ORIGINAL ARTICLE

Genotypic profiling of methicillin resistance and virulence in MRSA: Subtyping of SCC*mec* type I to V and PVL genes in clinical isolates from tertiary care hospital

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Abstract

Background: Methicillin-Resistant *Staphylococcus Aureus* (MRSA) remains a significant cause of healthcare- and community-associated infections. Molecular characterisation of resistance and virulence determinants is essential to understand its evolving epidemiology. *Material and Methods*: A total of 153 MRSA isolates from a tertiary care hospital were categorised as Community-Acquired (CA-MRSA, 63.3%) or Hospital-Acquired (HA-MRSA, 36.6%) based on clinical criteria. Methicillin resistance was confirmed by cefoxitin disk diffusion. Multiplex PCR was performed for SCCmec (types I–V) and Panton-Valentine Leukocidin (PVL) gene detection. Non-typable isolates (n = 21) underwent additional PCR assays. *Results:* All CA-MRSA isolates harboured *mecA* and *PVL* genes. Among HA-MRSA isolates, 16% were PVL-positive, including 1.3% *PVL*-positive/*mecA*-negative strains suggestive of PVL-positive MSSA. SCCmec type IVa was predominant (73.5%). Non-typable isolates exhibited high genetic variability. *Conclusion:* The co-occurrence of *mecA* and *PVL* genes and detection of PVL in HA-MRSA suggest blurring epidemiological boundaries between CA-MRSA and HA-MRSA. The findings highlight the importance of molecular surveillance and complementary genotyping methods to inform public health interventions against multidrug-resistant MRSA.

Keywords: Methicillin-Resistant Staphylococcus Aureus (MRSA), SCCmec typing, Panton-Valentine Leukocidin (PVL), Hospital-Associated MRSA (HA-MRSA), Community-Associated MRSA (CA-MRSA)

Introduction

Methicillin, introduced in 1959 as the first semisynthetic β-lactam, was developed to counter β-lactamase-producing *Staphylococcus aureus*. However, Methicillin-Resistant *S. aureus* (MRSA) emerged soon after its clinical introduction, marking a turning point in Antimicrobial Resistance (AMR) [1-3]. MRSA resistance is mediated by acquisition of the Staphylococcal Cassette Chromosome mec (SCCmec), a mobile genetic element ranging from 21 to 67 kb, which harbours the *mecA* gene encoding Penicillin-Binding Protein 2a (PBP2a), conferring resistance to nearly all β-lactams [4-7].

Originally a healthcare-associated pathogen, MRSA has evolved with the emergence of Community-associated clones (CA-MRSA), particularly over the past two decades [3,7]. These strains frequently carry smaller, more mobile SCCmec types mainly IV and V facilitating horizontal gene transfer and often encoding the Panton–Valentine leukocidin (PVL), a virulence factor linked to enhanced transmissibility and severe skin and soft tissue infections [3,8]. In contrast, hospital-Associated MRSA (HA-MRSA) typically carries larger, multidrug-resistant SCCmec types I to III.

SCCmec elements are structurally defined by two core components: the *mec* gene complex (*mecA*, IS431, and regulatory genes such as *mecI* and *mecR1*) and the *ccr* gene complex (*ccrA*, *ccrB*, or *ccrC*), which mediate site-specific recombination. These are flanked by variable "junkyard" (J) regions J1, J2, and J3 often containing resistance genes to non-β-lactam antimicrobials and heavy metals, supporting MRSA's adaptive success [5,9].

Although thirteen SCCmec types (I to XIII) have been characterised, types I–V remain the most studied and clinically relevant [5-6]. Conventional Polymerase Chain Reaction (PCR) based typing methods often lack resolution for newer subtypes, particularly types IV and V. Here, we employed a validated multiplex PCR assay [5-6] to detect SCCmec types I to V and key subtypes, alongside PVL gene detection (*lukF-PV*, *lukS-PV*), to explore the genetic landscape of MRSA across healthcare and community settings.

