Antimicrobial Resistance and Biofilm Formation among Clinical Staphylococcus aureus Isolates: A Cross-Sectional Study at a Tertiary Care Hospital in Northern India

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Abstract

Background: Staphylococcus aureus remains one of the most important agents of community- and hospital-acquired infections, and its capacity to acquire antimicrobial resistance together with the ability to form biofilm poses a major therapeutic challenge. This study was undertaken to determine the antimicrobial resistance pattern and biofilm-forming ability of S. aureus isolated from clinical samples of patients attending a tertiary care hospital in Bareilly, Uttar Pradesh, India.

Methods: A hospital-based cross-sectional study was conducted in the Department of Microbiology, Rajshree Medical Research Institute, Bareilly. A total of 500 clinical samples received from indoor and outdoor patients were processed by standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method as per CLSI guidelines, methicillin resistance was detected using the cefoxitin disc diffusion test, and biofilm production was assessed by the microtiter plate method.

Results: Of the 500 clinical samples processed, 59 (11.8%) yielded bacterial growth, of which 51 (86.4%) were identified as S. aureus. The isolates showed the highest resistance to penicillin and ampicillin (88.2% each), while all isolates (100%) remained sensitive to linezolid. Twenty-eight isolates (54.9%) were methicillin-resistant S. aureus (MRSA). Multidrug resistance (resistance to ≥3 antibiotic classes) was observed in 47 (92.2%) isolates. All 51 isolates produced biofilm *in vitro*, of which 13 (25.5%), 24 (47.1%), and 14 (27.5%) were weak, moderate, and strong biofilm producers, respectively. Strong biofilm producers retained the highest sensitivity to linezolid, gentamicin, and tetracycline.

Conclusion: A high prevalence of methicillin resistance, multidrug resistance, and biofilm production was observed among clinical S. aureus isolates in this tertiary care hospital. Linezolid, gentamicin, and tetracycline remain reliable therapeutic options. Regular antimicrobial surveillance and an institutional antibiotic stewardship programme are warranted to guide empirical therapy and curb the further spread of resistant strains.

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Keywords: Staphylococcus aureus, antimicrobial resistance, MRSA, biofilm, tertiary care hospital, Bareilly

Introduction

Staphylococcus aureus is a common bacterial pathogen responsible for a wide spectrum of community- and hospital-acquired infections, ranging from minor skin and soft-tissue infections to life-threatening bacteraemia, endocarditis, and device-associated infections. The organism poses a persistent clinical challenge owing to its remarkable ability to acquire resistance against antimicrobial agents. Infections caused by multidrug-resistant S. aureus are associated with prolonged hospital stay, increased

treatment cost, ongoing transmission of resistant strains within the hospital environment, and higher morbidity and mortality (Tarai *et al*. 2013; Chatterjee *et al*. 2018; Mehta *et al*. 2020) [19, 4, 13].

Factors such as irrational and over-the-counter use of antimicrobials, prescriptions by informal healthcare providers, polypharmacy, and inadequate antibiotic stewardship contribute significantly to the emergence and spread of resistant strains, a problem that is further compounded in resource-limited tertiary care settings where empirical therapy is often started before culture and sensitivity reports are available (Ansari *et al*. 2014) [1].

In addition to acquiring antimicrobial resistance, many strains of S. aureus, particularly methicillin-resistant S. aureus (MRSA), are capable of forming biofilms structured, sessile microbial communities encased in a self-produced extracellular polymeric matrix that adheres to living and non-living surfaces. Biofilm formation is an important virulence determinant that confers protection against host immune defences and antimicrobial agents, and is strongly associated with chronic and recurrent infections (Young *et al*. 2002) [23]. Biofilm-producing S. aureus isolates have been reported to exhibit significantly higher rates of multidrug resistance compared with non-biofilm producers; in one study, 86.7% of biofilm-producing isolates were multidrug resistant, whereas none of the biofilm-negative isolates were multidrug resistant (Neopane *et al*. 2018) [15].

Despite the well-recognised clinical importance of both antimicrobial resistance and biofilm production in S. aureus, data characterising these virulence properties from tertiary care hospitals of northern India, particularly the Bareilly region of Uttar Pradesh, remain limited. The present study was therefore undertaken to determine the antimicrobial resistance pattern, the prevalence of methicillin resistance, and the biofilm-forming ability of S. aureus isolated from clinical samples of patients attending Rajshree Medical Research Institute, Bareilly, so as to guide rational empirical antibiotic therapy and strengthen local antimicrobial stewardship efforts.

