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Original Article

Nasal colonization by methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* among medical students

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Abstract

Introduction: Nasal carriers of methicillin-resistant *Staphylococcus aureus* (MRSA) are common and play an important role in nosocomial infections. The prevalence rate and characterization of nasal carriers of MRSA among medical students in Jordan has not been investigated before.

Methodology: The resistance of *S. aureus* to several antibiotics was tested using disc diffusion method, automatic Vitek 2, and penicillin binding protein (PBP) 2 slide test. Bacterial species and resistance genes were confirmed using molecular analysis of three relevant genes by real-time PCR. Two hundred ninety nasal swabs were collected from medical students at Hashemite University from June 2015 to August 2016. All participants signed a voluntary consent form and filled a predesigned questionnaire.

Results: The mean age of participants was 19.7 ± 2 years and 61.7% of them were males. 63 out of the 290 (21.7%) samples were identified to have *S. aureus*, 56 (19.3%) were methicillin-sensitive *S. aureus* (MSSA) and 7 (2.4%) were MRSA. *S. aureus* nasal colonization significantly associates with male gender (OR = 1.7, CI = 0.94-3.18, P = 0.049) and chronic illnesses (OR = 4.0, CI = 1.52-10.65, P = 0.006). Consistency between disc diffusion, Vitek 2, and PBP 2 methods for MRSA screening were satisfactory compared to molecular analysis. All MRSA samples were positive for SCCmec:*orfx* junction gene (MRSA-specific), *nuc* gene (*S. aureus*- specific), mecA gene (PBP-mediated resistant), and PBP2 production. All MRSA isolates were multi-drug resistant and were sensitive to Linezolid, Vancomycin, and Tigecycline.

Conclusions: This study confirms that nasal colonization by MRSA among medical students necessitates further attention to prevent nosocomial infections.

Key words: *Staphylococcus aureus*; MRSA; MSSA; antibiotics resistance; mecA; Jordan.

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Introduction

Staphylococcus aureus (*S. aureus*) is a major cause of multiple serious human infections including skin and burn infections, surgical site infections, respiratory infections, food poisoning and bacteremia [1,2]. Carriers of *S. aureus* are common and play an essential role in transmission of infections [3]. A large proportion of *S. aureus* carriage is in the anterior nares of the nasal passages [2,4].

The emergence of antibiotic resistant strains of *S. aureus* is a worldwide concern since it is associated with high rate of morbidity and mortality [3,5,6]. Methicillin-resistant *S. aureus* (MRSA) infections were reported first in 1950 [7], however, the rate of infections with MRSA has increased dramatically in recent decades reaching up to 50%. Thirty percent of infected patients with MRSA will die within 30 days [5,6,8]. The MRSA rate is higher in hospitalized patients (HA-MRSA) [9]. However, recent reports indicate the growing frequency of community-acquired MRSA (CA-MRSA) [7, 10]. Vancomycin, the drug of choice for MRSA, is highly effective, but in recent years multiple new reports have indicated, for the first time, the appearance of Vancomycin resistant strains [11-13].

Antimicrobial resistance is genetically based and mediated by the acquisition of extra-chromosomal genetic elements containing resistance genes [14]. Multiple studies have indicated the critical role of multiple genes including mecA in mediating resistance to antibiotics in MRSA [15-17].

The Antibiotic Resistance Surveillance and Control in the Mediterranean Region (ARMed) project collected 5000 susceptibility test results of invasive isolates of *S. aureus* from blood cultures which were routinely processed within laboratories servicing 62 hospitals situated in Algeria, Cyprus, Egypt, Jordan,

Lebanon, Malta, Morocco, Tunisia and Turkey [18,19]. Overall, the median MRSA proportion was 39%. The highest proportions of MRSA were reported by Jordan, Egypt and Cyprus, where more than 50% of the invasive isolates were methicillin-resistant [18,19].

MRSA nasal carriage rate varied widely from 4.3% to 19% in the Jordanian healthy adult population [16,20,21], while children's MRSA nasal carriage rate ranged from 7.1% to 40% [21-23]. Isolates from clinical samples have higher rates of MRSA colonization that reach up to 62% [16,24,25]. The nasal carriage of MRSA was investigated in 550 hospital staff members of four hospitals in north Jordan. Of the 109 (19.8%) individuals who tested positive for nasal carriers of *S. aureus*, 32 (5.8%) were found to be carriers of MRSA [26,27]. A single report of persistent bacteraemia due to methicillin-resistant *S. aureus* with reduced susceptibility to Vancomycin in a patient with erythrodermic psoriasis was reported from Jordan University Hospital [28].

