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CONCISE ARTICLE

Prevalence and genetic relatedness of community-acquired methicillin-resistant *Staphylococcus aureus* in Israel

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Abstract The aims of the study presented here were to determine the prevalence of *Staphylococcus aureus* carriage and, specifically, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) carriage in children and their parents in Israel and to determine the genetic relatedness of these isolates. *S. aureus* was isolated from 580 of 3,373 (17.2%) individuals screened. The predominant type identified by pulsed-field gel electrophoresis was strain ST45-MSSA (25%). Five MRSA isolates were detected, and two of these were classified as CA-MRSA, based on the following criteria: no previous contact with a healthcare facility, absence of a multidrug-resistant (MDR) phenotype, and presence of SCC*mec* type IV. Isolates were negative for *pvl* and were classified as ST-45-MRSA. Although CA-MRSA is still rare in Israel, the genetic relatedness of the strains found in this study to a successful MSSA clone warrants close follow up.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have increased dramatically during the last several decades and have been shown to be associated with higher mortality and costs than those caused by methicillin-sensitive *S. aureus* (MSSA) isolates [1]. In the last decade, community-acquired MRSA (CA-MRSA) has emerged worldwide [2]. CA-MRSA can be distinguished from hospital-acquired MRSA (HA-MRSA) by its susceptibility to many non-β-lactam antibiotics, the presence of staphylococcal cassette chromosome *mec* (SCC*mec*) types IV and V (as opposed to types I–III), and lack of previous patient exposure to a healthcare setting. Many CA-MRSA strains have been reported to carry the Panton–Valentine leukocidin (*pvl*) genes and to cause invasive infections associated with increased mortality. Yet this last characteristic is not required to define CA-MRSA [2–4].

The objectives of this study were to determine the prevalence of CA-MRSA carriage among young children and their parents in Israel and to determine the genetic relatedness of these isolates to other *S. aureus* strains isolated from this population.

Patients and methods

The study was approved by the Sheba Medical Center Ethics Committee. Informed consent was obtained from all patients or their parents or guardians. During February 2002 and October 2002, children aged ≤40 months and their accompanying parents or guardians, who were visiting any of 53 participating primary-care pediatric clinics, were enrolled. The clinics were located in central Israel and belonged to a major health management organization (Maccabi Healthcare Services). Each participant was included only once.

Nasal swabs were obtained from each participant and data regarding demographic characteristics, possible contact with a healthcare facility, the physician's diagnosis of the child on the specific visit, and prior antibiotic treatment, were collected using questionnaires and patient files. *S. aureus* was identified by morphology, β-hemolysis, catalase, DNAase, and coagulase production. Antibiotic susceptibilities were determined using the VITEK-2 system with plate AST P536 (bioMérieux, Hazelwood, MO, USA). Oxacillin resistance was verified with MRSA screening agar, the broth microdilution MIC technique [5], E-test (AB Biodisk, Solona, Sweden) and PCR for the presence of the *mec*A gene (Promega, Madison, WI, USA). *S. aureus* isolates identified in children and their parents (150 isolates) were further analyzed by pulsed-field gel electrophoresis (PFGE) (New England BioLabs, Boston, USA) [6]. SCC*mec* typing [7] and multilocus sequence typing (MLST) [8] were assessed for all MRSA isolates and for the common MSSA clone. The following virulence factors were detected by PCR [9]: the Panton-Valentine leukocidin (*pvl*) locus (*lukS*-PV-*lukF*-PV), *sea*, *seb*, *sec*, *sed*, *see*, *seh*, *sej*, *sel*, *sep*, *egc*, *tst*, *eta*, *etb*, *etd*, *lukE-lukD*, *lukM*, *hlb*, *hlg*, and *hlg2*, *edin*, and *agr* genes. Doubling-times for the five MRSA isolates and the predominant MSSA clone were compared [6].

