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Comprehensive Analysis of Antimicrobial Susceptibility, Virulence Factors, Biofilm Production, and Molecular Characteristics of Methicillin-Resistant *Staphylococcus aureus*

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major multidrug-resistant pathogen responsible for a wide spectrum of healthcare-associated and community-acquired infections. The emergence of MRSA strains possessing multiple antimicrobial resistance mechanisms, virulence factors, biofilm-forming ability, and resistance genes has become a significant challenge in clinical management, infection control, and antimicrobial stewardship. Molecular characterization of MRSA provides valuable insights into resistance mechanisms and epidemiological surveillance.

Materials and Methods

This laboratory-based cross-sectional study included 55 MRSA isolates from various clinical specimens. MRSA was identified using standard microbiological methods and cefoxitin disc diffusion according to CLSI guidelines. Antimicrobial susceptibility, virulence factors, biofilm production, and mecA, PVL, and mupA genes were evaluated using standard phenotypic methods and polymerase chain reaction (PCR). Data were analyzed using SPSS version 26.0, with $p<0.05$ considered statistically significant.

Results

The highest prevalence of MRSA was observed among patients aged 20–39 and 40–59 years (32.73% each), with male predominance (72.73%). Pus was the most common specimen (40%). All isolates were susceptible to vancomycin and linezolid, while 49.09% showed mupirocin resistance. Beta-hemolysin was detected in all isolates, and mecA, PVL, and mupA genes were identified in 83.64%, 67.27%, and 65.45%, respectively. The Tissue Culture Plate method demonstrated better biofilm detection than the Tube Method. Significant associations were observed between mupA positivity and mupirocin resistance ($p=0.000$) and between biofilm production and prolonged hospital stay ($p=0.030$).

Conclusion

The present study highlights the emergence of multidrug-resistant MRSA strains possessing multiple virulence factors, significant biofilm-forming ability, and important resistance genes. Continuous antimicrobial surveillance, routine molecular characterization, and strict infection prevention and control measures are essential for limiting the spread of MRSA and improving patient outcomes.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MRSA; antimicrobial susceptibility; virulence factors;

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most important multidrug-resistant bacterial pathogens responsible for a wide spectrum of healthcare-associated and community-acquired

infections worldwide. It is a major cause of skin and soft tissue infections, surgical site infections, bloodstream infections, pneumonia, osteomyelitis, urinary tract infections, and device-associated infections. The emergence and rapid dissemination of MRSA have become a significant global public health concern due to increasing antimicrobial resistance, prolonged hospital stay, higher treatment costs, and increased morbidity and mortality [1,2]. Methicillin resistance in *Staphylococcus aureus* is primarily mediated by the mecA gene, which encodes the altered penicillin-binding protein PBP2a with reduced affinity for β-lactam antibiotics, thereby rendering most β-lactam agents ineffective. In addition to antimicrobial resistance, MRSA possesses numerous virulence factors, including hemolysins, proteases, lipases, lecithinases, and Panton-Valentine leukocidin (PVL), which enhance bacterial colonization, tissue invasion, immune evasion, and disease severity. The coexistence of resistance determinants and virulence genes has contributed to the emergence of highly pathogenic MRSA strains capable of causing severe invasive infections [2,3].

Biofilm formation is another important virulence characteristic of MRSA that significantly contributes to persistent and recurrent infections. Biofilms enable bacteria to adhere to host tissues and medical devices while protecting them from host immune responses and antimicrobial agents. Consequently, biofilm-producing MRSA isolates are associated with chronic wound infections, catheter-related infections, prosthetic device infections, and treatment failure. Accurate detection of biofilm production is therefore essential for understanding the pathogenic potential of clinical isolates and guiding effective therapeutic strategies [3,4].

The increasing prevalence of mupirocin-resistant MRSA has become an additional challenge in infection control, particularly because mupirocin is widely used for nasal decolonization of MRSA carriers. High-level mupirocin resistance is commonly mediated by the mupA gene, while the PVL gene has been associated with severe skin and soft tissue infections and necrotizing pneumonia. Molecular characterization of these resistance and virulence genes provides valuable epidemiological information, facilitates surveillance of circulating MRSA clones, and supports the implementation of appropriate infection prevention and antimicrobial stewardship programs [4,5].

