Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence, Antimicrobial Susceptibility Pattern, and Detection of *mec*A Gene among Cardiac Patients from a Tertiary Care Heart Center in Kathmandu, Nepal

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ABSTRACT

BACKGROUND: Methicillin Resistant *Staphylococcus aureus* (MRSA) is a significant human pathogen associated with nosocomial infections. *mec*A in the *S. aureus* is a marker of MRSA. The main objective of this study was to detect *mec*A and *vanA* genes conferring resistance in *S. aureus* among cardiac patients attending Sahid Gangalal National Heart Centre (SGNHC), Kathmandu, Nepal between May and November 2019.

METHODS: A total of 524 clinical samples (blood, urine, sputum) were collected and processed. Bacterial isolates were tested for antimicrobial susceptibility test (AST) and screening for MRSA was carried out by cefoxitin disc diffusion method. Minimum inhibitory concentration (MIC) of vancomycin for MRSA was established by agar dilution method and chromosomal DNA was extracted and used in polymerase chain reaction targeting the *mec*A *and van*A genes.

RESULTS: Out of 524 specimens, 27.5% (144/524) showed bacterial growth. Among 144 culture positive isolates, *S. aureus* (27.1%; 39/144) was the predominant bacteria. Among 39 *S. aureus* isolates, all isolates were found resistant to penicillin followed by erythromycin (94.9%; 37/39), gentamicin (94.9%; 37/39) and cefoxitin (87.2%; 34/39). Out of 39 *S. aureus*, 87.2% (34/39) were MRSA. Among 34 MRSA, 8.8% (3/34) were vancomycin intermediate *S. aureus* (VISA). None of the MRSA was resistant to vancomycin. All of the 3 VISA isolates were obtained from inpatients. Of 39 *S. aureus*, 82.1% (32/39) harbored *mecA* gene. Similarly, the entire VISA isolates and 94.1% (32/34) of the MRSA isolates were tested positive for *mecA* gene.

CONCLUSIONS: High prevalence of MRSA among the cardiac patients indicates the increasing burden of drug resistance among bacterial isolates. Since infection control is the crucial step in coping with the burgeoning antimicrobial resistance in the country, augmentation of diagnostic facilities with routine monitoring of drug resistance is recommended.

KEYWORDS: Antibiotic susceptibility pattern, MRSA, VISA, ***mec*A**

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Background

Infection stems from complex interactions between a host, pathogen, and the environment.[1] Among various infections, cardiac infections are life threatening and can affect all parts of the heart including its valves, endocardium, myocardium, and pericardium.[2] Nosocomial infections are the main cause of morbidity and mortality among the patients undergoing cardiac surgery.[3] Major causes of nosocomial infections in patients undergoing cardiac surgery are the presence of major surgical wounds and frequent use of invasive monitoring devices, which affect the normal host defense mechanisms.[4]

The most common pathogens involved in cardiac infections are *S. aureus*, viridians streptococci, coagulase negative staphylococci, and gram-negative bacilli.[5,6] Sternal infections are caused by coagulase-negative staphylococci (CONS).[7] The other predominant aerobic Gram-positive bacteria in cardiovascular infections is *S. aureus*.[8,9]

S. aureus is the most common pathogen in both community and hospital-associated infections.[10] After major heart surgery, *S. aureus* is the main cause of surgical site infection (SSI) with the patient's endogenous microbiota as the principal source.[11] MRSA is a special strain of the *S. aureus* that has developed antibiotic resistance, first to penicillin since 1948s, and later to methicillin and related anti staphylococcal drugs (such as flucloxacillin, nafcillin, and oxacillin). MRSA are resistant to penicillins, cephalosporins, monobactams, and carbapenems, cephems, and β-lactams and β-lactamase inhibitor

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combinations. Resistance to beta-lactam antibiotics by *S. aureus* is conferred by 2 different mechanisms.[12] The first mechanism consists of the production of beta-lactamase, an enzyme that hydrolyzes beta-lactam ring of the antibiotic. The second mechanism is mediated through an altered protein, referred to as low-affinity penicillin-binding protein (PBP2a). Unlike other PBPs, PBP2a—a unique transpeptidase—is not inhibited by β-lactams effectively. Hence, it can continue peptidoglycan crosslinking during the synthesis of bacterial cell wall. PBP2a is encoded by the *mec*A gene and is present in the chromosomal mobile genetic element called Staphylococcal cassette chromosome *mec* (SCC*mec*).[13]

