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Molecular characterization of the first community-acquired methicillin-resistant *Staphylococcus aureus* strains from Central Iran

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SUMMARY

Background: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has spread throughout the world with varying regional incidences and different staphylococcal cassette chromosome *mec* (SCC*mec*) elements in different genetic backgrounds. No information is available on CA-MRSA in Iran. A cross-sectional study was carried out among healthy students to investigate: (1) the prevalence of CA-MRSA in Central Iran, (2) the molecular epidemiology of such CA-MRSA strains, (3) the antimicrobial resistance patterns of the strains, and (4) the distribution of virulence genes in these CA-MRSA strains.

Methods: A total of 700 nasal swabs were collected and subjected to *S. aureus* and MRSA-specific isolation procedures. Antimicrobial resistance patterns were determined using the disk diffusion method, and molecular typing was carried out by multi-locus sequence typing (MLST), SCC*mec* typing, and Staphylococcus protein A (*spa*) typing for all CA-MRSA isolates. PCR was used to detect various virulence genes.

Results: One hundred fifty-four *S. aureus* strains were isolated from the anterior nares of 700 healthy students. According to the US Centers for Disease Control and Prevention definitions for CA-MRSA, seven (4.5%) isolates were confirmed as CA-MRSA. CA-MRSA isolates belonged to SCC*mec* types IV ($n = 6$) and V ($n = 1$). The predominant *spa*-type among the CA-MRSA isolates was t790 ($n = 3$), with single t660, t084, and t325 isolates; one isolate was not typeable. The predominant sequence type was ST22, t790, SCC*mec* IV in three isolates, and the four other sequence types were ST25, ST859, ST14, and ST15.

Conclusions: Iranian CA-MRSA strains are genetically diverse with an elevated prevalence of t790/ST22 SCC*mec* IV isolates. These findings support the need for more effective infection control measures to reduce nasal carriage and prevent dissemination of CA-MRSA in Iran.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and represents a challenge for public health, as community-acquired (CA) infections appear to be on the increase in both adults and children in various regions. CA-MRSA was first documented in children with infections in the USA in the late 1990s.[1,2]

The global epidemiology of CA-MRSA is remarkably heterogeneous. In some regions, a single clone dominates (e.g., USA300 in the USA), whereas in other regions, multiple clones have been identified (e.g., 100 different clones have been described in Australia).[3] Recently, virulence genes such as those encoding Panton–Valentine leukocidin (PVL, *pvl* gene) and exfoliative toxin (ET, *et* gene) have been associated with emerging CA-MRSA clones and specific clinical presentations.[4]

CA-MRSA containing the genes encoding PVL have spread all over the world with various incidences and characterizing different genetic backgrounds.[5] For instance, the sequence type (ST) 8 USA300 clone has been detected relatively frequently in North and South America and Spain, whereas the ST80 clone has been found to be the major European CA-MRSA clone, which is also frequently detected in Algeria.[6] Multiple additional PVL-positive CA-MRSA clones have been reported,[7] but no information is available on the epidemiology of CA-MRSA in Central Iran.[8] The aim of the current study was to evaluate the molecular

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epidemiology of CA-MRSA clones circulating in healthy Iranian carriers.

2. Methods

2.1. Bacterial isolates and MRSA detection

Seven hundred volunteers (437 females and 263 males; mean age 22.7 years) from the Arak University of Medical Sciences (Arak, Iran) campus, without symptoms or signs of clinical illness, were enrolled in the study over a 3-month period. Institutional ethical approval was obtained prior to study commencement. The healthy volunteers had not used antibiotics during the past 2 months and had no history of hospitalization, admission to a nursing home, skilled nursing facility, or hospice, dialysis because of renal failure, or recent surgery, and they had had no permanent indwelling catheters or medical devices that pass through the skin at least during the past year (which constitutes a risk factor for CA-MRSA).[2] In fact, these students were studying only in the medical faculty and were not being exposed to the hospital environment or patients.