Table 1 Characteristics of the MRSA strains and the common MSSA strain isolated in this study

Patient characteristic	Isolate					
	1	2	3	4	5	MSSA
Age	25 d	2.2 y	27 y	30 y	39 y	
Gender	Male	Female	Female	Female	Female	
Healthcare contact	Recent hospitalization (perinatal)	Recent hospitalization of a family member	Hospital nurse	None	None	
Isolate characteristics						
Strain identification	ST-247-MRSA-I	ST-5-MRSA-II	ST-5-MRSA-II	ST-45-MRSA-IV	ST-45-MRSA-IV	ST-45-MSSA
PFGE clone	X	Y	Z	F1	F2	F
*mec*A	+	+	+	+	+	Absent
OXA MIC	>256	8	>256	16	16	0.25
Antimicrobial resistance	MDR (PEN, OXA, RIF, TET, CMP, CLD, ERY, OFX, GEN)	MDR (PEN, OXA, CMP, GEN)	MDR (PEN, OXA, ERY, OFX)	β-lactam only (PEN, OXA)	β-lactam only (PEN, OXA)	Susceptible (PEN)
Mean doubling time (min)	25.7±2.5	26.3±3.7	42±2.3	28.8±5.9	28.6±5.9	46.0±3.5
agr type	1	2	2	1	1	1
Leukocidins: PVL gene cluster (*luks*-PV, *lukf*-PV) *lukE-lukD*, *lukM*	lukE-lukD	lukE-lukD	lukE-lukD	Absent	Absent	Absent
TSST-1	Absent	Absent	Absent	Absent	Absent	Absent
Hemolysins	*hlg-2*	*hlg-2*	*hlg-2*	*hlb*	*hlb*	*hlb*
EDIN A–C, Exfoliatin A, B and D	Absent	Absent	Absent	Absent	Absent	Absent
Enterotoxin genes	*egc*, *sea*	*egc*, *sed*	*egc*, *sed*	*egc*	*egc*	*egc*, *sec*, *sel, sep*

d days, *y* years, *NA* not applicable, *PEN* penicillin, *OXA* oxacillin, *RIF* rifampicin, *TET* tetracycline, *CMP* chloramphenicol, *CLD* clindamycin, *ERY* erythromycin, *OFX* ofloxacin, *GEN* gentamicin, *PVL* Panton-Valentine leukocidin, *Hlg* γ hemolysin gene, *hlg-v* γ hemolysin variant gene, *egc* enterotoxin gene cluster, which includes the *seg*, *sei*, *sem*, *sen* and *seo* genes.

Results and discussion

A total of 3,373 persons were sampled, including 1,768 children (55% male, median age 1.4 y, range 14 days–40 months) and 1,605 parents (86.7% mothers, median age 30 y, range 18–55 years). *S. aureus* was isolated from 201 (11.4%) children and from 378 (23.6%) parents. A predominant MSSA clone, exhibiting a common pulsotype, designated clone F, was found in 25% of 150 isolates tested by PFGE. Five MRSA isolates carrying the *mec*A gene were detected, two (1%) in children and three (0.8%) in parents. The MRSA carriage rate was thus 1.1/1,000 in children and 1.8/1,000 in their parents. Three MRSA isolates had a multidrug-resistant (MDR) phenotype (resistance to ≥3 antimicrobial groups). The carriers of these three isolates reported recent contact with a healthcare facility. The carriers of the two non-MDR MRSA isolates were unrelated adults (mothers of non-carrier children), who lived in different cities and who had no apparent contact with each other or with any healthcare facility (Table 1). All five MRSA carriers were healthy on the day of MRSA detection and they had not received antibiotic treatment within the prior month. The two non-MDR MRSA isolates possessed SCC*mec* type IV and were classified as MLST type 45 (ST45-MRSA-IV). Their PFGE pattern was similar to that of the common MSSA isolate, clone F, but their genotype differed in two bands; thus, they were subtyped as F1 and F2 (Fig. 1). The virulence profile was mostly similar to that of the common MSSA, but ST45-MRSA-IV carried only one enterotoxin gene, as compared to four, carried by the ST-45-MSSA strain. The three MDR-MRSA isolates had distinct PFGE patterns, all of which differed from the community *S. aureus* patterns observed and were similar to HA-MRSA clones (Fig. 1, Table 1).

The *pvl* locus was not carried by any of the CA-MRSA isolates. These CA-MRSA isolates grew significantly faster than the genetically related community MSSA isolates (Table 1) and at a similar rate to two of the MDR-MRSA isolates.

