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Original Article

Panton-Valentine leucocidin gene in methicillin resistant *Staphylococcus aureus* isolated from tertiary care hospital in Nepal

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Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) expresses the *Panton-Valentine leukocidin* (*PVL*) virulence gene, which is associated with community and hospital-acquired severe MRSA infections. The objective of this study was to determine the prevalence and antibiotic susceptibility profile with a focus on the presence of the *PVL* gene among MRSA isolates in healthcare settings.

Methodology: A total of 1,207 clinical specimens and 304 hospital environment swabs were collected in a tertiary care hospital in Nepal, and investigated following basic microbiological techniques. *S. aureus* was confirmed with the coagulase test. An antibiotic susceptibility test (AST) was performed by the Kirby-Bauer method and screening for MRSA was carried out by the cefoxitin disc diffusion method guided by the Clinical and Laboratory Standards Institute (CLSI), 2020. DNA was extracted and used in a polymerase chain reaction (PCR) to detect *mecA* and *PVL* genes.

Results: Of the 1,511 samples, 45 (2.9%) *S. aureus* (23 clinical and 22 environmental) were isolated. Among them, 69.6% (16/23) and 27.3% (6/22) were MRSA in clinical and environmental isolates, respectively. Twelve (52.2%) clinical isolates and seven (31.8%) environmental isolates were multidrug resistant. The majority of isolates were susceptible to vancomycin and linezolid. The *PVL* gene was detected in 18.2% (n = 4/22) of the MRSA isolates, of which three were from clinical sources and one was from an environmental swab.

Conclusions: The prevalence of MRSA, and PVL-producing *S. aureus* were higher in the hospital setting. Hence, immediate and urgent implementation of infection control and sanitation measures are needed in the hospital.

Key words: MRSA; MDR; hospital environment; mecA; PVL.

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Introduction

Staphylococcus aureus is a common commensal bacterium which is a frequent cause of healthcare-associated infections (HAI) and community-acquired infections. It is the most common cause of nosocomial pneumonia and surgical site infections [1]; and the second most common cause of bloodstream, cardiovascular, eye, ear, nose, and throat infections [2]. Drug resistance among *S. aureus* is increasing and the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) is a public threat globally. The resistance of MRSA to β-lactam antibiotics is associated with penicillin-binding protein (PBP) 2a, encoded by the *mecA* gene [3]. The primary mechanism by which MRSA acquires resistance against antibiotics is through the *mecA* gene, which results in the production of low-affinity penicillin-binding protein (PBP2a). The *mecA* gene is a component of the *SCCmec*, a large mobile genetic element that transfers by transduction or conjugation; but the clear mechanism of transmission has been debatable for over 50 years [4]. The MRSA strains in hospital and community settings have developed a significant level of antibiotic resistance [5,6]. Furthermore, there are reports that prevalence of MRSA has increased [7], even in those who have never been exposed to hospital risk factors [8].

MRSA can contaminate surfaces in the hospital environment that are frequently touched by hands of patients and healthcare personnel (HCP) who are nasal carriers of MRSA colonization/infection [3,9]. MRSA can be transmitted in a variety of ways in hospital/clinical settings, including (1) direct touch with clinical specimens (pus, blood, or saliva), (2) indirect contact with contaminated tools or ambient surfaces,

and (3) exposure to infected patients' microbiological aerosols. Additionally, HCPs who have MRSA but show no symptoms of infection can spread the bacteria to hospitalized patients. As a result, hospital surfaces and HCPs are likely to play a role in MRSA transmission to patients or other HCPs [10,11]. Analysis of the prevalence of MRSA contamination and probable environmental sites can help to focus on various hygienic measures, resulting in fewer cross transmissions and associated HAIs [12,13].

