Original Research Article Iran J Med Microbiol. 2025; 19(4): 254-264

Category: Molecular Epidemiology | Peer Reviewed | Open Access

10.30699/ijmm.19.4.254

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Molecular Characterization of Staphylococcal Cassette Chromosome *mec* types I to V in Methicillin-resistant *Staphylococcus aureus* by Multiplex PCR

Mrinmayee Umrotkar[1], Neetu Gupta[1*], Savita Vivekanand Jadhav[2]

1. *Symbiosis Medical College for Women (SMCW), Symbiosis University Hospital and Research Centre (SUHRC), Symbiosis International (Deemed University), Pune, India*
2. *Pacific Medical College and Hospital, Pacific Medical University (PMU), Udaipur, India*

ABSTRACT

Background and Aim: The staphylococcal cassette chromosome *mec* (SCC*mec*) is a mobile genetic element that acquires the *mec*A gene. There are five main categories (I-V) of SCC*mec* components in methicillin-resistant *Staphylococcus aureus* (MRSA). The majority of MRSA strains acquired in hospitals (HA-MRSA) have SCC*mec* types I, II, III, while strains acquired in the community (CA-MRSA) have SCC*mec* types IV or V. This study aimed to determine the presence of SCC*mec* types, subtypes, and their antibiotic sensitivity in clinical MRSA isolates.

Materials and Methods: This cross-sectional descriptive study was performed on MRSA isolates collected from different age groups of patients. The isolates identification and antibiotic sensitivity were performed using phenotypic and automated methods. Single-target PCR was performed for the presence of the *mec*A gene to confirm the MRSA. Multiplex PCR was performed to identify the types and subtypes of SCC*mec*.

Results: A total of 35 MRSA isolates were confirmed using single-target PCR for the *mecA* gene. Multiplex PCR showed *SCCmec* type IVd (63%) as the most commonly identified type, followed by *SCCmec* type V (23%). Few isolates (14%) remained non-typeable. The SCC*mec* type I-III/IVa-c was not detected in any of the isolates. Type IVd strains showed more than 50% resistance to erythromycin, cotrimoxazole, gentamicin and clindamycin. Type V strains showed more resistance to gentamicin and erythromycin.

Conclusion: MRSA SCC*mec* typing is a useful method to know the epidemiology of various SCC*mec* types and subtypes circulating in hospitals and communities and to understand the changing trend, if any. The use of other typing methods in combination with SCC*mec* typing may help further differentiate various clones circulating in these types and identify novel types.

Keywords: *mec*A Gene, MRSA, Multiplex PCR, SCC*mec* Typing

Received: 2025/06/21; **Accepted**: 2025/09/19; **Published Online:** 2025/10/10;

Corresponding Information: **Neetu Gupta**, *Symbiosis Medical College for Women (SMCW), Symbiosis University Hospital and Research Centre (SUHRC), Symbiosis International (Deemed University), Pune, India* & Email: neetu.gupta@smcw.siu.edu.in

Use a device to scan and read the article online

Umrotkar M, Gupta N, Jadhav S V. Molecular Characterization of Staphylococcal Cassette Chromosome mec types I to V in Methicillin-resistant *Staphylococcus aureus* by Multiplex PCR. Iran J Med Microbiol. 2025;19(4):254-64.

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks

Send citation to: Mendeley Zotero RefWorks

1. Introduction

Staphylococcus aureus is a Gram-positive, spherical-shaped bacterium that causes various types of infections, such as cutaneous infections, endocarditis, osteomyelitis, pneumonia and empyema (1). It also causes illnesses related to toxin production, such as food poisoning, toxic shock syndrome, and scalded skin syndrome. *Staphylococcus aureus* is associated with community-

associated bacterial infections worldwide and also acts as an opportunistic pathogen, one of the most common causes leading to nosocomial infections **(1-3)**.

Methicillin is semisynthetic penicillin that was first used in the medical field in 1960 and targets primarily the staphylococci producing beta-lactamases **(4)**. However, methicillin-resistant *Staphylococcus aureus* (MRSA) was also identified in that same year (1960) **(4, 5)**. MRSA produces a different penicillin-binding protein known as PBP2' (PBP2a), which was reported in the middle of the 1980s **(4)**. It encodes the gene *mec*A that has poor affinity for beta-lactams (semisynthetic penicillin and most cephalosporins) **(4, 5)**.

The emergence and worldwide spread of Methicillin-resistant *S. aureus* (MRSA) strains poses a significant challenge and severely hamper the treatment of infected patients. Staphylococcal cassette chromosome (SCC), a genomic island containing the *mec* gene, is a heterologous mobile genetic element that can be acquired by and integrated into the bacterial host chromosome, which confers and spreads the resistance traits among *Staphylococcus* species **(2, 6)**. These elements shared the following characteristics: i) *mecA* carriage in a *mec* gene complex, ii) *ccr* gene complex with *ccr* gene(s) (*ccrAB* and/or *ccrC*) iii) the integration *s*ite *s*equence (ISS) for SCC, which serves as a target for *ccr*-mediated recombination, and (iv) the flanking direct repeat (DR) sequences containing the ISS **(4)**.

The SCC*mec* elements are classified into various types and subtypes. SCC*mec* types are the combination of the *ccr* gene complex type and the class of the *mec* gene complex. Roman numerals can also be used for describing the types; such as SCC*mec* types I, II, and III. The junkyard region (J region) DNA segments are responsible for defining SCC*mec* subtypes because they contain defining genes, pseudogenes, non-coding areas, and mobile genetic elements like insertion sequences, plasmids, or transposons. The most common method of describing SCC*mec* subtypes is to represent the variations in the J region as small letters, such as IVa, IVb, and IVc **(4, 6, 7)**.