Following the spread of MRSA, glycopeptides (usually vancomycin and more recently teicoplanin) have become the mainstay of treatment for MRSA infections.[14] As vancomycin is commonly used for the treatment of MRSA infections, it has resulted in the development of vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus*. Vancomycin-resistant *S. aureus* is due to acquisition from *Enterococcus* spp. of the *van*A operon, carried by transposon Tn1546, resulting in high-level resistance.[15] The product of the *van*A gene is a ligase that leads to alteration of this dipeptide residue from D-Ala-D-Ala to D-alanyl-D-lactate (D-Ala-D-Lac), a dipeptide with substantially lower affinity for the antibiotic.[15] The presence of *mec*A gene generally indicates the potential resistance to beta lactam group and is used as a marker to identify MRSA.[16] The presence of *van*A gene indicates the potential resistance to glycopeptides and is used as a marker to identify VRSA.[17]

Currently, development in immunological methods and new advancement in molecular testing methods such as Polymerase Chain Reaction (PCR) have greatly reduced the time and labor required to detect MRSA and could help to improve the infection control and the management.[18] PCR methods target a portion of DNA where the MRSA-specific SCC*mec* gene of *S. aureus* are explored.[9] Immunochromatographic tests (Rapid test) use monoclonal antibody against PBP2a, a protein produced by the *mec*A gene.[19] Therefore, amplification of the *mec*A gene is considered as an important benchmark to diagnose MRSA in the nosocomial and community settings as these genes are largely detected among staphylococcal species.[20]

Detection of methicillin resistance in Nepal is largely based on cefoxitin and oxacillin disc diffusion method with fewer reports on MIC determination and *mec*A gene detection by PCR.[21] In Nepal, several studies have investigated the phenotypic prevalence of MRSA infections in the past.[21-27] Studies conducted at different hospital/settings in Nepal have shown the prevalence of MRSA ranging from 26.1% to 57.1%.[22-26,28] This study explores the prevalence of MRSA infections, antimicrobial susceptibility patterns of *S. aureus* and MRSA, by analyzing Minimum Inhibitory Concentration (MIC) of vancomycin by detecting *mec*A and *van*A gene using PCR among patients attending SGNHC.

Methods

Study design, study site, and sample size and population

This was a prospective laboratory based cross sectional study conducted between May and November 2019, among patients attending SGNHC, Bansbari, Kathmandu, Nepal. A total of 524 clinical specimens that included urine (n = 145), blood (n = 135), sputum (n = 126), pus (n = 34), body fluids (n = 34), wound swabs (n = 29), and aortic/mitral valve tissues (n = 21) were collected during the study period; and were analyzed by standard microbiological methods.[29]

Inclusion and exclusion criteria

Patients suspected of bacterial infection from age range: 1 month to 90 years visiting SGNHC were enrolled in this study. The clinical specimens were collected from cardiac patients who attended the hospital for treatment and provided consent for the study. In cases of children below 16 years, assents were collected from their parents. Cardiac patients who had confirmed other chronic diseases based on the diagnosis made by clinicians and had the recent history of antibiotic treatment after the admission were excluded.

Sample collection, transportation, and processing

A total of 524 clinical specimens were collected aseptically and were labeled with the date, time, and method of collection and patient's history. All collected clinical specimens were processed based on the standard microbiological procedure.[29,30] The information of patients included signs and symptoms, history of prior infection, any underlying diseases, and history of past antibiotic use.

Culture of the sample

Blood: About 5 ml of blood was mixed with 45 ml of Brain Heart Infusion (BHI) broth for adult and 1 ml of blood with 9 ml of BHI broth for children. The bottle was incubated at 37°C for 24 hours. After 24-hours, blind subculture was performed and the subculture was repeated after viewing turbidity, hemolysis, and pellicle formation. The broths were kept incubated till 96 hours/8 days then discarded after blind subculture. If the growth occurred, then the isolated colony was identified based on colony morphology, Gram staining, and biochemical test results.[30]

Urine samples: The urine samples were cultured into Cysteine Lactose Electrolyte Deficient (CLED) agar by semi-quantitative culture techniques using a standard calibrated loop (4 mm). A loop full of urine was streaked on the plate and then incubated at 37°C in aerobic condition for 18 to 24 hours. Colony count was performed to calculate the number of Colony Forming Unit (CFU) per ml of urine and bacterial

count was reported as "not significant" (bacterial count <10^4 CFU/ml of organisms), "doubtful" (10^4-10^5 CFU/ml of organisms) and significant (bacterial colony count >10^5 CFU/ml organisms).[31] Uropathogens showing significant growth were processed for antimicrobial susceptibility testing.