Material and Methods

The study protocol was reviewed and approved by the Independent Institutional Ethics Committee of LNCT Medical College and Sewakunj Hospital, Indore (VU/LNCT/IEC/2024/13). Various clinical samples were processed in the Department of Microbiology, where *S. aureus* was isolated and identified using standard conventional and biochemical methods [8-9]. Phenotypic confirmation of MRSA was performed using the Cefoxitin Disk Diffusion Test [10]. Cefoxitin was chosen over methicillin or oxacillin for this assay due to its greater stability and reliability. Cefoxitin effectively induces the *mec*A gene, which confers methicillin resistance in MRSA. In this test, a cefoxitin disk is placed on an agar plate inoculated with the bacteria to be tested. Resistance is indicated by the growth of bacteria near the disk, with a zone of inhibition ≤21 mm, confirming MRSA. Conversely, a zone of inhibition ≥22 mm suggests susceptibility, indicating MSSA. Results were interpreted following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [11].

Quality control: MRSA Control Strain (e.g., *S. aureus* ATCC 43300), MSSA Control Strain (e.g., *S. aureus* ATCC 25923).

Clinical history and demographic information

Clinical history and demographic data were obtained from the patient's medical records including prior hospitalization, major comorbid conditions (e.g., diabetes mellitus, renal dysfunction, post-surgical status, malignancy, solid organ or stem cell transplantation, neutropenia, trauma, or burn injury), and antibiotic exposure within the preceding year.

Definition and classification of MRSA

MRSA isolates were classified as HA-MRSA if the source patient exhibited any of the following risk factors: a history of hospitalization, residence in a long-term care facility (e.g., nursing home), dialysis, or surgical procedures within one year prior to specimen collection; MRSA growth occurring 48 hours or more after hospital admission; presence of a permanent indwelling catheter or percutaneous device at the time of culture; or a prior positive MRSA culture report. In contrast, if none of these risk factors were present, infections occurring in otherwise healthy individuals who had not been recently hospitalized or undergone medical procedures were categorized as CA-MRSA. CA-MRSA infections typically manifest as skin infections, such as abscesses or boils [12-14].

Confirmation of genomic DNA integrity

The quality and integrity of genomic DNA (gDNA) extracted from all 153 MRSA isolates were evaluated by electrophoresis on 1% (w/v) agarose gels prepared in Tris-acetate-EDTA (TAE) buffer. DNA was visualised using ethidium bromide staining under Ultraviolet (UV) transillumination. High molecular weight, intact DNA without visible degradation was considered suitable for downstream molecular analysis. Representative electrophoretic profiles are shown in Figure 1, with lanes 1–8 depicting gDNA from selected MRSA isolates.

SCCmec typing by multiplex PCR

Multiplex PCR was performed to detect the mecA gene and classify SCCmec elements into types I–V, including subtypes IVa to IVd, using primer sets and optimised reaction conditions described previously by Zhang *et al*. and Jadhav *et al*. [5,12]. PCR reactions were carried out in 25 µl volumes containing appropriate concentrations of each primer (Table 1), with thermal cycling parameters and reagent concentrations adapted from the referenced protocols. A no-template control was included in each run to ensure specificity and rule out contamination. PCR products were resolved by electrophoresis on 1.5% (w/v) agarose gels in TAE buffer, stained with ethidium bromide, and visualised under UV illumination using a gel documentation system.

Supplementary SCCmec typing and detection of mecA and PVL genes

Twenty-one MRSA isolates that remained non-typeable using the Zhang *et al*. [5] primer scheme were further characterised by multiplex PCR using primer sets described by Oliveira *et al*. [6]. Primers were synthesised commercially (Sigma), and PCR was conducted following the general methodology of Zhang *et al*. and Jadhav *et al*. [5,12], with optimised primer concentrations to enhance amplification specificity.

