Methicillin Resistant *Staphylococcus aureus* in Health Care Workers of a Tertiary Care Infectious Disease Hospital in Nepal

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ABSTRACT

Objectives: Acquisition of *mecA* gene in infectious strains of Methicillin resistant *Staphylococcus aureus* (MRSA) are considered as one of the potential virulence factors that enables the host bacteria to carry out several nosocomial and community-acquired infections. The main aim of this study was to determine the prevalence of MRSA, their antibiogram and *mec*A gene in the bacterial isolates obtained from the asymptomatic healthcare workers (HCWs) working in Sukraraj Tropical and Infectious Disease Hospital (STIDH), Kathmandu Nepal.

Methods: This prospective cross-sectional study involved the collection of nasal and hands swab of 125 randomly selected HCWs from December 2019 to February 2020. Conventional microbiological methods were used to isolate and identify *S. aureus*. Antimicrobial susceptibility testing was done by modified Kirby Bauer disc diffusion method. MRSA was confirmed by using cefoxitin disc. Detection of mecA gene in the chromosome which was extracted by Phenol: Chloroform: isoamyl alcohol DNA extraction method, amplified by using PCR and visualized by running agarose gel electrophoresis.

Results: The overall and MRSA carriage rate among the HCWs was found to be 28% (35/125) and 10.4% (13/125) respectively. *S. aureus* carriage rate was highest among sanitation staffs (34.2%) followed by pharmacy staffs (33.3%), laboratory personnel (18.8%), doctors (9.1%) and nurses (7.5%). Similarly, 34.2% (13/38) of the *S. aureus* isolates were resistant to methicillin, 31.6% (12/38) were inducible-clindamycin resistant and 63.2% (24/38) of them were multi-drug resistant (MDR). All the 13 MRSA isolates harbored the *mecA* gene.

Conclusion: Carriage rate of MRSA among HCWs was high and alarming, indicating the prompt need of intervention measures to curb the growth and spread of resistant isolates in the hospital settings. Effective surveillance (of infectious diseases) and establishment of advanced diagnostic facilities can assist in estimating the actual burden of the MRSA which in turn helps to formulate and implement the appropriate policies and infection-control programs to address the increasing antimicrobial resistance in the country.

Keywords: MRSA, Health care workers, *Staphylococcus aureus,* Nasal carriage

INTRODUCTION

Staphylococcus aureus—a human commensal and opportunistic pathogen—constitutes the major causative agent of several bacterial infections. Despite the advancement and availability of several antibiotic therapies, staphylococcal infection is still remains as one of the most frequent infections in hospitalized patients causing a wide variety of clinical manifestations ranging in severity from superficial infections such as cutaneous infections to severe invasive diseases like bacteremia (Chakolwa et al. 2019).

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Antibiotics are the miracle drugs in treatment of the infections caused by pathogenic strains of bacteria. However, due to extensive and irrational use of antibiotic has led to the emergence of antimicrobial resistance (AMR), a condition in which bacteria shows the resistance against the antibiotics prescribed against it (Mazzon 2016). Like all other AMR problems, methicillin-resistant *S. aureus* (MRSA) has also emerged as a major barrier in the management of nosocomial and community-acquired infections. Following the introduction of penicillin in 1940, *S. aureus* showed resistance against penicillin due to its ability to produce penicillinase, an enzyme that hydrolyses and inactivates the penicillin group of antibiotics. First case of penicillin-resistant *S. aureus* strain was detected in 1942. Methicillin, a semi synthetic penicillin was then developed in the late 1950s to treat penicillin resistant *S. aureus* but the widespread use and misuse of methicillin and other semi-synthetic penicillin led to the emergence of methicillin-resistance, first case reported in 1961 (Barber 1961). Methicillin resistance is chiefly mediated due to the acquisition of a new penicillin binding protein PBP-2' (expressed by an exogenous gene, *mec*A) which has low affinity to the most β-lactam antibiotics (Archer 1998). There occurs a wave in the outbreaks associated with MRSA strains which is associated with higher mortality rates, prolonged hospital stays and unwanted economic burden due to the increased cost of treatment (Lakhundi and Zhang 2018). An individual colonized with MRSA has a fourfold increased risk of subsequent infection than by other susceptible strains of *S. aureus* (Safdar and Bradley 2008).

MRSA once confined to hospitals, health care environments and patients frequenting such facilities, now has become a serious problem for communities due to its emergence as a major cause of the community-associated infections (Khatri et al. 2017; Lakhundi and Zhang 2018). Since mid-1990s, sudden increase in the number of MRSA infections reported in the communities has been associated with the recognition of new MRSA strains. Such novel strains are often called as community-associated MRSA (CA-MRSA) strains. These strains which principally used to cause skin and soft tissue infections are now responsible for hospital care-associated infection as well (David and Daum 2010).

