ORIGINAL ARTICLE

Typing of staphylococcal cassette chromosome *mec* encoding methicillin resistance in *Staphylococcus aureus* isolates in Ahvaz, Iran

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen. We sought to determine the frequency of the different types of SCC*mec* in MRSA isolates by performing a cross-sectional study. A total of 72 *S. aureus* isolates were collected from Imam Khomeini and Golestan hospitals and analysed for MRSA and SCC*mec* typing by multiplex PCR. The pattern of antibiotic resistance among *S. aureus* isolates was determined by disc diffusion analysis. Of the 72 *S. aureus* isolates, 29 (40.27%) were recognized as MRSA. SCC*mec* type III was the most common type, with 55.17% (16/29), followed by type II with 27.58% (8/29); type IV with 10.34% (3/29); and type I with 6.89% (2/29). All 29 MRSA isolates were resistant to chloramphenicol and erythromycin. In addition, resistance to cephalothin, gentamicin, clindamycin, ciprofloxacin, tetracycline and rifampicin was seen in 24 (75%), 26 (63.4%), 17 (94.4%), 27 (71.05%), 10 (71.42%) and 13 (68.42%) MRSA isolates, respectively. A decreased sensitivity of MRSA to the antibiotics used was observed, with type III SCC*mec* being the predominant isolate.

Keywords: *mec*A, methicillin, MRSA, SCC*mec* typing
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Introduction

Staphylococcus aureus is an important human pathogen in nosocomial infections. In addition, it can cause skin and soft tissue infections in the community [1]. Methicillin as a β-lactamase-resistant antimicrobial agent first was introduced in 1959 for staphylococcal infection therapy [2]. However, during a brief period in 1961, the first methicillin-resistant *S. aureus* (MRSA) strain was reported from London [1,2].

MRSA now is a major nosocomial pathogen that causes severe morbidity and mortality around the world. MRSA strains are endemic in many countries, including Iran, and account for over 50% of clinical isolates [3]. MRSA strains have distinct microbiologic and therapeutic patterns compared to methicillin-susceptible *S. aureus* strains [4].

Resistance to methicillin is due to acquiring the *mec*A gene. This gene is not native for the *S. aureus* genome, and its expression is due to the production of a special penicillin-binding protein called PBP2a, which has a low affinity to β-lactam antibiotics in compression with PBPs [5]. The *mec*A gene is widely distributed in both coagulase-positive and -negative staphylococci and is usually carried on a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) [6].

SCC*mec* consist of two main components: the *ccr* gene complex (*ccr*) and the *mec* gene complex (*mec*). Moreover, the cause of the mobility of SCC*mec* is the *ccr* genes complex, which encodes site-specific recombinases and the surrounding open reading frames. The *mec* gene complex is composed of the *mec*A gene, regulatory genes of *mecR1-mecI* and the insertion elements for the potential integration of some unrelated resistance determinants [5]. According to the combination of *ccr* allotypes with the *mec* gene complex, 11 types (I–XI) SCC*mec* have already been reported [5,6].

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In general, MRSA strains are divided two main groups: hospital associated (HA) and community associated (CA) [7]. The infections caused by HA-MRSA have been associated with an increase in length of hospitalized time and healthcare costs [2]. Clinically, the infections caused by HA-MRSA strains are associated with high mortality and morbidity. These strains are usually multidrug resistant, a feature that could limit the selection of a proper antibiotic to treat staphylococcal infections [7].

A growing population of CA-MRSA strains express some virulence factors, such as Panton-Valentine leukocidin, which is associated with serious diseases such as severe necrotizing infections [3]. CA-MRSA strains are usually resistant to fewer non-β-lactam classes of antimicrobials [8].

HA-MRSA isolates typically belong to SCC*mec* types I to III, while types IV and V are usually associated with CA-MRSA isolates [7]. In the United States most HA-MRSA isolates carry SCC*mec* type II, whereas in other countries these isolates usually carry SCC*mec* type III [8]. SCC*mec* typing has provided strong evidence for an origin HA-MRSA distinct from CA-MRSA strains.

We investigated the frequency of the different types of SCC*mec* in MRSA isolates in Ahvaz, Iran.