Detection of mecA and PVL genes by multiplex PCR

All 153 MRSA isolates were screened for the presence of the mecA gene and PVL genes using a validated multiplex PCR assay. Primer sequences were adapted from Zhang *et al*. for mecA [5] and Lina *et al*. for PVL [13], and synthesised by Sigma. DNA extraction, thermal cycling conditions, and primer annealing parameters were performed as described by Jadhav et al. [3,12, 14]. Reference strains of MRSA and MSSA were included as positive and negative controls to ensure assay validity and reproducibility.

Results

Of the 153 phenotypically confirmed MRSA isolates, 97 (63.3%) were initially classified as CA-MRSA and 56 (36.6%) as HA-MRSA based on clinical metadata. All isolates, confirmed mecA-positive by PCR, were subjected to molecular characterisation to delineate transmission dynamics and refine epidemiological classification. SCCmec typing was performed using multiplex PCR with eight primers grouped to optimise amplicon resolution, as per Zhang *et al*. [5] Screening with Group 1 primers identified subtype IVa (776 bp) in 16 isolates (36%) and type II (398 bp) in 6 isolates (13%), while subtype IVc (200 bp) was not detected.

All 153 samples demonstrated positive amplification for the mecA gene using the mec147A forward and reverse primer set, producing an amplified product of 147 bp (Figure 2). For the typing and subtyping analysis of MRSA isolated

from clinical samples, genomic DNA (gDNA) from these 153 isolates, which had previously shown the presence of the mecA gene by PCR (Figure 1), was selected for further analysis as detailed in our earlier work.

SCCmec typing by multiplex PCR

Multiplex PCR using Group 1 primers targeting SCCmec subtypes IVa (776 bp), II (398 bp), and IVc (200 bp) yielded specific amplicons in multiple MRSA isolates. Lane L contained a 100 bp DNA ladder; Lane N served as the non-template control. Distinct bands corresponding to IVa and II were observed in isolates M8–M31, MR1–MR4, and M47–M14 (Figure 2). No amplification was detected for subtype IVc.

Group 2 primers, targeting subtypes IVb (493 bp) and III (280 bp), produced no detectable amplicons, indicating their absence in all tested isolates. In contrast, screening with Group 3 primers identified 9 isolates (5.8%) positive for SCCmec type V (325 bp). No amplification was observed for types I (613 bp) or IVd (881 bp) (Figure 3).

Typing efficiency of SCCmec-specific multiplex PCR

Of the 153 MRSA genomic DNA samples screened using eight primer sets targeting SCCmec types I to V (Table 1), 132 (86.2%) were successfully typed into defined types and subtypes. The remaining 21 isolates (13.7%) were non-typeable using the Zhang *et al.* scheme. [5] These were further analysed using an alternative multiplex PCR assay incorporating eight primer sets described by Oliveira *et al*. (Table 2), combined in a single reaction mixture to improve type resolution. [6]

As shown in Figure 4, multiplex PCR with the eight primer sets (Oliveira *et al*. [6]) identified dual amplicons in 15 MRSA gDNA samples (9.8%). A 342 bp amplicon (types I, II, IV) alongside a 381 bp band indicated type IA. Sample C7 yielded a 243 bp amplicon, confirming type III. Five isolates (3.2%) exhibited mixed typing patterns (Figure 5). Isolate M33 showed multiple unclassifiable amplicons; MR6 and MR9 produced bands consistent with types IA and II; M6 showed bands

