Avicenna Journal of Clinical Microbiology and Infection
doi:10.34172/ajcmi.2019.18 2019December;6(4):100-105 http://ajcmi.umsha.ac.ir

Original Article

Molecular Typing of *ccrB* Gene in Methicillin-resistant *Staphylococcus aureus* by Restriction Fragment Length Polymorphism

Fatemeh Noorbakhsh[1*], Parvane Atayifar[1], Shohreh Zare Karizi[2]

[1]Department of Microbiology, Biological Science College, Varamin-Pishva Branch, Islamic Azad University, Varamin-Pishva, Iran
[2]Department of Genetics, Biological Science College, Varamin-Pishva Branch, Islamic Azad University, Varamin-Pishva,Iran

*Corresponding author:
Fatemeh Noorbakhsh, Ph.D.
Department of Microbiology,
Islamic Azad University,
Varamin-Pishva Branch,
Varamin, Iran, P.O. BOX:
33817-74895,
Tel: 09122043654,
Email: niloofar_noorbakhsh@yahoo.com

Received: 16 Nov 2019
Accepted: 10 Dec 2019
ePublished: 31 Dec 2019

Abstract

Background: *Staphylococcus aureus* is one of the most important pathogens acquired from the hospital and community. Increasing the resistance of *S. aureus* to antibiotics is a major health concentration, and thus the study of antibiotic resistance in *S. aureus* is very important. The aim of this study is to determine the typing of methicillin-resistant *S. aureus* (MRSA) in the region of the *ccrB* gene by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Methods: One hundred and six *S. aureus* were isolated from urine, blood, sputum, wound, and the trachea of patients hospitalized in Tehran during (March-April) 2016. Antibiotic susceptibility test was done by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI). In addition, molecular typing for staphylococcal cassette chromosome *mec* (Sccmec) type I-V was performed in MRSA isolates, followed by conducting PCR-RFLP by restriction enzymes *BsmI* and *HinfI* in the *ccrB* gene area.

Results: PCR and typing showed that type II SCC*mec* was 40% (n=20), followed by types III (28, 56%), IVc (12, 24%), I (11, 22%), V (9, 18%) IVa (7, 14%), and IVb (5, 10%). However, SCC*mec* type IVd was not observed in the isolates. Finally, after the amplification of *ccrB* gene and RFLP, all isolates the same as the typing method represented types I, II, III, IVa, IVb, and IVc while no type V was detected by this method.

Conclusions: The results of this study demonstrated that SCC*mec* (type I-IV) can be detected by PCR-RFLP in the *ccrB* gene, but this method identified no type V SCC*mec* in MRSA.

Keyword: *Staphylococcus aureus*, SCCmec, Typing, ccrB, PCR-RFLP

Background

Staphylococcus aureus is considered as one of the most essential bacterial pathogens in humans that can cause various infections in patients ranging from skin infections to fatal necrotizing pneumonia, bacteremia, and endocarditis (1). Methicillin-resistant *S. aureus* (MRSA) is one of the important pathogens that is responsible for many nosocomial infections. First, MRSA was identified only in the hospitals, but 30 years later, the first virulent MRSA was acquired from the community (2). The resistance to methicillin in *S. aureus* is induced by the presence of the *mecA* gene, encoding low-affinity penicillin-binding protein PBP2a (78 KD) (3,4). MRSA has a mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*) carrying the *mecA* gene. (5- 7) SCC*mec* elements in *S. aureus* are as unique genomic islands with 2 essential components (i.e., the *ccr* and the *mec* gene complexes) and J region (5,8,9). The *ccr* gene complex is composed of *ccr* genes encoding 2 site-specific recombinases (*ccrA* and *ccrB*), and the *mecA* gene complex contains *mecA* and regulatory genes *mecI* and *mecR. (*6,10,11) Zhang et al defined 8 different types of SCC*mec* in the combination of *ccr* and *mec* complex. While types I-V were widespread (12), other types existed in the strains of the country from which they were originated (13,14). SCC*mec* exchange between species is related to the *ccr* gene expression (15). Several allotypes of *ccr* and *mec* gene are classified in SCC*mec*. The 5 allotypes of the *ccr* gene complex include *ccrAB1*, *ccrAB2*, *ccrAB3*, *ccrAB4*, and *ccrC* (16,17), and 5 classes of the *mec* gene complex (types I-V) were described and SCC*mec* type IV has 8 individual subtypes (18,19).

