PREVALENCE AND MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AND VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS (VRSA) IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Resistance shown by *Staphylococcus aureus* to methicillin; mediated by mecA, and vancomycin; mediated by vanA, has led to difficulty in treatment of related infections. Despite reports showing methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* (VRSA) in Nepal, and need for their regular surveillance, no study has been conducted on it in our hospital. So, this study is aimed to determine prevalence of MRSA, VRSA and their molecular characterization along with antibiogram. A descriptive cross-sectional study was conducted from August to December, 2022 in Clinical Microbiology Laboratory of NMCTH among *S. aureus* (n=160) isolated from various clinical specimens after receiving ethical approval from NMC-IRC. AST was done by modified Kirby-Bauer's disc diffusion method. MRSA and VRSA were detected by cefoxitin disc method and agar dilution method respectively. Inducible clindamycin resistance was detected by D-test. Resistant genes (mecA, PVL, and vanA) were detected using conventional PCR. Prevalence of MRSA was found to be 31.2% (50/160) but none of the isolates were resistant to vancomycin. Total 7 (46.6%) mecA and 7 (46.6%) PVL genes were detected among 15 selected MRSA isolates but vanA was not found. All the MRSA isolates were susceptibile to co-trimoxazole, tigecycline, chloramphenicol, vancomycin, teicoplanin and linezolid. The resistance rate against ciprofloxacin, ofloxacin, and clindamycin was 52.0%, 44.0%, and 68.0% (20.0% iMLSB, 28.0% cMLSB and 16.0% MS-phenotypes) respectively. Prompt implementation of hospital antibiotic policy and AMR Act by government along with regular surveillance of MRSA and VRSA seems essential to contain MRSA infections. Co-trimoxazole could be treatment option against MRSA in our setting, keeping vancomycin in reserve. However, large scale studies are required to establish this conclusion.

KEYWORDS

Staphylococcus aureus, mecA, MRSA, PVL, vanA, VRSA, Nepal

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INTRODUCTION

Resistance developed by bacteria to various antibiotics has created common global challenge for treatment of bacterial infections.[1] Resistance to methicillin and vancomycin among *Staphylococcus aureus* leading to difficulty for treatment of related infections has included methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* (VRSA) as high priority pathogens by World Health Organization.[2]

MRSA was reported first time in Nepal in 1990 by Rai *et al.*[3] After that, MRSA have been reported frequently along with recent VRSA infection in our country.[4-8] Resistance genes like mecA and vanA responsible for methicillin and vancomycin resistance respectively, have been reported from various countries including Nepal.[8-11] Severe infections with MRSA, mediated by PVL gene, also have been reported.[9] However, such extensive studies have not been conducted in our context yet.

So, this study was aimed to determine the prevalence of MRSA and VRSA along with their molecular characterization and AST pattern in a tertiary care hospital of Nepal, findings of which will give view on selection of suitable antibiotics against MRSA and VRSA related infections. Results of this study also could be helpful for containment of MRSA and VRSA infections in Nepalese scenario.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted at Clinical Microbiology Laboratory of NMCTH from August to December, 2022. The study was done on non-repeated bacterial isolates of *S. aureus* from various clinical specimens from patients attending NMCTH after receiving Ethical approval from Nepal Medical College-Institutional Review Committee (NMC-IRC, Ref no: 05-079/080).

All the clinical samples received for culture and sensitivity were inoculated into culture plates (urine: CLED agar, pus/wound swab: blood agar and MacConkey agar, sputum and body fluids: blood agar, MacConkey agar and chocolate agar) and incubated at 37^0 C for 24 hours aerobically. Blood culture bottles received were incubated at 37^0 C and after 24 hours, sub-cultured on blood agar and MacConkey agar every alternate days for 7 days. Total 160 *S. aureus* isolates included in this study, were then identified by studying colony characters, Gram staining and other biochemical tests according to standard microbiological technique.[12] AST was done by modified Kirby Bauer's disc diffusion method, and MRSA, inducible clindamycin resistance and vancomycin resistance were detected as per CLSI guideline.[13,14] *S. aureus* ATCC 25923 was used as control strain for AST.

