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Original Research Article

Molecular identification of SCCmec lineages in methicillin resistant *Staphylococcus aureus* from a tertiary care hospital

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Abstract

Aim and Objective: The aim of this study is to evaluate and analyses the mecA gene and its lineages in methicillin resistant *Staphylococcus aureus* isolates from tertiary care hospital.

Material and Methods: This is a cross-sectional molecular study conducted at tertiary care hospital Vijayapur. A total of 152 *S.aureus* isolates were collected from clinical specimens and screened for methicillin resistance using the cefoxitin disc diffusion method, following Clinical and Laboratory Standards Institute guidelines. Molecular characterization of SCCmec types was performed through polymerase chain reaction to detect the mecA gene and its associated subtypes. Demographic data, including age and sex, were analysed to identify trends and correlations in MRSA prevalence.

Results: Among the 152 isolates 55(36%) were identified as MRSA. Molecular analysis revealed SCCmec type IVa as the most predominant subtype, comprising 72.7% of the MRSA isolates. Other subtypes, including SCCmec types I, II, III and IVb, were observed with prevalence rates of 3.6%, 10.9% 7.3% and 5.5% respectively.

Conclusion: This study highlighted the dominance of SCCmec type IVa among MRSA isolates, indicating the likely incursion of community- associated MRSA strains into healthcare settings. The findings underscored the genetic diversity and adaptability of MRSA, presenting significant challenges for infection control. These results emphasise the urgent need for enhanced surveillance robust infection control measures, and optimized antimicrobial stewardship to stop the impact of MRSA in healthcare environments.

Keywords: *Staphylococcus aureus*, Methicillin-resistant" SCCmec lineages, Drug resistance, Infection control, Polymerase chain reaction.

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1. Introduction

The rise of methicillin-resistant *Staphylococcus aureus* continues to be a critical concern in public health, particularly within hospital environments, where it frequently leads to severe and hard-to-treat infections. Central to MRSA resistance mechanism is the staphylococcal cassette chromosome mec, a genetic element responsible for conferring resistance to beta-lactam antibiotics.[1] The diversity and adaptability of SCCmec lineages play a Pivotal role in the pathogen's evaluation and its ability to spread across healthcare settings. By conducting a molecular investigation of SCCmec lineages from clinical specimens in tertiary care hospital, this study seeks to uncover valuable insights into their genetic variations and epidemiological patterns.[2]

In hospital settings, MRSA remains a major contributor to healthcare-associated infections, including bloodstream infections, Pneumonia, and surgical site infections. Tertiary care hospitals serve as hotspots for MRSA transmission due to their high patient turnover and the presence of vulnerable patient populations.[3] The interplay of environmental, clinical, and microbial factors in such settings often results in a diverse array of MRSA strains, carrying SCCmec elements of varying complexity. Larger SCCmec types (e.g, types I, II, III) are associated with hospital-acquired MRSA, while smaller, more mobile types (e.g, types IV and V) are community-acquired MRSA.[4] Studying these genetic

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variations within healthcare facilities is critical for identifying transmission patterns and designing targeted.

The SCCmec element exhibits remarkable variability in size, genetic composition and structural organization, leading to its classification into distinct types and subtypes. These variations have profound implications for the clinical and epidemiological characteristics of MRSA strains, influencing their transmissibility, virulence, and outbreak potential.[5] The classification of SCCmec is based on the combination of the mec complex, which determines antibiotic resistance, and the CCR (cassette chromosome recombinase) complex, responsible for the mobility of the elements.[6] Molecular characterization of these elements provides critical insights into the evolutionary pathways and adaptive mechanisms of MRSA, facilitating a deeper understanding of its global and local epidemiology.[7]

