Community-acquired meticillin-resistant Staphylococcus aureus in Palestine

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Community-acquired meticillin-resistant Staphylococcus aureus (CA-MRSA) is becoming an important public-health problem. This study attempted to investigate S. aureus and MRSA colonization in nasal swabs obtained from 843 patients without a history of hospitalization at the time of hospital admission and from 72 health-care workers chosen for comparison. Of the patients, S. aureus was detected in 218/843 (25.9 %) and MRSA in 17/843 (2.0 %). Of the health-care workers, S. aureus was detected in 15/72 (20.8 %) and MRSA in 10/72 (13.9 %). The majority of the 27 MRSA isolates exhibited a sensitivity pattern expected for CA-MRSA. Multilocus restriction fragment typing resolved the isolates into eight restriction fragment types. The predominant restriction fragment types were AAACCAA and AAAAAAA, accounting for 51.9 % (14/27) of the MRSA isolates and included CC5 and CC1 groups, respectively. This study thus demonstrated the transmission of CA-MRSA strain types into a health-care setting, emphasizing the need for implementation of a revised set of control measures in both hospital and community settings.

INTRODUCTION

Hospitals worldwide are increasingly concerned with infection by meticillin-resistant Staphylococcus aureus (MRSA). Whereas hospital-acquired MRSA has been a concern for in-patients since the 1960s, the threat of community-acquired MRSA (CA-MRSA) has recently been associated with healthy people without traditional risk factors (Carleton et al., 2004; Seybold et al., 2006; Zetola et al., 2005).

Strains of CA-MRSA are more frequently susceptible to a variety of non-β-lactam antibiotics. Although a small percentage contain SCCmec type V, these strains predominantly carry SCCmec type IV, which is smaller in size than the gene cassette found in most strains of hospital-acquired MRSA (types I, II and III). This observed differential in SCC size may allow more efficient transfer of resistance among different bacteria (Albrich & Harbarth, 2008; Boyle-Vavra et al., 2005; Carleton et al., 2004; File, 2007; Oliveira et al., 2006; Weber, 2005; Zetola et al., 2005). Several reports have described the entry of CA-MRSA strain types into health-care settings (Albrich & Harbarth, 2008; Carleton et al., 2004; File, 2007; Klevenst et al., 2006; Zetola et al., 2005). No articles have documented CA-MRSA emergence in Palestine. The objectives of this investigation were to describe trends in S. aureus and MRSA infections in Palestine, to explore the transmission of these strains into health-care settings and to characterize the CA-MRSA strains circulating in Palestine.

METHODS

Clinical specimens. This study was conducted over a period of 1 year (2003) to determine the carriage rate and characteristics of CA-MRSA in patients admitted to Ramallah Governmental Hospital, Palestine. Ramallah Governmental Hospital is the biggest hospital in the West Bank. It is located in the city of Ramallah and serves 16 municipalities, 53 villages and five refugee camps. A total of 843 nasal swabs were obtained from patients who had no contact with health-care workers at the time of hospital admission and analysed for the presence of S. aureus. For the purpose of comparing the carriage ratio of S. aureus in the study group, 72 volunteer health-care workers working in close contact with patients in the Internal Medicine Wards were also included in this study. S. aureus isolates were defined as catalase-producing Gram-positive cocci that were positive for coagulase activity.

Antimicrobial susceptibility testing. All S. aureus isolates were tested for meticillin resistance. The disc-diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 1990) was used with a 1 μg oxacillin disc (Oxoid). Zone sizes were read after incubation at 35 °C for 24 h. Isolates with zone sizes ≤10 mm were considered to be meticillin resistant. Isolates giving a zone of ≤10 mm were confirmed as meticillin resistant by the ability of the isolates to grow on Mueller–Hinton agar (Oxoid).

Abbreviations: CA-MRSA, community-acquired meticillin-resistant Staphylococcus aureus; MLRFT, multilocus restriction fragment typing; RFT, restriction fragment type.
supplemented with 4 % sodium chloride and 4 μg oxacillin l⁻¹ (McDougal & Thornsberry, 1986). Genetic resistance to meticillin was verified by detection of the meCA gene (Predari et al., 1991). A standard strain of S. aureus (ATCC 25923) was used as a control. Meticillin-resistant isolates were tested against other antibiotics using the disc-diffusion method (NCCLS, 1990).

