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Biofilm-Associated Multidrug-Resistant and Methicillin-Resistant Staphylococcus aureus Infections

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ABSTRACT

Background: The ability of *Staphylococcus aureus* to form biofilms—architectural complexes that cause chronic and recalcitrant infections—along with its notorious variant, methicillin-resistant *Staphylococcus aureus* (MRSA), leads to multidrug-resistant (MDR) infections that are challenging to treat with antibiotics. This cross-sectional study investigated the prevalence of *S. aureus* infections in Kanti Children's Hospital and characterized the antibiograms of MDR, MRSA, and biofilm-forming strains, along with their coexistence.

Methods: *S. aureus* strains were isolated and identified from clinical samples and tested for antibiograms following standard microbiology guidelines. MDR strains were non-susceptible to at least one agent in three antimicrobial categories, whereas MRSA strains were cefoxitin-resistant. The microtiter plate method was used to detect biofilms. Statistical analyses were performed using SPSS version 17.0.

Results: *S. aureus* was detected in 9.0% (11.4-6.6%, 95% Confidence Interval) of 543 samples, primarily from pus (79.6%, 39/49). Children aged 1 to <3 years most commonly contracted infections (30.6%, 15/49), and males (67.4%, 33/49) had twice as many infections as females (32.7%, 16/49). As high as 84.7% (83/98) of strains were penicillin-resistant, while 18.4% (27/147) were aminoglycoside-resistant. MDR accounted for 79.6% (39/49) of all *S. aureus* infections, while MRSA and biofilm-formers accounted for 67.6% (33/49) and 24.5% (12/49), respectively. Fluoroquinolone resistance in non-MDR-MRSA-biofilm-formers, MDR-MRSA, MDR-biofilm-formers, and MRSA-biofilm-formers was 31.3%, 46.8%, 58.3%, and 60.0%, respectively, while aminoglycoside resistance was 0%, 32.3%, 50.0%, and 45.0%, and penicillin resistance was 87.5%, 85.5%, 100.0%, and 100.0%.

Conclusions: MDR-isolates and MRSA caused nearly four-fifths of *S. aureus* infections. Compared to MDR and MRSA strains, biofilm-formers triggered higher levels of antimicrobial resistance.

Keywords: Antibiotics; biofilms; children; resistance; staphylococcus aureus.

INTRODUCTION

Staphylococcus aureus, a gram-positive commensal of human skin and mucous membrane, is among the top three pathogens of clinical significance due to its inherent virulence, ability to exchange genetic information, and resistance to multiple antibiotics.[1] It was not until 1961 that methicillin-resistant *S. aureus* (MRSA) strains arose, and by the late 1990s, they had transcended gentamicin and vancomycin resistance.[2,3]

While most antimicrobial studies on staphylococcal strains concentrate on planktonic cultures, limited literature exists on the prevalence of biofilms—architectural complexes embedded in extracellular polymeric substances—associated with MDR and MRSA infections.[4] Reports on prevalence are important

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to clinicians, as they help them identify the root cause of a disease and implement new treatment strategies. Hence, this study assessed the prevalence of *S. aureus* infections in a tertiary care hospital and examined the antibiograms of MDR, MRSA, and biofilm-forming isolates.

METHODS

This analytical cross-sectional study was conducted between January and June 2021 in the Department of Microbiology at Kanti Children's Hospital (KCH), Kathmandu, Nepal. KCH is a prominent pediatric hospital dedicated to children at the federal level, providing promotional, preventive, specialized, and super-specialized child health services and referrals nationwide. Study participants were hospital-visiting children suspected of bacterial infections who underwent bacteriological examinations.

Institutional Review Committee approval (Registration No.: 09/2020-2021) was obtained from Kanti Children's Hospital, Kathmandu, Nepal. Patient consent or assent (from the patient's guardian) was obtained before sample collection by the involved healthcare professionals. Simple random sampling was used to collect the sample. Herein, a unique number for each individual in a study population was assigned from 1 to 406, and then, using a random number generator, a subset of those numbers was selected so that each individual had an equal chance of being selected for the sample.