Materials and Methods

Study design and setting

This hospital-based, cross-sectional, observational study was conducted in the Department of Microbiology, Rajshree Medical Research Institute, Bareilly, Uttar Pradesh, India. Clinical samples received from patients attending the indoor (IPD) and outdoor (OPD) departments of the hospital, with clinical suspicion of bacterial infection, were included in the study. The study was approved by the Institutional Ethics Committee, and informed consent was obtained from patients or their attendants prior to sample collection.

Sample collection and processing

A total of 500 clinical samples (blood, pus/wound swabs, and other relevant clinical specimens) were collected aseptically and processed using standard microbiological techniques. Samples were inoculated onto blood agar and MacConkey agar and incubated aerobically at 37°C for 24–48 hours. Bacterial colonies were identified based on colony morphology, Gram's staining, and standard biochemical tests, including catalase and coagulase tests, to confirm S. aureus.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of all confirmed S. aureus isolates was performed by the Kirby–Bauer disc diffusion technique on Mueller–Hinton agar, and results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI 2018) [6]. The antibiotic panel included penicillin (10 µg), ampicillin (10 µg), erythromycin (15 µg), azithromycin (15 µg), cefoxitin (30 µg), co-trimoxazole (25 µg), clindamycin (30 µg), teicoplanin (30 µg), ciprofloxacin (5 µg), vancomycin (30 µg), chloramphenicol (30 µg), levofloxacin (5 µg), tetracycline (30 µg), gentamicin (10 µg), and linezolid (30 µg). Methicillin-resistant S. aureus (MRSA) was identified using the cefoxitin (30 µg) disc diffusion test as per CLSI criteria (Weinstein and Lewis 2020) [20]; isolates with a zone of inhibition ≥22 mm, 21–22 mm, and ≤21 mm were classified as sensitive, intermediate, and resistant, respectively. Isolates resistant to agents from three or more antibiotic classes were classified as multidrug resistant (MDR).

Detection of biofilm formation

Biofilm production by all S. aureus isolates was assessed by the quantitative microtiter plate method described by Aslantaş and Demir (2016) [2]. Isolates were grown overnight in tryptic soy broth (TSB) at 37°C, diluted 1:10 in TSB containing 1% dextrose, and 200 µl of the suspension was added to wells of a 96-well flat-bottomed polystyrene microtiter plate in triplicate; wells containing sterile TSB served as negative controls. Plates were incubated at 37°C for 48 hours without shaking, after which planktonic cells were removed and wells were washed twice with sterile phosphate-buffered saline (PBS). Adherent biofilm was fixed with methanol, stained with 0.1% (w/v) crystal violet, washed, air-dried, and solubilised with 30% (v/v) acetic acid. The optical density (OD) of the solubilised stain was measured at 570 nm using a microplate reader. Isolates were classified as non-, weak, moderate, or strong biofilm producers based on comparison with the cut-off OD (ODc) of the negative control, following the criteria of Stepanović *et al*. (2007) [18].

Statistical analysis

Data were entered and analysed using descriptive statistics. Categorical variables, including antimicrobial susceptibility profiles and biofilm-forming categories, were expressed as frequencies and percentages.

Results

Of the 500 clinical samples processed during the study period, 59 (11.8%) samples showed significant bacterial growth. Among these culture-positive samples, 51 (86.4%) were identified as S. aureus based on colony morphology, Gram's staining, and biochemical characteristics, indicating this organism as a predominant pathogen among culture-positive clinical isolates in the study population.

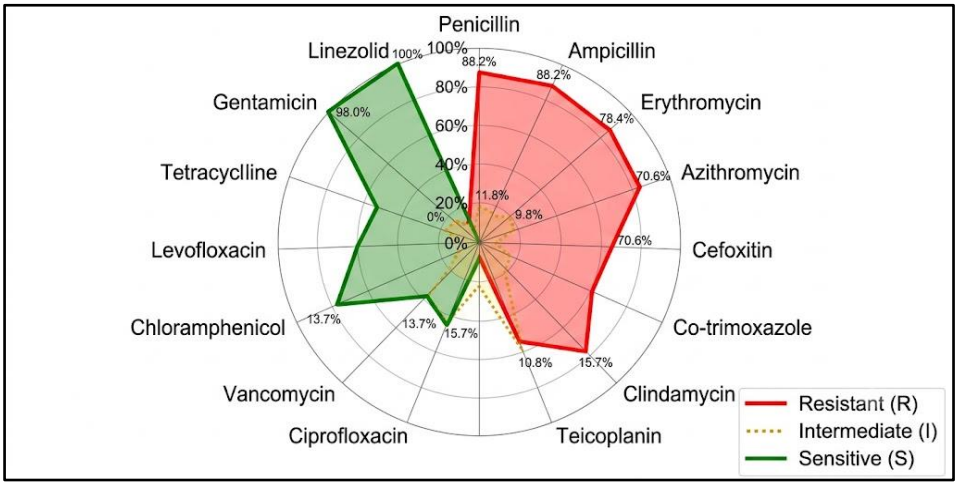

Fig 1: Radar Chart of Antibiotic susceptibility Profiles (n=51)