Numerous cytolysins, such as bicomponent leukocidins, homolysin, α-toxin, and phenol-soluble modulins (PSMs), are produced by *S. aureus* and directly lyse leukocytes [14]. Panton-Valentine leukocidin (PVL) is one of the most important virulence factors of *S. aureus*. The *PVL* gene that encodes cytotoxin comprises two exoprotein subunits, encoded by *LukS-PV* and *LukF-PV* [15]. These destroy leukocytes and alter the immune system by creating beta pores in the mitochondrial membrane, which eventually leads to mitochondrial cell membrane lysis and death [16]. The PVL toxin is responsible for many of the mild to severe clinical symptoms of infection caused by MRSA such as severe necrotizing pneumonia, furunculosis, invasive osteomyelitis, and necrotic lesions of the skin and soft tissues [17,18]. PVL positive MRSA are more virulent and highly transmissible compared to *PVL*-negative MRSA. Patients with *PVL*-positive MRSA infections had a greater mortality rate than those with *PVL*-negative MRSA, with a 1.56-fold increased chance of mortality (95% confidence interval) [19].

Moreover, recent studies suggest an alarmingly high prevalence of MRSA (33–55.6%) in clinical specimens causing skin and soft-tissue infections (SSTI) in Nepal between 2013 and 2021 [20–24]. The *PVL* gene carrying MRSA strains have been increasingly linked to outbreaks of hospital illnesses in reports from several countries [25–27]. However, there are rare studies in Nepal that have collected comprehensive data on the prevalence and molecular characteristics of *PVL*-positive MRSA in hospitalized patients and the hospital environment. The frequency of *PVL* varies across studies, and it is not frequently examined in diagnostic laboratories. The *PVL*- positive isolates have varying levels of antibiotic susceptibility. Therefore, the aim of this study was to determine the prevalence, drug resistance patterns, and molecular characteristics of *PVL*-positive *S. aureus* isolated from patients and patients' room (hospital environment) in tertiary care hospitals of Nepal.

Methodology

Study site, design and sampling

This is a cross-sectional descriptive laboratory-based study including environmental swabs and clinical specimen collected in a tertiary care hospital in Nepal. The study was conducted from April to November 2021.

Clinical samples including pus, blood, sputum, urine, and wound swab were collected from in-patients and out-patients, and environmental swabs were collected from the surfaces of medical instruments, beds, door handles, and floor/walls in general medical ward of KIST Medical College and Teaching Hospital of Lalitpur district, Nepal. A written consent was obtained from each patient who provided clinical specimen for this study.

All the clinical samples like pus, blood, sputum, urine, and wound swabs were collected with standard clinical management and the results were anonymized for analytical purpose. Sterile moistened swabs were used to collect specimens from surfaces of highly touched items (bed surfaces, medical instrument, door knobs, and floor), which were conveniently chosen from the rooms of studied patients.

The research was carried out at the Microbiology unit of Clinical Service laboratory, KIST Medical College and Teaching Hospital, Lalitpur; and Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal.

Isolation and identification

The clinical samples (pus, blood, sputum, urine, wound swabs) and environmental swab samples were inoculated in 5% blood agar (Hi-Media Laboratory Mumbai, India) and incubated at 37.0 °C for 24 hours aerobically. Single beta-hemolytic colonies of cultures from blood agar were inoculated into mannitol salt agar (MSA) plates and incubated at 37.0 °C for 24 hours before checking for fermentation of mannitol [28]. All positive isolates from MSA were sub-cultured into nutrient agar (HiMedia Laboratory, Mumbai, India) using a single discrete colony per plate (sample) and incubated at 37.0 °C in ambient air for 24 hours to obtain pure colonies.

Identification of the bacterial isolates was carried out using standard microbiological procedures [28]. The isolates were identified by observation of colony morphology; Gram staining; enzymatic reaction; and fermentation tests, including catalase and tube coagulase tests [29].

The pure cultures of *S. aureus* that were obtained were placed in tryptic soy broth (TSB) with 40%

glycerol and kept at −80 °C until needed for further analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by Kriby-Bauer disc diffusion method guided by the Clinical Laboratory Standard Institute (CLSI) guidelines 2020 [30] using the following antibiotics: cefoxitin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), amikacin (30 µg), ampicillin (10 µg), oxacillin (30 µg), tetracycline (30 µg), linezolid (30 µg), and vancomycin (30 µg).

The quality control of antimicrobial susceptibility testing was carried out using the coagulase positive methicillin susceptible *S. aureus* (MSSA) ATCC 25923.