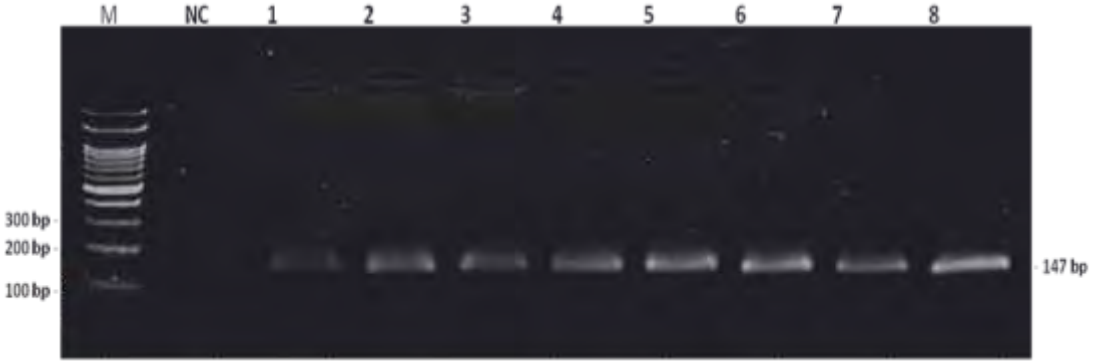

Figure 1: Detection of mecA by single-target PCR. Agarose gel electrophoresis (2% w/v) showing amplification of the mecA gene (147 bp). Lane M: 3 kb DNA marker; Lane NC: negative control; Lanes 1–8: representative MRSA isolates positive for mecA using mecA147 forward and reverse primers.

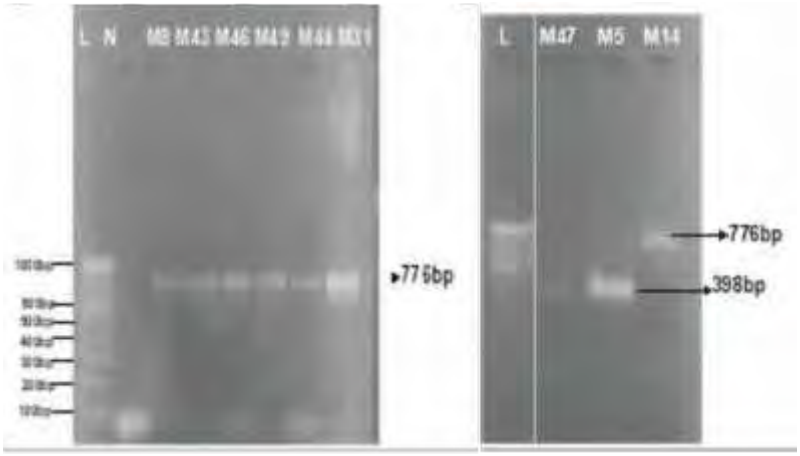

Figure 2: Multiplex PCR using Group 1 primer sets for the detection of subtypes IVa (776 bp), II (398 bp), and IVc (200 bp). L: 100 bp DNA ladder; N: Non-template control; M8-M31, MR1-MR4, M47-M14: Amplicons from multiplex PCR with MRSA genomic

aligning with types I, II, and IV. All isolates were successfully typed, with type IVa and type IA being the most common subtype and type, respectively (Table 1; Figure 5).

Of the 153 MRSA isolates analysed, 97 (73.5%) were identified as SCCmec subtype IVa. Based on phenotypic criteria consistent with established CA-MRSA definitions, and further supported by genotypic confirmation (Figure 6), these isolates were classified as CA-MRSA (Figure 6).

Amplification of mecA and PVL Genes by PCR

PCR parameters were optimised using genomic DNA from MRSA and MSSA reference strains. Amplification products were visualised on a 1.8% agarose gel. The mecA gene was amplified exclusively from MRSA DNA, while PVL was detected in both strains, with amplicon sizes matching expected lengths. The MRSA reference strain, positive for both targets, was subsequently used as the positive control for patient isolate testing (Figure 6).

Of the 153 MRSA isolates analysed, 104 (69.2%) harboured the PVL gene, while 2 isolates lacked the mecA gene, indicating MSSA strains carrying PVL. The gene distribution was as follows: 44 isolates (28.7%) were mecA-positive/PVL-negative; 2 isolates (1.3%) were PVL-positive/mecA-negative; 104 isolates (67.9%) were positive for both genes; and 3 isolates (1.9%) were negative for both mecA and PVL (Figure 8). The optimised multiplex PCR conditions were further employed to amplify mecA and PVL from genomic DNA extracted from MRSA isolates of 54 clinical samples, yielding distinct amplicons as shown in (Figure 6).

