AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

68 (2021) 2, 121–127

DOI:
10.1556/030.2021.01365

ORIGINAL RESEARCH PAPER

Genetic diversity of healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates from Southern Iran

SOROUR FARZI[1], MOHSEN REZAZADEH[2], AHMADREZA MIRHOSSEINI[2], MOHAMMAD AMIN REZAZADEH[3], FARHAN HOUSHYAR[2] and MOHAMMAD HOSSEIN AHMADI[1]*

[1] Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran

[2] Department of Medicine, Faculty of Medicine, Moscow State University M. V. Lomonosov, Moscow, Russia

[3] Department of Dentistry, Faculty of Dentistry, First Moscow State Medical University, Moscow, Russia

Received: December 10, 2020 • Accepted: May 6, 2021
Published online: June 2, 2021

ABSTRACT

Staphylococcus aureus is a common pathogen causing hospital infections. The increasing rate of healthcare-associated infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in developing countries has led to many public health problems. This study aimed to investigate the molecular epidemiology as well as the antibiotic resistance pattern of clinical isolates of MRSA from Southern Iran. A total of 135 *S. aureus* isolates were collected from the patients referred to three hospitals in South Iran. The phenotypic and genotypic diagnosis of MRSA isolates was performed by disk diffusion and PCR methods, respectively. The antibiotic resistance pattern for MRSA isolates was performed using Kirby–Bauer method. The molecular epidemiology of isolates was performed by MLST, Spa typing and SCC*mec* typing. From 135 *S. aureus* isolates, 50 (37%) MRSA strains were detected from which two different sequence types including ST239 and ST605 were identified. SCC*mec* type III was the most common profile (50%) and t030 was the predominant spa type (48%) among the strains. The MRSA isolates had the highest resistance to penicillin (100%), tetracycline (88%), levofloxacin (86%), ciprofloxacin (84%), erythromycin (82%), gentamicin (80%), and clindamycin (78%). The results of this study show that the most common genetic type among the MRSA isolates was ST239-SCC*mec* III/t030. The rapid and timely detection of MRSA and the administration of appropriate antibiotics according to the published antibiotic resistance patterns are essential. Furthermore, the continuous and nationwide MRSA surveillance studies are necessary to investigate clonal distribution and spreading of MRSA from community to hospitals.

KEYWORDS

MRSA, *mecA* gene, SCC*mec* typing, *Spa* typing, MLST

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium causing a wide variety of suppurative infections in both humans and animals. In humans, it is one of the most common causes of skin and soft tissue infections as well as bacteremia. *S. aureus* is an opportunistic pathogen carried by approximately 30–50% of the human population worldwide [1]. The anterior nares are the primary niche for *S. aureus*, although the throat and perineum are also important reservoirs [2]. Moreover, methicillin-resistant *Staphylococcus aureus* (MRSA)

*Corresponding author.
E-mail: mhahmadi@shahed.ac.ir

AKJournals

has emerged as a major threat to human health. MRSA strains are generally classified into healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) and community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), which differ with respect to several characteristics in addition to the source of infection [3]. The infections are predominantly due to healthcare-associated (HA) strains linked to the advanced age, comorbidities, surgical procedures or indwelling medical devices [4]. MRSA isolates are resistant to the majority of the antibiotics commonly used to treat *S. aureus* infections in humans. It is a significant cause of severe nosocomial infections and a major public health concern globally [5, 6]. One of the interesting conclusions that have emerged from large international surveillance studies using molecular typing techniques is that a relatively few MRSA clones have been responsible for a disproportionately large fraction of all MRSA disease worldwide [7–9]. Modern MRSA has evolved from several successful clonal lineages of methicillin-susceptible *S. aureus* strains via acquisition of a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*). This element contains the *mecA* gene, which encodes penicillin-binding protein 2 (PBP2) with significantly reduced affinity for β-lactams. Other *mec* genes such as *mecB* and *mecC* can also lead to methicillin resistance in *S. aureus* [10]. Increased emergence of multidrug resistance among MRSA strains has become a major concern in the hospital environment, as it invokes a tremendous financial burden and enhanced morbidity and mortality due to difficulties in treatment of systemic infections [11]. Genotyping data from large international studies have shown that a limited number of major clones of MRSA display an enhanced propensity to spread and cause opportunistic human infections in various parts of the world [7]. There are some studies reporting the prevalence of HA-MRSA in some area of Iran, including Tehran [12], Isfahan [13], the West [14], and Southwest [15] of the country. However, due to lack of information on the prevalence and molecular epidemiology of MRSA in the South of Iran, the present study was performed. The aim of this study was to investigate the antibiotic resistance pattern and evaluate the molecular epidemiology of clinical isolates of HA-MRSA from three hospitals in the Southern Iran.