Identification of MRSA strains

MRSA was detected by using a cefoxitin disk of 30µg on a Mueller Hinton Agar (MHA) plate by Kirby-Bauer disc diffusion method. If the diameter of the zone of inhibition around the cefoxitin disk was ≤ 21mm after overnight aerobic incubation, the isolate was considered to be MRSA positive [30].

Rapid DNA extraction method

Total DNA from isolates was extracted by the boiling method [31]. Briefly, a number of bacterial colonies were inoculated in 10 mL of Luria-Bertani (LB) broth and incubated at 37 °C for 18 hours. A loopful of overnight culture was then suspended in 100 µL of 50 mM NaOH taken in a sterile microfuge tube. Such a prepared suspension was placed in a boiling water bath for 5 minutes. Then, the suspension was placed in a refrigerator set at 4 °C for 5 minutes. Following refrigeration, 16 µL of 1 M Tris-HCl was added to suspension and centrifuged at 8,000 rpm for 2 minutes. The supernatant was then transferred into a new sterile microfuge tube and used as template DNA for polymerase chain reaction (PCR) reactions or stored at -20 °C until further use.

PCR amplification of mecA and PVL genes

The detection of *mecA* and *PVL* genes of *S. aureus* was done by PCR amplification assay using gene-specific primer pairs (Table 1). The PCR procedure was carried out with 25 µL volume in a PCR tube and each of the reaction mixtures contained: 5 µL PCR master mix (5X; which contained reaction buffer (0.4 M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 0.1% w/v Tween-20), 1.5 mM $MgCl_2$, *Taq* DNA polymerase, dNTPs (200 mM each)); 1 µL of each primer (10 pmol/ µL); 3 µL (10 ng/µL) of template DNA; and 15 µL PCR grade water. The amplification took place by the process of PCR thermocycling in a thermal cycler (Bio-Rad, Hercules, USA), commencing with an initial denaturation at 95 °C for 10 minutes; followed by 30 cycles, each comprising 94 °C for 1 minute, annealing at 53 °C for 50 sec, and 56 °C for 45 sec for *mecA* and *PVL* gene, respectively, and a common extension of 72 °C for 1 minute; with a final extension at 72 °C for 5 minutes.

Then, 10 µL of PCR amplicons were loaded in agarose gel (1.5%) containing ethidium bromide (0.5 g/mL) followed by electrophoresis at 70 V for 1 hour, and visualized under Azure™ gel documentation system (Azure Biosystems, Dublin, USA) for image analysis. The 163 bp and 433 bp fragments of DNA corresponded to *mecA* and *PVL* gene, respectively (Table 1).

Statistical analysis

The raw data was tabulated in Microsoft Excel, cleaned, and then exported to Statistical Package for Social Science (SPSS) software version 16.0 (IBM corporation, Armonk, New York, USA) for analysis. The values were expressed as percentages and frequencies. Categorical variables were analyzed using descriptive statistics and Chi square test for the significant differences in their association. Differences were considered statistically significant when $p < 0.05$.

Ethical review and approval

The study received ethical approval from the Ethical Institutional Review Committee (IRC) of KIST Medical College and Teaching Hospital (Ref. no. 077/078/54).

Table 1. Specific primers used in this study for the amplification of target genes.

Genes	Primer sequence	Amplicon size	Reference
mecA	F: 5-ACTGCTATCCCACCCTCAAAC-3 R: 5- CTGGTGAAGTTGTAATCTGG-3	163 bp	[24,32]
PVL	F: 5-ATCATTAGGTAAAATGTCTGGACATGATCCA-3 R: 5-GCATCAAGTGTATTGGATAGCAAAAGC-3	433 bp	[20,33]

Table 2. Distribution of *Staphylococcus aureus* isolates with *mecA* and *PVL* in clinical specimens from patients.