Panel 9a: PVL gene amplification on 1% agarose gel. Lane 1: reference MRSA; Lanes 2–3: clinical isolates; Lane 4: Non-Template Control (NTC).

Panel 9b: mecA gene amplification on 1.8% agarose gel. Lane 1: reference MRSA; Lanes 2–3: clinical isolates; Lane 4: NTC.

Table 1 summarises the SCCmec typing and subtyping results of 153 MRSA isolates. Among the 132 typable isolates (Zhang *et al.* [5] method), subtype IVa was predominant (n = 97; 73.5%), followed by subtype II (n = 26; 19.7%) and subtype

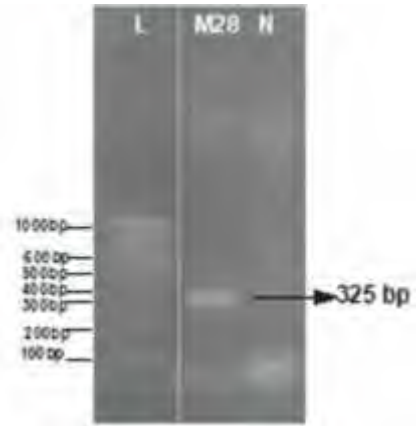

Figure 3: Multiplex PCR with Group 3 primers. Lane L: 100 bp ladder; N: Non-template control; M28: MRSA gDNA showing 325 bp amplicon for type V

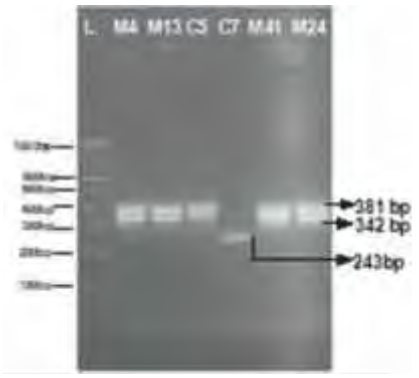

Figure 4: Multiplex PCR with eight primer sets for MRSA typing. Lane L: 100 bp DNA ladder; M4–M24, M16–M42, M25–M29: MRSA gDNA showing type-specific amplicons.

V (n = 9; 6.8%). Of the 21 non-typable isolates, subsequent analysis using the Oliveira *et al.* [6] method classified 15 (71.4%) as type IA, 1 (4.7%) as type III, and 5 (23.8%) as mixed types.

Of the 153 MRSA isolates analysed, the distribution of mecA and PVL gene presence was as follows: 44 isolates (28.7%) were positive for mecA but negative for PVL; 2 isolates (1.3%) were positive for

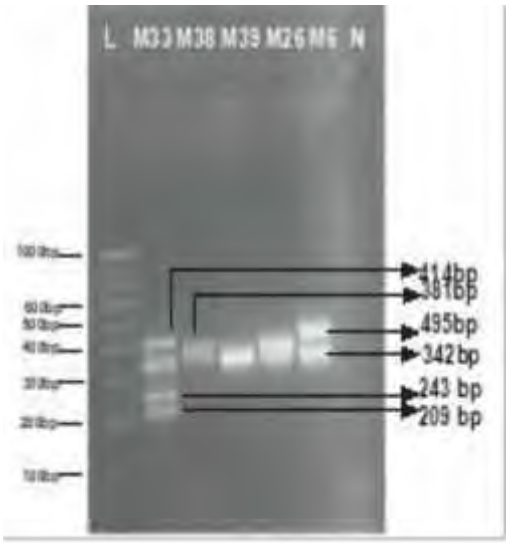

Figure 5: Typing analysis of MRSA using multiplex PCR with eight primer sets. L: 100 bp DNA ladder; M33-M6, MR6-MR9: Amplicons from gDNA of MRSA isolates from clinical samples.

