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Major Article

Methicillin-resistant *Staphylococcus aureus* nasal carriage between healthy students of medical and nonmedical universities

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Key Words:
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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a challenge for public health, and community-acquired (CA) infections seem to be increasing among people in different areas.
Methods: A total of 700 healthy student volunteers residing in dormitories of universities in Urmia, Iran, were enrolled in this study. After identification of the isolates, antibiotic susceptibility, presence of *mec*A and *pvl* genes, and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were evaluated.
Results: Nasal screening identified 137 (19.6%) carriers of *S aureus*, and 18 (13.14%) were MRSA isolates. The antimicrobial susceptibility patterns of isolates revealed high resistance to penicillin (93.4%). All isolates were sensitive to vancomycin. The SCC*mec* typing showed that most MRSA strains belonged to SCC*mec* type IV (n = 14; 77.8%). Only 1 (5.56%) MRSA isolates carried the *pvl* gene.
Conclusions: Our findings revealed the relatively high frequency of *S aureus* nasal carriers and the advent of multidrug resistance among these isolates. Most MRSA isolates were SCC*mec* type IV; the transfer of such MRSA strains from carriers to other individuals in crowded living conditions such as dormitories can act as a risk factor for outbreak of CA MRSA and is a serious threat for the study groups.

The excessive use and abuse of antibiotics has been increasing with the rapid of resistant strains; >20,000 potential resistance genes exist among bacterial genome.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious risk to patients in health care (HA) and a challenge for public health, and community-acquired (CA) infections seem to be increasing among people in different areas.[2] MRSA strains harbor the *mec*A gene, encoding penicillin-binding protein 2a, which is poorly acylated by β-lactam antibiotics[3] and is also carried on mobile genetic elements inserted into the staphylococcal chromosome, designated staphylococcal cassette chromosome *mec* (SCC*mec*) elements.[4] Of 12 different and known SCC*mec* types, types IV (mainly) and V have been found in CA MRSA strains.[5] These types tend to be less resistant but more transmissible. The first report of CA MRSA among children was reported in the United States in the late 1990s,[6] and after that, outbreaks of CA MRSA infections have been documented in correctional facilities, among athletic teams and military recruits, in newborn nurseries, and among homosexuality.[7,8] The presence of an association between virulence genes, such as the *pvl* gene encoding Panton-Valentine leukocidin toxin and *et* gene encoding exfoliative toxin, and emerging CA MRSA clones with specific clinical presentations has been demonstrated in some studies.[9] Because *S aureus* strains that reside in the anterior nares serve as a reservoir for future infection[10] via acquiring antimicrobial resistance and can play an important role in the spread of such resistance within the community, this study aimed to investigate the prevalence, antimicrobial susceptibility, and molecular characteristics of CA MRSA between 2 groups of college students (medical and nonmedical) in the universities in Urmia, Iran.

METHODS AND MATERIALS

Study population

From 2012-2015, a total of 700 healthy student volunteers who were residing in dormitories of state-run universities in Urmia, Iran, which are under the direct supervision of Iran's Ministry of Science, Research and Technology (for nonmedical universities [NMU]) and Ministry of Health and Medical Education (for medical universities [MU]), were enrolled in this study. Included MU students were

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in the basic medical sciences course and not in health care practice or internship programs.

Informed consent was obtained from all individual participants included in the study. Data from each case were collected to distinguish HA MRSA from CA MRSA strains, as defined by the Centers for Disease Control and Prevention.[11] Every student with a history of antibiotic consumption during the last 2 months; hospitalization; admission to a nursing home, skilled nursing facility, or hospice; dialysis for renal failure; recent surgery; and permanent indwelling catheters or medical devices during the last year were excluded from this study.

Bacterial isolates

The volunteers were screened for nasal carriage of *S aureus* by streaking both anterior nares with sterile moistened cotton swabs to a depth of approximately 1 cm, and rotated 5 times.[12] Nasal swabs were placed in tubes containing sterile normal saline and quickly transported to the microbiology laboratory at the faculty of medicine in Urmia and inoculated onto mannitol salt agar and blood agar plate media and incubated at 35°C for 48 hours. The isolates were presumptively identified as *S aureus* by standard biochemical tests, such as gram stain, catalase test, and coagulase test. In addition, polymerase chain reaction (PCR) was used for detection of species-specific genes to confirm their identities.[13] Isolates were stored separately in tryptic soy broth medium with 15% glycerol at –20°C for further phenotypic and genotypic analysis.

