



## Original Research Article

# MOLECULAR EPIDEMIOLOGY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN A TERTIARY CARE HOSPITAL: AN OBSERVATIONAL STUDY

Arun Aravind<sup>1</sup>, Divya M B<sup>2</sup>, R.C. Krishna Kumar<sup>3</sup>, L Ravichandran<sup>4</sup>

<sup>1</sup>Associate Professor, PK Das Institute of Medical Sciences, Kerala, India.

<sup>2</sup>Associate Professor, PK Das Institute of Medical Sciences, Kerala, India.

<sup>3</sup>Medical Director, PK Das Institute of Medical Sciences, Kerala, India.

<sup>4</sup>Professor, PK Das Institute of Medical Sciences, Kerala, India.

Received : 10/07/2024  
Received in revised form : 08/09/2024  
Accepted : 23/09/2024

### Corresponding Author:

**Dr. L Ravichandran,**  
Professor, PK Das Institute of Medical Sciences, Kerala, India.  
Email: ravichandranlara71@gmail.com

DOI: 10.70034/ijmedph.2024.3.180

Source of Support: Nil,  
Conflict of Interest: None declared

**Int J Med Pub Health**  
2024; 14 (3); 1001-1005

### ABSTRACT

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a persistent global health concern due to its role in hospital-acquired infections (HAIs) and its resistance to commonly used antibiotics, particularly beta-lactams. The emergence of MRSA clones in healthcare settings presents a significant challenge to infection control, leading to high morbidity, mortality, and increased healthcare costs. **Purpose:** This observational study explores the molecular epidemiology of MRSA within a tertiary care hospital, identifying the prevalent MRSA strains, molecular characteristics, and potential risk factors associated with colonization and infection.

**Materials and Methods:** A cross-sectional observational study was conducted over one year at a tertiary care hospital. Clinical specimens were obtained from patients with suspected MRSA infections across various wards. Standard microbiological methods confirmed MRSA presence. Molecular typing, including pulsed-field gel electrophoresis (PFGE) and staphylococcal cassette chromosome mec (SCCmec) typing, was performed to identify genetic relationships between strains. STROBE guidelines were strictly adhered to in designing and reporting the study. Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method.

**Results:** Of the 500 clinical specimens collected, 150 were identified as MRSA-positive. Molecular analysis revealed three dominant MRSA clones circulating within the hospital, with SCCmec type IV accounting for the majority of isolates. Prolonged hospitalization, antibiotic use, and ICU admissions emerged as significant risk factors for MRSA acquisition. All isolates exhibited high resistance to commonly used antibiotics, with 100% susceptibility to vancomycin.

**Conclusion:** This study underscores the importance of continuous molecular surveillance of MRSA to control its spread in healthcare settings. Identifying dominant clones and associated risk factors is vital for tailoring infection control measures and optimizing antibiotic stewardship.

**Keywords:** molecular epidemiology, Methicillin-Resistant, *Staphylococcus Aureus*, Treatment Measures.

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a significant pathogen responsible for a wide range of infections, particularly in

hospital environments. Its ability to acquire resistance to beta-lactam antibiotics, including methicillin, has created a persistent challenge in healthcare settings.<sup>[1-3]</sup> MRSA contributes significantly to the global burden of HAIs, resulting

in prolonged hospital stays, increased healthcare costs, and adverse patient outcomes. Despite various infection control programs and antibiotic stewardship initiatives, MRSA continues to thrive in healthcare settings due to its capacity for rapid genetic adaptation and clonal dissemination.<sup>[4,5]</sup> Understanding the molecular epidemiology of MRSA is critical to improving hospital infection control measures and preventing outbreaks.<sup>[6]</sup> The ability to identify prevalent MRSA clones and their transmission dynamics can inform targeted interventions and optimize patient care. The present study seeks to provide an in-depth examination of the molecular epidemiology of MRSA in a tertiary care hospital, with a focus on identifying prevalent strains, their molecular characteristics, and the risk factors associated with colonization and infection.

## MATERIALS AND METHODS

This study was designed and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines, ensuring rigorous data collection, analysis, and interpretation. The observational nature of the study allowed for the comprehensive evaluation of MRSA epidemiology within the hospital setting.

### Study Design and Setting

This cross-sectional observational study was conducted over a one-year period (August 2023 - August 2024) in Department of Microbiology, P K Das Institute of Medical Sciences. The hospital caters to a diverse population, including critical care, medical, surgical, and outpatient services. The study was conducted across all hospital wards, with a particular focus on the Intensive Care Unit (ICU), general medical wards, and surgical wards, where MRSA infections are most prevalent.