Sputum, wound swab or pus: Samples were inoculated in Blood agar (BA), Chocolate agar (CA) and MacConkey agar (MA) plates. The plates were incubated aerobically overnight at 37°C for 24 to 48 hours. Then the isolated bacterial colony were identified by colony morphology, Gram staining and biochemical test.[31]

Pericardial fluids and other body fluids: The citrated sample in a sterile tube was centrifuged for about 20 minutes to sediment the bacteria. The supernatant fluid was removed and the sediment was re-suspended. The sediment sample was then inoculated in BA and MA plates. The plates were incubated aerobically overnight at 37°C.[30] Then the isolated colonies were identified by colony morphology, Gram staining, and biochemical test.[31]

Identification of bacteria

For identification of Gram-positive bacteria, catalase, oxidase, and coagulase tests were performed based on the ASM guidelines.[29,31] *S. aureus* were confirmed using following tests: golden-yellow colored on mannitol salt agar (MSA), slide and tube coagulase positive, beta-hemolysis positive on blood agar, gelatin hydrolysis positive, catalase positive, methyl red positive, Voges–Proskauer positive, nitrate reduction positive, lactose, mannitol, maltose, sucrose, and trehalose mannose fermenting, DNase producing, and alkaline phosphatase positive.[27,32]

Antibiotic susceptibility test of isolated organisms

Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion technique following clinical and laboratory standards institute (CLSI) guidelines.[33] The antibiotics disc consisting of penicillin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), erythromycin (15 µg), cefoxitin (30 µg), and clindamycin (30 µg) **(**Hi-media Laboratories Pvt. Limited, Bombay, India**)** were used for Gram positive bacteria. Based on the susceptibility pattern of the isolates, bacterial isolates resistant to ⩾3 classes of antibiotics were considered as multidrug resistant.[34]

Screening of methicillin resistance Staphylococcus aureus *(MRSA)*

Screening for methicillin resistant *S. aureus* was carried out by cefoxitin disc diffusion method and interpreted according to CLSI guidelines. The growth of *S. aureus* with zone of inhibition around cefoxitin disc (ZOI) ⩽21 was identified as methicillin resistant *S. aureus* and the isolates with zone of inhibition of ⩾ 22 mm were identified as methicillin susceptible *S. aureus* (MSSA).[35]

Determination of minimum inhibitory concentration of vancomycin to MRSA

Minimum inhibitory concentration (MIC) was determined to find the vancomycin intermediate and resistant strains of *S. aureus* isolates. MIC of vancomycin for MRSA was determined by agar dilution method following CLSI M07-A11, 2018 guidelines.[36] Different concentrations ranging from 0.0625 to 32 µg/ml of vancomycin incorporated plates were prepared. Positive controls were kept for each isolate and *S. aureus* (ATCC 25923) of known MIC was also included in each test as control for antibiotic potency. *S. aureus* that showed MIC value of vancomycin ⩽2 µg/ml was considered susceptible; ⩾4 to 8 µg/ml was considered intermediate resistant and ⩾16 ug/ml was considered resistant.[37,38]

Preservation of isolates

All phenotypically confirmed *Staphylococcus aureus* isolates were preserved in 20% glycerol containing tryptic soya broth and kept at −70°C until further processing for molecular analysis of the possible genes conferring antimicrobial resistance.

DNA extraction and PCR amplification

Chromosomal DNA was extracted from the MRSA isolates by chloroform: phenol extraction method as described for gram-positive bacteria.[39] Primer pairs used for mecA gene were Forward Primer (FP): (5′-ACT GCT ATC CAC CCT CAA AC-3′) and reverse primer (RP): (5-′CTG GTG AAG TTG TAA TCT GG-3′) (Eurofins Genomics) of amplicon size 163 bp.[40,41] Primer pairs used for *van*A gene were (Forward Primer): (5′-ATG AAT AGA ATA AAA GTT GC-3′) and revers primer (RP): (5′-TCA CCC CTT TAA CGC TAA TA-3′) of amplicon size 1032 bp.[42] PCR amplification reactions were carried out in a 25 µL volume in which master mix containing 200 µM of $dNTP_s$ (dATP, dCTP, dGTP, and dTTP), 120nM of each primer 0.5 U/µL of *Taq polymerase* in 1× PCR buffer, 25 mM $MgCl_2$, and 1 µL of DNA was added. Amplification reactions were performed under thermal and cycling conditions for the *mecA* gene: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 30 seconds for *mecA*, extension at 72°C for 3 minutes, and final extension at 72°C for 2 minutes. For *van*A gene the process followed: initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 1 minutes, annealing at 56°C for 1 minutes, extension at 72°C for 1 minutes and final extension at 72°C for 5 minutes.