The site-specific recombination of SCC*mec* is catalyzed by its encoded *ccr* recombinases, *ccrA* and *ccrB* for types I to IV and *ccrC* for type V. In addition, *ccrA* and *ccrB* belong to a family of large serine invertase and resolvases which consist of resolvases, invertases, phage integrases, and transposases (6,20).

Further, *ccrB* gene as a target gene is often chosen because the sequence is highly conserved compared to the

ccrA gene (21).

Molecular techniques for the typing of the used microbes include PFGF, methods based on restriction enzyme, the analysis of plasmid, and DNA typing method based on polymerase chain reaction (PCR) (22). Pulse-field gel electrophoresis and multi-locus sequence typing are the best techniques for phenotypic and genotypic studies, but the most important problems of this method are the technical complexity, high cost, as well as a longer process (23). Studies suggest that PCR restriction fragment length polymorphism (PCR-RFLP) can be replaced by these expensive and time-consuming techniques (22). Antibiogram is regarded as one of the most important typing methods in many hospitals since it is easily standardized (24).

Objectives

The aim of this study was the molecular typing of *ccrB* gene in MRSA by RFLP.

Materials and Methods

Bacterial Isolates

A total of 106 specimens doubtful to *S. aureus* were isolated from blood, urine, wound, nasal fluid, and the sputum of patients hospitalized in Milad hospital during March-April 2016. *S. aureus* isolates were identified based on gram-staining, catalase, coagulase test, and growth on the mannitol salt agar.

Antibiotic Susceptibility Test

The presence of the *mecA* gene and resistance to methicillin in *S. aureus* was confirmed by the oxacillin/cefoxitin-resistant in all isolates. In addition, the antibiotic susceptibility test was performed by the disk diffusion method according to CLSI 2017. Further, the tested antibiotics were oxacillin (30 µg)/cefoxitin (30 µg) prepared from Padtan Teb. *S. aureus* ATCC 25923 was used as the control strain in the antibiotic susceptibility test.

DNA Isolation

MRSA isolates were cultured on brain heart infusion agar and incubated overnight in 37°C. The bacteria were harvested from the medium and washed by the saline buffer. Then, DNA was extracted by the boiling method and the quality of the DNA was determined by the electerophores (25).

Amplification of SCC*mec* Type

Multiplex PCR for the detection of MRSA isolates SSC*mec* Type (I-V) were previously optimized for standard strains by Ito and Katayama (10). The primers of SCCmec types I–V including type IV subtypes (Table 1) were previously selected and reported (26). Further, the PCR mixture (Cinnagen) contained 12.5 µL of master mix (400 µm of dNTPs, 3 mM mgcl2, and 1.2 u Taq polymerase), 9.5 µL nuclease-free water, 10 pM each primer, and 1 µg DNA. Furthermore, the amplification for SCC*mec* types I, II, III, and V was performed by initial denaturation at 95°C for 5 minutes, followed by 30-cycle denaturation at 95°C for 30 seconds, annealing at 58°C for 1 minute, and extension at 72°C for 2 minutes. Moreover, the multiplex PCR carried out for IV subtypes encompassed the initial denaturation at 95°C for 5 minutes, followed by 30-cycle denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds and extension at 72°C for 2 minutes, as