The study population was categorized into five groups based on their ages, <1 month: Neonate, 1 month to <1 year: infant, 1 year to <3 years: Toddler, 3 years to <5 years: Pre-school, 5 years to 17 years: school.[5] Demographic information and laboratory findings were collected using a patient information sheet and recorded using Microsoft Excel version 10.0.

This study included clinical samples from children (<17 years) submitted for bacteriological culture analysis and antibiotic susceptibility testing. Samples with incomplete labeling were excluded, as were those with repeated positive cultures for similar bacterial agents and urine that contained multiple bacteria (≥3).

Clinical samples, such as pus, body fluids, blood, and urine, were included in the study. Blood samples were collected at a 1:10 ratio of brain-heart infusion broth, whereas pus samples were collected using a leak-proof sterile container (2 ml) when discharged or a sterile cotton wool swab if not discharged. Sterile, dry, wide-necked, leak-proof containers were used to collect purulent sputum (5 ml) and urine (10–20 ml) samples. To collect urine, patients were instructed to clean the genital area with clean water and dry the area with a sterile gauze pad. Whenever possible, samples were collected before antimicrobial treatment. Samples were collected aseptically, labeled properly, and delivered to the Department of Microbiology, maintaining a cold chain (4-6°C) (except for blood culture), with a requisition form (age, gender, sample number, and date and time of collection).

The non-repetitive midstream urine samples were streaked onto cysteine lactose electrolyte-deficient agar plates with a calibrated inoculating loop and incubated aerobically for 24 hours at 37°C. Other samples were streaked on blood agar and MacConkey agar and incubated at 37°C for 24 hours. Before passing the report as sterile, body fluids were reincubated for another 24 hours (up to 48 hours) and blood culture samples for 48 hours (up to 72 hours). The colony-forming unit (CFU) of the urine sample was quantitatively enumerated, and culture growth was reported as insignificant growth for less than 10^4 CFU/ml organisms, doubtful significance for 10^4-10^5 CFU/ml organisms (repeat specimens), and significant bacteriuria for more than 10^5 CFU/ml organisms. Significant growth of the bacteria was observed following the colonial morphological study, including shape, size, surface, texture, edge, elevation, color, and opacity. *S. aureus* colonies were identified by Gram staining (gram-positive cocci predominantly in grape-like clusters) and biochemical tests (golden yellow colony on mannitol salt agar, catalase positive, tube coagulase positive).[6]

Kirby Bauer's disc diffusion method was used to assess antibiotic susceptibility patterns, which were interpreted as susceptible, intermediate, and resistant based on the CLSI 30th edition criteria.[7] Multidrug resistance (MDR) was defined as an acquired non-susceptibility to at least one agent in three or more antimicrobial categories, [8] while MRSA were strains with a cefoxitin disc zone of inhibition ≤21 mm.[9]

Biofilms were detected using the gold standard method, i.e., the microtiter plate method, described by Christensen et al.[10] The cut-off OD (ODc) was defined as equivalent to three standard deviations above the mean OD of the negative control. Test isolates' OD (OD_{test}) was calculated from the average of triplicates. The criteria for the interpretation of the final result in the microtiter plate method were as follows:

Table 1. Interpretation criteria for biofilm production by the microtiter plate method.		
Criteria	**Result**	**Interpretation**
$OD_{test} < OD_c$	<0.4	Non-biofilm-formers
$OD_c < OD_{test} < 2 \times OD_c$	0.4-0.8	Weak biofilm-formers
$2 \times OD_c < OD_{test} < 4 \times OD_c$	0.8-1.6	Moderate biofilm-formers
$4 \times OD_c < OD_{test}$	≥1.6	Strong biofilm-formers

The data was analyzed using descriptive statistics in SPSS, version 17.0, providing frequencies and percentages as key indicators. Quantitative variables were analyzed by an independent student *t*-test, while qualitative variables were analyzed by a chi-square test. The threshold for determining statistical significance was established as $p<0.05$.