Antibiotic susceptibility patterns of the isolates

The antimicrobial susceptibility of the isolates was determined using the disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines,[14] for the following antimicrobial agents: penicillin (10 U), gentamicin (10 μg), erythromycin (15 μg), rifampin (5 μg), mupirocin (200 μg), ciprofloxacin (5 μg), tetracycline (30 μg), clindamycin (2 μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg). *S aureus* ATCC 35923 (American Type Culture Collection, Manassas, VA) has been used as a quality control strain for susceptibility testing.

Initially, MRSA isolates were identified using cefoxitin (30 μg) disks (all antibiotic disks were from Mast Diagnostics, Merseyside, UK) based on the 2014 CLSI recommendations and following that correlated with the presence of the *mec*A gene using PCR.[15] All the MRSA isolates were assessed regarding their susceptibility to vancomycin, using BHI-vancomycin (brain heart infusion) screening agar (in both 4- and 6-μg/mL concentrations)[14] and Etest (Liofilchem, Roseto degli Abruzzi, Italy) for determination of minimum inhibitory concentrations.

*SCC*mec *typing and detection of virulence genes among MRSA*

The genomic DNA of MRSA isolates was extracted using a commercial kit (CinnaClon, Tehran, Iran) for gram-positive bacteria. Multiplex PCRs were performed on MRSA for separate detection of SCC*mec* type (I-V).[15] PCR was used for amplification of virulence genes encoding *pvl* and *et*a and *et*b as described previously.[16,17] Reactions were performed in a thermal cycler (bioer, Hangzhou, China). Amplified PCR products were analyzed using gel electrophoresis on a 1.5% agarose gel with 0.5× Tris-borate buffer. The gels with safe stains were photographed under ultraviolet illumination (G:BOX; Syngene, Cambridge, UK). Each PCR assay included positive control strains (provided by Dr Mohammad Ahangarzadeh Rezaee, Department of Microbiology, Tabriz University of Medical Sciences) and a negative control, which contained all the reagents but not the template DNA. All molecular analyses were performed at the Laboratory of Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran.

Statistical analysis

The prevalence of *S aureus*, MRSA, and virulence genes were compared using Fisher exact test. Statistical analysis was performed with SPSS version 16 (SPSS Inc, Chicago, IL). $P < .05$ was regarded as statistically significant.

RESULTS

During the study period, nasal screening identified 137 (19.6%) carriers of *S aureus*, and their distributions are shown in Table 1.

Eighteen out of 137 (13.14%) isolates were defined as MRSA using a cefoxitin 30-μg disk screening test and PCR (for *mec*A gene) (Fig 1). The isolates have been considered as CA MRSA via the lack features suggesting hospital-associated acquisition (Centers for Disease Control and Prevention criteria).[11]

The antimicrobial susceptibility patterns of MRSA and methicillin-sensitive *S aureus* (MSSA) isolates are shown in Table 2.

The highest rate of resistance was shown with penicillin (93.4%). All isolates in both groups were sensitive to rifampin, with the exception

Table 1
Distributions of the isolates among study population

Characteristic	Value
Age, y, mean ± SD (range)	22.29 ± 2.49 (18-46)
Male (NMUs: n = 285, 40.7%; MUs: n = 225, 32.1%)	510 (72.8)
Female (NMUs: n = 65, 9.3%; MUs: n = 125, 17.9%)	190 (27.1)
No. of *Staphylococcus aureus* nasal carrier among students	
MUs (men: n = 52, 82.5%; women: n = 11, 17.5%)	63 (46)
NMUs (men: n = 66, 89.2%; women: n = 8, 10.8%)	74 (54)
Total	137 (19.6)
MRSA isolates	
MUs	7 (2.0)
NMUs	11 (3.14)
Total (all were from men)	18 (2.57)

NOTE. Values are n (%) or as otherwise indicated.
MRSA, methicillin-resistant *S aureus*; *MU*, medical university; *NMU*, nonmedical university.

