Rani et al | Article 10800
J Pure Appl Microbiol. 2026;20(1):289-295. doi: 10.22207/JPAM.20.1.14
Received: 24 July 2025 | Accepted: 30 September 2025
Published Online: 30 January 2026

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Prevalence and Correlation of intercellular adhesion *icaA* and *icaB* Genes with Biofilm Formation in MRSA Isolates from Clinical Samples

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Abstract

Biofilm formation is a major virulence factor in methicillin-resistant *Staphylococcus aureus* (MRSA), contributing to chronic infections and antibiotic resistance. The intercellular adhesion *icaA* and *icaB* genes are key determinants involved in biofilm synthesis and stabilization. Understanding the prevalence and correlation of these genes with biofilm formation is essential for improved diagnostic and therapeutic strategies. This study aimed to evaluate the prevalence of *icaA* and *icaB* genes and their correlation with phenotypic biofilm formation in MRSA isolates from clinical samples. An observational study was conducted over two years in a tertiary care hospital in India. A total of 102 MRSA isolates were phenotypically assessed for biofilm formation using the tissue culture plate method. Genotypic detection of *icaA* and *icaB* was performed by real-time PCR. Statistical correlation between gene presence and biofilm formation was analyzed using the chi-square test. Among the 102 MRSA isolates, 36.3% were biofilm producers, including 15.7% strong, 11.8% moderate, and 8.8% weak producers. Genotypic analysis showed that 66.7% of isolates harbored both *icaA* and *icaB*, while 20% carried only *icaB*. A strong correlation was observed between *icaA* presence and strong biofilm production ($p < 0.05$), whereas *icaB* alone did not correlate significantly with biofilm formation. The findings highlight the critical role of *icaA* in initiating biofilm formation, while *icaB* may contribute to biofilm stabilization. Comprehensive phenotypic and genotypic screening is essential for understanding MRSA pathogenicity and improving infection control strategies.

Keywords: MRSA, Biofilm, *icaA*, *icaB*, Real-time PCR, Antibiotic Resistance

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Citation: Rani R, Khandait M, Sardar M, Sharma M, Devi LS, Phogat R. Prevalence and Correlation of intercellular adhesion *icaA* and *icaB* Genes with Biofilm Formation in MRSA Isolates from Clinical Samples. *J Pure Appl Microbiol.* 2026;20(1):289-295. doi: 10.22207/JPAM.20.1.14

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a major global health threat, primarily due to its resistance to beta-lactam antibiotics and its ability to cause a spectrum of infections, ranging from minor skin and soft tissue infections to life-threatening conditions such as pneumonia, endocarditis, osteomyelitis, and septicemia.[1,2] The burden of MRSA is particularly concerning in healthcare settings, where it is a leading cause of hospital-acquired (nosocomial) infections and is associated with significant morbidity, mortality, and healthcare costs.[3,4] A key factor contributing to the persistence and pathogenicity of MRSA is its ability to form biofilms, which are complex microbial communities adhered to surfaces and embedded in a self-produced extracellular matrix.[5] These biofilms can develop on both biotic surfaces (such as tissues) and abiotic surfaces (like catheters, prosthetics, and implants), thereby complicating clinical management.[6] Biofilm formation not only enhances bacterial survival under hostile conditions but also significantly increases resistance to antibiotics and evasion from host immune responses.[7,8]

Central to the biofilm-forming capacity of MRSA is the intercellular adhesion (*ica*) operon, particularly the *icaA* and *icaB* genes. The *icaA* gene encodes N-acetylglucosaminyltransferase, which catalyzes the synthesis of polysaccharide intercellular adhesin (PIA), a key component of the biofilm matrix.[9,10] The *icaB* gene, in turn, encodes a deacetylase that modifies the PIA structure, enhancing its adhesive and structural properties, thereby contributing to the robustness and maturity of biofilms.[11]

Recent studies have extended this focus to the entire *icaADBC* operon, showing a high prevalence of *icaA*, *icaB*, *icaC*, and *icaD* among multidrug-resistant *S. aureus* isolates, particularly those with strong biofilm-forming ability.[12] Moreover, investigations in both clinical and environmental isolates indicate that biofilm gene carriage varies geographically and may be influenced by selective antibiotic pressure.[13] Additionally, regulatory networks such as the *agr* quorum-sensing system and global regulators like *sarA* and *sigB* have been shown to interact with the *ica* operon, suggesting that biofilm formation in MRSA may result from both *ica*-dependent and *ica*-independent mechanisms.[14]

The expression and prevalence of these genes have been directly linked to the strength and density of biofilm formation, making them important molecular markers for understanding biofilm-associated infections. Investigating the presence and functional role of *icaA* and *icaB* genes in MRSA isolates can therefore provide critical insights into biofilm-associated pathogenesis and guide the development of targeted anti-biofilm therapies. In this context, the present study seeks to evaluate the prevalence of *icaA* and *icaB* genes in MRSA isolates from clinical samples and explore their correlation with biofilm formation capacity, thereby contributing to the increasing evidence on biofilm-related virulence mechanisms in MRSA and supporting efforts for better infection control and treatment strategies.