In the early 2000s, after the SCC*mec* I, II, and III types were reported, researchers identified other SCC*mec* elements also and adopted this SCC*mec* typing as a molecular epidemiology tool in the research to understand the evolution of *Staphylococcus* **(4)**. Various studies have shown that SCC*mec* types I-V are the most commonly observed types globally. SCC*mec* types I, II and III are large in size. MRSA isolates with these types are generally associated with nosocomial infection and are Hospital-acquired MRSA (HA-MRSA). Types IV and V are relatively smaller in size and MRSA isolates with these types are associated with the community that are known as community-acquired MRSA (CA-MRSA) **(8, 9)**. Over the past few years, CA-MRSA strains carrying SCC*mec* type IV have been increasingly prevalent in certain regions around the globe, posing significant challenges for the management and treatment of MRSA infections **(1)**. The emergence of CA-MRSA strains within hospitals also represents a significant public health threat to future treatment **(10)**.

Currently, I-XV SCC*mec* types have been classified in *S. aureus* **(11, 12)**. SCC*mec* types and subtypes can be identified by using PCR-based methods, DNA microarray, and Whole-Genome Sequencing-Based Methods. PCR-based methods continue to be widely used methods because of the easy access of reagents, availability of equipment in various research institutes, hospitals and because of the cost effectiveness than the Whole-Genome Sequencing-Based Method **(4)**.

Staphylococcal cassette chromosome (SCC) elements are the vectors for genetic exchange of the *mec*A gene encoding methicillin resistance in *Staphylococcus*. Due to constant changes in epidemiology of MRSA strains, there is a need to pay attention to these mobile genetic elements and to know the epidemiology of various geographical regions. SCC*mec* typing is an important tool for molecular epidemiological studies to investigate the evolution of MRSA, which can help timely start the infection control measures to prevent spread and effectively treat MRSA infections.

This study was designed to identify MRSA strains from various clinical specimens using phenotypic and automated methods and to confirm the presence of the *mecA* gene in their genome by PCR. We also categorized the types and subtypes of SCC*mec* using multiplex PCR technique. The antibiotic resistance pattern of these isolates was also identified.

2. Materials and Methods

In this cross-sectional descriptive study samples were collected from different age groups of patients. Various clinical specimens (blood, pus, sputum, etc.) of infected patients attending the outpatient department and admitted to a tertiary care hospital were included. Sampling was conducted from October to December 2023. All clinically significant MRSA strains isolated during the study period were included in the project.

2.1 Inclusion and Exclusion Criteria of MRSA

Inclusion criteria: Isolates identified as MRSA by conventional (cefoxitin disc diffusion method) and automated (BD Phoenix, MIC) methods according to the mentioned guidelines in the clinical laboratory and the standard institute (CLSI) **(13)**. All those identified MRSA isolates confirmed for the presence of the *mec*A gene by genotypic (PCR) method were included in the study. Some isolates from the same patients were also included if they were from different samples or different sites.

Exclusion criteria: Isolates that did not meet the criteria of MRSA according to CLSI and showed discrepancies in the phenotypic and genotypic methods.

2.2 Isolation of *S. aureus* Isolates

Standard laboratory procedures, i.e morphology on Gram staining, colony characteristics, biochemical reactions (catalase and coagulase test) and an automated system (BD Phoenix) were used for identification of *S. aureus* isolated from various specimens **(14-16)**.

2.3 Identification of Methicillin Resistance

2.3.1 Cefoxitin Disc Diffusion Method

The cefoxitin (30 µg) disc and Kirby-Bauer disc diffusion method were used. Reported cefoxitin resistance in case of inhibition zone was ≤21 mm, and sensitive if ≥22 mm as recommended by Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines **(13)**.

2.3.2 Minimum Inhibitory Concentration

Minimum inhibitory concentration was checked using an automated method (BD Phoenix) following the CLSI guidelines. Oxacillin MIC≥4 µg/mL was considered MRSA, and ≤2 µg/mL as methicillin-sensitive *S. aureus* (MSSA) **(13)**.

2.3.3 Confirmatory test

PCR amplification of the *mec*A gene was done for all isolates using specific primers **(17, 18)**.

2.4 Analysis of Antibiotic Susceptibility Profiles

The BD Phoenix automated method was used to check the sensitivity to antimicrobials, and the antibiotics included were penicillin, cefoxitin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, gentamicin, tetracycline, rifampin, linezolid, teicoplanin, and daptomycin **(13)**.

Two methods were used for detection of Inducible Clindamycin Resistance (ICR) in *Staphylococcus*: an automated system (BD Phoenix) and disc diffusion method.

Disc diffusion method: Discs containing 15 µg erythromycin and 2 µg clindamycin were placed at 15 mm apart on Muller-Hinton agar plate after the standard inoculation procedure for the disc diffusion test and incubated at 37ºC for 16-18 hr. Flattening of the inhibition zone adjacent to the erythromycin disc was referred to as D-zone, and Inducible clindamycin resistance **(13)**.

The control strains *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used for the conventional and automation tests, respectively **(13)**.

2.5 Definitions of HA-MRSA and CA-MRSA

The case definition by Center for Disease Control and Prevention (CDC) was used to distinguish between CA-MRSA and HA-MRSA. The isolates were considered CA-MRSA when identified in a patient sample from an outpatient department setting or within 48 hr after hospital admission and in the absence of the healthcare-associated risk factors such as history of hospitalization, surgery, dialysis, long-term care facility (e.g nursing home) within the previous year of MRSA culture-positive date; a permanent indwelling catheter or percutaneous device; or a prior MRSA isolation report. All MRSA isolates that did not meet the mentioned criteria of CA-MRSA were considered HA-MRSA **(19, 20)**.