Investigating the role of medical students as nasal carriers of MRSA has attained recent interest as medical students constitute an important but forgotten part of the healthcare workforce that interacts frequently with patients and are less informed and possibly not well trained about infection control measures [29]. Several previous studies have investigated the prevalence of MRSA carriers among medical students [30-33] which proved their important role in nosocomial infections [34,35]. The main aim of the present study was to investigate the prevalence, antimicrobial susceptibility pattern, antibiotics resistance genes, and risk factors of MRSA-carrying medical students in Jordan.

Methodology

Study participants

In the present cross-sectional study, 290 Medical students from the first to the fourth year in the Hashemite University's Faculty of Medicine were recruited voluntarily from June 2015 to August 2016. Each participant signed a consent form and completed a designed questionnaire that included questions regarding demographic data (age, gender, residency, number of family members currently living with the participant and occupation). Relevant clinical data were collected individually from each participant including recent upper respiratory tract infections (URTI), fever, history of chronic medical illnesses, previous surgeries, hospitalizations, and the use of medications, including antibiotics. Possible risk factors for nasal MRSA colonization including hospital-based clinical training, smoking, sports, and history of skin infections were also included in the questionnaire. This study was approved by the Institutional Review Board of the Hashemite University.

Nasal swabs collection

Nasal swabs were collected from each participant under sterile conditions. A dry polyester sterile swab was inserted about 2.5 cm into the nostril, parallel to the palate, and rolled in place for a few seconds. It was then slowly withdrawn with a rotating motion. Specimens from both nostrils were obtained with the same swab [17].

Microbiological identifications

All swabs were processed within 1 hour of collection. Swabs were streaked on nutrient media (Mast, Bootle, Merseyside, UK), sheep blood agar (Mast, Bootle, Merseyside, UK), and mannitol salt agar (Biolab, Budapest, Hungary) and incubated at 37° C for 24-48 hours. Positive cultures were analyzed by colony morphology, Gram stain (GCC diagnostics, Deeside, Flintshire, UK) and biochemical tests. Isolates were considered positive for *S. aureus* if they showed Gram-positive cluster shape, produced yellow colonies on mannitol salt agar, produced creamy colonies with beta hemolytic zone on sheep blood agar, and tested positive for coagulase and catalase enzymes (Biolife, Milano, Italy) [36]. All positive isolates including isolates with a weak coagulase test were further confirmed using automatic Vitek 2 compact system for Gram-positive identification (GP card, REF 21342) according to manufacturer instructions. Isolates were stored appropriately at -80° C for further analysis of antibiotics sensitivity and molecular analysis. The *S. aureus* strains ATCC 33591 (MRSA) were used as positive control and ATCC 25923 (MSSA) were used as negative control throughout study.

Antibiotics susceptibility tests

S. aureus strains were tested for antibiotic susceptibility using the standard disc diffusion method according to the current CLSI guidelines (Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing, twenty fifth informational supplements, Jan 2015). Briefly, 3-4 fresh colonies were diluted in 2 mL of sterile normal saline to prepare a suspension of 0.5 McFarland barium sulphate standard unit. The surface of Mueller-Hinton Agar (Liofilchem, Roseto degli Abruzzi, Italy) was covered with required inoculums followed by impregnation of small antibiotics filter disks, namely Oxacillin 1 μg and Cefoxitin 30 μg (Bioanalyse,

London, UK) and incubated for 18-24 hours at 35° C. The zone of inhibition was measured carefully and interpreted using the last recommendation by CLSI for Oxacillin (resistant ≤ 10 mm, sensitive > 10 mm) and for Cefoxitin (resistant ≤ 21 mm, sensitive > 21 mm). Furthermore, to confirm the antibiotic sensitivity pattern, automated susceptibility testing was performed with the Gram-positive susceptibility card (AST P592, REF 22287) using the Vitek 2 compact system according to the manufacturer's recommendation. The interpretation for Oxacillin followed the breakpoint recommendation (resistant, ≤ 0.5 µg/mL; susceptible, ≥ 0.25 µg/mL) [37].