MATERIALS AND METHODS

This observational study was conducted over a period of two years (January 2023 to December 2024) in the Department of Microbiology, Faculty of Medicine and Health Sciences, SGT University, Gurugram, India. The protocol of the study was approved by the Institutional Ethics Committee, Faculty of Medicine and Health Sciences, SGT University (IEC/FMHS/MD/MS/2023-27).

A total of 127 non-duplicate *Staphylococcus aureus* isolates were recovered from various clinical specimens, including pus, blood, Bronchoalveolar lavage (BAL), and high vaginal swabs, received in the microbiology laboratory. Identification of *S. aureus* was performed based on colony morphology, Gram staining, catalase, and coagulase tests. Methicillin resistance was determined using the cefoxitin disc diffusion method (30 µg) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Out of the total isolates, 102 were confirmed as MRSA and included for further analysis.[15]

Phenotypic detection of biofilm formation

Biofilm production was assessed by the tissue culture plate (TCP) method. MRSA isolates were cultured in BHI broth supplemented

with 2% sucrose and incubated at 37 °C for 24 h. Cultures were diluted 1:100 and inoculated into 96-well microtiter plates. After incubation, wells were washed with PBS, stained with 0.1% crystal violet, and OD was measured at 450 nm.[16] Biofilm formation was categorized as non, weak, moderate, or strong based on OD values relative to the negative control.

Genotypic detection of *icaA* and *icaB* genes

Genomic DNA was extracted using the HiPurA™ Bacterial Genomic DNA Purification Kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Real-time PCR was performed using gene-specific primers and Hi-SYBr Green master mix (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Cycling conditions included initial denaturation at 94 °C (10 min), followed by 40 cycles at 94 °C (10 s), 55 °C (45 s), and 72 °C (30 s).

Statistical analysis

Data were analyzed using the chi-square test (SPSS v25), and $p < 0.05$ was considered statistically significant to determine the association between biofilm production and the presence of *icaA* and *icaB* genes.

RESULTS

In this study, 102 confirmed methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were analyzed to evaluate their biofilm-forming potential and the prevalence of the *icaA* and *icaB* genes associated with biofilm synthesis.

Phenotypic biofilm formation

Phenotypic assessment making use of the tissue culture plate (TCP) method revealed that 37 of the 102 MRSA isolates 37 (36.3%) exhibited biofilm-forming ability to varying degrees. Specifically, 16 isolates (15.7%) demonstrated strong biofilm production, 12 (11.8%) were moderate producers, and 9 (8.8%) were weak biofilm formers. The majority of isolates (n = 65; 63.7%) were non-adherent, showing no detectable biofilm production (Table 1).

Genotypic detection of *icaA* and *icaB* genes

Genotypic screening by real-time PCR targeting the *icaA* and *icaB* genes showed that 20 (66.7%) isolates were positive for both genes. Notably, 6 (20.0%) isolates harboured only the *icaB* gene, and 1 (3.3%) isolate carried only *icaA*. A total of 3 (10.0%) isolates were negative for both genes (Figure 1).

The predominance of dual gene presence suggests a synergistic role of *icaA* and *icaB* in biofilm development, particularly in isolates with strong biofilm-forming phenotypes.

Table 1. Phenotypic Biofilm Formation in MRSA Isolates

Biofilm Strength	MRSA isolates	
	Number	Percentage (%)
Strong Biofilm	16	15.7
Moderate Biofilm	12	11.8
Weak Biofilm	9	8.8
Non-Biofilm	65	63.7

