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Major article

Molecular analysis and susceptibility patterns of methicillin-resistant *Staphylococcus aureus* strains causing community- and health care-associated infections in the northern region of Palestine

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The aim of our study was to investigate the prevalence of nasal carriage of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) strains among 360 healthy university students at An-Najah National University, Palestine. For the purpose of comparing the staphylococcal cassette chromosome methicillin resistant determinant (SCCmec) type of MRSA, 46 clinical MRSA isolates were also included in this study. Nasal carriage of *S aureus* was found in 86 of 360 students (24%.) MRSA accounted for 9% of *S aureus* isolates. All 86 strains of *S aureus* were sensitive to vancomycin. Resistance to penicillin G, amoxicillin/clavulanic acid, ciprofloxacin, erythromycin, and clindamycin was found in 98%, 93%, 33%, 23%, and 12% of the isolates, respectively. Resistance rates of the MRSA isolates were as follows: 100% resistant to penicillin G and amoxicillin/clavulanic acid, 96% to ethromycin, 52% to clindamycin, and 48% to ciprofloxacin. No vancomycin-resistant isolates were identified. In our study, nearly half (52%) of the MRSA isolates belonged to SCC*mec* types IVa and V. However, SCC*mec* types II and III are represented by 48%, whereas SCC*mec* type I was completely absent. These findings indicate the existence of SCC*mec* type IVa in both student nasal carriers and health care settings. This emphasizes the need for implementation of a revised set of control measures in both settings. Moreover, the rational prescription of appropriate antibiotics should also be considered.

The expanding community reservoir of community-acquired Q5 methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has led to the inevitable infiltration of CA-MRSA in hospitals.[1-4] Several reports further suggest that CA-MRSA may be replacing the traditional hospital-acquired MRSA (HA-MRSA). This event is a considerable concern because strains of CA-MRSA had staphylococcal Q6 cassette chromosome methicillin resistant determinant (SCC*mec*) Q7 type IV or V. SCC*mec* types IV and V have increased mobility; therefore, there is a greater potential for horizontal spread to diverse *S aureus* genetic backgrounds compared with other SCC*mec* types.[5-8]

Nasal carriage of MRSA represents a major risk factor for subsequent infection and transmission of this pathogen.[8,9] Although several studies have reported the prevalence of MRSA nasal carriage in patients in health care settings,[8-10] this subject has not been investigated in healthy individuals very much, and practically no articles have documented MRSA nasal carriage emergence in Palestine.

To our knowledge, no epidemiologic surveillance studies in Palestine have investigated the molecular nature of MRSA strains circulating in the community and health care settings. The objectives of our study were to obtain a snapshot on the prevalence of nasal carriage of *S aureus* and MRSA in a Palestinian university, to explore transmission of these strains in health care settings, and to molecularly characterize MRSA strains circulating in Palestine.

MATERIALS AND METHODS

This study was performed between March and June 2011 to determine the prevalence of nasal carriage of *S aureus* and MRSA in

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university students at An-Najah National University, Palestine. Study participants were 360 healthy students. For the purpose of comparing the SCC*mec* type of MRSA in the study group, 46 clinical MRSA isolates obtained from 3 different health centers in northern Palestine in the same time period of the study were also included in this study.

Nasal samples were collected from both nostrils by the use of a collection swab. The tip of the swab was inserted approximately 1 in into the anterior vestibule of the nose and rolled 5 times in each nostril. Each swab was inoculated into enrichment broths to increase the isolation rate of *S aureus.* After incubation, the broths were streaked onto a mannitol salt agar (Oxoid Ltd, City, State) plate, were further incubated aerobically for 48 hours at 35°C, and subsequently examined for growth. *aureus* was identified based on its Gram stain morphology, colonial morphology, and production of catalase. The Staphytect plus tests (Oxoid Ltd) was used to determine the presence of Protein A and bound coagulase that are specific for *S aureus.*[4]

All *S aureus* isolates were tested for methicillin resistance. The disc-diffusion method outlined by the National Committee for Clinical Laboratory Standards,[11] was used with a 1 μg oxacillin disc (Oxoid Ltd). Zone sizes were read after incubation at 35°C for 24 hours. Isolates with zone sizes ≤10 mm were considered to be methicillin resistant.