2.6 Data Collection

Medical records of the patients and laboratory records were reviewed for the above-mentioned risk factors and antibiotic sensitivity pattern, respectively.

2.7 Statistical Analysis

Descriptive statistics based on relative frequency w ere used to present the findings. The chi-square test was used to find the association between SCC*mec* types and antibiotic resistance patterns. P value <0.05 was considered statistically significant.

2.8 Molecular Typing

Bacterial DNA samples were isolated from fresh cultures of MRSA strains using the NucleoSpin Tissue Mini kit (MN) according to the manufacturer's instructions. Quality check of DNA was performed on 1% (w/v) agarose gel using agarose gel electrophoresis. Purity of DNA was assessed by LT-291 UV-VIS Spectrophotometer (Labtronics, India). Concentration calculated from OD 260 using the dsDNA factor (50 ng/µL per OD unit) (Supplementary Table).

Single-target PCR was performed for the *mec*A gene on all isolates. The multiplex PCR amplification of the genes of the isolate was performed using the Zhang et al **(17)** method with primer sequences specific for SCC*mec* types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V with the *mec*A gene **(17)** (Table 1).

For MRSA strains that were non-typeable using Zhang et al **(17)**. Primers typing were carried out again using the method and primers described by Oliveira et al **(18)** by the multiplex PCR method (Table 2). Analysis of the PCR products was completed on a 2% agarose gel through Agarose Gel Electrophoresis, and the size of the amplicons was evaluated against a reference ladder. The 2% agarose gel was mixed with LabSafe Nucleic Acid Stain to achieve a final concentration of 0.5 µg/mL. Bands were visualized using UV transilluminator. Images of the gel were captured using the BIO-RAD GelDoc XR documentation system.

Table 1. Primers specifications for SCC*mec* types and subtypes.

Name	Seq (5'-3')	Amplicon size	Specificity	Reference
Type I-F	GCTTTAAAGAGTGTCGTTACAGG	613 bp	SCC*mec* I	Zhang et al **(17)**
Type I-R	GTTCTCTCATAGTATGACGTCC			
Type II-F	CGTTGAAGATGATGAAGCG	398 bp	SCC*mec* II	
Type II-R	CGAAATCAATGGTTAATGGACC			
Type III-F	CCATATTGTGTACGATGCG	280 bp	SCC*mec* III	
Type III-R	CCTTAGTTGTCGTAACAGATCG			
Type IVa-F	GCCTTATTCGAAGAAACCG	776 bp	SCC*mec* IVa	
Type IVa-R	CTACTCTTCTGAAAAGCGTCG			
Type IVb-F	TCTGGAATTACTTCAGCTGC	493 bp	SCC*mec* IVb	
Type IVb-R	AAACAATATTGCTCTCCCTC			
Type IVc-F	ACAATATTTGTATTATCGGAGAGC	200 bp	SCC*mec* IVc	
Type IVc-R	TTGGTATGAGGTATTGCTGG			
TypeIVd-F5	CTCAAAATACGGACCCCAATACA	881 bp	SCC*mec* IVd	
TypeIVd-R6	TGCTCCAGTAATTGCTAAAG			
Type V-F	GAACATTGTTACTTAAATGAGCG	325 bp	SCC*mec* V	
Type V-R	TGAAAGTTGTACCCTTGACACC			
MecA147-F	GTGAAGATATACCAAGTGATT	147 bp	*mecA*	
MecA147-R	ATGCGCTATAGATTGAAAGGAT			

Table 2. Primers specifications for multiplex PCR.

Locus	Name	Seq (5'-3')	Amplicon size	Specificity	References
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495 bp	I	Oliveira et al **(18)**
	CIF2 R2	ATTTACCACAAGGACTACCAGC			
B	KDP F1	AATCATCTGCCATTGGTGATGC	284 bp	II	
	KDP R1	CGAATGAAGTGAAAGAAAGTG			
C	MECI P2	ATCAAGACTTGCATTCAGGC	209 bp	II, III	
	MECI P3	GCGGTTTCAATTCACTTGTC			
D	DCS F2	CATCCTATGATAGCTTGGTC	342 bp	I, II, IV	
	DCS R1	CTAAATCATAGCCATGACCG			
E	RIF4 F3	GTGATTGTTCGAGATATGTGG	243 bp	III	
	RIF4 R9	CGCTTTATCTGTATCTATCGC			
F	RIF5 F10	TTCTTAAGTACACGCTGAATCG	414 bp	III	
	RIF5 R13	GTCACAGTAATTCCATCAATGC			
G	IS431 P4	CAGGTCTCTTCAGATCTACG	381 bp	----*	
	pUB110 R1	GAGCCATAAACACCAATAGCC			
H	IS431 P4	CAGGTCTCTTCAGATCTACG	303 bp	----*	
	pT181 R1	GAAGAATGGGGAAAGCTTCAC			
mecA	MECA P4	TCCAGATTACAACTTCACCAGG	162 bp	Internal control	
	MECA P7	CCACTTCATATCTTGTAACG			

***Loci G and H added to distinguish variant IA from I and IIIA from III respectively.**

3. Results

All 35 isolates included in the study were confirmed as MRSA using phenotypic and automated methods. All 35 MRSA were also confirmed by genotypic method using single-target PCR for the *mecA* gene. Figures 1 and 2 show the bacterial DNA isolation results and single PCR results of some isolates, respectively.

Of 35 MRSA isolates, 30 isolates (86%) were from the admitted patients in the hospital, and 5 (14%) isolates were from patients attending the outpatient department. Out of 35 MRSA isolates, the maximum 27 strains (77%) were from pus samples, followed by blood 6 (17%) and sputum 2 (6%). The isolates were classified as CA-MRSA 26/35 (74%) and HA-MRSA 9/35 (26%) based on the definition mentioned above. Nine HA-MRSA isolates were collected from 7 patients, and 26 CA-MRSA isolates from 24 patients.