HCWs serve as the link between hospitals and the communities, which plays a significant role in cross-contamination of nosocomial and community acquired infections (El Aila et al. 2017). Asymptomatic colonization of MRSA among HCWs is a prerequisite for subsequent endogenous infection and dissemination of the strains to the hospital environment (Abimana et al. 2019). Early identification of MRSA carriers among HCWS may help to minimize the potential outbreaks in several hospitals.

Drug-resistant, often multidrug-resistant strains of the commensal and opportunistic bacteria including MRSA, methicillin resistant *S. epidermidis* (MRSE), vancomycin resistant *Staphylococcus aureus* (VRSA) and some strains of *Pseudomonas* spp, *Haemophilus* spp, *Streptococcus* spp are the major problem in the infection control strategies. Aside from socioeconomic burden and augmented risk of dissemination, these resistant strains may go undetected in the resource poor settings of Low- to lower-middle income countries (LMICs) which results the emergence of novel strains furthering the challenges in the fight with AMR (Thapa et al. 2020). A number of studies are conducted in Nepal which have estimated the prevalence of MRSA ranging from 26.1% to 57.1% (Ansari et al. 2014; Kumari et al. 2008; Rijal et al. 2008; Khanal and Jha 2010; Raut et al. 2017; Bhomi et al. 2016; Rijal et al. 2008; Shahi et al. 2018; Thapa et al. 2020; Kandel et al. 2020). Although there seems a large number of studies are based on MRSA but almost all studies are merely relied on the phenotypic detection. Therefore, there is still a paucity of researches to estimate the molecular detection and characterization of the resistant genes. This study explores the prevalence of MRSA strains, their antibiogram and the molecular detection of *mec*A gene in order to corroborate the need and importance of molecular detection techniques and their reliability in the precise detection of resistant strains.

MATERIALS AND METHODS

Study design, study site and sample population

This prospective cross-sectional study was conducted for a period of three months from December 2019 to February 2020. Sample collection and processing was conducted at Sukraraj Tropical and Infectious Disease Hospital (STIDH), Teku while the molecular detection of the *mec*A gene was carried out at Central Department of Microbiology, Tribhuvan University, Kathmandu. All purposively selected HCWs above 18 years of age from the hospital who consented to provide socio-demographic information along with nasal and hand

swab samples, were included in the study. Similarly, HCWs under 18 years of age, who were unable provide written consent for socio-demographic information were excluded from the study. A total of 250 (n=125 nasal; n=125 hand) swab samples were obtained from 125 HCWs, each of the study participant provided a nasal swab and a hand swab.

Collection and transport of samples

Swab samples were collected aseptically by using sterile cotton swabs pre-moistened with sterile normal saline, following standard methodology (Cheesbrough 2012). Briefly, the swab was rotated inside the anterior nares of each nostril for 2-3 times with slight finger pressure on the outside of the nose in order to assure good contact between swab and the chamber of the nostril. Without contaminating the swab, the procedure was repeated for the second nostril and the swabs were transported to the laboratory with the labels of subject's identification number and other required information (Cheesbrough 2012). No transport medium was used as the microbiology department and sample collection sites were adjacent to each other. Drying of swab was prevented by keeping the swab in tubes containing nutrient broth.

Isolation and identification of *S. aureus*

Swab samples were inoculated into mannitol salt agar (MSA) and blood agar (BA) and were incubated at 37^{o}C for 24 hrs. *S. aureus* was identified on the basis of colony characteristics, Gram's staining, and biochemical assays such as catalase, oxidase and coagulase test (Cheesbrough 2012).

Antibiotic susceptibility testing and screening of MDR *S. aureus*

Antimicrobial susceptibility test (AST) was performed in-vitro using modified Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) as per CLSI guidelines (2019). Following antibiotic discs (Hi Media Laboratories, Pvt. Limited, India) were used: amoxicillin (AMX 10µg), cefoxitin (CX 30µg), ciprofloxacin (CIP 5µg), clindamycin (CD 2µg), cotrimoxazole (COT 25µg), erythromycin (E 15µg), gentamicin (GEN 10µg), penicillin (P 10µg), tetracycline (TE 30µg), cloxacillin (COX 10µg) and ceftriaxone (CTR 30µg). Results were interpreted on the basis of CLSI guidelines (2019). Isolates showing non-susceptibility (either resistant or intermediate) to at least one agent in three or more antimicrobial categories were identified as MDR (Magiorakos et al. 2011). Confirmed *S. aureus* isolates were preserved by using 20% glycerol in TSB for further analysis.

Screening of MRSA

Methicillin resistance was detected by using cefoxitin disk of 30µg on MHA plates by Kirby-Bauer disc diffusion method and was interpreted according to CLSI guidelines (2019). Isolates showing zone of inhibition of ≤ 21 mm around cefoxitin disc (30 µg) were considered as MRSA (CLSI 2019).

