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Emergence of staphylococcal cassette chromosome *mec* type I with high-level mupirocin resistance among methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Objective: To investigate the molecular epidemiology and antimicrobial resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthcare workers and patients.

Methods: MRSA isolates were recovered from nasal swabs collected at a tertiary care hospital of Nepal and confirmed on the basis of Gram staining, conventional biochemical tests, and PCR amplification of *mecA* gene. PCRs were also used for detection of the different resistance genes and staphylococcal cassette chromosome (SCC) *mec* types. Antibiotic susceptibility patterns of isolates were assessed by disc diffusion method and minimum inhibitory concentrations were determined by E-test.

Results: A total of 29 MRSA were isolated from 536 nasal swabs (5.4%) of health care workers and patients at a tertiary care hospital in Nepal. All isolates were susceptible to amikacin, gentamicin, vancomycin (minimal inhibitory concentrations < 2 μg/mL), tigecycline, tetracycline, nitrofurantoin, rifampicin, quinupristin-dalfopristin, and linezolid. Among the 29 MRSA isolates, resistance to erythromycin (72%), ciprofloxacin (75%), co-trimoxazole (62%), clindamycin (10%), and chloramphenicol (10%) was found, and fifteen isolates (51%) exhibited high-level mupirocin resistance (minimal inhibitory concentrations > 1 024 μg/mL). Fourteen isolates were found harboring the *mupA* gene and one isolate was found carrying the novel *mupB* gene. High prevalence (68%) of SCC*mec* I type was found, followed by SCC*mec* V (13%) and SCC*mec* III (3%) among all the MRSA isolates.

Conclusions: We found the emergence of SCC*mec* type I with high-level mupirocin resistance among MRSA in Nepal. Data also suggest that MRSA SCC*mec* type V strain has spread from the community to the hospital.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a major pathogen that causes nosocomial and community-acquired infections. Therapeutic options for these infections have been reduced due to increased resistance to many classes of antimicrobial agents [1]. In clinical settings, methicillin-resistant *S. aureus* (MRSA) was one of the most challenging pathogens to treat because of its resistance to many antibiotic classes. Normally, penicillin-binding proteins of the *S. aureus* cell wall have a high affinity for β-lactam antibiotics. In MRSA, the penicillin-binding protein 2a encoded by the *mecA*

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The study protocol was performed according to the Helsinki declaration and approved by the Nepal Health Research Council. Written informed consent was obtained from all study participants before specimen collection or interview.

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gene contained within the staphylococcal cassette chromosome (SCC) reduces this affinity, resulting in methicillin resistance. SCC*mec* typing is a method available for the molecular epidemiological study of MRSA [2]. There are altogether 11 major SCC*mec* types, some of which are further classified into subtypes. The majority of hospital-acquired MRSA (HA-MRSA) carry SCC*mec* types I, II, and III, whereas community-acquired MRSA (CA-MRSA) usually carry unique SCC*mec* types IV and V [3,4]. Mupirocin is approved for nasal decolonisation of MRSA. It inhibits isoleucyl-tRNA synthetase necessary for bacterial protein synthesis. High-level mupirocin resistance (HMR) is defined as an minimal inhibitory concentration (MIC) 512 μg/mL and low-level mupirocin resistance (LMR) as an MIC between 8 and 256 μg/mL [5]. HMR is mediated by the acquisition of plasmid carrying *mupA* gene and LMR is due to a point mutation of the chromosomal encoded native *ileS-1* gene [6,7]. Increased incidence of mupirocin resistance in MRSA has been reported. Also, decolonization treatment with mupirocin has been found to be a risk factor in the development of MRSA [8,9]. Health care workers and patients may serve as vectors of MRSA by cross-transmission as they are at the interface between hospital and community. Although the prevalence of *S. aureus* nasal carriage has been reported from Nepal [10], the molecular analysis of SCC*mec* types and mupirocin resistance in MRSA is still unclear. We investigated the molecular characteristics and antimicrobial resistance patterns of MRSA strains isolated from health care workers and patients.