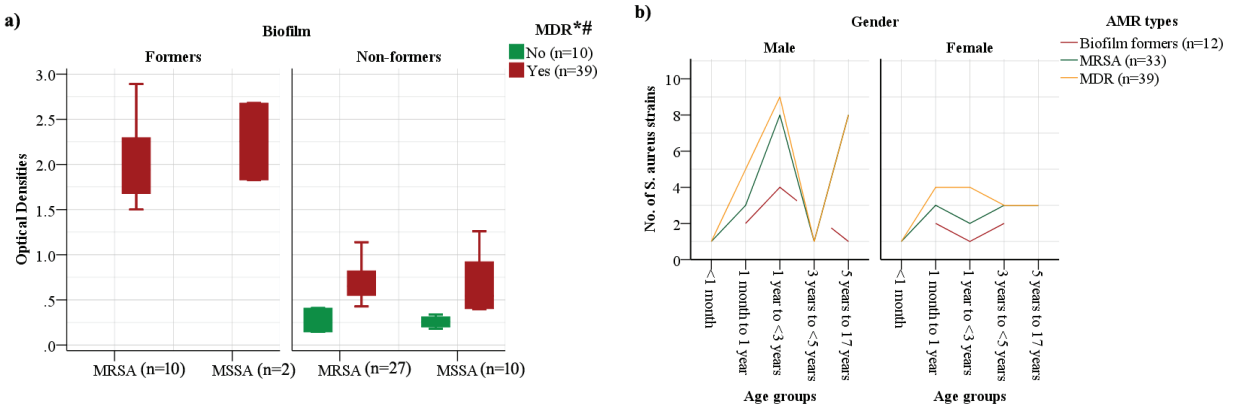
RESULTS

A total of 49 (9.0%) (11.4-6.6%, 95% Confidence Interval) out of 543 clinical samples from 406 children were culture-positive for *S. aureus*. Infected patients had a median age (interquartile range) of 2 years (0.9-5.0). Toddlers (30.6%, 15/49), who were males (33.3%, 11/33), and inpatients (38.5%, 10/26) were most likely to be infected. Thirty-nine (79.6%) strains of *S. aureus* were isolated from pus. Blood (40.0%, 2/5) and pus (25.6%, 10/39) were the only specimens from which biofilm-formers were isolated. MRSA was mostly isolated from pus (71.8%, 28/39) (Table 2).

Table 2. Patients' demographics, samples, biofilm-formers/non-formers, and methicillin resistant/susceptible *S. aureus*.									
Variables		**Gender**		**Patient types**		**Samples**			
		Male (n=33)	**Female (n=16)**	**Inpatient (n=26)**	**Outpatient (n=23)**	**Pus (n=39)**	**Blood (n=5)**	**Abscess (n=4)**	**Urine (n=1)**
Age	Median age (interquartile range)	2 (0.9-5.0)				-	-	-	-
Age groups	<1 month: Neonate (n=3)	2 (6.1)	1 (6.3)	3 (11.5)	0 (0.0)	2 (5.1)	0 (0.0)	1 (25.0)	0 (0.0)
	1 month to <1 year: Infant (n=12)	7 (21.2)	5 (31.3)	7 (26.9)	5 (21.7)	8 (20.5)	2 (40.0)	2 (50.0)	0 (0.0)
	1 year to <3 years: Toddler (n=15)	11 (33.3)	4 (25.0)	10 (38.5)	5 (21.7)	13 (33.3)	1 (20.0)	0 (0.0)	1 (100.0)
	3 years to <5 years: Pre-school (n=6)	3 (9.1)	3 (18.8)	3 (11.5)	3 (13.4)	4 (10.3)	1 (20.0)	1 (25.0)	0 (0.0)
	5 years to 17 years: School (n=13)	10 (30.3)	3 (18.8)	3 (11.5)	10 (43.5)	12 (30.8)	1 (20.0)	0 (0.0)	00 (0.0)
Methicillin resistance	Yes/MRSA (n=33)	21 (63.6)	12 (75.0)	25 (96.2)	8 (34.8)	28 (71.8)	3 (60.0)	1 (25.0)	1 (100)
	No/MSSA (n=16)	12 (36.4)	4 (25.0)	1 (3.9)	15 (65.2)	11 (28.2)	2 (40.0)	3 (75.0)	0 (0.0)
Biofilm	Non-formers (n=37)	26 (78.8)	11 (68.8)	16 (61.5)	21 (91.3)	29 (74.4)	3 (60.0)	2 (50.0)	1 (100)
	Formers (n=12)	7 (21.2)	5 (31.3)	10 (38.5)	2 (8.7)	10 (25.6)	2 (40.0)	0 (0.0)	0 (0.0)