Tertiary care hospital represent an ideal environment for molecular epidemiological studies aimed at characterizing SCCmec diversity. These institution often encounter a mix endemic and imported MRSA strains providing a unique opportunity to investigate the distribution and genetic diversity of SCCmec lineages. Such studies are essential not only for understanding the genetic makeup of MRSA in specific settings but also for informing clinical decision-making and optimising antimicrobial stewardship programs.[8-10]

Advancements in molecular techniques, such as polymerase chain reaction and whole-genome sequencing, have revolutionized the field of molecular epidemiology. These tools enable precise identification and characterization of SCCmec elements, facilitating the detection of novel or atypical variants and their potential role in resistance transmission. By elucidating the genetic relationships between MRSA strain and mechanisms underlying their resistance, these methodologies offer valuable insights into the horizontal transfer of resistance genes.[11,12] This study aims to investigate the molecular diversity of SCCmec lineages in MRSA isolates collected from a tertiary care hospital. Through this targeted approach, the study seeks to uncover the distribution patterns of SCCmec types, explore their association with clinical and demographic factors, and assess their impact on resistance profiles.

2. Materials and Methods

The samples were collected from a tertiary care centre. This investigation was designed as a cross-sectional molecular epidemiological study conducted in a tertiary care hospital setting. Combining conventional cefoxitin disc diffusion methods with PCR molecular techniques, the study systematically characterised the SCCmec subtypes and analysed their prevalence. By correlating these findings with demographic and clinical variables, the study provided valuable insights into the transmission dynamics of MRSA. This comprehensive approach aims to support evidence-based infection control practices and optimize antimicrobial stewardship programs addressing the pressing challenge of methicillin resistance in healthcare environments.

2.1. Inclusion criteria

The inclusion criteria for this study were meticulously designed to ensure the selection of clinically significant *Staphylococcus aureus* isolates with confirmed methicillin resistance. Only isolates identified as methicillin-resistant *S.aureus* based on the cefoxitin disc diffusion assay, according to the standards set by the Clinical and Laboratory Standards Institute, were included. Specifically, isolates exhibiting inhibition zone diameter of less than 22mm were classified as MRSA and considered eligible.

2.2. Exclusion criteria

Isolates of *Staphylococcus aureus* that did not meet the criteria for methicillin resistance based on the cefoxitin disc diffusion method.

Non MRSA isolates or any isolates not meeting the study's strict diagnostic standards.

2.3. Data collection

This study was conducted at a prominent tertiary care centre, where clinical specimens like pus, blood, exudates, urine samples were collected to isolate *Staphylococcus aureus*. Suspected colonies of *S.aureus* were cultured on blood agar, followed by precise identification using Gram staining and standard biochemical assays including catalase and coagulase tests. From this screening process, 152 isolates were confirmed as *S.aureus*. Percentage of various samples from which *S.aureus* was isolated are Pus 75%, blood 23%, and exudates 36% urine 18%. These isolates served as the foundational data set for advanced molecular characterisation.

2.4. Screening of MRSA by cefoxitin disc diffusion method

S.aureus isolates which showed resistant to cefoxitin (30mg) according to CLSI guidelines (CLSI performance standards for Antimicrobial susceptibility Testing. 33rd CLSI supplement M100 clinical and Laboratory Standards Institute; 2023) were screened as MRSA. Further the screened MRSA isolates were subjected to molecular method i.e. Polymerase chain reaction for detection of mecA gene.

2.5. Data analysis

Total bacterial DNA extraction

The bacterial DNA extraction will be performed using a modified phenol-based DNA extraction method (Sambrook & Ressell, 2001) with 5µL of lysozyme (50mg ml^{-1}) (Roche molecular Diagnostics, Germany) 50µL of 20% sodium dodecyl sulphate stock solution (Promega Corporation) and 10µL of proteinase K (20gm ML^{-1}) (Finnzymes, south

Africa) *Staphylococcus aureus* DNA was then re-suspended in 20µL of TE.

