DETECTION OF *MECA* GENE IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM HEALTH CARE WORKERS AT A TERTIARY CARE CENTER IN KATHMANDU

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ABSTRACT

Staphylococcus aureus is a frequent cause of bacterial infections ranging from minor skin infections to fatal necrotizing infections and infections are on the rise particularly the hospital-acquired infections. The infections becomes more complicated when the infection are caused by drug (methicillin) resisant *S. aureus*. Therefore, detection of methicillin-resistant *S. aureus* (MRSA) becomes important. In this study, thus, we investigated the mecA gene in 53 *S. aureus* isolates isolated from health care workers (n= 213). Of the total 53 *S. aureus* isolates tested in this study, 24 (45.3%) were positive for *mecA* gene (533bp). Present study showed a relatively higher rate *mecA* gene positivity.

KEYWORDS

S. aureus, *mecA* gene, health care workers, Nepal.

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INTRODUCTION

Staphylococcus aureus is a frequent cause of bacterial infections ranging from minor skin infections to fatal necrotizing infections.[1] Although, it is naturally susceptible to almost every antibiotic developed so far, it is an adaptable pathogen with remarkable ability to acquire resistance.[2,3] Methicillin-resistant *S. aureus* (MRSA) was reported first in 1961 by Jevons.[4] In Nepal, first cases of MRSA among patients, staffs and hospital environment was reported by Rai *et al*[5] in 1990. Since then many reports on MRSA have been published from Nepal, and as reviewed by Khanal *et al*,[6] over 25 reports are available only after 2008 with the prevalence of MRSA ragning from 14.6% to 72.4%.

MRSA can cause wide range of infections such as impetigo, boils, cellulitis, folliculitis, abscesses including rarer but more severe infections like necrotizing pneumonia, surgical site infection, necrotizing fasciitis, bacteremia with possible complications of endocarditis or severe sepsis.[3,7] Such severe infections demand complicated medical care, and even then, high morbidity and mortality are reported.[3,8] MRSA is the most commonly identified antimicrobial-resistant pathogen in most parts of the world.[9]

As reviewed by Dulon *et al*[10] the MRSA carriage rate among health care workers (HCWs) in non-outbreak settings in Europe and USA ranged 0.2% to 15.0%. However, in another review, by Pathare *et al*[11] the prevalence of MRSA among HCWs ranges in between 18.3% to 43.8%. The prevalence of MRSA among HCWs in Nepal ranged from 5.2% to 37.1%.[12-16] In South Asian (SAARC) countries, it is reported to be 9.2%[17] and 41.2% in north-western part of India[18] and Kurdistan region of Iraq.[19] Iyer *et al*[20] from Saudi Arabia and El Aila *et al*[21] from Gaza Strip have reported a very high incidence of MRSA among HCWs; 73.0% and 82.3%, respectively. On the other hand, the MRSA among the *S. aureus* isolates from different clinical sampels have been reported to be 14.6% to 72.4% in Nepal.[6] We, therefore, in this paper report the incidence of MRSA among HCWs as detected by *mecA* gene PCR.

MATERIALS AND METHODS

After obtaining the ethical approval from Institutional Review Committee of Nepal Medical College, Kathmandu (Ref. No. 014/2072-73), nasal and hand swabs were collected from 213 HCWs working at Nepal Medical College Teaching Hospital, Gokarneswor-8, Kathmandu (Nepal) in between January to June, 2018. The swabs were screened for the presence of *S. aureus* using mannitol salt agar (MSA) as selective media. Colony morphology, Gram stain, catalase and coagulase tests were done to identify the *S. aureus* isolates. Identified carriers were decolonized with 2% mupirocin nasal ointment and re-sampling was done every month for five months to assess re-colonization.[22] Altogether, 53 *S. aureus* were isolated and all isolates were subjected to detection of *mecA* gene to identify MRSA isolates.

DNA extraction from S. aureus: The isolates were swabbed on Mueller-Hinton agar. Standard vancomycin disk was placed over the inoculated area, followed by incubation at 37ºC for overnight. The vancomycin disk weakens the thick cell wall of *S. aureus*. The weakened bacterial cell wall was then rapidly lysed simply by heating. The bacterial colonies from the edges of the inhibition zone was re-suspended in sterile distilled water and matched to 0.5 McFarland standard (approximately 10^8 cfu/ml). This bacterial suspension was heated at 95ºC for 15 min and cooled at room temperature. The crude lysate was used as a DNA template for all isolates during PCR.