Detection of penicillin binding protein

A slide latex agglutination test (BioMerieux, Marcy l'Etoile, France) using a highly specific monoclonal antibody for the detection of penicillin binding protein 2 (PBP2) was performed according to manufacturer instructions. *S. aureus* colonies from a fresh blood agar plate were suspended in a 1.5 mL microcentrifuge tube containing 4 drops (each drop equal 50 µL) of extraction reagent (0.1 M NaOH). The suspension was boiled for 3 min at 95-100 °C. One drop of extraction reagent (0.5 MKH_2PO_4) was added to the suspension, mixed well, and then centrifuged at 1500G for 5 min. Supernatant was placed on slide and mixed with 1 drop of anti-PBP2 monoclonal antibody sensitized latex. If agglutination is observed within 3 min, the test was considered positive for MRSA producing PBP2 [15,37].

Molecular characterization

Samples which tested positive for *S. aureus* were further subjected to molecular analysis by PCR in order to confirm the species and to identify genes and plasmids that mediate antibiotic resistance. The mecA gene (methicillin resistance gene), the *nuc* gene (*S. aureus*-specific genome fragment), and the SCCmec:*orfx* junction (MRSA-specific amplicon) were identified using multiplex real-time PCR using reagents and controls supplied by Q-MRSA detection kit (QuanDx, San Jose, CA, USA) according to the supplier's instructions. Briefly, bacterial DNA was extracted from each sample using commercially available DNA extraction kit (Qiagen, Hilden, Germany), purity and concentration of DNA obtained were measured using NanoDrop Spectrophotometer (ThermoFisher, Waltham, MA, USA). Later on, extracted DNA was amplified as follows; PCR master mix was prepared by mixing the 10 µL of QD Fast supermix with 5 µL of Primer/Probe mix and 1 µL of internal control. 16 µL of master mix was mixed with 4 µL of samples or controls. qPCR was run using the manufacturer recommended program using 4 detection channels; FAM for SCCmec:*orfx* junction, HEX for mecA, CY5 for *nuc*, and ROX for internal control. Specific fluorescent probes will illuminate in the presence of targeted genes. Amplification blot and threshold cycle were used to identify MRSA-positive samples [17] ATCC strains of MRSA and MSSA were used as positive and negative controls beside the controls which were provided in the Kit.

Statistical analysis

Statistical analysis was performed using SPSS version 16. Differences in proportions were tested by Chi-square test and presented as numbers and frequency percentage. Associations with potential risk factors were assessed using Chi-square test and presented as odds ratio (OR), 95% Confidence Interval (95% CI), and P value. Correlations with possible risk factors using bivariate Pearson's correlation test and linear regression model accounting for possible cofactors were used for confirmation of statistical significance. P values less than 0.05 were considered statistically significant.

Results

Characteristics of study participants

A total of 290 medical students in preclinical (first to third year) and clinical years (fourth year) were recruited in the present study. The mean age of the participants were 19.7 ± 2 years, males constituted 61.7% of the participants and 38.3% were females. The majority of participants were residing in Amman, Jordan, the capital city, (70.7%), followed by the city of Zarqa (15.5%). The distribution of the students throughout the years of their medical study was as follows: 27.6% were in the first year, 18% were in the second year, 19.6% were in third year, and 34.8% were in the fourth year. Most of the participants are living within families of four to six members (58.6%). 35.5% indicated they worked or attended training in hospitals for different periods of time. 39.3% reported playing different types of sports. Among the participants, 23.4% were smokers, 25.9% were previously admitted to hospital/s for more than 24 hours, and 22% had a history of surgery, while only 6.2% had history of chronic medical illnesses. Furthermore, skin infections were reported in 5.2% of the study participants, while 12.1% used prescription or OTC medications, 8.3% used antibiotics in the last 2 weeks, 15.2% reported recent

URTI infections, and 2.4% had fever within the last 48 hours (see Table 1).

