

Clonal Diversity and Genetic Landscape of *mecC*-Positive *S. aureus* in Human Samples from Iran: First Report of CC/ST423/t742

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ABSTRACT

Background: The emergence of *mecC*-carrying *Staphylococcus aureus* strains is a serious concern in public health. Our study investigated the clonal diversity, genetic characteristics, antimicrobial resistance, virulence determinants, biofilm-forming ability, and molecular epidemiology of *mecC*-positive *Staphylococcus aureus* isolates obtained from hospitalized patients in Iran.

Methods: We analyzed 45 *mecC*-carrying *S. aureus* strains isolated from 1,590 *S. aureus* samples collected from different clinical sources. The characterization included antimicrobial susceptibility tests, assessment of biofilm formation, *spa* typing, multilocus sequence typing (MLST), and PCR analysis to detect resistance, biofilm-related, and virulence genes.

Results: Our results showed that 17.8%, 17.8%, and 64.4% of the isolates were classified as weak, intermediate, and strong biofilm producers, respectively. The prevalence of the *ant(4')-Ia* and *tet(M)* was found in 80% and 73.3% of the isolates, respectively. MLST revealed that the 45 isolates belonged to four clonal complexes: CC8 (44.4%), CC130 (24.5%), CC121 (17.8%), and CC425 (13.3%). The majority of *S. aureus* isolates belonged to the CC8/ST239 lineage (44.4%). Also, *tst*-positive strains belonged to CC121/ST599/t5930 (17.8%) and CC8/ST239/t037 (11.1%), while *eta*-positive isolates belonged to CC/ST130/t843 (15.6%). Fusidic acid-resistant *S. aureus* isolates primarily belonged to CC8/ST239/t037 lineage (11.1%), followed by CC8/ST239/t7688 (6.7%), both carrying the *fusB* gene. Vancomycin-intermediate *S. aureus* strains were classified into CC/ST599/t5930 (6.7%) and CC/ST130/t843 (4.4%) clones.

Conclusion: This study highlights the need for close and continuous monitoring of the genetic diversity and characteristics of the *mecC*-carrying *S. aureus* strains. Besides, urgent measures are required to prevent the transmission of these strains and to effectively treat associated infections.

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1. INTRODUCTION

Staphylococcus aureus is a significant pathogen that contributes to both hospital-acquired and community-associated infections worldwide. It is implicated in a wide spectrum of clinical presentations, ranging from superficial skin infections to severe, life-threatening conditions^[1]. A critical challenge in managing *S. aureus* infections is the emergence and widespread dissemination of multidrug-resistant (MDR) strains, which consistently compromise therapeutic efficacy. Among these strains, methicillin-resistant *S. aureus* (MRSA) is responsible for the majority of *S. aureus*-related infections^[1,2].

The resistance in MRSA is primarily mediated by the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with a reduced affinity for β -lactam antibiotics, rendering these treatments ineffective^[2]. Recently, a novel genetic determinant, initially identified as *mecALGA251* and now referred to as *mecC*, has been discovered^[3,4]. This gene encodes a transpeptidase and is transferable between livestock-associated MRSA or other Staphylococci species and human MRSA strains. Notably, *mecC* shares only 69% nucleotide and 63% protein sequence identity with the conventional *mecA*/PBP2a^[3]. This divergence renders *mecC* undetectable by standard *mecA*-specific PCR assays or PBP2a slide agglutination tests, often leading to its misidentification as methicillin-sensitive *S. aureus*^[3,5-7]. Following its initial identification in Europe, *mecC*-positive MRSA has subsequently been documented in multiple European countries, including the United Kingdom, Denmark, Germany, France, and the Netherlands, as well as in regions beyond Europe, such as Australia and parts of Asia^[4-6]. These strains have been isolated from both human clinical specimens and various animal sources, particularly livestock and wildlife, underscoring their zoonotic nature and the significance of interspecies transmission in their spread^[4,6,8].

While the prevalence of *mecC*-positive MRSA is generally lower than that of *mecA*-mediated MRSA, growing attention has been directed toward their clinical importance due to diagnostic limitations and the risk of unrecognized circulation in healthcare environments. Although the emergence, epidemiology, and genetic variability of the *mecA* gene have been extensively studied, the prevalence and clinical implications of *mecC*-carrying MRSA isolates remain insufficiently understood^[3,5]. In Iran, information on *mecC*-positive *S. aureus* remains scarce, as the majority of surveillance and molecular studies have primarily addressed methicillin resistance associated with the *mecA* gene. As a result, limited data are available regarding the occurrence, genetic features, and virulence-related traits of *mecC*-carrying *S. aureus* isolates in clinical settings across the country. This lack of evidence

underscores the necessity for focused investigation on enhancing epidemiological insight and improving diagnostic recognition of these emerging MRSA variants in the region.

2. MATERIAL AND METHODS

2.1. Sample collection

This study was conducted over eleven years, from 2013 to 2024, in Tehran, Iran. *S. aureus* strains were isolated from various clinical samples (skin wounds, burn wounds, surgical wounds, blood, pus, and urine) referred to the clinical laboratories of hospitals affiliated with Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran. The identification process involved catalase testing and tube coagulase testing. For additional confirmation, the isolates were subcultured on DNase agar and mannitol-salt agar. To genetically confirm the isolates, we amplified the *nucA* gene using PCR. The forward primer sequence was GCGATTG ATGGTGATACGGTT, and the reverse primer was AGCCAAGCCTTGACGAACTAAAGC, as described previously^[9]. The PCR products were examined through electrophoresis on 1.5% agarose gels. The confirmed *S. aureus* isolates, supplemented with 20% glycerol, were stored at -70°C for further analysis, as described formerly^[9].

2.2. Screening for MRSA strains and *mecC* gene detection

MRSA strains were screened using the disk diffusion method with cefoxitin (30 μ g) disks on Mueller-Hinton agar (Merck, Germany), following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2022). The presence of the *mecC* gene in all isolates was further investigated through PCR analysis, using forward (5'-GTCCCTAACAAAAC ACCCAAAGA-3') and reverse (5'-GAAGATCTTTTC CGTTTTCAGC-3') primers, as outlined before^[9].

2.3. Determination of isolate susceptibility

All *mecC*-carrying isolates were evaluated for the determination of minimum inhibitory concentration (MIC) values for various antibiotics using the microbroth dilution method, in accordance with the CLSI guidelines. The antibiotics tested included fusidic acid, ciprofloxacin, erythromycin, clindamycin, nitrofurantoin, gentamicin, penicillin, chloramphenicol, tetracycline, rifampin, vancomycin, and mupirocin (including both low- and high-level resistance)^[9]. Additionally, the D-zone test was conducted in accordance with the CLSI standards to further characterize resistance patterns. For quality control, the reference strains of *S. aureus* (ATCC 29213, ATCC 25923, and ATCC 43300) were included in each experiment to ensure the reliability and accuracy of the testing procedures.

2.4. Detection of biofilm formation

The biofilm formation was evaluated using a microtiter plate assay as explained earlier^[9]. Biofilm formation by *mecC*-carrying strains was determined by measuring the optical density (OD) at 490 nm. The cut-off OD was calculated by adding three times the standard deviation (SD) to the mean OD of the blank control wells. Isolates were then categorized according to their biofilm-forming capacity as follows: non- (OD $\leq$ 0.059), weak (OD_c < OD $\leq$ 2 $\times$ OD_c), moderate (2 $\times$ OD_c < OD $\leq$ 4 $\times$ OD_c), and strong (OD > 4 $\times$ OD_c) biofilm producers. The *S. epidermidis* ATCC 35984 strain was used as the positive control in the biofilm formation assays.