Samples were collected from both anterior nares of the volunteers by rotating a sterile Transwab with Amies clear transport medium (MW170 Transwabs; Medical Wire and Equipment Company, Corsham, UK). Swabs were transported to the Department of Medical Microbiology and Immunology at Arak University of Medical Sciences and cultured.

The isolates were presumptively identified as *S. aureus* by standard biochemical tests for catalase, coagulase, clumping factor, DNase, and thermostable nuclease. All isolates were also evaluated for the presence of the *sa442* gene by PCR.[9]

Methicillin resistance was detected by disk diffusion testing with a cefoxitin 30-μg disk (Mast, UK) and an oxacillin 10 μg disk (Mast, UK), in accordance with the guidelines issued by the Clinical and Laboratory Standards Institute (CLSI).

Isolates were also tested by MRSA screen latex agglutination (Denka Seiken Co., Ltd, Tokyo, Japan), and oxacillin minimum inhibitory concentrations (MICs) were determined using the Etest system (bioMérieux, Marcy l'Etoile, France). The presence of the *mec*A gene was verified by PCR only for phenotypically oxacillin-resistant isolates.

2.2. Antimicrobial susceptibility testing

For all CA-MRSA isolates, antimicrobial resistance patterns were determined using a panel of 17 antibiotic disks in accordance with the Kirby–Bauer method (CLSI and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, 2011). Antibiotics included gentamicin, amikacin, netilmicin, levofloxacin, co-trimoxazole, mupirocin, quinupristin–dalfopristin, ciprofloxacin, cefoxitin, oxacillin, rifampin, linezolid, fusidic acid, clindamycin, tetracycline, tigecycline, and erythromycin. The MIC to vancomycin was determined by Etest (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.

2.3. Detection of virulence genes by PCR

A DNA extraction kit (BioFlux, Bioer, Korea) was used for purification of genomic DNA from all CA-MRSA strains; the manufacturer's instructions were followed. PCR was used for amplification of genes encoding PVL (*pvl*), *arcA* as an indicator of the arginine catabolic mobile element (ACME), exfoliative toxins A and B (*eta* and *etb*), staphylococcal enterotoxins A, B, C, G, H, and I (*sea*, *seb*, *sec*, *seg*, *seh*, and *sei*), and toxic shock syndrome toxin (*tsst*). All primers and programs can be found in Table 1.[10,11]

2.4. Molecular diversity of CA-MRSA based on spa typing

The short sequence repeat (SSR) X region of the *spa* gene was amplified using the primers forward: 5′-AGACGATCCTTCGGTGAGC-3′, and reverse: 5′-GCTTTTGCAATGTCATTTACTG-3′, as described by Shopsin et al.[12] A ready-to-use PCR master mixture (Sina Clone, Iran) was used in a final volume of 50 μl, consisting of 10 μl of master mix, 0.1 μl of forward primer and 0.1 μl of reverse primer, 38.8 μl of distilled water, and 1 μl of DNA template in a 0.5-ml microcentrifuge tube. PCR incubation mixtures were subjected to a quick spin before being placed in an Eppendorf thermocycler (Germany). PCR was carried out as described by Harmsen et al.[13] using the following cycling parameters: initial denaturation of 10 min at 95 °C, followed by 30 cycles of

Table 1
PCR primers and cycling parameters for genes presented in this study

Gene	Primer/sequence	PCR conditions	PCR size	Reference
sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	120 bp	[10]
seb	F: TCGCATCAAACTGACAAACG R: GCAGGTACTCTATAAGTGCC	1 min 94 °C,1 min 55 °C, 1 min 72 °C	478 bp	[10]
sec	F: GCATAAAAGCTAGGAATTT R: AAATCGGATTAACATTATCC	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	257 bp	[10]
seg	F: AATTATGTGAATGCTCAACCCGATC R: AACTTATATGGAACAAAAGGTACTAGTTC	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	642 bp	[10]
seh	F: CAATCACATCATATGCGAAAGCAG R: CATCTACCCAAACATTAGCACC	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	372 bp	[10]
sei	F: CTCAAGGTGATATTGGTGTAGG R: AAAAAACTTACAGGCAGTCCATCTC	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	576 bp	[10]
eta	F: CTAGTGCATTTGTTATTCAA R: TGCATTGACACCATAGTACT	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	119 bp	[10]
etb	F: ACGGCTATATACATTCAATT R: TCCATCGATAATATACCTAA	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	200 bp	[10]
tsst	F: ATGGCAGCATCAGCTTGATA R: TTTCCAATAACCACCCGTTT	1 min 94 °C, 1 min 55 °C, 1 min 72 °C	350 bp	[10]
pvl	F: ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAASTGTATTGGATAGCAAAAGC	30 s 94 °C, 30 s 55 °C, 30 s 72 °C	433 bp	[11]
*arc*A	F: GAGCCAGAAGTACG R: CACGTAACTTGCTAGAACGAG	30 s 94 °C, 30 s 55 °C, 30 s 72 °C	770 bp	

