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Research Article

Virulence Potential and Antibiotic Resistance of *Staphylococcus aureus* Isolates from Clinical, Colonizing and Environmental Sources

Azra Naseem[1], **Malini Shariff**[2*]

[1]Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi- 110007, India

[2]Professor and Head, Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110007, India

***Correspondence author**: Malini Shariff, Professor and Head, Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110007, India; Email: malini.shariff@gmail.com

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Abstract

Introduction: *Staphylococcus aureus* (*S. aureus*) is a facultative anaerobe commonly colonizing the nose, skin and other sites. It causes localized and systemic infections, is a major agent of hospital-acquired disease and methicillin resistance complicates treatment. We analyzed isolates from patients, healthy carriers and environmental sources.

Materials and methods: A total of 100 *S. aureus* isolates were analyzed, comprising 50 clinical, 40 colonizing and 10 environmental samples. The study assessed antibiotic resistance profiles, virulence determinants and the presence of associated genetic markers.

Results: Based on cefoxitin resistance testing, 46 isolates were classified as methicillin-resistant *S. aureus* (MRSA) and 54 as methicillin-susceptible *S. aureus* (MSSA). The *mecA* gene was detected in 36 (78%) MRSA isolates and, unexpectedly, in 27 (50%) MSSA isolates. Among clinical isolates, 22/50 (44%) were positive for Panton-Valentine Leukocidin (*PVL*), 34/50 (68%) for α-hemolysin (*Hla*), 44/50 (88%) for fibronectin-binding protein A (*FnbA*) and 43/50 (86%) for staphylococcal protein A (*SpA*) genes. In colonising isolates, the prevalence was lower for *PVL* (8/40, 20%), *Hla* (14/40, 35%) and *SpA* (25/40, 62.5%). However, *FnbA* showed a higher frequency of 39/40 (97.5%). Environmental isolates showed lower rates, with *PVL* present in 1/10 (10%), *Hla* in 5/10 (50%), *FnbA* in 9/10 (90%) and *SpA* in 2/10 (20%).

Conclusion: MRSA isolates exhibiting virulence properties that are frequently encountered in hospital settings pose significant therapeutic challenges, contributing to increased morbidity and mortality. Effective infection control and robust surveillance strategies are essential to monitor and limit their transmission. Furthermore, restricting the availability of over-the-counter antibiotics is critical to curbing the spread of MRSA and may aid in its long-term control, if not eradication.

Keywords: Methicillin-Resistant *Staphylococcus aureus*; MSSA; Virulence Factors; Colonizer; Environmental Isolates; *HLA*; *PVL*; *FnbA*; *SpA*

Introduction [1]

Staphylococcus aureus (*S. aureus*) is a facultative anaerobe that thrives in the nasal cavity, on the skin and across various mucosal surfaces in humans. This bacterium can cause localized and systemic infections, including skin and soft tissue infections, bacteremia, endocarditis and osteomyelitis. Its pathogenicity is attributed to various virulence factors, including protein A, which aids in evading host immune responses and exotoxins that enhance its virulence [2]. *S. aureus* can produce several virulence factors, including hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins and elements that modulate or evade the

immune system. The diverse virulence factors of *S. aureus* enable it to adhere to surfaces, infiltrate or evade the immune system and induce harmful effects on the host, which is associated with the wide range of infections it causes. Additionally, they can be horizontally transferred to other *S. aureus* strains and are encoded by Mobile Genetic Elements (MGEs). The pathophysiology of illnesses caused by *S. aureus* is complex; however, certain clinical features and the expression of specific virulence factors are correlated, suggesting their role in particular diseases such as tissue necrosis and Panton-Valentine Leukocidin (*PVL*) infections.