SCC*mec* typing of 35 isolates revealed that *SCCmec* type IVd strain 22 (63%) was the most frequently isolated strain, followed by *SCCmec* type V: 8 (23%). Five (14%) isolates did not show positive amplification with Zhang et al study primers used for *SCCmec* typing and retested using Oliveira et al primers, but remained non-typeable. No isolate proved positive for SCC*mec* I, II, III, IVa, IVb, IVc.

Figure 3 shows the multiplex PCR results of *SCCmec* type IVd, *SCCmec* type V and notypeable strains.

SCC*mec* Type IV d strains were isolated from different types of specimens: Pus culture isolates 15/35 (43%) followed by blood culture isolates 6/35 (17%), and sputum samples 1/35 (3%), while the SCC*mec* type V strains were only identified from pus samples 8/35 (23%). Of 5 non-typeable isolates, 4 were from pus samples and 1 from a sputum sample. The frequency of various SCC*mec* types in different sample types is also mentioned in Table 3.

All HA-MRSA and CA-MRSA isolates were identified with *SCCmec* IVd and *SCCmec* V type. Of 9 HA-MRSA isolates, 7 (78%) carried *SCCmec* type IVd, 1 (11%) *SCCmec* type V, and 1 (11%) was non-typeable. Of 26 CA-MRSA, 15 (58%) carried *SCCmec* type IVd, 7 (27%) SCC*mec* type V, and 4 (15%) remained non-typeable. These findings suggest high prevalence of the same SCC*mec* type (IVd) in both settings.

Antimicrobial resistance rates among SCC*mec*-IVd, V and non-typeable isolates are shown in Table 4. Type IVd strains showed more than 50% resistance to erythromycin (64%), gentamicin (59%), clindamycin

(59%), cotrimoxazole (55%) and type V strains showed more resistance to gentamicin (88%) and erythromycin (88%), followed by clindamycin (13%), cotrimoxazole (13%). Non-typeable strains showed 100% resistance to gentamicin. All clindamycin-resistant isolates showed inducible clindamycin resistance. The assessment of the association between the presence of different SCC*mec* types and antibiotic resistance in 35 MRSA strains showed a significant relationship (P<0.05) with cotrimoxazole and clindamycin.

Table 3. Frequency distribution of SCC*mec* types between different sample types.

Sample Type	No. (%)	*SCCmec* Type		
	35 (100%)	**IV d**	**V**	**Non-typeable**
Pus	27 (77.14%)	15 /27 (55.55%)	8/27 (29.62%)	4/27 (14.81%)
Blood	6 (17.14%)	6/6 (100%)	0/6 (0%)	0/6 (0%)
Sputum	2 (5.71%)	1/2 (50%)	0/2 (0%)	1/2 (50%)

Table 4. Antimicrobial resistance of *S. aureus* according to the presence of *mecA* gene chromosomal cassette type (n =35).

Drugs	Resistance	MRSA n = 35(%)		
		SCC*mec* type IV d	**SCC*mec* type V**	**Not-typeable**
Penicillin	35 (100%)	22/22 (100%)	8/8 (100%)	5/5 (100%)
Gentamicin	25 (71%)	13/22 (59%)	7/8 (88%)	5/5 (100%)
Erythromycin	23 (66%)	14/22 (64%)	7/8 (88%)	2/5 (40%)
Clindamycin	16 (46%)	13/22 (59%)	1/8 (13%)	2/5 (40%)
Trimethoprim-sulfamethoxazole	14 (40%)	12/22 (55%)	1/8 (13%)	1/5 (20%)
Tetracycline	1 (3%)	0/22 (0%)	1/8 (13%)	0/5 (0%)
Rifampin	0 (0%)	0/22 (0%)	0/8 (0%)	0/5 (0%)
Vancomycin	0 (0%)	0/22 (0%)	0/8 (0%)	0/5 (0%)
Linezolid	0 (0%)	0/22 (0%)	0/8 (0%)	0/5 (0%)
Daptomycin	0 (0%)	0/22 (0%)	0/8 (0%)	0/5 (0%)

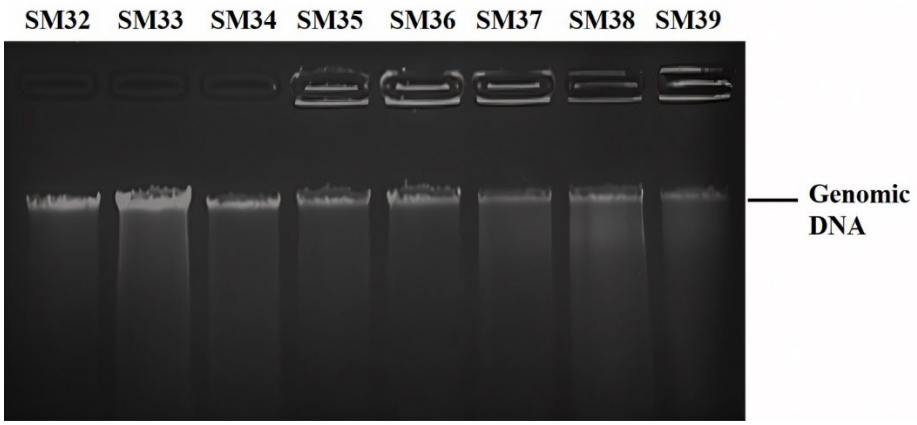

Figure 1. Bacterial genomic DNA isolation QC results **(Prepared by Authors, 2023-2025)**.