**Multilocus restriction fragment typing (MLRFT).** MLRFT was performed as described previously (Diep et al., 2003).

**DNA extraction.** DNA was isolated as described previously (Unal et al., 1992). One loopful of S. aureus cells was harvested from agar plates. Cells were resuspended in 50 μl lysostaphin (Sigma Chemical) at 100 μg ml⁻¹ and incubated for 10 min at 37 °C. Proteinase K at a concentration of 100 μg ml⁻¹ and 150 μl 0.1 M Tris/HCl (pH 7.5) were then added, followed by incubation for 10 min at 37 °C and for 5 min at 95 °C. The cell debris was pelleted by centrifugation at 8000 g for 5 min and the supernatant containing the released DNA was transferred to a fresh microcentrifuge tube. PCR amplification was performed in a 50 μl reaction volume containing 4 μl of the boiled whole-cell lysate, 1 μM each forward and reverse primer, 0.2 mM each dNTP, 1.25 U Taq DNA polymerase and 5 μl 10 × buffer B with 1.5 mM MgCl₂ supplied with the polymerase (MBI Fermentas). Seven housekeeping genes were amplified in PCRs using primers and PCR cycling conditions described previously for MLRFT (Enright et al., 2000). Amplicons were subjected directly to digestion with restriction endonucleases by adding 10 μl DNA amplicon to 20 μl of a reaction mixture containing 3 μl 10 × digestion buffer and 5 U restriction enzyme. Complete digestion was achieved without prior purification of the PCR amplicon. The restriction enzymes used alone or in combination for each locus were HinfI, AluI, Tsp509I, CfoI, RsaI, BbuI, MboI, VspI and DdeI. CfoI and DdeI were purchased from Roche and the other restriction enzymes were purchased from MBI Fermentas.

Restriction fragments were electrophoresed on 4.0 % agarose gels from Roche and the other restriction enzymes were purchased from MBI Fermentas. Seven housekeeping genes were amplified in PCRs using primers and PCR cycling conditions described previously for MLRFT (Enright et al., 2000). Amplicons were subjected directly to digestion with restriction endonucleases by adding 10 μl DNA amplicon to 20 μl of a reaction mixture containing 3 μl 10 × digestion buffer and 5 U restriction enzyme. Complete digestion was achieved without prior purification of the PCR amplicon. The restriction enzymes used alone or in combination for each locus were HinfI, AluI, Tsp509I, CfoI, RsaI, BbuI, MboI, VspI and DdeI. CfoI and DdeI were purchased from Roche and the other restriction enzymes were purchased from MBI Fermentas.

Restriction fragments were electrophoresed on 4.0 % agarose gels (Sigma) stained with ethidium bromide alongside a 50 bp DNA ladder (Roche). The gels were visualized on a UV transilluminator and photographed.

Restriction fragment types (RFTs) were defined by the combination of alleles at the seven loci in the order arcC–aroE–glpF–gmk–pta–tpi–yqiL (e.g. RFT-BBBBBA).

**RESULTS**

**Bacteriological screening**

A total of 218 (25.9 %) S. aureus isolates from 843 nasal samples were obtained from patients at the time of hospital admission during the study period. MRSA was detected in 17 samples (2.0 %). S. aureus was identified in 15/72 (20.8 %) nasal swabs of health-care workers and MRSA was isolated in 10/72 (13.9 %).

The 27 MRSA isolates in our sample population had a broad range of antibiotic-resistance patterns (Table 1). All isolates were fully resistant to ampicillin. Rates of resistance to non-β-lactam antibiotics were 29.6 % to ciprofloxacin (eight isolates), 18.5 % to erythromycin (five isolates), 11.1 % to clindamycin (three isolates) and 7.4 % to tetracycline (two isolates). No vancomycin-resistant isolates were identified.

Analysis of the 27 MRSA isolates revealed eight RFTs by MLRFT. The predominant RFTs, AAACCAA and AAAAAAA, were represented by eight (29.6 %) and six (22.2 %) of the isolates, respectively. Other less-frequent MRSA RFTs were CAACAC and AABBCB (four isolates each), AAJBCBC (two isolates), and AAAAAAA, AABBBAB and BAAACAC (one isolate each) (Table 2). Because of the translational property between MLRFT and multilocus sequence typing, the common MRSA RFTs could provisionally be identified as belonging to sequence types corresponding to known MRSA clonal lineages: RFT-AAAACAA to the new CC5 strain and AAAAAAA to the CC1 group (Enright et al., 2002).

