

## Nasal carriage of methicillin and inducible clindamycin resistant *Staphylococcus aureus* among healthcare workers in a tertiary care hospital, Kathmandu, Nepal

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### Abstract

**Introduction and Objectives:** Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) from healthcare workers is one of the most frequent causes of nosocomial infections globally. There is a significant burden of nosocomial MRSA infections in low and low-middle income countries (LMICs), including Nepal. The present study investigated the rate of nasal carriage of MRSA among the healthcare workers in a tertiary care hospital, in Kathmandu, Nepal with emphasis on inducible macrolide-lincosamide-streptogramin B (iMLSB) resistance.

**Material and method:** The study was conducted at Star Hospital, Lalitpur, Nepal, from September 2022 to November 2022. Healthcare workers (HCWs) working at the different departments of the hospital were enrolled. Nasal swabs from both anterior nares of HCWs were collected aseptically and cultured on Mannitol Salt agar. *S. aureus* was identified by Gram stain and standard biochemical tests. Antibiotic susceptibility of *S. aureus* was performed by disc diffusion method. MRSA isolates were detected phenotypically by disc diffusion method using cefoxitin disc (30 µg), and inducible clindamycin resistance was detected phenotypically by the D-zone test.

**Results:** Total 105 HCWs were enrolled in the study. Out of 105 HCWs, 14 (13.3%) were positive for *S. aureus* among which 6 (5.7%) were MRSA carriers. The nasal carriage of MRSA was highest among doctors (16.7%) and the HCWs of the post-operative department (14.3%). All the isolated MRSA were susceptible to chloramphenicol and vancomycin. Inducible MLSB resistance was detected in 33.3% MRSA while the rate was 21.4% in all isolated *S. aureus*.

**Conclusion:** The study demonstrated that HCWs could be the potential source of nosocomial infection by methicillin and inducible clindamycin resistant *S. aureus*. Thus, preventive measures should be initiated to mitigate the risk of its spread and the test for detection of inducible clindamycin resistance should be incorporated into the routine antibiotic susceptibility testing in hospital settings.

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### Introduction

*Staphylococcus aureus* is a Gram-positive coccus, arranged in clusters and is ubiquitously present as

normal flora in humans and animals [1]. *S. aureus* is a highly infectious human pathogen that, despite being a normal component of the floral biota, has

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the potential to cause a wide variety of infections ranging from minor cutaneous symptoms to fatal sepsis [2]. Its adaptive versatility to alternating host and environmental conditions has rendered it a clinically important bacterium.

Methicillin was frequently used in 1959 to treat infections caused by penicillin resistant *S. aureus*. After two years, the first reports of methicillin-resistant *S. aureus* strains came from the United Kingdom in 1961 [3]. The  $\beta$ -lactam drugs including methicillin, penicillin, oxacillin, and amoxicillin are not effective against MRSA [4].

Macrolide-lincosamide-streptogramin B (MLSB) antibiotics are commonly used for the management of infection by MRSA [5]. The category of antibiotics known as MLSB includes the macrolides (such as erythromycin, azithromycin, and spiramycin), lincosamides (such as clindamycin, and lincomycin), and streptogramin B (such as quinupristin). Clindamycin is a popular choice for various staphylococcal infections, notably skin and soft tissue infections, and it is an alternative for people who are allergic to penicillin. This has caused clinicians to become more interested in MLSB antibiotics to treat *S. aureus* infections rather than penicillin derivatives [6,7]. However, with time and overuse, *S. aureus* has also acquired resistance against MLSB antibiotics. Resistance to MLSB antibiotics is mediated by methylation of rRNA, active efflux and enzymatic inactivation [8]. The expression of the bacterial resistance to MLSB antibiotics may either be constitutive or inducible. Therefore, clinical failures may result if resistance to MLSB antibiotics is not sufficiently investigated in the laboratory [6,8].