Detection of inducible-clindamycin resistance (ICR)

Inducible-clindamycin resistant was detected by double disk approximation test (*D*-test) as per CLSI (2019) guidelines. In this test, a 0.5 McFarland's standard suspension of *S. aureus* was prepared and plated onto MHA. An erythromycin disk (15 µg) and clindamycin disk (2 µg) were placed 15 mm apart edge-to-edge on MHA plate. Plates were analyzed after 18 hours of incubation at 37°C. Isolates were considered inducible-clindamycin resistant when an isolate was resistant to erythromycin but sensitive to clindamycin showing flattening of the zone of inhibition of ≥21mm around clindamycin producing a "*D*" shaped blunting towards erythromycin disk (*D*-test positive).

Extraction of DNA

All phenotypically confirmed MRSA isolates were treated under Phenol: chloroform: isoamyl alcohol extraction method for the detection of *mec*A gene. In this method, isolates were grown in Luria Bertani (LB) broth at 37°C in an orbital shaker at 120rpm for 24 hours. 1.5 ml of liquid culture was transferred to microfuge tube of 1.5ml volume. Then, the bacterial cells were lysed with 3-5 mg/ml lysozyme in the presence of 1/10 volume of 10 % Sodium Dodecyl Sulfate (SDS) at high P^{H} and the lysate was then neutralized. Subsequent deproteinization with 1:1 Phenol: Chloroform was done and then genomic DNA was precipitated with ethanol by spinning at high speed (Shrestha and Adhikari 2014).

PCR amplification of mecA gene

Thus extracted mecA gene was further amplified by polymerase chain reaction (PCR). In PCR test, the crude lysates were used as a DNA template whereas (*mecA* PF1)5′- ACT GCT ATC CAC CCT CAA AC-3′ and (*mecA* PR1) 5′- CTG GTG AAG TTG TAA TCTGG-3′ were used as forward and reverse primer respectively (Vatansever et al. 2016). A final 10 µl

solution was prepared by mixing up of 5 µl master mix, 1 µl each of forward and reverse primer, 1 µl DNA, and 2 µl nuclease free water. The amplification cycle consisted of initial denaturation at 95°C for 120 seconds, denaturation at 95°C for 30 seconds, annealing at 56.2°C for 30 seconds, extention at 72°C for 20 seconds, and 29 cycles of amplification at 72°C for 5 minutes. The amplified products were then subjected to gel electrophoresis using 1.5 % agarose gel stained with ethidium bromide.The final product (163-bp DNA fragment) was then visualized under UV light. The presence of the gene was confirmed by comparing with a positive control using 100 bp DNA ladder (Molecular Biology, Thermo Fisher Scientific Company) in the gel run. The band of 163-bp was considered positive for the *mecA* gene (Vatansever et al. 2016; Oliveira and de Lencastre 2011).

Statistical analysis

All the data were analyzed by using IBM SPSS statistics 23.0 version software. Frequency and percentage for descriptive and Chi Square test with cross tab for inferential statistics were used. A p-value of <0.05 was considered as statistically significant.

RESULTS

Distribution of bacterial growth in swab samples

Among the 250 swabs processed, 241 (96.4%) samples showed the growth of bacteria in which 81.2% (203/250) of them were coagulase-negative Staphylococci (CONS) while 15.2% (38/250) showed growth of *S. aureus* (Figure 1). Out of 38 positive samples, 42.1% (n=16) were isolated from nasal swab while 57.9% (n=22) were isolated from hands swab. About 10.4% (13/125) of the HCWs had colonization with *S. aureus* only, 15.2% (19/125) of them had hands colonization alone while 2.4% (3/125) of them had both nasal and hands colonization. However, 72% of the staff's both nasal and hand swabs were free from *S. aureus.*

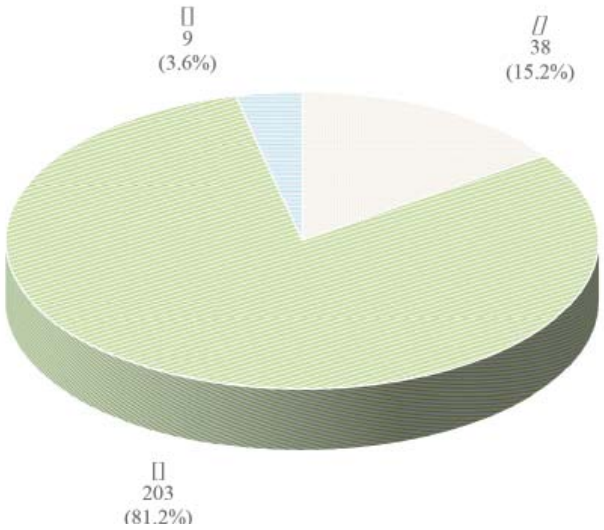

Figure 1: Distribution of bacterial growth

Antibiotic resistance of *S. aureus*

Regarding antimicrobial susceptibility pattern of all 38 *S. aureus* isolates, a high proportion of *S. aureus* isolates (73.7%) showed resistance towards amoxycillin. Also, 68.4%, 60.5%, 47.3%, 36.8%, 34.2%, 15.8%, 12%, 7.9% and 7.9% of the isolates were resistant to erythromycin, cloxacillin, ciprofloxacin, ceftriaxone, cefoxitin, gentamicin, clindamycin, cotrimoxazole and tetracycline respectively (Figure 2).