Genetic resistance to methicillin was verified by detection of the *mecA* gene. Susceptibility testing was performed by disk diffusion susceptibility tests following the method recommended by the Clinical and Laboratory Standards Institute.[11]

DNA was extracted following the boiling method described by Zhang et al.[12] One to 5 colonies from an 18-24 hour MRSA culture grown in nutrient agar plate were suspended in 50 μL distilled water, and boiled for 10 minutes. The supernatant with DNA was harvested after centrifugation at 20,000 × g for 5 minutes.

SCC*mec* types were determined by the use of specific primers for amplification of the key genetic elements as described by Ghaznavi-Rad et al.[13] Polymerase chain reaction (PCR) was performed with a Ready Mix PCR kit (Sigma-Aldrich Co, City, State). Reaction mixtures contained 2.5 μL template DNA, 12.5 μL master mix with, 2.5 μL primer mix (1 μM for each primer) (Syntezza Bioscience Ltd, Jerusalem, Israel) and ribonuclease-free water to a final volume of 25 μL. The reaction was carried out in an Eppendorf Mastercycler gradient according to the following program: 94°C for 4 minutes; 35 cycles of 94°C for 30 seconds, 48°C for 30 seconds, 72°C for 2 minutes, and a final extension at 72°C for 4 minutes. PCR products were separated by electrophoresis in agarose 2% gels and stained with ethidium bromide.

Three nasal MRSA and 3 clinical MRSA samples obtained from health care settings were comprehensively used to amplify and sequence *mecA*. Primers used were 5'-TGGCTATCGTGTCACAATCG-3' and 5'-CTGGAACTTGTTGAGCAGAG-3', yielding 310-bp fragment.[14] The PCR products were purified using the MinElute PCR purification kit (Qiagen, Hilden, Germany) and the inserts were sequenced by a dideoxy chain termination method on an ABI PRISM Model 3130 Sequence Instrument (Manufacturer, City, State) at Bethlehem University, Bethlehem, Palestine. The phylogenic relationships between the MRSA isolates were conducted using the CLC Main Workbench software (version 5.6.1, 2009, Manufacturer, City, State). The phylogenic tree was rooted with the *S sciuri* (GenBank accession No. Y13096).

The nucleotide sequences of the three nasal MRSA isolates (19, 32, and 89) and three clinical MRSA isolates (3, 7, and 8) reported here were assessed with the following GenBank accession Nos.: JN108029, JN108030, and JN108031, and JN108026, JN108027, and JN108028, respectively.

Table 1
Antibiotic resistance of 86 *Staphylococcus aureus* isolates from nasal swabs collected from healthy students

Antibiotic	Number of resistant isolates	Percentage of resistant isolates
Vancomycin	0	
Ciprofloxacin	28	33
Penicillin G	84	98
Amoxicillin/clavulanic acid	80	93
Erythromycin	20	23
Clindamycin	10	12
Methicillin	8	0

RESULTS

Out of the total 360 nasal swabs obtained from healthy students at An-Najah National University during the study period, *S aureus* was isolated in 24% (n = 86). All 86 strains of *S aureus* were sensitive to vancomycin. Resistance to penicillin G, amoxicillin/clavulanic acid, ciprofloxacin, erythromycin, and clindamycin was found in 98%, 93%, 33%, 23%, and 12% of the isolates, respectively (Table 1). Methicillin resistance was detected in 8 of 86 (9%) isolates. Nearly 35% of isolates were noted to be multiply resistant; that is, resistant to β-lactam plus 2 or more antibiotics of ciprofloxacin, erythromycin, and clindamycin.

The 54 MRSA isolates in our sample population (ie, the 8 nasal MRSA and the 46 clinical MRSA isolates had a broad range of antibiotic-resistance patterns [Table 2]). All isolates were fully resistant to penicillin G and amoxicillin/clavulanic acid. Rates of resistance to non−β-lactam antibiotics were 96% to erythromycin (n = 52), 52% to clindamycin (n = 28), and 48% to ciprofloxacin (n = 26). In addition, 40 (74%) isolates were noted to be multiply resistant; that is, typically resistant to β-lactam plus 2 or more antibiotics of ciprofloxacin, erythromycin, and clindamycin. No vancomycin-resistant isolates were identified.