2.5. DNA extraction, virulence, resistance, and biofilm genes

The phenol-chloroform assay was used for the extraction of chromosomal DNA as previously described^[9]. The detection of toxin-encoding genes (*pvl*, *etb*, *tst*, and *eta*) and resistance determinants (*mupA*, *msr(A)*, *vanA*, *erm(A)*, *fusA*, *fusB*, *msr(B)*, *vanB*, *mupB*, *erm(B)*, *erm(C)*, *fusC*, *tetL*, *tetK*, *tetO*, *tetM*, *aph* (3')-IIIa, *aac* (6')-Ie/*aph* (2''), and *ant* (4')-Ia) was performed using PCR. All *mecC*-carrying isolates were evaluated for the presence of *clfA*, *clfB*, *fnbA*, *fnbB*, *ebp*, *can*, and *bap* genes by conventional PCR^[9-11]. Specific primers were designed for each target gene, and their sequences were verified using the BLAST tool available on the NCBI GenBank database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

2.6. Genotypic characterization *spa* typing

The *spa* typing was performed to classify different types of *S. aureus* protein A, following the protocol established previously^[12]. Specific primers and optimized PCR conditions were used for the assay, and each PCR reaction included a negative control that replaced genomic DNA with water. Following amplification, the PCR products were purified using the QIAquick PCR Purification Kit (Toronto, ON, Canada). The purified products were then sequenced using an ABI Prism 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were processed and edited with Chromas software (version 1.45, Australia). Finally, the sequences were submitted to the Ridom SpaServer database (<http://www.spaserver.ridom.de>) for comprehensive analysis and *spa* profile assignment.

2.7. Multilocus sequence typing (MLST)

MLST was performed on all *mecC*-carrying strains following the method described before^[13]. To determine sequence types (STs), the sequences of seven housekeeping genes (*aroE*, *gmK*, *pta*, *tpiA*, *yqiL*, *arcC*,

and *glpF*) were compared with the allelic profiles available in the PubMLST database (<https://pubmlst.org/>). ST clustering was analyzed using the eBURST program (version 3), which identifies related STs and groups them into clonal complexes. The clonal complex for *S. aureus* was further verified by cross-referencing the ST with the PubMLST database.

2.8. Phylogenetic analysis

The bioinformatics data were thoroughly analyzed. The multilocus sequences of *ArcC*, *AroE*, *GlpF*, *Gmk*, *Pta*, *Tpi*, and *YqiL* were aligned using ClustalW on the CIPRES Science Gateway platform. The final alignment consisted of 3,186 bp for the combined analysis of seven genes. Phylogenetic tree construction was performed using the maximum likelihood (ML) method implemented in the IQTREE web server (<https://iqtree.github.io/>). The selection of the evolutionary model and ML phylogenetic analysis, with 10,000 ultrafast bootstrap replicates, was conducted on the IQTREE web server. The best-fitting evolutionary model was determined to be TPM2u+F+I, and the analysis was run with 10,000 bootstrap replicates (parameters set to '-alrt 10,000 -bb 10,000 -nt AUTO' with all other options set to default).

3. RESULTS

3.1. Sample collection

A total of 45 *mecC*-carrying *S. aureus* strains were obtained from 1,590 *S. aureus* isolates collected from different clinical samples. All *mecC*-carrying *S. aureus* isolates were confirmed to be MRSA. Among these isolates, 25 (55.6%) were obtained from female patients, and the remaining 20 (44.4%) were from male patients. A total of six different clinical sample types were collected in this study. Skin wound specimens were the most common, accounting for 33.3% of all samples, followed by burn wound samples (20.0%), surgical wound samples (15.6%), blood samples (13.3%), pus samples (11.1%), and urine samples (6.7%). Our findings showed that a low percentage of *mecC*-carrying strains (13.3%) were isolated from invasive infections, whereas the remaining isolates (86.7%) were related to non-invasive infections. The mean age of the patients was 40 years. Patients were divided into four age groups: 5 (11.1%) aged $\leq$ 20 years, 23 (51.1%) aged 21–45 years, 12 (26.7%) aged 46–65 years, and 5 (11.1%) aged $\geq$ 65 years.

3.2. Antibiotic susceptibility testing

According to our MIC results, the highest and lowest resistance rates among the 45 *mecC*-carrying *S. aureus* isolates were observed for penicillin (100%) and chloramphenicol (11.1%), respectively (Table 1). Notably, none of the tested isolates were resistant to

Table 1. Antimicrobial susceptibility patterns of *mecC*-carrying *S. aureus* isolated from different clinical samples

Antibiotic	MIC (µg/ml)			N (%)
	Range	50%	90%	
Chloramphenicol	1-64	4	8	5 (11.1)
Ciprofloxacin	0.25-32	1	4	12 (26.7)
Clindamycin	0.25-64	4	4	24 (53.3)
Erythromycin	0.5-64	16	16	31 (68.9)
Fusidic acid	0.125-4	1	2	8 (17.8)
Gentamicin	1-128	32	32	40 (88.9)
Mupirocin	4-512	128	256	10 (22.2)
Nitrofurantoin	8-512	32	128	6 (13.3)
Penicillin	0.125-16	1	4	45 (100)
Rifampin	0.5-32	2	4	10 (22.2)
Tetracycline	0.5-128	16	32	42 (93.3)
Vancomycin	0.125-64	2	4	5 (11.1)

vancomycin; however, five isolates had an MIC value of 4 µg/mL, which confirmed them as vancomycin-intermediate *S. aureus* (VISA) isolates. Our results also showed that 10 isolates (22.2%) were resistant to mupirocin, and all exhibited high-level resistance, which was classified as high-level mupirocin-resistant *S. aureus* (HLMUPR). Overall, 88.9% of the *mecC*-carrying *S. aureus* isolates were classified as MDR, with MDR defined as resistance to three or more antimicrobial agents. According to our results (Table 1), 24 isolates (53.3%) exhibited constitutive macrolide-lincosamide-streptogramin B (cMLSB) phenotypes, and 7 (15.6%) isolates indicated inducible macrolide-lincosamide-streptogramin B (iMLSB) phenotypes.

The results of the micro broth dilution method indicated 12 distinct resistance profiles. The top three resistance profiles included resistance to clindamycin, erythromycin, gentamicin, penicillin, and tetracycline, which were observed in 24.4% (11/45) of the isolates. In addition, resistance to clindamycin, erythromycin, gentamicin, penicillin, tetracycline, and rifampicin was detected in 11.1% (5/45) of the isolates. Resistance patterns to ciprofloxacin, erythromycin, gentamicin, mupirocin, penicillin, and tetracycline, as well as to gentamicin, penicillin, and tetracycline, were observed in 8.9% (4/45) of the isolates. Details of the simultaneous resistance patterns and the distribution of clinical samples are summarized in Table 2.