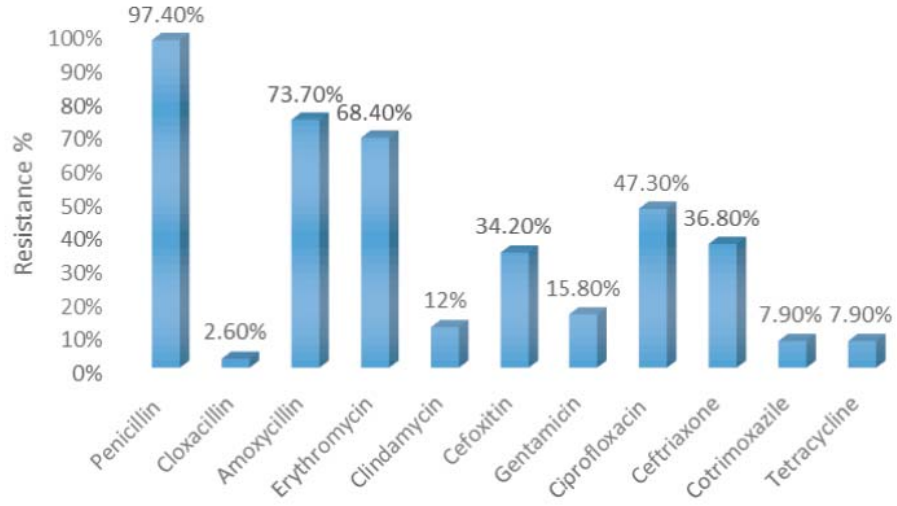

Figure 2: Antibiotic resistance pattern of *S. aureus* isolates

Phenotypic screening of MRSA

Out of 38 *S. aureus* isolates, 34.2% (13) were resistant to methicillin. Among which, 61.5% (8/13) isolates were obtained from nasal swab whereas 38.5% (5/13) were obtained from hand swab (Figure 3).

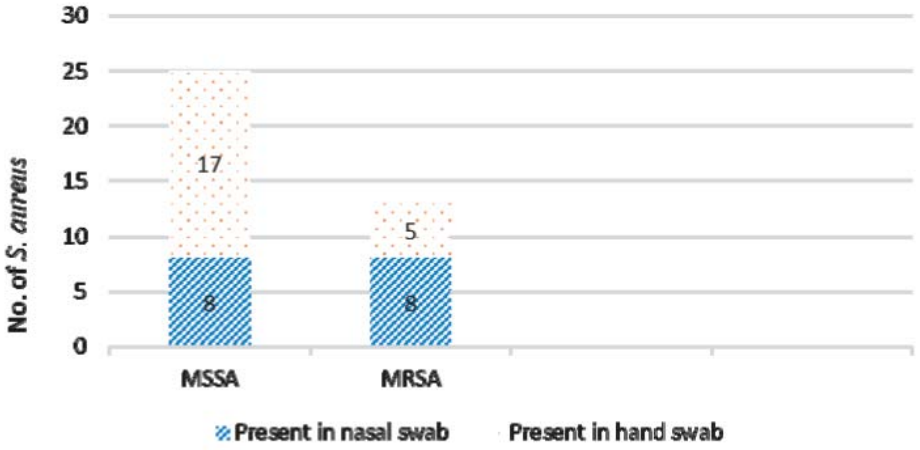

Figure 3: MSSA and MRSA among *S. aureus* isolates

Distribution of *S. aureus* and MRSA according to age and gender of the subjects

Among the total studied participants, 24 were males and 101 were females. Respectively 32.7% (33/101) and 20.8% (5/24) female and male participants were found to have harbored *S. aureus*. Similarly, 10.9% (11/101) and 8.35% (2/24) of the male and female subjects respectively harbored MRSA strains.

This study included HCWs of the age between 16-80 years old. Colonization with MRSA was highest (6.3%; 2/32) among the age group >50 years, followed by the age group of 26-50 years (5.6%; 8/144) and 15-25 years (4.1%; 3/74). However, there was no significant association between the bacterial load (MRSA) and, gender and age groups (p=0.67) (Table 1).