Table 1. The Profile of SCC*mec* Type Specific Primers

Primer	Orientation	Primer sequence	Target Gene	Size (bp)
Type I	Forward Reverse	GCTTTAAAGAGTGTCGTTACAGG GTTCTCTCATAGTATGACGTCC	ORF E008 of strain NCT C10442	613
Type II	Forward Reverse	GATTACTTCAGAACCAGGTCAT TAAACTGTGTCACACGATCCAT	kdpE of strain N315	287
Type III	Forward Reverse	CATTTGTGAAACACAGTACG GTTATTGAGACTCCTAAAGC	J1 region of SCC*mec* Type III	243
Type Iva	Forward Reverse	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	ORF CQ002 of strain CA05	776
Type IVb	Forward Reverse	AGTACATTTTATCTTTGCGTA AGTCATCTTCAATATGGAGAAAGTA	J1 region of SCC*mec* type IVb	994
Type IVc	Forward Reverse	TCTATTCAATCGTTCTCGTATT TCGTTGTCATTTAATTCTGAACT	IVc element of strain 81/108	677
Type IVd	Forward Reverse	AATTCACCCGTACCTGAGAA AGAATGTGGTTATAAGATAGCTA	CD002 in type IVd	1242
Type V	Forward Reverse	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	ORF V011 of strain JCSC3624	325
ccrB	Forward Reverse	GGCTATTATCAAGGCAATTTACC ACTTTATCACTTTTGACTATTTCG		643

well as ending by final extension at 72ºC for 8 minutes and hold step at 5ºC. Finally, PCR products were electrophoresed in 1.5% gel agarose containing 1 µL safe stain and image supplied by UV transilluminator and gel document.

Amplification of *ccr*B Gene

The primer sequence for the amplification of the *ccrB* gene was selected from the study by Yang et al (22) (Table 1). The total volume of PCR master mix was 50 µL containing 25 µL of master mix (400 µm of dNTPs, 3 mM mgcl2, and 1.2 u Taq polymerase), 20 µL nuclease-free water, 1.5 µL (10 pM) each primer, and 2 µL chromosomal DNA. Moreover, the cycling conditions included an initial step at 94ºC for four minutes, followed by 30 cycles of 94°C for 30 seconds, 59°C for 1 minute, 72°C for 2 minutes, and the final step 72°C for 8 minutes.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

RFLP was used to detect the molecular typing of SCC*mec* (22). After the amplification of the *ccrB* gene in the MRSA strain, the PCR product was digested by *HinfI* and *BsmI* enzymes. Additionally, the digestion reaction was performed in two steps as follows.

Step 1: PCR products were mixed with 17 µL nuclease-free water, one unit *HinfI* (Fermentas), and a two-unit buffer in a final reaction volume of 30 µL and then incubated at 37°C for 16 hours.

Step 2: A total of 10 µL of step 1 product was mixed with 17 µL nuclease-free water, one unit *BsmI* (Fermentas), and a two-unit buffer in a final reaction volume of 30 µL and incubated at 37°C for 10 minutes, followed by adding 2 µL ethylenediaminetetraacetic acid (EDTA) (0.5 M) and incubating at 80°C for 20 minutes. The restriction fragments were separated by 2% agarose gel electrophoresis in Tris-acetate-EDTA buffer for 50 minutes at 90 V and stained by the ethidium bromide. Eventually, SPSS Statistics, version 22 was used to analyze the data.

Results

Bacterial Isolates

Staphylococcus aureus from the clinical samples in Milad hospital were isolated from different sources including (n=32, 30.1%) urine, (n=18, 17%) blood, (n=21, 19.8%) wound, (n=15, 14.15%) trachea, (n= 9, 8.5%) nasal fluid, and (n=11, 10.4%) sputum.

The average age of the evaluated patients was 45 years with a minimum of 15 and a maximum of 75 years. In addition, 45 and 61 strains were collected from men (42.45%) and women (57.54%), respectively.

