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ORIGINAL ARTICLE

mecA and *PVL* genes in methicillin-resistant *Staphylococcus aureus* from clinical specimens: a cross-sectional hospital based study from Nepal

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ABSTRACT

Background and Objectives: *Staphylococcus aureus* has increasingly been associated with community and healthcare-associated infections worldwide and contributes to treatment failures due to the emergence of multidrug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains. We aimed to determine the prevalence and antibiotic susceptibility pattern of MRSA isolated from patients attending a burn center and to detect *mecA* and *PVL* genes among MRSA isolates.

Materials and Methods: A cross-sectional hospital based study was conducted on 1950 clinical samples collected from hospital inpatients and outpatients of Kirtipur Hospital, which is a burn specialist hospital in Kathmandu, Nepal. Each sample underwent conventional cultural methods for bacterial isolates identification.

Results: Out of 1950 samples, 452 (23.2%) samples showed bacterial growth, of which 109 isolates (24.1%) were identified as Gram positive and 343 (75.9%) as Gram negative bacteria. Among the Gram positive bacteria, 53 (48.62%) were *Staphylococcus aureus*. Of the total *S. aureus* isolates, 40 (75.5%) were MRSA and 48 (90.6%) were MDR. Of the 40 MRSA isolates, 29 (72.5%) carried the *mecA* gene and 3 (7.5%) harbored *PVL* gene.

Conclusion: The high prevalence of MRSA in a burn unit underscores the need for more rigorous infection control practices that follow standard protocols to reduce MRSA transmission in both individuals and the hospital environment.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MDR; *mecA* gene; *PVL* gene; Wound infection

INTRODUCTION

Staphylococcus aureus is a Gram positive bacterium commonly found on human skin, with the anterior nares being a major reservoir. While approximately 30% of the human population may be asymptomatic carriers, colonization increases the risk of symptomatic infection in others (1). Three types of carriers exist: including persistent carriers (10-35%), intermittent carriers (20-75%), and non-carriers (5-10%) (2). *S. aureus* is involved in various infections such as infective endocarditis, bacteremia, pneumonia, osteomyelitis, infective arthritis, skin and soft tissue infections (3).

Initially effective antibiotics are being challenged by the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. MRSA, which is associated with increased morbidity and mortality, has become a major global health concern. Its prevalence has spread beyond healthcare settings into the com-

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munity, causing skin and soft tissue infections (4). MRSA is resistant to all β-lactams due to the presence of *mecA*, a gene that produces a pencillin binding protein (PBP2a) with low affinity for β-lactam antibiotics. In low-income countries such as Nepal, MRSA has gained attention despite initial overlook. Studies in Nepal show variable MRSA incidence rates, highlighting the need for surveillance (5). The rise of multidrug-resistant (MDR) bacteria further limits therapeutic options (6-8). Therefore, the aim of this study was to evaluate the antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from different clinical specimens of patients attending a burn unit and to detect *mecA* and *PVL* genes among MRSA isolates. Identification and surveillance of drug resistant isolates are essential to address the challenges posed by MDR MRSA.

MATERIALS AND METHODS

Study design and setting. A hospital based cross sectional prospective study was conducted from January 2023 to July 2023 at Kirtipur Hospital located in Kirtipur Municipality, Kathmandu, Nepal. Kirtipur Hospital is a community hospital with burn and plastic surgery as super specialty services.

Sample size and sampling technique. A nonprobability purposive sampling was done. A total of 1950 non-duplicated clinical samples were included in this study.

Study population and selection criteria. This study included all outpatient and inpatient specimens of all age groups and both sexes received at the microbiology laboratory for culture and antibiotic susceptibility testing at Kirtipur Hospital. Samples from patients receiving antibiotics prior to sample collection were excluded from the study. Improperly stored samples, dried samples, unlabeled samples, inadequate information, contaminated samples and sample leakage during transportation were excluded from the study.

Sample collection and processing. Clinical samples including pus, wound swab, urine, blood, central line tips, catheter tips, sputum, throat swab, vaginal swab and pleural fluid were collected by experienced medical personnel in a clean leak- proof sterile container according to the specimen type and hospital policy (9). The specimens were immediately sent to the microbiology laboratory for routine culture and antibiotic susceptibility testing.

Isolation and identification of *S. aureus*. All the samples were inoculated on blood agar and mannitol salt agar and incubated at 37°C for 24 hours. Identification of *S. aureus* was done based on colony characteristics, Gram staining and biochemical tests including catalase, oxidase, oxidative-fermentative and coagulase (9).