MRSA has been a common cause of nosocomial infections in 5–10% of hospitalized patients [9]. Effective therapy has been difficult due to an increase in infections by MRSA strains that are multi-drug resistant [10]. Healthcare workers (HCWs) frequently serve as MRSA transmission and dissemination vectors. MRSA is known to colonize primarily in the anterior nares and other body sites including skin, axillae, and intestinal tract, allowing a high risk of exposure and transmission in admitted patients [11,12]. Specifically, the carriage of MRSA in the nose, a site of frequent touch, appears to be the chief reason behind the

escalating prevalence of nosocomial MRSA infections [13,14].

Hospital-acquired infections (HAIs) are a major problem in the world today and healthcare workers are an important reservoir of infectious agents. Undoubtedly, HAIs are an important interface between healthcare centers and the community [15,16]. HAIs due to MRSA is associated with significant morbidity, mortality and cost burden [15]. HCWs are more frequently viewed as vectors, rather than being the main source of MRSA transmission [17]. The commonest mode of MRSA transmission has been through the hands of HCWs contaminated with colonizer MRSA. Several case reports have documented symptomatic clinical MRSA infections among carrier HCWs [18].

MRSA has been a major threat to public health in developing nations, particularly Nepal, because of poor infection control practices and excessive antibiotic use [2]. According to data from published research, the proportion of HCWs who had nasal MRSA carriage ranged from 20.37% to 43.80% [19]. These figures demonstrate the necessity and significance of screening HCWs for MRSA nasal carriage, which is a key element in the prevention of nosocomial infections. Monitoring and locating MRSA carriers among healthcare workers (HCWs) enables effective preventive measures against transmission to clients and coworkers.

This study examined the nasal carriage of MRSA in a tertiary care hospital in Kathmandu, Nepal, and studied their antibiotic susceptibility pattern with emphasis on inducible macrolide-lincosamide-streptogramin B (iMLSB) resistance. The findings of this project are aimed at bringing forth the importance of antimicrobial procedures and infection control strategies by and within healthcare workers.

## Materials and Methods

**Study site and sample collection:** This cross-sectional study was conducted in Star Hospital, Lalitpur, Nepal, from September 2022 to November 2022. The Institutional Research Committee of Star Hospital Research Center granted ethical approval to this study (Registration No.: 323/078/079).

Informed written consent was taken from the participants.

Samples were taken by the non-probability purposive sampling method. Following informed written consent, volunteer healthcare workers were enrolled.

The sample size was determined as:

$n = Z^2 pq / l^2$ , where,  $n$ = required sample size,  $Z$ = 1.96 at 95% confidence interval,  $p$ = expected prevalence = 38.2% [2],  $l$ = allowable error= 20% of  $p$  (38.2%) = 7.64,  $q$ = 100- $p$  = 100 - 38.2 = 61.8%. Therefore,  $n = Z^2 p(100-p) / l^2$ ;

$$n = (1.96)^2 \times 38.2 \times 61.8 / (7.64)^2 = 155$$

Nasal swabs from both anterior nares of HCWs working at Star Hospitals which included doctors, nurses, laboratory professionals, pharmacists, and health assistants were collected. The nasal swab samples were then transported to the Microbiology Laboratory of Modern Technical College, Lalitpur, Nepal for processing and examination.

**Isolation and characterization of *S. aureus*:** Nasal swabs were streak on Mannitol Salt Agar plates and incubated overnight at 37°C. The colony morphology, Gram stain, and biochemical tests namely catalase, coagulase, deoxyribonuclease (DNase) were used to identify *S. aureus*.

**Antimicrobial susceptibility test:** On Muller-Hinton agar (MHA), antimicrobial susceptibility test (AST) was carried out by Kirby-Bauer disc diffusion method. Bacterial suspension turbidity was adjusted to 0.5 McFarland standard. Antimicrobial discs used were amoxicillin (10µg), chloramphenicol (30µg), clindamycin (2µg), cotrimoxazole (25µg), ceftiofur (30µg), erythromycin (15µg), gentamicin (10µg), ofloxacin (5µg), tetracycline (30µg) and vancomycin (30 µg). Antimicrobial agents were selected based on clinical significance and were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines [20,21].