Antibiotic Susceptibility Test

Antibiotic susceptibility of 106 strains of *S. aureus* to cefoxitin/oxacillin showed that 50 (47.1%), 36 (34%), and 20 (18.9%) isolates were resistant, susceptible, and intermediate, respectively. Fifty strains as MRSA were used for the molecular typing of SCC*mec*.

SCC*mec* Typing

A clear discriminated band pattern was obtained for all five types and subtypes of the main SCC*mec* using the PCR (Figure 1) and multiplex PCR (Figure 2). The individual PCR band size of each fragment for SCC*mec* types I, II, III, V, IVa, IVb, IVc, and IVd were 613, 287, 200, 325, 776, 994, 677, and 1242 bp, respectively. All the MRSA strains were positive in a certain SSC*mec* type. The most common type was type III (56%) while IVd subtype was not detected in this study (Figure 3).

Rapid Typing

After the amplification of the *ccrB* gene, the PCR product was used to digest the two steps of PCR-RFLP (22). SCC*mec* typing method was applied for 50 strains of MRSA isolates. The results in electrophoresis were shown as type I with 1 band of 404 bp, type II with 1 band of 530 bp, type III with 2 bands of 218 and 225 bp, type Iva with 2 bands of 311 and 200 bp, type IVb with 2 bands of 530 and 600 bp, as well as type IVc with 3 bands of 311, 530, and 600 bp (Figure 4). Type IVd established 3 bands of 154, 225, and 264 bp, but type IVd was not detected in this study. This protocol is a suitable method for the rapid typing of the SCC*mec* element types I to IV, but the PCR-RFLP method is not likely to identify type V.

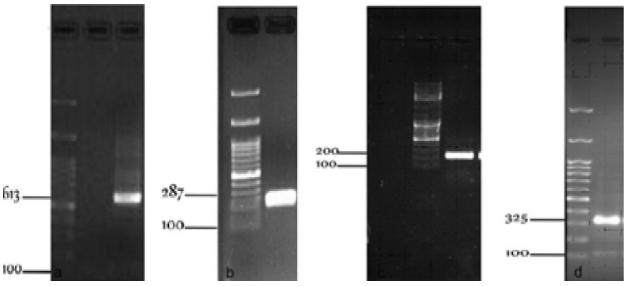

Figure 1. The Amplification of *S. aureus* SSC*mec* Type: (a) Type I, (b) Type II, (c) Type III, and (d) Type V.

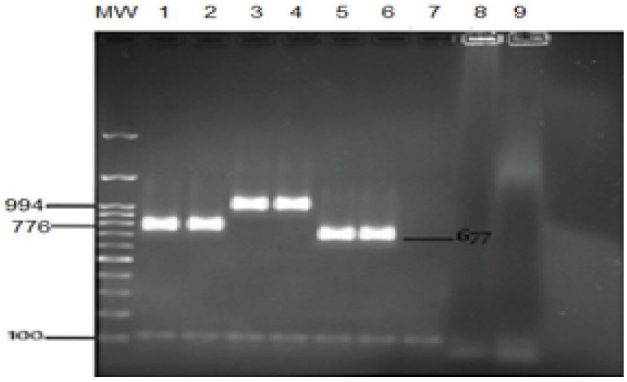

Figure 2. *Staphylococcus aureus* SCC*mec* Type IV: MW: Marker 3kb + 100 bp, 1, 2: Type IVa; 3, 4: Type IVb; 5, 6: Type IVc; 7, 8: IVd, 9: Negative control.

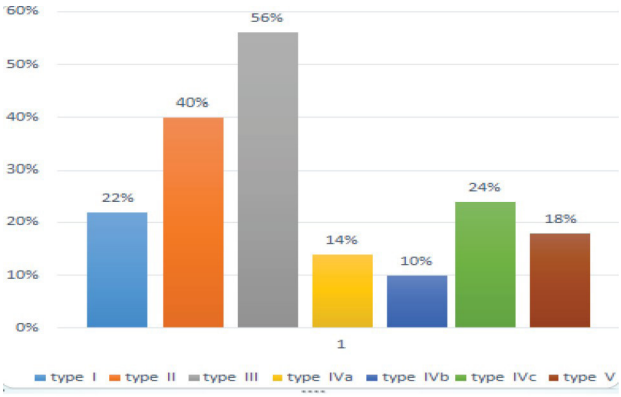

Figure 3. The Frequency of SSC*mec* Type and Subtype.