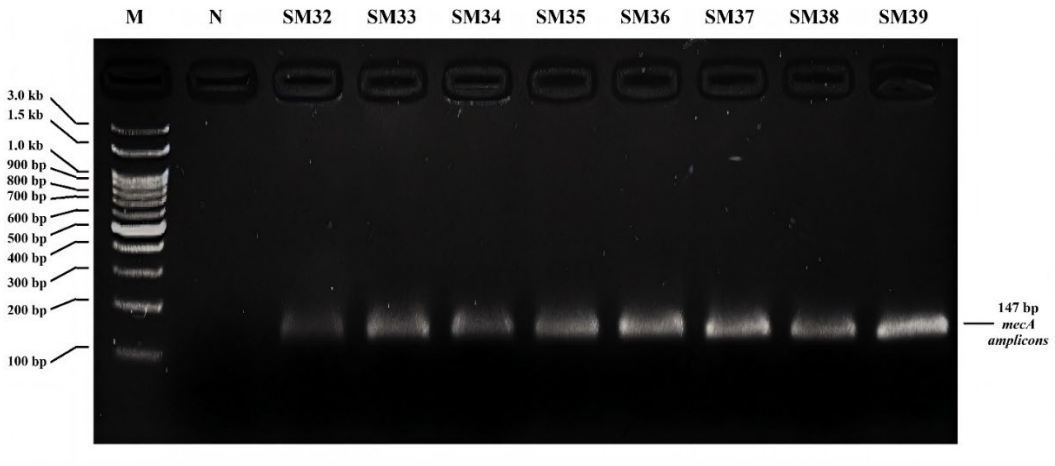

Figure 2. Single-Target PCR image: 2% (W/V) agarose gel electrophoresis: Lane M: 3kb DNA marker; Lane N: negative control; Lanes SM32-SM39: samples PCR Products (147 bp) **(Prepared by Authors, 2023-2025)**.

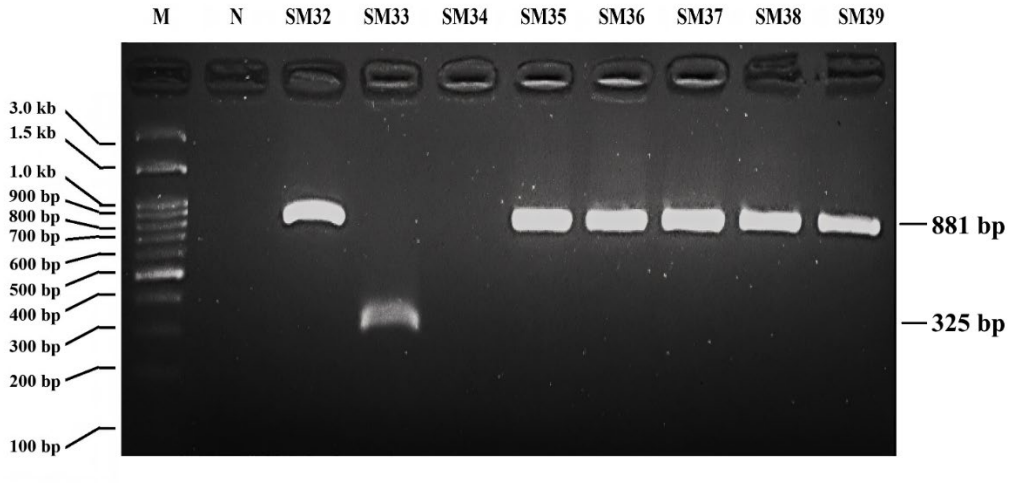

Figure 3. Multiplex PCR image: 2% (W/V) agarose gel electrophoresis: Lane M: 3kb DNA marker; Lane N: negative control; Lanes SM32-SM39: Samples PCR Products (881 and 325 bp) **(Prepared by Authors, 2023-2025)**.

4. Discussion

SCC*mec* typing is a useful tool in any surveillance system to understand the molecular epidemiology and evolution of MRSA strains. This study found that SCC*mec* type IVd (63%) was the most commonly identified type, followed by SCC*mec* type V (23%) among MRSA strains isolated from the patients of a tertiary care hospital. Among the SCC*mec* types, type IV is difficult to manage because is more mobile and spreads in the community more quickly than the other SCC*mec* types due to its smaller structure, which carries functional recombinases **(17)**. Kishk et al **(21)** from Egypt reported predominantly MRSA strains carrying SCC*mec* types IVa (63.7%) and V (23.6%), type I and III strains were not detected and type II (12.7%) was detected less commonly. In the Present study also, SCC*mec* types I to III were not detected. Alkharsah et al **(22)**, also identified in their study from Saudi Arabia, the SCC*mec*-IV (IVa and IVc) was frequently detected (77.3%), followed by type V (13.2%) and III (9.4%) **(22)**. These studies results match with our study, but in the present study, IVd type was comparatively identified as predominant. A study conducted by Ali et al **(10)** in Sudan, identified SCC*mec* typing results: type I (9.6%), III (12.8%), IVb (1.61%), IVc (3.22%), IVd (20.9%) and type V (16.1%), but type II and IVa were not identified. In this study, likewise, the present study IVd type and V were found predominantly. In the study of Kavitha and Srikumar **(23)** from India, SCC*mec* type V among MRSA was 53% followed by SCC*mec* type III (47%) and SCC*mec* type IV (37.6%). In Alfouzan et al study **(24)** in Kuwait, the SCC*mec* type IV (39.5%) was commonly identified, followed by type III (34.4%), type V (25.8%), and type VI (3.8%).

