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Molecular characteristics of community-associated methicillin-resistant *Staphylococcus aureus* colonizing surgical patients in Greece☆,☆☆

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ABSTRACT

Fifty-one of 925 patients screened for methicillin-resistant *Staphylococcus aureus* (MRSA) upon admission to a surgical unit were MRSA carriers; 15 were classified as community- and 36 as hospital-associated-MRSA. Fourteen of 22 isolates typed by molecular methods belonged to the European clone ST80-IVc, 3 of which exhibited resistance to ≥3 non–β-lactam antibiotics.

Community-associated methicillin-resistant *S. aureus* (CA-MRSA) is an important cause of recurrent skin infections and severe necrotizing pneumonia in healthy individuals in the community (David and Daum, 2010). Although CA-MRSA strains are usually susceptible to many non–β-lactam antibiotics, emergence of resistance to multiple classes of antibiotics has become increasingly recognized in several community-associated clones, such as those of ST8, ST80, ST59, and ST772 genetic lineages, adding to the overall burden of infection caused by this pathogen (Ellington et al., 2009; Francois et al., 2008; Hanssen et al., 2005).

Since its first identification in Greece in 1998 (Aires de Sousa et al., 2003), the so-called European clone (ST80-IVc, PVL-positive) has been recognized with increasing frequency as a cause of CA-MRSA infections both in children and in adults (Katopodis et al., 2010; Niniou et al., 2008; Vourli et al., 2009). More importantly, MRSA clones of community origin could become endemic in the healthcare setting causing severe infections in hospitalized patients (Popovich et al., 2008). According to a previous study, PVL-positive community-associated clones account already for 45% of healthcare-associated (HA) MRSA infections at several hospitals in Greece (Chini et al., 2006).

It is well established that the colonizing strains of *S. aureus* are the ones that usually cause infection in surgical patients. Therefore, it is important to know what proportion of MRSA colonizing surgical patients in our geographic region belongs to community clones and what are the characteristics of the strains colonizing this patient population. Herein, we report the molecular typing and the antimicrobial susceptibility patterns of MRSA isolates from surgical patients screened upon admission to surgical wards.

Consecutive patients admitted to a surgical unit of a tertiary care hospital in Athens, Greece, between January and July 2009, were screened within 48 h of admission for MRSA carriage. The study was approved by the Institutional Review Board of Laiko General Hospital. The nares and inguinal area were sampled with a sterile swab, and

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samples were inoculated onto BBL CHROMagar MRSA II medium. Isolates were classified as CA-MRSA if patients did not have surgery, hospitalization, or hemodialysis within the previous year; did not reside in a chronic-care facility during the previous year; did not have indwelling catheters at the time of sampling; and had no previous isolation of MRSA, according to Centers for Diseases Control and Prevention epidemiologic definition (Klevens et al., 2007). Antimicrobial susceptibility to ciprofloxacin, erythromycin, clindamycin, fusidic acid, co-trimoxazole, gentamicin, tetracycline, and rifampin was determined for all CA-MRSA isolates, by disk-diffusion testing, using Kirby–Bauer disks and interpreted according to Clinical and Laboratory Standards Institute criteria (CLSI, 2011). Genomic DNA for polymerase chain reaction analysis was prepared using the QIAamp DNA Mini and Blood Mini kit (Qiagen, Hilden, Germany). All MRSA isolates were examined for the presence of Panton–Valentine leukocidin (PVL) and *mecA* genes as previously described (Krziwanek et al., 2007). SCC*mec* typing and subtyping, *spa* typing, and multilocus sequence typing (MLST) were performed for all CA-MRSA isolates and for a selected number of HA-MRSA as described previously (Enright et al., 2000; Harmsen et al., 2003; Milheirico et al., 2007; Zhang et al., 2005). The amplicons were purified, and sequence analysis was performed with the BigDye Terminator kit v.3.1 (Applied Biosystems, CA, USA) using the 3730 DNA Analyser (Applied Biosystems, CA, USA), Biogenomica, Athens, Greece. DNA alignment was performed with the BLAST software (http://www.ncbi.nlm.nih.gov). The sequence types (ST) of MRSA isolates were assigned through the MLST database (http://saureus.mlst.net/sql/multiplelocus.asp), and the *spa* types were determined using the BioNumerics Demo Web Server (http://bnas.applied-maths.com/spaupload.aspx). All MRSA-colonized patients were followed prospectively for development of infection. The infection was presumed to be due to the same strain if the colonizing and infecting isolates had the same ST, *spa* type, and SCC*mec* type or subtype.

A total of 925 patients (51% males; median age, 62.8 years) were screened, of whom 51 (5.5%) were positive for MRSA at the time of admission. Fifteen (29.4%) of 51 MRSA isolates fulfilled the epidemiologic criteria for CA-MRSA of which 9 belonged to community MRSA clones and 6 to hospital MRSA clones. The community clones were ST80-IVc ($n = 7$) and ST30-IVc ($n = 2$), whereas the hospital clones were ST239-III ($n = 2$), ST5-II ($n = 2$), ST105-II ($n = 1$), and ST22-IVc ($n = 1$) as shown in the Table 1. Of note, one of the PVL-negative community MRSA clones (ST30-IVc) was of a novel *spa* type, t8767 (http://spa.ridom.de/spatypes.shtml). Of 36 HA-MRSA isolates, as designated by epidemiologic criteria, 7 of the 8 PVL-positive isolates that were analyzed belonged to the community MRSA clone, ST80-IVc (Table 1). The remaining 28 PVL-negative HA-MRSA isolates were examined only for the presence of SCC*mec* elements: 4 isolates carried the SCC*mec* II element; 6, the SCC*mec* III; 13, the SCC*mec* IV; 2, the SCC*mec* V; 2, the SCC*mec* VI; and 1 was not typable.