MATERIALS AND METHODS

Sample collection and bacterial isolates

During six months (from December 2018 to May 2019), a total of 135 *S. aureus* isolates, were collected from different clinical samples of patients referred to three hospitals in the South of Iran, one of which located in Bushehr city (Shohadaye Khalije Fars hospital), and two others in Bandar Abbas city (Khalije Fars and Shahid Mohammadi hospital). The majority of the isolates was recovered from blood and wound samples (Table 1). In order to identification, the isolates were cultured on Blood Agar (Merck, Germany) medium, and then single colony was inoculated on Mannitol Salt Agar (Merck, Germany) at 37 °C for 24 h. The suspicious colonies were subjected to biochemical tests including catalase, coagulase, clumping factor, DNase, thermo-stable nuclease, as well as Gram staining. Then, all isolates were also evaluated for the presence of the *sa442* gene by polymerase chain reaction (PCR) using the forward: 5′AATCTTTGTCGGTACACGATATTCTTCACG-3 and reverse: 5′CGTAATGAGATTTCAGTAGATAATACAACA3′ primers [16]. The *S. aureus* ATCC 29213 strain was used as the reference strain [17]. Finally, the colonies, after approval, were stored in Luria Bertani Broth (LBB) supplemented with 20% Glycerol (Merck, Germany) and kept at −80 °C until use.

MRSA detection

Methicillin resistance was detected by disk diffusion testing with an oxacillin 10 µg disk (Mast, UK) and a cefoxitin 30-µg disk (Mast, UK), in accordance with the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI); The minimum inhibitory concentrations (MICs) of the isolates were also determined for oxacillin using the E-test system (bioMérieux, Marcy l'Etoile, France). *S. aureus* ATCC 29213 strain was used as a positive control and distilled water as a negative control [18]. The DNA of strains was extracted using an extraction kit prepared by Bioflux-bioer (South Korea), according to the manufacturer's instructions. The presence of the *mecA* gene was evaluated using the PCR amplification. *mecA* gene was amplified by two specific primers: *mecA* F (5′ TCCAGATTACAACTTCACCAGG 3′) and *mecA* R (5′- CCACTTCATATC-

Table 1. Characteristics of MRSA strains isolated from patients

Department	Clinical sample isolate no. (%)							Total
	Blood	Urine	Abscesses	Wounds	Synovial fluid	CSF	Lungs	
Surgery	5 (10%)	2 (4%)	2 (4%)	5 (10%)	0 (0%)	0 (0%)	1 (2%)	15 (30%)
Orthopedic	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	2 (4%)
Pediatrics	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Emergency	2 (4%)	0 (0%)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)	4 (8%)
Infectious diseases	8 (16%)	2 (4%)	1 (2%)	2 (4%)	0 (0%)	0 (0%)	3 (6%)	16 (32%)
Neurology	2 (4%)	2 (4%)	0 (0%)	3 (6%)	0 (0%)	3 (6%)	0 (0%)	10 (20%)
ICU	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	2 (4%)
Total	18 (36%)	6 (12%)	4 (8%)	14 (28%)	1 (2%)	3 (6%)	4 (8%)	50 (100%)

TTGTAACG -3′) as previously described, and *S. aureus* ATCC 29247 strain was used as a positive control [16].