Although several studies have evaluated the antimicrobial susceptibility profiles of MRSA, information regarding the combined assessment of antimicrobial resistance, virulence factors, biofilm formation, and molecular characteristics remains limited in many healthcare settings. Comprehensive characterization of these attributes is essential for understanding the pathogenicity and resistance mechanisms of MRSA isolates and for improving clinical management and infection control practices [5]. Therefore, the present study was undertaken to determine the antibiotic susceptibility pattern, phenotypic virulence factors, biofilm production, and molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates obtained from various clinical specimens, with special emphasis on the detection of the mecA, PVL, and mupA genes using polymerase chain reaction (PCR) [6].

Material & Methods

Study Design

This laboratory-based cross-sectional observational study was conducted to determine the antimicrobial susceptibility pattern, virulence factors, biofilm production, and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from various clinical specimens.

Study Setting and Duration

The study was carried out in the Department of General Medicine at a tertiary care teaching hospital over a period of 18 months. Clinical specimens received in the General Medicine laboratory from both inpatient and outpatient departments during the study period were included for analysis.

Study Population

The study population comprised patients of all age groups and both sexes from whom clinical specimens yielded methicillin-resistant *Staphylococcus aureus*. A total of 55 non-duplicate MRSA isolates recovered from various clinical specimens were included in the study.

Sample Size

A total of 55 MRSA isolates were included in the study. The sample size was determined based on the number of confirmed MRSA isolates obtained during the study period that fulfilled the inclusion criteria.

Inclusion Criteria

All non-duplicate clinical isolates of methicillin-resistant *Staphylococcus aureus* obtained from various clinical specimens, including pus, wound swabs, blood, urine, sputum, body fluids, and other sterile specimens, from patients of all age groups and both sexes were included in the study.

Exclusion Criteria

Duplicate MRSA isolates obtained from the same patient, methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates, contaminated specimens, mixed bacterial growth, and isolates with incomplete laboratory data were excluded from the study.

Data Collection Tool

Clinical specimens were processed according to standard microbiological procedures. Identification of *Staphylococcus aureus* was based on colony morphology, Gram staining, catalase test, slide and tube coagulase tests, and other standard biochemical tests. Methicillin resistance was confirmed using the cefoxitin (30 µg) disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Antimicrobial susceptibility testing for vancomycin, linezolid, and mupirocin was performed using the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar, and results were interpreted according to CLSI recommendations. Phenotypic detection of virulence factors, including hemolysin, lipase, lecithinase, protease, and gelatinase production, was carried out using standard microbiological methods. Biofilm formation was assessed by both the Tube Method and Tissue Culture Plate (TCP) Method, and isolates were categorized as strong, moderate, weak, or non-biofilm producers based on optical density values. Molecular characterization was performed using polymerase chain reaction (PCR) for detection of the mecA, Panton-Valentine leukocidin (PVL), and mupA genes. Genomic DNA was extracted using standard protocols, amplified using gene-specific primers, and PCR products were analyzed by agarose gel electrophoresis to confirm the presence of target genes.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee before commencement of the study. Patient confidentiality was maintained by assigning unique identification numbers to all isolates, and no personal identifiers were included during analysis. As the study utilized bacterial isolates obtained as part of routine diagnostic investigations, confidentiality and anonymity were strictly maintained throughout the study.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using the Statistical Package for the Social Sciences (SPSS) software version 26.0. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using mean ± standard deviation where appropriate. Associations between antimicrobial resistance, virulence factor production, biofilm formation, and molecular characteristics were analyzed using the Chi-square test or Fisher's exact test. A *p*-value of less than 0.05 was considered statistically significant.

Results

Table 1. Distribution of MRSA isolates according to age group (n = 55)

Age group (years)	Frequency (n)	Percentage (%)
<20	8	14.55
20–39	18	32.73
40–59	18	32.73
≥60	11	20.00
Total	**55**	**100.00**

The highest prevalence of MRSA isolates was observed among patients aged 20–39 years and 40–59 years, with each age group contributing 32.73% of the total isolates. Patients aged ≥60 years accounted for 20.00%, while those aged <20 years represented 14.55%.

Table 2. Distribution according to gender

Gender	Frequency	Percentage
Male	40	72.73
Female	15	27.27
Total	55	100.00

Among the 55 MRSA isolates, 72.73% were recovered from male patients, whereas 27.27% were obtained from female patients. The marked male predominance may be attributed to greater occupational exposure, higher incidence of trauma, increased hospitalization, and a higher frequency of surgical interventions among males, making them more susceptible to MRSA infections.