**Phenotypic testing of MRSA:** The MRSA was detected phenotypically by disc diffusion method using ceftiofur disc (30 µg). The inoculated MHA plates were incubated aerobically at 35°C for 18 hours. *S. aureus* yielding zone diameter of

≤21 mm was phenotypically confirmed as MRSA, as per CLSI M100-S28.

**Detection of iMLSB resistance:** Inducible clindamycin resistance was detected by D-zone test [22]. Briefly, 0.5 McFarland standard bacterial suspension of *S. aureus* was lawn cultured on MHA plate. On the MHA plate, erythromycin disc (15 µg) were placed at a distance of 15 mm from the clindamycin disc (2 µg) and incubated for 18 to 24 hours at 37°C. After incubation, organisms were determined to be iMLSB resistant if the clindamycin zone of inhibition adjacent to the erythromycin disc flattened out. Resistance to erythromycin and clindamycin indicated a constitutive MLSB resistance (cMLSB). Susceptibility to clindamycin and resistance to erythromycin defined the macrolide–streptogramin B (MSB) phenotype.

**Statistical Analysis:** The 2016 version of Microsoft Excel was used to compile the data, and Statistical Package for Social Sciences (SPSS) version 25 was used to analyse the data.

## Results

A total of 105 healthcare workers were enrolled and out of which 28 (26.7%) and 77 (73.3%) were male and female respectively. The age range was 18 to 52 years. Out of 105 nasal samples, 14 (13.3%) yielded growth of *S. aureus* of which 6 (5.7%) were MRSA and 8 (7.6%) were MSSA. Laboratory personnel had the highest prevalence of *S. aureus* (24%, (6/25)) while doctors had the highest prevalence of MRSA (16.7%, 2/12). Detail is shown in Table-1.

Similar to department-wise, the general ward's HCWs had the highest prevalence of *S. aureus* 20% (4/20). A post-operative ward was found to have the largest distribution of MRSA at 14.3% (1/7), whereas the general ward had the highest distribution of MSSA at 20.0% (4/20) (Table 2). Detail distribution of carrier rates of *S. aureus* and MRSA in HCWs from different departments of the hospital is shown in Table-2. HCW from post-operative ward was the highest carrier of MRSA (14.3%) followed by HCWs from ICU and OPD. The carrier rate of MRSA was 7.1% and 5.2% (Table-3) in male and female HCWs respectively ( $p > 0.05$ ).

**Table-1:** Profession-wise distribution of isolated *S. aureus*

Profession	No. of samples (%)	<i>S. aureus</i> n (%)	MRSA n (%)	MSSA n (%)	Non-carriers n (%)
Doctor	12 (11.4)	2 (16.7)	2 (16.7)	0	10 (83.3)
Nurse	30 (28.5)	5 (16.7)	1 (3.3)	4 (13.3)	25 (83.3)
Laboratory professionals	25 (23.8)	6 (24.0)	2 (8.0)	4 (16.0)	19 (76.0)
HA	12 (11.4)	0	0	0	12 (100.0)
Pharmacist	7 (6.7)	0	0	0	7 (100.0)
Others*	19 (18.0)	1 (5.3)	1 (5.3)	0	18 (94.7)
Total	105	14 (13.3)	6 (5.7)	8 (7.6)	91 (86.7)

Others\* = Certified Medical Assistants (CMAs), Housekeepers, and Security guards; *S. aureus*= *Staphylococcus aureus*, MRSA= methicillin resistant *S. aureus*; MSSA= methicillin sensitive *S. aureus*.