Antimicrobial susceptibility testing of the 51 S. aureus isolates revealed the highest resistance to penicillin and ampicillin (45 isolates each, 88.2%), followed by erythromycin (40 isolates, 78.4%) and azithromycin (36 isolates, 70.6%). All isolates (51, 100%) were sensitive to linezolid, and a majority remained sensitive to gentamicin (50, 98.0%) and tetracycline (47, 92.2%). The complete antimicrobial susceptibility profile of the isolates is presented in Table I, Figure 1.

Table 1

Antibiotic	Sensitive (S)	Intermediate (I)	Resistant (R)
Penicillin	6 (11.8%)	0 (0%)	45 (88.2%)
Ampicillin	6 (11.8%)	0 (0%)	45 (88.2%)
Erythromycin	6 (11.8%)	5 (9.8%)	40 (78.4%)
Azithromycin	13 (25.5%)	2 (3.9%)	36 (70.6%)
Cefoxitin	23 (45.1%)	0 (0%)	28 (54.9%)
Co-trimoxazole	25 (49.0%)	1 (2.0%)	25 (49.0%)
Clindamycin	36 (70.6%)	8 (15.7%)	7 (13.7%)
Teicoplanin	38 (74.5%)	5 (9.8%)	8 (15.7%)
Ciprofloxacin	41 (80.4%)	2 (3.9%)	8 (15.7%)
Vancomycin	41 (80.4%)	7 (13.7%)	3 (5.9%)
Chloramphenicol	44 (86.3%)	3 (5.9%)	4 (7.8%)
Levofloxacin	44 (86.3%)	3 (5.9%)	4 (7.8%)
Tetracycline	47 (92.2%)	0 (0%)	4 (7.8%)
Gentamicin	50 (98.0%)	0 (0%)	1 (2.0%)
Linezolid	51 (100%)	0 (0%)	0 (0%)

Based on the cefoxitin disc diffusion test, 28 of the 51 (54.9%) S. aureus isolates were identified as MRSA, while the remaining 23 (45.1%) were methicillin-sensitive S. aureus (MSSA). On further analysis of the number of antibiotic classes to which isolates were resistant, 4 (7.8%) isolates were resistant to two antibiotic classes, 26 (51.0%) were resistant to three to five classes, 20 (39.2%) were resistant to six to nine classes, and 1 (2.0%) isolate was resistant to more than nine antibiotic classes (Table II). Overall, 47 (92.2%) isolates met the criteria for multidrug resistance.

Table 2

Degree of resistance	No. of isolates	Percentage
Resistant to 2 antibiotic classes	4	7.8%
Resistant to 3–5 antibiotic classes	26	51.0%
Resistant to 6–9 antibiotic classes	20	39.2%
Resistant to >9 antibiotic classes	1	2.0%

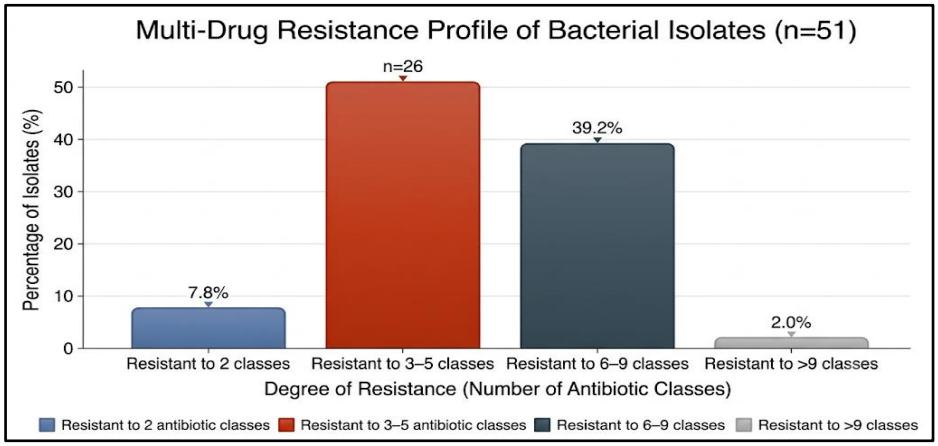

Fig 2

All 51 (100%) S. aureus isolates demonstrated the ability to form biofilm *in vitro*. Of these, 13 (25.5%) were weak biofilm producers, 24 (47.1%) were moderate biofilm producers, and 14 (27.5%) were strong biofilm producers (Table III). Among the strong biofilm-producing isolates, the majority retained sensitivity to linezolid, gentamicin, and tetracycline, whereas resistance to penicillin, ampicillin, and erythromycin remained high, mirroring the overall resistance trend observed in the total isolate pool.