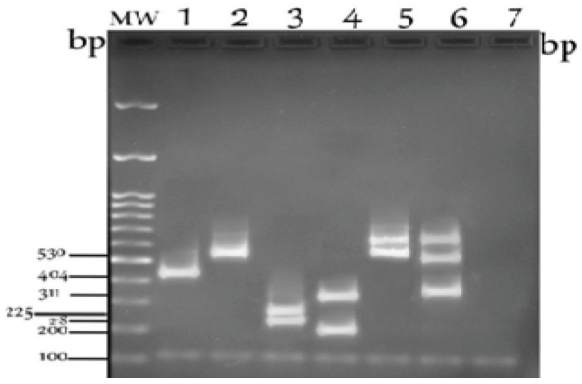

Figure 4. Agarose Gel Electrophoresis Showing 6 PCR-RFLP Patterns of Types I to IV Digested by *HinfI* (one step), as well as *HinfI* and *BsmI* (two steps).

Discussion

Methicillin-resistant (MRSA) gene in *S. aureus* is coded by chromosomal cassette *mec* and contains five main types of SCC*mec*. These organisms cause severe rates of disease and mortality worldwide. In the present study, 100 samples of *S. aureus* strains were isolated from hospitalized patients. The antibiotic susceptibility test revealed that 50 strains were resisted to oxacillin/cefoxitin. Further, methicillin resistance in staphylococci isolated from clinical samples by Rahbar was 53% in Tehran and 51% of MRSA were also reported in Turkey (27).

In this study, SCC*mec* typing was performed on 50 MRSA strains and all strains were typeable encompassing types III (28 cases), II (20), IVc (12), I (11), V (14), IVa (7), IVb (5), and IVd (0). Types III and IV were identified as the dominant types in this study, which mainly cause multi-drug resistant leading to increased health problems, especially in the hospital. Furthermore, types III and II exist together in 20% of MRSA strains and types II, III, IVb, and V were present together only in one bacterium although SCC*mec* type was observed in none of the cases 4, 5, 6,... Four subtypes of SCC*mec* type IV were not detected together in any strains.

Amiri et al evaluated 3 type I SCC*mec*, 12 type II SCC*mec*, 8 type IVb SCC*mec*, 4 type IVd SCC*mec*, and 3 type V SCC*mec* isolates. The findings revealed different types of SCC*mec* carries in MRSA strains in a hospital in Kashan and two dominant types in this study were types II and IV SCC*mec* (28). In another study, Abdollahi et al identified that 15 cases related to types I, IV, and V and 63 cases belonged to types II and III. Moreover, the highest type related to different types of II (34 cases), III (29 cases), and other types were I (6 cases), V (six), and IV (three) (29). The typing of MRSA using M-PCR included types III (33.33%), IV (43.33%), and V (23.33%), (12), which is somewhat similar to our study. Several studies indicated that SCC*mec* type III was to be circulating in Iranian hospitals and other Asian countries (30-32) and SCC*mec* type IV was the most frequent isolation of MRSA in healthy carries (33) whereas SCC*mec* type V was dominant in methicillin-resistant *S. haemolyticus* (31) in Iran.

In Slovenia, from 31 strains of MRSA, 16 isolates (51.6%) of SCC*mec* type IV, 7 (22.5%) and 2 (5.6%) strains were of SCC*mec* types I and III, respectively, and 6 strains (19.4%) were classified as non-typed. In Malaysia, among the 66 observed strains of MRSA, 52 cases (78.8%) were of SCC*mec* type III and 12 cases (18.18%) belonged to SCC*mec* type II (34). Chongtrakool et al showed that SCC*mec* type III was the most common type of *mecA* in 8 Asian countries (35). These differences in the literature indicate that different locations and the variety of treatment may affect the epidemic distribution of SCC*mec* type in the world.