Variables	Isolates (n = 23) Number (%)	MRSA (n = 16) Number (%)	*mecA* (n = 12) Number (%)	*PVL* (n = 3) Number (%)
Age (years)				
0–10	1 (4.3)	1 (6.3)	1 (8.3)	
11–20	1 (4.3)	-	-	
21–30	6 (26.1)	4 (25.0)	3 (25.0)	1 (33.3)
31–40	5 (21.7)	3 (18.7)	2 (16.7)	1 (33.3)
41–50	2 (8.7)	-	-	
51–60	-	-	-	-
61–70	2 (8.7)	2 (12.5)	2 (16.7)	1 (33.3)
71–80	2 (8.7)	2 (12.5)	2 (16.7)	
> 81	4 (17.4)	4 (25.0)	2 (16.7)	
Gender				
Male	8 (34.8)	3 (18.7)	2 (16.7)	-
Female	15 (65.2)	13 (81.3)	10 (83.3)	3 (100)
Type of specimen				
Pus	9 (39.1)	6 (37.5)	5 (42.7)	2 (66.7)
Blood	5 (21.7)	3 (18.7)	1 (8.3)	-
Sputum	4 (17.4)	4 (25.0)	4 (33.3)	1 (33.3)
Urine	3 (13.0)	2 (12.5)	1 (8.3)	-
Wound swab	2 (8.7)	1 (6.3)	1 (8.3)	-

MRSA: methicillin-resistant *Staphylococcus aureus*; PVL: *Panton-Valentine leucocidin.*

Table 3. Distribution MRSA, and *mecA* and *PVL* genes among environmental sampling sites before and after disinfection.

Sampling site	Isolates (n = 22) Number (%)		MRSA (n = 6) Number (%)		*mecA* (n = 2) Number (%)		*PVL* (n = 1) Number (%)	
	Before Dis. (n = 14)	After Dis. (n = 8)	Before Dis. (n = 4)	After Dis. (n = 2)	Before Dis. (n = 2)	After Dis. (n = 0)	Before Dis. (n = 1)	After Dis. (n = 0)
Medical instrument	2 (14.3)	2 (25.0)	0	1 (50.0)	-	-	-	-
Bed surfaces	4 (28.6)	1 (12.5)	1 (25.0)	-	-	-	-	-
Floor/wall of wards	4 (28.6)	3 (37.5)	3 (75.0)	-	2 (100.0)	-	1 (100.0)	-
Door handles	4 (28.6)	2 (25.0)	-	1 (50.0)	-	-	-	-
Total	14 (63.6)	8 (36.4)	4 (66.7)	2 (33.3)	2 (100.0)	-	1 (100.0)	-

*Dis.: disinfection; MRSA: methicillin-resistant *Staphylococcus aureus*; PVL: *Panton-Valentine leucocidin.*

Table 4. Antibiotic resistance in *Staphylococcus aureus* isolates.

Antibiotics	Clinical isolates (n = 23) (n %)			Environmental isolates (n = 22) (n %)			*p* value*
	S	I	R	S	I	R	
Ampicillin	-	-	23 (100)	4 (18.2)	-	18 (81.8)	0.032
Chloramphenicol	12 (52.2)	5 (21.7)	6 (26.1)	15 (68.2)	4 (18.2)	3 (13.6)	0.491
Cefotaxime	11 (47.8)	4 (17.4)	8 (34.8)	19 (86.4)	2 (9.1)	1 (4.5)	0.016
Ceftriaxone	10 (43.5)	1 (4.3)	12 (52.2)	14 (63.6)	7 (31.8)	1 (4.5)	0.001
Gentamycin	11 (47.8)	6 (26.1)	6 (26.1)	21 (95.5)	1 (4.5)	-	0.002
Amikacin	16 (69.6)	5 (21.7)	2 (8.7)	22 (100)	-	-	0.019
Cefoxitin	7 (30.4)	-	16 (69.6)	16 (72.7)	-	6 (27.3)	0.005
Oxacillin	7 (30.4)	-	16 (69.6)	16 (72.7)	-	6 (27.3)	0.005
Ciprofloxacin	10 (43.5)	5 (21.7)	8 (34.8)	17 (77.3)	2 (9.1)	3 (13.6)	0.069
Ofloxacin	12 (52.2)	2 (8.7)	9 (39.1)	17 (77.3)	-	5 (22.7)	0.139
Levofloxacin	12 (52.2)	1 (4.3)	10 (43.5)	15 (68.2)	3 (13.6)	4 (18.2)	0.143
Erythromycin	4 (17.4)	5 (21.7)	14 (60.9)	15 (68.2)	1 (4.5)	6 (27.3)	0.002
Clindamycin	14 (60.9)	1 (4.3)	8 (34.8)	15 (68.2)	3 (13.6)	4 (18.2)	0.03
Co-trimoxazole	6 (26.1)	4 (17.4)	13 (56.5)	20 (90.9)	1 (4.5)	1 (4.5)	0.0001
Cefepime	5 (21.7)	5 (21.7)	13 (56.5)	15 (68.2)	2 (9.1)	5 (22.7)	0.007
Tetracycline	22 (95.7)	-	1 (4.3)	20 (90.9)	2 (9.1)	-	0.215
Linezolid	22 (95.7)	-	1 (4.3)	22 (100)	-	-	0.323
Vancomycin	23 (100)	-	-	22 (100)	-	-	NA