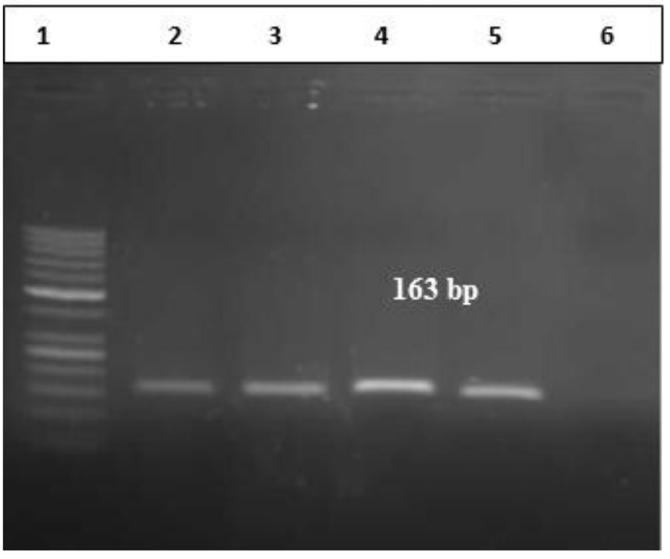

Fig 1. Agarose gel electrophoresis of amplified *mec*A gene by polymerase chain reaction. Column 1 shows ladder 50 bp; column 2, positive control strain; columns 3-5, *mec*A-positive strains in the study; and column 6, negative control.

Table 2
Antibiotic susceptibility patterns of *Staphylococcus aureus* isolates from nasal carriage

	S aureus isolates						
	MSSA			MRSA			
Antibiotics	S	I	R	S	I	R	Total no. resistant
Penicillin	9 (7.6)	0 (0)	110 (92.4)	0 (0)	0 (0)	18 (100)	128 (93.4)
Gentamicin	118 (99.2)	0 (0)	1 (0.8)	14 (77.8)	2 (11.1)	2 (11.1)	3 (2.2)
Erythromycin	90 (75.6)	2 (1.7)	27 (22.7)	7 (38.9)	1 (5.6)	10 (55.6)	37 (27.0)
Rifampin	118 (99.2)	0 (0)	1 (0.8)	18 (100.0)	0 (0)	0 (0)	1 (0.7)
Mupirocin	114 (95.8)	0 (0)	5 (4.2)	17 (94.4)	0 (0)	1 (5.6)	6 (4.4)
Ciprofloxacin	99 (83.2)	14 (11.8)	6 (5.0)	13 (72.2)	2 (11.1)	3 (16.7)	9 (6.6)
Clindamycin	96 (80.7)	1 (0.8)	22 (18.5)	12 (66.7)	2 (11.1)	4 (22.2)	26 (19.0)
Trimethoprim-sulfamethoxazole	106 (89.1)	0 (0)	13 (10.9)	14 (77.8)	0 (0)	4 (22.2)	17 (12.4)

NOTE. Values are n (%).
I, intermediate; *MRSA*, methicillin-resistant *S aureus*; *MSSA*, methicillin-sensitive *S aureus*; *R*, resistant; *S*, sensitive.

Table 3
Distribution of subtypes of SCC*mec* type IV among isolates

	Isolates from students		
Subtypes of SCC*mec* type IV	NMU	MU	Total
IVa	6 (42.86)	3 (21.43)	9 (64.29)
IVb	0 (0)	0 (0)	0 (0)
IVc	3 (21.43)	1 (7.14)	4 (28.57)
IVd	0 (0)	0 (0)	0 (0)
IVh	1 (7.14)	0 (0)	1 (7.14)

NOTE. Values are n (%).
MU, medical university; *NMU*, nonmedical university; *SCCmec*, staphylococcal cassette chromosome *mec*.

of 1 MSSA strain. None of the isolates could grow in BHI- vancomycin screening agar contained 4 or 6 μg/mL of vancomycin. Most MRSA isolates belonged to SCC*mec* type IV (n = 14; 77.8%), and a few strains were included in SCC*mec* type V (n = 4; 22.2%). Distribution of subtypes of SCC*mec* type IV among isolates has been demonstrated in Table 3. Meanwhile, SCC*mec* I, SCC*mec* II, and SCC*mec* III were not identified in any MRSA isolates. Only 1 (5.56%) MRSA isolates carried the *pvl* gene, and none of them harbored *et*A and *et*B genes.

DISCUSSION

Drug-resistant bacteria are an increasing threat to public health, as highlighted by an estimate that in the United States, MRSA may contribute to more deaths than HIV.[18] A disturbing trend related to such resistant bacteria is the possibility of their spread within the community. This study showed that *S aureus* nasal carriage among enrolled students was 19.6% (137/700). The analysis of distribution of *S aureus* between the 2 study groups revealed a slight increase in the isolates from NMU (n = 74; 54%) than MU (n = 63; 46%) students; however, this difference was not statistically significant ($P = .616$). The prevalence of CA MRSA was relatively low (n = 18/137; 13.14%). The frequency of MRSA isolates was also higher among NMU (n = 11/74; 14.86%) than MU (n = 7/63; 11.11%) students; however, the difference was not significant ($P = .349$).