Methicillin-Resistant *S. aureus* (MRSA) has emerged as a concern in hospitals, where it is linked to severe infections and presents challenges for treatment [3]. MRSA strains carry the *mecA* gene, which encodes a modified penicillin-binding protein that confers resistance to beta-lactam antibiotics [4]. Moreover, MRSA strains can show resistance to many other antibiotics. The spread of MRSA in healthcare environments is often associated with factors such as inadequate infection control practices and patient-to-patient transmission [5]. In addition to healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA) strains have become a significant source of infections in society. The nasal carriage of *S. aureus* is a well-documented phenomenon, with approximately 20-30% of healthy individuals being persistent carriers [6]. Colonizers can act as reservoirs for the bacterium, potentially leading to infection. The colonization of *S. aureus* is influenced by various factors, including host genetics, immune response and environmental conditions [7]. Colonizing strains can be either methicillin-sensitive or methicillin-resistant and their presence can impact the dynamics of infection spread within communities and healthcare settings. Environmental isolates of *S. aureus* are found in diverse locations, including hospitals, community environments and natural habitats [8]. Healthcare environments, surfaces, medical equipment and the hands of healthcare workers can harbor *S. aureus*, facilitating its spread [9]. The ability of *S. aureus* to survive in various environmental conditions and its potential to colonize both human and environmental surfaces necessitate effective cleaning and disinfection protocols. The environmental persistence of *S. aureus* is a challenge to public health, highlighting the need for continuous surveillance and preventive measures [10].

Hence, we aimed to study the isolates' antibiotic susceptibility to various classes of antibiotics, detect the penicillin resistance genes in methicillin-resistant isolates and detect virulence factor genes, by PCR on clinical, colonizing and environmental isolates of *S. aureus*.

Materials and Methods

Study Design

This retrospective analytical study.

Study Isolates

A hundred *S. aureus* isolates from various sources stored in the institutional repository, which is a collection of all the clinical, colonizing and environmental isolates identified in the laboratory, preserved in 16% glycerol broth at -80°C. Isolates were obtained from: Clinical samples (e.g., sputum, bronchial aspirate, blood, endotracheal tube tips, Foley's catheter tips and pleural fluid) collected from patients in Outpatient Departments (OPD), emergency units, High-Dependency Units (HDU) and Intensive Care Units (ICU). Those isolates obtained from clinical samples showing significant counts only were included to rule out colonizers. Surveillance swabs from healthcare workers and patients (e.g., nasal and hand swabs). Isolates from nasal swabs and throat swabs from patients were considered colonizers irrespective of the infection/colonization status of the patient Environmental surfaces within hospital settings (e.g., bed railings, switchboards, side tables and stethoscopes).

Ethical Statement

Ethical approval was obtained from the Institutional Human Ethics Committee of Vallabhbhai Patel Chest Institute, University of Delhi, Delhi (Ref: VPCI/DIR/IHEC/2023/249). The study adhered to the Indian Council of Medical Research's National Ethical Guidelines for Biomedical and Health Research Involving Human Participants and was conducted in accordance with the principles of the Declaration of Helsinki. Data confidentiality was strictly maintained and the authors declare no conflicts of interest.

All *S. aureus* isolates were maintained in the departmental repository in 16% glycerol broth at -80 °C. The isolates were revived and reconfirmed using conventional biochemical tests and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Confirmed isolates were subsequently used for molecular characterization.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) [11]. The antibiotics evaluated included penicillin, oxacillin, cefoxitin, ceftriaxone, erythromycin, clindamycin, gentamicin, ciprofloxacin, linezolid and vancomycin. A single colony was inoculated into peptone broth and adjusted to a 0.5 McFarland standard. From this suspension, a lawn culture was prepared on MHA plates. Antibiotic discs were applied and the plates were incubated overnight at 37°C under ambient conditions. The diameters of the inhibition zones were measured and interpreted as sensitive, intermediate or resistant according to CLSI guidelines [12].

For methicillin resistance screening, a 30 μg cefoxitin disk was employed. Isolates with inhibition zones ≤21 mm were classified as methicillin-resistant *S. aureus* (MRSA), whereas those with zones ≥22 mm were identified as Methicillin-Susceptible *S. aureus* (MSSA). Cefoxitin is preferred over oxacillin because it induces the production of PBP2a, serving as a more reliable surrogate marker for *mecA*-mediated resistance. Previous studies have demonstrated that the cefoxitin disk diffusion test correlates strongly with molecular detection of *mecA* and is recommended by the Clinical and Laboratory Standards Institute (CLSI) for routine use [12].