All 54 MRSA isolates were positive for *mec*A and a certain type of SCC*mec.* Four different SCC*mec* types were detected. In our study, 28 (52%) of MRSA isolates belonged to SCC*mec* type V (n = 12) or type IVa (n = 16), which are traditionally associated with CA-MRSA. However, 26 (48%) of the isolates showed the traditional nosocomial SCC*mec* types II (n = 10) and III (n = 16), whereas SCC*mec* type I was completely absent. In addition, SCC*mec* type IVa was found to be circulating in both students' nasal carriers and health care settings (Table 2).

The rates of resistance to ciprofloxacin, clindamycin, and erythromycin among the MRSA SCC*mec* types were as follows: 60%, 80%, and 100%, respectively, among SCC*mec* type II isolates; 88%, 63%, and 100%, respectively, among SCC*mec* type III; 25%, 25%, and 88%, respectively, among SCC*mec* type IVa; and 17%, 50%, and 100%, respectively, among SCC*mec* type V. Without exception, all MRSA isolates were fully resistant to penicillin G and amoxicillin/clavulanic acid (Table 2). On the other hand, CA-MRSA isolates were less resistant than HA-MRSA isolates to ciprofloxacin (21% and 77%, respectively) and clindamycin (36% and 69%, respectively). However, by taking into account the SCC*mec* types as well, the dissimilarities in the antimicrobial resistance patterns were small. In fact, CA-MRSA isolates (n = 8) and HA-MRSA strains (n = 8) bearing SCC*mec* type IVa did not show considerable differences in their resistance profiles. This could be explained by the presence of some CA-MRSA resistance profiles in the HA-MRSA such as resistance to clindamycin, erythromycin, amoxicillin/clavulanic acid, and penicillin G (2 isolates), and resistance to erythromycin, amoxicillin/clavulanic acid, and penicillin G (4 isolates), which together accounted for 75% of the CA-MRSA isolates.

Table 2
Distribution of 54 clinical and community methicillin-resistant *Staphylococcus aureus* (MERSA) isolates by staphylococcal cassette chromosome methicillin resistant determinant (SCC*mec)* type and resistance profile

SCC*mec* type	No. of MRSA strains	No. of clinical MRSA strains	No. of nasal MRSA strains	No. (%) of strains resistant to					
				VAN	CIP	CLI	ERY	AMC	PEN
II	10	10	0	0	6 (60)	8 (80)	10 (100)	10 (100)	10 (100)
III	16	16	0	0	14 (88)	10 (63)	16 (100)	16 (100)	16 (100)
IVa	16	8	8	0	4 (25)	4 (25)	14 (88)	16 (100)	16 (100)
V	12	12	0	0	2 (17)	6 (50)	12 (100)	12 (100)	12 (100)
Total	54	46	8	0	26 (48)	28 (52)	52 (96)	54 (100)	54 (100)

VAN, vancomycin; *CIP*, ciprofloxacin; *CLI*, clindamycin; *ERY*, erythromycin; *AMC*, amoxicillin/clavulanic acid; *PEN*, penicillin G.

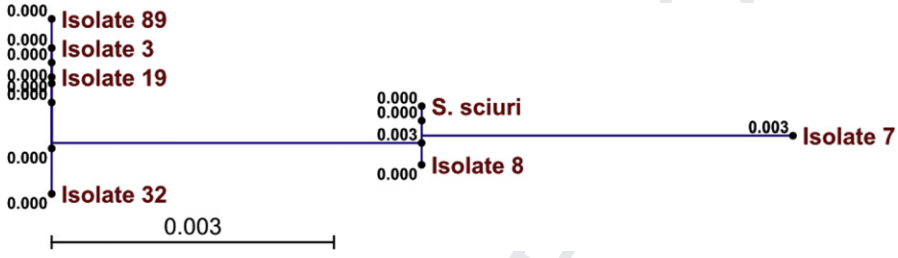

Fig 1. Phylogenetic tree based on the partial nucleotid sequences of the *mec*A gene of 3 selected community-acquired methicillin-resistant *Staphylococcus aureus* isolates (19, 32, and 89), 3 health care settings methicillin-resistant *Staphylococcus aureus* isolates (3, 7, and 8). The phylogenic tree was rooted with the *S sciuri (*GenBank accession No. Y13096). Numbers above branches are bootstrap values. The tree is rooted by the version 5.6.1 of CLC Main Workbench (Manufacturer, City, State).

Partial DNA sequencing of the *mecA* gene was determined in 6 MRSA isolates representing both nasal and clinical isolates. Nucleotide sequence analyses have revealed that the *mecA* gene is much more conserved among all investigated isolates (Fig 1).