denaturation for 30 s at 95 °C, 30 s annealing at 60 °C, and 45 s extension at 72 °C, and a final extension at 72 °C for 10 min. *S. aureus* ATCC 25923 was used as positive control, while sterile deionized water was used as negative control. Amplified PCR products were visualized after agarose gel electrophoresis (60 Volts, 500 mA, and duration of 60 min). Purified PCR products were sequenced (DNA Sequencer ABI, model 3730-XL) commercially (Gene Fanavaran, Iran) using the *spa* primers. The sequences obtained were subjected to *spa* repeat analysis and *spa* typing using the SpaServer (http://www.spaserver.ridom.de).

2.5. Multi-locus sequence typing (MLST)

For all CA-MRSA isolates, the ST was determined by MLST. Partial gene sequences of seven housekeeping genes (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi*, and *yqi*L) were determined by PCR and direct sequencing, and their allelic profile (allele numbers) and ST were obtained using the *S. aureus* MLST database (http://www.mlst.net) hosted by Imperial College in London, UK.[14]

2.6. SCCmec typing

The structure of SCC*mec* was determined by the method developed by Ghaznavi-Rad et al.[15]

3. Results

One hundred fifty-four *S. aureus* strains were isolated from the anterior nares of 700 healthy students. Overall, seven isolates (IR1 to IR7) were resistant to methicillin and were confirmed as CA-MRSA based on the detection of the *mec*A gene and the overall lack of features suggesting hospital-related acquisition. The antimicrobial resistance patterns of these CA-MRSA isolates are shown in Table 2.

According to the results of the vancomycin Etest, all isolates were susceptible to this agent. The highest rates of susceptibility were observed with amikacin (100%, $n = 7$), gentamicin (100%, $n = 7$), netilmicin (100%, $n = 7$), and co-trimoxazole (100%, $n = 7$). Six of seven isolates (85.8%) were susceptible to ciprofloxacin, levofloxacin, rifampin, and mupirocin, while four of seven isolates (57.1%) were susceptible to erythromycin, clindamycin, and tetracycline.

Five different profiles were identified by MLST: ST22, ST14, ST15, ST25, and ST859. *spa* typing discriminated four different types: t790, t084, t325, and t660; one strain was not *spa*-typeable (Table 3). Six isolates belonged to SCC*mec* type IV (85.8%) and one isolates showed SCC*mec* type V (14.3%).

ST22, SCC*mec* type IV, *spa*-type t790 strains (IR4, IR5, and IR6) were determined as the predominant clone in Central Iran. The prevalence of *seb*, *eta*, and *pvl* genes in CA-MRSA strains were all 14.3%, and the prevalence of *sec*, *sei*, *tsst*, *arcA*, *seg*, and *seh* genes were 28.5%, 28.5%, 28.5%, 28.5%, 42.8%, and 42.8% respectively, whereas the *sea* and *etb* genes were not found in our strains. The frequencies of virulence factor genes in CA-MRSA isolates are presented in Table 3.

Two of the ST22 strains were isolated from students participating in the same course. The strains also shared identical antibiogram profiles but displayed different virulence gene patterns.