The molecular analysis was done by M- polymerase chain reaction (PCR) techniques to detect the presence of the mecA gene and its associated subtypes within the isolates. The isolates were systematically categorized into SCCmec types I, I, III, IVa and IVb based on their genetic composition.

A volume of 2.5µL of the extracted DNA was added to 22.5µL PCR reaction mixture. The reaction mixture contained 12.5µL of the Qiagen Multiplex PCR Master mix (Promega Corporation). The Qiagen Multiplex PCR Master mix (Promega Corporation) consisted of HotstarTaq DNA polymerase supplied in 10x PCR buffer with 3mM MgCl2 and 400µM of each dNTP (Promega Corporation). The assay included six set of primers prepared in a 10x primer mix. Five microliters of the primer mix was added to the Qiagen M-PCR Mastermix. In addition, 2.5µL of the Qiagen Q-solution was added to the reaction mixture. To obtain a final volume of 25µL, 2.5µL of RNAse-Free water supplied with the Qiagen M-PCR Mastermix kit was added to the reaction mixture.

The M-PCR amplification was performed using a PX2 Thermal cycler (Thermo Electron Corporation) with an initial denaturation step at 94°C for 15 min followed by 10 cycles of 94°C for 30s, 60◦C for 90s and extension at 72°C for 90s followed by another 25 cycles of 94°C FOR 45s, annealing at 55˚C for 45s, extension at 72°C for 90s and a final extension step at 72°C for 10 min. The PCR amplification was performed using a perkin Elner thermocycler (Thermo Electron Corporation) with an initial activation step at 94°C for 5 min followed by 30 cycles of denaturation at 92°C for 30s annealing at 52°C for 40s and extension at 72°C for 5 min.

Prevalence and distribution data were analysed across demographic variables such as age and gender, offering critical insights into the epidemiological patterns and genetic variability of *S.aureus* strains. Statistical tools were applied to contextualize the findings within the broader scope of infection control and antimicrobial resistance trends.

2.6. Sequence of oligonutleotide primers

Target Gene Primer Nucleotide sequence (5'-3') Amplicon (bp)
MecA gene MecA1 GTA GAA ATG ACT GAA CGT CCG ATA A 310
MecA2CCA ATT CCA CAT TGT TTC GGT CTAA
SCCmec I I-F GCT TTA AAG AGT GTC GTT ACA GG 613
I-R GTTCTCTCATAGTATGACGTC
SCCmec II II-F CGTTGAAGATGATGAAGCG 398
II-R CGAAATCAATGGTTAATGGAC
SCCmec III III-F CCATATTGTGTACGATGCG 280
III-R CCTTAGTTGTCGTAACAGATC
SCCmec IVa IVa-F GCCTTATTCGAAGAAACCG 776
IVa-R CTACTCTTCTGAAAAGCGTCG
SCCmec IVb IVb-F TCTGGAATTACTTCAGCTGC 493
IVb-R AAACAATATTGCTCTCCCTC

3. Results

A total of 200 isolates were collected from the clinical samples Among them 55 isolates showed positive results for Methicllin resistant *Staphylococcus aureus* with a Zone measuring <22mm according to CLSI recommendations, shows that 29.4% were in the age group of >60 yrs, 52%(n-78) were found to posses mecA gene and various subtypes like SCCmec type I, II, III, IVa, IVb on molecular typing.

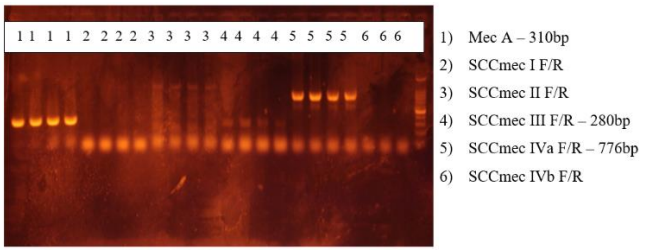

Figure 1:

This figure depicts molecular findings detailing the amplification of specific DNA fragments during the characterization of SCCmec elements and the mecA gene. The mecA gene fragment was successfully amplified, producing a size of 310 base pairs, confirming its presence in the MRSA isolates analysed. Similarly, SCCmec type two yielded a fragment size of 398 bp, indicative of its structural characteristics. Further differentiation within SCCmec type IV was achieved, with type IVa generating a 776 bp fragments, though appearing as a faint band, while type IVb produced a distinct fragment of 493 bp. These results, derived through PCR- based amplification, provided crucial insights into the genetic diversity and structural variations of SCCmec elements in the studied MRSA strains. Such findings are integral to advancing the understanding of the molecular epidemiology of MRSA and its resistance mechanism.

Table 1: Prevalence of SCC gene among MRSA

SCCmec Type	Numbers	Prevalence in %
I	2	3.6 %
II	6	10.9
III	4	7.3%
IVa	40	72.7%
IVb	3	5.5%
Total	55	100

The **Table 1** illustrated the distribution and prevalence of various SCCmec types identified among MRSA isolates, providing valuable insights into their epidemiological significance. SCCmec type I was detected in 2 isolates, representing a low prevalence of 3.6%, indicating its rare

occurance. Type II accounted for 10.9% of the isolates, being identified in 6 cases, while type III was observed in 4 isolates, comprising 7.3% of the total population. Notably, the majority of the isolates carried SCCmec type IVa, which was overwhelmingly dominant, being found in 40 isolates and constituting a significant 72.7% of the total. In contrast, SCCmec type IVb was identified in 3 isolates, with a prevalence of 5.5%. These findings underscored the predominance of SCCmec type IVa among the MRSA strains studied, highlighting its critical role in the molecular epidemiology and potential transmission dynamics of MRSA within the analysed cohort.

Table 2: Distribution of *S.aureus* according to age

Age	Number	Percentage %
1-20	37	24.3 %
21 -40	34	22.4%
41-60	38	25.0 %
>60	43	28.3 %
Total	152	100

Table 2 provides a detailed overview of the age-wise distribution of MRSA isolates within the study cohort. The highest prevalence was prevalence was observed among individuals aged 60 years and above, comprising 44 isolates (28.3% of the total). The 41-60 age group followed with 38 isolates (25.3%), indicating a significant burden within middle- aged to elderly to populations, The younger age groups, 1-20 years and 21-40 years, accounted for 34 isolates (23.3%) and 33 isolates (22.0%), respectively. Overall, the dataset included 152 isolates, representing a comprehensive age distribution. These results underscore the disproportionate impact of MRSA infections on older individuals, emphasizing the critical need for age specific infection prevention and control strategies in healthcare settings.

Table 3: Distribution of *S.aureus* according to sex

Sex	Number	Percentage
Male	80	52.6%
Female	72	47.4%
Total	152	100%

This **Table 3** provides an in depth analysis of the distribution of MRSA isolates by gender within the study population. Males accounted for a slightly higher proportion of cases, with 80 isolates representing 52.6% of the total. Females contributed 72 isolates, making up 47.4% of the cases. In total, 152 MRSA isolates were analysed, reflecting a near- equitable distribution between genders. While the prevalence among males was marginally higher, this finding emphasises the need for further investigation into potential gender- related biological, behavioural, or environmental factors influencing the prevalence and transmission dynamics of MRSA in healthcare settings.

Age and gender specific classification is important because it minimise mutations, sustain lineages genotypic properties and allow for resistance pattern comparisons and confirmations.

4. Discussion

This study provided significant insights into the molecular epidemiology of *Staphylococcus aureus* and the distribution of SCCmec lineages in a tertiary care hospital setting. The molecular typing revealed that SCCmec subtype IVa was the most dominant, accounting for an overwhelming 72.7% of the MRSA isolates analysed. This remarkable prevalence of SCCmec type IVa, traditionally associated with community-acquired MRSA, indicated a potential infiltration of community-associated strains into the hospital environment. Such findings underscored the dynamic and evolving nature of MRSA transmission and highlighted the critical need for robust surveillance systems to track these trends.