Table 1: Age and gender wise distribution of *S. aureus* and MRSA

Age of health care workers	Total no. of samples N	Gender of the health care workers				Total MRSA
		Male (n =24)		Female (n =101)		
		S. aureus N (%)	MRSA N (%)	S. aureus N (%)	MRSA N (%)	N (%)
18-25	74	0	0	6 (8.1)	3 (4.1)	3 (4.1%)
26-50	144	3 (2.03)	1 (0.7)	23 (16)	7 (4.9)	8 (5.6%)
Above 50	32	2 (6.3)	1 (3.1)	4 (12.5)	1 (3.1)	2 (6.3%)
Total	**250**	**5**	**2**	**33**	**11**	**13 (5.2%)**

***S. aureus* and MRSA carriage among different groups of health profession**

Among the total staffs, the highest percentage of sanitation staffs (34.2%; 13/38) were colonized with *S. aureus* followed by pharmacy staffs (33.3%; 2/6) and lab personnel (18.8%; 6/32). However, MRSA carriage was observed highest in pharmacy staffs (16.7%; 1/6) followed by lab personnel (12.5%; 4/32), sanitation staffs (7.9%; 3/38) and nurses (5%; 4/80). This distribution of the bacterial load and MRSA were not found to be statistically associated with the various professions (wards) of the HCWs (p= 0.152) (Table 2).

Table 2: Distribution of *S. aureus* and MRSA carriage among different groups of health professions

Profession of health care workers	Total no. of samples N	No. of *S. aureus* N (%)	MRSA N (%)
Doctor	22	2 (9.1 %)	0
Nurse	80	6 (7.5 %)	4 (5 %)
Lab Personnel	32	6 (18.8 %)	4 (12.5 %)
Pharmacist	6	2 (33.3 %)	1 (16.7 %)
Health Assistant	18	3 (16.7 %)	0
Sanitation Staff	38	13(34.2 %)	3 (7.9%)
Interns	30	3 (10 %)	0
Others	24	3 (12.5 %)	1 (4.2%)

Distribution of isolates in different departments of the hospital

In this study, the rate of *S. aureus* carriage was highest among pharmacy staffs (33.3 %; 2/6) followed by laboratory staffs (20.3 %; 13/64) and ICU staffs (16.7 %; 1/6) and staffs from the emergency department (16.7%; 3/18). However, MRSA colonization was higher in HCWs from pharmacy (16.7%; 1/6) and ICU (16.7%; 1/6) followed by HCWs from ward staffs (6.9%; 5/72) and lab staffs (6.3%; 4/64) respectively. This distribution was not statistically significant with p-value of 0.628, which suggests that MRSA carriage rate and departments of duty of HCWs are independent of each other (Table 3).

Table 3: Distribution of *S. aureus* and MRSA isolates in different departments

Department of duty	Total no. of samples N	No. of *S. aureus* N (%)	MRSA N (%)
Emergency	18	3(16.7 %)	1(5.6 %)
OPD	36	4 (11.1 %)	0
Ward (Gastro + Male + Cabin)	72	11(15.3 %)	5(6.9 %)
ICU	6	1 (16.7 %)	1(16.7 %)
ART Department	26	1 (3.8 %)	0
Laboratory	64	13 (20.3 %)	4(6.3 %)
Pharmacy	6	2 (33.3 %)	1(16.7 %)
Immunization	10	1 (10 %)	0
Others	12	2 (16.7 %)	1(8.3 %)

Distribution of MRSA and MSSA on the basis of duration of employment

Highest number of *S. aureus* was seen in those HCWs who served the hospital for less than 10 years as 52.6% (20/38) of the *S. aureus* and 35% (7/20) of the MRSA isolates were recovered from them. This was followed by the service year of 20-30 years with 23.7 % (9/38) *S. aureus* load and 22.2 % (2/9) of MRSA. Those who served for 20-30 years showed the rate of 23.7% (9/38) of *S. aureus* while the rate was 22.2% (2/9) of MRSA. It was then followed by 10-20 years and 30-40 years of employment with 21.1% (8/38) and 2.6% (1/38) of *S. aureus* carriage respectively. However, the distribution was statistically insignificant as (p=0.532) (Figure 4).