Table 2. Antibiotic resistance profiles of *mecC*-carrying *S. aureus* strains and their distribution in different samples

No. of antibiotics	Resistance pattern	No. of isolates (%)	Type of samples (N; %)
Eight	CLI, ERY, FUS, GEN, MUP, NIT, PEN, TET	3 (6.7)	BW (3; 100)
Seven	CHO, ERY, GEN, MUP, PEN, RIF, TET	3 (6.7)	B (1; 33.3), SuW (1; 33.4), SW (1; 33.3)
	CLI, ERY, FUS, GEN, PEN, RIF, TET	2 (4.4)	SW (2; 100)
	CIP, CLI, ERY, FUS, GEN, PEN, TET	3 (6.7)	SuW (1; 33.3), SW (1; 33.4), BW (1; 33.3)
Six	CLI, ERY, GEN, PEN, TET, RIF	5 (11.1)	BW (2; 40), SuW (1; 20), B (1; 20), P (1; 20)
	CIP, ERY, GEN, MUP, PEN, TET	4 (8.9)	SW (2; 50), U (1; 25), B (1; 25)
	CIP, GEN, NIT, PEN, TET, VAN	3 (6.7)	SuW (1; 33.3), SW (1; 33.4), U (1; 33.3)
	CHO, CIP, GEN, PEN, TET, VAN	2 (4.4)	B (1; 50), U (1; 50)
Five	CLI, ERY, GEN, PEN, TET	11 (24.4)	SW (4; 36.4), BW (3; 27.2), SuW (2; 18.2), B (2; 18.2)
Three	GEN, PEN, TET	4 (8.9)	SW (2; 50), P (2; 50)
Two	PEN, TET	2 (4.4)	SuW (1; 50), SW (1; 50)
One	PEN	3 (6.7)	SW (1; 33.3), P (2; 66.7)

CLI: clindamycin; ERY: erythromycin; FUS: fusidic acid; GEN: gentamicin; MUP: mupirocin; NIT: nitrofurantoin; PEN: penicillin; TET: tetracycline; CHO: chloramphenicol; RIF: rifampin; CIP: ciprofloxacin; VAN: vancomycin; B: blood; BW: burn wounds; SuW: surgical wounds; SW: skin wounds; U: urine; P: pus

3.3. Resistance-related genes

Antibiotic resistance gene screening indicated that of 45 tested isolates, 36 (80%) had *ant(4')-Ia* gene, while 33 (73.3%) *tet(M)*, 14 (31.1%) *erm(A)*, 12 (26.7%) *aac(6')-Ie/aph(2'')*, 10 (22.2%) *mupA*, 8 (17.8%) *fusB*, 8 (17.8%) *erm(B)*, 6 (13.3%) *tet(L)*, 5 (11.1%) *tet(K)*, 5 (11.1%) *msrB*, 4 (8.9%) *msrA*, 4 (8.9%) *tet(O)*, 3 isolates (6.7%) *tet(S)*, 3 isolates (6.7%) *erm(C)*, and 1 *aph(3')-IIIa* (2.2%), as shown in Figure 1. All isolates were found to carry the *mecA* gene. Regarding antibiotic resistance genes, 14 profiles were detected. The three most frequently detected profiles were: *ant(4')-Ia*, *tet(M)*, *erm(A)* (11.1%; 5/45), *ant(4')-Ia*, *tet(M)*, *tet(K)*, *aac(6')-Ie/aph(2'')* (11.1%; 5/45), and *ant(4')-Ia*, *tet(M)* (11.1%; 5/45). All HLMUPR strains were found to carry the *mupA* gene, and none of the VISA isolates harbored the *vanA* gene.

3.4. Biofilm-related genes

The biofilm assay showed that all *mecC*-carrying *S. aureus* strains were biofilm producers. Among the 45 total isolates, 29 (64.4%) were classified as strong biofilm producers, while 8 (17.8%) were categorized as moderate producers, and 8 (17.8%) as weak biofilm producers. All *mecC*-carrying *S. aureus* strains with iMLSB phenotypes were strong biofilm producers (15.6%; 7/45). Of the 24 cMLSB isolates, 17 (70.8%) indicated strong biofilm production, 4 (16.7%) moderate ability, and 3 (12.5%) weak ability. All fusidic acid-resistant *S. aureus* isolates carrying the *mecC* gene, including those resistant to HLMUPR, exhibited strong ability in biofilm formation. The distribution of

resistance patterns among different biofilm-producing isolates is presented in Table 3.

As depicted in Table 4, the prevalence of biofilm carriage genes was recorded as follows: *clfA* in all isolates (100%), *clfB* in 40 isolates (88.9%), *fnbA* in 37 isolates (82.2%), *fnbB* in 24 isolates (53.3%), *cna* in 23 isolates (51.1%), *bap* in 10 isolates (22.2%), and *ebp* in 5 isolates (11.1%). Six patterns of biofilm-related genes were detected. Among these genes, *clfA*, *clfB*, *fnbA*, *fnbB*, and *cna* had a higher frequency (26.7%; 12/45). Figure 2 indicates the presence of biofilm genes in *mecC*-carrying *S. aureus* strains. Toxigenic *mecC*-carrying *S. aureus* strains were detected in 20 isolates (44.4%), with the majority of *tst* gene (28.9%, 13/45), followed by the *eta* (15.6%, 7/45).

3.4. Molecular typing

All isolates were analyzed using *spa* and MLST typing methods. In the present survey, five *spa* types and four STs were identified. The invasive *mecC*-carrying *S. aureus* strains were associated with *spa* types t742 (6.7%; 3/45) and t7688 (6.7%; 3/45). The TST-carrying isolates belonged to *spa* types t5930 and t037, accounting for 17.8% (8/45), and 11.1% (5/45), respectively. All the isolates carrying *eta* toxin belonged to *spa* type t843, representing 15.6% (7/45) of the total isolates. MLST analysis revealed four STs among *mecC*-carrying *S. aureus* strains: ST239 (44.4%, 20/45), ST130 (24.5%, 11/45), ST599 (17.8%, 8/45), and ST425 (13.3%, 6/45). These STs were categorized into clonal complexes: CC8 (44.4%), CC130 (24.5%), CC121 (17.8%), and CC425 (13.3%).