Materials and methods

Bacterial strains

We analysed 72 nonduplicate *S. aureus* strains from a previous study for SCC*mec* typing [9]. Briefly, the strains were collected from patients referred to Imam Khomeini and Golestan hospitals. Patients' mean age was 29.1 ± 4.55 years; men comprised 42 (58.33%) of the subjects and women 30 (41.66%). The strains were isolated from clinical samples including pus, burn, wound, catheter, blood, sputum and cerebrospinal fluid. All isolates of *S. aureus* were identified by catalase, tube coagulase and DNase tests as well as fermentation of mannitol.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disc diffusion method on Mueller-Hinton agar (Merck GmbH, Darmstadt, Germany) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [10]. We used antibiotic discs of oxacillin (1 μg), cephalotin (30 μg), gentamicin (10 μg), clindamycin (2 μg), ciprofloxacin (5 μg), tetracycline (30 μg), chloramphenicol (30 μg), rifampicin (5 μg) and erythromycin (15 μg). We used *S. aureus* ATCC 25923 as the quality-control strain.

Screening for methicillin resistance

Resistance to methicillin was detected by growth on agar screen plates (Mueller-Hinton agar) containing 6 μg/mL oxacillin with 4% NaCl. All plates were incubated at 35°C for 24 hours according to CLSI recommendations [10]. The presence of the *mec*A gene was evaluated in all 72 isolates by its amplification. Sequences of primers used for amplification of the *mec*A gene are listed in Table 1.

The amplification process was performed by the MasterCycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany), with one cycle of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing 52°C for 30 seconds, extension at 72°C for 45 seconds and a cycle of final extension at 72°C for 7 minutes. All PCR products were visualized on a 1% agarose gel stained with ethidium bromide.

Screening for vancomycin resistance

Resistance to vancomycin was detected by growth on agar screen plates (Mueller-Hinton agar) containing 6 μg/mL vancomycin. All plates were incubated at 35°C for 24 hours. Minimum inhibitory concentration (MIC) values of vancomycin were determined by the agar dilution method according to CLSI recommendations [10]. Briefly, MIC $\leq$ 2 μg/mL was proposed as sensitive, MIC 4 to 8 μg/mL intermediate and MIC $\geq$ 16 resistant.

PCR-based assignment of SCC*mec* elements

Before this work, chromosomal DNA from MRSA isolates was extracted using High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) according to the manufacturer's directions. The design of this multiplex PCR was described by

TABLE 1. Primers used for determining SCC*mec* types

Name	Primer sequence (5′ to 3′)	Length (bp)	Target	SCC*mec* I	II	III	IV	V
β α3	F: ATTGCCTTGATAATAGCCYTCT R: TAAAGGCATCAATGCACAAACACT	937	ccrA2-B		*		*	
ccrF ccrR	F: CGTCTATTACAAGATGTTAAGGATA R: CCTTTATAGACTGGATTATTCAAAA	518	ccrC			*		*
1272F1 1272R1	F: GCCACTCATAACATATGGAA R: CATCCGAGTGAAACCCAAA	415	IS1272	*			*	
5R*mec*A 5R431	F: TATACCAAACCCGACAACTAC R: CGGCTACAGTGATAACATCC	359	*mec*A-IS431					*

Boye et al. [11]. An assay of multiplex PCR was performed in 50 μL reactions with 1 μ of AmpliTaq DNA polymerase, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM deoxyribonucleotide triphosphate and 2 μL genomic DNA and distilled water to a final volume of 50 μL. The primer concentrations were as follows: 0.2 pmol/μL each of primers β and α3; 0.25 pmol/μL each of primers ccrCF and ccrCR; 0.08 pmol/μL each of primers 1272F1 and 1272R1; and 0.1 pmol/μL each of primers 5R*mec*A and 5R431.

The sequences of primers used for amplification of SCC*mec* types are provided in Table 1. A multiplex PCR reaction was performed for 1 cycle at 94°C for 4 minutes, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 60 seconds at 72°C, with a final extension for 4 minutes at 72°C. The PCR products were visualized on a 1% agarose gel stained with ethidium bromide. Five MRSA strains—NCTC10442 (SCC*mec* I), NCTC N315 (SCC*mec* II), NCTC 85/2082 (SCC*mec* III), NCTC CA05 (SCC*mec* IVa) and JCSC3624 (SCC*mec* V)—were used as the standard strains with SCC*mec* elements [12].

Results

Seventy-two *S. aureus* strains were collected from different clinical samples. Resistance to oxacillin was found in 29 isolates (40.2%) and was confirmed by the amplification of the *mec*A gene. SCC*mec* typing of these 29 isolates was performed by multiplex PCR. SCC*mec* type III was the most common type, with a frequency of 55.1% (16/29), followed by type II, with frequency of 27.5% (8/29); type IV, with a frequency of 10.3% (3/29); and type I, with a frequency of 6.8% (2/29) (Fig. 1).

