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SCC*mec* Typing of Methicillin-Resistant *Staphylococcus aureus*: An Eight Year Experience

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Background: Methicillin resistant *Staphylococcus aureus* strains (MRSA) are important pathogens that cause serious diseases in humans. Throughout the recent years, the spread of these strains has increased in medical environments and society, and has become a serious challenge in health systems. Therefore, it is vital to investigate the various MRSA types to identify the origins of the infections and to control the spread of these infections in hospitals.

Objectives: The current study aimed to evaluate the different SCC*mec* types in MRSA isolates from hospitals of Tabriz, by staphylococcal cassette chromosome *mec* (SCC*mec*) typing.

Materials and Methods: The present descriptive and retrospective study was performed on 151 selected *S. aureus* isolates obtained from clinical specimens who were referred to Tabriz university of medical sciences educational-health care centers from April 2005 to September 2012. MRSA isolates were identified by agar disk diffusion and *mec*A PCR assays. Ultimately, they were typified according to the genetic diversity of the chromosome cassette of SCC*mec* and *ccr* regions.

Results: Of the 151 isolates, 53 were recognized as MRSA. All of these 53 samples were sensitive to teicoplanin and vancomycin. Antibiotic resistance patterns were as follows: azithromycin 56.6%, ciprofloxacin 28.3%, imipenem 11.3%, meropenem 9.4%, ofloxacin 13.2%, ceftriaxone 66%, cotrimoxazole 49.1%, gentamicin 52.8%, linezolid 11.3%, penicillin 90.6%, and rifampicin 5.7%. The majority of MRSA isolates belonged to *SCCmec* III (69.8%) followed by SCC*mec* IVc (7.5%), SCC*mec* IVa (3.8%), and SCC*mec* I (1.9%). Other types of SCC*mec* were not observed in the present study. Moreover, from the 53 MRSA samples, 9 were recognized as non-typable. However, staphylococcal cassette chromosome recombinase (*ccr*) genetic complex analysis revealed that among the 53 studied samples, 4 isolates had *ccr* type 1 pattern, and 11 and 32 isolates had *ccr* type 2 and *ccr* type 3 pattern, respectively. Furthermore, 6 isolates were considered as non-typable with *ccr*-typing.

Conclusions: As about 70% of methicillin-resistant isolates belonged to SCC*mec* III, the present study can conclude that, over an 8-year period, only one dominant and stable clone of MRSA strain was found in Tabriz hospitals. This finding could be a result of incorrect medical orientations, inadequate infection controlling policies, and insufficient preventive approaches.

Keywords: MRSA, SCC*mec* Typing, *ccr* Typing, Multiplex-PCR

1. Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have long been recognized as one of the major human pathogens responsible for a wide range of infections. Even presently, beta-lactams, such as penicillins, have fundamental roles in the treatment of staphylococcal infections. However, one of the most important causes of the penicillin resistance in *S. aureus* is the expression of the *mec*A gene. The *mec*A gene is encoded inside a *mec* operon, which is carried by the staphylococcal cassette chromosome, SCC*mec* (1). In addition to the mecA gene, all MRSA strains have other genes such as mecR1, *ccr* AB, *ccr* C, and j connective regions in SCC*mec* regions. Two essential parts in the SCC*mec* elements of staphylococci are the *ccr* and mec gene complexes. Furthermore, antibiotic resistance genes may possibly be present in the SCC*mec* region. Thus, *ccr* typing is a suitable method to determine the presence of SCC*mec* genes (2). The SCC*mec* chromosome cassette is divided into 8 different types (I-VIII). Generally types IV, V, VII are named as community acquired MRSA (CA-MRSA), and types I, II, III, VI, and VIII are called hospital acquired MRSA or HA-MRSA (2-5).

With most studies focusing on the evolutionary relationships between different MRSA strains (6-11), various molecular typing techniques were used for the epidemiological study of MRSA. Several studies have been conducted globally to determine the prevalence of various SCC*mec* types in different regions of the world (12-14). However, to the best of our knowledge, no comprehensive investigations have been performed regarding MRSA strain typing in Northwest Iran.

2. Objectives

The present study was designed and carried out in Tabriz hospitals to evaluate the prevalence of different SCC*mec* types in MRSA isolates over time (from April 2005 to September 2012). Furthermore, antimicrobial susceptibility patterns of the MRSA isolates were also determined. Such monitoring can determine the conditions of MRSA distribution in hospital communities.