The prevalence of CA-MRSA is increasing, but it varies among different countries [2]. Several reports have related the rapid spread to a rapid growth rate [10], and to super-adapted strains, due to the combination of the *pvl* determinant with the *mec*A gene [2]. In our study population we found a low prevalence of MRSA carriage (15/10,000 population), and an even lower "true" CA-MRSA rate.

There is controversy regarding the origin of CA-MRSA strains [11–13]. Recent evidence supports a rapid evolution of CA-MRSA from common MSSA [14, 15]. The two CA-

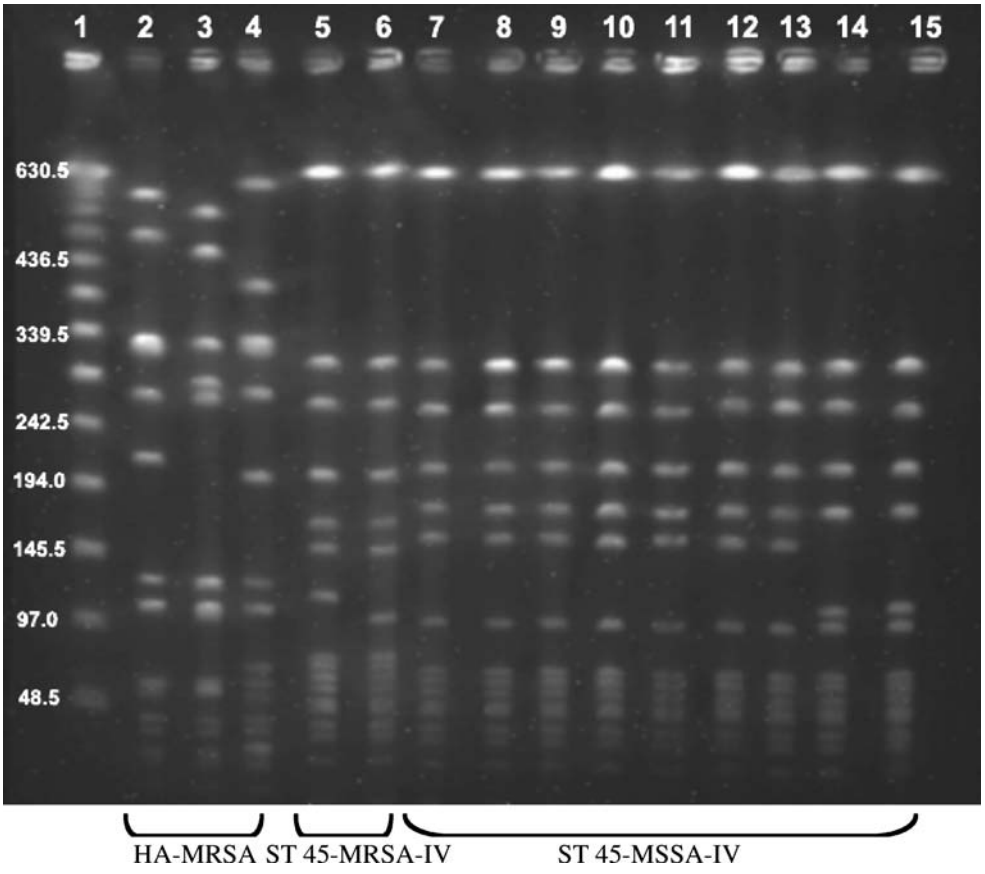

Fig. 1 Pulsed-field gel electrophoresis (PFGE) pattern following *Sma*I restriction of the MRSA and MSSA clones isolated from the community. *Lane 1* lambda DNA ladder molecular-weight marker; *lanes 2–4* HA-MRSA clones (clones X, Y, Z); *lanes 5 and 6* CA-MRSA ST45-MRSA-IV strains (subtypes F1 and F2) possessing a subtype of the common ST 45-MSSA clone — F (*lanes 7–15*)

MRSA isolates described in this study were genetically related to the common MSSA clone (ST45-MSSA) identified in this population, thus supporting the idea of strain evolution. These isolates could either represent two independent incidents of *mec*A gene acquisition or, conversely, spread of a single clone, which could represent a hospital escape. Both scenarios are equally worrisome.

