

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.

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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; Klevens *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 μ g oxacillin disc (Oxoid). Zone sizes were read after incubation at 35 °C for 24 h. Isolates with zone sizes \leq 10 mm were considered to be meticillin resistant. Isolates giving a zone of \leq 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 I⁻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 (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-BBBBBAB).

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

Table 1. Patterns of resistance to individual antibiotics among 27 MRSA isolates

Resistance pattern*	Resistance profile	No. of isolates (%)
Amp	I	17 (63.0)
Amp, Cip	II	4 (14.8)
Amp, Cip, Ery, Cli	III	3 (11.1)
Amp, Ery	IV	1 (3.6)
Amp, Cip, Ery, Tet	V	1 (3.6)
Amp, Tet	VI	1 (3.7)

*Amp, Ampicillin; Cip, ciprofloxacin; Ery, erythromycin; Cli, clindamycin; Tet, tetracycline.

AAAAAAA, were represented by eight (29.6%) and six (22.2%) of the isolates, respectively. Other less-frequent MRSA RFTs were CAAACAC and AAABCBC (four isolates each), AAJBCBC (two isolates), and AAAACAA, BBBBAB 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-AAACCAA 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, AAACCAA, AAAAAAA, AAABCBC and AAJBCBC, 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-AAACCAA 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-AAAAAAA. The RFT-AAAAAAA 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

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

Multilocus RFT*							Total	Patients	HCWs	Resistance profile (no. of isolates)
A	A	A	C	C	A	A	8	5	3	I (4), II (1), III (2), IV (1)
A	A	A	A	A	A	A	6	5	1	I (6)
C	A	A	A	C	A	C	4	2	2	II (3), III (1)
A	A	A	B	C	B	C	4	3	1	I (4)
A	A	J	B	C	B	C	2	1	1	I (2)
A	A	A	A	C	A	A	1	0	1	VI (1)
B	B	B	B	B	A	B	1	1	0	I (1)
B	A	A	A	C	A	C	1	1	0	V (1)

*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-BBBBBAB).

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; Vivoni *et al.*, 2006), showing that these RFTs have the ability to spread to distant areas.

The majority of the 27 MRSA isolates exhibited sensitivity patterns expected for CA-MRSA, i.e. typically less resistant to non- β -lactam antimicrobial agents such as clindamycin, erythromycin, ciprofloxacin and tetracycline (Table 1), a finding that has been mirrored elsewhere (Albrich & Harbarth, 2008; Carleton *et al.*, 2004; File, 2007; Zetola *et al.*, 2005).

This was a cross-sectional study with a relatively small sample size. Additionally, staphylococcal chromosomal cassette *mec* (SCC*mec*) typing was not performed. However, according to the high correlation between the genotype and antibiogram, we could assume that at least some of these MRSA strains had SCC*mec* type IV/V. SCC*mec* type IV/V has increased mobility and therefore greater potential for horizontal spread to diverse *S. aureus*

genetic backgrounds compared with other SCC*mec* 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 methicillin-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 (one isolate), AAABCBC (four isolates), AAACCAA (three isolates), AAJBCBC (two isolates) and BBBBBAB (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.

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