3. Materials and Methods

In the present study, the sample population consisted of 151 non-duplicate *S. aureus* strains that were selected randomly from stock isolated during eight years from April 2005 to September 2012 in five educational hospitals of the Tabriz University of Medical Sciences, Iran. The isolates were collected from the various clinical specimens including, wounds, blood, urine, sputum, synovial and other body fluids of patients. All *S. aureus* isolates were identified with previously described standard morphological and biochemical methods such as growth on mannitol salt agar, colony morphology, gram staining, catalase, slide, or tube coagulase, and DNase tests (15).

3.1. Antimicrobial Susceptibility Tests

All *S. aureus* isolates were tested against 14 antibiotics using disc agar diffusion including: penicillin (10 U), oxacillin (1 μg), gentamicin (10 μg), trimethoprim-sulfamethoxazole (25 μg), rifampin (5 μg), vancomycin (30 μg), linezolid (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), imipenem (10 μg), meropenem (10 μg), teicoplanin (30 μg), and azithromycin (15 μg) (10). *S. aureus* ATCC 29213 was used as a control strain for the antibacterial susceptibility testing (16).

3.2. DNA Extraction

The extraction was carried out by applying the DNG kit (CinnaGen Co.) with a slight modification. Initially, each strain was incubated for 24 hours in LB broth medium and then transferred to a microtube and centrifuged at 8,000 rpm. Supernatant was discarded and the pellet was re-suspended in 100 μl of TE buffer, and heated to 95°C for 10 minutes. Next, 400 μl of CinnaGen DNG extraction solution was added, vortexed, and then 600 μl of isopropanol was added. It was then frozen at -20°C for 30 minutes, and centrifuged at 12,000 g. The supernatant was discarded, and the product was washed twice using 75% ethanol. Ultimately, the final product was re-suspended in 50 μl of TE buffer.

3.3. Amplification of nuc, mec, SCCmec, and ccr Complex Genes

3.3.1. Detection of nuc and mecA Genes

To confirm the identity of *S. aureus* isolates and methicillin resistance, polymerase chain reaction (PCR) assay was performed using the primers for the *nuc* and *mec*A genes. Table 1 shows and describes the process.

3.3.2. Amplification of SCCmec and the ccr Complex

SCC*mec* typing was performed by multiplex PCR using eight set of primers as previously described (Table 1). The PCR thermal cycling conditions included initial denaturation at 94°C for 5 minutes, followed by 10 cycles at 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 minutes, and another 25 cycles at 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 minutes, ending with a final extension step at 72°C for 10 minutes, followed by a hold at 4°C.

The PCR assay for the staphylococcal *ccr* complex was performed using three single primers to distinguish the types 1 or 2 (Table 1) (17). Electrophoresis of products was carried out by 2% agarose gel containing 0.5 μg/ml ethidium bromide and visualized by a gel documentation system.

4. Results

4.1. Bacterial Isolation

A total of 151 *S. aureus* isolates collected over eight years were processed for their typing by SCC*mec* and *ccr*. From these, 53 *S. aureus* were confirmed as MRSA by PCR. Isolates were cultured from blood, wounds, urine, dialysis samples, sputum, pleural and synovial fluids, catheters, tracheal, abscesses, and bile aspirates.

4.2. MRSA Typing

As shown in Table 2, and Figures 1 and 2, four types of SCC*mec* and three different types of *ccr* complex were identified among the studied isolates. Accordingly, SCC*mec* III was the most prevalent, followed by type IVc, type Via, and type I. Nine (17%) of the MRSA isolates could not be typed in this manner. On *ccr* typing, the *ccr* -complex III was recognized as the dominant type, followed by type II and type I, while 6 MRSA isolates were non-typable by *ccr* region polymorphism.

4.3. Antimicrobial Resistance

Table 3 briefly explains the antimicrobial resistant profiles of the MRSA isolates. As shown, there was complete susceptibility to teicoplanin and vancomycin in all 53 investigated MRSA isolates. Our results were showed a reduced gradient of MRSA susceptibility to ofloxacin, ceftriaxone, cotrimoxazole, gentamicin, azithromycin, rifampicin, ciprofloxacin, and linezolid. All strains were resistant to penicillin; however, only less than 10% were resistant to meropenem and rifampicin.