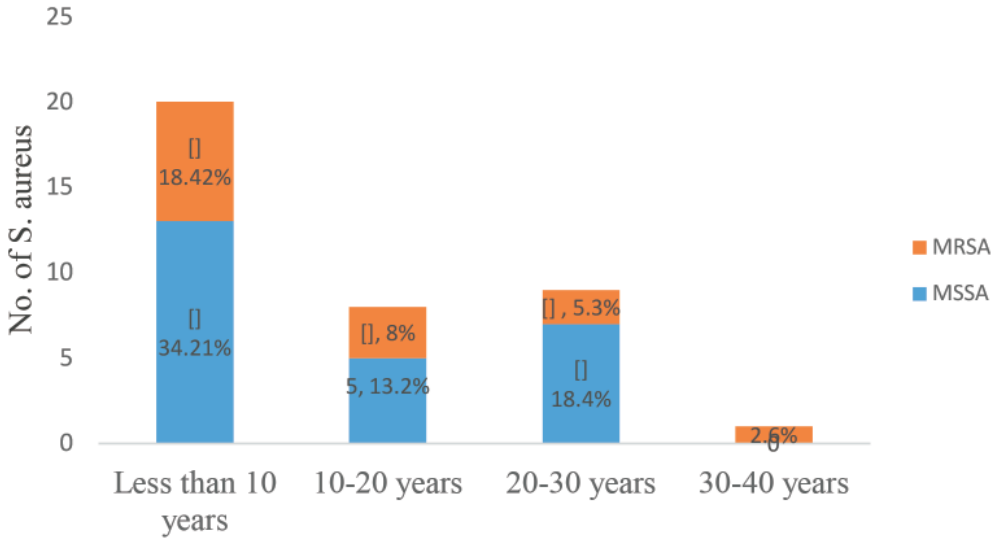

Figure 4: MSSA and MRSA distribution according to years of employment

Antibiotic susceptibility pattern of MSSA and MRSA

All (13) of the MRSA isolates were resistant towards penicillin, amoxycillin, cefoxitin and ceftriaxone. 12 (92.3%) isolates were resistant to erythromycin. 92.3% (12/13) of the total MRSA isolates were susceptible to cloxacillin, clindamycin and gentamicin. Almost equal portion (84.6%; 11/13) of the MRSA isolates were susceptible to both Cotrimoxazole and Tetracycline. However, 60% (15/25) of MSSA isolates were resistant to amoxycillin followed by 56% (14/25) for erythromycin. All of the MSSA isolates were susceptible towards cefoxitin and cloxacillin. 96% (24/25) of MSSA were susceptible for ceftriaxone, cotrimoxazole and tetracycline. 92% (23/25) and 80% (20/25) of them were susceptible towards clindamycin and gentamicin respectively (Table 4).

Table 4: Antibiotic susceptibility patten of MSSA and MRSA

Antibiotic disc	MSSA (N=25), n (%)		MRSA (N=13), n (%)	
	Sensitive (S) N (%)	Resistant (R) N (%)	Sensitive (S) N (%)	Resistant (R) N (%)
Penicillin	1 (4%)	24 (96%)	0	13 (100%)
Cloxacillin	25 (100%)	0	12 (92.3%)	1 (7.7%)
Amoxycillin	10 (40%)	15 (60%)	0	13 (100%)
Erythromycin	11 (44%)	14 (56%)	1 (7.7%)	12 (92.3%)
Clindamycin	23 (92%)	2 (8%)	12 (92.3%)	1(7.4%)
Cefoxitin	25 (100%)	0	0	13 (100%)
Gentamicin	20 (80%)	5 (20%)	12 (92.3%)	1 (7.7%)
Ciprofloxacin	14 (56%)	11 (44%)	6 (46.2%)	7 (53.8%)
Ceftriaxone	24 (96%)	1 (4%)	0	13 (100%)
Cotrimoxazole	24 (96%)	1 (4%)	11 (84.6%)	2 (15.4%)
Tetracycline	24 (96%)	1 (4%)	11 (84.6%)	2 (15.4%)

Prevalence of multi-drug resistant and inducible -clindamycin resistant *S. aureus* isolates

Among 38 *S. aureus* isolates, 24 (63.2%) of them were MDR. Similarly, all of the MRSA isolates were MDR. Out of 38 *S. aureus* isolates, 12 (31.5%) isolates were screened as inducible-clindamycin resistance. Among 12 isolates, 7 were MRSA (Table 5).

Table 5: Prevalence of multi-drug resistant and inducible -clindamycin resistant *S. aureus*

No. of *S. aureus*	MRSA N (%)	MDR N (%)	MDR isolates excluding MRSA, N (%)	D-test Positive N (%)	MRSA with Positive D-test, N (%)	MSSA with Positive D-test,N (%)
38	13 (34.2%)	24 (63.2%)	11 (29%)	12 (31.6%)	7 (18.4%)	5 (13.2%)

Prevalence of *mec*A gene among MRSA isolates

All of the 13 MRSA isolates were confirmed to have harbored *mec*A gene (Table 6). The *mec*A gene was detected under gel electrophoresis with product size 163 bp (Figure 5).