Among 72 *S. aureus* isolates, resistance to cephalotin, gentamicin, clindamycin, ciprofloxoacin, tetracycline, chloramphenicol, rifampicin and erythromycin was seen in 32 (44.4%), 41 (56.9%), 18 (25%), 38 (52.7%), 14 (19.4%) 11 (15.2%), 19 (26.3%) and nine (12.5%) isolates, respectively. In addition, according to results obtained from the screen agar study, all isolates showed sensitivity to vancomycin. All 29 MRSA isolates were resistant to chloramphenicol and erythromycin. In addition, resistance to cephalotin, gentamicin, clindamycin, ciprofloxoacin, tetracycline and rifampicin was seen in 24 (75%), 26 (63.4%), 17 (94.4%), 27 (71.05%), ten (71.42%) and 13 (68.42%) MRSA isolates, respectively.

The association between SCC*mec* types and antimicrobial resistance pattern is shown in Table 2.

Discussion

S. aureus infections are increasingly reported in public-health arenas. MRSA infections are one of the main causes of

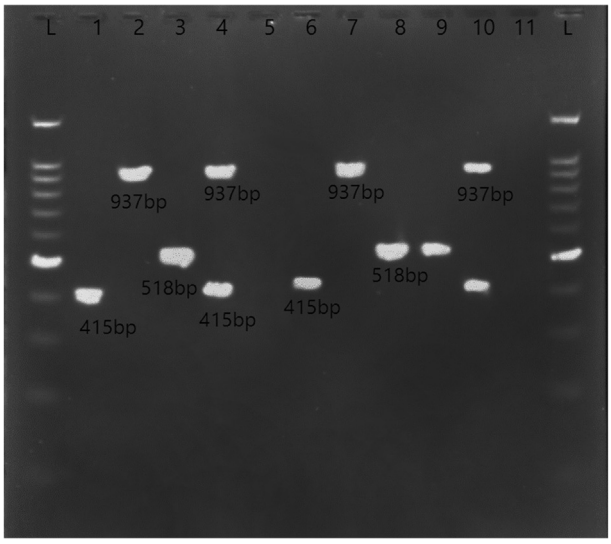

FIG. 1. Amplification results of SCC*mec* typing in MRSA isolates. L, ladder of 100 bp (cinnagene_Iran); lanes 1 and 6: MRSA type I; lanes 2 and 7, MRSA type II; lanes 3, 8 and 9, MRSA type III; lanes 4 and 10, MRSA type IV; lanes 5 and 11, negative control. MRSA, methicillin-resistant *Staphylococcus aureus*.

marked morbidity and mortality, which can impose a high burden on healthcare costs [5]. Annually, HA-MRSA infections occur in approximately 19 000 hospitalized American patients; this number is similar to the frequency of deaths caused by AIDS and tuberculosis [13].

In our study, the *mec*A gene was found in 29 isolates (40.2%). In addition, all of these isolates showed phenotypic resistance to oxacillin. The prevalence of this gene with the different frequencies has been reported in other regions of Iran [14–16]. The differences in the distribution of the *mec*A gene can be explained by the populations studied or by the diversity types of the clinical specimens. Furthermore, in the study conducted by Goudarzi et al. [16] in Tehran, most MRSA isolates were obtained from hospitalized patients in intensive care units. Furthermore, intensive care units are considered to be high-risk areas for dissemination of MRSA infections [17].

However, the prevalence of the *mec*A gene in our study was comparable to previously reported studies from other countries: 36.6% in Greece, 46% in Israel, 38.3% in Italy and 45.76% in the Philippines [18].

Molecular typing of MRSA is an essential approach for the identification of the origin of strains, epidemiologic investigation and antibiotic therapy [19].

In our study, SCC*mec* typing recognized 55.1% of MRSA isolates as type III. According to the data, most MRSA isolates in the present study may have originated from HA-MRSA isolates. SCC*mec* typing was performed in other regions of Iran, and in

TABLE 2. Association between SCC*mec* types and antimicrobial resistance patterns of MRSA isolates

Type of SCC*mec*	GEM (n = 41), n (%)	ERY (n = 9), n (%)	CIP (n = 3), n (%)	CLINDA (n = 18), n (%)	CEFA (n = 32), n (%)	TET (n = 14), n (%)	CHLO (n = 11), n (%)	RIF (n = 19), n (%)
I (n = 2)	2 (100)	1 (50)	2 (100)	1 (50)	2 (100)	1 (50)	1 (50)	1 (50)
II (n = 8)	5 (62.5)	2 (25)	7 (87.5)	4 (50)	6 (75)	4 (50)	3 (37.5)	2 (25)
III (n = 16)	16 (100)	5 (31.25)	15 (93.75)	11 (68.75)	14 (87.5)	4 (25)	6 (37.5)	8 (50)
IV (n = 3)	3 (100)	1 (33.3)	3 (100)	1 (33.3)	2 (66.6)	1 (33.3)	1 (33.3)	2 (66.6)
MRSA total	26 (63.4)	9 (100)	27 (71.05)	17 (94.4)	24 (75)	10 (71.42)	11 (100)	13 (68.42)