After PCR amplification, 2.5 µL of each reaction was separated by electrophoresis in 1.0% agarose gel for 40 minutes at 100 V in 0.6 × TAE buffer. DNA was stained with ethidium bromide (EtBr) (1 µg/mL) and the bands were detected using UV transilluminator (Cleaver Scientific Ltd).[43,44]

Table 1. Demographic characteristics and distribution of culture positive bacterial isolates among patients attending Shahid Gangalal National Heart Center (n = 144).

CHARACTER	TOTAL SAMPLE PROCESSED	CULTURE POSITIVE		*P*-VALUE
		NUMBER	%	
Clinical samples processed	524	144	27.5	
Type of clinical samples				
Urine	145	57	39.6	0
Blood	135	24	16.7	
Sputum	126	30	20.8	
Pus	34	19	13.2	
Wound Swab	29	12	8.3	
Body fluids	34	1	0.7	
Aortic value tissues	21	1	0.7	
Gender				
Male	297	78	54.2	.52
Female	227	66	45.8	
Age group (in years)				
0-15	86	25	17.4	.83
16-45	161	38	26.4	
46-60	137	38	26.4	
>60	140	43	29.8	
Type of patients				
Inpatients department (IPD)	371	115	79.9	.01
Out patients department (OPD)	153	29	20.1	

Quality control

For quality control, *S. aureus* ATCC 29213 (*mec*A negative), *S. aureus* ATCC 700699 (*mec*A positive) and vancomycin resistant *Enterococcus faecalis* ATCC 51299 were used. For PCR controls, sterile water (negative) and the known positive DNA and negative controls from previous extraction (positive) were processed to ensure the accuracy of PCR process.

Statistical analysis

The data were entered in Microsoft excel sheet and analyzed using Statistical Package for Social Sciences (SPSS) version 24.0 software. Statistical analysis was conducted using Chi-squared test for association and *P*-value $<.05$ was considered statistically significant.

Results

Growth pattern of bacterial isolates

Out of 524 clinical specimens, 27.5% (144/524) showed the growth of bacteria. Among 144 bacterial isolates, 39.6% (57/144) were obtained from urine specimens followed by sputum (20.8%; 30/144). Male patients (54.2%; 78/144) were more in number than the female (45.8%; 66/144) ones in our study. In terms of age wise distribution of cardiac patients, 29.8% (43/144) were from age group >60 years followed by age group (46-60) years and (16-45) years. Among 144 cardiac patients, admitted patients at in-patient department (79.9%; 115/144) were more prone to bacterial infection than the patients visiting out-patients department (20.1%; 29/144) (P = .01) (Table 1)

Distribution of bacterial genera among culture positive isolates

Among 144 culture positive isolates, 12 different bacterial genera were identified. Among them, *S. aureus* (27.1%; 39/144) was the predominant bacteria in cardiac patients followed by *K. pneumoniae* (23.6%; 34/144), *E. coli* (20.1%; 29/144), and *Acinetobacter* spp. (10.4%; 15/144) (Figure 1).

Antibiotic susceptibility pattern of S. aureus *and MRSA isolates*

Among 39 *S. aureus* isolates, all isolates were found resistant to penicillin followed by erythromycin (94.9%; 37/39),

