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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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Background: Community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is a major global problem. This study attempted 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 (SCC*mec*) type of MRSA, 46 clinical MRSA isolates were also included in this study.

Methods: Susceptibility testing was performed by the disc diffusion method. The genetic association of MRSA isolates was investigated by SCC*mec* typing. A selected number of isolates were also used to amplify and sequence *mecA*.

Results: 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.

Conclusion: The findings of this study 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 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 cassette chromosome methicillin resistant determinant (SCC*mec*) 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

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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 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 inch 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, Basingstoke, UK) plate, were further incubated aerobically for 48 hours at 35°C, and subsequently examined for growth. *S 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, St Louis, MO). 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 (Hitachi Ltd, Tokyo, Japan) 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, CLC bio, Aarhus, Denmark). 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	0
Ciprofloxacin	28	33
Penicillin G	84	98
Amoxicillin/clavulanic acid	80	93
Erythromycin	20	23
Clindamycin	10	12
Methicillin	8	9

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%,