CEF, cefalotin; CIP, ciprofloxacin; CLINDA, clindamycin; ERY, erythromycin; GEM, gentamycin; MRSA, methicillin-resistant *Staphylococcus aureus*; RIF, rifampicin; TET, tetracycline.

all of these published studies, the most frequent SCC*mec* type among nosocomial MRSA strains was type III [14,15,20–22]. The frequency of SCC*mec* type III was reported as 74.3% in Shiraz [20], 98% in Tehran [14], 69.8% in Tabriz [15], 91% in Isfahan [21] and 45% in the provinces of western Iran [22].

In concordance with several studies from Iran, SCC*mec* type III has been reported to be the predominant type among MRSA strains isolated from most Asian countries. However, in Japan and Korea, the predominant SCC*mec* type among MRSA strains was type II [6].

Some researchers have reported an increase in the prevalence of HA-MRSA strains, with SCC*mec* type IV occurring in hospital settings [23,24]. Valsesia et al. [25] in Switzerland reported SCC*mec* type IV as the most frequent type among HA-MRSA strains (76.6%), but surprisingly, SCC*mec* types I and II represented a minority, with frequencies of 5% and 8.3%, respectively. In addition, SCC*mec* type III was completely absent. It is unclear why SCC*mec* type IV strains are common in the hospital setting. Some evidence indicates that the replication of MRSA strains with SCC*mec* type IV is more rapid than SCC*mec* type II/III, resulting in first strains that may have had enhanced fitness compared to SCC*mec* type II/III strains [25].

In this study, according to antibiotic susceptibility testing, all MRSA isolates were recognized as multidrug resistant. Also, all MRSA isolates were sensitive to vancomycin and resistant to chloramphenicol and erythromycin. Resistance to clindamycin was observed in more than 90% of MRSA isolates, whereas the rate of resistance to cefalotin, tetracycline, rifampicin, gentamicin and ciprofloxoacin was more than 60%. In concordance with our results, Japoni et al. [20], Rahimi et al. [21] and Dibah et al. [4] reported a high incidence of resistance to rifampicin, gentamicin, tetracycline, clindamycin and ciprofloxoacin. However, Mohammadi et al. [22] and Amirkhiz et al. [15] found a relatively low prevalence of antibiotic resistance among MRSA isolates. On the other hand, the study of Dibah et al. [4] found that most MRSA isolates were resistant to chloramphenicol, while studies by Fatholahzadeh et al. [14] and Rahimi et al. [21] found that most MRSA isolates were sensitive to chloramphenicol. In our study, all MRSA isolates were susceptible to vancomycin, a finding similar to other reports in Iran [4,14–16,20–22].

One of the benefits SCC*mec* typing of MRSA isolates is differentiation of antibiotic susceptibility patterns. We thus investigated the association between SCC*mec* types and antimicrobial resistance patterns. According to our results, most MRSA type III isolates were resistant to cephalotin, clindamycin and ciprofloxacin, while all isolates were resistant to gentamicin. These findings are similar to those of Japoni et al. [20] in Shiraz, although they found higher rates tetracycline resistance than we did.

In our study, all type IV isolates showed resistance to ciprofloxacin and gentamicin and were relatively resistant to other antibiotic agents. This finding is contrary to the research of Rahimi et al. [21], who also reported that most type IV isolates were sensitive to all antibiotic agents except the β-lactam group. This finding in our study might have been due to the acquisition of resistance determinants to non-β-lactam antibiotics through exposure of these strains with theses antibiotics, or to their survival in the hospital environment. In our study, the frequency of type I, II and IV isolates was low. A discussion on their antibiotic resistance is thus unreliable.

One of the main limitations of our study was the low numbers of MRSA isolates. For this reason, the association of antibiotic resistance with SCC*mec* types was difficult. Also, unfortunately, we did not study the antibiotic sensitivity of MRSA isolates to new agents such as mupirocin and linezolid.

Conclusions

We found a decreased sensitivity of MRSA isolates to common antibiotics. In addition, SCC*mec* type III was recognized as the predominant type. These results suggest that efficient control protocols ought to be adopted to prevent the transfer of MRSA strains among patients in hospital settings. In addition, the use of antibiotics with low resistance, such as vancomycin, is only recommended as a last treatment option for multidrug-resistant strains.

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Conflict of interest

None declared.

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