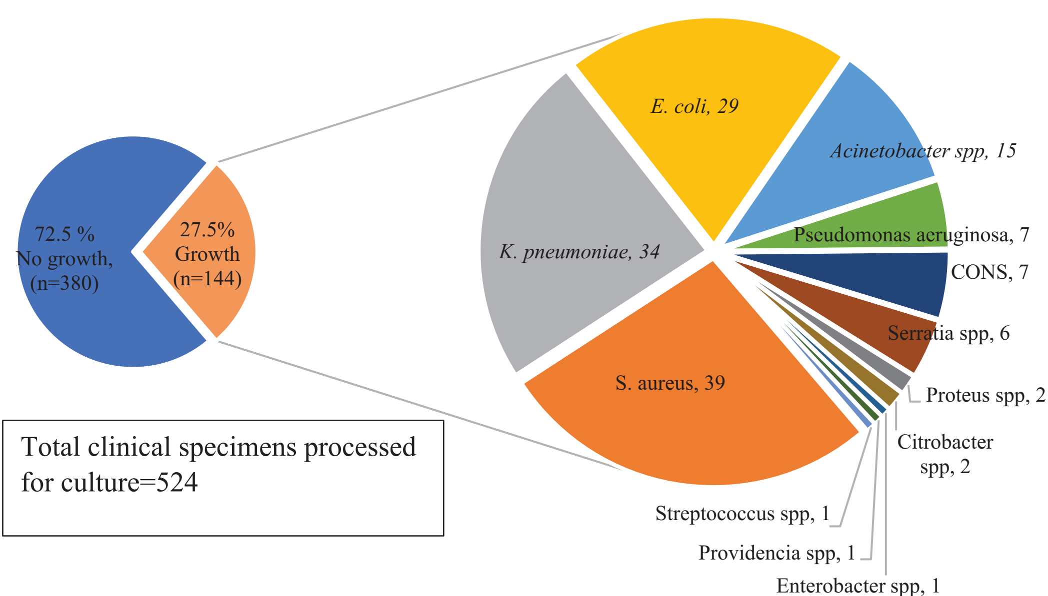

Figure 1. Growth pattern and distribution of bacterial genera among patients attending Gangalal National Heart Center, Kathmandu.

Table 2. Antibiotic susceptibility pattern of *S. aureus*.

ANTIBIOTICS	*S. AUREUS* (N=39)		MRSA (N=34)	
	SENSITIVE N (%)	RESISTANT N (%)	SENSITIVE N (%)	RESISTANT N (%)
Penicillin (30 µg)	0 (0)	39 (100)	0	34 (100)
Gentamicin (10 µg)	2 (5.1)	37 (94.9)	13 (38.2)	21 (61.8)
Ciprofloxacin (30 µg)	17 (43.6)	22 (56.4)	12 (35.4)	22 (64.6)
Erythromycin (15 µg)	2 (5.1)	37 (94.9)	1 (2.9)	33 (97.1)
Clindamycin (30 µg)	17 (43.6)	22 (56.4)	12 (35.4)	22 (64.6)
Cefoxitin (30 µg)	5 (12.8%)	34 (87.2)	0	34 (100)

gentamicin (94.9%; 37/39) and cefoxitin (87.2%; 34/39). All 34 MRSA isolates were found resistant to penicillin and cefoxitin followed by erythromycin (97.1%; 33/34), clindamycin (64.6%; 22/34), ciprofloxacin (64.6%; 22/34), and gentamicin (61.8%; 21/34) (Table 2).

Distribution of MRSA, MSSA, VSSA, VISA according to clinical specimens, gender, age, and type of patients

Among 39 *S. aureus* isolated, 87.2% (34/39 isolates) were MRSA and 12.8% (5/39) were MSSA. All 39 *S. aureus* were multidrug resistant (Figure 2).

Out of 39 *S. aureus* isolated, 43.6% (17/39) were isolated from blood followed by sputum (23.1%; 9/39) and pus (15.4%; 6/39). Male (66.7%; 26/39) were more prone to *S. aureus* infection than the female (33.3%; 13/39). Among 34 MRSA isolates, 35.3% (12/34) of the isolates were from blood followed by sputum (26.8%; 9/34) (Table 3).

MRSA isolates were predominant among the isolates obtained from male patients (70.6%; 24/34) in comparison to female ones (29.4%; 10/34). Patients of the age above 60 years harbored more MRSA (35.4%; 12/34) isolates followed by the age group (46-60) years (29.4%; 10/34). Similarly, 91.2% (31/34) of patients from in-patient's department harbored MRSA. Among 5 MSSA, all were isolated from the blood specimens. Among 34 MRSA isolates, 8.8% (3/34) were VISA and remaining (91.2%; 31/32) were VSSA. Among 3 VISA isolates, all isolates were isolated from in-patients. VISA isolates were isolated more in children (66.7%; 2/3) than the adults (33.3%; 1/3) (Table 3).

Minimum Inhibitory Concentration (MIC) of vancomycin to MRSA

Out of 34 MRSA, 8.8% (3/34) organisms were VISA and the remaining (91.2%; 31/34) were susceptible to vancomycin. None of MRSA were resistant to antibiotic vancomycin.