4. Discussion

The present study describes the detection and molecular characterization of CA-MRSA from healthy Iranian students without an obvious CA-MRSA acquisition risk. Data obtained during this study show that the *S. aureus* nasal carriage rate among healthy students was 22%, which is within the reported range.[16]

MRSA screening revealed seven CA-MRSA isolates (7/700 subjects (1%) or 7/154 *S. aureus* isolates (4.5%)) colonizing normal healthy students who had no risk factors. This is slightly higher than the rate reported in many previous reports. Despite the global increase in community-associated infections, rates of MRSA carriage in the healthy population remain low in most parts of the world. Pooled data from 10 studies (8350 patients) showed a prevalence of 1.3% for CA-MRSA colonization. After excluding those with MRSA acquisition risk factors, the prevalence of CA- MRSA was only 0.2%.[17]

It has been reported that CA-MRSA from different parts of the world have different genetic backgrounds.[18] ST8 SCC*mec* type IV (USA300 clone) is the predominant clone in North America, while community-associated MRSA in Europe is characterized by clonal heterogeneity; several clones seem to have emerged in Europe such as the ST398-V pig-associated clone (which was reported first in The Netherlands[19] and Denmark[20]), a PVL-positive ST152-V clone in the Balkan region,[21] ST150 clone in Sweden, PVL-negative clones causing infections in injecting drug users in Switzerland (ST45) and the UK (ST1),[22] and an ST377-V clone in Greece.[1]

Evaluation of CA-MRSA in Asian countries showed that the most predominant clones in these regions included: ST59-MRSA-SCC*mec* type IV-*spa*-type t437 in Taiwan, Hong Kong, Vietnam, and Sri Lanka; ST30-MRSA-SCC*mec* type IV-*spa*-type t019 in the Philippines; and ST72-SCC*mec* type IV-*spa* type t324 in Korea.[23]

By application of various typing methods, three CA-MRSA strains (IR4, IR5, and IR6) were characterized as *spa*-type t790, ST22, SCC*mec* type IV, being PVL-negative in this study. One of these t790/ST22 strains (IR6) was found to contain the *sec*, *she*, and *tsst* virulence genes, which is similar to a strain isolated from our neighboring country Abu Dhabi.[24] Another t790/ST22 strain (IR5) was found to carry *seg* and *seh* enterotoxin genes; this is the first ST22-ACME *arc*A-positive strain from Iran. It is similar to the ST22-CA-MRSA that has previously been reported from Dublin, Ireland.[25] The ST22/t790 strain (IR4) was found to carry *sec*, *sei*, and *seg* enterotoxin genes. The ST22 clone has previously been reported as a predominant CA-MRSA clone in Germany.[26]

Table 2
Antimicrobial resistance patterns of CA-MRSA isolates

MRSA isolates	AK	GEN	NET	CIP	LZD	FUS	MUP	TGC	LEV	TS	CLI	E	SYN	T	FOX	OXA	VAN	RP
IR1	S	S	S	S	S	S	S	S	S	S	R	R	S	S	R	R	S	S
IR2	S	S	S	R	S	S	S	R	R	S	R	R	S	S	R	R	S	S
IR3	S	S	S	S	R	R	R	S	S	S	R	R	R	R	R	R	S	R
IR4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S
IR5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S
IR6	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	R	S	S
IR7	S	S	S	S	S	R	S	S	S	S	S	S	S	R	R	R	S	S

CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus*; R, resistant; S; susceptible; AK, amikacin; GEN, gentamicin; NET, netilmicin; CIP, ciprofloxacin; LZD, linezolid; FUS, fusidic acid; MUP, mupirocin; TGC, tigecycline; LEV, levofloxacin; TS, co-trimoxazole; CLI, clindamycin; E, erythromycin; SYN, quinupristin–dalfopristin; T, tetracycline; FOX, cefoxitin; OXA, oxacillin; VAN, vancomycin; RP, rifampin.