*Chi-square (χ^2) test; S: susceptible; I: intermediate; R: resistant.

Results

A total 45 *Staphylococcus aureus* (23 isolates from all clinical specimens — pus, blood, sputum, urine, wound swabs; and 22 isolates from environmental swabs were isolated from a total of 1,511 samples which included 1,207 clinical specimens and 304 hospital environmental swabs. A total of 22 *S. aureus* isolates were considered MRSA by cefoxitin disc diffusion method; out of which, 72.7 % (n = 16/22) were clinical isolates and 27.3% (n = 6/22) were from environmental swabs. The number of samples from different clinical specimens and hospital environmental sections, according to the gender, are presented in Tables 2 and 3, respectively.

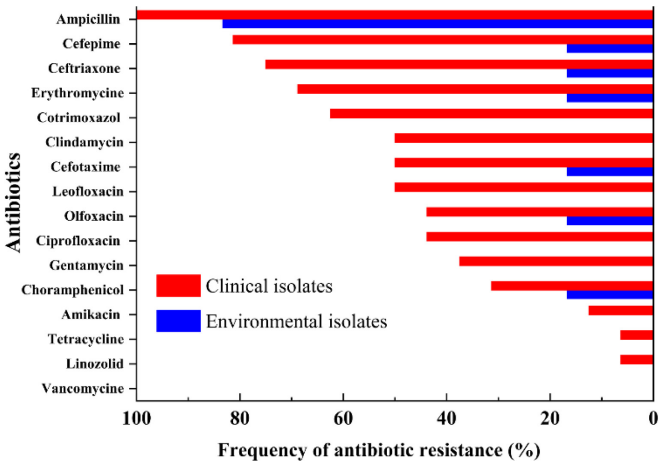
The *mecA* (163 bp) and *PVL* (433 bp) genes were tested in all the MRSA isolates (n = 22) after confirming the identity of MRSA. Clinical MRSA (n = 16) that was isolated from pus, had the highest prevalence of *mecA* gene (n = 5, 42.7%), followed by sputum (n = 4, 33.3%) (Table 2); while 33.3% (n = 2/6) of MRSA from hospital environment swab were positive for *mecA* gene (Table 3).

Both *mecA* and *PVL* genes were detected in 18.8% (n = 3/16) of the MRSA isolates from clinical specimens. The majority of the *PVL* positive isolates were obtained from pus specimen accounting for 2/3 (66.7%). The remaining *PVL* gene positive clinical isolates were detected in sputum specimen. However, among the MRSA from hospital environment swabs, only one of the isolates were found positive for *PVL* (Table 2 and 3).

The results of antimicrobial susceptibility testing of all 45 isolates are shown in Table 4. Our analysis found statistically significant differences in the susceptibility patterns of clinical isolates and environment swab isolates, except towards quinolones, chloramphenicols, tetracyclines, and linezolids. Ampicillin showed the highest rate of antimicrobial resistance, while vancomycin exhibited no evidence of resistance. The prevalence of antibiotic resistance among the MRSA isolates is illustrated in Figure 1.