DNA Isolation

Genomic DNA was extracted using HIMedia's Genomic DNA Purification Kit (Cat. No. MB505) following the manufacturer's protocol. Briefly, 1.5 ml of overnight *S. aureus* broth culture was centrifuged at 13,000 rpm for 2 minutes at room temperature. The pellet was resuspended in 200 μl lysozyme solution and incubated at 37 °C for 30 minutes, followed by the addition of 20 μl each of Proteinase K and RNase A. After 5 minutes of incubation at room temperature, 200 μl of Lysis solution was added, vortexed and incubated at 55 °C for 10 minutes. Ethanol (200 μl, 95-100%) was added and the lysate was transferred to a HiElute Miniprep Spin Column. After centrifugation, the column was washed sequentially with Prewash and Wash Solutions, then dried by centrifugation. DNA was eluted with 200 μl of Elution Buffer and stored at -20 °C for further analysis.

Detection of Methicillin Resistance Genes by PCR

The *mecA* gene encodes an additional penicillin-binding protein (Pbp2a) with reduced affinity for β-lactam antibiotics, thereby conferring methicillin resistance. Detection of the *mecA* gene was performed using established protocols [13]. PCR amplification was carried out in a final reaction volume of 25 μl, containing 1.25 U of Taq polymerase, 1× PCR buffer (with $MgCl_2$), 10 μM of each primer, 200 μM of dNTPs and 2 μl of DNA template. The primer sequences and PCR cycling conditions employed for *mecA* detection are provided in Table 1.

Detection of Virulence Factor

PCR was performed to detect genes encoding the virulence factors Panton-Valentine Leukocidin (*PVL*), α-hemolysin (*Hla*), fibronectin-binding protein A (*FnbA*) and staphylococcal protein A (*SpA*), following published protocols [13,14]. Each amplification reaction was carried out in a final volume of 25 μl, containing 1.25 U of Taq polymerase, 1× PCR buffer (with $MgCl_2$), 10 μM of each primer, 200 μM of dNTPs and 2 μl of DNA template. The primer sequences and PCR cycling conditions are summarized in Table 1 [13,14].

Amplified PCR products were visualized on a 1.8% agarose gel stained with non-toxic Hi-SYBR safe gel stain (Himedia, Catalog No ML053). A 100 bp DNA ladder (New England BioLabs Inc. Category No N3231S) was used as a molecular weight marker.

Statistical analysis was performed using SPSS software. Chi-square or Fisher's exact test was performed to compare categorical variables between the two groups. The P-value of <0.05 was significant.

Targeted Gene	Primers	Product size (bp)	PCR conditions
MecA	F:5'-GGGATCATAGCGTCATTATTC-3' R:5'-AACGATTGTGACACGATAGCC-3′	527	Initial denaturation 5 min at 94 °C Number of cycles: 35 Denaturation: 30s at 94 °C Hybridization: 30s at 56 °C Elongation: 1 min at 72 °C Final elongation 10 min at 72 °C
Panton-Valentine leukocidin (*PVL*)	F:5'ATCATTAGGTAAAATGTCTGGACATGATCCA3' R: 5'-GCATCAASTGTATTGGATAGCAAAAGC-3'	433	Initial Denaturation, 94^{0}C, 5 Min Denaturation, 94^{0}C, 30 Sec Hybridization, 55^{0}C, 30 Sec Elongation, 72^{0}C,1Min Final elongation,72^{0}C,5 Min Number of cycles,30
α-Hemolysin (*Hla*)	F: 5'-CTGATTACTATCCAAGAAATTCGATTG-3' R: 5'-CTTTCCAGCCTACTTTTTTATCAGT-3'	209	Initial Denaturation, 94^{0}C, 7 Min Denaturation, 94^{0}C, 1 Min Hybridization, 65^{0}C, 1 Min Elongation, 72^{0}C, 1 Min Final elongation,72^{0}C,7 Min Number of cycles,35
Fibronectin-binding protein A (*FnbA*)	F: 5'-CATAAATTGGGAGCAGCATCA-3' R: 5'-ATCAGCAGCTGAATTCCCATT-3'	128	Initial Denaturation, 94^{0}C, 1 Min Denaturation, 94^{0}C, 45 Sec Hybridization, 55^{0}C, 45 Sec Elongation, 72^{0}C, 1 Min Final elongation,72^{0}C,7 Min Number of cycles,30
Staphylococcal protein A(*SpA*)	F: 5'-ATCTGGTGGCGTAACACCTG-3' R: 5'-CGCTGCACCTAACGCTAATG-3'	1150-1500	Initial Denaturation, 94^{0}C, 2 Min Denaturation, 94^{0}C, 30 Sec Hybridization, 60^{0}C, 30 Sec Elongation, 72^{0}C, 1 Min Final elongation,72^{0}C,5 Min Number of cycles,35

Table 1: Primers and PCR conditions for *MecA* and virulence factors.