In our research, the frequency of nasal carriage was notably higher in male (n = 118; 86.1%) than female (n = 19; 13.9%) students ($P = .001$). Meanwhile, MRSA was not detected among female students of both groups. It seems that the male sex is a risk factor for *S aureus* nasal colonization. This finding may relate to an increased attention to health issues, especially personal hygiene by the female sex. On the other hand, it may also be affected by the small sample size for women in comparison with men (190 vs 510, respectively). The various prevalence of CA MRSA has been reported from different studies around the world.[19,20] It has been shown that nasal carriers have an increased risk of acquiring an infection with this bacterium.[21] CA MRSA can cause skin and soft tissue infections and, in some cases, respiratory tract infection, bacteremia, and septic shock. A study showed that 80% of strains causing bacteremia in carriers were endogenous.[22] Based on this evidence, detection of MRSA among the study population is a threat to the patients and a source of transmission to other susceptible individuals, particularly on campus.

The antibiotic susceptibility patterns of the isolates revealed that multidrug resistance (resistance against at least 2 different classes of antimicrobials) was more common among MRSA (n = 15/18; 83.33%) isolates than MSSA (n = 40/119; 33.61%). In vitro antimicrobial susceptibility tests of MRSA isolates showed that 3 (16.67%) isolates were susceptible to non–β-lactam agents, whereas insensitivity to 5, 4, 3, and 1 non–β-lactam antibiotics was observed in 1 (5.56%), 3 (16.67%), 5 (27.78%) and 6 (33.33%) MRSA isolates, respectively. Despite these findings, our study revealed less resistance in CA MRSA to rifampin, mupirocin, gentamicin, and trimethoprim-sulfamethoxazole. This is consistent with other studies that indicated CA MRSA strains are less resistant to non–β-lactam antibiotics.[23] However, multidrug resistance between CA MRSA and MSSA isolates has obviously developed. Easy access to antibiotics without doctors' prescriptions may cause development of such resistance among staphylococcal isolates in our region.

A notable point in this research is the sensitivity of all the isolates (except 1) to rifampin. This reduction may partly relate to the decreased prescription of rifampin by clinicians for long periods in our region. Although this antibiotic is rarely used as a single agent to treat CA MRSA infection because resistance can occur rapidly; however, it can be used synergistically with other antibiotics like vancomycin (in this study, all the isolates were sensitive) for treatment of serious *S aureus* infection, such as endocarditis, as recommended previously.[24] Mupirocin is used to eliminate nasal colonization of *S aureus*, particularly MRSA.[25] In this study, resistance to mupirocin (n = 6; 4.4%) was also low, and only 1 isolate was CA MRSA. Based on the 2014 CLSI criteria, none of the mupirocin-resistant isolates were considered as high-level resistance because all isolates exhibited zones of inhibition. Therefore, mupirocin can be used for decreasing nasal carriage if necessary.

Most studies on CA MRSA strains revealed that their genetics differ with each other. In this study, SCC*mec* typing showed that SCC*mec* type IV is dominant among MRSA isolates. In addition, few isolates harbored type V. The lack of types I, II, and III were predictable among isolates because these types are frequently found in HA MRSA.[5] However, we detected the mentioned types among the study population because both CA MRSA and HA MRSA strains are now circulating in the community.[26]

Only 1 MRSA strain was found to carry the *pvl* gene. Although CA MRSA strains with the *pvl* gene are predominate in Western countries,[26] a lower rate of the *pvl* gene has been seen in Eastern countries, where most CA MRSA isolates lack the gene.[27,28] Absence

or less frequency of the *pvl* gene among MRSA isolates have also been reported in other studies from different parts of Iran.[29,30]

CONCLUSIONS

The relatively high frequency of *S aureus* nasal carriage, especially considering that some isolates were CA MRSA, is a reason for concern. Susceptibility patterns of the isolates demonstrated that multidrug resistance is emerging among CA MRSA, and individuals who are harboring these isolates can act as reservoirs. Most MRSA isolates had SCC*mec* type IV, and few harbored type V (the types tend to be highly mobile); the transfer of such MRSA strains from carriers to other individuals in crowded living conditions, such as dormitories, may act as a risk factor for outbreaks of CA MRSA and is a serious threat for the study groups. The continuously surveillance of CA MRSA is essential to prevent transmission of *S aureus* from the infected carriers to others and also to apply effective therapeutic options for treatment of them.

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