Cefoxitin (30 µg) for the detection of methicillin resistance, and erythromycin (15 µg) and clindamycin (2 µg) discs (Hi-media, India) at 15 mm apart were also used on AST plate for the detection of inducible clindamycin resistance.[13] After applying antibiotic discs, the plates were incubated aerobically at 37^0 C for 18 hours. The isolates showing zone of inhibition of <or =21 mm for cefoxitin were considered as MRSA.[13]

Clindamycin resistance was detected as:(i) Inducible resistance phenotypes ($_iMLS_B$): resistant to erythromycin and having a clindamycin zone ≥21 mm with a D-shaped zone, (ii) constitutive resistance phenotypes ($cMLS_B$): resistant to both erythromycin and clindamycin, and (iii) MS phenotype: isolates resistant to erythromycin and susceptible to clindamycin without D-zone.[13]

On agar dilution, *S. aureus* isolates showing minimum inhibitory concentration (MIC) of vancomycin ≥16 mcg/ml were considered as VRSA, ranging from 4-8 mcg/ml were considered as vancomycin intermediate *S. aureus* (VISA), and ≤2 mcg/ml were considered as VSSA.[14] *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 52199 strains were used as controls while detecting MRSA and VRSA.

Detection of mecA, vanA, and PVL genes among the selected (n=15) MRSA isolates, selected according to convenience sampling technique, was done by using conventional PCR. In brief, DNA was extracted.[15] Then, it was run in PCR after mixing DNA with forward and reverse primers, master-mixture, and distilled water according to mentioned standard guideline.[9] Positive control for mecA and PVL genes and negative control (Distilled water) were also run in each series of PCR reaction. The PCR product was subjected to electrophoresis in 1.5% agarose gel containing DNA safe stain (ethidium bromide) and documented using gel documentation UV GeNei™ (UVI TEC, Cambridge, UK) for detection of mecA (162 bp), PVL (433 bp), and vanA (125 bp).[16,17]

Primers used were as follows:[16,17]

mecA F- TCCAGATTACAACTTCACCAGG,

mecA R- CCACTTCATATCTTGTAACG

PVL F- ATCATTAGGTAAAATGTCTGGACATGA,

PVL R- GCATCAASTGTATTGGATAGCAAAAGC

and **vanA F**- AATAGCGCGGACGAATTGGAC, **vanA R**- AACGCGGCACTGTTTCCCAA.

Data were analysed using SPSS software-version 17. Chi-square test and p- value were used as statistical tools.

RESULTS

Of the 7,674 various clinical specimens processed for culture and sensitivity, 160 *S. aureus* were isolated. Out of which, prevalence of MRSA was found to be 31.2% (50/160).

The isolation rate of MRSA were highest in pus (60.0%) followed by, wound swab (16.0%), blood (16.0%), and sputum (8.0%) and from out-patients (76.0%) compared to in-patients (24.0%) (Table 1).

The highest rate of resistance among MSSA was found in ampicillin followed by, erythromycin, ciprofloxacin, ofloxacin, clindamycin, gentamycin, and co-trimoxazole. Beside these, MRSA isolates showed marked resistance to clindamycin, erythromycin, ciprofloxacin, ofloxacin, gentamycin, followed by levofloxacin. All MRSA isolates were susceptible to co-trimoxazole, tigecycline, chloramphenicol, vancomycin, teicoplanin, and linezolid (Table 2).

Prevalence of inducible clindamycin resistance ($_{i}MLS_{B}$), $cMLS_{B}$, and MS- phenotype was found to be 20.0%, 28.0% and 16.0%, respectively among MRSA (Table 3). MecA gene was found among 7/15 (46.6%) MRSA (Fig. 1). Panton valentine leucocidin (PVL) gene was also found among 7/15 (46.6%) MRSA (Fig. 2).