Results

Study Isolates

A total of 100 *S. aureus* isolates from the institutional repository were revived and analyzed. These comprised 50 isolates from clinical samples, 40 colonizing isolates from patients and healthcare workers and 10 environmental isolates obtained from hospital beds, bed railings and side tables.

Antibiotic Susceptibility of the Isolates to Various Classes of Antibiotics

The antibiotic susceptibility profile of the isolates is presented in Table 2. *S. aureus* demonstrated varying degrees of resistance across all classes of antibiotics tested. Vancomycin is showing high resistance (n-22, 22%), it could be because we have used disc diffusion test which may give false resistance. Linezolid exhibited comparatively low resistance rates, at 5%, emerging as the most effective agent. Overall, linezolid, chloramphenicol and tetracycline were identified as the most effective antibiotics against isolates. In contrast, cefoxitin, penicillin and azithromycin showed high levels of resistance.

Penicillin Resistance Gene in Methicillin-Resistant Isolates by PCR.

Of the 100 isolates, 46 were MRSA based on cefoxitin resistance. Among these, only 36 (78%) possessed the *mecA* gene. Fifty-four were MSSA and 27 (50%) possessed the *mecA* gene, despite being cefoxitin-sensitive (Table 3).

Presence of Virulence Factor Genes by PCR

The data reveals that clinical isolates exhibited the highest prevalence of virulence factors genes, *PVL* (n-22; 44%), *Hla* (n-34; 68%) and *SpA* (n-43; 86%), underscoring their strong pathogenic potential. Fibronectin-binding protein A (*FnbA*) gene was detected in nearly all isolates, highlighting its critical role in colonization. Statistical analysis confirmed that *PVL*, *Hla* and *SpA* genes were significantly associated with clinical isolates (Table 4).

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Cefoxitin	46	0	54
Penicillin	46	0	54
Azithromycin	47	0	53
Gentamicin	36	0	64
Clindamycin	25	0	75
Vancomycin*	22	1	77
Linezolid	5	0	95
Ciprofloxacin	51	3	46
Cotrimoxazole	43	3	54
Tetracycline	18	0	82
Chloramphenicol	10	0	90
*Disc diffusion test			

Table 2: Antimicrobial susceptibility of *S. aureus* isolates by the Kirby-Bauer disk diffusion method (n=100).

Gene/MRSA	Clinical (n = 50) (%)	Colonizers (n = 40) (%)	Environmental (n=10) (%)
Cefoxitin resistance (MRSA) (46)	29 (63)	15 (32.6)	2 (4)
MecA gene (+v) MRSA (36)	26 (72)	9 (25)	1 (2.7)

Table 3: Presence of phenotypic MRSA and *MecA* gene in *S. aureus* isolates (n = 100).

Virulence Factor Genes	Clinical (n = 50) (%)	Colonizers (n = 40) (%)	Environmental (n=10) (%)	P-value Clinical vs colonizers
Panton-Valentine leukocidin (*PVL*)	22 (44%)	8 (20%)	1 (10%)	0.016
α-Hemolysin (*Hla*)	34 (68%)	14 (35%)	5 (50%)	0.008
Fibronectin-binding protein A (*FnbA*)	44 (88%)	39 (97.5%)	9 (90%)	0.198
Staphylococcal protein A(*SpA*)	43 (86%)	25 (62.5%)	2 (20%)	<0.001

Table 4: Presence of genes responsible for virulence factors in isolates of *S. aureus*.

Discussion

Staphylococcus aureus is a gram-positive bacterium that forms part of the normal human microbiota, predominantly colonizing the skin and nasal passages. However, it has the potential to become pathogenic, causing a wide spectrum of infections ranging from mild dermatological conditions to severe, life-threatening diseases such as pneumonia, endocarditis and sepsis [15]. The organism's remarkable ability to acquire resistance to multiple antibiotics, most notably in the form of methicillin-resistant *S. aureus* MRSA), poses a major global public health challenge [16]. The increasing prevalence of MRSA and multidrug-resistant strains in both hospital and community settings complicates treatment and creates significant obstacles for healthcare providers and public health authorities [17].