The epidemiology of MRSA strains is ever-evolving, and it is very well known that even in different

hospitals within the same nation or wards within the same hospital may have different prevalence and features. Therefore, it is critical to type MRSA isolates and contrast them with the strains acquired from other medical facilities across the nation **(24)**.

As mentioned in the present study, type IVd predominance, followed by V and types I-III, was not detected. This may be the regional trend, but further studies with larger sample size are required to explain the actual prevalence of these SCC*mec* types. In this study, all blood sample isolates were identified with SCC*mec* type IVd. Nakano et al **(25)**, in their study, reported that the bloodstream infection rate was significantly higher with SCC*mec* type IV MRSA isolates than in SCC*mec* type II MRSA. Recent studies reported that SCC*mec* type IV MRSA utilize the fibrin and fibrinogen brought in by coagulase and result in bloodstream infections by creating a biofilm structure in the host blood **(25)**.

In this study, almost all CA-MRSA and HA-MRSA isolates carried SCC*mec* IVd, followed by SCC*mec* V. Various studies showed that in recent years, discrimination of CA-MRSA and HA-MRSA isolates using SCC*mec* typing has become more difficult due to changing epidemiology because community-acquired MRSA SCC*mec* types may infiltrate the hospital setting and vice versa **(26)**. Like this study, various studies reported that in recent years, SCC*mec* types IV and V are taking the place of SCC*mec* types I, II and III in hospital settings. Davis SL et al. in their study quoted many studies explaining the presence of SCC*mec* IV or IV variants in hospital settings **(27)**. Boye et al **(28)** reported SCC*mec* type IV in 86% of the CA-MRSA and in 84% of HA-MRSA isolates. In a study of Trindade et al **(29)**, 95% of nosocomial oxacillin resistant isolates were identified with SCC*mec* type IV. Dhawan et al **(30)** reported dissemination of SCC*mec* IV/V genotype in hospitals. These findings suggest that although SCC*mec* typing is a valuable epidemiological tool, it is not very effective at differentiating between CA-MRSA and HA-MRSA. In the study of Prakash et al **(31)** from India, a high prevalence of SCC*mec* type IV (20.5%) and V (12%) reported from device-associated strains suggests the role of community strains in device-associated infections. Although many studies showed the emergence of SCC*mec* types IV and V in hospitals, the use of other typing methods in combination with the SCC*mec* typing may help further differentiate various clones circulating in these types.

Five (14%) isolates in this study were non-typeable by SCC*mec* typing. These results highlighted that other or new SCC*mec* types are also circulating in this region, although less commonly. The limitations of the multiplex PCR are known and based on primer sequence mismatches, the result of point mutation and the presence of a still not-typed SCC*mec*. In comparison, whole-genome sequencing is a more precise epidemiological tool and should be widely used in clinical settings for identification of SCC*mec* types and subtypes, novel SCC*mec* types, to reveal the relatedness of the SCC*mec* elements, which helps identifying the transmission and outbreaks of pathogens **(4)**.

SCC*mec* type IVd strains showed resistance to erythromycin (64%), gentamicin (59%), clindamycin (59%), cotrimoxazole (55%), and Type V strains showed more resistance to gentamicin (88%) and erythromycin (88%) followed by clindamycin (13%), cotrimoxazole (13%). Non-typeable strains showed 100% resistance to gentamicin. Japoni et al **(32)** in their study reported more antibiotic resistance to cotrimoxazole, clindamycin, gentamicin erythromycin cephalexin, ciprofloxacin, and tetracycline, in SCC*mec* type IVc strains and the lowest resistance in type V **(32)**. In the study of Kishk et al **(21)**, SCC*mec* type IVa isolates were found with resistance to clindamycin (60%) and erythromycin (35%) and SCC*mec* type V strains showed resistance to aminoglycosides **(21)**. The present study found clindamycin and cotrimoxazole resistance significantly associated and SCC*mec* type IVd. El-Ashry et al **(33)** study reported SCCmec type V was most common with emergence of SCC*mec* type III among CA-MRSA colonizers with high gentamicin resistance **(33)**. A High rate of gentamicin resistance in MRSA has been reported in many studies. Similarly, we also found it with SCC*mec* type V. Gentamicin resistance in non-typeable suggests the presence of novel or recombined SCC*mec* cassettes that carry the *mecA* and aminoglycoside resistance genes. To reveal any epidemiological and clinical significance in these strains requires further studies with a larger sample size, primers for other SCC*mec* types and subtypes, and the involvement of other genotypic methods.

Limitation

This study limitation was that the project included fewer samples with MRSA strains, making it difficult to generalize the true prevalence of IVd and V types in this population, even if it exists. SCC*mec* was not identified in five (14%) MRSA isolates with the primers included in the study. There was a lack of use of WGS in this study, which was able to characterize non-typeable isolates.

5. Conclusion

SCC*mec* types IVd and V are dominant in this geographical region with no detection of other SCC*mec* types I, II, III, IVa, IVb, IVc. As the five isolates in this study were not typed by these multiplex methods, other types might be missed. SCC*mec* elements are described as important gene acquisition vectors that

serve as a genetic shuttle between *Staphylococcus* species, thus continuous and expanded surveillance is essential in order to understand the epidemiology, evolution, and mobilization of their variants to control their spread and infection. The multiplex PCR using primers for maximum types, with other typing methods and whole genome sequencing must be included in the studies to check all rarely identified and novel types, to know their true prevalence and significance. SCC*mec* typing cannot be used as a sole marker for HA-MRSA and CA-MRSA detection. Therefore, there is a need for further studies with involvement of other methods to know various clone complexes circulating in the different regions.

6. Declarations

6.1 Acknowledgment

The authors thank dedicated technical team, especially Mrs. Swati Raut and Mr. Siddharam Hirake from the Department of Microbiology, Symbiosis Medical College for Women.