MRSA=Methicillin-resistant *S. aureus*, MSSA=Methicillin-susceptible *S. aureus*

A total of 39 *S. aureus* were MDR strains, encompassing 100.0% (12/12) ($p=0.044$) of biofilm-formers and 93.9% (31/33) ($p<0.001$) of MRSA. The median (Q1-Q3) OD of MDR-MRSA-Biofilm-formers was 1.9 (1.7-2.3), while that of MDR-MSSA-Biofilm-formers was 2.3 (1.8-N/A) (Figure 1a). The incidence of MDR strains was 33.3% (13/39) among children aged 1 year to < 3 years. Notably, two cases of MDR-MRSA were detected in children aged less than one month. Males (33.3%, 4/12) aged 1 year to 3 years and females (16.7%, 2/12) aged 1 month to 1 year or 3 years to 5 years were mostly infected with biofilm-formers (Figure 1b).



*=statistically significant (p<0.05) with biofilm formation, #= statistically significant (p<0.05) with methicillin resistance, MDR=multidrug-resistance, MRSA=methicillin-resistant *S. aureus*, MRSA=methicillin-susceptible *S. aureus*, AMR=antimicrobial resistance

Figure 1. a) exhibits statistical correlation of biofilms and methicillin resistance with multidrug resistance, b) exhibits incidences of MDR, MRSA, and biofilm-formers based on patients' demographics.

S. aureus exhibited variable antibiotic resistance (Table 3). Bloodstream isolates exhibited 100% ciprofloxacin resistance, while pyogenic isolates showed 71.8% ciprofloxacin resistance, 69.2% cloxacillin resistance, and 41.0% gentamicin resistance. *S. aureus* was resistant to 100.0% (49/49) of amoxicillin and 73.5% (147/262) of ciprofloxacin tested. *S. aureus* exhibited >60.0% resistance to cloxacillin (34/49) and cefoxitin (33/49). *S. aureus* was 100% susceptible to chloramphenicol, doxycycline, teicoplanin, and vancomycin. The isolates showed the highest cumulative resistance to penicillins (84.7%), followed by cephalosporins (52.0%), fluoroquinolones (43.9%), and aminoglycosides (18.4%) (Table 3).

Table 3. Antimicrobial resistance profile of *S. aureus* strains (n=49).