A comprehensive understanding of the relationship between resistance mechanisms and virulence factors is critical for the development of targeted interventions and improved patient outcomes. Previous studies have often focused either on phenotypic antibiotic resistance profiles or on specific virulence genes of *S. aureus*, without adopting an integrated approach. For example, Mukim, et al., reported a high prevalence of MRSA with substantial resistance to commonly used antibiotics but did not investigate the genetic basis of this resistance or its association with virulence determinants [17]. Similarly, Gitau, et al., described the antibiotic susceptibility profile of *S. aureus* isolates but did not assess virulence factors, which are central to the pathogenic potential of the bacterium [18].

To address these gaps, the present study integrates antibiotic susceptibility testing with the detection of penicillin resistance genes and key virulence factors in clinical, colonizing and environmental isolates. This comprehensive approach provides valuable insights into the dynamics of *S. aureus* infections and contributes to the development of more effective infection control measures and therapeutic strategies.

Significance of *S. aureus* in Colonization and Infection: *S. aureus* is an important human pathogen capable of causing a wide range of infections, from mild skin conditions to severe systemic diseases. Its adaptability and diverse virulence factors enable colonization of multiple body sites and survival in varied environments, particularly within healthcare settings, where it poses a significant risk to patient safety. The emergence and spread of Methicillin-Resistant *S. aureus* (MRSA) have further complicated treatment and infection control measures [3].

The findings of this study contribute to a deeper understanding of the distribution and characteristics of *S. aureus* across clinical, colonizing and environmental isolates, which is essential for designing targeted interventions. Supporting evidence from previous research underscores the importance of such integrated approaches. For instance, Xie, et al., investigated the prevalence and characteristics of *S. aureus*, including MRSA, among medical laboratory staff in hospitals in Guangzhou, China. They reported that 20% (87/434) of staff carried *S. aureus* in their nasal passages, with a higher prevalence among microbiology laboratory staff (34/130; 26.2%) compared to others (53/304; 17.4%) [19]. Additionally, MRSA colonization was more frequent among other healthcare workers (8/304; 2.6%).

Similarly, Horváth, et al., examined *S. aureus* isolates recovered from public playground equipment in Hungary, assessing their prevalence, antibiotic resistance, virulence patterns and clonal diversity. They found that 2.8% (10/355) of samples contained *S. aureus*, specifically Methicillin-Susceptible Strains (MSSA) and no MRSA isolates were found. This highlights the importance of maintaining environmental hygiene and implementing surveillance measures to reduce the risk of community-acquired infections [20].

A comprehensive study by Paling, et al., investigated the relationship between *S. aureus* colonization and the development of *S. aureus* pneumonia (SAIP) in patients receiving mechanical ventilation in the Intensive Care Unit (ICU). The authors concluded that prior colonization with *S. aureus* at the time of ICU admission was the sole independent risk factor for SAIP, with colonized patients being 3.6 times more likely to develop pneumonia compared to non-colonized individuals [21].

Distribution of *S. aureus* Isolates

A total of 100 *S. aureus* isolates were collected for analysis, comprising 50 clinical, 40 colonizers and 10 environmental isolates. The predominance of isolates from clinical specimens- including blood, sputum and other bodily fluids-underscores the critical role of *S. aureus* as a pathogen in healthcare settings. Colonizing isolates, particularly those obtained from healthcare workers, highlight the potential contribution of asymptomatic carriers to nosocomial transmission. Environmental isolates further demonstrate the organism's ability to persist on hospital surfaces, facilitating indirect spread. The detection of *S. aureus* on bed railings, mobile phones and stethoscopes indicates that inanimate objects can serve as reservoirs for hospital-acquired infections. These findings emphasize the urgent need for stringent infection control measures, including routine disinfection protocols and strict adherence to hand hygiene practices among healthcare workers.

Comparable observations have been reported in other settings. Liu, et al., in Tianjin, China, documented *S. aureus* carriage in 19% (15/79) of hospital personnel, underscoring their role as reservoirs and the associated risk of cross-contamination within healthcare environments [22]. Yu, et al., further investigated the genetic relationship between environmental and clinical

MRSA/MSSA isolates in hospitalized patients, demonstrating that 15% (15/100) of MRSA-infected patients had environmental samples genetically similar to their clinical isolates. Their study also identified the USA300 strain (SCCmec IV, ST8, *PVL*-positive) as the most prevalent, accounting for 7/18 (39%) of environmental and 7/21 (33%) of clinical MRSA isolates [23].