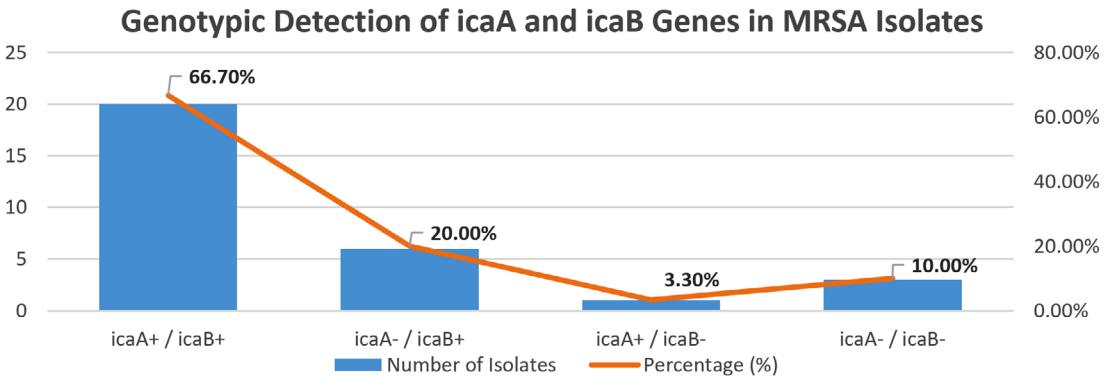

Figure 1. Genotypic Detection of *icaA* and *icaB* Genes in MRSA Isolates

Table 2. Correlation Between Biofilm Formation and *icaA*/*icaB* Gene Presence

Biofilm Strength	*icaA* Positive	*icaA* Negative	*icaB* Positive	*icaB* Negative
Strong Biofilm	14 (70%)	3 (15%)	20 (100%)	0 (0%)
Moderate Biofilm	4 (20%)	1 (5%)	4 (20%)	1 (5%)
Non-Biofilm	0 (0%)	0 (0%)	3 (15%)	0 (0%)

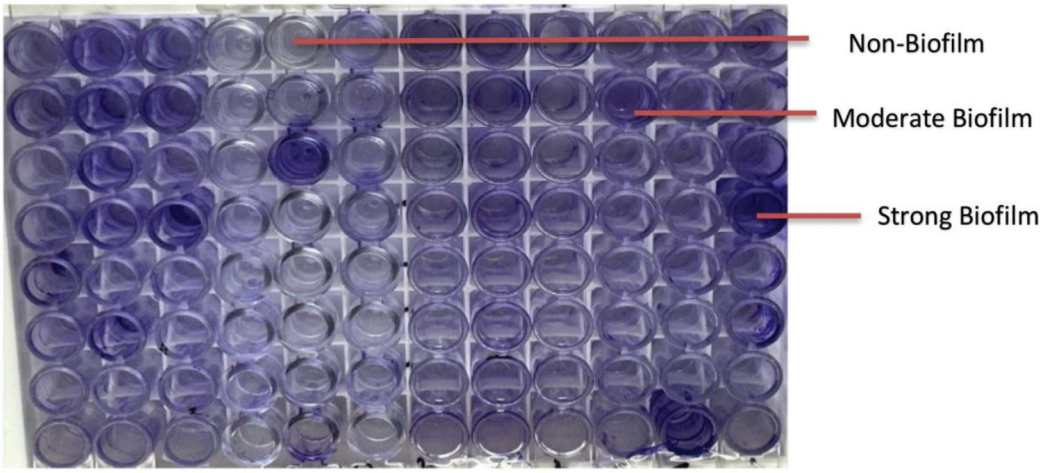

Figure 2. Tissue culture plate method showing biofilm production by *Staphylococcus aureus*

Correlation between gene presence and biofilm formation

A comparative analysis of gene presence and phenotypic biofilm strength revealed a strong correlation between *icaA* gene detection and high-level biofilm formation. Among the 16 strong biofilm producers, 14 were positive for *icaA*, and all were positive for *icaB* (Table 2). Interestingly, *icaB* was also detected in a subset of non-biofilm-producing isolates (n = 3), indicating that its presence alone may not be sufficient to drive biofilm formation in the absence of *icaA* (Figure 2). This suggests a primary role for *icaA* in the initiation of biofilm synthesis, with *icaB* likely contributing to PIA modification and structural reinforcement.

DISCUSSION

This study highlights a significant correlation between the presence of the *icaA* gene and strong biofilm formation among MRSA isolates, reinforcing its critical role in initiating the synthesis of the polysaccharide intercellular adhesin (PIA) that constitutes the biofilm matrix. Consistent with our findings, recent studies have demonstrated that *icaA*-positive *S. aureus* strains exhibit enhanced adherence and biofilm density due to active production of PIA. A study by Armoon et al.[17]; Alibegli et al.,[12] confirmed that *icaA* expression was significantly associated with increased biofilm biomass and structural integrity in clinical MRSA isolates, particularly in device-associated infections.[17] This finding also aligns with the study by Manandhar *et al.*, who reported that *icaA*-positive MRSA isolates exhibited significantly enhanced biofilm formation.[15] Similarly, Nourbakhsh and Namvar found that 70% of biofilm-forming MRSA isolates in their study were *icaA*-positive, further emphasizing the gene's central role in biofilm development.[18,19]