### Population and Sample

The study population consisted of all patients admitted to the hospital during the study period who presented with clinical signs of infection and were suspected of harboring *Staphylococcus aureus*. Inclusion criteria included patients of all ages, sexes, and comorbid conditions who had not previously been diagnosed with MRSA. Patients who received treatment in the hospital for less than 48 hours were excluded, as were those who had recent antibiotic exposure unrelated to the suspected infection.

A total of 500 clinical samples were collected from these patients. The samples included blood, wound swabs, pus, respiratory secretions, and urine. Ethical approval was obtained from the institutional review board (IRB), and informed consent was obtained from all participants or their legal guardians.

### Microbiological Procedures

Microbiological identification of *Staphylococcus aureus* was carried out using Gram staining, catalase testing, and coagulase tests. Confirmation of methicillin resistance was conducted using the

cefoxitin disc diffusion method following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). MRSA isolates were confirmed by the presence of the *mecA* gene, detected through polymerase chain reaction (PCR).<sup>[6]</sup>

### Molecular Typing

Pulsed-field gel electrophoresis (PFGE) was used to establish clonal relationships among MRSA isolates. The genomic DNA of each MRSA isolate was digested using *SmaI* restriction enzyme, and the resulting fragments were separated using PFGE. Banding patterns were analyzed and compared to identify distinct clones circulating within the hospital. Additionally, staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed to determine the types of SCC*mec* elements present in each MRSA isolate. PCR was utilized for SCC*mec* typing, focusing on the identification of types I-V, which are commonly associated with healthcare-associated MRSA strains.<sup>[7]</sup>

### Data Collection and Variables

Demographic data (age, gender), clinical information (comorbidities, previous hospitalizations, and antibiotic use), and hospitalization characteristics (ward, length of stay, and ICU admission) were collected from patient records. Data on infection outcomes, including complications, treatment, and discharge status, were also recorded. The primary outcome was the identification of MRSA colonization or infection. Secondary outcomes included the determination of MRSA prevalence and identification of molecular characteristics of the isolates.

### Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method, with results interpreted based on CLSI guidelines. The antibiotics tested included cefoxitin, clindamycin, erythromycin, tetracycline, ciprofloxacin, gentamicin, linezolid, and vancomycin. The minimum inhibitory concentration (MIC) for vancomycin was determined using the E-test method. The resistance profiles of MRSA isolates were analyzed to identify multidrug-resistant strains and assess the effectiveness of current antibiotic regimens.<sup>[4]</sup>

### Statistical Analysis

Data analysis was performed using SPSS software version 25. Descriptive statistics, including means, medians, frequencies, and percentages, were used to summarize the data. The prevalence of MRSA was calculated as the proportion of MRSA-positive samples among all clinical samples collected. Chi-square tests and logistic regression analysis were conducted to determine the significance of risk factors associated with MRSA colonization or infection. P-values <0.05 were considered statistically significant.

## RESULTS

### Patient Demographics and Clinical Characteristics

A total of 500 patients were enrolled in the study, and 150 patients (30%) were confirmed to have MRSA infections. The median age of MRSA-positive patients was 61 years (IQR: 45-75), with a slight male predominance (58%). Most of these patients presented with multiple comorbidities, such as diabetes (45%), chronic obstructive pulmonary disease (COPD) (27%), and chronic kidney disease (21%). Table 1 summarizes the demographic and clinical characteristics of MRSA-positive patients.

### MRSA Prevalence and Specimen Source

The study identified MRSA in 150 (30%) of the clinical specimens, distributed across various departments of the hospital. Wound swabs were the most common sample type (40%), followed by blood cultures (30%) and respiratory secretions (20%). The MRSA-positive cases were heavily concentrated in the ICU (68%), with medical and surgical wards accounting for 18% and 14%, respectively. The distribution of MRSA-positive specimens across different sources is presented in Table 2.

### Molecular Typing

Molecular typing of MRSA isolates using PFGE revealed the presence of three major clones (A, B, and C). Clone A was the most prevalent, accounting for 48% of the isolates, followed by Clone B (36%) and Clone C (16%). Further SCCmec typing showed that the majority of isolates (67%) belonged to SCCmec type IV, with SCCmec type II (21%) and type V (12%) also detected. The results of the molecular typing are detailed in Table 3.