Table 3
Molecular characterization and virulence factor genes of CA-MRSA isolates

MRSA isolates	*spa* type	SCC*mec*	ST	Allelic profile	PVL	*arc*A	*eta*	*etb*	*sea*	*seb*	*sec*	*sei*	*seg*	*seh*	*tsst*
IR1	T660	IV	ST25	4-1-4-1-5-5-4	–	–	–	–	–	–	–	–	+	–	+
IR2	Unknown	V	ST-14	1-13-1-1-12-11-13	–	–	–	–	–	–	–	+	–	–	–
IR3	t084	IV	ST15	13-13-1-1-12-11-13	+	+	+	–	–	+	–	–	–	–	–
IR4	t790	IV	ST22	7-6-1-5-8-8-6	–	–	–	–	–	–	+	+	+	–	–
IR5	t790	IV	ST22	7-6-1-5-8-8-6	–	+	–	–	–	–	–	–	+	+	–
IR6	t790	IV	ST22	7-6-1-5-8-8-6	–	–	–	–	–	–	+	–	–	+	+
IR7	t325	IV	ST859	79-1-14-23-12-4-31	–	–	–	–	–	–	–	–	–	+	–

CA-MRSA, community-acquired methicillin-resistant *Staphylococcus aureus*; SCC*mec*, staphylococcal cassette chromosome *mec*; ST, sequence type; PVL, Panton–Valentine leukocidin.

In vitro antimicrobial susceptibility tests showed that the IR4 and IR5 strains were susceptible to non-beta-lactam agents, while the IR6 strain was resistant to tetracycline and fusidic acid.

ST22-MRSA-IV (EMRSA-15) is a pandemic CC22-MRSA strain. It emerged in the UK in 1991. Since then, it has become one of the dominant strains in that region and is currently considered one of the global hospital-acquired (HA)-MRSA pandemic clones. In Dresden, Portugal, Malta, and the Azores it accounted for 50%, 54%, 66%, and 80% of MRSA isolates, respectively.[27] Nevertheless, ST22-MRSA-IV occurs in hospitals as well as in outpatient settings.[28] Recently, it was reported to be carried asymptomatically by a few (5/879) healthy individuals without associated health care risk factors in Ireland.[29] Another study reported carriage of EMRSA-15 in people using urban Portuguese public buses.[30] In addition a single ST22-MRSA-IVa, *spa* t223, *pvl* gene-negative strain was detected in 64% of MRSA strains isolated from the noses of healthy children and their parents throughout the Gaza Strip.[31] It should be noted that seven strains of ST22 methicillin-susceptible *S. aureus* (MSSA) were recently found as the causative agents of nosocomial infections that occurred in Alzahra Hospital in Isfahan, Iran; no ST22 MRSA was detected in that study.[32] This finding confirms the fact that transmission of *S. aureus* from the community to hospitals and vice versa could easily happen. This state could gradually cause the loss of a clear-cut border between the two populations (CA and HA).

The fourth CA-MRSA strain (IR7) in our study belongs to t325, ST859, SCC*mec* type IV; this was found to be *she*-positive and only resistant to tetracycline and fusidic acid. ST859 IV has already been reported as PVL-negative HA-MRSA in Iran.[32] The presence of this particular strain in both the community and hospital suggests that this strain is actively circulating in the region.[33]

The fifth strain (IR1) belongs to ST25, SCC*mec* type IV, t660; this strain was found to be *tsst*- and *seg*-positive, and resistant to erythromycin and clindamycin. ST25 has been identified as CA-MRSA, but only very rarely. However, MSSA of ST25 has been encountered as a predominant clone in Algeria, the USA, France, and Paraguay.[34] In a study from China, ST25-V-t081, ST25-V-t078, and ST25-t078 were isolated from the noses of students on a medical campus.[35]

Only one out of the seven CA-MRSA (IR3 strain; t084, ST15, SCC*mec* type IV) was found to be both PVL- and ACME-positive. This strain was found to harbor *eta* and *seb* virulence genes. ST15 has been reported to represent a successful lineage among CA- and HA-MSSA isolates and is the most disseminated clone; it has been identified in 11 different European countries.[36] Nevertheless, Rasigade et al. found five PVL-positive ST15 strains among the 211 *S. aureus* isolates that were collected from 19 different countries in a multicenter study.[34] Currently PVL-positive CA-MRSA have become a serious public health concern because of their virulence, their ability to cause outbreaks in households and close contact social groups, and their rapid spread in many countries. Importantly, in our study, this strain was found to be linezolid- and quinupristin–dalfopristin-resistant, which is the first report from Iran. In addition this strain was also resistant to erythromycin, clindamycin (constitutive), rifampin, tetracycline, fusidic acid, and mupirocin.

