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 *mecA* 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: isoa myl 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).

**Date of Submission:** August 16, 2020  
**Date of Acceptance:** October 5, 2020  
**Published Online:** December, 2020  
**DOI:** https://doi.org/10.3126/tujm.v7i0.33786
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 \textit{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, \textit{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 \textit{S. aureus} strain was detected in 1942. Methicillin, a semi synthetic penicillin was then developed in the late 1950s to treat penicillin resistant \textit{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, \textit{mecA}) 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 \textit{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 \textit{S. epidermidis} (MRSE), vancomycin resistant \textit{Staphylococcus aureus} (VRSA) and some strains of \textit{Pseudomonas} spp, \textit{Haemophilus} spp, \textit{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 \textit{mecA} 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 \textit{mecA} 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°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 meca 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 PEI 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, extension 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.

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 1: Distribution of bacterial growth
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 2: Antibiotic resistance pattern of S. aureus isolates

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

<table>
<thead>
<tr>
<th>Age of health care workers</th>
<th>Total no. of samples N</th>
<th>Gender of the health care workers</th>
<th>Male (n=24)</th>
<th>Female (n=101)</th>
<th>Total MRSA</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-25</td>
<td>74</td>
<td>S. aureus</td>
<td>0</td>
<td>0</td>
<td>6 (8.1)</td>
<td>3 (4.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRSA</td>
<td>3 (2.03)</td>
<td>1 (0.7)</td>
<td>23 (16)</td>
<td>7 (4.9)</td>
</tr>
<tr>
<td>26-50</td>
<td>144</td>
<td>S. aureus</td>
<td>2 (6.3)</td>
<td>1 (3.1)</td>
<td>4 (12.5)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRSA</td>
<td></td>
<td></td>
<td></td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>Above 50</td>
<td>32</td>
<td>S. aureus</td>
<td></td>
<td></td>
<td>13 (34.2)</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRSA</td>
<td></td>
<td></td>
<td></td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>S. aureus</td>
<td>5</td>
<td>2</td>
<td>33 (13)</td>
<td>11 (5.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MRSA</td>
<td></td>
<td></td>
<td></td>
<td>13 (5.2)</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Department of duty</th>
<th>Total no. of samples N</th>
<th>No. of S. aureus N (%)</th>
<th>MRSA N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergency</td>
<td>18</td>
<td>3(16.7 %)</td>
<td>1(5.6 %)</td>
</tr>
<tr>
<td>OPD</td>
<td>36</td>
<td>4 (11.1 %)</td>
<td>0</td>
</tr>
<tr>
<td>Ward (Gastro + Male + Cabin)</td>
<td>72</td>
<td>11(15.3 %)</td>
<td>5(6.9 %)</td>
</tr>
<tr>
<td>ICU</td>
<td>6</td>
<td>1 (16.7 %)</td>
<td>1(16.7 %)</td>
</tr>
<tr>
<td>ART Department</td>
<td>26</td>
<td>1 (3.8 %)</td>
<td>0</td>
</tr>
<tr>
<td>Laboratory</td>
<td>64</td>
<td>13 (20.3 %)</td>
<td>4(6.3 %)</td>
</tr>
<tr>
<td>Pharmacy</td>
<td>6</td>
<td>2 (33.3 %)</td>
<td>1(16.7 %)</td>
</tr>
<tr>
<td>Immunization</td>
<td>10</td>
<td>1 (10 %)</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>2 (16.7 %)</td>
<td>1(8.3 %)</td>
</tr>
</tbody>
</table>
Distribution of MRSA and MSSA on the basis of duration of employment

The 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) of *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, amoxyccillin, 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 amoxyccillin 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 pattern of MSSA and MRSA

<table>
<thead>
<tr>
<th>Antibiotic disc</th>
<th>MSSA (N=25), n (%)</th>
<th>MRSA (N=13), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive (S) N (%)</td>
<td>Resistant (R) N (%)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1 (4%) 24 (96%)</td>
<td>0</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>25 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Amoxyccillin</td>
<td>10 (40%) 15 (60%)</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>11 (44%) 14 (56%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>23 (92%) 2 (8%)</td>
<td>12 (92.3%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>25 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20 (80%) 5 (20%)</td>
<td>12 (92.3%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>14 (56%) 11 (44%)</td>
<td>6 (46.2%)</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>24 (96%) 1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>24 (96%) 1 (4%)</td>
<td>11 (84.6%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>24 (96%) 1 (4%)</td>
<td>11 (84.6%)</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>No. of S. aureus</th>
<th>MRSA N (%)</th>
<th>MDR N (%)</th>
<th>MDR isolates excluding MRSA, N (%)</th>
<th>D-test Positive N (%)</th>
<th>MRSA with Positive D-test, N (%)</th>
<th>MSSA with Positive D-test, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>13 (34.2%)</td>
<td>24 (63.2%)</td>
<td>11 (29%)</td>
<td>12 (31.6%)</td>
<td>7 (18.4%)</td>
<td>5 (13.2%)</td>
</tr>
</tbody>
</table>

Prevalence of meca gene among MRSA isolates

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

Table 6: Detection of meca gene in MRSA

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of MRSA isolates N</th>
<th>No. of meca positive MRSA N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal swab</td>
<td>8</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Hand swab</td>
<td>5</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

Figure 5: Confirmation of meca 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 meca 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 Shafaya 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 Shayka 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 aforementioned 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 *meca* 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 \( \text{mecA} \) in MRSA isolates in large number of study findings (Kandel et al. 2020). Globally, a number of studies have documented the absence of \( \text{mecA} \) in MRSA isolates (Aziz et al. 2014).

A study from Nigeria reported the absence of 5 major SCCmec types, gene products of PBP2 and \( \text{mecA} \) genes in phenotypically confirmed MRSA isolates. This finding informs the existence of other intrinsic factors such as the probability of hyper-production of \( \beta \)-lactams, responsible for conferring the resistance (Olayinka et al. 2009). Hence, the existence of several other intrinsic factors aside for \( \text{mecA} \) 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 \( \text{mecA} \) 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.

**ACKNOWLEDGEMENTS**

The authors acknowledge the concerned persons at Sukraraj Tropical and Infectious Disease Hospital (STIDH) and Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal for their contributions.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

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