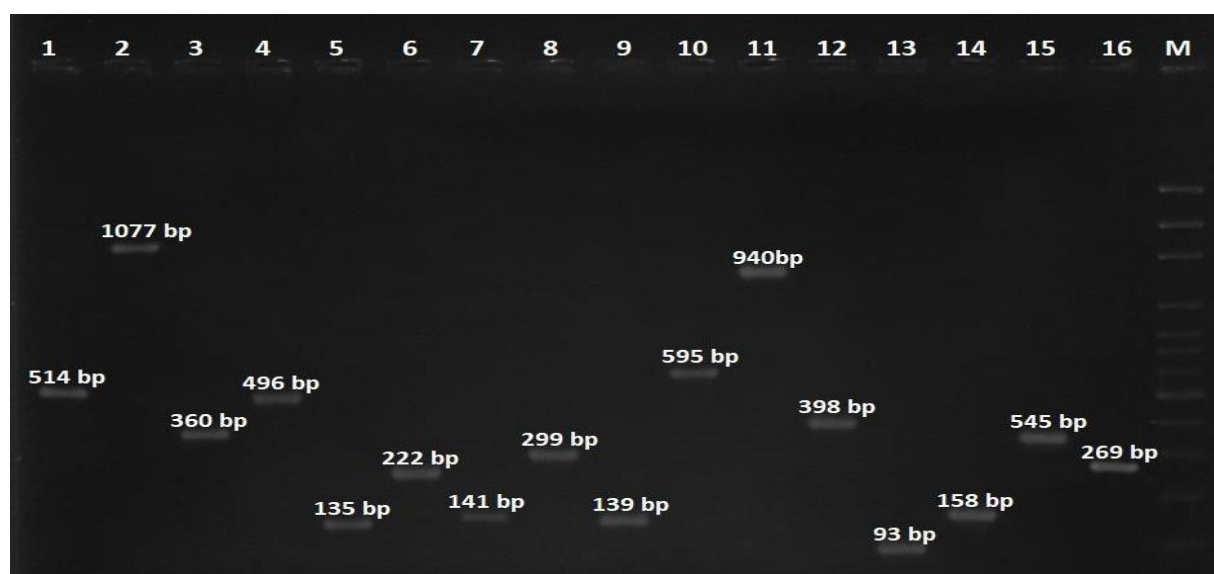


Fig. 1. PCR products of antibiotic resistance and toxin genes. Lane 1: *tetO* gene; lane 2: *tetL* gene; lane 3: *tetK* gene; lane 4: *fusB* gene; lane 5: *ant(4')-Ia* gene; lane 6: *aac(6')-Ie/aph(2'')* gene; lane 7: *erm(B)* gene; lane 8: *erm(C)* gene; lane 9: *erm(A)* gene; lane 10: *msrB* gene; lane 11: *msrA* gene; lane 12: *tst* gene; lane 13: *eta* gene; lane 14: *tetM* gene; lane 15: *mecC* gene; lane 16: *aph(3')-IIIa* gene; lane M: 100 bp DNA ladder (Thermo Fisher Scientific, UK).

Table 3. Distribution of resistance patterns among biofilm-producing *mecC*-carrying *S. aureus* strains

Biofilm status	Phenotypic resistance (N; %)	N (%)
Strong producer	CLI, ERY, FUS, GEN, MUP, NIT, PEN, TET (3; 10.3)	29 (64.4)
	CIP, CLI, ERY, FUS, GEN, PEN, TET (3; 10.3)	
	CHO, ERY, GEN, MUP, PEN, RIF, TET (3; 10.3)	
	CLI, ERY, FUS, GEN, PEN, RIF, TET (2; 6.9)	
	CIP, ERY, GEN, MUP, PEN, TET (4; 13.9)	
	CHO, CIP, GEN, PEN, TET, VAN (2; 6.9)	
	CIP, GEN, NIT, PEN, TET, VAN (3; 10.3)	
	CLI, ERY, GEN, PEN, TET, RIF (3; 10.3)	
	CLI, ERY, GEN, PEN, TET (6; 20.8)	
Moderate producer	CLI, ERY, GEN, PEN, TET, RIF (2; 25)	8 (17.8)
	CLI, ERY, GEN, PEN, TET (2; 25)	
	GEN, PEN, TET (3; 37.5)	
	PEN (1; 12.5)	
Weak producer	PEN (2; 25)	8 (17.8)
	PEN, TET (2; 25)	
	GEN, PEN, TET (1; 12.5)	
	CLI, ERY, GEN, PEN, TET (3; 37.5)	

CLI: clindamycin; ERY: erythromycin; FUS: fusidic acid; GEN: gentamicin; MUP: mupirocin; NIT: nitrofurantoin; PEN: penicillin; TET: tetracycline; CIP: ciprofloxacin; RIF: rifampin; CHO: chloramphenicol; VAN: vancomycin;

3.5. Analysis of *mecC*-positive clones

3.6.1. CC8

In the present study, the CC8, corresponding to CC8/ST239/t7688 (33.3%; 15/45) and CC8/ST239/t037 (11.1%; 5/45), was the most predominant clone among *mecC*-carrying *S. aureus* strains, as obtained by spa typing and MLST. The CC8/ST239-SCC*mec* XI strain

was recovered from skin wounds (35%), burn wounds (20%), and surgical wounds, blood, and urine (each 15%). All the CC8 isolates were resistant to mupirocin and indicated the HLMUPR phenotype carrying the *mupA* gene. Additionally, all the CC8/ST239/t037 isolates were found to harbor the *tst* gene. Of eight fusidic acid-resistant *S. aureus* isolates, three (37.5%;

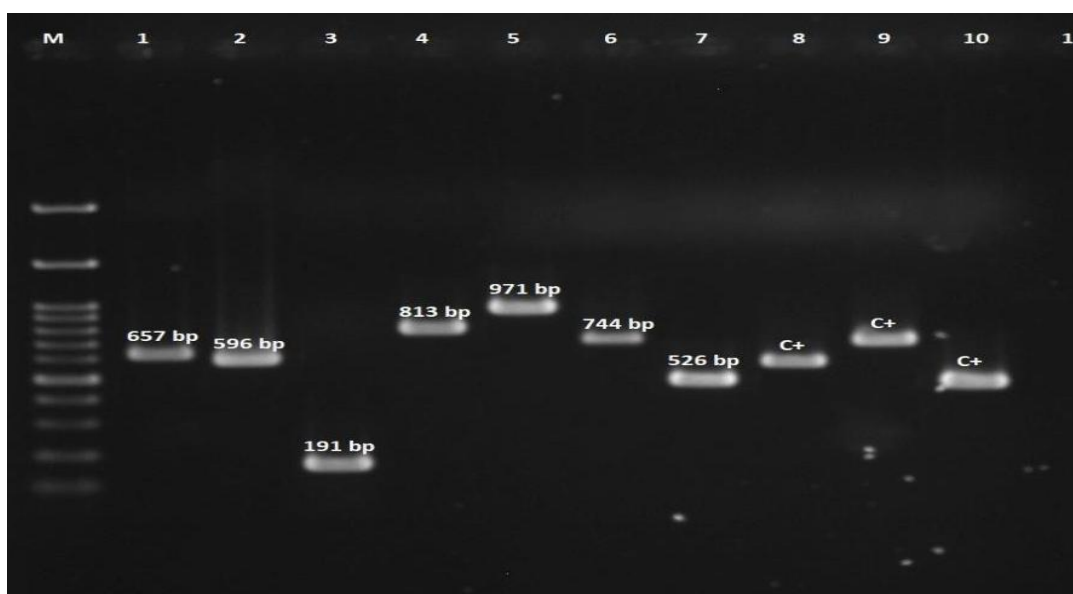


Fig. 2. Representative amplification of biofilm encoding genes from *mecC*-carrying *S. aureus* isolates. Lane M: 100-bp DNA ladder (Thermo Fisher Scientific, UK); lane 1: *clfA*-encoding gene; lane 2: *clfB*-encoding gene; lane 3: *fnbA*-encoding gene; lane 4: *fnbB*-encoding gene; lane 5: *bap*-encoding gene; lane 6: *can*-encoding gene; lane 7: *ebp*-encoding gene; lane 8: positive control of *clfB*-encoding gene; lane 9: positive control of *can*-encoding gene; lane 10: positive control of *ebp*-encoding gene; lane 11: negative control.

3/8) elongated to the CC8/ST239/t7688 and five (62.5%; 5/8) belonged to CC8/ST239/t037, both of which carried the *fusB* gene. All CC8/ST239/t037 isolates were identified as strong biofilm producers. In contrast, among the CC8/ST239/t7688 isolates, 66.7% were strong biofilm producers and 33.7% were weak biofilm producers. The present research indicated that the iMLSB phenotype was more prevalent in CC8/ST239/t7688 strains compared to the cMLSB phenotype (46.6% vs. 20%). Conversely, CC8/ST239/t037 strains displayed a 100% prevalence of the cMLSB phenotype, with none exhibiting the iMLSB phenotype. Regarding resistance genes, *ant(4')-Ia*, *tet(M)*, and *fusB* were detected in all the CC8/ST239/t037 isolates. More than three-fourths of the CC8/ST239/t7688 isolates carried *ant(4')-Ia* and *tet(M)* genes. The distribution of biofilm-related genes among the CC8/ST239 isolates was diverse. Figure 3 details the distribution of resistance profiles and biofilm genes among the ST239 clonal lineage.