Antibiotic susceptibility test of *S. aureus*. Antibiotic susceptibility testing of *S. aureus* isolates was performed using modified Kirby Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines (10). The antibiotic disks used were ampicillin (10 µg), cefoxitin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), clindamycin (2 µg), erythromycin (15 µg), cotrimoxazole (sulphamethoxozole 23.75µg + trimethoprim 1.25 µg), tetracycline (30 µg) and linezolid (10 µg) as per the hospital's protocol. Multidrug resistance was defined as resistance to three or more classes of antibiotics tested.

Screening of MRSA. If the zone of inhibition around the cefoxitin disc was ≤ 21 mm, it was considered to be MRSA (11).

DNA extraction and quantitation. All phenotypically confirmed MRSA were tested for the presence of *mecA* and *PVL* genes. Chromosomal DNA from *S. aureus* was obtained by boiling method (12). Briefly, fresh bacterial culture was suspended in 100 µl of 50 mM NaOH and heated at 100°C for 20 minutes to extract the DNA, subsequently the tube was immediately transferred to 4°C for 5 min. Then 20 µl Tri-HCl (pH 7.5) was added and gently mixed by inversion for several times and centrifuged at 13,000 rpm for 10 minutes. Supernatant was then transferred to a sterile tube and stored at -20°C until use. Quantification of extracted DNA was performed by using nanodrop spectrophotometer.

Detection of *mecA* gene by conventional PCR. The forward primer 5'-ACTGCTATCCACCCTCAAAC-3' and reverse primer 5'-CTGGTGAAGTTGTAATCTGG-3' was used to amplify

mecA gene (13). The reaction mixture for the *mecA* gene was 25 µl and consisted of 5 µl of 5× Qiagen Master Mix, 0.5 µl of 10 pmolar primers (forward and reverse), 16 µl nuclease free water and 3 µl of extracted DNA template. Amplification was performed under the following conditions: initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 3 minutes for 35 cycles and final extension at 72°C for 2 minutes. The PCR amplification products were further processed for agarose gel electrophoresis using 2% agarose gel and visualized by staining with ethidium bromide. The PCR product size was 163 bp.

Detection of *PVL* gene by conventional PCR. The forward primer 5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3'and reverse primer-5'GCATCAAGTGTATTGGATAGCAAAAGC-3' was used to amplify the *PVL* gene (14). The reaction mixture for the *PVL* gene was 25 µl and consisted of 5 µl of 5× Qiagen Master Mix, 1 µl of 10 pmolar primer (forward and reverse), 16 µl nuclease free water and 3 µl of extracted DNA template. Amplification was performed under the following conditions: initial denaturation at 97°C for 6 minutes, denaturation at 92°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds for 35 cycles and final extension at 72°C for 10 minutes. PCR amplification products were fractionated by means of 2% agarose gel electrophoresis and visualized by staining with ethidium bromide staining. The size of the PCR product was 433bp.

Variables. The outcome variables in this study were *S. aureus* (MRSA and MSSA), while age, sex, specimen type, antibiotic susceptibility, presence of *mecA* and *PVL* genes were predictor variables.

Ethical issues. Ethical approval of this study was obtained from Institutional Review Committee (IRC) of the Public Health Concern Trust, Nepal (IRC application number: 107-2023). Samples were collected only after taking written informed consent from the patients.

Data analysis. All the data obtained were analyzed using the Statistical Package of Social Sciences (SPSS version 21.0). A Chi- square test was used to determine the association of independent variables and a p-value ≤ 0.05 was considered as statistically significant.

Ethical statement. This study was performed in accordance with the Declaration of Helsiki. Ethical approval of this study was obtained from the Institutional Review Committee (IRC) of the public Health Concern Trust, Nepal (IRC application number:107-2023). At the time of enrolment, written informed consent was obtained from each patient or their guardians/parents on behalf of the patient. The participants were informed that the collected information will not be disclosed and will be used solely for data analysis without disclosing the name or identity of cases.

RESULTS

Growth pattern and distribution of bacteria. Of the1950 samples processed, 452 (23.2%) showed bacterial growth, of which 109 (24.1%) were identified as Gram positive and 343 (75.9%) as Gram negative bacteria. Out of 24.1% of Gram positive isolates, *S. aureus* 53 (11.7%) was predominant (Table 1).