The MRSA infection, labeled as HA-MRSA if patients were hospitalized >48 h prior to the current infection (i.e., patient was not infected with MRSA at the time of hospitalization but culture and infection were identified >48 h after admission) [19].

Antimicrobial susceptibility testing

Antimicrobial resistance patterns were determined for all isolates using a panel of 14 antibiotic disks (Table 2) with Kirby–Bauer method, according to the CLSI instruction. The MIC to vancomycin was determined by E-test (bioMérieux). *S. aureus* ATCC 25923 was used as a control strain in the antibacterial susceptibility testing. Intermediate sensitivity was scored as resistance. *Enterococcus faecalis* ATCC 29212 and *Enterococcus faecium* BM4147 were used as controls for vancomycin susceptibility and vancomycin resistance, respectively [18].

Conventional *Spa* typing

Spa typing of strains was performed by amplification of variable short sequence repeat (SSR) X region of the *spa* gene using the primers forward: 5′-AGACGATCCTTCGGTGAGC-3′, and reverse: 5′-GCTTTTGCAATGTCATTTACTG-3′, as described previously by Shopsin et al. [20]. A ready-to-use PCR master mixture (SinaClone, Tehran, Iran) was used in a final volume of 50 µL, consisting of 10 µL of master mix, 0.5 µL of forward primer and 0.5 µL of reverse primer, 38 µL of distilled water, and 1 µL of DNA template in a 0.5-mL microcentrifuge tube. PCR program was carried out as described by Japoni-Nejad et al. [21]. The sequences obtained were subjected to *spa* repeat analysis and *spa* typing using the SpaServer (http://www.spaserver.ridom.de).

Conventional Multi-locus sequence typing (MLST)

The sequence type (ST) for each MRSA isolate was characterized by MLST as described by Enright et al. [22]. The test was carried out by sequencing an internal fragment of seven unlinked housekeeping genes to identify the following allelic profiles: phosphate acetyltransferase (*pta*), carbamate kinase (*arcC*), triosephosphate isomerase (*tpi*), shikimate dehydrogenase (*aroE*), guanylate kinase (*gmk*), acetyl-coenzyme A acetyltransferase (*yqiL*), and glycerol kinase (*glp*). The entire PCR products were purified and then sequenced (Bioneer Co., Korea). Finally, STs were obtained using the *S. aureus* MLST database (http://www.mlst.net).

Table 2. The antimicrobial resistance patterns of 50 healthcare-associated MRSA isolates

Antibiotic	Resistant (%)	Sensitive (%)
Teicoplanin	8 (16%)	42 (84%)
Gentamicin	40 (80%)	10 (20%)
Clindamycin	39 (78%)	11 (22%)
Erythromycin	41 (82%)	9 (18%)
Tetracycline	44 (88%)	6 (12%)
Cotrimoxazole	6 (12%)	44 (88%)
Penicillin	50 (100%)	0 (0%)
Levofloxacin	43 (86%)	7 (14%)
Netilmicin	3 (6%)	47 (94%)
Chloramphenicol	3 (6%)	47 (94%)
Ciprofloxacin	42 (84%)	8 (16%)
Vancomycin	0 (0%)	50 (100%)
Linezolid	2 (4%)	48 (96%)
Quinupristin/dalfopristin	0 (0%)	50 (100%)

Screening for of SCC*mec* elements

The presence of SCC*mec* genes in *S. aureus* isolates was determined by the method developed previously by Ghaznavi-Rad et al. [16].

RESULTS

A total of 50 strains (37%) of MRSA were detected over a six-month period all of which had the *mecA* and *sa442* genes and were resistant to cefoxitin. The antibiotic resistance pattern of these strains is shown in Table 2.