Among clinical isolates, 43.5% (n = 10/16) of MRSA were multidrug resistant (MDR) (those that were resistant to three or more classes of antimicrobials); and 30% (n = 3/10) of MDR were *PVL* gene positive (Table 5). 9.1 % (n = 2/6) of MRSA isolated from the environment swabs were MDR and one non-MDR isolate was *PVL* gene positive. The rate of MDR MRSA isolates was significantly higher among clinical than environment swab isolates. ($p < 0.005$) (Figure 2).

Figure 1. Frequency of antibiotic resistant MRSA isolates from clinical and environmental samples. The figure shows high rate of antibiotic resistance among clinical samples.



MRSA: methicillin-resistant *Staphylococcus aureus*.

Table 5. Association between *mecA* and *PVL* genes and the frequency of antibiotic resistance.

Sample code	Specimen / sampling site	Resistant antibiotics	Genes	
			mecA	*PVL*
P-568	Sputum	AMP, C, E, CD, CTX, CTR, CIP, GEN, AK, OF, LE, CPM	+	+
P-1191	Pus	Amp, C, COT, CTR, CPM	+	+
31/28	PUS	AMP, CTR, CIP, OF, LE, CPM	+	+
B-728	Blood	AMP, C, CTX, CTR, CPM, E, CD, OF LE GEN, AK, COT,	+	-
P-590	Pus	AMP, CTR, COT, CPM, E	+	-
P-592	Wound Swab	AMP, CTR, CTX COT, CPM, E, CD	+	-
P-629	Sputum	AMP, CTX, CTR, CPM, E, CD, GEN, CIP, OF, LE	+	-
P-633	Sputum	AMP, COT, E	+	-
691	Pus	AMP, C, CTX, CTR, CIP, E, CD, GEN, COT, TE, CPM, CIP, OF, LE	+	-
1280	Sputum	AMP, C, CTR, CTX, CIP, COT, CPM, CD	+	-
1231	Pus	AMP, C, CTX, CTR, GEN, COT, CPM, E, CD	+	-
1398	Pus	AMP, CIP	+	-
S-13	Floor	AMP, C, CTX, CTR, CPM	+	-
S-14	Floor	AMP, E, CIP, GEN	+	-
S-15	Floor	-^	-	+

PVL: *Panton-Valentine leucocidin;* AK: amikacin (30 µg); AMP: ampicillin (10 µg); C: chloramphenicol (30 µg); CD: clindamycin (2 µg); CIP: ciprofloxacin (5 µg); COT: cotrimoxazole (25 µg); COX: oxacillin (30 µg); CPM: cefepime (30 µg); CTR: ceftriaxone (30 µg); CTX: cefotaxime (30 µg); CX: cefoxitin (30µg); E: erythromycin (15 µg); GEN: gentamicin (10 µg); LE: levofloxacin (5 µg); LZ: linezolid (30 µg); OF: ofloxacin (5 µg); TE: tetracycline (30 µg); VA: vancomycin (30 µg). ^ The isolate was susceptible to all tested antibiotics.

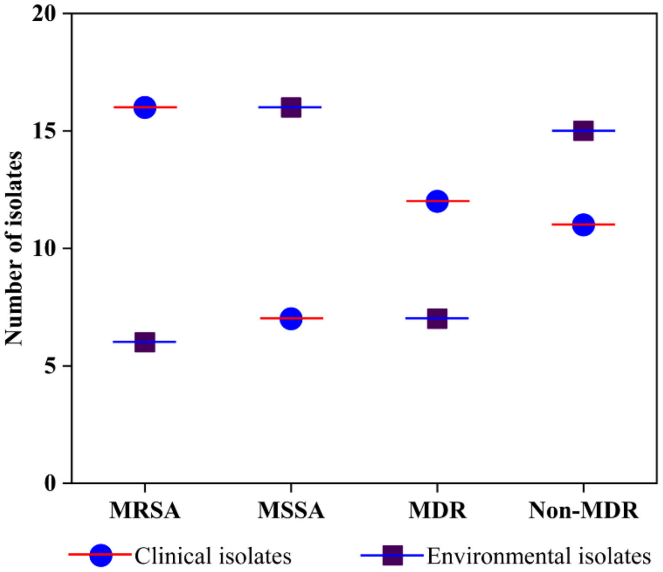
Discussion

The spread of MRSA in the healthcare setting is associated with public health concerns due to treatment challenges. MRSA may lead to infection in vulnerable populations in surgical post-operative units and intensive care units. MRSA can be transferred from person to person or from person to frequently touched objects in the hospital environment, and vice versa [3].