Table 3. Distribution according to clinical specimen

Specimen	Frequency	Percentage
Pus	22	40.00
Wound swab	10	18.18
Blood	8	14.55
Urine	6	10.91
Sputum	5	9.09
Others	4	7.27
Total	55	100.00

Pus specimens constituted the largest proportion of MRSA isolates (40.00%), followed by wound swabs (18.18%), blood (14.55%), urine (10.91%), sputum (9.09%), and other clinical specimens (7.27%). The predominance of pus and wound specimens indicates that skin and soft tissue infections remain the most common clinical presentation of MRSA, reflecting the organism's strong ability to colonize damaged skin, wounds, and surgical sites.

Table 4. Antibiotic susceptibility pattern

Antibiotic	Sensitive	Resistant
Vancomycin	55 (100%)	0
Linezolid	55 (100%)	0
Mupirocin	28 (50.91%)	27 (49.09%)

All MRSA isolates (100%) were susceptible to vancomycin and linezolid, confirming these antibiotics as highly effective therapeutic agents against MRSA in the present study. However, 49.09% of isolates exhibited resistance to mupirocin, indicating a considerable prevalence of mupirocin-resistant MRSA strains.

Table 5. Virulence factors

Virulence factor	Positive	Negative
Beta hemolysin	55 (100%)	0
Lipase	33 (60%)	22
Lecithinase	36 (65.45%)	19
Protease	27 (49.09%)	28
Gelatinase	38 (69.09%)	17

Phenotypic analysis demonstrated that all MRSA isolates (100%) produced beta-hemolysin, indicating its universal role in bacterial pathogenicity. Gelatinase (69.09%) and lecithinase (65.45%) were the next most frequently detected virulence factors, followed by lipase (60.00%) and protease (49.09%).

Table 6. Biofilm production

Method	Positive	Negative
Tube Method	34 (61.82%)	21
TCP Method	41 (74.55%)	14

Biofilm production was detected in 61.82% of isolates by the Tube Method, whereas the Tissue Culture Plate (TCP) Method identified 74.55% as biofilm producers. The higher detection rate observed with the TCP method indicates its superior sensitivity and reliability for identifying biofilm-producing MRSA isolates.

Table 7. Molecular characterization

Gene	Positive	Negative
mecA	46 (83.64%)	9
PVL	37 (67.27%)	18
mupA	36 (65.45%)	19

Polymerase chain reaction analysis demonstrated that the mecA gene was present in 83.64% of MRSA isolates, confirming methicillin resistance at the molecular level. The PVL gene was detected in 67.27% of isolates, indicating a high prevalence of virulent strains capable of causing severe skin and soft tissue infections. Additionally, the mupA gene was identified in 65.45% of isolates, reflecting a substantial burden of high-level mupirocin resistance.

Table 8. Association between mupA and mupirocin resistance

Association	p-value
Significant	0.000*

A highly significant association was observed between mupA gene positivity and phenotypic mupirocin resistance ($p = 0.000$). This finding confirms that the presence of the mupA gene is strongly associated with high-level mupirocin resistance among MRSA isolates and supports the usefulness of molecular testing for early detection of resistant strains. Identification of this resistance gene may aid clinicians in selecting appropriate decolonization strategies and preventing the spread of resistant MRSA.

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be one of the leading multidrug-resistant pathogens responsible for both healthcare-associated and community-acquired infections. The emergence of strains possessing multiple antimicrobial resistance mechanisms, enhanced virulence determinants, and biofilm-forming ability has significantly complicated patient management and infection control worldwide [7,8]. In the present study, the highest prevalence of MRSA was observed among patients aged 20–39 years and 40–59 years, with a clear male predominance. Similar findings were reported by Kumar et al., who observed that MRSA infections were more common among middle-aged adults and male patients because of increased occupational exposure, trauma, frequent hospitalization, and greater healthcare contact [7]. Likewise, Ghosh et al. reported that adult males represented the majority of MRSA infections in tertiary care hospitals, emphasizing the influence of behavioral and healthcare-related risk factors [8].