The strain type ST45-MRSA-IV, described herein, differs from the commonly reported CA-MRSA in that it does not carry the *pvl* gene or *agr* type 3. ST45 was previously found in both MRSA and MSSA strains [11, 15]. ST45-MRSA, also known as the Berlin epidemic MRSA or USA600 strain, has been shown to carry different SCC*mec* types (II and IV). Recently, Wannet et al. [15] reported that ST45-MRSA has rapidly emerged in the Netherlands, where it was first detected in 2000 and became endemic by 2002. They found four different SCC*mec* types carried by this clone, and six of the SCC*mec*IV isolates were from different geographical regions, thus suggesting that multiple introductions of SCC*mec* into MSSA have occurred. We recently reported an outbreak caused by this particular strain (non-MDR, SCC*mec* IV, *pvl*-negative, ST-45-MRSA), in a neonatal ICU [4]. This implies that although it is rare in the community (and previously not detected in this hospital), this strain has the potential to spread rapidly and to cause lethal infections. Furthermore, the rapid in vitro growth of these isolates compared to their suspected progenitor MSSA strain, suggests a competitive advantage for rapid spread. Interestingly, the CA-MRSA strain also differed from the ST-45-MSSA strain in its enterotoxin gene profile. The significance of this observation has yet to be determined.

The results of this study should be interpreted with respect to two components of its design: (1) the population was sampled from primary-care clinics, which may not accurately represent the healthy population; (2) the reference MSSA strain was obtained from children and their parents, who both carried *S. aureus*. This strain might not be fully representative of the common MSSA strain(s) in the general population. Further surveillance and continuous follow-up is required in order to determine the actual rate at which such strains are evolving as well as their pathogenic potential.

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References

1. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y (2003) Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylocccus aureus* bacteremia: a meta-analysis. Clin Infect Dis 36:53–59
2. Netola N, Francis JS, Nuermberger EL, Bishai WR (2005) Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis 5:275–286
3. Vandenesch F, Naimi T, Enright MC et al (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton–Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9:978–984
4. Regev-Yochay G, Rubinstein E, Barzilai A, Carmeli Y, Kuint J, Etienne J, Blech M, Smollen G, Maayan-Metzger A, Leavitt A, Rahav G, Keller N (2005) Methicillin-resistant *Staphylococcus aureus* in neonatal intensive care unit. Emerg Infect Dis 11:453–456
5. National Committee for Clinical Laboratory Standards (2005) Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement. M100-S15. NCCLS, Wayne, Pa
6. Schwaber MJ, Navon-Venezia S, Leavitt A, Mekuzas Y, Hammer-Munz O, Schlesinger J, Schwartz D, Carmeli Y (2004) Failure of broth-based tests to detect methicillin-resistant *Staphylococcus aureus* in a clinical specimen. Eur J Clin Microbiol Infect Dis 23:348–351
7. Oliveira DC, de Lencastre H (2002) Multiplex PCR strategy for rapid identification of structural types *anc* variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 46:2155–2161
8. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38:1008–1015
9. Jarraud S, Mougel C, Thiolouse J, Lina G, Meugnier H, Forey F et al (2002) Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles) and human disease. Infect Immun 70:631–641
10. Okuma K, Iwakawa K, Turnidge JD et al (2002) Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol 40:4289–4294
11. Enright MC, Ashley Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG (2002) The evolutionary history of methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci USA 99:7687–7692
12. Aires de Sousa M, de Lencastre H (2003) Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. J Clin Microbiol 42:3806–3815
13. Charlebois ED, Perdreau-Remington F, Kreiswirth B, Bangsberg DR, Ciccarone D, Diep BA, Ng VL, Chansky K, Edlin B, Chambers HF (2004) Origins of community strains of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 39:47–54
14. Holden MT, Feil EF, Lindsay JA et al (2004) Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci USA 101:9786–9791
15. Wannet WJB, Spalburg E, Heck MEOC, Pluister GN, Willems RJL, de Neeling AJ (2004) Widespread dissemination in the Netherlands of the epidemic Berlin methicillin-resistant *Staphylococcus aureus* clone with low-level resistance to oxacillin. J Clin Microbiol 42:3077–3082