2. Materials and methods

2.1. Population studied

A cross-sectional study was carried out among health care workers and patients at a 100-bed tertiary care hospital in Kathmandu in Nepal, between October 2014 and April 2015. Nasal swabs from 427 patients [164 from intensive care unit (ICU) and 263 from wards] and 109 health care workers were collected. Health care workers from different departments participated as follows: nurses ($n = 44$), ward attendants ($n = 28$), doctors ($n = 23$) and laboratory workers ($n = 14$). The study protocol was approved by the Nepal Health Research Council. Written informed consent was obtained from all study participants before specimen collection or interview.

2.2. Bacterial isolation and identification

Non-duplicate nasal swabs were collected from study participants using a sterile screw capped cotton wool swab (Hi-Media, India). *S. aureus* was identified based on Gram staining, catalase test, coagulase test and confirmed by detection of the organism specific 16s rRNA gene and *femA* gene [11,12]. MRSA isolates were identified by growing on mannitol salt agar containing 4 μg/mL of oxacillin and cefoxitin (30 μg) (Hi-Media, India). MRSA isolates were confirmed by the detection of the *mecA* gene [13].

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by a disc diffusion test on Mueller-Hinton agar in accordance with the Clinical and Laboratory Standard Institute [14]. The antibiotics tested included teicoplanin (30 μg), gentamicin (10 μg), amikacin (30 μg), erythromycin (15 μg), ciprofloxacin (5 μg), tetracycline (30 μg), tigecycline (30 μg), clindamycin (2 μg), co-trimoxazole (1.25/23.75 μg), nitrofurantoin (300 μg), chloramphenicol (30 μg), rifampin (5 μg), quinupristin-dalfopristin (15 μg) and linezolid (30 μg) (Hi-Media, India). The MICs of oxacillin, vancomycin, and mupirocin (Hi-Media, India) were determined by E-test. MRSA COL strain, *Enterococcus faecalis* ATCC 29212 and transconjugant *S. aureus* containing the pMG1 plasmid were used as positive controls for oxacillin, vancomycin and mupirocin resistance, respectively. Inducible clindamycin resistance was detected for those isolates that were susceptible to clindamycin and resistant to erythromycin, by the double disc diffusion test (D-test) [14].

2.4. PCR amplification of ileS-1, mupA, and mupB genes

PCR assays were used to detect mupirocin resistant genes (*mupA* and *mupB*) and mutations in the *ileS-1* gene as described previously [15]. All primers and temperatures used to detect these genes are shown in Table 1. PCR products were analyzed by electrophoresis in 2% agarose gel containing 0.3 μg/mL ethidium bromides.

Table 1
PCR primers and thermal cycling parameters for genes detected in this study.

Gene	Primer/sequence	Temperature (°C)	PCR size (bp)	Ref
16s-rRNA	F: CGAAAGCCT GACGGAGCAA R: AACCTTGCG GTCGTACTCCC	57	597	[11]
femA	F: CTTACTTACTG GCTGTACCTG R: ATGTCGCTTGT TATGTGC	59	686	[12]
mecA	F: AAAATCGATGG TAAAGGTTGGC R: AGTTCTGGAG TACCGGATTTGC	53	533	[13]
ileS-1	F: ATAAAGGTAAAA AGCCAGTTTATTGGT R: CAACATACTC CAATTCCTTAC	55	360	[15]
mupA	F: TATATTATGCG ATGGAAGGTTGG R: AATAAAATCAGC TGGAAAGTGTTG	57	457	[15]
mupB	F: CTAGAAGTCGAT TTTGGAGTAG R: AGTGTCTAAAATGA TAAGACGATC	55	674	[15]

2.5. SCCmec typing

All *mecA* positive MRSA strains were subjected for multiplex PCR assay for SCC*mec* typing (type I–type V) as reported previously [16]. Control strains used included NCTC 10422 (SCC*mec* type I), clinical isolates from Thailand (SCC*mec* type II), JCSC 10442 (SCC*mec* type IV) and WIS (SCC*mec* type V).

3. Results

3.1. Demographic characteristic and prevalence of methicillin-sensitive S. aureus (MSSA) and MRSA nasal carriage

One hundred and thirty-five *S. aureus* isolates were recovered from the anterior nares of 536 study participants. We found 106 of 538 health care workers and patients (19.8%) harboring MSSA and 29 subjects (5.4%) were MRSA carriers (Table 2). MRSA nasal carriage rate among 13.6% of nurses and 6.4% of ward patients were found in this study. Low rate of MRSA nasal carriage were found in doctor, ward attendants, laboratory workers and patients from ICU (Table 2).