Table 6: Detection of *mec*A gene in MRSA

Sample	No. of MRSA isolates N	No. of *mec* A positive MRSA N (%)
Nasal swab	8	8 (100%)
Hand swab	5	5(100%)

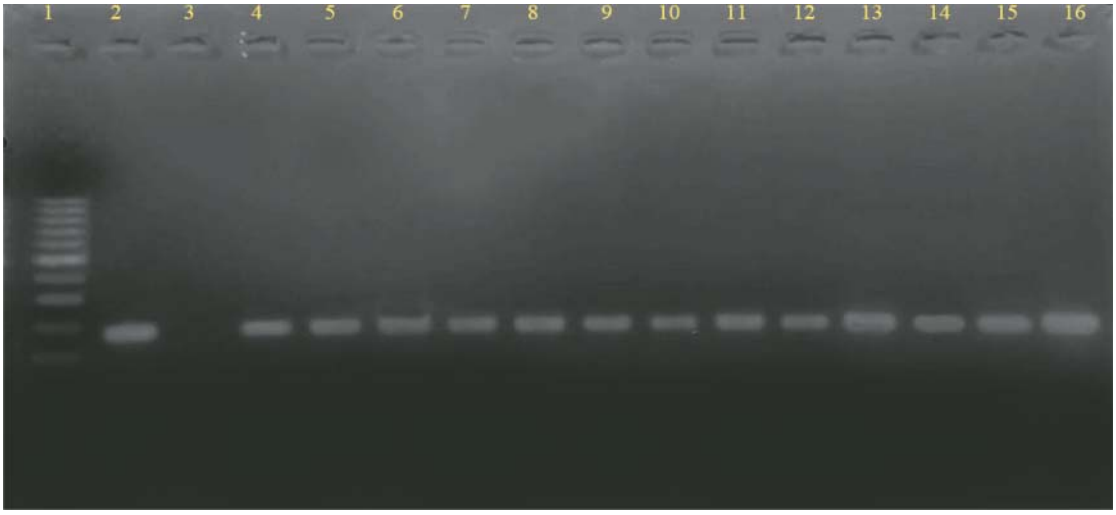

Figure 5: Confirmation of *mec*A gene by gel documentation of PCR products. Lane 1: 100bp DNA ladder, Lane 2: Positive control, Lane 3: Negative control, Lane 4-16: isolates with positively amplified *mec*A gene (163bp).

DISCUSSION

Healthcare workers (HCWs) are one of the major reservoirs of *S. aureus* as they often serve as the interface between hospitals and communities. Therefore, HCWs are required to screened for carriage of pathogenic and resistant strains of *S. aureus* so that potential nosocomial and community acquired infections and can be prevented and the chain of transmission can break at the earliest. As MRSA has emerged as a serious public health problem, screening of HCWs allows appropriate management of the colonized staff members. In this study, nearly one fifth of the HCWs were colonized with the commensal, *S. aureus*. Of the positive individuals, one-third (34.2%) of the isolated bacteria were the strains of MRSA—all of those isolates harbored the *mecA* gene.

Some previous studies have reported the prevalence of *S. aureus* and that of MRSA among the HCWs as 23.7% and 4.6% respectively (Albrich and Harbarth 2008; Khatri et al. 2017). In this study, the overall prevalence was slightly higher than the average rate which may be due to negligence on infection control guidelines, safety and sanitation measures among them. Moreover, nasal carriage rate of *S. aureus* and MRSA among the HCWs

was found to be 12.8% (16/125) and 6.4% (8/125) respectively. Similarly, the carriage rates of *S. aureus* and MRSA in the hands of HCWs were 17.6% (22/125) and 4% (5/125) respectively. The findings of our study are in line with the previous study by Khatri et al. (2017), in which the nasal carriage rates of *S. aureus* and MRSA were 18.3% and 7.5% respectively. Also, 72.7% of *S. aureus* colonization was reported by Lama et al. (2017), 15.7% by Khanal et al. (2015), 20.37% by Sah et al. (2013), 25% by Shakya et al. (2010). The nasal carriage rate of *S. aureus* in this study was lower than the studies conducted elsewhere in Nepal. Prevalence of *S. aureus* in this study when compared internationally also shows variation. A study conducted by Shibabaw et al. (2013) from Northeast Ethiopia and Abimana et al. (2019) from Central Uganda reported the rate of 28.8% from each of the studies. Lower prevalence than our study was reported from Kenya with 18.3%; Zambia (17.1%), Kuwait (21%) and India (21.4%) (Omuse et al. 2012). Higher prevalence was from Iran (31%), Gaza (31%), Germany (33.8%), Chile (34.9%), Libya (39%), Central Uganda (41.9%), Tanzania (41.4%) and Nigeria (64%) (Shibabaw et al. 2013; Abimana et al. 2019; El Aila et al. 2017). Similarly, nasal MRSA carriage rate was also lower than the findings by Shakya et al. (2010) with 10% and Khatri et al. (2017) with 7.5% but was higher than those reported by Shrestha et al. (2009) with 2.3% and Khanal et al. (2015) with 3.4% from Nepal. MRSA prevalence in present study is higher than some overseas findings from Iran (5.3%) and Zambia (5.7%) but lower than Ethiopia (12.7%), Libya (19%), Egypt (13.5%), and Gaza (25.5%) (Chakolwa et al. 2019). These differences in the prevalence of *S. aureus* and its strains between countries and hospitals are probably due to differences in the quality and size of samples, variation in sampling techniques, microbiological procedures, different interpretation guidelines, local infection control standards and the local prevalence of MRSA. Moreover, different levels of commitment while performing laboratory works by the investigator also contribute to these differences (El Aila et al. 2017, Chakolwa et al. 2019).