3.6.2. CC130

The present survey showed that all the isolates belonging to the CC130 clone were classified as CC/ST130/t843. The CC/ST130-SCC*mec* XI/t843 strain

was isolated from burn wounds (27.3%), pus (27.3%), skin wounds (27.3%), and surgical wounds (18.1%). Also, two VISA isolates (40%; 2/5) were identified within this clone. The cMLSB phenotype was detected in 81.8% of isolates (9/11). Notably, none of the CC130 isolates exhibited the iMLSB phenotype. Resistance gene analysis showed the presence of *ant(4')-Ia* (81.8%), *tet(M)* (90.9%), *tet(K)* (27.3%), *tet(S)* (9.1%), *aac(6')-Ie/aph(2'')* (45.4%) among the tested isolates (Table 4). Virulence gene analysis indicated that the *eta* gene was found only in CC/ST130/t843 (63.6%; 7/11) isolates. According to our results, all the isolates exhibited strong biofilm production. The distribution of resistance profiles and biofilm-related genes is presented in Figure 4 (A and B). Surprisingly, we detected a relatively high prevalence of *bap* (36.4%) among these isolates.

3.6.3. CC121

The third most common *mecC*-carrying *S. aureus* clone was ST599/t5930, which exhibited VISA in 37.5% of isolates (3/8). The CC/ST599-SCC*mec* XI/t5930 strain was recovered from skin wounds (50%), burn wounds (25%), and pus and surgical wounds (each 12.5%). Notably, none of these isolates exhibited the HLMUPR

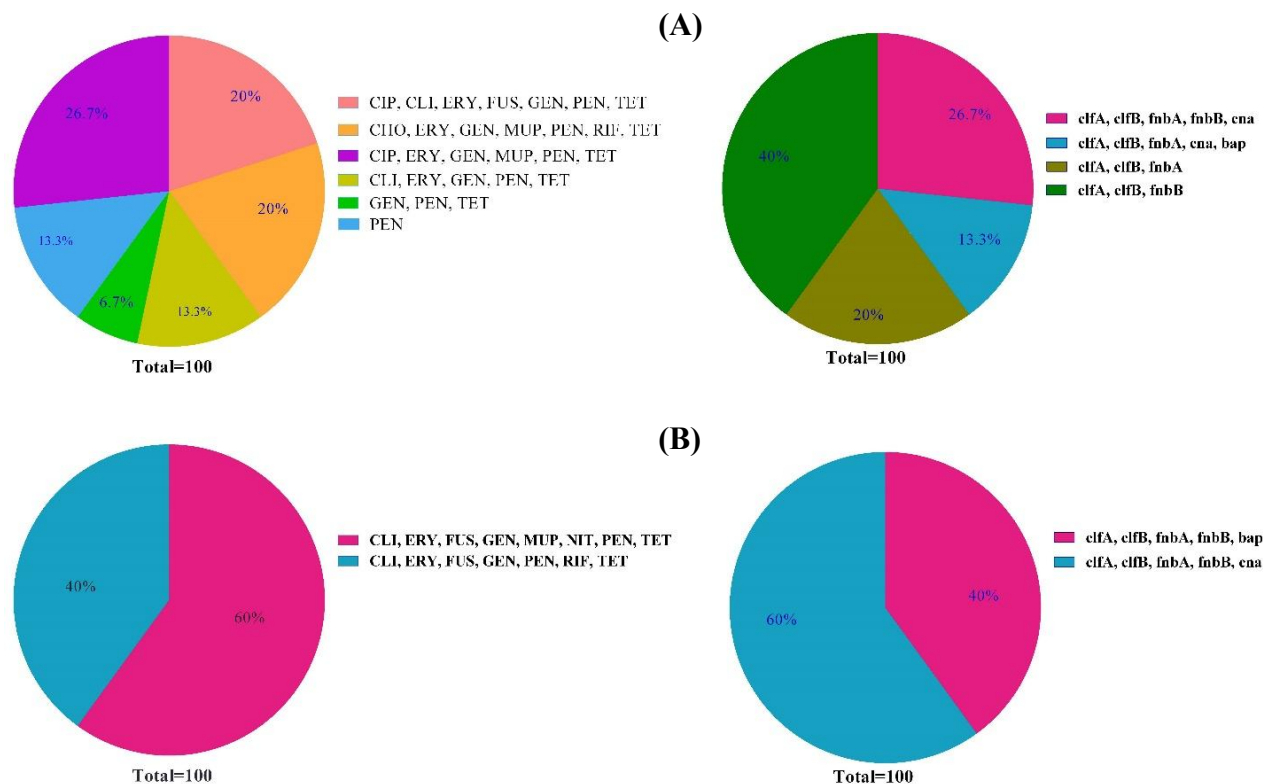


Fig. 3. Distribution of resistance profiles and biofilm encoding genes among CC8 lineages. (A) Left: distribution of phenotypic resistance patterns in CC8/ST239/t7688 isolates; right: distribution of biofilm genes in CC8/ST239/t7688 isolates; (B) left: distribution of phenotypic resistance patterns in CC8/ST239/t037 isolates; right: distribution of biofilm genes in CC8/ST239/t037 isolates.

Table 4. Characteristics of the 45 *mecC*-positive *S. aureus* strains obtained from clinical isolates

Clonal lineage	Toxin genes	Biofilm formation degree (N; %)	Genotype (N; %)	Adhesion genes (N; %)	Total N (%)
CC/ST130/t843	<i>eta</i> (7; 63.6)	Strong (11; 100)	<i>ant(4')-Ia</i> (9; 81.8), <i>tet(M)</i> (10; 90.9), <i>tet(K)</i> (3; 27.3), <i>tet(S)</i> (1; 9.1), <i>aac</i> (6')- <i>Ie/aph</i> (2'') (5 45.4)	<i>clfA</i> (11; 100), <i>clfB</i> (11; 100), <i>fnbA</i> (9; 81.8), <i>fnbB</i> (7; 63.6), <i>cna</i> (9; 81.8), <i>bap</i> (4; 36.4)	11 (24.5)
CC8/ST239/t7688	-	Strong (10; 66.7), Weak (5; 33.3)	<i>ant(4')-Ia</i> (13; 86.7), <i>tet(M)</i> (13; 86.7), <i>fusB</i> (3; 20), <i>erm(A)</i> (6; 40), <i>mupA</i> (7; 46.7), <i>erm(C)</i> (3; 20), <i>erm(B)</i> (7; 46.7), <i>tet(O)</i> (2; 13.3), <i>aac</i> (6')- <i>Ie/aph</i> (2'') (2; 13.3), <i>msr(B)</i> (2; 13.3), <i>msrA</i> (3;20)	<i>clfA</i> (15; 100), <i>clfB</i> (15; 100), <i>fnbA</i> (9; 60), <i>fnbB</i> (10; 66.7), <i>cna</i> (6; 40), <i>bap</i> (2; 13.3)	15 (33.3)
CC8/ST239/t037	<i>tst</i> (5; 100)	Strong (5; 100)	<i>ant(4')-Ia</i> (5; 100), <i>tet(M)</i> (5;100), <i>mupA</i> (3; 60), <i>fusB</i> (5; 100), <i>erm(A)</i> (3; 60), <i>msr(B)</i> (2;40)	<i>clfA</i> (5; 100), <i>clfB</i> (5;100), <i>fnbA</i> (5; 100), <i>fnbB</i> (5; 100), <i>bap</i> (2; 40), <i>cna</i> (3; 60)	5 (11.1)
CC/ST425/t742		Moderate (5; 83.3), Weak (1; 16.7)	<i>ant(4')-Ia</i> (3; 50), <i>tet(M)</i> (6;100), <i>tet(L)</i> (1; 16.7), <i>tet(O)</i> (1;16.7), <i>aac</i> (6')- <i>Ie/aph</i> (2'') (2;33.3), <i>erm(B)</i> (1; 16.7), <i>msr(A)</i> (1; 16.7), <i>aph</i> (3')- <i>IIIa</i> (1; 16.7), <i>erm(A)</i> (3;50)	<i>clfA</i> (6; 100), <i>fnbA</i> (6; 100), <i>clfB</i> (4; 66.7), <i>ebp</i> (2; 33.3), <i>cna</i> (2; 33.3)	6 (13.3)
CC121/ST599/t5930	<i>tst</i> (8; 100)	Strong (3; 37.5), Moderate (3; 37.5), Weak (2; 25)	<i>ant(4')-Ia</i> (6; 75), <i>tet(M)</i> (4; 50), <i>tet(K)</i> (2; 25), <i>tet(O)</i> (1; 12.5), <i>tet(S)</i> (2; 25), <i>aac</i> (6')- <i>Ie/aph</i> (2'') (3; 37.5), <i>msr(B)</i> (1; 12.5), <i>erm(A)</i> (2; 25), Without resistance gene (1; 12.5)	<i>clfA</i> (8; 100), <i>clfB</i> (5; 62.5), <i>fnbA</i> (8; 100), <i>fnbB</i> (2; 25), <i>bap</i> (2; 25), <i>ebp</i> (3; 37.5), <i>cna</i> (3; 37.5)	8 (17.8)