Table 2
Prevalence of MSSA and MRSA among health care workers and patients [*n* (%)].

Subjects	Number	MRSA	MSSA	*S. aureus*
Nurse	44	6 (13.6)	12 (27.3)	18 (40.9)
Doctor	23	0 (0.0)	8 (34.8)	8 (34.8)
Laboratory workers	14	0 (0.0)	6 (42.9)	6 (42.9)
Ward attendants	28	2 (7.1)	13 (46.4)	15 (53.6)
ICU patients	164	4 (2.4)	24 (14.6)	28 (17.1)
Ward patients	263	17 (6.4)	43 (16.3)	60 (22.8)
Total	536	29 (5.4)	106 (19.8)	135 (25.2)

3.2. Antibiotic susceptibility

Antibiotic susceptibility patterns, SCC*mec* types and resistant genes of MRSA are shown in Table 3. MRSA isolates were resistant to erythromycin (*n* = 21; 72%), ciprofloxacin (*n* = 22; 75%), co-trimoxazole (*n* = 18; 62%), clindamycin (*n* = 3; 10%) and chloramphenicol (*n* = 3; 10%). Fifteen isolates were resistant to high-level mupirocin (51% MICs > 1 024 μg/mL). All 29 strains were fully susceptible to amikacin, gentamicin, vancomycin (MICs < 2 μg/mL), tigecycline, tetracycline, nitrofurantoin, rifampicin, quinupristin-dalfopristin and linezolid. Among 12 isolates tested, 7 isolates also exhibited inducible clindamycin resistance patterns.

3.3. SCCmec types and mupirocin resistant genes of MRSA

Amplified product with 533 bp of *mecA* gene was detected in all 29 MRSA isolates. Three different profiles were identified by SCC*mec* typing: SCC*mec* type I (*n* = 20; 68%), SCC*mec* type III (*n* = 1; 3%) and SCC*mec* type V (*n* = 4; 13%). Four (*n* = 4) strains were non-typeable. The *femA* gene was detected in all isolates. Mupirocin resistant strains harboring *mupA* gene were found in 14 isolates and *mupB* gene positive strain was found in 1 isolate. Mutation in *ileS-1* gene conferring LMR was not detected in all strains.

Table 3
Antibiotic susceptibility patterns, SCC*mec* types and resistance genes of MRSA.

Isolate	SCC*mec* type	Resistance patterns	Mupirocin resistance genes			*femA*	*mecA*
			ileS-1	*mupA*	*mupB*		
MRSA-1	NT	E/CIP/MU/CD	–	+	–	+	+
MRSA-2	NT	E/CIP/COT/MU/CD	–	+	–	+	+
MRSA-3	I	E/CIP/COT/MU	–	+	–	+	+
MRSA-4	I	E/CIP/COT/MU/CD	–	+	–	+	+
MRSA-5	I	E/CIP/COT/MU	–	+	–	+	+
MRSA-6	I	CIP/COT	–	–	–	+	+
MRSA-7	I	E/CIP/MU	–	+	–	+	+
MRSA-8	V	E/CIP	–	–	–	+	+
MRSA-9	NT	E/CIP	–	–	–	+	+
MRSA-10	NT	E/CIP/COT	–	–	–	+	+
MRSA-11	I	COT	–	–	–	+	+
MRSA-12	I	E/CIP/COT/C/MU	–	+	–	+	+
MRSA-13	V	CIP/COT	–	–	–	+	+
MRSA-14	I	CIP/MU	–	+	–	+	+
MRSA-15	III	E/CIP/COT/C	–	–	–	+	+
MRSA-16	V	CIP/MU	–	+	–	+	+
MRSA-17	I	E/COT	–	–	–	+	+
MRSA-18	I	CIP/MU	–	+	–	+	+
MRSA-19	I	E/CIP/C/MU/CD	–	+	–	+	+
MRSA-20	I	E/CIP/COT	–	–	–	+	+
MRSA-21	V	E	–	–	–	+	+
MRSA-22	I	E	–	–	–	+	+
MRSA-23	I	E/COT/MU	–	+	–	+	+
MRSA-24	I	E/COT/MU	–	+	–	+	+
MRSA-25	I	E/CIP/MU	–	+	–	+	+
MRSA-26	I	CIP/COT/MU	–	–	+	+	+
MRSA-27	I	E/COT	–	–	–	+	+
MRSA-28	I	CIP/COT	–	–	–	+	+
MRSA-29	I	E/CIP/COT	–	–	–	+	+

NT: Non-typeable; E: Erythromycin; CIP: Ciprofloxacin; COT: Co-trimoxazole; MU: Mupirocin; CD: Clindamycin; +: Positive; –: Negative.