None of the MRSA isolates were found resistant or intermediate sensitive to vancomycin by

Table 1: Showing sample, in-patient and OPD wise distribution of MRSA

Clinical sample	In-patient n (%)	Out-patient n (%)	Total n (%)
Pus	10 (20.0%)	20 (40.0%)	30 (60.0%)
Wound swab	0 (0.0%)	8 (16.0%)	8 (16.0%)
Blood	0 (0.0%)	8 (16.0%)	8 (16.0%)
Sputum	2 (4.0%)	2 (4.0%)	4 (8.0%)
Total	12 (24.0%)	38 (76.0%)	50 (100.0%)

Table 2: Showing resistance rate of MRSA and MSSA to various antibiotics

Name of Antibiotics	**MRSA** n=50, (%)	**MSSA** n=110, (%)
Ampicillin (10 mcg)	50 (100.0%)	34 (49.1%)
Cefoxitin (30 mcg)	50 (100.0%)	0 (0.0%)
Cloxacillin (30 mcg)	50 (100.0%)	0 (0.0%)
Cephalexin (30 mcg)	50 (100.0%)	0 (0.0%)
Erythromycin (15 mcg)	32 (64.0%)	40 (36.3%)
Clindamycin (2 mcg)	34 (68.0%)	26 (23.6%)
Chloramphenicol(25 mcg)	0 (0.0%)	0 (0.0%)
Gentamycin (10 mcg)	12 (24.0%)	10 (9.1%)
Co-trimoxazole (1.25/23.75 mcg)	0 (0.0%)	4 (3.6%)
Ciprofloxacin (5 mcg)	26 (52.0%)	40 (36.3%)
Ofloxacin (5 mcg)	22 (44.0%)	34 (30.9%)
Levofloxacin(5 mcg)	6 (12.0%)	0 (0.0%)
Tigecycline (15 mcg)	0 (0.0%)	0 (0.0%)
Vancomycin (30 mcg)	0 (0.0%)	0 (0.0%)
Teicoplanin (30 mcg)	0 (0.0%)	0 (0.0%)
Linezolid (10 mcg)	0 (0.0%)	0 (0.0%)

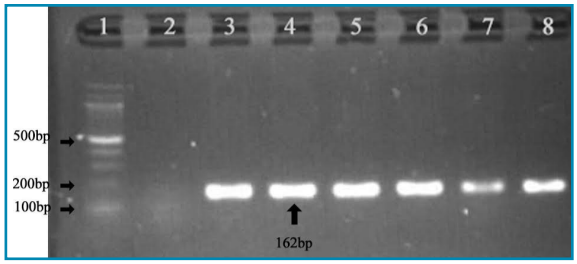

Fig. 1: Showing mecA gene among MRSA

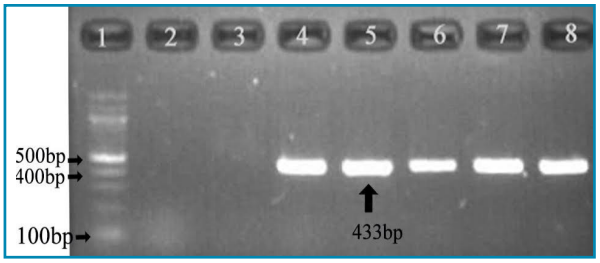

Fig. 2: Showing PVL gene among MRSA.

Table 3: Showing prevalence of $_{i}MLS_{B}$, $cMLS_{B}$, and MS-phenotypes among MRSA (n=50)

Clindamycin resistance type	n (%)
$iMLS_{B}$	10 (20.0%)
$cMLS_{B}$	14 (28.0%)
MS-phenotype	8(16.0%)

agar dilution technique. VanA gene was not detected among any of the selected 15 MRSA isolates using conventional PCR.

DISCUSSION

Staphylococci are common cause of skin, soft tissue and various other infections.[18] In this study, majority of the MRSA were isolated from pus sample which is in accordance with findings of other studies in various places and time duration.[7-9,15,16] Staphylococci are predominant etiology of suppurative infections that signifies reason for its high prevalence among pus specimen.[18]

According to various literatures, MRSA prevalence is more common among inpatients.[8,18,19] However, we found contrasting result from outpatients. This result showed the presence of MRSA in our community setting. PVL gene is one of the indicator of community acquired (CA)-MRSA.[20] In this study, we found PVL genes among 7/15 selected MRSA (46.6%), which further supports the presence of CA-MRSA in our community setting, however, less number of MRSA obtained in this study during short study-period may not reflect the scenario. This study shows the need of further studies in large scale over a long period of time to establish this finding. Dissimilarity of our finding with other researches might be due to unregulated use of antibiotics in our context which is not same for other countries where these findings have been obtained.[18,19]

As compared to previous studies, infections with MRSA is in increasing trend in our country.[6-8] We found prevalence of MRSA to be 31.2% in our study. This must be due to unregulated use of antibiotics in our setting which shows necessity for prompt implementation of antimicrobial resistance (AMR) policies in hospitals, and implementation of AMR act by Government of Nepal to contain AMR; including MRSA in our context.