Table 1. Set of Primers Used In This Study [a]

Primer	Sequence (5'→3')	Amplicon size, bp	Target gene	References
Type I		613	SCC*mec* I	(10)
F	GCTTTAAAGAGTGTCGTTACAGG			
R	CGAAATCAATGGTTAATGGACC			
Type II		398	SCC*mec* II	(10)
F	CGTTGAAGATGATGAAGCG			
R	CGAAATCAATGGTTAATGGACC			
Type III		280	SCC*mec* III	(10)
F	CCATATTGTGTACGATGCG			
R	CCTTAGTTGTCGTAACAGATCG			
Type IVa		776	SCC*mec* IVa	(10)
F	GCCTTATTCGAAGAAACCG			
R	CTACTCTTCTGAAAAGCGTCG			
Type IVb		493	SCC*mec* IVb	(10)
F	TCTGGAATTACTTCAGCTGC			
R	AAACAATATTGCTCTCCCTC			
Type IVc		200	SCC*mec* IVc	(10)
F	ACAATATTTGTATTATCGGAGAGC			
R	TTGGTATGAGGTATTGCTGG			
Type IVd		881	SCC*mec* IVd	(10)
F5	CTCAAAATACGGACCCCAATACA			
R6	TGCTCCAGTAATTGCTAAAG			
Type V		325	SCC*mec* V	(10)
F	GAACATTGTTACTTAAATGAGCG			
R	TGAAAGTTGTACCCTTGACACC			
nuc		279	*nuc*	(15)
F	GCGATTGATGGTGATACGGTT			
R	AGCCAAGCCTTGACGAACTAAAGC			
***Mec*A147**		147	*mec*A	(10)
F	GTGAAGATATACCAAGTGATT			
R	ATGCGCTATAGATTGAAAGGAT			
***ccr*AB-β2**	ATTGCCTTGATAATAGCCITCT			(16)
***ccr*AB-α2**	AACCTATATCATCAATCAGTACGT	700	Type 1 *ccr*	(16)
***ccr*AB-α3**	TAAAGGCATCAATGCACAAACACT	1000	Type 2 *ccr*	(16)
***ccr*AB-α4**	AGCTCAAAAGCAAGCAATAGAAT	1600	Type 3 *ccr*	(16)

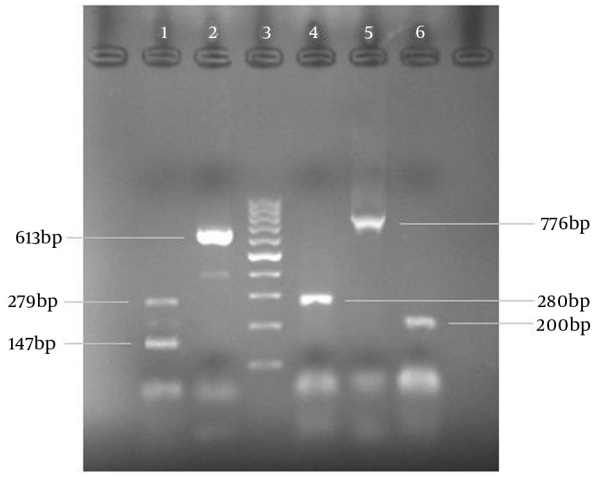
[a] Abbreviations: F, forward; R, reverse.

Table 2. Frequency of Distinguished SCC*mec* With Their *ccr* Elements [a]

SCC*mec* type	*ccr* type	Number of Isolates	MRSA type
I	1	1 (1.9%)	HA-MRSA
III	1	2 (3.8%)	HA-MRSA
III	2	5 (9.4%)	HA-MRSA
III	3	30 (56.6%)	CA-MRSA
IVa	2	2 (3.8%)	CA-MRSA
IVc	2	4 (7.5%)	CA-MRSA
-	1	1 (1.9%)	Non-typable
-	3	2 (3.8%)	Non-typable
-	-	6 (11.3%)	Non-typable
		Total = 53 (100)	

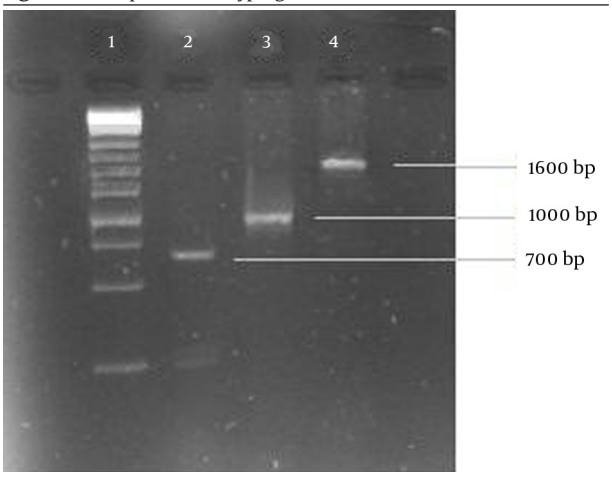
[a] Values are presented as No. (%).

Figure 1. Multiplex PCR SCC*mec* Typing of MRSA Isolates



Lane 3: 100 bp ladder (Fermentas); lane 1: *mec*A (147 bp) and *nuc* gene (279 bp); lane 2: SCC*mec* I (613 bp); lane 4: SCC*mec* III (280 bp); lane 5: SCC*mec* IVa (776 bp); lane 6: SCC*mec* IVc (200 bp).