N: number; strong and weak refer to clonal lineage.

phenotype (Fig. 4C and 4D). All of these isolates carried the *tst* gene. Additionally, 37.5% of the CC121 isolates were high-level biofilm producers. More than three-fourths of the isolates harbored the *ant(4')-Ia*. Biofilm-related genes detected in the isolates of this clone included *clfA* (100%), *fnbA* (100%), *clfB* (62.5%), *fnbB* (25%), *bap* (25%), *ebp* (37.5%), and *cna* (37.5%), as shown in Figure 5. Table 5 shows details of different clonal lineages based on *spa* and MLST typing methods.

3.6.4. CC425

CC/ST425/t742 (13.3%) was a clone identified in *mecC*-carrying *S. aureus* strains. The CC/ST425-SCC*mec* XI/t742 strain was recovered from blood

(50%), as well as surgical wounds, skin wounds, and pus (16.7% each). More than three-fourths (75%) of the isolates were moderate biofilm producers. The most prevalent resistance genes included *tet(M)* (100%), *ant(4')-Ia* (50%), and *erm(A)* (50%). A phylogenetic tree based on seven combined loci is presented in Figure 4 (E and F). The tree was constructed using the RAxML method with a dataset of 3,186 bp. The phylogenetic analysis revealed that all STs were classified into four well-supported clades. The ST599 clade occupied a basal position relative to the other STs (Fig. 5). The ST239 clade formed a highly supported group together with ST425, and they were placed as sister taxa in the ML tree. Notably, ST239 included two strains that showed different genetic resistance patterns.

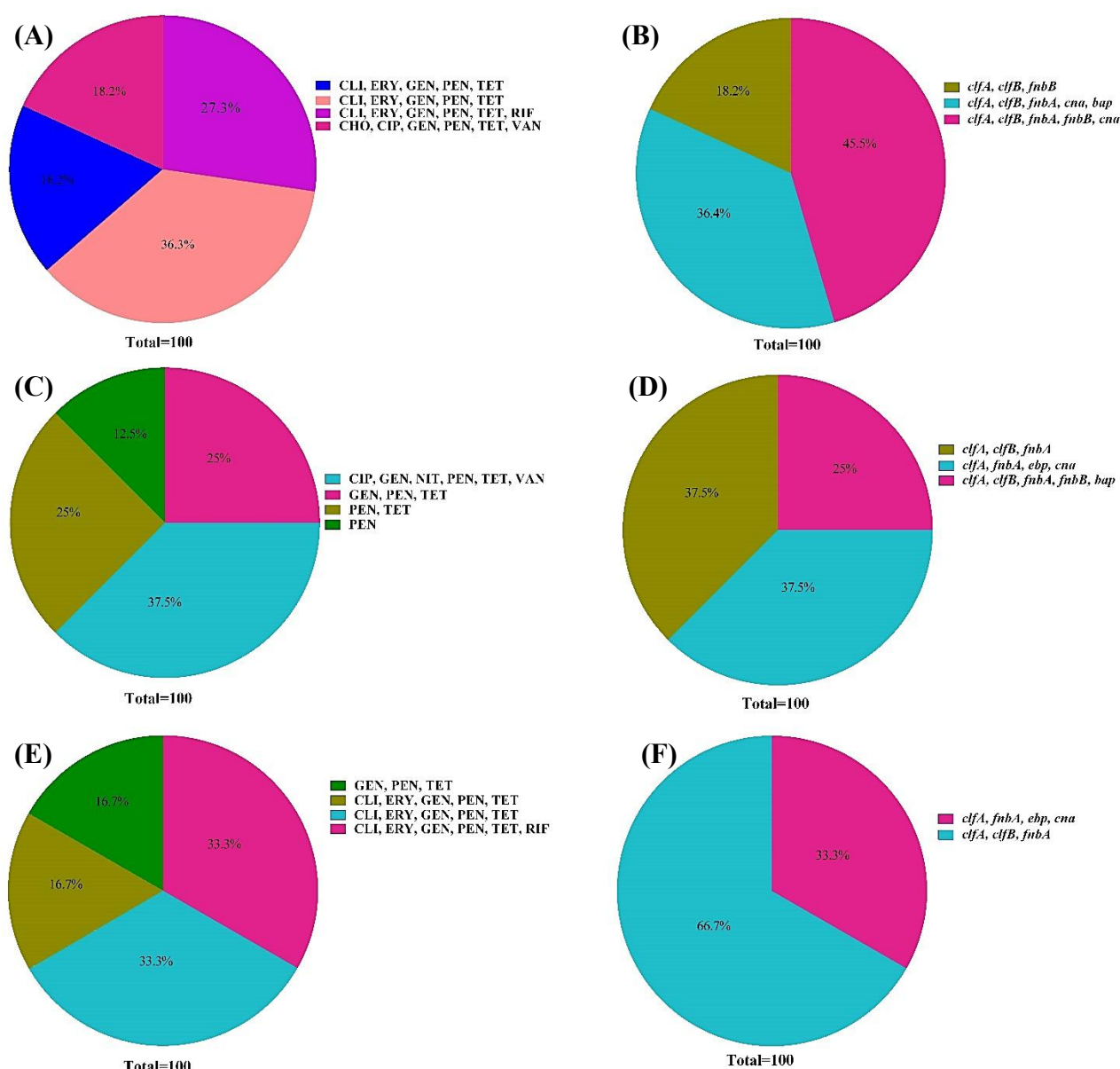


Fig. 4. Distribution of resistance profiles and biofilm encoding genes among CC lineages. Distribution of (A) resistance profiles and (B) biofilm encoding genes among CC130 lineages. Distribution of (C) resistance profiles and (D) of biofilm-encoding genes among CC121/ST599/t5930 isolates. Distribution of (E) resistance profiles and (F) biofilm-encoding genes among CC/ST425/t742 isolates.