Similarly with a previous report (Katopodis et al., 2010), the most common antimicrobial resistance pattern observed in the ST80-IVc clone was resistance to fusidic acid and tetracycline. Three of the 14 PVL-positive isolates of the ST80-IVc genetic lineage exhibited resistance to 3 or more non–β-lactam classes of antibiotics (Table 1; isolate nos. 6, 7, and 21). As expected, the isolates belonging to hospital MRSA clones (Table 1; isolates nos. 11, 15, 17–19) exhibited more extensive antimicrobial resistance profile. Two of the patients colonized with the ST80-IVc community clone at the time of admission (Table 1; patient nos. 1 and 24) developed clinical infection by the same MRSA strain during their hospital stay.

This work provides important information on the molecular characteristics of MRSA that had colonized patients prior to admission to a surgical unit of a Greek tertiary care hospital. At least one-third of MRSA-colonizing isolates belonged to the ST80-IVc clone, indicating that this clone is the most predominant among the community MRSA clones circulating in our region, as it seems to be in most European countries (Otter and French, 2010). Moreover, 2 patients colonized with this particular clone developed clinical infection by the same strains, as was evidenced by the identical ST, *spa* type, and SCC*mec* of the respective colonizing and infecting isolates. These findings suggest that the ST80-IVc clone not only is an epidemiologically successful clone but also has the potential to become a nosocomial pathogen. In view of the spread of community MRSA clones in the healthcare setting and the probable diffusion of hospital clones to the community, the molecular typing of MRSA isolates appears to be not so useful a method for designating MRSA isolates as community or hospital acquired.

More worrisome is the emergence of multidrug resistance in several community MRSA clones and particularly in the ST80-IVc clone. This is in accordance with other studies that have observed multiresistant isolates belonging to the ST80 lineage (Hanssen et al.,

Table 1
Genetic characteristics and phenotypes of MRSA colonizing surgical patients.

No.	PVL	ST	SCC*mec*	*spa*	Ciprofloxacin	Erythromycin	Clindamycin	Fusidic acid	Co-trimoxazole	Gentamicin	Tetracycline	Rifampin
CA-MRSA isolates												
1	+	80	IVc	t044	S	S	S	R	S	S	R	S
2	+	80	IVc	t044	S	S	S	R	S	S	R	S
3	+	80	IVc	t044	S	S	S	R	S	S	R	S
4	+	80	IVc	t044	S	S	S	R	S	S	R	S
5	+	80	IVc	t044	S	S	S	R	S	S	R	S
6	+	80	IVc	t044	S	R	R	R	S	S	R	S
7	+	80	IVc	t131	R	R	R	R	S	S	R	S
8	+	30	IVc	t8026	S	S	S	S	S	S	S	S
11	−	239	III	t421	R	R	R	R	R	S	R	S
12	−	22	IVc	t005	S	S	S	S	S	S	S	S
13	−	30	IVc	t8767	S	S	S	S	S	S	S	S
15	−	105	II	t002	R	R	R	R	S	S	S	S
17	−	5	II	t002	R	R	R	S	R	S	S	S
18	−	5	II	t002	R	R	R	S	R	S	S	S
19	−	239	III	t1152	R	R	R	R	R	R	R	S
HA-MRSA isolates												
20	+	80	IVc	t044	S	S	S	R	S	S	R	S
21	+	80	IVc	t044	S	S	S	R	S	R	R	S
22	+	80	IVc	t044	S	S	S	R	R	S	R	S
23	+	80	IVc	t131	S	S	S	R	S	S	R	S
24	+	80	IVc	t044	S	S	S	R	S	S	R	S
25	+	80	IVc	t044	S	S	S	R	S	S	R	S
26	+	80	IVc	t044	S	S	S	R	S	S	R	S

PVL = Panton–Valentine leukocidin; ST = multilocus sequence type; S = susceptible; R = resistant.

2005; Katopodis et al., 2010). In Greece, one of the most common resistance patterns exhibited by the ST80-IV clone is resistance to fusidic acid, tetracycline, erythromycin, and clindamycin (Katopodis et al., 2010). Notably, in the present report, 1 isolate belonging to the ST80-IVc clone and to a unique *spa* type (t131) expressed a further increase in its resistance repertoire including 5 different classes of non–β-lactam antibiotics, namely, quinolones, macrolides, clindamycin, fucidic acid, and tetracyclines.

In conclusion, a significant proportion of the MRSA colonizing surgical patients in our geographic region belongs to the epidemiologically successful ST80-IVc European clone which has the potential to become multidrug resistant and cause nosocomial infections. These findings raise serious concerns and cause new challenges in infection control practitioners and clinicians.

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