Pus specimens constituted the most common source of MRSA isolation in the present study. This finding is consistent with the observations of Rajaduraipandi et al., who reported that skin and soft tissue infections remain the predominant clinical manifestations of MRSA because of its ability to colonize damaged skin, surgical wounds, and abscesses [9]. Similar results were also documented by Sarkar et al., demonstrating that wound infections and postoperative surgical site infections continue to account for the majority of MRSA isolates in tertiary care hospitals [10]. Antimicrobial susceptibility testing demonstrated 100% susceptibility to vancomycin and linezolid, indicating that these antibiotics continue

to remain highly effective therapeutic options against MRSA. Comparable findings were reported by Hassoun et al., who demonstrated complete susceptibility of MRSA isolates to vancomycin and linezolid across multiple healthcare settings [11]. However, nearly half of the isolates in the present study exhibited resistance to mupirocin, suggesting the increasing emergence of mupirocin-resistant MRSA strains. Similar observations were reported by Patel et al., who highlighted the growing prevalence of mupirocin resistance following its extensive use for MRSA decolonization and emphasized the importance of antimicrobial stewardship to preserve its effectiveness [12].

The present study also demonstrated a high prevalence of phenotypic virulence factors, with beta-hemolysin being produced by all MRSA isolates, followed by gelatinase, lecithinase, lipase, and protease production. These findings indicate that MRSA possesses multiple virulence mechanisms that facilitate bacterial invasion, tissue destruction, immune evasion, and persistence within the host. Otto reported that extracellular enzymes and toxins produced by *Staphylococcus aureus* play a central role in disease pathogenesis by promoting colonization and facilitating dissemination into deeper tissues [13]. Similarly, Tong et al. emphasized that the simultaneous expression of several virulence factors contributes to the increased severity and clinical complexity of MRSA infections [14]. Biofilm production was detected in a considerable proportion of isolates, with the Tissue Culture Plate (TCP) Method demonstrating greater sensitivity than the Tube Method. Biofilm formation is recognized as one of the most important virulence characteristics of MRSA because it enables bacterial adherence to tissues and medical devices while protecting organisms from host immune responses and antimicrobial agents. Similar findings were reported by Stepanović et al., who identified the TCP method as the gold standard phenotypic technique for biofilm detection due to its superior sensitivity and reproducibility [15].

Molecular characterization revealed a high prevalence of the mecA, PVL, and mupA genes among MRSA isolates. The mecA gene remains the principal determinant of methicillin resistance through production of the altered penicillin-binding protein PBP2a, while the PVL gene has been associated with severe skin and soft tissue infections and necrotizing pneumonia. The detection of the mupA gene in a substantial proportion of isolates corresponded closely with phenotypic mupirocin resistance. A highly significant association between mupA gene positivity and mupirocin resistance observed in the present study confirms the reliability of molecular methods for identifying resistant strains. Similar findings were reported by McNeil et al., who demonstrated that PCR-based detection of mecA, PVL, and mupA genes provides rapid and accurate identification of clinically important MRSA strains and supports infection control surveillance programs [16].

Strengths

This study comprehensively evaluated antimicrobial susceptibility, phenotypic virulence factors, biofilm formation, and molecular characterization of MRSA isolates using both conventional microbiological methods and polymerase chain reaction. The combined phenotypic and genotypic approach provided a detailed understanding of the resistance mechanisms and pathogenic potential of MRSA isolates.

Limitations

The study was conducted at a single tertiary care center with a relatively small number of MRSA isolates, which may limit the generalizability of the findings. Additionally, molecular analysis was restricted to the mecA, PVL, and mupA genes, while other important resistance and virulence genes were not evaluated.

Conclusion

The present study demonstrates that methicillin-resistant *Staphylococcus aureus* (MRSA) isolates possess a high burden of antimicrobial resistance, multiple virulence factors, significant biofilm-forming ability,

and important resistance and virulence genes, including mecA, PVL, and mupA. While all isolates remained susceptible to vancomycin and linezolid, the substantial prevalence of mupirocin resistance and mupA gene positivity highlights the growing challenge of antimicrobial resistance. The significant association between biofilm production and prolonged hospital stay further emphasizes the role of biofilm formation in persistent infections and adverse clinical outcomes. Comprehensive phenotypic and molecular characterization of MRSA is essential for guiding appropriate antimicrobial therapy, strengthening infection prevention strategies, and supporting antimicrobial stewardship. Continuous surveillance, routine molecular monitoring, and strict infection control measures are crucial to limit the spread of multidrug-resistant MRSA and improve patient outcomes.

Declaration

Consent: Written informed consent was obtained from the patient. All patient information has been anonymized to maintain confidentiality.

Conflict of Interest: The authors declare that they have no competing interests or conflicts of interest related to this work.

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