Out of the 50 MRSA isolates, 47 belonged to ST239 and 3 isolates to ST605, identified by MLST. Also nine different *spa* types were identified including t030 (n = 24, 48%), t037 (n = 13, 26%), t7685 (n = 2, 4%), t459 (n = 4, 8%), t275 (n = 3, 6%), t790 (n = 1, 2%), t701 (n = 1, 2%), t325 (n = 1, 2%), and t122 (n = 1, 2%). Moreover, four different types of SCC*mec* were identified, including type I (n = 5, 10%), type II (n = 18, 36%), type III (n = 25, 50%), and type IV (n = 2, 4%). SCCmec type III was the most common profile (50%) and t030 was the predominant spa type (48%) among the strains. The detailed results are shown in Table 3 and the association among SCC*mec* types, spa types, and sequence types is shown in Table 4.

DISCUSSION

In our study, the *mec*A gene was found in 50 isolates (37%). The prevalence of this gene has been reported in other regions of Iran among HA-MRSA with different frequencies [13, 15, 23]. For example, in the Southwest, it has been reported to be 34% and 32% by Mousavian et al. [24] and Tajik et al. [12], respectively. This rate in the West [14] and central [21] Iran was reported to be 39% and 32%, respectively. These results indicate an almost similar prevalence of MRSA in the South to West of Iran, as well as the predominance of similar molecular types in these regions.

There is a continuous increase in the global prevalence of MRSA in European countries. In Belgium and Spain, 19% and 29% of the *S.aureus* strains were methicillin-resistant, respectively [5, 9]. However, the prevalence of the *mecA* gene in our study was comparable to previously studies reported from other countries including 36.6% in Greece, 46% in Israel, 38.3% in Italy, and 45.76% in the Philippines [25]. The differences in the distribution of the *mecA* gene can be

Table 3. Molecular characterization of MRSA isolates from patients

Isolate	sa442	*MecA*	*Spa*Type	ST	SCC *mec*	Source	Ward
IR1	+	+	t030	ST 239	III	Blood	Infectious diseases
IR2	+	+	t030	ST 239	III	Urine	Surgery
IR3	+	+	t030	ST 239	II	Abscesses	Surgery
IR4	+	+	t030	ST 239	III	Wounds	Neurology
IR5	+	+	t030	ST 239	I	Synovial fluid	Orthopedic
IR6	+	+	t037	ST 239	II	Blood	Surgery
IR7	+	+	t030	ST 239	III	Blood	Infectious Diseases
IR8	+	+	t7685	ST 239	II	Blood	Infectious Diseases
IR9	+	+	t030	ST 239	III	Blood	Pediatrics
IR10	+	+	t030	ST 239	III	Wounds	ICU
IR11	+	+	t030	ST 239	I	Blood	Surgery
IR12	+	+	t459	ST 239	III	Blood	Neurology
IR13	+	+	t275	ST 605	II	Abscesses	Infectious diseases
IR14	+	+	t790	ST 239	II	Blood	Infectious diseases
IR15	+	+	t037	ST 239	III	Wounds	Orthopedic
IR16	+	+	t701	ST 239	I	CSF	Neurology
IR17	+	+	t325	ST 239	II	CSF	Neurology
IR18	+	+	t037	ST 239	II	Blood	Infectious diseases
IR19	+	+	t030	ST 239	III	Wounds	Infectious diseases
IR20	+	+	t037	ST 239	IV	CSF	Neurology
IR21	+	+	t037	ST 239	III	Blood	Neurology
IR22	+	+	t037	ST 239	II	Blood	Infectious diseases
IR23	+	+	t030	ST 239	III	Lungs	Infectious diseases
IR24	+	+	t030	ST 239	III	Wounds	Infectious diseases
IR25	+	+	t030	ST 239	II	Urine	Neurology
IR26	+	+	t030	ST 239	II	Blood	Surgery
IR27	+	+	t030	ST 239	III	Abscesses	Surgery
IR28	+	+	t030	ST 239	III	Wounds	Surgery
IR29	+	+	t037	ST 239	IV	Urine	Neurology
IR30	+	+	t030	ST 239	III	Blood	Infectious diseases
IR31	+	+	t122	ST 239	II	Urine	Infectious diseases
IR32	+	+	t7685	ST 239	II	Wounds	Surgery
IR33	+	+	t459	ST 239	II	Wounds	Surgery
IR34	+	+	t030	ST 239	III	Wounds	Emergency
IR35	+	+	t037	ST 239	II	Wounds	ICU
IR36	+	+	t037	ST 239	III	Wounds	Neurology
IR37	+	+	t459	ST 239	III	Lungs	Infectious diseases
IR38	+	+	t037	ST 239	I	Urine	Infectious diseases
IR39	+	+	t030	ST 239	III	Wounds	Neurology
IR40	+	+	t030	ST 239	III	Blood	Emergency
IR41	+	+	t459	ST 239	II	Blood	Infectious Diseases
IR42	+	+	t030	ST 239	III	Blood	Surgery
IR43	+	+	t037	ST 239	I	Wounds	Emergency
IR44	+	+	t275	ST 605	III	Lungs	Infectious diseases
IR45	+	+	t030	ST 239	II	Wounds	Neurology
IR46	+	+	t030	ST 239	III	Blood	Surgery
IR47	+	+	t037	ST 239	II	Blood	Emergency
IR48	+	+	t275	ST 605	III	Wounds	Surgery
IR49	+	+	t030	ST 239	III	Urine	Surgery
IR50	+	+	t037	ST 239	II	Lungs	Surgery