Antibiotics		Susceptible n (%)	Resistance						
			n (%)	Cumulative (%)	MAR index	Samples			
					Median (range)	Abscess (n=4)	Blood (n=5)	Pus (n=39)	Urine (n=1)
Penicillins	Amoxicillin	0 (0)	49 (100.0)	84.7	0.3 (0.1-0.6)	4 (100.0)	5 (100.0)	39 (100.0)	1 (100.0)
	Cloxacillin	15 (30.6)	34 (69.4)			2 (50.0)	4 (80.0)	27 (69.2)	1 (100.0)
Aminoglycosides	Gentamicin	31 (63.3)	18 (36.7)	18.4		0 (0.0)	2 (40.0)	16 (41.0)	0 (0.0)
	Amikacin	40 (81.6)	9 (18.4)			0 (0.0)	2 (40.0)	7 (17.9)	0 (0.0)
	Doxycycline	49 (100.0)	0 (0)			0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fluoroquinolones	Ciprofloxacin	13 (26.5)	36 (73.5)	43.9		2 (50.0)	5 (100.0)	28 (71.8)	1 (100.0)
	Levofloxacin	42 (85.7)	7 (14.3)			0 (0.0)	1 (20.0)	6 (15.4)	0 (0.0)
Cephalosporins	Cephalexin	31 (63.3)	18 (36.7)	52.0		2 (50.0)	4 (80.0)	12 (30.8)	0 (0.0)
	Cefoxitin	16 (32.7)	33 (67.4)			1 (25)	3 (60.0)	28 (71.8)	1 (100.0)
Glycopeptides	Teicoplanin	49 (100.0)	0 (0.0)	0.0		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Vancomycin	49 (100.0)	0 (0.0)			0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others	Clindamycin	40 (81.6)	9 (18.4)	-		0 (0.0)	1 (20.0)	8 (20.5)	0 (0.0)
	Cotrimoxazole	39 (79.6)	10 (20.4)			0 (0.0)	0 (0.0)	10 (25.6)	0 (0.0)
	Chloramphenicol	49 (100.0)	0 (0.0)			0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

MAR index=multiple antibiotic resistance index

MRSA exhibited 42.4% (p=0.235), 24.2% (p=0.339), and 15.2% (p=0.804) resistance to gentamicin, cotrimoxazole, and

levofloxacin, respectively, while MSSA exhibited 25.0%, 12.5%, and 12.3%. MRSA and MSSA exhibited similar resistance rates for cloxacillin (69.0%), amikacin (18.0%), and clindamycin (18.0%). As compared to biofilm-non-formers (59.5%) (p=0.008), biofilm-formers exhibited 100% resistance to cloxacillin. Biofilm-formers exhibited resistance rates of 91.7% (p=0.100) and 41.7% (p=0.016) to ciprofloxacin and amikacin, respectively, while biofilm-non-formers exhibited resistance rates of 67.6% and 10.8% (Table 4).

Table 4. Antimicrobial resistance between methicillin-resistant and susceptible *S. aureus* and biofilm-formers and non-formers.

Antibiotics		MRSA (n=33)		MSSA (n=16)			Biofilm non-formers (n=37)		Biofilm-formers (n=12)		
		Resistant		Resistant		p-value	Resistant		Resistant		p-value
		No	Yes	No	Yes		No	Yes	No	Yes	
Penicillins	Amoxicillin	0 (0.0)	33 (100)	0 (0.0)	16 (100.0)	-	0 (0.0)	37 (100.0)	0 (0.0)	12 (100.0)	-
	Cloxacillin	10 (30.3)	23 (69.7)	5 (31.3)	11 (68.8)	0.946	15 (40.5)	22 (59.5)	0 (0.0)	12 (100.0)	0.008
Cephalosporins	Cephalexin	23 (69.7)	10 (30.3)	8 (50.0)	8 (50.0)	0.180	25 (67.6)	12 (32.4)	6 (50.0)	6 (50.0)	0.273
Fluoroquinolones	Ciprofloxacin	9 (27.3)	24 (72.2)	4 (25.0)	12 (75.0)	0.866	12 (32.4)	25 (67.6)	1 (8.3)	11 (91.7)	0.100
	Levofloxacin	28 (84.9)	5 (15.2)	14 (87.5)	2 (12.5)	0.804	33 (89.2)	4 (10.8)	9 (75.0)	3 (25.0)	0.222
Aminoglycosides	Gentamicin	19 (58.6)	14 (42.4)	12 (75.0)	4 (25.0)	0.235	26 (70.3)	11 (29.7)	5 (41.7)	7 (58.3)	0.074
	Amikacin	27 (81.8)	6 (18.2)	13 (81.3)	3 (18.8)	0.962	33 (89.2)	4 (10.8)	7 (58.3)	5 (41.7)	0.016
	Doxycycline	33 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	-	37 (100.0)	0 (0.0)	12 (100.0)	0 (0.0)	-
Glycopeptides	Teicoplanin	33 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	-	37 (100.0)	0 (0.0)	12 (100.0)	0 (0.0)	-
	Vancomycin	33 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	-	37 (100.0)	0 (0.0)	12 (100.0)	0 (0.0)	-
Others	Chloramphenicol	33 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	-	37 (100.0)	0 (0.0)	12 (100.0)	0 (0.0)	-
	Clindamycin	27 (81.8)	6 (18.2)	13 (81.3)	3 (18.8)	0.962	34 (91.9)	3 (8.1)	6 (50.0)	6 (50.0)	0.001
	Cotrimoxazole	25 (75.8)	8 (24.2)	14 (87.5)	2 (12.5)	0.339	33 (89.2)	4 (10.8)	6 (50.0)	6 (50.0)	0.003