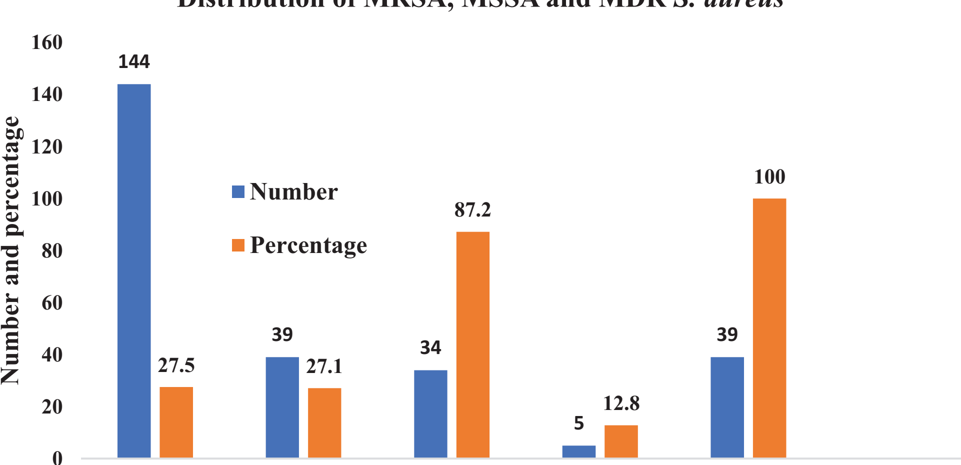

Figure 2. Distribution of MRSA, MSSA, and MDR *S. aureus* among clinical specimens of patients.

Table 3. Distribution of *S. aureus*, MRSA, MSSA, VSSA, VISA according to clinical specimens, gender, age, and type of patients (n = 39).

CHARACTER	*S. AUREUS*	MRSA	MSSA	VSSA	VISA
	N (%)				
Clinical specimens					
Blood	17 (43.6)	12 (35.3)	5 (100%)	11 (35.5)	1 (33.4)
Sputum	9 (23.1)	9 (26.8)	0	9 (29.1)	0
Pus	6 (15.4)	6 (17.6)	0	5 (16.1)	1 (33.3)
Wound swab	5 (12.7)	5 (14.7)	0	4 (12.9)	1 (33.3)
Body fluids	1 (2.6)	1 (2.9)	0	1 (3.2)	0
Aortic value tissue	1 (2.6)	1 (2.9)	0	1 (3.2)	0
	39	34	5	31	3
Gender					
Male	26 (66.7)	24 (70.6)	2 (40)	22 (70.9)	2 (66.7)
Female	13 (33.3)	10 (29.4)	3 (60)	9 (29.1)	1 (33.3)
Age groups (in years)					
0-15	6 (15.4)	6 (17.6)	0	4 (12.9)	2 (66.7)
16-45	11 (28.2)	6 (17.6)	4 (80)	6 (19.4)	0
46-60	10 (25.6)	10 (29.4)	1 (20)	10 (32.3)	0
>60	12 (30.8)	12 (35.4)	0	11 (35.4)	1 (33.3)
Type of patients					
In patients	35 (89.7)	31 (91.2)	4 (80)	28 (90.3)	3 (100)
Out patients	4 (10.3)	3 (8.8)	1 (20)	3 (9.7)	0

Prevalence of mecA gene and vanA gene in S. aureus

Out of 39 *S. aureus*, 82.1% (32/39) showed *amplified mecA gene (167 bp)* (Figure 3A). None of the *S. aureus* had *van*A gene (Figure 3B).

Among 34 MRSA, 94.1% (32/34) showed *mec*A gene and all VISA isolates (100%; 3/3) had *mec*A gene. Among 5 MSSA isolates, none of them had *mec*A gene (Table 4).