Socio-demographic characteristics including age, gender, profession, length of healthcare services, and services in different departments within the same hospital have been reported to influence the carriage of *S. aureus* (Kandel et al. 2020). Also in this study, significant differences in the prevalence of *S. aureus* among HCWs have been well documented with the variation in such characteristics listed in the afore-mentioned sentence.

In the antimicrobial susceptibility assay, most of the isolates were susceptible to carbapenems and gentamicin. Higher susceptibility of *S. aureus* isolates towards these antibiotics was also reported in previous studies (Thapa et al. 2020; Kandel et al. 2020; Sah et al. 2013) and augmented resistance to gentamicin was also reported in another studies (Thulunga et al. 2015). Similarly, AST of MRSA isolates showed resistance to most of the antibiotics used except carbapenems, vancomycin and gentamicin. Our findings resonate well with some of other findings reported earlier (Thapa et al. 2020; Kandel et al. 2020; Sah et al. 2013; Belbase et al. 2017; Rijal et al. 2008). However, in another study, ciprofloxacin was effective against MRSA isolates (Shrestha 2013).

Furthermore, this study revealed nearly one-third (31%) of *S. aureus* isolates as inducible-clindamycin resistance which could be easily misidentified as clindamycin susceptible in Kirby-Bauer disk diffusion method. Therefore, D-test should be routinely performed to all the *S. aureus* isolates in clinical microbiology laboratory to guide clinicians for appropriate use of clindamycin. The prevalence of inducible-clindamycin resistance among *S. aureus* observed in this study is higher than the prevalence reported by many other researches done in Nepal as well as done internationally. From Nepal, Adhikari et al. (2017) reported 11.48%, 12.4% by Ansari et al. (2016), 12.1% by Sah et al. (2013), 3% by Mishra et al. (2013), 28.7% by Kumari et al. (2008). Varying prevalence rates of inducible-clindamycin resistance have been reported from worldwide.

mecA gene responsible for conferring the drug-resistant to the MRSA and MDR isolates of S. aureus. Detection of *m*ecA gene serves as an evidence of the presence of MRSA among entire *S. aureus* isolates. This statement has been further reinforced by various findings from Sudan (Maimona et al. 2014), Saudi Arabia (Meshref and Omer, 2011), Iraq (Al-Zu'bi et al. 2004), Japan (Hotta et al. 1999), India (Mehndiratta et al. 2009), Australia (Cloney et al. 1999) and USA (Murakami et al. 1991). In comparison to those studies, burden of *mecA* was similar in our study as all of the MRSA isolates were tested positive for the gene. This could be due to the increased awareness, augmented infection controls

in the health facility under the study. However, other intrinsic factors also need to be assessed in future studies which might have been competing and inhibiting the expression of *mecA* in MRSA isolates in large number of study findings (Kandel et al. 2020). Globally, a number of studies have documented the absence of *mecA* in MRSA isolates (Aziz et al. 2014).

A study from Nigeria reported the absence of 5 major SCCmec types, gene products of PBP2 and mecA genes in phenotypically confirmed MRSA isolates. This finding informs the existing of other intrinsic factors such as the probability of hyper-production of β-lactams, responsible for conferring the resistance (Olayinka et al. 2009). There can also be the possibility of the alterations in various amino acids in protein binding proteins cascade (PBPs 1, 2 and 3). Such alterations have been reported to cause 3 amino acid substitutions (with identical or different amino acids) in all variants of PBP (Ba X et al. 2014). Hence, the existence of several other intrinsic factors aside for mecA suggests that detection of the gene alone cannot assure the detection of resistance. This important point needs to be pondered by the regional and reference laboratories while formulating and implementing the policy. Moreover, a more strict policy should be considered to discourage the over-the-counter (OTC) use of drugs and irrational prescriptions and use of antibiotics among HCWs and patients. Furthermore, HCWs, irrespective of the absence of clinical complaints are advised to be routinely screened for the potential carriage and transfer of the pathogenic strains of the bacteria.

CONCLUSION

More than one-fifth of the HCWs were colonized with *S. aureus* while one in ten isolates were MRSA and all of the MRSA tested positive for *mecA* gene in this study. The findings in this study reinforce the need for more commitment towards infection control measures that meet the standard protocols and aims at reducing the spread of infection by MRSA among susceptible individuals. Augmentation of diagnostic facilities along with antimicrobial stewardships can be recommended to combat the burgeoning spread of resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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