The distribution of SCC*mec* in nature is limited to relatively few clonal complexes of related MRSA (36). The majority of epidemic H-MRSAs carries SCCmec types I, II or III (18). SCC*mec* subtype IVa or IVb carries non-oxacillin-resistant *S. aureus* strains (26), but SCC*mec* subtype IVc was observed in the hospital-acquired strains (37). Additionally, SCC*mec* IV is present in diverse genetic backgrounds, which suggests that type IV is a mobile element (38).

In this study, rapid SCC*mec* typing was investigated by the PCR-RFLP for the *ccrB* gene using two *HinfI* and *BsmI* enzymes in MRSA, which matched the results obtained by the PCR assay. Similarly, SCC*mec* types II and IV were identified by one-step digestion with *HinfI*. In addition, 24 (12 IVc, 7 IVa, and 5 IVb subtypes, respectively) isolates of SCC*mec* type IV and 20 isolates of SCC*mec* type II were successfully discriminated by one-step digestion. The second step with *BsmI* digestion is necessary for discriminating of SCC*mec* type I and III. In general, 28 isolates of SCC*mec* type III and 11 isolates of SCC*mec* type I were detected in the second step.

Zhang et al detected SCC*mec* types IV, II, and III by the PCR-RLFP method. However, no SCC*mec* type I strains was found among the clinical MRSA isolates in this study (14). Type V cannot be identified by the PCR-

RFLP method since the *ccrB* gene of types I to IV has 37.4% homology to the *ccrC* gene of type V at the nucleic acid level (39,40).

Conclusions

The findings revealed that using the PCR-RFLP method can be used to identify different types of staphylococcal cassette chromosome *mec* (SCC*mec*). This method can replace the original method with eight pairs of primers for SCC*mec* typing whereas the PCR-RFLP method is only performed with one pair of primers and one or two restriction enzymes.

Ethical Approval

Not applicable.

Conflict of Interest Disclosures

The authors declare that there is no financial or commercial conflict of interest in this study.

Acknowledgments

The authors would like to thank the microbiology laboratory personnel of Islamic Azad University, Pishva Branch for their cooperation.