DISCUSSION

Nasal carriage of *S aureus* has been demonstrated to be a significant risk factor for nosocomial and community acquired infection in a variety of populations.[8,10,15] The prevalence of nasal carriage of *aureus* in our university student community was 24% and of MRSA was 2%. Although the reduced number of tested samples limits generalizations, the estimated prevalence in our study of *S aureus* nasal carriage and MRSA were within the range reported previously.[15,16]

In our study, maximum resistance of *aureus* was observed toward penicillin G and amoxicillin/clavulanic acid followed by ciprofloxacin, erythromycin, and clindamycin. To our knowledge, there has not been any previous similar study from this region to evaluate the susceptibility of nasal carrier isolates of *S aureus.* However, an increasing trend of resistance is probably due, in part, to the selective pressure resulting from uncontrolled and inappropriate use of erythromycin, ciprofloxacin, and amoxicillin/clavulanic acid antibiotics. This is promoted by the lack of an antibiotic policy and the availability of antibiotics sold over the counter in Palestine. The high rate of resistance has major therapeutic implications, insofar as our population of *S aureus* is associated with multiresistance. Nearly 35% of isolates were noted to be multiply resistant.

Our MRSA strains were often resistant to 5 of the 6 antibiotics, namely erythromycin, clindamycin, ciprofloxacin, and 2 of which were β-lactams, a finding mirrored elsewhere.[4] The total resistance to the β-lactam antibiotics is unsurprising because all isolates described in Table 2 are MRSA, and therefore, inherently resistant to this class of antibiotic. The high levels of resistance seen to erythromycin and clindamycin may in part be due to a single resistance mechanism that affects these antibiotics.

In our health care settings erythromycin, ciprofloxacin, and amoxicillin/clavulanic acid are extensively used for prophylaxis and treatment of MRSA infection; however, the data of this study suggests that these antibiotics are not suitable for use in clinical practice because sustained antibiotic pressure on these less-sensitive isolates could result in the emergence of resistant isolates. Once again using the data recorded in this study, the use of amoxicillin/clavulanic acid, ciprofloxacin, and erythromycin would be limited because resistance to these antibiotics was demonstrated to be 93%, 33%, and 23% among the nasal carrier isolates of *S aureus*, respectively. The data presented in this article highlight the need for a clear understanding of the dynamics of local antibiogram profiles that can then inform the local prescribing policy. The results of this work showed that vancomycin as a form of treatment would work in all cases of *S aureus* and MRSA investigated in this study.

In our study, nearly half (52%) of MRSA isolates belonged to SCC*mec* types IVa and V, which are traditionally associated with CA-MRSA. However, classical nosocomial SCC*mec* types II and III are represented by 48%, whereas SCC*mec* type I was completely absent. The data confirms the tendency of CA-MRSA SCC*mec* type IVa strains to spread in hospital settings as mentioned previously.[17,18]

Our data shows that CA-MRSA strains were less resistant than HA-MRSA strains to non-β-lactam antimicrobial agents such as ciprofloxacin, clindamycin, and erythromycin, (Tables 1 and 2), a finding that has been mirrored elsewhere.[5] However, by taking into account the SCC*mec* types as well, the presence of some CA-MRSA carrying SCC*mec* type IVa resistance profiles within health care settings was observed. This is based on the observation that 75% of CA-MRSA strains carrying small SCC*mec* type IVa showed identical resistance profiles to HA-MRSA.

Sequence analysis shows that the *mecA* genes of the 3 CA-MRSA isolates were identical to that found in health care settings, and therefore the possibility of horizontal transfer must be considered. Moreover, the sequence analysis of *mecA* genes in this study can establish a base for epidemiologic studies, management of outbreaks, and eradication programs of MRSA infections in this region. This is the first report of the *mecA* gene sequence of MRSA in Palestine.

A high level of resistance of MRSA to the commonly used antibiotics has been observed; with SCC*mec* type IVa circulating in both

clinical and community settings in Palestine. These results suggest that efficient control protocols should be adopted in both clinical and community settings. Moreover, rational use of antibiotics and preventing sale of antibiotics without prescriptions should also be considered. Application of this protocol, along with surveillance for antimicrobial resistance of MRSA strains, could prevent the emergence of multidrug-resistant strains.

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