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
Original Submission: 5 September 2017; **Revised Submission:** 14 November 2017; **Accepted:** 22 November 2017
Article published online: 28 November 2017

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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].

https://doi.org/10.1016/j.nmni.2017.11.006

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					*