HA-MRSA: Healthcare-associated methicillin-resistant *Staphylococcus aureus*; SCCmec: staphylococcal cassette chromosome *mec*; ST: sequence type.

explained by the various studied populations or by the different types of the clinical specimens.

Our study showed that the prevalence of MRSA and resistance to antibiotics used in this study was higher in the infectious disease (32%), surgical (30%), and neurological (20%) sections rather than other wards. In the study conducted by Khosravi et al. in Ahvaz, most MRSA isolates were detected from hospitalized patients in intensive infectious disease, intensive care unit (ICU), and outpatient (OP) departments [23]. The potential risk of MRSA in the intensive

Table 4. Comparison among different types of MRSA isolates

spa types	SCCmec I	SCCmec II	SCCmec III	SCCmec IV	Total	ST 239	ST 605
t030	2 (4%)	4 (8%)	18 (36%)	0 (0%)	24 (48%)	24 (48%)	0 (0%)
t037	2 (4%)	6 (12%)	3 (6%)	2 (4%)	13 (26%)	13 (26%)	0 (0%)
t7685	0 (0%)	2 (4%)	0 (0%)	0 (0%)	2 (4%)	2 (4%)	0 (0%)
t459	0 (0%)	2 (4%)	2 (4%)	0 (0%)	4 (8%)	4 (8%)	0 (0%)
t275	0 (0%)	1 (2%)	2 (4%)	0 (0%)	3 (6%)	0 (0%)	3 (6%)
t790	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)
t701	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)
t325	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)
t122	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (2%)	1 (2%)	0 (0%)
Total	5 (10%)	18 (36%)	25 (50%)	2 (4%)	50 (100%)	47 (94%)	3 (6%)

infectious disease and surgical units has been considered continuously due to the greater problems of patients and the present study has reported an abundance of these strains in this section. Furthermore, intensive infectious disease departments are considered to be high risk areas for spreading of MRSA infections.

The present study found that the resistance of *S. aureus* to cefoxitin in the South of Iran was very high, and all MRSA isolates were recognized as multidrug resistant. Resistance to, erythromycin, clindamycin, tetracycline, gentamicin, levofloxacin, and ciprofloxacin was observed in more than 70% of MRSA isolates. Furthermore, all MRSA isolates were sensitive to vancomycin and quinupristin/dalfopristin. In the study of Mousavian et al., the highest sensitivity of MRSA was to vancomycin and linezolid and the lowest one was to clindamycin [24]. In 2010, Javan et al. isolated a total of 150 samples of *S. aureus* from hospitalized patients in Tehran hospitals, of which 68 strains (45%) were MRSA strains all of which were resistant to penicillin, amikacin, kanamycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, and clindamycin [26].