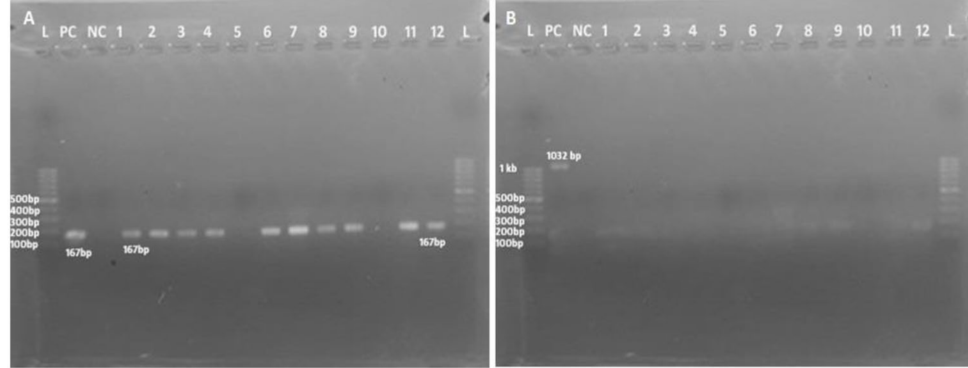

Figure 3. (A) Agarose gel electrophoresis showing PCR amplified *mec*A gene (167 bp). Lane L, 100 bp DNA ladder (thermo scientific); Lane PC, positive control, Lane NC, negative control; Lane 1, 2, 3, 4, 6, 7, 8, 9, 11, 12: *mec*A gene (167 bp). (B) Agarose gel electrophoresis showing *vanA* positive control (1032 bp) and *van*A gene negative in MRSA isolates. Lane L: 100 bp DNA ladder (thermo scientific), Lane PC, positive control; Lane NC: negative control, Lane 1, 2, 3, 4,. . . 12: *van*A gene negative.

Table 4. Prevalence of *mec*A gene detected by PCR (n = 39).

ORGANISM	NO. OF ISOLATES	*MECA* GENE DETECTED	
		N	%
S. aureus	39	32	82.1
MRSA	34	32	94.1
MSSA	5	0	0
VSSA	31	29	93.5
VISA	3	3	100

Discussion

One fourth of the clinical samples received in laboratory of SGNHC showed bacterial growth. Commonest bacteria from clinical specimens were *S. aureus* followed by *K. pneumoniae*, and *E. coli*. One fourth of the culture positive isolates were *S. aureus*. All the isolated *S. aureus* were MDR and nearly 90% of them were MRSA. Out of 34 MRSA isolates, 94% showed *mec*A gene and none of the MRSA had *van*A gene. Bacterial growth pattern in this study was consistent with the previous studies conducted in different clinical settings of Nepal.[45-48] In this study the predominant bacteria identified was *S. aureus*, however, most of the past studies reported *E. coli* as the main bacteria. This might be due to variation in study sites, nature of illnesses and the clinical specimens.

MRSA has emerged as an important human pathogen with increasing trend of resistance toward currently used antimicrobial therapy.[49] Globally and in Nepal, the prevalence of MRSA is heterogeneous with varying prevalence (past studies from Nepal: 15.4%-26.0%),[23,50] however, recent studies from Nepal have shown higher prevalence (26%-69%) indicating the rising trend over the years.[51,52] Most of the studies investigating MRSA in Nepal utilized just cefoxitin and oxacillin for screening. Compared to oxacillin, cefoxitin is a better drug to detect *mec*A gene in MRSA and is considered as a substitute marker. Detection of *mec*A gene or its product PBP2a by cefoxitin is considered as the gold standard for MRSA confirmation.[53]

In our study, 87.2% of *Staphylococcus aureus* isolates were MRSA and was consistent with previous reports from Chitwan,[21] and Pakistan.[54,55] However, other previous studies from Nepal reported low prevalence (<50%) of MRSA.[23,50-52] These differences may have been due to the length of study period, sample size, number of study sites, sample type, and the laboratory procedures utilized for estimation of MRSA. Furthermore, our study was conducted in a tertiary heart center, where large numbers of open-heart surgeries are conducted on a daily basis, along with other interventions such as angiography and angioplasty.

PCR assay was performed using a single set of primers for the amplification of *mec*A gene. Out of 34 MRSA isolates, 32 *mec*A positive isolates and 2 *mec*A negative isolates were identified. All 34 strains were confirmed to be methicillin resistant disc diffusion susceptibility method. This finding may have resulted from a false negative PCR reaction that can arise from point mutation or deletion in *mec*A gene or due to the presence of inhibitors. Studies have suggested that a variety of genes regulatory elements such as fem factors (factors essential for methicillin resistance) *mecI* and *mecR1* regulatory genes are involved in *mec*A expression or repression.[44,56] Cefoxitin is used as a more reliable marker than oxacillin for methicillin resistance. However, resistance to cefoxitin does not mean detection of the *mec*A gene or its PBP2a product. Screening with cefoxitin will determine which isolates will be tested by other methods, phenotypic or genotypic, for the detection of methicillin resistance markers, the *mec*A gene or its product. Furthermore, it is not uncommon for cefoxitin-resistant strains lacking the *mec*A gene. The isolates that lack the *mec*A gene may have other methicillin resistance mechanisms such as the novel *mec*A homologous, *mec*C as change in affinity of penicillin-binding protein for oxacillin.[44,52,56]