In contrast, the detection of isolates carrying *icaB* without *icaA* suggests the presence of ica-independent or compensatory pathways for biofilm formation. The *icaB* gene encodes a deacetylase that modifies PIA, enhancing its

adhesive capacity, but alone may not initiate biofilm synthesis. This observation aligns with the findings by El-Jakee et al.,[20] who found that *icaB* may stabilize the biofilm matrix but is unlikely to independently initiate biofilm formation.[19] More recently, Rimi et al.[13] also reported discrepancies between ica genotype and biofilm phenotype, demonstrating that some MRSA isolates carrying ica genes remained weak or non-biofilm producers. Moreover, studies by Cafiso et al. and Fitzpatrick et al. have highlighted that ica-independent mechanisms, such as the *agr* quorum-sensing system and *sigB* stress response pathways, can facilitate biofilm development, indicating that *icaA*-negative strains may still possess robust biofilm-forming capabilities.[21,22]

Moreover, the existence of weak or moderate biofilm-forming *icaA*-negative isolates supports the theory of alternative biofilm formation mechanisms. The *agr* quorum-sensing system, *sarA* regulon, and sigma factor B (*sigB*) have been implicated in ica-independent biofilm development. Study demonstrated that MRSA strains lacking *icaA* could still form biofilms through the action of surface-associated proteins such as fibronectin-binding proteins (FnBPs), clumping factors (ClfA/ClfB), and extracellular DNA release.[22,23]

Comparatively, this study's findings align closely with investigations in developing countries, where a higher prevalence of *icaB* without *icaA* has been observed. This variation suggests potential environmental or geographical influences on MRSA biofilm-forming behaviors. For instance, studies in Iran and India have reported similar trends where *icaB*-positive strains were frequently found among non-biofilm producers, highlighting *icaB*'s likely role in biofilm maturation rather than initiation. Additionally, the identification of *icaA*- / *icaB*+ isolates suggests that biofilm development in these strains may involve alternative pathways independent of the ica operon. A study by O'Gara has proposed that MRSA may utilize surface proteins like fibronectin-binding proteins (FnBPs) or clumping factor proteins (Clf) to promote biofilm formation in the absence of *icaA*.[24] This alternative pathway may explain the presence of biofilm-forming *icaA*-negative isolates in this study. The findings underscore the importance of incorporating both phenotypic and genotypic methods for detecting biofilm-forming MRSA strains. Relying solely on genotypic screening for *icaA* and *icaB* may overlook ica-independent biofilm pathways, which could compromise diagnostic accuracy and hinder effective treatment strategies. These findings stress the importance of regional surveillance, as local environmental factors and antibiotic usage patterns may drive the evolution of distinct biofilm-forming strategies.

The clinical relevance of biofilm formation in MRSA cannot be overstated. Biofilm-associated infections are notoriously difficult to eradicate due to their resistance to antibiotics and immune clearance, often leading to chronic infections, treatment failures, and device-related complications. Therefore, combining phenotypic biofilm assays with genotypic screening for *icaA* and *icaB* provides a more comprehensive understanding of a strain's biofilm-forming potential.

Overall, this study's results contribute to the growing body of evidence indicating that *icaA* plays a crucial role in biofilm initiation, while *icaB* may serve more of a stabilizing function. The variability observed across different geographic regions and clinical settings highlights the need for region-specific surveillance to guide effective infection control practices and therapeutic interventions.

Routine screening of *icaA* and *icaB* genes in MRSA isolates should be integrated into clinical microbiology practices. Anti-biofilm agents and combination therapies should be explored to improve treatment outcomes for biofilm-associated MRSA infections. Further research should investigate alternative biofilm mechanisms to explore regulatory networks controlling ica expression, identify novel genes involved in ica-independent biofilm formation, and assess their implications in virulence, antimicrobial resistance, and persistence.

CONCLUSION

This study demonstrates a high prevalence of *icaA* and *icaB* genes among MRSA isolates from clinical samples, with a strong correlation between *icaA* presence and robust biofilm formation. While

icaB appears to play a supporting role in biofilm maturation, it is insufficient in the absence of *icaA*. The presence of *icaA*-negative biofilm-forming isolates suggests alternative mechanisms are at play, highlighting the need for comprehensive diagnostic strategies that include both phenotypic and genotypic assessments.

These findings reinforce the clinical importance of biofilm detection in MRSA infections and advocate for the integration of biofilm-targeted diagnostics and therapeutics into infection control protocols. Continued research into ica-independent pathways may uncover novel targets for anti-biofilm therapies, offering promising avenues to manage persistent MRSA infections.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Faculty of Medicine and Health Sciences, SGT University (IEC/FMHS/MD/MS/2023-27).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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