Table 1. Growth status of Gram positive and Gram-negative bacteria (n=452)

Organism isolated	Gram positive (Number)	Gram negative (Number)	Total number (%)
S. aureus	53		53 (11.7)
CoNS	46		46 (10.2)
S. saprophyticus	6		6 (1.3)
E. faecalis	4		4 (0.9)
E. coli		160	160 (35.4)
K. pneumoniae		53	53 (11.7)
P. aeruginosa		48	48 (10.6)
C. koseri		26	26 (5.8)
ACBC		18	18 (4)
P. mirabilis		11	11 (2.4)
A. lwoffeii		9	9 (2)
K. oxytoca		8	8 (1.8)
P. vulgaris		3	3 (0.7)
C. freundii		2	2 (0.4)
E. aerogenes		2	2 (0.4)
E. cloaceae		2	2 (0.4)
Salmonella Typhi		1	1 (0.2)
Total	109	343	452 (100)

Distribution of *S. aureus* in various clinical samples. The highest number of *S. aureus* was isolated from wound swabs 15.1% (37/245), followed by pus 25.0% (12/48), bone 100% (1/1), endotracheal aspirate 25.0% (1/4), tissue 8.3% (1/12), and catheter tip 5.6% (1/18) (Table 2).

Antibiotic susceptibility profile of *S. aureus* isolates. All the isolates were resistant to ampicillin, and they were all susceptible to linezolid. More than 90% of the isolates were resistant to ciprofloxacin and erythromycin (Table 3).

Phenotypic detection of MRSA and its distribution. Of the total *S. aureus* isolates, 40 (75.5%) were MRSA and 13 (24.5%) were MSSA. Among the total *S. aureus* isolates, 48 (90.6%) were MDR of which 39 (97.5%) were MRSA and 9 (69.2%) were MRSA.

Detection of *mecA* and *PVL* genes in MRSA isolates by PCR. Among the 40 MRSA isolates, 29 (72.5%) carried the *mecA* gene and 3 (7.5%) carried the PVL gene. There was no significant association between the occurrence of *mecA* and *PVL* genes in MRSA isolates (Table 4).

DISCUSSION

The growing prevalence of multidrug-resistant bacteria requires a multifaceted approach including surveillance, antimicrobial stewardship, infection control practices, and continued research into new therapeutic options. By leveraging these insights, healthcare providers can optimize patient outcomes and mitigate the public health impact of antimicrobial resistance.

Our study showed a relatively high growth rate of bacteria in urine samples as compared to other clinical samples which was similar to previous reports (6). Gram negative isolates were almost three times more than Gram positive bacteria in our study. The distribution of *S. aureus* among different clinical samples in our study showed the highest prevalence in wound swabs, followed by pus samples, bone, endotracheal aspirate, tissue, and catheter tips. This highlights the diverse clinical manifestations of *S. aureus* infections and the importance of targeted surveillance and management strategies. The capacity of bacteria to form biofilms on a variety of inhospitable surfaces,

Table 2. Bacteria isolated from different clinical specimens

Sample type	Number of bacteria (n=452)																Growth %
	E. coli	*C. freundii*	*K. oxytoca*	*K. pneumonia*	*E. aerogenes*	*P. mirabilis*	*P. vulgaris*	*A. lwoffeii*	ACBC	*C. koseri*	*P. aeruginosa*	*S. Typhi*	*E. faecalis*	CoNS	*S. aureus*	*E. cloacae*	*S. saprophyticus*	
Wound swab (245)	16	0	2	5	0	3	2	6	12	10	30	0	2	27	37	1	0	62.4
Blood (393)	0	1	0	5	0	1	0	0	2	1	3	1	0	0	0	0	0	3.6
Urine (1108)	137	1	5	26	0	7	1	0	1	10	2	0	0	0	0	0	6	17.7
Central line tips (11)	0	0	0	2	0	0	0	0	0	0	2	0	0	5	0	0	0	81.8
Tissue (12)	0	0	0	1	0	0	0	2	1	1	0	0	1	3	1	0	0	83.3
Bone (1) Sputum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	100
(94) Vaginal	0	0	0	10	0	0	0	0	2	1	7	0	0	0	0	0	0	21.3
swab (8) Pleural	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	12.5
fluid (7) Pus (48)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Foley tip (1) Catheter tip	3	0	1	0	2	0	0	0	0	2	1	0	0	7	12	0	0	58.3
(18) Endotracheal	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
aspirate (4)	3	0	0	3	0	0	0	1	0	0	3	0	0	3	1	1	0	83.3
Total (1950)	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	100
	160	2	8	53	2	11	3	9	18	26	48	1	4	46	53	2	6	