MRSA=methicillin-resistant *S. aureus*, MSSA=methicillin-susceptible *S. aureus*

As well as being 100.0% resistant to penicillins, MRSA-biofilm-formers (n=10) and MDR-biofilm-formers (n=12) were 50.0% resistant to clindamycin and cotrimoxazole. The resistance to gentamicin and cephalexin was 58.3% and 50.0% for MDR-biofilm-formers, respectively, while the resistance to MRSA-biofilm-formers was 50.0% and 40.0% (Table 5).

Table 5. Comparison of antimicrobial resistance between MRSA-biofilm-formers and MDR-biofilm-formers.

Antibiotics		non-(MDR-MRSA-Biofilm) (n=8)	MDR-MRSA (n=31)	MRSA-Biofilm-formers (n=10)	MDR-Biofilm-formers (n=12)
Penicillins	Amoxicillin	8 (100.0)	31 (100.0)	10 (100.0)	12 (100.0)
	Cloxacillin	6 (75.0)	22 (70.9)	10 (100.0)	12 (100.0)
Aminoglycosides	Gentamicin	0 (0.0)	14 (45.2)	5 (50.0)	7 (58.3)
	Amikacin	0 (0.0)	6 (19.4)	4 (40.0)	5 (41.7)
Fluoroquinolones	Ciprofloxacin	4 (50.0)	24 (77.4)	9 (90.0)	11 (91.7)
	Levofloxacin	1 (12.5)	5 (16.1)	3 (30.0)	3 (25.0)
Cephalosporins	Cephalexin	1 (12.5)	9 (29.0)	4 (40.0)	6 (50.0)
	Cefoxitin	0 (0.0)	-	-	10 (83.3)
Other	Clindamycin	0 (0.0)	6 (19.4)	5 (50.0)	6 (50.0)
	Cotrimoxazole	0 (0.0)	8 (25.8)	5 (50.0)	6 (50.0)

MRSA-methicillin-resistant *S. aureus*, MDR=multidrug resistance

DISCUSSION

The infection rate of *S. aureus* was 9.0% (95% CI, 6.6%-11.4%) in this study, which was lower than other studies in Nepal (17.4%-20.9%).[11,12] Because this study involved children (<17 years) instead of general hospital-visiting patients, the prevalence of *S. aureus* infections was comparatively lower. Pretreatment with antibiotics may also explain the lower prevalence of *S. aureus*. Herein, *S. aureus* infections were twice as common in males as females. It could be attributed to estrogen's ability to protect against Gram-positive infections and Hla's role in pathogenesis that females in this study had a lower infection rate.[13] Furthermore, most of the infected children were inpatients (53.1%), especially infants (38.5%) and neonates (11.5%). A similar study conducted in Nepal also found that inpatients had a higher prevalence of *S. aureus* (7.7%) compared to outpatients (5.1%).[14] Nosocomial infections explain the higher incidence of *S. aureus* among inpatients.