Interestingly, 17 (63.0 %) of the isolates were noted to be susceptible to non-β-lactam antibiotics such as ciprofloxacin, erythromycin, tetracycline and clindamycin. These isolates were detected in six RFT groups. It is noteworthy that, among these six RFT groups, four groups, AAACCA, AAAAAA, AAABCB and AAJBCB, were found to be circulating in both community and health-care settings.

In the present study, three of the eight isolates in the common RFT-AAAACCA group were resistant to ciprofloxacin, a much higher proportion than that found in any of the other RFT groups containing MRSA isolates. Furthermore, these MRSA strains exhibited resistance to only a few antibiotics (to erythromycin in three of the eight isolates and to clindamycin in two of the eight isolates); this was in contrast to the typical pattern of resistance seen in the second most common group, RFT-AAAAAA. The RFT-AAAAAA isolates were fully susceptible to ciprofloxacin, erythromycin, tetracycline and clindamycin.

**DISCUSSION**

CA-MRSA infections are becoming more widely reported, although the prevalence of MRSA carriage overall remains low in healthy persons in the community (Harbarth et al., 2005; Salgado et al., 2003).

In our study, the rate of colonization of S. aureus among healthy patients at the time of hospital admission was
25.9% and carriage of MRSA was 2.0%. Data reported in other studies worldwide show a similar incidence among healthy persons in the community (Salgado et al., 2003; Shobha et al., 2005).

Of the 72 health-care workers screened, *S. aureus* was detected in 20.8% and MRSA was detected in 13.9%. The prevalence of *S. aureus* carriage was within the range reported previously. In 41 studies, the mean carriage of *S. aureus* was 23.7% [2508/10 589 health-care workers; range 0–40%, 95% confidence interval (CI) 10.7–36.7%] (Albrich & Harbarth, 2008; Shobha et al., 2005; Dimitrov et al., 2003). Although the small number of tested samples limits generalizations, MRSA carriage in the health-care workers in this study was relatively high compared with that reported in many other countries. In 127 studies, the mean prevalence of MRSA was 4.6% (1545/33 318 HCW; range 0–59%, 95% CI 1.0–8.2%) (Albrich & Harbarth, 2008; Dimitrov et al., 2003; Rioux et al., 2007; Shobha et al., 2005).

Analysis of the 27 MRSA isolates by MLRFT placed the isolates into eight RFTs with worldwide distribution as described previously by others (Diep et al., 2003). However, according to the high correlation between the genetic backgrounds compared with other SCCmec types (Albrich & Harbarth, 2008; Carleton et al., 2004; File, 2007; Ito et al., 2001; Robinson & Enright, 2003; Zetola et al., 2005). The emergence of CA-MRSA and the growing presence of a community reservoir for meticillin-resistant strains threaten the future control of antimicrobial resistance in the health-care setting; it is possible that they may develop additional antimicrobial resistance (Klevens et al., 2006; Seybold et al., 2006). This phenomenon could be explained by the presence of some CA-MRSA RFTs within health-care settings such as AAAAAAA (six isolates), AAAACAA (three isolates), AAABCBC (four isolates), AAACCAA (three isolates), and BBBCBC (two isolates) and BBBBBB (one isolate), defined by MLRFT analysis, which together accounted for 63.0% of the isolates (Table 2).

In conclusion, this study has described the emergence of CA-MRSA in Palestine. In addition, similar to the situation that is occurring in other countries, we demonstrated CA-MRSA strain type transmission within a health-care setting, a finding that emphasizes the need for implementation of a revised set of control measures in both hospital and community settings.

**REFERENCES**


### Table 2. Distribution of multilocus RFTs and resistance profiles of 27 MRSA isolates from healthy patients and health-care workers (HCWs)

<table>
<thead>
<tr>
<th>Multilocus RFT*</th>
<th>Total</th>
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<th>HCWs</th>
<th>Resistance profile (no. of isolates)</th>
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<td>5</td>
<td>3</td>
<td>I (4), II (1), III (2), IV (1)</td>
</tr>
<tr>
<td>A A A A A A A</td>
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<td>5</td>
<td>1</td>
<td>I (6)</td>
</tr>
<tr>
<td>C A A A C A C</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>II (3), III (1)</td>
</tr>
<tr>
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<td>3</td>
<td>1</td>
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<tr>
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<td>1</td>
<td>1</td>
<td>I (2)</td>
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<td>1</td>
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</table>

*An RFT was defined by the combination of alleles at the seven loci tested in the order arcC–aroE–glpF–gmk–pta–tpi–yqiL (e.g. RFT-BBBBBBAB).