4. DISCUSSION

MRSA infections are widespread in both healthcare settings and the community. The *mecC* gene has emerged as a novel determinant of methicillin resistance in *Staphylococcus* species^[3]. This study investigates the molecular epidemiology and genetic characterization of clinical *mecC*-carrying *S. aureus* strains isolated from the selected hospitals in Tehran, providing novel insights into circulating *mecC*-carrying *S. aureus* strains previously unreported in Iran. The findings include the first report on the prevalence and genetic diversity of *mecC*-positive *S. aureus* isolates in

clinical samples from Iran. Notably, *mecC*-positive *S. aureus* strains predominantly exhibited strong biofilm-forming capabilities. Additionally, a high prevalence of the CC8 clonal complex was observed among these isolates, highlighting their significant role in infections and outbreaks in healthcare settings.

The *mecC* gene has been detected in *S. aureus* isolates from both humans and dairy cattle^[3]. Studies have indicated an increasing prevalence of *mecC*-carrying *S. aureus*^[3,14]. In our research, a 2.8% prevalence of *mecC*-carrying *S. aureus* isolated from clinical samples was found, with all being MRSA. Over the past two decades,

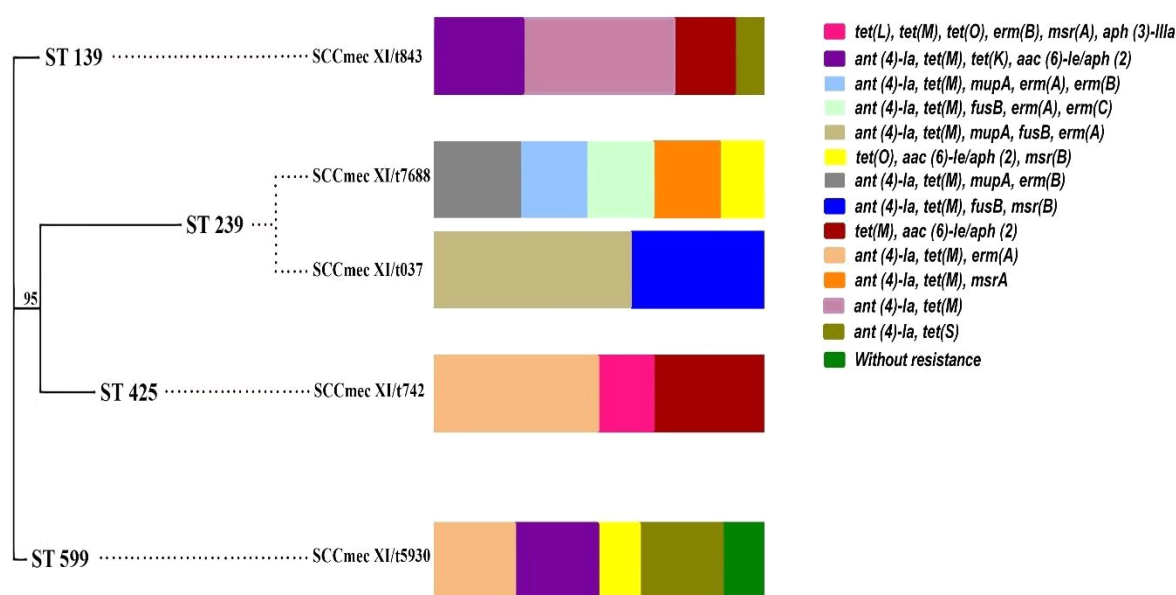


Fig. 5. A phylogenetic tree generated through ML analysis of four STs, using a combination of seven loci (*ArcC*, *AroE*, *GlpF*, *Gmk*, *Pta*, *Tpi*, and *YqiL*). The analysis was conducted with the IQTREE software, applying the TPM2u+F+I model and performing 10,000 ultrafast bootstrap replicates. Bootstrap values greater than 95% are indicated on the tree. ST 239 included two strains that showed different genetic resistance patterns.

the prevalence of *mecC* among *S. aureus* in Iran has increased from 0.6% to 0.9%^[9,15]. In 2015, a systematic review and meta-analysis study by Diaz et al. showed a low level of *mecC*-carrying *S. aureus* strains originated from human samples (0.004%; 95% CI = 0.002-0.007) but also highlighted a higher prevalence in animal samples (0.10%; 95% CI = 0.033-0.174)^[14]. Research from different geographic area, such as Pakistan (96.8%), Egypt (6%), the UK (0.45%), Denmark (5.9%), Germany (0.9%), Belgium (0.18%), Spain (15%), Austria (2%), Finland (0.74%), and Slovenia (1.5%), has reported various prevalence rates of *mecC*-carrying *S. aureus* isolated from human samples, indicating a wide geographical spread of these strains^[14,16,17]. Notably, our findings revealed a concerning rise in the prevalence of *mecC*-carrying *S. aureus* in clinical settings in recent years^[18]. However, *mecC* remains more prevalent in Iran than in several other European and some Asian countries^[5,19-21]. Although the origin of *mecC*-carrying *S. aureus* remains unclear, earlier evidence suggests that horizontal gene transfer from non-*aureus* *Staphylococcus* species to *S. aureus* may have significantly contributed to the increasing incidence and prevalence of *mecC*-mediated resistance in clinical settings in recent years^[3]. As a result, clinical microbiologists should remain vigilant about the potential transfer of *mecC* from other methicillin-resistant *Staphylococcus* pathogens^[3,14,19].

Earlier research has indicated that livestock serves as a

reservoir of *mecC*-carrying *S. aureus* and a major source of zoonotic infections in humans^[19]. It seems reasonable to attribute the high prevalence rate of *mecC* in our study to contact with livestock and the growing trend of keeping pets, which could facilitate the transmission of *mecC*-carrying *S. aureus* strains from animals to humans. In our study, MRSA isolates exhibited high resistance to erythromycin, penicillin, tetracycline, gentamicin, and clindamycin and appeared to be multidrug-resistant, consistent with previous reports from this region^[9,15]. We also showed the resistance profile of clinical *mecC*-carrying *S. aureus* isolates for various antibiotics. These findings suggest either an overuse of antibiotics in human and veterinary medicine or potential evolutionary adaptations of the bacteria. Notably, intermediate resistance to vancomycin has emerged in our clinical settings, with 11.1% of isolates showing this resistance. This is particularly concerning as the emergence of intermediate or resistant strains poses a significant threat, given that vancomycin remains the primary treatment for MRSA infections. A systematic review and meta-analysis study conducted by Shariati et al., from 1997 to 2019, indicated a 3.6-fold increase in the prevalence of VISA strains from 1.2% before 2010 to 4.3% after 2010^[22]. This increase in VISA strains may result from several genetic and phenotypic alterations in wild-type bacteria, changes in cell wall thickness, easy availability of these strains, and a lack of guidelines for antibiotic use. Additional evidence shows an increasing

trend in resistance to fusidic acid among MRSA strains. In a study performed in Kuwait, Udo and colleagues reported an increasing trend of resistance to fusidic acid from 22% in 1994 to 92% in 2004^[23]. In contrast to earlier surveys in Iran indicating resistance rates of 2.5%-8% for fusidic acid^[11,24], our study showed a prevalence rate of 17.8% for fusidic acid-resistant isolates, all of which carried the *fusB* gene. Consistent with other research^[25], our study highlights a relatively high prevalence of resistance to fusidic acid in MRSA strains. This increase reflects a broader shift in antibiotic usage patterns and a growing prevalence of staphylococcal infections, underscoring the urgent need for revised treatment protocols and enhanced antimicrobial stewardship to combat rising resistance.