References

1. Mahon CR, Lehman DC, Manuselis G Jr. Textbook of Diagnostic Microbiology. 3th ed. Saunders; 2007. p. 369.
2. Turlej A, Hryniewicz W, Empel J. Staphylococcal cassette chromosome *mec* (SCC*mec*) classification and typing methods: an overview. Pol J Microbiol. 2011;60(2):95-103.
3. Song MD, Wachi M, Doi M, Ishino F, Matsuhashi M. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. 1987;221(1):167-71. doi: 10.1016/0014-5793(87)80373-3.
4. Namvar AE, Afshar M, Asghari B, Rastegar Lari A. Characterisation of SCC*mec* elements in methicillin-resistant *Staphylococcus aureus* isolated from burn patients. Burns. 2014;40(4):708-12. doi: 10.1016/j.burns.2013.09.010.
5. Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. Drug Resist Updat. 2003;6(1):41-52. doi: 10.1016/s1368-7646(03)00003-7.
6. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, *Staphylococcus cassette* chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2000;44(6):1549-55. doi: 10.1128/aac.44.6.1549-1555.2000.
7. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH, et al. Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J Dairy Sci. 2007;90(3):1176-85. doi: 10.3168/jds.S0022-0302(07)71604-1.
8. Cookson BD, Robinson DA, Monk AB, Murchan S, Deplano A, de Ryck R, et al. Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant *Staphylococcus aureus* strains: the HARMONY collection. J Clin Microbiol. 2007;45(6):1830-7. doi: 10.1128/jcm.02402-06.
9. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. 2001;9(10):486-93. doi: 10.1016/s0966-842x(01)02175-8.
10. Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob Agents Chemother. 1999;43(6):1449-58.
11. Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle-Vavra S, et al. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. Antimicrob Agents Chemother. 2002;46(4):1147-52. doi: 10.1128/aac.46.4.1147-1152.2002.
12. Sadeghi Y, Salami SA, Kananizadeh P, Mozhgani SH, Pourmand MR. Real-time PCR followed by high-resolution melting analysis - a new robust approach to evaluate SCC*mec* typing of methicillin-resistant *Staphylococcus aureus*. Future Microbiol. 2019;14:155-64. doi: 10.2217/fmb-2018-0193.
13. Oliveira DC, Milheirico C, de Lencastre H. Redefining a structural variant of staphylococcal cassette chromosome *mec*, SCC*mec* type VI. Antimicrob Agents Chemother. 2006;50(10):3457-9. doi: 10.1128/aac.00629-06.
14. Zhang K, McClure JA, Elsayed S, Conly JM. Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A *mec* and type 4 *ccr* gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2009;53(2):531-40. doi: 10.1128/aac.01118-08.
15. Ghaznavi-Rad E, Fard-Mousavi N, Shahsavari A, Japoni-Nejad A, Van Belkum A. Distribution of staphylococcal cassette chromosome *mec* types among methicillin-resistant coagulase negative staphylococci in central Iran. Iran J Microbiol. 2018;10(1):7-13.
16. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. Antimicrob Agents Chemother. 2009;53(12):4961-7. doi: 10.1128/aac.00579-09.
17. Oliveira DC, Tomasz A, de Lencastre H. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb Drug Resist. 2001;7(4):349-61. doi: 10.1089/10766290152773365.
18. Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, et al. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2001;45(5):1323-36. doi: 10.1128/aac.45.5.1323-1336.2001.
19. Lim TT, Chong FN, O'Brien FG, Grubb WB. Are all community methicillin-resistant Staphylococcus aureus related? a comparison of their mec regions. Pathology. 2003;35(4):336-43.
20. Noto MJ, Archer GL. A subset of Staphylococcus aureus strains harboring staphylococcal cassette chromosome mec (SCCmec) type IV is deficient in CcrAB-mediated SCCmec excision. Antimicrob Agents Chemother. 2006;50(8):2782-8. doi: 10.1128/aac.00032-06.
21. Goh SH, Byrne SK, Zhang JL, Chow AW. Molecular typing of Staphylococcus aureus on the basis of coagulase gene polymorphisms. J Clin Microbiol. 1992;30(7):1642-5.
22. Yang JA, Park DW, Sohn JW, Kim MJ. Novel PCR-restriction fragment length polymorphism analysis for rapid typing of staphylococcal cassette chromosome mec elements. J Clin Microbiol. 2006;44(1):236-8. doi: 10.1128/jcm.44.1.236-238.2006.
23. Nasonova ES. [Pulsed field gel electrophoresis: theory, instruments and applications]. Tsitologiia. 2008;50(11):927-