Table 3

Biofilm-forming category	No. of isolates	Percentage
Weak biofilm producer	13	25.5%
Moderate biofilm producer	24	47.1%
Strong biofilm producer	14	27.5%
Total biofilm producers	51	100%

Discussion

Access to high-quality diagnostic and antimicrobial susceptibility testing services remains a challenge in several tertiary care settings in India, often contributing to delayed diagnosis and empirical, rather than evidence-based, antibiotic prescribing (Wilson *et al*. 2018) [21]. In the present study, S. aureus was identified in 51 of 59 (86.4%) culture-positive clinical samples, reaffirming its position as a predominant pathogen among clinical isolates at this tertiary care hospital. This proportion is comparable to a study from a tertiary healthcare hospital in India, which reported S. aureus in 36.26% of culture-positive clinical samples, although the higher proportion observed in our study may reflect differences in the sample types and patient population studied (Pappu *et al*. 2020) [16]. A study of hospital-attending patients in Nepal similarly reported S. aureus in 20% of culture-positive samples (Sapkota *et al*. 2019) [17].

A high rate of resistance to the penicillin group of antibiotics observed in our study is consistent with previous reports and is most likely attributable to widespread and often unsupervised use of these agents (Yılmaz and Aslantaş 2017; Islam *et al*. 2021) [22, 11]. The resistance rates to ampicillin and erythromycin observed in the present study were also comparatively high, a finding that may be specific to the local prescribing practices and antibiotic-use patterns in this region (Duran *et al*. 2012; Yılmaz and Aslantaş 2017) [8, 22]. Notably, more than half (54.9%) of the S. aureus isolates in our study were found to be MRSA, a proportion that warrants close attention and reinforces the need for stringent infection-control measures and continuous surveillance within the hospital. A study conducted in a tertiary care hospital in Nepal reported an MRSA prevalence of 47.4% and noted that the rates of multidrug and methicillin resistance were higher among biofilm-producing isolates compared with non-biofilm producers (Belbase *et al*. 2017) [3], a trend broadly consistent with our findings of high multidrug resistance among biofilm-producing isolates.

Biofilm formation among clinical S. aureus isolates has been extensively studied because of its established association with disease chronicity and antimicrobial resistance. A study from India reported that 67.07% of MRSA isolates obtained from various clinical samples were biofilm producers (Chaudhary *et al*. 2021) [5], while another Indian study on febrile patients found 84.28% of Staphylococcal isolates to be biofilm producers, of which 21.4%, 62.8%, and 15.8% were strong, moderate, and non-biofilm producers, respectively (Gupta *et al*. 2015) [10]. In the present study, all S. aureus isolates (100%) demonstrated biofilm-forming ability, with 27.5% classified as strong producers — a considerably higher overall prevalence than previously reported, which may reflect differences in patient population, sample type, or the specific clinical setting of a tertiary referral hospital. Similar to earlier reports, strong biofilm producers in our study retained the highest sensitivity to linezolid, gentamicin, and tetracycline (Mottola *et al*. 2016) [14], and linezolid has previously been demonstrated to be an effective agent against biofilms formed by MRSA *in vitro* (Martínez *et al*. 2016) [12], although the precise anti-biofilm mechanism of linezolid warrants further investigation.

The high burden of methicillin resistance, multidrug resistance, and biofilm production observed among clinical S. aureus isolates in this study is of considerable clinical concern. Judicious prescription of antibiotics such as penicillin, ampicillin, and erythromycin, together with strengthening of institutional antimicrobial stewardship programmes, is essential to curb the further emergence and spread of resistant strains. Routine antimicrobial susceptibility testing and biofilm assessment of clinical S. aureus isolates would aid clinicians in selecting appropriate empirical and definitive antibiotic therapy, particularly in patients with device-associated or chronic infections.

Conclusion

This study demonstrates a high prevalence of methicillin resistance, multidrug resistance, and biofilm production among clinical S. aureus isolates at a tertiary care hospital in Bareilly, Uttar Pradesh. Linezolid, gentamicin, and

tetracycline remain the most reliable therapeutic options against these isolates, including strong biofilm producers. These findings highlight the urgent need for continuous antimicrobial resistance surveillance, judicious antibiotic prescribing, and strengthened infection-control practices to limit the spread of resistant and biofilm-forming S. aureus within the hospital setting.

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