PVL but negative for mecA; 104 isolates (67.9%) were positive for both mecA and PVL; and 3 isolates (1.9%) were negative for both genes (Table 2)

Discussion

This study characterised 153 MRSA isolates to determine SCCmec types I toV and assess the distribution of mecA and PVL genes, aiming to correlate methicillin resistance with PVL-associated virulence.

Of these, 97 (63.3%) were classified CA-MRSA and 56 (36.6%) as HA-MRSA based on medical records and phenotypic confirmation. All CA-MRSA isolates harboured both mecA and PVL, while 9 HA-MRSA isolates carried PVL, of which 2 lacked mecA, representing genotypic MSSA. This genotypic convergence between CA-MRSA and HA-MRSA suggests an evolving epidemiological scenario in which PVL-positive, highly virulent strains may increasingly emerge in healthcare settings, posing

enhanced risks for resistance dissemination. Among the 132 isolates typable using the method by Zhang *et al*. [5], SCCmec subtype IVa predominated

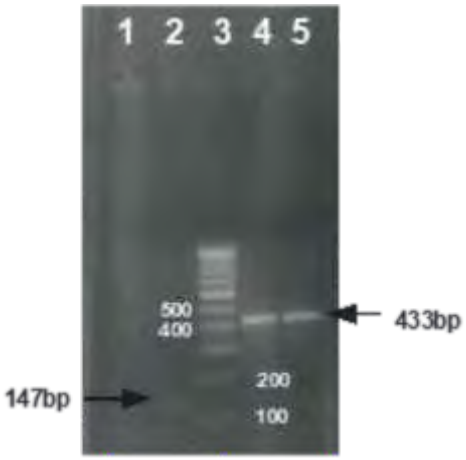

Figure 6: PCR amplification of mecA (147 bp) and PVL (433 bp) genes from reference MSSA and MRSA strains. Lane 3: 100 bp ladder; Lanes 1–2: mecA amplicons; Lanes 4–5: PVL amplicons.

(73.48%), followed by subtype II (19.69%) and subtype V (6.82%). The 21 non-typable isolates were resolved using the method by Oliveira *et al*. [6], which identified subtype IA in 71.4%, mixed subtypes in 23.8%, and subtype III in 4.7%. These findings align with earlier reports [5-6], although the prevalence of subtype II appears to vary regionally, as also noted by Makgotlho *et al*. [20]. The predominance of SCCmec IVa, a subtype frequently associated with CA-MRSA, reinforces its widespread global distribution, while the diversity among non-typable isolates highlights the need for multiple complementary molecular approaches for accurate typing.

Comparable observations from Western Nepal reported PVL in 90.4% of CA-MRSA and 7.1% of HA-MRSA [14-16], consistent with our findings. Similar patterns of community-associated genotypes infiltrating healthcare environments and exhibiting heightened virulence and multidrug resistance have been reported in India, Ireland, and Finland [17-19]. Such epidemiological shifts underscore the necessity for vigilant surveillance to track MRSA strain movement across community and hospital boundaries.

The gene distribution in our isolates revealed that 104 (67.9%) were positive for both mecA and PVL, 44 (28.7%) for mecA only, 2 (1.3%) for PVL only, and 3 (1.9%) were negative for both. Co-positivity was notably higher than reported by Muto *et al*. (45%) and Daum *et al*. (38%) [26-27]. The mecA only proportion in our study (28.7%) closely matched those of Muto et al. (30%) [26] and Daum *et al*. (23%) [27], while the PVL only rate (1.3%) was markedly lower than the 5–6% in earlier reports [26-27]. The double-negative rate (1.9%) was substantially below the 33–35% previously observed [26-27]. Similar comparisons with Frazee *et al*. [28] and Cassat and Thomsen [29] demonstrated consistent patterns for mecA only isolates but significantly lower rates of PVL only and double-negative isolates in our population. These discrepancies may reflect geographical variability in MRSA epidemiology or methodological differences in sampling and molecular detection.