PCR protocol: For PCR, a mixture of distilled water 7.8 µl, KOD One master mix (TOYOBO, Japan) 10 µl, primers (Forward, 5'-AAAATCGATGGTAAAGGTTGGC-3' and Reverse, 5'-AGTTCTGGAGTACCGGATTTGC-3') 0.6 µl (0.3 µM) each and crude lysate 1 µl was prepared. PCR cycle consisted of stage 1 at 95ºC for 1 minute, stage 2 with 33 cycles of denaturation at 98 ºC for 10 seconds, annealing at 55ºC for 5 seconds followed by extension at 68ºC for 5 seconds, and stage 3 at 68ºC for 1 minute. The final products were subjected to 1% agarose gel electrophoresis with Midori Green as nucleic acid stain and interpretation was done. Quality control was maintained using *S. aureus* ATCC 33591 (*mecA*-positive) and *S. aureus* ATCC 25923 (*mecA*-negative).

RESULTS

Among the 213 participants (HCWs) 39 (18.3%) were identified as *S. aureus* carriers, i.e. 35 were nasal carriers, four were both nasal and hand carriers. After successful decolonization with 2% mupirocin nasal ointment twice a day for five days, 10 of the nasal carriers were recolonized. In total, 53 isolates were obtained out of which 24 (45.3%) were *mecA* gene positive (MRSA isolates) (Table-1). Of the nose and hands, nose was colonized *S. aureus* more (n=49) than hands (n=4) and among the *S. aureus*, MRSA among nasal isolates was 48.9% (24/49) whereas none (0/4) in hand isoaltes (p=0.058).

Table 1: Distribution of *S. aureus* isolates with *mecA* gene among the site of carriage				
Site of carriage	***S. aureus isolates* (n)**	***S. aureus* with *mecA* gene (n)**	**%**	**P-value**
Nose	49	24	48.9	
Hands	4	0	0.0	0.058
Total	**53**	**24**	**45.3**	

Of the total 53 *S. aureus* isolates, 43 were isolated from carriers and 10 from re-colonized carrier (after successful treatment with mupirocin). Out of 43 cariers, 55.8% (24/43) were MRSA as detected by *mecA* gene. Interestingly, none (0/10) of the re-colonized carrier were MRSA (p=0.001) (Table-2).

Table 2: Distribution of *S. aureus* isolates with *mecA* gene among the type of carrier				
Type of carrier	***S. aureus isolates* (n)**	***S. aureus* with *mecA* gene (n)**	**%**	**P-value**
Carrier	43	24	55.8	
Re-colonized carrier	10	0	0.0	0.001
Total	**53**	**24**	**45.3**	

Fig. 1 shows the agar gel electrophoresis results of *mecA* gene (533 bp) PCR product bands (Lane A: molecular markers in on the left, Lane B, F, K and L: *mecA* gene negative, Lane C, D, E, G, H, I and J: *mecA* gene positive, Lane M: *mecA* gene negative control and Lane N: *mecA* gene positive control). Of the total 53 *S. aureus* isolates isolated from HCWs (cariers n=43 and re-colonized carrier n=10), 24 isolates were found to be positive for *mecA* gene and all there *mecA* gene positive isolates were isolated carriers (and not from re-colonised carriers).

DISCUSSION

Among the 213 participants included in this study, 39 (18.3%) were identified as *S. aureus* carriers (35 were nasal carriers, 4 were both nasal and hand carriers). Reprtedly, the nasal carriage rate of *S. aurues* among HCWs range from 20.4-43.8%.[23] Relatively, higher prevalence nasal carrier rate of *S. aureus* (41.3%) among HCWs in Kathamndu has also been reorted.[8] Elsewhere, Lin *et al*[24] have reported a very high (67.2%) carrier rate of *S. aureus* among HCWs. In this study, however, little lower nasal carrier rate has been found. Giri *et al*[15] have reported nasal carriage MRSA of 5.2% among HCWs in Kathmandu. After successful decolonization with 2% mupirocin nasal ointment twice a day for five days,[22] 10 of the nasal carriers were re-colonized after 3 months. This occurs as a result of hiding *S. aureus* in the posterior vestibula where mupirocin can hardly reached.[25]