**Table-2:** Department-wise distribution of isolated *S. aureus*

HCW from	No. of samples n (%)	<i>S. aureus</i> isolated n (%)	MRSA n (%)	MSSA n (%)	Non-carriers n (%)
ER	12 (11.4)	0	0	0	12(100.0)
ICU	9 (8.6)	1(11.1)	1(11.1)	0	8(88.9)
Laboratory	32 (30.5)	6(18.8)	2(6.3)	4(12.5)	26(81.3)
Pharmacy	8 (7.6)	0	0	0	8(100.0)
PO-ward	7 (6.6)	1(14.3)	1(14.3)	0	6(85.7)
General ward	20 (19.0)	4(20)	0	4(20)	16(80.0)
OPD	17 (16.2)	2(11.8)	2(11.8)	0	15(88.2)
Total	105	14(13.3)	6(5.7)	8(7.6)	91(86.7)

ER = emergency room, ICU = intensive care unit, PO = post operative, OPD = out patient department. MRSA= methicillin resistant *Staphylococcus aureus*, MSSA= methicillin sensitive *Staphylococcus aureus*

**Table-3:** Distribution of *S. aureus* by gender

Gender	No. of samples, n (%)	<i>S. aureus</i> isolated n (%)	MRSA n (%)	MSSA n (%)	Non-carriers n (%)
Male	28 (26.7)	4 (14.3)	2 (7.1)	2 (7.1)	24 (85.7)
Female	77 (73.3)	10 (12.9)	4 (5.2)	6 (7.8)	67 (87.0)

*S. aureus*= *Staphylococcus aureus*; MRSA= methicillin resistant *S. aureus*; MSSA= methicillin sensitive *S. aureus*.

As shown in Table-4, none of the MRSA isolate was resistant to chloramphenicol and vancomycin, while resistance to clindamycin, cotrimoxazole and tetracycline was 16.7% for each. Similarly, none of the MSSA was resistant to vancomycin and

resistance to tetracycline, ofloxacin, cotrimoxazole, chloramphenicol, and gentamicin was same (each 12.5%). Similar to this, clindamycin and erythromycin were shown to have the lowest sensitivity against MSSA strains (75 % each).

**Table-4:** Results of antibiotic susceptibility of isolated *S. aureus*

	MRSA (n=6)	MSSA (n=8)	Total <i>S. aureus</i> (n=14)
Antibiotic	Resistant n (%)	Resistant n (%)	Resistant n (%)
Amoxicillin	6 (100)	4 (50)	10 (71.4)
Chloramphenicol	0	1 (12.5)	1 (7.1)
Clindamycin	1 (16.7)	2 (25)	3 (21.4)
Cotrimoxazole	1 (16.7)	1 (12.5)	2 (14.3)
Erythromycin	3 (50)	2 (25)	5 (35.7)
Gentamicin	2 (33.3)	1 (12.5)	3 (21.4)
Ofloxacin	2 (33.3)	1 (12.5)	3 (21.4)
Tetracycline	1 (16.7)	1 (12.5)	2 (14)
Vancomycin	0	0	0

**Table-5:** MLSB resistance phenotypes among *S. aureus* by D test

D test result	MRSA (N=6), n (%)	MSSA (N=8), n (%)	Total (N=14), n (%)
ER-R, CL-S, D test +ve (iMLSB phenotype)	2 (33.3)	1 (12.5)	3 (21.4)
ER-R, CL-R (cMLSB phenotype)	0	2 (25)	2 (14.3)
ER-R, CL-S, D test –ve (MSB phenotype)	1 (16.7)	2 (25)	3 (21.4)
ER-S, CL-S	3 (50)	3 (37.5)	6 (42.9)

ER: Erythromycin, CL: Clindamycin, R: Resistant, S: sensitive

Out of total isolated *S. aureus*, 21.4% (3/14) exhibited iMLSB trait (D test positive) and 14.3% was cMLSB phenotype. MRSA isolates exhibited higher iMLSB trait (33.3%) compared to MSSA isolates (12.5%). Detail pattern is shown in Table-5.

## Discussion

*S. aureus* has become one of the most prevalent multi-drug resistant nosocomial bacteria and is responsible for a wide variety of life-threatening infections in both hospital and community settings. HCWs should be thoroughly screened for the presence of MRSA strains because they are one of the main *S. aureus* reservoirs and frequently act as a bridge between hospital and the general population. In our study, 13.3% of HCWs was carrier of *S. aureus*. The finding is comparable to the rate of carriers (14.7% to 18.3%) among the HCWs working in different

hospital in Nepal [16,23-26]. However, Shrestha et al. [27] reported the carrier rate as 27.1% among HCWs working in a hospital in Nepal.