MSSA and MRSA nasal colonization among participants

Out of 290 nasal swab samples, 66 (22.7%) were identified as *S. aureus*, of them 54 (18.6%) were MSSA and 12/290 (4.1%) were MRSA using traditional microbiological assays and disc diffusion method only. All 54 samples identified by disc diffusion method as MSSA were also identified as MSSA using Vitek 2 analysis. However, among the 12 MRSA samples identified by disc diffusion method, Vitek 2 analysis identified 3 samples as other closely related species (2 samples for *Staphylococcus pseudintermedius* and 1 sample for *Streptococcus thoraltensis*), 2 samples were MSSA, and 7 samples were MRSA. Molecular analysis of these 12 MRSA samples using multiplex real-time PCR confirmed that isolates identified by Vitek 2 as MRSA (n = 7) were positive for SCCmec:*orfx* junction, mecA gene, and *nuc* gene, and PBP2 while the other 5 samples were negative for mecA gene and PBP2. All MSSA isolates have the *S. aureus nuc* gene, none of them had the mecA gene and all of them tested negative for PBP2. As mecA gene is considered the gold standard for MRSA identification [15,17,38], only the 7/290 samples (2.4%) that were identified by Vitek 2 as MRSA, have SCCmec:*orfx* junction, mecA gene, and

Table 1. Study participant's information. Demographic, social and clinical characteristics of study participants.

		Number	**Frequency%**
Age (mean)	years 19.7 ± 2	290	100
Gender	Male	179	61.7
	Female	111	38.3
Governorate	Amman	205	70.7
	Zarqa	45	15.5
	Others	40	13.8
MD program study year	First year	80	27.6
	Second year	52	18
	Third year	57	19.6
	Fourth Year	101	34.8
Number of family members living with participant	≤ 3	49	16.9
	4-6	170	58.6
	> 6	71	24.5
Work or training in hospital	Yes	103	35.5
	No	187	64.5
Sport	Football	21	7.2
	Fitness	57	19.7
	Walking	29	10.0
	No	176	60.7
Smoking	Yes	68	23.4
	No	222	76.6
Previous hospitalization	Yes	75	25.9
	No	215	74.1
Previous surgeries	Yes	64	22
	No	226	78
Chronic diseases	Yes	18	6.2
	No	272	93.8
Skin infections	Yes	15	5.2
	No	275	94.8
Medications	Yes	35	12.1
	No	255	87.9
Antibiotic use in the past 2 weeks	Yes	24	8.3
	No	266	91.7
URTI symptoms	Yes	44	15.2
	No	246	84.8
Fever within last 48 hours	Yes	7	2.4
	No	283	97.6
Total		**290**	100

Table 2. *S. aureus* positive isolates laboratory identification. Laboratory identification using disc diffusion, automatic Vitek 2 compact system, molecular characterization of three relevant genes, and PBP2 slide test.

MSSA				MRSA			
Culture and disc diffusion	Vitek 2	Molecular mecA	PBP2	Culture and disc diffusion	Vitek 2	Molecular mecA, nuc, and orfx	PBP2
54	56	0	0	12	7	7	7

nuc gene, and tested positive for PBP2 were considered MRSA while 56/290 (19.3%) were considered as MSSA and 3/290 (1%) were considered as other closely related species (see Table 2).

Nasal colonization with S. aureus association with potential risk factors

Associations with possible risk factors for nasal *S. aureus* colonization including hospital based clinical training, prolonged patient encounters, smoking, sports, use of antibiotics, and history of skin infections, were all analyzed. Nasal colonization with *S. aureus* was significantly higher in male participants (OR = 1.7, 95% CI = 0.94-3.18, P = 0.049) with an odds ratio of 1.7 in favor of colonization by male gender. Also, colonization was significantly higher with participants who had chronic illnesses (OR = 4.0, 95% CI = 1.52-10.65, P = 0.006). Participants with chronic illnesses had a 4 times higher risk for colonization with *S. aureus* compared to participants with no chronic illnesses. Correlation analysis using bivariate Pearson's correlation test confirmed positive correlation between nasal *S. aureus* colonization and male gender (R^2 = 0.011, Pearson's correlation = 0.105, P = 0.037) (Figure 1) and between nasal *S. aureus* colonization with chronic diseases (R^2 = 0.031, Pearson's correlation = 0.176, P = 0.003) (Figure 2). There was a trend toward a decrease in *S. aureus* colonization in participants who participated in regular sports but with no significant association (OR = 0.6, 95% CI = 0.33-1.09, P = 0.061). Other factors did not show any significant association with *S. aureus* nasal colonization including URTI, fever, hospitalization, antibiotics use, hospital-based clinical training, smoking, residency, number of family members, and history of skin infections (See Table 3). There was no significant association between nasal colonization with MRSA and all possible risk factors including age (OR = 0.7, 95% CI = 0.13-4.36, P = 0.53), gender (OR = 0.25, 95% CI = 0.05-1.25, P = 0.09), fever (OR = 1.0, 95% CI = 0.98-1.05, P = 0.88), URTI (OR = 2.4, 95% CI = 0.39-14.55, P = 0.30), antibiotics use (OR = 2.1, 95% CI = 0.20-22.68, P = 0.45), skin infection (OR = 2.9, 95% CI = 0.26-32.96, P = 0.38), chronic diseases (OR = 1.0, 95% CI = 0.10-9.44, P = 0.74), hospitalization (OR = 0.38, 95% CI = 0.04-3.42, P = 0.34), smoking (OR = 0.61, 95% CI = 0.06-5.57, P = 0.55), sport practicing (OR = 0.91, 95% CI = 0.16-5.20, P = 0.64), and hospital training (OR = 0.66, 95% CI = 0.11-3.74, P = 0.49).