6.2 Ethical Considerations

The study was approved by the Institutional Ethical Committee under the proposal number SIU/IEC/595.

6.3 Authors' Contributions

Gupta Neetu and Jadhav Savita Vivek designed the study. Umrotkar Mrinmayee collected the isolates and patient data. All authors collectively wrote the manuscript, analyzed data, reviewed, edited, and approved the final draft.

6.4 Conflict of Interests

The authors have no conflict of interest.

6.5 Financial Support and Sponsorship

Not applicable.

6.6 Using Artificial Intelligence Tools (AI Tools)

All authors declare that there is no use of AI Tools in this study, including the writing of this manuscript.

References

1. Hesari Y, Sohrabi N, Abiri R, Babaei S, Amiri Z. staphylococcal cassette chromosome mec typing of meticillin-resistant Staphylococcus aureus in Kermanshah Province, West of Iran. Jundishapur J Microbiol. 2020;13(3):1-7. [DOI:10.5812/jjm.98852]
2. Bitrus AA, Zunita Z, Khairani-Bejo S, Othman S, Nadzir NA. Staphylococcal cassette chromosome mec (SCCmec) and characterization of the attachment site (attB) of methicillin resistant Staphylococcus aureus (MRSA) and methicillin susceptible Staphylococcus aureus (MSSA) isolates. Microb Pathog. 2018;123:323-9. [DOI:10.1016/j.micpath.2018.07.033] [PMID]
3. Zaid Tariq Ahmed R, Mujahid Abdullah R. Prevalence of Multidrug Resistant Staphylococcus aureus and their Pathogenic Toxins Genes in Iraqi Patients, 2022-2023. Iran J Med Microbiol. 2023;17(5):559-70. [DOI:10.30699/ijmm.17.5.559]
4. Uehara Y. Current status of staphylococcal cassette chromosome mec (SCC mec). Antibiotics. 2022;11(1):86. [PMCID] [PMID] [DOI:10.3390/antibiotics11010086]
5. Nandhini P, Kumar P, Mickymaray S, Alothaim AS, Somasundaram J, Rajan M. Recent developments in methicillin-resistant Staphylococcus aureus (MRSA) treatment: a review. Antibiotics. 2022;11(5):606. [PMCID] [DOI:10.3390/antibiotics11050606] [PMID]
6. Ito T, Hiramatsu K, Oliveira D, De Lencastre H, Zhang K, Westh H, et al. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother. 2009;53:4961-7. [DOI:10.1128/AAC.00579-09] [PMID] [PMCID]
7. Shore AC, Coleman DC. Staphylococcal cassette chromosome mec: recent advances and new insights. Int J Med Microbiol. 2013;303(6-7): 350-9. [DOI:10.1016/j.ijmm.2013.02.002] [PMID]

8. Rebić V, Budimir A, Aljicević M, Vranić SM, Rebić D. Panton-Valentine leukocidin and staphylococcal cassette chromosome mec characterization of community acquired methicillin-resistant Staphylococcus aureus. Cent Eur J Public Health. 2019;27(1):58-63. [DOI:10.21101/cejph.a4979] [PMID]

9. Sağlam M, Kılıç İH, Zer Y. Investigation of SCCmec types using the real time PCR method in cefoxitin-resistant Staphylococcus aureus isolates. Indian J Med Microbiol. 2024;50: 100649. [DOI:10.1016/j.ijmmb.2024.100649] [PMID]

10. Ali AN, Hassan T, Elhag W, Abdel Rahim ME, Elkhidir IM, Enan KA. Molecular Characterization of Methicillin-Resistant Staphylococcus aureus Isolated from Khartoum Hospitals. J Bacteriol Mycol. 2020;7(3):1135.

11. John J, Narendrakumar L, Thomas S, Nelson-Sathi S. Hybrid genome assembly and annotation of multidrug-resistant Staphylococcus aureus genotype ST672-SCCmec type IVd (2B). J Glob Antimicrob Resist. 2023;32:74-7. [DOI:10.1016/j.jgar.2022.12.011] [PMID]

12. Huang J, Xiao J, Wang X, Xue X, Ma Y, Zhang Z, et al. Unearthing new ccr genes and staphylococcal cassette chromosome elements in staphylococci through genome mining. J Infect Dis. 2024;230(1):231-8. [DOI:10.1093/infdis/jiae044] [PMID]

13. CLSI. Performance standards for antimicrobial susceptibility testing, M100. 33rd ed. Clinical and Laboratory Standards Institute, Wayne, PA. 2023.

14. Morad N, Misbah S. vicK gene as potential identification marker for Staphylococcus aureus. J Biosci Med. 2018;6(11):98-110. [DOI:10.4236/jbm.2018.611010]

15. Prihandani SS, Wibawan IW, Noor SM, Putri AF, Nuradji H. Phenotypic and Genotypic Identification to S. aureus isolates from Cattle in Bogor. InIOP Conference Series: Earth and Environmental Science. Vol. 1359, No. 1, pp. 012145. 2024. Bristol, United Kingdom: IOP Publishing. [DOI:10.1088/1755-1315/1359/1/012145]

16. Carroll KC, Borek AP, Burger C, Glanz B, Bhally H, Henciak S, et al. Evaluation of the BD Phoenix automated microbiology system for identification and antimicrobial susceptibility testing of staphylococci and enterococci. J Clin Microbiol. 2006;44(6):2072-7. [DOI:10.1128/JCM.02636-05] [PMID] [PMCID]

17. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2005; 43(10):5026-33. [PMID] [PMCID] [DOI:10.1128/JCM.43.10.5026-5033.2005]