Our finding indicated a lower prevalence of MRSA in hospital environment compared to clinical specimens. Similar results were obtained in previous studies from Nepal [3,34]. However, incidence of infection is not associated with prevalence of MRSA and MSSA [35,36]. *S. aureus* and MRSA are commonly spread through the hands of healthcare workers and visitors. Patient load as a factor for contamination has also been implicated elsewhere [1]. Previous studies have concluded that more than 20% of infection within the departments or units are cross-transmitted through the hands of healthcare workers in the hospital environment [10,37]. *S. aureus* has been identified as an important nosocomial pathogen due to its ability to survive on inanimate objects for several days. The isolation of *S. aureus* from the surfaces of medical instruments, floor, bed sheet, and door handles, indicate that human hands are an important source of spread of *S. aureus* to the patient (Table 3). The presence of *S. aureus*, including MRSA, on these surfaces increases the risk of transmission and may subsequently result in secondary bacterial infections such as sepsis and pneumonia. In this study, 27.3% (n = 6/22) MRSA were hospital environment isolates. Among them, four were isolated before disinfection (1 in bed surface and 3 in floor/wall of wards), and two isolates were obtained after disinfection (one each from a medical instrument and door handle). This result implies that some of the MRSA isolates had potential to escape disinfection. Previous research has shown that using preventives and highly concentrated disinfectants on a regular basis can help to decrease MRSA in hospital settings [38]. In addition, the daily use of disinfectants is ineffective against MRSA and has the potential to develop increased tolerance to these agents by efflux pump expression [39].

In this study, we observed 69.6% of MRSA among the clinical isolates; most of them (43.8%), were found in pus/wound swabs. This observation is consistent with the results of previous studies conducted in different parts of Nepal and other countries [20–23,40]. This result indicates that *S. aureus* is responsible for pyogenic infection in soft tissue and skin during cuts, surgical incisions, and burns [41]; and produces

Figure 2. Frequencies of MRSA and MDR isolates from clinical and environmental samples. There was a significant difference between MRSA and MDR in clinical and environmental samples.



Chi- square (χ^2) test p value = 0.005. MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin susceptible *S. aureus*; MDR: multi-drug resistant.

cytolytic exotoxins that form small pores in the plasma membrane, leading to the lysis of the host's targeted cell. The most well-known cytolytic toxins are the leucocidins, including Panton-Valentine leucocidin (PVL) [20,42].

MRSA has developed a number of defense mechanisms against antimicrobial therapy. They include modification of the target with lower affinity for the antibiotic, genetic variations, and enzyme inactivation of the antibiotics [43]. The results of antimicrobial susceptibility testing revealed higher resistance among clinical isolates compared to environmental isolates; however, some groups of antibiotics did not have statistically significant differences (Table 4). Similar finding was observed in previous studies from Nepal [20,24]. In 2018, Bhatta *et al.* reported that *S. aureus* isolated from frequently touched objects in hospitals was highly susceptible to amikacin, gentamycin, ciprofloxacin, and clindamycin. These findings are identical to the observations from environmental isolates of our study [3]. The reasons for differences in antibiotic resistant rates between clinical isolates and environmental isolates are likely to be multifactorial. Some of the potential reasons for the increasing antimicrobial resistance in clinical isolates are the ineffective empiric therapy, antibiotic exposure, overuse of antimicrobials agents in treatment of patients, and the hospital environment and infections;

and preventions control strategies (IPCs) in the case of HAI [44]. For instance, when antibiotics are overused, vulnerable bacteria are killed; however, more resistant pathogens can survive. These resistant bacteria then multiply causing the number of antimicrobial-resistance bacteria to rapidly increase in healthcare settings.