Our findings of MRSA carrier rate of 5.7% were in agreement with the results reported by Giri et al [23] 5.2%, Khatri et al [26] 7.5%, Shakya et al [28] 7.1%, and Shrestha et al [27] 2.3%. In contrast, our investigation revealed a decreased prevalence of nasal carriage of MRSA compared to studies by Rongpharpi et al [11] and Vinodhkumaradithyaa et al [29] who reported MRSA carrier rate as 11.4% and 15.4%, respectively. The rate of nasal carriage of *S. aureus* and MRSA among healthcare personnel varies depending on sampling methodology, demography, hospital environment and patient load. Our results emphasize that to lower the incidence of MRSA infections in the hospital setting, proper surveillance of carriage must be implemented, and HCWs' expertise must be improved [30].

According to our study, doctors had the highest MRSA carriage rate (16.7%). Similar to this, Rongpharpi et al [11] showed that doctors had the highest MRSA colonization rate of 25.0%. MRSA colonization among laboratory workers was found to be 10.5% in a study by Khatri et al [26]. However, Shah et al [31] reported highest MRSA carrier rate of 16.7% among the pharmacists. Frequent physical contact with patients may contribute to higher MRSA colonization among doctors, which further suggests higher patient infection risk during treatment, resulting in lengthier hospital stays, prolonged antibiotic administration, and higher expenses [23].

In our study HCWs in the post-operative ward had the highest MRSA carriage rates (14.3%). The finding is similar to the studies by Giri et al [23] and Khatri et al [26] who reported the rate as 18.2% and 14.3% respectively. The increased carriage rate in these HCWs could be caused by surgical immune suppression and traumatic stress [32]. Patients are more susceptible to developing MRSA infections at surgical sites, which complicates care and lengthens healing time [23]. In contrast, study by Shah et al [31] found greater MRSA colonization rates in HCWs in ICUs and pharmacies, at 16.7% each.

In our study, all the MRSA and MSSA were susceptible to chloramphenicol, and vancomycin. Shrestha et al [27] have also reported similar susceptibility of both MRSA and MSSA to vancomycin, teicoplanin, chloramphenicol, rifamycin, and gentamicin. However, according to Karimi et al [33], only 58.5% of *S. aureus* was susceptible to tetracycline. Similar to this, MRSA isolates also demonstrated greater susceptibility to tetracycline, clindamycin, and cotrimoxazole (83.3%), indicating that these antibiotics are advantageous choices for empirical therapy for MRSA infections. Vancomycin was the only drug that all MSSA were sensitive to (100%), and it was followed by gentamicin, ofloxacin, tetracycline, chloramphenicol, and cotrimoxazole (each 88.0%), suggesting that those antibiotics could be used in the empirical treatment of MSSA infections.

In the present study, 21.4% of isolated *S. aureus* tested positive for the D-test while 33.3% (2/6) of MRSA strains exhibited iMLSB resistance which was in line with the results of other reported studies

from Nepal [8,23,34-37]. However, our result did not agree with the findings of Khanal et al [24] who found iMLSB resistance as 66.7% in MRSA. In the present study, constitutive MLSB (cMLSB) resistance was 14.3% among the isolated *S. aureus*. This rate was low compared to the rates reported by other studies [8,34,35,37]. The difference might be due to difference in study populations, study periods, and different hospital settings. The presence of iMLSB and cMLSB in *S. aureus* poses a significant risk in using clindamycin as a therapeutic drug in staphylococcal infections. Therefore, D test should be routinely carried out on all *S. aureus* isolates in clinical microbiology laboratories and the practitioner should be made aware of the potential limitations of clindamycin.

## Declarations

## Author Contributions

GP: Conceptualization, designing, supervision and approval of SOP of the study, validation of the results, writing, editing and reviewing the manuscript.

ASG, LM, BM, AU and AS: involved in writing SOP for the study, sample collection, performing tests, analysis and reporting of the results, manuscript writing and literature search.

## Conflict of Interest

The authors have no conflicts of interest to declare.

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