In this study, thus a total of 53 *S. aureus* isolates obtained from 39 HCWs were subjected for *mecA* gene (MRSA) detection. Of the *S. aureus* 53 isolates, 24 (45.3%) were *mecA* gene positive. Similar positive rate of *mecA* gene has also been reported by Khanal *et al*[26] in clinical MRSA positive *S. aureus* isolates from Nepal. The overall prevalence of MRSA with or without detection of *mecA* gene have been reported to be ranged from 14.6% to 72.4%.[6] Hussein *et al*[19] has reported the *mecA* gene positive in 50.4% of *S. aureus* isolated from hospital staffs in Kurdistan region of Iraq. Sililalry, Khan *et al*[18] have also found that 41.2% of *S. aureus* isolates isolated from hospital personnels

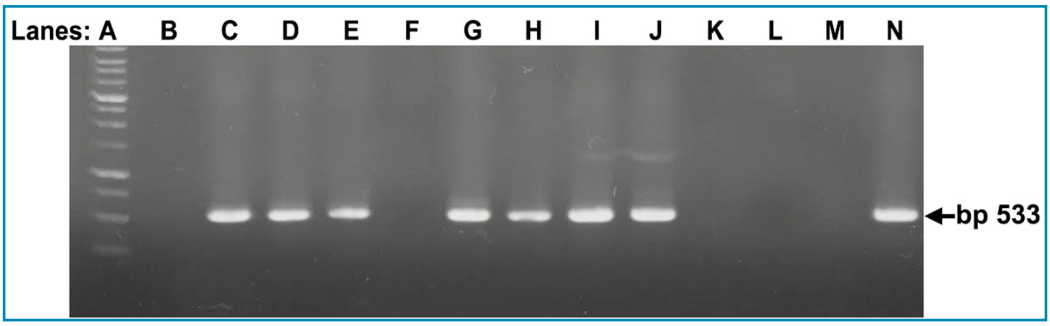

Fig. 1: Bands of molecular markers and *mecA* gene of *S. aureus* isolated from HCWs and *mecA* gene positive/negative controls in agar gel electrophoresis of PCR procucts together with negative and positive controls (**Lane A**: molecular markers in on the left, **Lane B, F, K** and **L**: *mecA* gene negative, **Lane C, D, E, G, H, I** and **J**: *mecA* gene positive, **Lane M**: *mecA* gene negative control and **Lane N**: *mecA* gene positive control)

in North-West part of India. Almost similar positive rate (37.1%; 13/35) *mecA* gene positive has bee reported by Shah *et al*[16] among HCWs from Nepal recently. Nearly similar findings (36.2%) has been reported by Salmanov *et al*[27] from Ukraine. Adhikari *et al*[28] have reported the *mecA* gene in 29.1% of *S. aureus* isolated from patients in Nepal. Dhungel *et al*,[29] Nepal *et al*[30] and Shrestha *et al*[31] from Nepal have also reported a positive rate of *mecA* gene in *S. aureus* isolated from clinical samples in 82.1%, 84.1% and 75.7%, respectively. Kandel *et al*[32] have also reported the *mecA* gene positive rate of 72.2%, however, in MRSA isolated from clinical samples in Nepal. Earlier, El Aila *et al*[21] have also reported very high rate (82.3%; 51/62) of MRSA (as detected by *mecA* gene) among the *S. aureus* isolates isolated from HCWs in Gaza Strip. Reportedly, in eastern Nepal, *mecA* gene was detected in 71.1% of the total *Staphylococcus* isolates.[33] Bhatta *et al*[34] from western Nepal have reported a 56.8% prevalence of *mecA* gene in *S. aureus* isolates.

However, on the otherhand, low prevalence (27.1%) of *mecA* gene positivity has been reported in *S. aureus* isolated from nasal samples of HCWs in Nepal.[35] In Egypt, Allam *et al*[36] have reported 27.6% of MRSA isolates among HCWs working at a tertiary care hospital. Khanal *et al*[13] reported a 21.9% of MRSA among 32 *S. aureus* isolated from health care workers in western Nepal. On the otherhand, Shokravi *et al* (2020)[37] from Iran have reported *mecA* gene in 38.0% of coagulase-negative *Staphylococcus* (CONS) indicating the importance of methicillin-resistant CONS.

The *mecA* gene positive *S. aureus* is on the rise in the HCWs and well as in the community. Therefore, keeping in view of wide range of infections caused by *S. aureus*, it is necessary to take all precaution to prevent the infections in health care center as well as in the community as a whole.

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