections is highly recommended.

It should be noted that the positive control strains for SCC*mec* typing were donated by Amir Azimian (department of pathobiology and anatomy, school of medicine, North Khorasan University of Medical Sciences, Bojnord, Iran).

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