Figure 2. Multiplex PCR *ccr* typing of MRSA isolates



Lane 1: 1 kbp ladder (Fermentas); lane 2: *ccr* type 1 (700 kbp); lane 3: *ccr* type 2 (1000 kbp), lane 4: *ccr* type 3 (1600 kbp).

Table 3. Antimicrobial Resistance Profiles of MRSA Isolates

Antibiotic	Resistant	Intermediate	Sensitive
Ciprofloxacin	15 (28.3%)	20 (37.7%)	18 (34%)
Azithromycin	30 (56.6%)	2 (3.8%)	21 (39.6%)
Meropenem	5 (9.4%)	-	48 (90.6%)
Teicoplanin	-	-	53 (100%)
Imipenem	6 (11.3%)	1 (1.9%)	46 (86.8%)
Ofloxacin	7 (13.2%)	1 (1.9%)	45 (84.9%)
Ceftriaxone	35 (66%)	-	18 (34%)
Cotrimoxazole	26 (49.1%)	-	27 (50.9%)
Gentamicin	28 (52.8%)	-	25 (47.2%)
Linezolid	6 (11.3%)	-	47 (88.7%)
Oxacillin	53 (100%)	-	-
Penicillin	53 (100%)	-	-
Rifampicin	3 (5.7%)	-	50 (94.3%)
Vancomycin	-	-	53 (100%)

5. Discussion

SCC*mec* is the most important factor defining the origin and source of MRSA infections in society. If we could determine HA-MRSA or CA-MRSA, then we could manage MRSA infections and select the best treatment protocol (1). Each type of SCC*mec* has unique genetic elements. Eight types of SCC*mec* and 3 types of *ccr* genes were investigated in this research (12-19). We were recognized 4 types of SCC*mec* and among them, type III was the most prevalent in our isolates.

Recently, MRSA infections have raised global concerns. Therefore, its prevalence in most countries has been highlighted (20-23) because the determination of MRSA prevalence or spread through transmission within communities may influence how this problem will be addressed.

In the present investigation, the rate of methicillin resistance among *S. aureus* isolates was 33.8%, which is lower than the prevalence of MRSA in previous studies from Shahid Beheshti Hospital in Kashan, and Imam Hussein Hospital in Tehran (24, 25). This could be from the correct usage of antibiotic in our province. The prevalence of MRSA in Greece, Italy, France, and Turkey was similar to our results, but in Germany, Poland, Spain, Sweden, and Switzerland the prevalence of these isolates was lower than our results (26). This could be a result of their developed health systems. Regarding Table 3, the antibiotic susceptibility test revealed that all samples were sensitive to teicoplanin and vancomycin. Resistances to rifampin and meropenem were less than 10%, and resistances to cotrimoxazole, azithromycin, gentamicin, ceftriaxone, penicillin, oxacillin, clindamycin, and erythromycin were between 50% and 100%. However, some antibiotics, such as rifampicin, imipenem, meropenem, ofloxacin, and linezolid, were still effective against MRSA in this work; indicating that these agents may be applicable in the treatment of MRSA infections. SCC*mec* analysis identified 69.8% of MRSA isolates as type III, suggesting that most of the MRSA isolates in the present study may have originated from HA-MRSA or clonal diversion of MRSA. These results were consistent with previous reports about the predominance of SCC*mec* III in most Asian countries (27), and in Iranian cities. The next predominant type was SCC*mec* IVc (7.5%) followed by type IVa (3.8%), which was also confirmed by the present study and is supported by other research (13). However, we could investigate other genes correlated with SCC*mec* to determine more types of SCC*mec*, and this could be a limitation of the current research.

Obtained data showed that in the Iranian East-Azerbaijan Province, SCC*mec* III was the predominant SCCi type. As a result, multidrug-resistance in our province could be high. Furthermore, typing of isolates using *ccr*-complex verified our data about SCCmec.

The data indicate that multidrug-resistant MRSA in our isolates has caused serious problems that result from the inappropriate use of antibiotics. Therefore, physicians should prescribe suitable antibiotics based on their effectiveness, price, and accessibility.

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Authors' Contributions

Mohammad Ahangarzadeh Rezaee and Alka Hasani: Study concept and design, development of the study, data interpretation, and manuscript revision. Mohammad Fateh Amirkhiz: Phenotypic and molecular studies, and manuscript drafting. Mohammad Aghazadeh: Helped with experimental procedures. Behrooz Naghili: Medical consultation. Mohammad Ahangarzadeh Rezaee: Study supervision.

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