In this study, SCC*mec* typing recognized 50% of MRSA isolates as type III. SCC*mec* typing was performed in other regions of Iran, and in all of these published studies, the most frequent SCC*mec* type among nosocomial MRSA strains was type III [27–31]. The frequency of SCC*mec* type III was reported as 74.3% in Shiraz [29], 98% in Tehran [28, 32], 69.8% in Tabriz [27], 91% in Isfahan [31], and 45% in the West provinces of Iran [30]. One of the benefits of SCC*mec* typing of MRSA isolates is differentiation of antibiotic susceptibility patterns. Therefore, we investigated the association between SCC*mec* types and antibiotic resistance patterns. According to our results, most MRSA isolates with type III SCC*mec* were resistant to gentamycin, clindamycin, and ciprofloxacin, while all isolates were resistant to penicillin. These findings are similar to those of Japoni et al. [29] in Shiraz, although they found higher rates of tetracycline resistance than we did.

According to the data, MRSA isolates in the hospital (HA-MRSA) could be CA-MRSA isolates. In the present study, two isolates from the neurology department were SCC*mec* type IV with similar genetics which indicates the dissemination of CA-MRSA in this department. Some researchers have reported an increase in the prevalence of MRSA strains with SCC*mec* type IV occurring in hospital settings [33, 34]. Valsesia et al. reported SCC*mec* type IV as the most frequent type (76.6%) among HA-MRSA strains in Switzerland. Some evidences indicate that the replication of MRSA strains with SCC*mec* type IV is more rapid than the strains with SCC*mec* type II/III, perhaps due to their enhanced fitness compared to SCC*mec* type II/III strains [35]. Our results are similar to those of the previous studies conducted in Iran and other parts of the world [10, 25].

According to our results from *spa* typing, t030 (48%), and t037 (26%), were recognized as the most common *spa* types. Moreover, this study showed that the *SCCmec*III-t030 and *SCCmec*II-t037 are spread in the South Iran. These *spa* types were previously reported from Saudi Arabia, China, Iran, as well as among HA-MRSA isolates found in Europe, America, and other regions of Asia [36–39]. Similar to our results, *SCCmec* type III was the most common type (91.4%) and t030 was the most common spa type (85.1%) among MRSA strains isolated from Turkish hospitals [24, 40].

In our study, 94% of MRSA isolates were ST-239. The review of the literature reveals that the multi-resistant ST239 clone is an important HA-MRSA clone in Europe, United States, and some Asian countries including Kuwait and Malaysia [38]. Therefore, the presence of ST239-SCC*mec* III/t037 in our healthcare settings might be attributed to neighboring regions. In our study, ST239-SCC*mec* III/t030 and ST239-SCC*mec* II/t037, had the highest prevalence in the surgical and infectious diseases departments. Furthermore, t275, unlike other types, was reported with ST605 in the infectious diseases and surgical departments.

CONCLUSION

In conclusion, the results of the present study indicate that the most common genetic type among MRSA isolates was ST239-SCC*mec* III/t030. It is recommended to use quinupristin/dalfopristin, teicoplanin, netilmicin, or vancomycin, or a combination of these antibiotics in treatment of the diseases caused by MRSA according to the treatment protocols. The rapid and timely detection of MRSA, as well as the administration of appropriate antibiotics according to the published antibiotic resistance pattern of *S. aureus*, is essential. Continuous and nationwide MRSA surveillance

studies are necessary to investigate clonal distribution and spreading of MRSA strains from community to hospitals.

Conflict of interest: None.

Funding: None.