In contrast, our study detected MRSA and *PVL* positivity in only 1/10 (10%) environmental isolate, all of which belonged to novel sequence types (data not shown). Among colonizing isolates, 15/40 (37.5%) were MRSA, with only two derived from healthcare workers. Most colonizing and environmental isolates represented new sequence types (data not shown) and no predominant strain type was observed across the sample categories analyzed.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of 100 isolates revealed varying resistance levels among the isolates, with significant resistance observed against cefoxitin, penicillin and azithromycin (46%, 46% and 47%, respectively). These findings are consistent with previous reports indicating that β-lactam antibiotics, such as penicillin, are often ineffective against *S. aureus*, particularly Methicillin-Resistant Strains (MRSA) [4]. The high resistance rate to cefoxitin (46%) suggests a widespread presence of MRSA in both clinical and community settings, posing a considerable challenge to effective treatment.

In contrast, linezolid, chloramphenicol and tetracycline were identified as the most effective antibiotics against *S. aureus*, with resistance rates of only 5%, 10% and 18%, respectively. Linezolid demonstrated 95% sensitivity, underscoring its reliability as a treatment option for MRSA infections. These findings highlight the importance of continuous antibiotic susceptibility monitoring to guide empirical therapy and prevent inappropriate antibiotic use, which contributes to resistance development.

Comparable observations have been reported globally. A study from Egypt found linezolid to be highly effective , 9/159 (95%) against *S. aureus* [24]. In Ethiopia, the prevalence of MRSA was reported at 17.5% (34/194), with resistance patterns of Erythromycin 103/194 (53%) and clindamycin 23/194 (85.4%), while vancomycin remained effective, 10/194 (5%). Twenty-two percent of our isolates (22/100) were reported as vancomycin-resistant. This finding, however, should be interpreted with caution, as the disc diffusion method was employed. According to CLSI and EUCAST recommendations, disc diffusion is not a reliable method for vancomycin susceptibility testing and may overestimate resistance, leading to falsely elevated resistance rates. This was the case in the present study. More accurate methods, such as broth microdilution or E-test, are recommended for confirmation. Notably,98/194 (48.5%) of isolates were Multidrug-Resistant (MDR) [25]. Similarly, Qodrati, et al., reported that 216/576 (37.5%) of patient-derived isolates were MRSA, with a higher prevalence in infectious wards and ICUs. MRSA strains exhibited substantially greater resistance to gentamicin, cotrimoxazole and ciprofloxacin compared to MSSA and nearly half 279/576 (48.5%) were MDR. Importantly, none of the isolates showed resistance to newer agents such as daptomycin, linezolid or tigecycline, reinforcing their role as effective treatment options [26].

The findings of the present study agree with these reports, further emphasizing the global challenge posed by MRSA and the critical need for ongoing surveillance and judicious antibiotic use.

Detection of Methicillin Resistance Genes

The *mecA* gene is a critical marker for methicillin resistance. In our study, 36/46 (78%) of *S. aureus* isolates that were cefoxitin-resistant-a phenotypic indicator of MRSA-harbored the *mecA* gene. The observed discrepancy may be explained by several factors: 1. Heterogenous *mecA* expression wherein some strains carry *mecA* but express PBP2a at very low or variable levels, making them appear cefoxitin-sensitive despite being genotypically resistant. 2. Regulatory gene mutations where *mecA* expression is controlled by the mecI/mecR1 regulatory system. Mutations or partial deletions in these genes can alter expression, leading to discordant results. 3. Borderline Oxacillin-Resistant Strains (BORSA). These strains lack *mecA* but exhibit resistance due to hyperproduction of β-lactamase. They may appear resistant phenotypically but test negative for *mecA*. 4. mecC gene , a homologue of *mecA*, can confer resistance but may not be detected by *mecA*-specific PCR assays. Such isolates may appear cefoxitin-resistant but *mecA*-negative [27].

This discrepancy highlights that not all phenotypically methicillin-resistant isolates possess the *mecA* gene. Similar findings have been reported in other studies, suggesting that alternative mechanisms-such as β-lactamase hyperproduction or mutations in other penicillin-binding proteins (PBPs)-may also contribute to resistance [28].