35.
24. Ishino K, Tsuchizaki N, Ishikawa J, Hotta K. Usefulness of PCR-restriction fragment length polymorphism typing of the coagulase gene to discriminate arbekacin-resistant methicillin-resistant Staphylococcus aureus strains. J Clin Microbiol. 2007;45(2):607-9. doi: 10.1128/jcm.02099-06.
25. Oliveira CF, Paim TG, Reiter KC, Rieger A, D'Azevedo PA. Evaluation of four different DNA extraction methods in coagulase-negative staphylococci clinical isolates. Rev Inst Med Trop Sao Paulo. 2014;56(1):29-33. doi: 10.1590/s0036-46652014000100004.
26. Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, Bradbury R, et al. Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant Staphylococcus aureus in Central Sydney, Australia. J Antimicrob Chemother. 2002;49(5):793-801. doi: 10.1093/jac/dkf021.
27. Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F. Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant Staphylococcus aureus. J Infect Dev Ctries. 2008;2(1):46-50. doi: 10.3855/jidc.321.
28. Amiri Z, Safari D, Mousavi G. SCCmec gene cassette chromosome complex molecular characterization and typing of methicillin-resistant Staphylococcus aureus strains isolated from clinical samples martyr Beheshti Hospital during the year 1388. Quarterly J Grace. 2010;14:439-46. [Persian].
29. Abdollahi A, Koohpayeh SA, Najafipoor S, Mansoori Y, Abdollahi S, Jaafari S. Evaluation of drug resistance and staphylococcal cassette chromosome (SCCmec) types among methicillin-resistant Staphylococcus aureus (MRSA). Alborz University Medical Journal. 2012;1(1):47-52. doi: 10.18869/acadpub.aums.1.1.47. [Persian].
30. Ghaznavi-Rad E, Nor Shamsudin M, Sekawi Z, Khoon LY, Aziz MN, Hamat RA, et al. Predominance and emergence of clones of hospital-acquired methicillin-resistant Staphylococcus aureus in Malaysia. J Clin Microbiol. 2010;48(3):867-72. doi: 10.1128/jcm.01112-09.
31. Maleki A, Ghafourian S, Taherikalani M, Soroush S. Alarming and threatening signals from health centers about multi drug resistance Staphylococcus haemolyticus. Infect Disord Drug Targets. 2019;19(2):118-27. doi: 10.2174/1871526518666180911142806.
32. Mohammadi S, Sekawi Z, Monjezi A, Maleki MH, Soroush S, Sadeghifard N, et al. Emergence of SCCmec type III with variable antimicrobial resistance profiles and spa types among methicillin-resistant Staphylococcus aureus isolated from healthcare- and community-acquired infections in the west of Iran. Int J Infect Dis. 2014;25:152-8. doi: 10.1016/j.ijid.2014.02.018.
33. Japoni-Nejad A, Rezazadeh M, Kazemian H, Fardmousavi N, van Belkum A, Ghaznavi-Rad E. Molecular characterization of the first community-acquired methicillin-resistant Staphylococcus aureus strains from Central Iran. Int J Infect Dis. 2013;17(11):e949-54. doi: 10.1016/j.ijid.2013.03.023.
34. Thong KL, Junnie J, Liew FY, Yusof MY, Hanifah YA. Antibiograms and molecular subtypes of methicillin-resistant Staphylococcus aureus in local teaching hospital, Malaysia. J Microbiol Biotechnol. 2009;19(10):1265-70.
35. Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, et al. Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant Staphylococcus aureus strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. Antimicrob Agents Chemother. 2006;50(3):1001-12. doi: 10.1128/aac.50.3.1001-1012.2006.
36. Katayama Y, Robinson DA, Enright MC, Chambers HF. Genetic background affects stability of mecA in Staphylococcus aureus. J Clin Microbiol. 2005;43(5):2380-3. doi: 10.1128/jcm.43.5.2380-2383.2005.
37. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9(8):978-84. doi: 10.3201/eid0908.030089.
38. Daum RS, Ito T, Hiramatsu K, Hussain F, Mongkolrattanothai K, Jamklang M, et al. A novel methicillin-resistance cassette in community-acquired methicillin-resistant Staphylococcus aureus isolates of diverse genetic backgrounds. J Infect Dis. 2002;186(9):1344-7. doi: 10.1086/344326.
39. Francois P, Renzi G, Pittet D, Bento M, Lew D, Harbarth S, et al. A novel multiplex real-time PCR assay for rapid typing of major staphylococcal cassette chromosome mec elements. J Clin Microbiol. 2004;42(7):3309-12. doi: 10.1128/jcm.42.7.3309- 3312.2004.
40. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2002;46(7):2155-61. doi: 10.1128/aac.46.7.2155-2161.2002.