Table 3. Antibiotic susceptibility profile of *S. aureus* isolates (n=53)

Antibiotics	Sensitive number (%)	Resistant number (%)
Ampicillin	-	53 (100%)
Chloramphenicol	37 (69.8%)	16 (30.2%)
Clindamycin	7 (13.2%)	46 (86.8%)
Ciprofloxacin	5 (9.4%)	48 (90.6%)
Cotrimoxazole	28 (52.8%)	25 (47.2%)
Cefoxitin	13 (24.5%)	40 (75.5%)
Erythromycin	5 (9.4%)	48 (90.6%)
Gentamicin	21 (39.6%)	32 (60.4%)
Linezolid	53 (100%)	-
Tetracycline	29 (54.7%)	24 (45.3%)

Table 4. Association between *mecA* and *PVL* genes in MRSA isolates (n=40)

		PVL gene		Total	p- value
		Absent	Present		
mecA gene	Absent	11	0	11	0.267
	Present	26	3	29	
	Total	37	3	40	

as well as their abundance in the environment, may have contributed to the high frequency of *S. aureus* (15).

The main concern was about the prevalence of MRSA, which accounted for 75.5% of *S. aureus* isolates. Cefoxitin resistant *S. aureus* has emerged as an important bacterial human pathogen with an increasing trend of antibiotic resistance to currently used antimicrobials. The reported prevalence of MRSA reported in Nepal varies across different studies and healthcare settings. However, it generally ranges from approximately 21.1% to 68% (16-20). This range reflects the diversity of patient populations, healthcare facilities, and infection control practices in different regions of Nepal. It is important to note that these figures may change with ongoing surveillance efforts and changes in healthcare practices.

The higher proportion of MRSA in pus samples in our study may be due to patients' poor personal hygiene practices and inadequate wound care, making them more susceptible to infection and subsequent contamination. As data related to hygiene practices was not collected in this study, collecting such data could be useful to carry out preventive measures in hospital. Furthermore, a significant proportion of MRSA isolates were multidrug-resistant, posing a challenge for treatment and infection control efforts. Molecular analysis further elucidated the genetic determinants of resistance, indicating the presence of the *mecA* gene in the majority of MRSA isolates as well as the presence of the *PVL* gene in a subset.

In our study, 90.6% of *S. aureus* isolates were MDR. The high proportion of MDR among both MSSA and MRSA in our study was consistent with that reported from a tertiary care hospital in Nepal (20). Antibiotic resistance in bacteria occurs through spontaneous mutation and horizontal gene transfer between different bacterial species. Therefore, the higher prevalence may be due to indiscriminate and irrational use of antibiotics for treatment which is a common practice in developing countries (21). In our study, the *mecA* gene was detected in 72.5% of isolates, suggesting that the presence of this gene is very important but not mandatory for the development of resistance. Numerous intrinsic factors may contribute to the development of resistance and suppress the expression of this gene. Five major *SCC* (Staphylococcal Cassette Chromosome) *mec* types, *mecA*, and the gene product of PBP2 were completely absent in a previous investigation, but the isolates were still phenotypically resistant, indicating the likelihood of excessive beta lactamase synthesis (22). This study suggests that the *mecA* itself is a dominant but not mandatory factor to confer resistance among MRSA isolates; the existence of other intrinsic factors, other *mec* alphabets, mainly *mecB* and *mecC* and several allotypes could contribute to cefoxitin resistance despite the absence of *mecA* (23). Findings from this study also suggest that the existing diagnostic tools (both phenotypic and genotypic) cannot be a single factor for the detection of MRSA. The combination of these tools is likely to assist in the accurate detection of the burden and trend of MRSA, including guidance of antimicrobial therapy. The presence of the virulence factor PVL is associated with recurrent infection and clinical severity and has been linked to the community associated MRSA. Although our study showed a low prevalence of *PVL* gene among the isolates, which was consistent with that reported from Germany (24). However, recent studies show an alarmingly high prevalence of PVL (25). The major limitation of this study is that we didn't compare the results between burn patients and outpatients attending the hospital.

CONCLUSION

The high prevalence of MRSA (75.5%) in a burn specialty hospital emphasizes the need for more rigorous infection control practices that adhere to standard protocols to reduce MRSA transmission in both individuals and the hospital environment.

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