4. Discussion

A wide range of MRSA nasal carriage among health care workers (7.60%–43.58%) and patients (2.30%–11.8%) have been reported worldwide [17–20]. MRSA nasal carriage rate (5.40%) obtained from this study is in accordance with that previously reported from different hospitals in Nepal which was 2.3%–3.4% [10,18]. In agreement with previous studies, we found high prevalence of MRSA nasal carriage among nurses (13.6%) compare to other health care personals [18,21]. High prevalence of MRSA nasal carriage among nurses may be due to their regular contact with patients and hospital environments. This study is the first report of molecular analysis of SCC*mec* typing in MRSA from Nepal. The MRSA strains with different SCC*mec* types and subtypes are prevalent in different parts of the world. SCC*mec* element types I, II and III are main molecular marker of HA-MRSA and SCC*mec* type IV and V are molecular marker of CA-MRSA. However, in some study recently, SCC*mec* type IV has been associated with HA-MRSA and SCC*mec* type III with both HA- and CA-MRSA, which reflect the rapid spread of such pathogen in hospital and community environment [22]. The majority of MRSA isolates in our study were found to carry SCC*mec* type I. Accordingly, these data suggests most MRSA isolates in our study contracted HA-MRSA. The distribution of MRSA carried SCC*mec* III elements has been found predominant in Thailand, Korea, Vietnam, Japan, Taiwan, India, Philippines, Hong Kong, Iran and Sri Lanka; SCC*mec* II strains were found in Japan and Korea; and SCC*mec* I strains were found in Japan [23]. In some Asian countries, CA-MRSA strains with SCC*mec* types IV and V have spread from community to hospitals [24]. Four strains detected in our study was SCC*mec* type V that demonstrates the community origin of these strains. This finding suggests that CA-MRSA strains already have spread into Nepalese hospitals. However, the transmission of CA-MRSA to the hospital environment needs further investigation. Four isolates were non-typeable according to the multiplex PCR. These isolates may represent new or variant SCC*mec* types, and their SCC*mec* cassettes require further analysis. MRSA isolates susceptible to several antibiotics including glycopeptides represents their usefulness in treatment of MRSA infections. Mupirocin is broadly used to treat MRSA skin and soft tissue infections and to decolonize nasal MRSA. This study showed 86% of MRSA isolates were resistant to high-level mupirocin, which is the first reported case from Nepal. Similar results of high prevalence of mupirocin resistance among MRSA isolates were found in Malaysia and Iran [8,19]. Plasmid encoded *mupA* gene is a molecular marker of HMR in *S. aureus* [15]. We found high prevalence of mupirocin resistant MRSA carried *mupA* gene. This evidence was in agreement with previous report that HMR in MRSA is due to *mupA* gene [15]. We also found one MRSA isolate had *mupB* gene that conferred HMR. Increased resistance rates have been associated with increased mupirocin use, however, there is no report of mupirocin use for MRSA nasal decolonization among study subject. Data obtained from this study suggests the spread of mupirocin-resistant MRSA strains with variable genes in Nepalese hospitals. In order to determine the prevalence and understand risk factors and outcomes of *mupB* gene, further study will be needed.

In conclusion, we found MRSA containing SCC*mec* I element as an emerging pathogen in Nepalese hospital. SCC*mec* type V strain in this study suggested the spread of CA-MRSA into hospitals. Given the widespread endemicity of MRSA infections in most Nepalese hospital, further spread of mupirocin-resistant MRSA is anticipated. To understand the changing epidemiology of MRSA, continuous efforts are necessary for appropriate antimicrobial therapy and effective control of resistant clones.

Conflict of interest statement

We declare that we have no conflict of interest.

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