The last CA-MRSA strain in this study (IR2 strain) was non-typeable by the *spa* method and belongs to ST14 SCC*mec* type V; it was found to be only *sei*-positive. This strain was determined to be multiple drug resistant (MDR) and appears to be resistant to erythromycin, clindamycin (constitutive), ciprofloxacin, and levofloxacin. In addition this strain is the first tigecycline-resistant strain reported from Iran. It has been shown that ST14-t279-V belonging to CC15 is one of the lineages that cannot provide a stable genomic environment for the integration of SCC*mec*. However, this strain has been isolated from infection sites in patients in long-term care at a Spanish hospital.[37]

Although highly virulent CA-MRSA strains carrying PVL genes are known to prevail in the world,[38] in this study only one strain (IR3) was found to carry the *pvl* gene and the prevalence of PVL-positive strains was low. CA-MRSA pathogenesis has been linked to PVL, which is produced by the majority of CA-MRSA isolates; however, the absence of PVL is no proof against community origin. This was documented in Australia, Japan, and China, where the majority of CA-MRSA isolates lack PVL.[18,35,38]

A previous study showed that the toxin gene *seh* was associated with persistent *S. aureus* bacteremia.[39] This would suggest that three of the seven strains (IR5, IR6, and IR7) isolated in the present study carrying the *seh* gene have a high potential to cause bacteremia.

In this study, six of seven (85.8%) CA-MRSA strains were found to carry SCC*mec* type IV and the remaining strain (14.3%) to carry SCC*mec* type V. Data published from the different HA-MRSA clones indicate that ST239-III is the predominant HA-MRSA clone circulating in Iranian hospitals.[32,40,41] According to the results of the present study, SCC*mec* typing can be used as an epidemiological marker for differentiation between CA and HA MRSA.

Mupirocin is broadly used to treat MRSA skin and soft tissue infections and to eliminate nasal MRSA colonization among patients and medical staff. Our study shows that 14.3% of CA-MRSA strains were resistant to mupirocin, which is in agreement with mupirocin resistance rates observed in another study.[35] Therefore, mupirocin can be used as a decolonization agent for reducing nasal MRSA carriage in the Iranian community.

ST8 (USA300) and ST80 (European clone) have not been found in Central Iran. The ST8 and ST80 MRSA clones are obviously rare in Asian countries.[23] To confirm that these clones are not found in Iran, further studies should be performed in different parts of the country.

Further studies of the molecular epidemiology of CA-MRSA should be done in different population groups (such as sports teams and children) and at other body sites as well.

The present study was limited by the inclusion of healthy student volunteers. In addition, the evaluation of virulence determinants was based on their qualitative assessment by PCR.

We did not assess quantitative expression of these genes or the presence of single nucleotide polymorphisms, which may influence the function of gene products.[42]

Although the overall prevalence of CA-MRSA is presently low worldwide, there is clear evidence that this is increasing in most part of the world, such as the USA, Canada, Australia, and Europe.[17,43] HA-MRSA and CA-MRSA strains are often genotypically and phenotypically different, however it is difficult to distinguish between CA-MRSA and HA-MRSA on clinical and epidemiological assessment. Further investigations are needed to determine stringent criteria rather than SCC*mec* type, PVL gene, and antibiotic susceptibility patterns to establish a precise definition of CA-MRSA.

In conclusion, Iranian CA-MRSA clones are diverse, although clonal dissemination of the ST22 type strain indicates that the spread of ecologically successful European clones to the Asian continent continues. The emergence of linezolid, tigecycline, and quinupristin–dalfopristin resistance should be taken seriously.

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Conflict of interest: Nothing to declare.

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