MRSA antibiotics sensitivity pattern

All MRSA isolates were 100% resistant to the following antibiotics: Cefoxitin, Oxacillin, Benzylpenicillin, Ampicillin, and Imipenem. 45% were

Figure 1. Correlation analysis between frequency of nasal colonization with *S. aureus* and male gender. (R^2 = 0.011, Pearson's correlation = 0.105, P = 0.037).

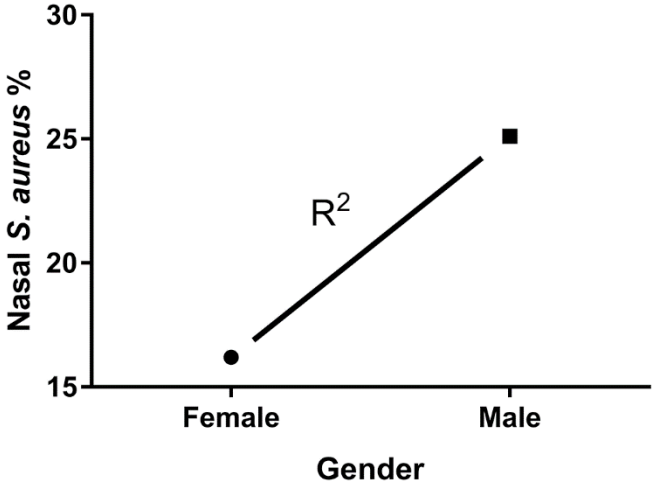

Figure 2. Correlation analysis between frequency of nasal colonization with *S. aureus* and chronic diseases. (R^2 = 0.031, Pearson's correlation = 0.176, P = 0.003).

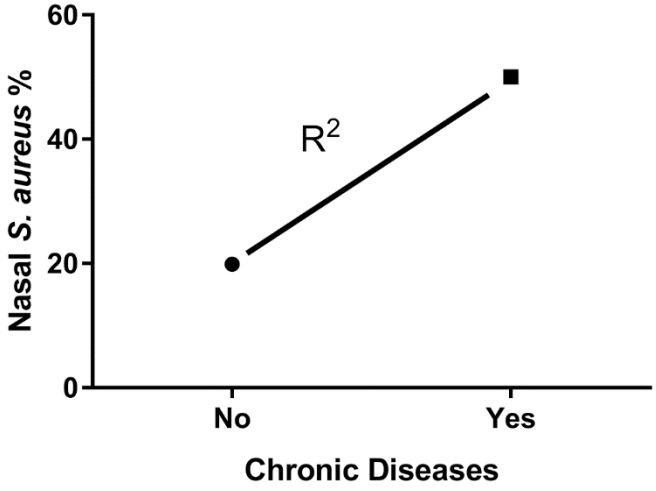

Table 3. Risk factors for *S. aureus* nasal colonization. Association with potential risk factors for *S. aureus* nasal colonization among study participants (n = 63).