In the current survey, 15.6% of the isolates indicated the iMLSB phenotype. The presence of this phenotype among *mecC*-carrying *S. aureus* strains has been reported by several researchers in the UK^[6], Iran^[15], India^[26], and Pakistan^[27]. Our findings, along with previous studies, support the widely accepted belief that the emergence of widespread resistance in clinical pathogens is driven by antibiotic use in both human and veterinary medicine. Notably, the *mecC*-positive CC8/ST239/t7688 strain accounted for 33.3% of the isolates and has been reported in Iran^[18], as well as other parts of Asia^[28]. Similar patterns have been observed in Iran and other countries, including Switzerland, where this strain was detected on the hands of healthcare workers and in hospital environments. Additionally, it has been identified as a successful pathogen responsible for infections in various hospital sites in Iran^[29]. Evidence suggests that the t7688/ST239 strain is a widely distributed endemic clone across many Asian countries^[18,23]. The presence of multiple resistance genes and an MDR profile underscores the need for close monitoring of the CC8/ST239/t7688 strain.

Our analysis indicated that more than three-fourths of the CC8/ST239/t7688 isolates carried *ant(4')-Ia* and *tet(M)* genes. Similar findings have been observed in a study conducted by Ghasemzadeh-Moghaddam et al. in Iran, which reported the presence of resistant genes in CC8/ST239/t7688 lineages^[18]. Consistent with previous studies in Iran that reported the presence of CC8/ST239-t7688 strains^[18], our findings confirmed that all *mecC*-positive CC8/ST239/t7688 isolates were MRSA. In this study, we identified CC8/ST239/t037 in 11.1% of *mecC*-positive strains, highlighting the persistence of ST239 in hospital settings, particularly in Iran, due to its multidrug resistance and rapid dissemination. All isolates exhibited strong biofilm formation, resistance to multiple antibiotic classes, and resistance to fusidic acid. In agreement with our results, an Iranian study on 308 *S. aureus* reported the presence

of ST239/t037 in *mecC*-positive strains of *S. aureus*^[15]. However, previous reports have documented the emergence of this clone in China, Kuwait, and Saudi Arabia^[30,31], further supporting the hypothesis that it may have been introduced from neighboring countries. An earlier study by Goudarzi et al. in Iran reported fusidic acid-resistant t030 strains^[10]. Similarly, between 2010 and 2013, spa type t030 carrying the *fusB* gene was detected in 7.1% of MRSA isolates from clinical samples in a hospital in China^[32]. In our study, spa type t037 (7.6%) emerged as the most prevalent genotype among fusidic acid-resistant *mecC*-positive strains, with a 100% prevalence of *fusB* carriage. These findings are consistent with earlier research by Chen et al. in Taiwan, which identified t037 (62%) and t002 (29%) as the most common genotypes among fusidic acid-resistant MRSA isolates from hospitalized patients^[33].

The second most common *mecC*-positive clone was CC/ST130/t843, characterized by the presence of multiple resistance genes, strong biofilm production, and the ETA toxin. A study by Lindgren et al. in Sweden, in which 45 *mecC*-positive *S. aureus* isolates from patients over 10 years were analyzed, found that 76% of *mecC*-carrying *S. aureus* belonged to spa types t373 and t843. The study also revealed a decline in the incidence of *mecC*-positive *S. aureus* over the past three years, dropping from 9.8% in 2014 to 3.4% in the same year^[21]. Similarly, research by Petersen et al. in Denmark, conducted between 2007 and 2011, identified CC30 (87.5%) and CC2361 (12.5%) as the predominant clones among *mecC*-positive *S. aureus* isolates, with no virulence genes detected^[4]. These findings align with those of García-Garrote et al., who reported that CC130 *mecC* MRSA is the most prevalent lineage among human isolates in Spain^[34]. Furthermore, a study by Dermota et al., analyzing 395 MRSA strains isolated from various clinical samples between 2006 and 2013, identified only six MRSA isolates carrying the *mecC* gene, all of which belonged to CC/ST130. In contrast, Dermota's research in Slovenia indicated that none of the *mecC*-positive MRSA isolates carried antibiotic resistance genes, and all were classified as CC/ST130^[35]. Although earlier epidemiologic research has infrequently reported *mecC*-carrying *S. aureus* isolates associated with ST599, we identified a *tst*-positive ST599 isolate that harbors the *mecC* gene and is characterized by spa type 5930. Previous studies have documented the presence of CC/ST599 isolates in Iran and many European countries, including Germany, Austria, the UK, and Belgium^[3,9,20]. Our observations regarding the *tst* carriage by ST599 isolates are comparable to the results obtained by Goudarzi et al. from Iran^[9], Kerschner et al. from Austria^[20], and Sabat et al. from France^[36].

In the present study, we identified the *mecC*-positive *S. aureus* clone ST425/t742, which belonged to CC425. This finding is supported by a study by Silva et al. on *S. aureus* isolates recovered from 78 surface water samples in Portugal. Their investigation into the clonal diversity of *mecC*-positive *S. aureus* strains reported the presence of CC/ST425^[37]. However, in contrast to our findings, they indicated that CC425 isolates were more prevalent than CC130 isolates in surface waters. Our results are also in line with the observations of Porrero et al., who showed that the CC425 lineage was the most common among *mecC*-MRSA strains isolated from animals and Urban Wastewater^[7]. Notably, our *mecC*-carrying *S. aureus* strains were negative for toxin-encoding genes, which corresponds with Silva's study^[37].

5. CONCLUSION

The present study describes the variability of clonal lineages of *mecC*-carrying *S. aureus* isolated from different clinical sources, including the first detection of CC/ST423/t742 in Iran. The identification of livestock-associated *S. aureus* lineages in healthcare settings underscores the zoonotic potential of this emerging resistant microorganism, along with other related animal-derived clades, which pose significant risks to human health. These findings underscore the importance of proper and rational clinical use of antibiotics in humans and livestock, which can contribute to the evolution of certain *mecC*-carrying *S. aureus* lineages. The widespread multidrug resistance observed in *mecC*-carrying *S. aureus* strains, especially in healthcare settings, highlights the need for continuous surveillance and robust antimicrobial management. Monitoring *mecC*-positive *S. aureus* strains in hospitals, veterinary practices, and food safety laboratories is crucial and should be included in preventive strategies to prevent potential health risks. Therefore, studying the detection and genetic diversity of *mecC*-positive *S. aureus* using robust phenotypic and molecular screening assays would be highly beneficial.

DECLARATIONS

Acknowledgments

Not applicable.

Generative AI and AI-assisted technologies

In this study, no artificial intelligence technology was used in the production of the submitted work.

Ethical approval

All the experimental procedures in this study were approved by the Research Ethics Committee of Shahid

Beheshti University of Medical Sciences, Tehran, Iran (ethical code: IR.SBMU.MSP.REC.1402.116).

Consent to participate

All participants voluntarily agreed to participate in this study. Written informed consent was obtained from all participants prior to enrollment in the study.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

MG: conceptualization, funding acquisition, methodology, project administration, resources, supervision, validation, writing—original draft, writing—review, editing; SSS and MR: data curation, formal analysis, methodology, writing original draft, writing—review, and editing; ZS: formal analysis, writing original draft, writing—review, editing; MD: formal analysis, investigation, methodology, project administration; MJN: formal analysis, writing original draft.

Data availability

All relevant data can be found within the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Supplementary information

The online version does not contain supplementary material.

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