REFERENCES

1. Brown AF, Leech JM, Rogers TR, McLoughlin RM. Staphylococcus aureus colonization: modulation of host immune response and impact on human vaccine design. Front Immunol 2014; 4: 507.
2. Islam MZ, Johannesen TB, Lilje B, Urth TR, Larsen AR, Angen Ø, et al. Investigation of the human nasal microbiome in persons with long-and short-term exposure to methicillin-resistant Staphylococcus aureus and other bacteria from the pig farm environment. PloS One 2020; 15(4): e0232456.
3. Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant Staphylococcus aureus (CA-MRSA). Curr Opin Microbiol 2012; 15(5): 588–95.
4. Coia J, Duckworth G, Edwards D, Farrington M, Fry C, Humphreys H, et al. Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities. J Hosp Infect 2006; 63: S1–44.
5. Benrabia I, Hamdi TM, Shehata AA, Neubauer H, Wareth G. Methicillin-resistant Staphylococcus aureus (MRSA) in poultry species in Algeria: long-term study on prevalence and antimicrobial resistance. Vet Sci 2020; 7(2): 54.
6. Sergelidis D, Angelidis A. Methicillin-resistant Staphylococcus aureus: a controversial food-borne pathogen. Lett Appl Microbiol 2017; 64(6): 409–18.
7. Chung M, Dickinson G, De Lencastre H, Tomasz A. International clones of methicillin-resistant Staphylococcus aureus in two hospitals in Miami, Florida. J Clin Microbiol 2004; 42(2): 542–7.
8. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). PNAS 2002; 99(11): 7687–92.
9. Oliveira DC, Tomasz A, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant Staphylococcus aureus. Lancet Infect Dis 2002; 2(3): 180–9.
10. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2003; 47(12): 3926–34.
11. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis 2003; 36(1): 53–9.
12. Tajik S, Najar-Peerayeh S, Bakhshi B. Hospital clones of Panton-Valentine leukocidin-positive and methicillin-resistant Staphylococcus aureus circulating in the Tehran community. J Glob Antimicrob Resist 2020; 22: 177–81.
13. Sedaghat H, Esfahani BN, Halaji M, Jazi AS, Mobasherizadeh S, Havaei SR, et al. Genetic diversity of Staphylococcus aureus strains from a teaching hospital in Isfahan, Iran: the emergence of MRSA ST639-SCCmec III and ST343-SCCmec III. Iran J Microbiol 2018; 10(2): 82.
14. Poormohammadi S, Farahani A, Mohajeri P. Genomic diversity and antimicrobial susceptibility profiling of nasal carriage Staphylococcus aureus isolated from pediatric ward in Western Iran. Saudi J Biol Sci 2019; 26(1): 1–7.
15. Parhizgari N, Khoramrooz SS, Malek Hosseini SAA, Marashifard M, Yazdanpanah M, Emaneini M, et al. High frequency of multidrug-resistant Staphylococcus aureus with SCC mec type III and Spa types t037 and t631 isolated from burn patients in Southwest of Iran. APMIS 2016; 124(3): 221–8.
16. Ghaznavi-Rad E, Shamsudin MN, Sekawi Z, van Belkum A, Neela V. A simplified multiplex PCR assay for fast and easy discrimination of globally distributed staphylococcal cassette chromosome mec types in meticillin-resistant Staphylococcus aureus. J Med Microbiol 2010; 59(10): 1135–9.
17. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG. Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus. J Clin Microbiol 1998; 36(3): 618–23.
18. CLSI MEE. Performance Standards for antimicrobial susceptibility testing: 29th informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
19. Parvez MAK, Ferdous RN, Rahman MS, Islam S. Healthcare-associated (HA) and community-associated (CA) methicillin resistant Staphylococcus aureus (MRSA) in Bangladesh–Source, diagnosis and treatment. J Genet Eng Biotechnol 2018; 16(2): 473–8.
20. Shopsin B, Gomez M, Montgomery S, Smith D, Waddington M, Dodge D, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 1999; 37(11): 3556–63.
21. 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.
22. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 2000; 38(3): 1008–15.
23. Khosravi AD, Jenabi A, Montazeri EA. Distribution of genes encoding resistance to aminoglycoside modifying enzymes in methicillin-resistant Staphylococcus aureus (MRSA) strains. Kaohsiung J Med Sci 2017; 33(12): 587–93.
24. Moosavian M, Dehkordi PB, Hashemzadeh M. Characterization of SCCmec, spa types and multidrug resistant of methicillin-resistant Staphylococcus aureus isolates in Ahvaz, Iran. Infect Drug Resist 2020; 13: 1033.
25. Sader HS, Watters AA, Fritsche TR, Jones RN. Daptomycin antimicrobial activity tested against methicillin-resistant staphylococci and vancomycin-resistant enterococci isolated in European medical centers (2005). BMC Infect Dis 2007; 7(1): 29.
26. Javan A, Falahati H, Saifi M, Talebi M, Ebrahimpour G, Pourshafi M. MECA gene among high level methicillin resistant Staphylococcus aureus isolated from Tehran hospitals. Iran J Infect Dis Trop Med 2010; 15(49): 17–22.