18. Oliveira DC, Lencastre HD. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2002;46(7): 2155-61. [PMID] [PMCID] [DOI:10.1128/AAC.46.7.2155-2161.2002]

19. Jiang JH, Cameron DR, Nethercott C, Aires-de-Sousa M, Peleg AY. Virulence attributes of successful methicillin-resistant Staphylococcus aureus lineages. Clin Microbiol Rev. 2023;36(4): e00148-22. [DOI:10.1128/cmr.00148-22] [PMID] [PMCID]

20. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010; 23(3):616-87. [DOI:10.1128/CMR.00081-09] [PMID] [PMCID]

21. Kishk RM, Mandour MF, Saleh RM. Staphylococcal Cassette Chromosome mec (SCCmec) gene typing in detection of methicillin-resistant staphylococcus aureus: toward precise detection in health care facility. Open J Med Microbiol. 2019;9(03):127. [DOI:10.4236/ojmm.2019.93013]

22. Alkharsah KR, Rehman S, Alkhamis F, Alnimr A, Diab A, Al-Ali AK. Comparative and molecular analysis of MRSA isolates from infection sites and carrier colonization sites. Ann Clin Microbiol Antimicrob. 2018;17(1):7. [PMCID] [DOI:10.1186/s12941-018-0260-2] [PMID]

23. Kavitha E, Srikumar R. SCC mec typing among the clinical isolates of Methicillin Resistant Staphylococcus aureus (MRSA) from a Tertiary Care Hospital, Puducherry. Res J Pharm Technol. 2024;17(6):2501-4. [DOI:10.52711/0974-360X.2024.00391]

24. Alfouzan W, Udo EE, Modhaffer A, Alosaimi AA. Molecular characterization of methicillin-resistant Staphylococcus aureus in a tertiary care hospital in Kuwait. Sci Rep. 2019;9(1):

18527. [DOI:10.1038/s41598-019-54794-8] [PMID] [PMCID]

25. Nakano Y, Murata M, Matsumoto Y, Toyoda K, Ota A, Yamasaki S, et al. Clinical characteristics and factors related to infection with SCCmec type II and IV Methicillin-resistant Staphylococcus aureus in a Japanese secondary care facility: a single-center retrospective study. J Glob Antimicrob Resist. 2022;31:355-62. [DOI:10.1016/j.jgar.2022.11.003] [PMID]
26. Arshad F, Saleem S, Tahir R, Jahan S, Jabeen K, Khawaja A, et al. Staphylococcal Cassette Chromosome mec Typing and Multilocus Variable Number Tandem Repeat Analysis of Methicillin Resistant Staphylococcus aureus Clinical Isolates with Vancomycin Creep Phenomenon. Infect Drug Resist. 2022;15: 3927-38. [DOI:10.2147/IDR.S368912] [PMID] [PMCID]
27. Davis SL, Rybak MJ, Amjad M, Kaatz GW, McKinnon PS. Characteristics of patients with healthcare-associated infection due to SCCmec type IV methicillin-resistant Staphylococcus aureus. Infect Control Hosp Epidemiol. 2006; 27(10):1025-31. [DOI:10.1086/507918] [PMID]
28. Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant Staphylococcus aureus SCCmec types I-V. Clin Microbiol Infect. 2007;13(7):725-7. [PMID] [DOI:10.1111/j.1469-0691.2007.01720.x]
29. Trindade PD, Pacheco RL, Costa SF, Rossi F, Barone AA, Mamizuka EM, et al. Prevalence of SCC mec type IV in nosocomial bloodstream isolates of methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2005;43(7):3435-7. [DOI:10.1128/JCM.43.7.3435-3437.2005] [PMID] [PMCID]
30. Dhawan B, Rao C, Udo EE, Gadepalli R, Vishnubhatla S, Kapil A. Dissemination of methicillin-resistant Staphylococcus aureus SCCmec type IV and SCCmec type V epidemic clones in a tertiary hospital: challenge to infection control. Epidemiol Infect. 2015; 143(2):343-53. [PMID] [PMCID] [DOI:10.1017/S095026881400065X]
31. Prakash PH, Rajan V, Gopal S. Predominance of SCCmec types IV and V among biofilm producing device-associated Staphylococcus aureus strains isolated from tertiary care hospitals in Mysuru, India. Enfermedades infecciosas y microbiologia clinica (English: Enferm Infecc Microbiol Clin). 2017;35(4):229-35. [DOI:10.1016/j.eimce.2017.03.005] [PMID]
32. 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. [DOI:10.7883/yoken.64.28][PMID]
33. El-Ashry AH, El-Mahdy RH, Gaballah MA, Talaat R. Staphylococcal cassette chromosome mec (SCCmec) typing and Gentamicin resistance in methicillin-resistant Staphylococcus aureus among children with atopic dermatitis in Egypt. Nov Res Microbiol J. 2022;6(6):1768-82. [DOI:10.21608/nrmj.2022.272044]

Supplementary Table. Reading of DNA QC.

Sample ID	OD 260	OD 280	OD 230	A260/A280	A260/A230	DNA conc. (ng/µL)*
SM32	2.29	1.25	1.09	1.83	2.10	114.7
SM33	2.43	1.30	1.15	1.87	2.12	121.6
SM34	1.95	1.03	0.94	1.90	2.08	97.5
SM35	1.77	1.01	0.89	1.75	1.99	88.3
SM36	2.05	1.15	1.01	1.78	2.02	102.4
SM37	2.18	1.21	1.06	1.80	2.05	109.1
SM38	1.90	1.06	0.95	1.79	2.00	95.2
SM39	2.14	1.16	0.99	1.85	2.15	106.8

***Concentration calculated from OD 260 using the dsDNA factor (50 ng/ µL per OD unit).**