Similarly, MRSA isolated from clinical specimens normally exhibited MDR, whereas MRSA isolated from hospital environments were mostly susceptible to antibiotics in our study. These outcomes are closely identical with those reported, previously [36,45]. Our findings indicate that MDR MRSA is associated with type of sample or specimens in healthcare settings. In the current study, all MDR MRSA isolates were susceptible to vancomycin. This is in accordance with other studies [22].

Among the environmental isolates, MRSA was comparatively higher before disinfection sampling than after disinfection. The antibiotic resistance trait does not affect the survival of bacteria with the use of disinfectant agent on environmental surfaces. However, survival rate of MRSA depends on the inoculum concentration and use of disinfecting materials in hospitals [46].

This study found a low prevalence of *mecA* genes among MRSA isolates, which could be due to intrinsic factors and other mechanisms. However, we were unable to perform *mecB* and *mecC* gene tests, and there was a lack of information on other *mec* genes responsible for methicillin resistance. However, previous research found that the *PBP2a* gene and the five major *SCCmec* types are completely absent in phenotypically MRSA isolates, suggesting that hyper β-lactamase production and mutations in specific amino acids on protein binding cascades are possible reasons [24,47–48].

A notably higher prevalence of *PVL* was observed among the MDR MRSA isolated from clinical specimens in the present study. Only one MRSA isolate was positive for the *PVL* gene among the environmental isolates (isolated from hospital floor/walls). These findings might have limited role of anti-leucocytic activity outside the host or inanimate objects of the hospital environment. The study conducted by Bhatta *et al*. in Pokhara, Nepal, found that MRSA isolates from hospital environment were negative for *PVL* genes [20], and similar finding was also reported from two different hospitals in Ghana by Donkor *et al.* [49]. However, research conducted in southern China and Taiwan found that approximately 40% of MRSA isolated from hospital environments were positive for the *PVL* gene [50,51]. This wide variation in the prevalence of *PVL* positive MRSA could be due to differences in sampling techniques, geographic distributions, and sanitation and hygiene practice.

Among the clinical isolates, 25.0% (3/12) of MRSA were positive for *PVL* genes, and all were *mecA* positive and MDR (Table 5). Similar results were reported from Kenya [31], South Africa [52], Cyprus [53], and Malaysia [16]. However, the global scenario of presence of *PVL* in MRSA isolates varies [54]. In previous studies from Nepal, the prevalence of the *PVL* gene was up to 35.6–57.7% among the MSRA [20,55]. However, the prevalence reported in the neighboring countries were 39.0% in China [56], 51.2% in Pakistan [57], 57.9% in Bangladesh [45], and 79% India [58]. In these studies, most of the *PVL*-positive MRSA was found in pus specimens. The results in the present study are in agreement with the findings of other studies [53,58]. Thus, detection of *PVL* in MRSA is crucial to understand the severity of the necrotizing diseases and has serious implications in antibiotic treatment.

Limitations of the study

This was a time-bound and single-center study. Further studies are needed for a better understanding of clonal diversity. *SCCmec* typing was not performed in the current study, but is planned for the future.

Conclusions

The prevalence of the MRSA strain (18.2%) in a healthcare setting in Lalitpur, Nepal, was established. The *PVL* gene was present in four out of twelve MRSA (25.0%) clinical isolates, indicating that these isolates were the source of the increased pathogenicity of MRSA. MRSA was present in the hospital environment at an incidence rate of 27.3% (6/22). The *PVL* gene is a virulence factor and a reliable genetic indicator of *S. aureus*. More consideration must be given to these isolates' genetic makeup and their acquisition of these virulence characteristics in Nepal. Continuous surveillance should be implemented in order to stop the spread of common *PVL*-positive MRSA in hospital settings.

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Authors' contributions

GS: study design, specimen collection and processing, data analysis, and manuscript drafting; SB, ABC, BLM, and PD: laboratory work such as specimen processing, identification of the isolates, antibiotic susceptibility testing, DNA extraction and PCR; LK, RT, SKR and DRJ: study design, definition of objectives, data analysis, manuscript drafting and refining. All authors have read and accepted the manuscript.

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