		Number (frequency %)	Odds Ratio (95% confidence Interval)	P value
Age	≤20 years	48 (76.2)	1.3 (0.68-2.50)	0.255
	>20 years	15 (23.8)		
Gender	Male	45 (71.4)	1.7 (0.94-3.18)	0.049
	Female	18 (28.6)		
Governorate	Amman	41 (65)		0.086
	Zarqa	8 (12.7)		
	Others	14 (22.3)		
Number of family members living with participant	≤ 3	8 (12.7)		0.33
	4-6	42 (66.7)		
	> 6	13 (20.6)		
Work or training in hospital	Yes	23 (36.5)	1.1 (0.59-1.88)	0.482
	No	40 (63.5)		
Sport	Yes	19 (30.2)	0.6 (0.33-1.09)	0.061
	No	44 (69.8)		
Smoking	Yes	13 (20.7)	0.8 (0.41-1.60)	0.34
	No	50 (79.3)		
Previous hospitalization	Yes	18 (28.6)	1.2 (0.64-2.22)	0.343
	No	45 (71.4)		
Chronic diseases	Yes	9 (14.3)	4.0 (1.52-10.65)	0.006
	No	54 (85.7)		
Skin infections	Yes	4 (6.3)	1.3 (0.40-4.33)	0.417
	No	59 (93.7)		
Medications	Yes	9 (14.3)	1.3 (0.57-2.91)	0.338
	No	54 (85.7)		
Antibiotic use in the past 2 weeks	Yes	5 (7.9)	0.9 (0.33-2.63)	0.575
	No	58 (92.1)		
URTI symptoms	Yes	10 (15.9)	1.1 (0.49-2.30)	0.499
	No	53 (84.1)		
Fever within last 48 hours	Yes	1 (1.6)	0.6 (0.07-5.02)	0.528
	No	62 (98.4)		
Total		63 (100)		

Table 4. Antibiotics susceptibility pattern of MRSA isolates. MRSA isolates Antibiotics susceptibility pattern as resistant, intermediate, and sensitive.

	MRSA		
	Resistant	**Intermediate**	**Sensitive**
Cefoxitin	100%	0%	0
Oxacillin	100%	0%	0
Benzylenicillin	100%	0%	0
Ampicillin	100%	0%	0
Imipinem	100%	0%	0
Erythromycin	45%	0%	55%
Clindamycin	45%	0%	55%
Fusidic acid	0%	15%	85%
Induced clindamycin resistance	28%	0%	72%
Cotrimoxazole	15%	0%	85%
Ciprofloxacin	0%	0%	100%
Gentamicin	0%	0%	100%
Moxifloxacin	0%	0%	100%
Fosfomycin	0%	0%	100%
Rifampicin	0%	0%	100%
Linezolid	0%	0%	100%
Teicoplanin	0%	0%	100%
Vancomycin	0%	0%	100%
Tigecycline	0%	0%	100%

resistant to Erythromycin and Clindamycin. 15% were intermediately sensitive to Fusidic acid and 15% were resistant to Co-trimoxazole. All MRSA isolates were 100% sensitive to the following antibiotics Ciprofloxacin, Gentamicin, Moxifloxacin, Fosfomycin, Rifampicin, Linezolid, Teicoplanin, Vancomycin, and Tigecycline. Moreover, 28% of collected samples were positive for induced Clindamycin resistance (see Table 4). The phenotype of resistance predicted using the MIC profile for antibiotics sensitivity were streptogramin B resistance and modification of PBP (mecA) for all samples and Macrolide, Lincosamid, streptogramin B (MLSB) inducible resistance among 28% of the collected samples (data not shown).

Discussion

Nasal colonization with *S. aureus* plays an essential role in transmission of infections [2,4]. Of particular importance are the nasal carriers of MRSA among medical staff who possess a higher risk for transmission of nosocomial infections [39-41]. MRSA is regarded as the most serious infection in hospitals [42]. Although medical students are not considered part of medical staff in hospitals, they interact frequently with patients during their training and play an important role for causing and transmitting nosocomial infections [30-32,34,35].

In the present study the prevalence rate of *S. aureus* nasal colonization among medical students was 21.7%, of them 19.3% were MSSA and 2.4% were MRSA. International studies indicated variable rates of *S. aureus* nasal colonization among medical students with *S. aureus* carriage rates of 14–45% and MRSA carriage rates of 0–14.3% [30-33,43]. Several previous studies that were conducted in Australia, Austria, Malaysia, Nigeria, and Thailand reported 0% MRSA nasal colonization among medical students, especially in the preclinical years [33,44-48]. Relatively high rates of MRSA nasal colonization were reported among medical students in China, Colombia and Iran [33,43,49]. In the Middle East, studies showed an MRSA carriers rate of 6.7% in Saudi Arabia [30], 13.14% in Iran [33] and 10% in Israel [35]. The rate reported in this study is lower than studies done in nearby countries [30,33,35]. No similar previous studies were conducted in Jordan.