In this study, most *S. aureus* were isolated from pus (73.5%)—indicating their predominance in pyogenic soft tissue and wound infections—followed by blood (10.2%) and abscesses (8.2%). Similarly, several studies conducted in Nepal found a high incidence of *S. aureus* isolates from pus (70.6%-78.9%).[15,16] This could be attributed to the fact that *S. aureus* colonizes skin as normal flora and can enter the body directly through skin trauma (burns, cuts, and sores) or penetrating the skin barrier under immunocompromised conditions, resulting in skin and soft tissue infections.[17]

In this study, doxycycline, teicoplanin, and vancomycin were 100.0% sensitive to *S. aureus*, while penicillins (84.7%) had the highest cumulative resistance, followed by cephalosporins (52.0%), fluoroquinolones (43.9%), and aminoglycosides (18.4%). There have been varying reports of *S. aureus* resistance to penicillins (26.0%-93.8%), cephalosporins (27%), fluoroquinolone (17.0%-61.7%), aminoglycosides (22.0%-96.0%), and chloramphenicol (94.9%) from different parts of the world.[18,19] The higher resistance rates could result from misuse or overuse of antibiotics, which leads to selective pressure favoring the dissemination of antibiotic-resistant bacteria. Despite their 100.0% effectiveness in this study, glycopeptides must always be considered a last resort and should not be considered a first-line drug. The susceptibility rate for clindamycin was 81.6% in this study, similar to Thapa et al. (76.3%), indicating its use as a first choice before using glycopeptides.[20] Increasing incidence of clindamycin resistance, which could be attributed to a high rate of spontaneous mutation during therapy, have been reported in Nepal.[20,21] Amikacin, levofloxacin, and chloramphenicol were also found effective against *S. aureus* in this study and therefore could be considered better options for treating *S. aureus* infection.

MRSA prevalence was 67.4% in this study, which is higher than other studies conducted in Nepal.[16,22] There is great variation in MRSA prevalence (17.0%-41.0%) worldwide.[8,23] These opposing data are hard to explain both in terms of time and location, but it is probably due to their differences in clonal expansion and local drug pressure. In this study, inpatients (67.9%) and infants (55.8%) were the primary sources of MRSA isolates. MRSA incidences have also been reported to range from 63.3% to 75.0% in Nepalese children, most frequently in inpatients.[24,25] MRSA is more common among inpatients because it could be nosocomial infections, often accompanied by hospital-associated risk factors, e.g., extended hospital stays, which increase the chances of secondary infection, or prolonged antibiotic treatment that cuts off its effectiveness.[25]

In this study, two-fifths of *S. aureus* strains formed biofilms, with moderate formers (76.9%) being predominant, followed by weak formers (20.2%) and strong formers (2.9%). A high incidence of biofilm-forming *S. aureus* has been reported in Nepal (21.1%) and India (55.0%-64.9%), with weak biofilm-formers (34.9%-74.4%) grading highest, followed by moderate (17.9%-27.9%) and strong biofilm-formers (6.9%-7.7%).[12,26] Several factors could have led to biofilm formation in *S. aureus*, including exposure to sub-inhibitory concentrations of antimicrobials or strain acquisition of biofilm-forming genes.[27]

This study found 7.6% of *S. aureus* to be MDR, substantially lower than another report (32.0%).[27] The reasons could be due to *S. aureus's* ability to produce biofilms that are intrinsically resistant to antibiotics or because their low growth rate leads to antibiotic degradation, preventing antibiotics from penetrating biofilms. In this study, all biofilm-formers and 34.0% of MRSA strains were MDR isolates. Moreover, this study also revealed a biofilm positivity of 30.0% among MRSA isolates.[32] Cross-transmission of pathogenic MDR strains between inpatients on high-antibiotic pressure wards or clinician misuse of antibiotics may be a major cause of such co-existences.