### Risk Factors for MRSA Acquisition

Significant risk factors for MRSA colonization and infection were identified through multivariable logistic regression. Prolonged hospital stay (>14 days) was the strongest predictor (OR: 3.5; 95% CI: 2.1-5.9), followed by prior antibiotic use within six months (OR: 2.9; 95% CI: 1.8-4.7), and ICU admission (OR: 5.2; 95% CI: 3.1-8.6). Comorbidities such as diabetes, COPD, and chronic kidney disease were common among MRSA patients, although they did not emerge as statistically significant predictors in the adjusted model. Table 4 presents the results of the risk factor analysis.

### Antibiotic Resistance Patterns

All MRSA isolates were subjected to antibiotic susceptibility testing (AST). The results revealed high resistance rates to erythromycin (90%), ciprofloxacin (85%), and gentamicin (78%). Resistance to clindamycin (60%) and tetracycline (55%) was also common. Importantly, no vancomycin-resistant isolates were identified, and all MRSA isolates remained susceptible to linezolid and vancomycin. The full antibiotic susceptibility profiles of the MRSA isolates are detailed in Table 5.

### MRSA Outcomes

Outcomes of patients with MRSA infections were also analyzed. The majority of MRSA-positive patients required intensive medical care, with 42% needing mechanical ventilation, and 25% developed sepsis during their hospital stay. The overall in-hospital mortality rate among MRSA patients was 18%. The outcome analysis is summarized in Table 6.

**Table 1: Patient Demographics and Clinical Characteristics (n = 150)**

Characteristic	n (%)
Age (median, IQR)	61 (45-75)
Male	87 (58%)
Diabetes mellitus	68 (45%)
COPD	40 (27%)
Chronic kidney disease	32 (21%)
ICU Admission	102 (68%)
Average hospital stay (days, range)	24 (10-60)

**Table 2: Distribution of MRSA-Positive Specimens by Source (n = 150)**

Specimen Source	n (%)
Wound Swabs	60 (40%)
Blood Cultures	45 (30%)
Respiratory Secretions	30 (20%)
Urine	15 (10%)

**Table 3: Molecular Typing of MRSA Isolates (n = 150)**

PFGE Clone	n (%)
Clone A	72 (48%)
Clone B	54 (36%)
Clone C	24 (16%)
SCCmec Type	
Type IV	101 (67%)
Type II	32 (21%)
Type V	17 (12%)

**Table 4: Multivariable Analysis of Risk Factors for MRSA Acquisition**

Risk Factor	OR (95% CI)
Prolonged Hospital Stay (>14 days)	3.5 (2.1-5.9)
Prior Antibiotic Use (within 6 months)	2.9 (1.8-4.7)
ICU Admission	5.2 (3.1-8.6)
Age > 65 years	1.4 (0.8-2.3)
Diabetes Mellitus	1.6 (0.9-2.7)
COPD	1.3 (0.7-2.1)
Chronic Kidney Disease	1.7 (0.8-2.8)

**Table 5: Antibiotic Susceptibility Patterns of MRSA Isolates (n = 150)**

Antibiotic	Resistant (%)
Erythromycin	135 (90%)
Ciprofloxacin	128 (85%)
Gentamicin	117 (78%)
Clindamycin	90 (60%)
Tetracycline	83 (55%)
Linezolid	0 (0%)
Vancomycin	0 (0%)

**Table 6: Clinical Outcomes of MRSA-Positive Patients (n = 150)**

Outcome	n (%)
Mechanical Ventilation	63 (42%)
Development of Sepsis	38 (25%)
Length of Stay > 30 Days	29 (19%)
In-Hospital Mortality	27 (18%)

## DISCUSSION

The results of this study highlight the continuing burden of MRSA in healthcare settings, particularly in tertiary care hospitals. The molecular epidemiology of MRSA in this study demonstrated the dominance of three clones, with clone A being the most prevalent. SCCmec typing further indicated the predominance of type IV, which is typically associated with healthcare-associated MRSA (HA-MRSA). The presence of clone A in such a significant proportion of isolates suggests a localized outbreak that requires immediate infection control measures.<sup>[7,8]</sup>

Risk factors associated with MRSA acquisition in this study were consistent with previous reports, with prolonged hospital stays and ICU admission being the most significant predictors. ICU patients often present with critical conditions and require invasive procedures, making them more susceptible to colonization and infection by MRSA [9,10].

Antibiotic resistance patterns among MRSA isolates confirmed the ongoing challenge of treating MRSA infections, with high levels of resistance to commonly used antibiotics. However, the absence of vancomycin resistance is a positive finding, ensuring that this antibiotic remains a viable option for treating MRSA infections in this setting.<sup>[11]</sup>

## CONCLUSION

In conclusion, the high prevalence of MRSA in this tertiary care hospital underscores the need for enhanced infection control practices, including stringent hand hygiene, antimicrobial stewardship, and continuous molecular surveillance to prevent

further transmission of MRSA and improve patient outcomes.