The high prevalence of SCCmec type IVa was consistent with its smaller, more mobile genetic structure, which facilitated horizontal gene transfer and enhanced adaptability. This adaptability likely contributed to its widespread presence and ability to thrive in both community and healthcare settings. Other SCCmec subtypes, including types I, II, III, and IVb, were detected at lower frequencies, with prevalence rates of 3.6% 10.9%, 7.3%, and 5.5% respectively. The presence of these subtypes demonstrated the considerable genetic diversity of MRSA strains circulating in the hospital, which posed challenges for infection control and treatment strategies.

Demographic analysis revealed that MRSA infections were slightly more prevalent among males (52%) than females (48%). Moreover, Ahmad et al., older individuals, particularly those aged 60 years and above, exhibited the highest prevalence of MRSA, accounting for 28.3% of the cases. These findings aligned with existing literature, suggesting that older populations and males were more susceptible to MRSA infections due to factors such as compromised immune systems.[13]

Furthermore, a study by Karahan et al, similarly identified SCCmec type IV as the most prevalent subtype, with a reported frequency of 65%.[9] This finding reinforced the adaptability of the smaller, more mobile SCCmec type IVa, which facilitated horizontal gene transfer and contributed to its widespread dissemination.[14] In comparison, the 72.7% prevalence of SCCmec type IVa in this study underscored its critical role in MRSA transmission dynamics in the Indian healthcare context. Other SCCmec types, including l (3.6%), II (10.9), III (7.3%), and IVb (5.5%), were detected at lower frequencies, consistent with global trends. Larger SCCmec elements, such as types I, II, and III have been commonly associated with HA-MRSA, as reported in a study by Stranden et al. in China, where SCCmec type III was identified as the predominant subtype in hospital settings.[15]

These findings illustrated the significant genetic diversity of MRSA isolates and highlighted regional differences in SCCmec type distribution.[16]

Demographic analysis revealed that MRSA infections were slightly more prevalent among males (52.6%) than females (47.4%), mirroring the findings of Bollet et al, who also observed a gender disparity in MRSA prevalence.[17] Additionally, individuals aged 60 years and above exhibited the highest prevalence of MRSA (28.3%), a trend consistent with the findings of Bollet et al, who attributed this increased susceptibility to factors such as advanced age, comorbidities, prolonged hospital stays, and weakened immune responses.

The observed predominance of SCCmec type lVa, along with the demographic trends, emphasised the need for enhanced infection control strategies tailored to the specific challenges posed by MRSA. Clinical and infection prevention implication regarding the spread of Sccmec IVa includes strengthened antimicrobial stewardship programs, Siddiqui et al., creating awareness among public regarding hygiene practices can help prevent the spread of these strains. Targeted surveillance particularly in community settings can help identify and control outbreaks of these strains.[18] The potential overlap between CA-MRSA and hospital-acquired MRSA (HA-MRSA) strains further highlighted the necessity of integrating community and healthcare-based prevention efforts to mitigate the transmission of MRSA effectively.

5. Conclusion

This study contributed valuable insights into the genetic and epidemiological characteristics of SCCmec lineages in MRSA isolates. The dominance of SCCmec type IVa, the observed genetic heterogeneity, and the demographic patterns highlighted the complex challenges posed by MRSA infections. These findings underscored the pressing need for sustained research, innovative approaches, and coordinated efforts to address the ongoing threat of antibiotic resistance in healthcare settings.