Interestingly, 27/54 (50%) MSSA isolates carried the *mecA* gene, which was likely not expressed. Under certain conditions, such as antibiotic exposure in the environment, these isolates may potentially evolve into MRSA. These findings underscore the importance of employing both phenotypic and genotypic methods for accurate MRSA identification. In the present study, MRSA was detected in 29/46 (63%) clinical isolates and 15/46 (32.6%) colonizing isolates, indicating that colonizing MRSA strains have the potential to cause infections in patients and pose significant therapeutic challenges.

Detection of Virulence Factors

The study examined the presence of key virulence factor genes, including Panton-Valentine Leukocidin (*PVL*), α-Hemolysin (*Hla*), Fibronectin-Binding Protein A (*FnbA*) and *Staphylococcal* protein A (*SpA*). Notably, the prevalence of these factors was significantly higher in clinical isolates, particularly methicillin-resistant strains, compared to surveillance and environmental isolates, indicating enhanced pathogenic potential. In contrast, the incidence of virulence factors in methicillin-sensitive isolates was similar between clinical and colonizing strains.

The *PVL* gene, associated with severe skin and soft tissue infections and frequently linked to community-acquired MRSA (CA-MRSA), was detected in 24/32 (75%) of clinical isolates, reflecting a substantial prevalence of *PVL*-positive strains. HA-MRSA isolates from infection sites harbored *PVL* in 12/19 (63.1%). This indicates that *PVL*-positive strains are invading hospitals and causing infections, Interestingly, 26/55 (47%) of colonizing isolates also carried *PVL*, demonstrating the presence of highly virulent strains within colonizing populations [29]. However in the present study *PVL* was seen in 22/50 (44%) of clinical, 8/40 (20%) colonizing and 1/10 (10%) of environmental isolates. Twenty-two out of 31 (71%) isolates that were *PVL* positive were MRSA. These findings were similar to the study by Alkharsah, et al. [29].

In the present study, *Hla* gene, known for its role in tissue damage, was predominantly observed in 34/50 (68%) in clinical isolates, but was also present in 14/40 (35%) of colonizing and 5/10(50%) of environmental isolates. The *FnbA* gene, essential for bacterial adhesion and colonization, was the most prevalent, detected in 92/100 (92%) of isolates with 44/50 (88%) clinical and 39/40 (97.5%) in colonizing isolates underscores the potential of colonizing strains to cause infection. The *SpA* gene, which facilitates immune evasion, was found in 43/50 (86%) and 25/40 (62.5%) of clinical and colonizing isolates respectively, Tilouche, et al., reported a somewhat lower prevalence of *SpA* (45.7%) [13].

Urrego, et al., further demonstrated the importance of virulence factors in colonizing strains, reporting *PVL* in 23/32 (71.9%) of dialysis patient colonizers and 18/18 (88.9%) of their household contacts, along with TSST-1 in 21/32 (67.2%) and 12/18 (66.7%,) respectively. Household colonizers more frequently harbored virulent isolates, thereby transmitting them to patients [30]. Taken together, our findings indicate that *PVL*, *Hla* and *SpA* were highly significant in clinical isolates, however their presence in colonizing isolates reinforces their role in pathogenicity and infection dynamics.

Limitations of the Study

This retrospective study was conducted using *S. aureus* isolates from the institutional repository. As a result, comprehensive clinical information, including patient demographics, treatment history and outcome data, was not available. We tested for the presence of virulence genes; however, their detection may not necessarily correspond to the expression of virulence proteins or the manifestation of phenotypic characteristics. We assessed vancomycin sensitivity using the disc diffusion technique; however, this method may have yielded erroneously elevated resistant results.

Conclusion

We undertook this comprehensive study to investigate the antibiotic susceptibility and virulence mechanisms of *S. aureus* isolated from clinical, colonizing and environmental specimens. Colonizing isolates were found to be not only methicillin-resistant but also carriers of key virulence factors, underscoring their potential to cause disease. These findings highlight the critical importance of strict adherence to infection control measures to limit the transmission of pathogenic strains and colonizers with the capacity to evolve into clinically significant infections.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement
Not applicable.

Ethical Statement
The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore was exempt.

Informed Consent Statement
Informed consent was taken for this study.

Authors' Contributions
All authors contributed equally to this paper.

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