Clindamycin is regarded as one of the treatment option against MRSA infections.[21] This study showed high rate of clindamycin resistance (Only 16.0% MS-phenotypes), indicating to rethink about use of clindamycin as an alternate to keep vancomycin in reserve for MRSA. However, this can be used if AST report shows susceptibility.

According to previous findings, vancomycin seems as good treatment option against MRSA related infections.[6-8] Routine AST report using modified Kirby Bauer's disc diffusion method for reporting resistance to vancomycin is not recommended and should be confirmed by phenotypic dilution technique.[14] We performed agar dilution method for determining minimum inhibitory concentration (MIC) of vancomycin and found that, no MRSA isolate was resistant to it (MIC ≤ 2 mcg/ml for all MRSA isolates). This shows it as a suitable antibiotic against MRSA till date. However, VRSA has been reported in recent past in Nepalese context.[9] This could be due to more ICU patients included in those previous studies compared to our study in which most of the MRSA isolates are from outpatient's clinical specimens. Further, gene silencing may lead to un-expression of resistance property among bacteria despite being resistant to antibiotics.[22] We tried to detect most common vancomycin resistant gene (vanA) by using conventional PCR among MRSA according to convenience sampling. No MRSA isolate in this study contained vanA gene indicating that, all MRSA isolates were susceptible to vancomycin.

In addition, we found reversion of co-trimoxazole resistance showing susceptibility to MRSA compared to high resistance against it ranging from 47.5 to 74.0% reported in various studies in Nepal.[8] This could be interesting finding showing efficacy of co-trimoxazole

as treatment option for MRSA in our set-up, however, it could be too early to reach to this conclusion because of our less sample size of MRSA obtained in this study. Further, large scale studies on MRSA showing similar findings to that of us and its good clinical efficacy in vivo also only could establish that, co-trimoxazole could be a good treatment option against MRSA in our setting, keeping vancomycin in reserve for future.

Various study reports have shown that, tigecycline could be an alternate to vancomycin against complicated MRSA infections.[23] We also found that, all MRSA isolates were susceptible to tigecycline in this study. This suggests that, it could be one of the treatment option against severe MRSA infections, if future studies also could establish similar findings to us.

Genotypic characterization of MRSA by detecting mecA and PVL gene was performed in this study using conventional PCR. Our findings showed only 7/15 isolates (46.6%) contained mecA gene. Similar findings have been reported in other few research studies too.[24,25] However, dissimilar findings to that of us also are available.[2,26] These dissimilar findings could be due to methicillin resistance mediated by other mec-types of MRSA (other than mecA) and efflux pump mechanism.[24,25] Similarly, panton valentine leucocidin (*PVL*) gene is believed to be responsible for severity of infection among MRSA infection cases.[20] we found 7/15 MRSA (46.6%) showing *PVL* gene also in our study. All MRSA isolates showing *PVL* gene were isolated from cases of deep suppurative infections in our study proving severe MRSA infections.

In conclusion, prevalance of MRSA is high in our set up and also in increasing trend. So, prompt implementation of AMR act by Government and implementation of hospital antibiotic policies in the hospitals should be done to contain MRSA infections in our set-up. Regular surveillance detection of MRSA and VRSA also seems essential in our context to control MRSA infections. No detection of vanA gene among MRSA isolates shows ray of hope for treatment of MRSA infections till date. Further, co-trimoxazole could be one of the treatment option against MRSA, keeping vancomycin in reserve for future, if similar findings to that of our's could be established by future large scale studies too.

We obtained only 50 MRSA isolates in our study duration, among which all isolates were susceptible to co-trimoxazole. This small sample size could be limitation of our study for establishing this new finding, which was not kept in mind during study plan. Future large scale studies are needed to establish our finding.

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