## REFERENCES

- Lakhundi S, Zhang K. Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev.* 2018 Sep 12;31(4):e00020-18. doi: 10.1128/CMR.00020-18. PMID: 30209034; PMCID: PMC6148192.
- David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev.* 2010 Jul;23(3):616-87. doi: 10.1128/CMR.00081-09. PMID: 20610826; PMCID: PMC2901661.
- Tkadlec J, Capek V, Brajerova M, Smelikova E, Melter O, Bergerova T, Polivkova S, Balejova M, Hanslianova M, Fackova D, Neradova K, Tejkalova R, Vagnerova I, Bartonikova N, Chmelarova E, Drevinek P, Krutova M. The molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Czech Republic. *J Antimicrob Chemother.* 2021 Jan 1;76(1):55-64. doi: 10.1093/jac/dkaa404. PMID: 33118033.
- Tomao P, Pirolo M, Agnoletti F, Pantosti A, Battisti A, Di Martino G, Visaggio D, Monaco M, Franco A, Pimentel de Araujo F, Palei M, Benini N, Motta C, Bovo C, Di Renzi S, Vonesch N, Visca P. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* from dairy farms in North-eastern Italy. *Int J Food Microbiol.* 2020 Nov 2; 332:108817. doi: 10.1016/j.ijfoodmicro.2020.108817. Epub 2020 Aug 6. PMID: 32777624.
- Moreno Mochi P, Vargas JM, Vivaldo S, Bottiglieri M, López C, Mochi S, Cobos M, Castillo M, Del Campo R, Jure MA. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* from different population groups in Argentina. *J Glob Antimicrob Resist.* 2020 Dec; 23:82-86. doi: 10.1016/j.jgar.2020.07.016. Epub 2020 Aug 5. PMID: 32763358.
- Sánchez-Serrano A, García-González N, Bonillo D, Ruiz-Hueso P, Villanova R, Campo-Bes I, Tormo N, Salvador C, Gimeno C, González-Candelas F. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Tertiary Hospital from the Comunidad Valenciana (Spain). *Microb Drug Resist.* 2022 Dec;28(12):1071-1078. doi:

- 10.1089/mdr.2022.0027. Epub 2022 Oct 11. PMID: 36251890.
7. Wang WY, Chiu CF, Lee YT, Hsueh PR, Tsao SM. Molecular epidemiology and phenotypes of invasive methicillin-resistant vancomycin-intermediate Staphylococcus aureus in Taiwan. *J Microbiol Immunol Infect.* 2022 Dec;55(6 Pt 2):1203-1210. doi: 10.1016/j.jmii.2021.09.003. Epub 2021 Oct 1. PMID: 34635425.
  8. Blanc DS, Grandbastien B, Bally F, Lienhard R, Tritten ML, Clerc O, Fracheboud D, Pfister S, Chuard C, Burr M, Schmiedel Y, Liassine N, Jost G, Togni G, Di Lorenzo V, Jayol A, Prod'hom G, Greub G, Senn L. Staphylococcus aureus résistant à la méticilline : 15 ans de surveillance moléculaire en Suisse romande [Methicillin-resistant Staphylococcus aureus: 15 years of molecular epidemiology in Western Switzerland]. *Rev Med Suisse.* 2022 Apr 13;18(777):724-728. French. doi: 10.53738/REVMED.2022.18.777.724. PMID: 35417102.
  9. Otto M. Community-associated MRSA: what makes them special? *Int J Med Microbiol.* 2013 Aug;303(6-7):324-30. doi: 10.1016/j.ijmm.2013.02.007. Epub 2013 Mar 19. PMID: 23517691; PMCID: PMC3729626.
  10. Dotel R, O'Sullivan MVN, Davis JS, Newton PJ, Gilbert GL. Molecular epidemiology of methicillin-resistant Staphylococcus aureus isolates in New South Wales, Australia, 2012-2017. *Infect Dis Health.* 2019 Aug;24(3):134-140. doi: 10.1016/j.idh.2019.04.002. Epub 2019 May 13. PMID: 31097401.
  11. Pereira-Franchi EPL, Barreira MRN, da Costa NSLM, Riboli DFM, Abraão LM, Martins KB, Victória C, Cunha MLRSD. Molecular epidemiology of methicillin-resistant Staphylococcus aureus in the Brazilian primary health care system. *Trop Med Int Health.* 2019 Mar;24(3):339-347. doi: 10.1111/tmi.13192. Epub 2019 Jan 11. PMID: 30549385.