27. Amirkhiz MF, Rezaee MA, Hasani A, Aghazadeh M, Naghili B. SCCmec typing of methicillin-resistant Staphylococcus aureus: an eight year experience. Arch Pediatr Infect Dis 2015; 3: e30632.
28. Fatholahzadeh B, Emaneini M, Gilbert G, Udo E, Aligholi M, Modarressi MH, et al. Staphylococcal cassette chromosome mec (SCC mec) analysis and antimicrobial susceptibility patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolates in Tehran, Iran. Infect Drug Resist 2008; 14(3): 217–20.
29. Japoni A, Jamalidoust M, Farshad S, Ziyaeyan M, Alborzi A, Japoni S, et al. Characterization of SCCmec types and antibacterial susceptibility patterns of methicillin-resistant Staphylococcus aureus in Southern Iran. Jpn J Infect Dis 2011; 64(1): 28–33.
30. Mohammadi S, Sekawi Z, Monjezi A, Maleki M-H, 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.
31. Rahimi F, Katouli M, Pourshafie MR. Characteristics of hospital- and community-acquired meticillin-resistant Staphylococcus aureus in Tehran, Iran. J Med Microbiol 2014; 63(6): 796–804.
32. Goudarzi M, Goudarzi H, Sá Figueiredo AM, Udo EE, Fazeli M, Asadzadeh M, et al. Molecular characterization of methicillin resistant Staphylococcus aureus strains isolated from intensive care units in Iran: ST22-SCC mec IV/t790 emerges as the major clone. PloS One 2016; 11(5): e0155529.
33. Huang Y, Tseng S, Hu J, Tsai J, Hsueh P, Teng L. Clonal spread of SCCmec type IV methicillin-resistant Staphylococcus aureus between community and hospital. Clin Microbiol Infec 2007; 13(7): 717–24.
34. Strandén A, Frei R, Adler H, Flückiger U, Widmer A. Emergence of SCCmec type IV as the most common type of methicillin-resistant Staphylococcus aureus in a university hospital. Infection 2009; 37(1): 44.
35. Valsesia G, Rossi M, Bertschy S, Pfyffer GE. Emergence of SCCmec type IV and SCCmec type V methicillin-resistant Staphylococcus aureus containing the Panton-Valentine leukocidin genes in a large academic teaching hospital in central Switzerland: external invaders or persisting circulators? J Clin Microbiol 2010; 48(3): 720–7.
36. Goudarzi M, Eslami G, Rezaee R, Heidary M, Khoshnood S, Nia RS. Clonal dissemination of Staphylococcus aureus isolates causing nosocomial infections, Tehran, Iran. Iran J Basic Med Sci 2019; 22(3): 238.
37. Goudarzi M, Seyedjavadi SS, Nasiri MJ, Goudarzi H, Nia RS, Dabiri H. Molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from patients with bacteremia based on MLST, SCCmec, spa, and agr locus types analysis. Microb Pathog 2017; 104: 328–35.
38. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant Staphylococcus aureus. PloS One 2011; 6(4): e17936.
39. Monecke S, Skakni L, Hasan R, Ruppelt A, Ghazal SS, Hakawi A, et al. Characterisation of MRSA strains isolated from patients in a hospital in Riyadh, Kingdom of Saudi Arabia. BMC Microbiol 2012; 12(1): 146.
40. Bozdoğan B, Yıldız O, Oryaşın E, Kırdar S, Gülcü B, Aktepe O, et al. t030 is the most common spa type among methicillin-resistant Staphylococcus aureus strains isolated from Turkish hospitals. Mikrobiyol Bul 2013; 47(4): 571–81.