The high prevalence of mecA and PVL co-positivity in both CA-MRSA and selected HA-MRSA isolates supports the hypothesis of an ongoing convergence between resistance and virulence traits. This convergence challenges traditional distinctions between CA- and HA-MRSA and may necessitate revised classification frameworks. Goudarzi *et al*. [22] emphasised the importance of integrating

Table 1: Typing and subtyping analysis of 153 MRSA isolates

Subtype	Positive Isolates	Percentage (%)
IV a (Zhang *et al.*)	97/132	73.48
II (Zhang *et al.*)	26/132	19.69
V (Zhang *et al.*)	9/132	6.8
Total 132 typable by Zhang *et al*.		
I A (Oliveira *et al.*)	15/21	71.4
III (Oliveira *et al.*)	1/21	4.7
Mixed (Oliveira *et al.*)	5/21	23.8
Total 21non typable by Zhang *et al*. [5] were typed by Oliveira *et al*. [6]		

Table 2: Presence of mecA and PVL genes in genomic DNA of MRSA isolated from clinical samples of 153 patients

mecA/PVL presence	Total number of isolates	Percentage (%)
***mecA* (+ve), *PVL* (-ve)**	44	28.7
***mecA* (-ve), *PVL* (+ve)**	2	1.3
mecA* (+ve), *PVL	104	67.9
***mecA* (-ve), *PVL* (-ve)**	3	1.9
Total	153	100

Multilocus Sequence Typing (MLST), SCCmec typing, and spa typing to identify strains with overlapping genetic signatures of CA- and HA-MRSA. Frazee *et al*. [28] underscored the clinical relevance of PVL in skin and soft tissue infections, while Cassat and Thomsen [29] highlighted the role of comprehensive molecular typing in elucidating the pathogenicity resistance interplay in MRSA.

The diversity observed in non-typable isolates with 71.4% subtype IA, 23.8% mixed, and 4.7% subtype III emphasises the limitations of single-method SCCmec typing. Giulieri *et al*. [21] demonstrated that genomic approaches can uncover adaptive mutations and resistance determinants undetectable by conventional methods. Likewise, Makgotlho *et al*. [20] and subsequent studies [23–25] have shown the value of alternative molecular techniques for identifying subtypes overlooked by standard assays. This reinforces the need for an integrative genotyping approach, combining SCCmec typing with high-resolution genomic tools to fully capture MRSA diversity.

The predominance of SCCmec IVa in our setting, together with a high co-occurrence of mecA and PVL, indicates a dynamic MRSA population with significant public health implications. These traits, particularly when combined in HA-MRSA, may facilitate the spread of highly virulent, multidrug-resistant strains in healthcare environments. Such strains not only complicate infection control but also increase the likelihood of treatment failure.

In summary, this study reveals (i) SCCmec IVa dominance with regional variation in subtype II prevalence; (ii) high mecA–PVL co-positivity, including in HA-MRSA; and (iii) subtype diversity in non-typable isolates requiring complementary molecular methods. Together, these findings highlight an evolving MRSA epidemiology marked by the convergence of resistance and virulence determinants, blurring traditional epidemiological boundaries. This warrants robust molecular surveillance, integration of multiple genotyping techniques, and adaptive public health strategies encompassing antimicrobial stewardship, targeted infection control, and continuous genomic monitoring.

Conclusion

This study of 153 Staphylococcus aureus isolates demonstrates the predominance of SCCmec subtype IVa (73.48%) and a notable co-occurrence of mecA and PVL genes (67.9%), reflecting the convergence of methicillin resistance and virulence determinants. The presence of PVL in HA-MRSA underscores a shifting epidemiology in which the traditional distinctions between community- and hospital-associated lineages are increasingly indistinct. The genetic heterogeneity observed, including non-typable isolates, highlights the limitations of single-locus typing and the need for complementary molecular approaches. These findings reinforce the necessity of integrated molecular surveillance, rigorous antimicrobial stewardship, and context-specific infection control measures. Elucidating the genetic drivers of resistance–virulence convergence will be essential for the development of effective therapeutic and preventive interventions.

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