In the diagnostic laboratories, detection of *mec*A gene confirms the MRSA strains, which has been established by

previous studies from Sudan,[57] Iraq,[58] Saudi Arabia,[59] India,[60] Australia,[61] Japan,[62] and USA.[63] Although the detection of genes had long been regarded as the gold standard among resistant isolates, a number of studies suggest the absence of *mec*A among resistant isolates.[64] Studies have also shown that the non-*mec*A resistance can be caused by *mec*C.[65] Additionally, isolates that lacked the *mec*A gene showed moderate methicillin resistance.[66]

In our study, 94% of isolates were tested positive for *mec*A, suggesting the presence of such gene to be crucial but not mandatory in the development of resistance. A number of intrinsic factors could play role in developing resistance which can suppress the expression of such gene(s). In a previous study reported from Nigeria, 5 major SCC *mec* types, *mec*A, and the gene product of PBP2 were completely absent but the isolates were still phenotypically resistant, suggesting the probability of hyper production of β-lactamase.[67] Another study suggests the probable role of specific alterations in amino acids on protein binding cascades (PBPs 1, 2, and 3) for the development of MRSA. These alterations can occur by both identical and non-identical substitution of amino acids, which was observed in the isolates of different sequence types by multilocus sequence typing (MLST).[68] These studies suggest that the *mec*A itself is a predominant but not mandatory factor to confer resistance in MRSA isolates; the existence of other intrinsic factors and mechanisms may explain the methicillin resistance in this bacterial species. Findings from this study suggest that the existing diagnostic tools (conventional and molecular) cannot be a standalone test for the detection of MRSA. Combination of these tools probably aids in accurately detecting the burden and trend of MRSA including in guiding the antimicrobial therapy.

In our study, 8.8% of MRSA isolates were found VISA (vancomycin MIC of 4-8 μg/ml) and remaining 92.2% were VSSA (showed MIC value of 2 μg). The findings of this study are consistent with the studies reported from Kashmir, India,[69] and Mangalore, India.[70] The resistance mechanism in VISA is not fully known. Although, the sequestration of glycopeptides (vancomycin and teicoplanin) from accumulation of peptidoglycan precursor, acyl-D-alanyl-D-alanine dipeptide, have been attributed to reduce the drug's penetration to its target site. No gene or operon has been linked to VISA, although frequent patient exposure to high doses of various antibiotics including vancomycin, may cause chromosomal mutations.[71] In our study, none of the MRSA isolates had *van*A gene. The threat of vancomycin resistance in *S. aureus* has been a great concern since the first report of VISA in the 1997[72] and VRSA in 2002.[73] Nonetheless, globally, there are limited reports of clinical infections caused by VRSA.[74-76] while infections caused by VISA are increasing.[77-79]

Strengths and limitations

This is the first comprehensive study which explored the prevalence of MRSA using both phenotypic and molecular methods among patients attending a tertiary cardiac centre of Nepal. The findings and conclusions of this study are useful for major tertiary centers where nosocomial infections are high. These findings can inform the antimicrobial policy for tertiary care centers including preparing the management of hospital infections, treatment protocol, and diagnostic procedure. There are few limitations of this study that includes the shorter duration of the study, smaller sample size and the study was conducted in a single hospital. Future studies can build on it to conduct a longitudinal study at multiple tertiary care centers to strengthen the findings. Nonetheless, as a first study of its types triangulating the phenotypic and molecular methods, this will be a valuable reference for future studies on MRSA in Nepal.

Conclusion

High prevalence of MRSA among cardiac patients is suggestive of the medical emergency as MDR strains can lead to various extent of treatment failures, uncontrolled nosocomial infections, and mortalities. Although PCR- based detection technique outweighs other conventional techniques, combination of these methods can offer diagnostic accuracy.

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Author's Contributions

All the authors made substantial contribution to the study. SD, KRR, BY, PG conceived and designed the study. SD collected samples, investigated and recorded the laboratory findings. BY, NA, UTS, KRR, and PG supervised the laboratory work. KRR, MRB, BA and PG advised and formulated the methodology for the study. SD, BD and KRR drafted the original draft. KRR and BA are responsible for reviewing several versions of manuscript. Others helped to review and amend this manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

All data pertaining to this study are within the manuscript. A raw data of excel sheet was attached in supplementary file.

Ethical Approval and Consent to Participate

Ethical approval was obtained from Institutional Review Committee, Institute of Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal (Reg No: 5/2019). Written informed consents or assents in case of minors were obtained from all the participants. In case of Children below 16 years, a written informed consent was obtained from a parents or guardian attending hospital along with the patients.

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