Molecular analysis was used to confirm the bacterial species in the nasal isolates. *Nuc* gene is widely used to specifically identify *S. aureus* [50,51]. All *S. aureus* isolates in this study were confirmed by real-time PCR to have the *nuc* gene. MRSA is mediated through specific genes/plasmids, with mecA being the most important of them [15-17]. All MRSA isolates in this study have MRSA specific junction gene, SCCmec:*orfx* junction, covering both SCCmec and the *S. aureus* specific *orfx* region [52]. Also, all MRSA strains in this study have mecA gene responsible for production of PBP as illustrated by positive PBP2 slide test. While this is the first study to identify the resistance gene mecA among medical students in Jordan in preclinical years, other studies conducted in different populations identified mecA gene in all nasal MRSA isolates in healthy adults in Jordan [16,21]. MRSA identification using three relevant genes is important to confirm species (other *Staphylococcus* species commonly present in nose may carry mecA genes).

Three different methods were used in the present study for the identification of antibiotic sensitivity, namely disc diffusion, automatic Vitek 2, and PBP2 production. Considering molecular analysis with mecA as the gold standard [15,17,38], consistency and prediction ability of different methods were satisfactory. There was an excellent prediction ability of Vitek 2 automatic system for MRSA. Traditional microbiological procedures and Cefoxitin disc diffusion missed 5 out of the 68 positive samples, 3 samples were closely related species and 2 samples were actually MSSA. This is mostly due to the presence of normal flora in the nose that has biochemical and cultural characteristics similar to *S. aureus,* including coagulase-negative *Staphyllococci*. While the performance of Cefoxitin diffusion disc is considered superior to Oxacillin diffusion disc in MRSA identification, using the manual and automatic MIC methods and/or molecular analysis would be important in difficult-to-isolate samples [15,38].

In the present study, male gender and chronic illnesses appeared to be the only factors which played a significant role in *S. aureus* nasal colonization. Many previous studies did not show any significant association between *S. aureus* nasal colonization and potential risk factors among medical students [31,45,47], while other studies reported significant association with gender, smoking, URTI, contact with pets, and antibiotic use [32,33,44,45,49,53,54]. The significant association of *S. aureus* nasal colonization among medical students with male gender reported in this study is consistent with other studies [33,44,45,54]. A single study reported significant correlation between nasal MRSA colonization and chronic sinusitis only [49]. As most factors have no significant correlation with *S. aureus* nasal colonization among medical students, it suggests that other yet to be identified

factors play role in transmission and pathogenesis [2]. There was no significant association between nasal colonization with MRSA and all possible risk factors analyzed in this study. Low number of positive MRSA isolates (n = 7) might under estimate the importance of some risk factors.

While most study participants were in their preclinical years, many students reported a history of previous hospitalization, surgery, clinical training, or short clinical rotation of less than 8 weeks (fourth year students). None of these factors were found to play a significant role in *S. aureus* nasal colonization. This is in agreement with most other studies which reported no correlation between *S. aureus* nasal colonization and clinical exposure [29,31,33,43,47,48,54,55]. To the contrary, some studies suggested possible correlation between clinical exposure and *S. aureus* nasal colonization [35,44,49], while a few studies suggested strong correlation [56,57].

Antibiotic susceptibility pattern of MRSA isolates indicated that all isolates are actually multi-drug resistant (resistance to 3 antibiotics of 3 different classes). The susceptibility pattern is similar to other reports [33,35,43,49]. All isolates in this study were sensitive to Vancomycin in agreement with all other studies [31-33,43,49,56].

Conclusions

In conclusion, this study indicated the presence of nasal colonization with *S. aureus* in 21.7% of medical students, 2.4% of them having MRSA. *S. aureus* nasal colonization significantly associates with male gender and chronic illnesses. All MRSA isolates were multi-drug resistant and tested positive for SCCmec:*orfx* junction gene, *nuc* gene, mecA gene, and production of PBP2. Further analysis of the medical students' role in transmission of nosocomial infections and the importance of medical students' screening, prophylactic treatment, and proper training in infection control is necessary.

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