6. Ethical Approval

This study was approved by Institute ethical committee with ref. no. BLDE (DU)/IEC/809/2022-23.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

1. Lakhundi S, Zhang K. Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev*. 2018;31(4):e00020–18.
2. Makgotlho PE, Kock MM, Hoosen A, Lekalakala R, Omar S, Dove M, et al. Molecular identification and genotyping of MRSA isolates. *FEMS Immunol Med Microbiol*. 2009;57(2):104–15.
3. Ekinci B, Edgunlu TG, Bayramoglu G, Atay GU. Molecular investigation of staphylococcal cassette chromosome mec types and genotypic relations of methicillin-resistant staphylococci isolated from before and after hospital exposed students. *Indian J Med Microbiol*. 2018;36(2):257–64.
4. Abebe AA, Birhanu AG. Methicillin Resistant Staphylococcus aureus: Molecular Mechanisms Underlying Drug Resistance Development and Novel Strategies to Combat. *Infect Drug Resist.* 2023;16:7641–62.
5. Uehara Y. Current Status of Staphylococcal Cassette Chromosome *mec* (SCC *mec*). *Antibiotics (Basel)*. 2022;11(1):86.
6. Miragaia M. Factors contributing to the Evolution of *mecA*-Mediated β-lactam resistance in staphylococci: update and new insights from whole genome sequencing (WGS). *Front Microbiol*. 2018;9:2723.
7. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI document. Wayne, PA, USA: Clinical Laboratory Standards Institute; 2017.
8. 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.
9. Karahan ZC, Tekeli A, Adaleti R, Koyuncu E, Dolapci I, Akan OA. Investigation of Panton-Valentine leukocidin genes and SCC mec types in Clinical Staphylococcus aureus isolates from Turkey. *Microb Drug Resist*. 2008;14(3):203–10.
10. Aggrawal S, Jena S, Panda S, Sharama S, Dhawan B, Nath G, et al. Antibiotic susceptibility, virulence pattern, and typing of Staphylococcus aureus strains isolated from variety of infections in India. *Front Microbiol*. 2019;10:2763.
11. Ullah A, Tara T, Maqbool A, Zeb MA, Jamal SF, Saidal A. Staphylococcus cassette chromosome mec typing of methicillin resistant Staphylococcus aureus strains prevailing in Hayatabad Medical Complex, Peshawar. *Rehman J Health Sci*. 2022;4(1):20–5.
12. Kumar S, Anwer R, Azzi A. Molecular typing methods & resistance mechanisms of MDR *Klebsiella pneumoniae*. *AIMS Microbiol*. 2023;9(1):112–30.
13. Ahmad N, Ruzan IN, Abd Ghani MK, Hussin A, Nawi S, Aziz MN, et al. Characteristics of Community- and hospital-acquired methicillin-resistant Staphylococcus aureus strains carrying Scc mec typeIV isolated in Malaysia. *J Med Microbiol.* 2009;58(9):1213–8.
14. Raheema RH, Abed KA. Molecular identification of Virulence genes of Staphylococcus aureus isolated from different clinical isolates. *Indian J Public Health Res Dev*. 2019;10(2):212–8.
15. Stranden AM, Frei R, Adler H, Fluckiger U, Widmer AF. Emergence of Sccmec type IV as the most common type of methicillin-resistant Staphylococcus in a university hospital. *Infection*. 2009;37(1):44-8.
16. Vieira G, Leal N, Rodrigues A, Chaves C, Rodrigues F, Osorio N. MRSA/MSSA causing infections: prevalence of *mecA* gene. *Eur J Public Health*. 2020;30(Suppl 2):ckaa040-052.
17. Bollet C, Gevaudan MJ, de Lamballerie X, Zandotti C, de Micco P. A simple method for the isolation of chromosomal DNA from gram positive or acid-fast bacteria. *Nucleic Acids Res*. 1991;19(8):1955.
18. Siddique A, Mohmood S, Thair S, Tariq I, Shabbir CA, Arfat Y. Characterization and Prevalence of Antibiotic Resistance Staphylococcus aureus in Street Food: A Public Health Concern. *TSF J Biol.* 2024;2(2):5–20.

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