In this study, MRSA strains exhibited higher resistance to gentamicin [42.4% versus (vs.) 25.0%] ($p>0.05$), cotrimoxazole (24.2% vs. 12.5%) ($p>0.05$), and levofloxacin (15.2% vs. 12.5%) ($p>0.05$) compared to

MSSA. Numerous studies conducted in Nepal concur with this finding.[16,28] In contrast, Sanjana et al.[16] reported a lower resistance to gentamicin (38.0%), and Kumari et al.[28] reported a higher resistance to ciprofloxacin (67.8%). These strains harbor the *mecA* gene, which encodes a PBP 2a with low affinity for all ß-lactam antibiotics as well as increased resistance to other antibiotics.[3] Similarly, biofilm-formers in this study showed higher resistance to cloxacillin (100.0% vs. 59.5%) ($p<0.05$), ciprofloxacin (91.7% vs. 67.8%) ($p>0.05$), and amikacin (41.7% vs. 10.8%) ($p<0.05$) compared to biofilm non-formers. Moreover, cloxacillin was effective against a small percentage of MSSA isolates (31.3%) ($p>0.05$) and biofilm non-formers (40.5%) ($p<0.05$). Antibiotics such as these are relatively cheaper and easily accessible over the counter in Nepal, which has led to the emergence of resistant strains in Nepal.[28] Similarly, a study by Neopane et al. reported increased resistance to cloxacillin (72.0% vs. 28.0%) and ciprofloxacin (54.0% vs. 46.0%) in biofilm-formers compared to non-formers.[12] Herein, chloramphenicol, and glycopeptides were 100% effective against MRSA and biofilm-formers and can be prescribed for the treatment of infections caused by *S. aureus*. It is important to note that while gentamicin (58.6%) and levofloxacin (84.9%) exhibit good effectiveness against MRSA, they should not be used empirically to treat MRSA-associated infections, as these drugs select and yield resistant mutants and result in relapse and treatment failure.

Biofilm-forming MDR strains (50.0%) exhibited the highest aminoglycoside resistance in this study, followed by biofilm-forming MRSA (45.0%), MDR-MRSA (32.3%), and biofilm non-forming non-MDR MSSA strains (0.0%). Resistance patterns for cephalexin and cloxacillin were also similar. In contrast, fluoroquinolone resistance was highest in biofilm-forming MRSA isolates (60.0%), followed by biofilm-forming MDR (58.3%), MDR-MRSA (46.8%), and biofilm non-forming non-MDR-MSSA strains (31.3%). In biofilm-forming MDR and MRSA stains, clindamycin, and cotrimoxazole resistance were highest and similar. High resistance in biofilm-formers, irrespective of MRSA or MDR association, may be related to *S. aureus* biofilms' protective layer, which hinders antibiotic penetration. Moreover, studies suggest that *SCCmec* elements (types I-III in hospital-acquired or IV-V in community-acquired) alter the biofilm phenotype in *S. aureus*, increasing biofilm strength and supporting *SCCmec* genes as a pivotal factor in biofilm-associated infections.[29,30]

This study suffers from limitations. Firstly, a report on *S. aureus* prevalence, particularly conducted on children, from a single hospital may either underestimate or overestimate it. Secondly, antibiotic resistance genes were not correlated with heightened resistance in the strains. Nonetheless, this study indicates that people visiting this hospital are more likely to contract *S. aureus* infections with MDR and biofilm-formers, which is why antimicrobial stewardship requirements need to be strictly adhered to.

CONCLUSIONS

Paediatric *S. aureus* infections were rare. They mainly caused pyogenic infections in toddlers, males, and inpatients. The infection rates of MRSA strains were higher than those of biofilm-forming strains. More than 95% of MDR strains comprised of biofilm-formers and MRSA. Penicillins were the least effective antibiotics against *S. aureus*, followed by cephalosporins and fluoroquinolones. Regardless of association with MDR or MRSA, biofilm-forming strains had the highest antimicrobial resistance. Clindamycin, chloramphenicol, or glycopeptides were 100% effective.

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