ORIGINAL ARTICLE BACTERIOLOGY

High prevalence of methicillin-resistant *Staphylococcus aureus* clone ST80-IV in hospital and community settings in Algiers

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Abstract

USA300 is an epidemic community-acquired methicillin-resistant *Staphylococcus aureus* (C-MRSA) clone in the USA, whereas the European C-MRSA clone ST80-IV has mainly a sporadic diffusion in Europe. The prevalence of European clone ST80-IV in Algeria is poorly documented. We prospectively studied *S. aureus* infections at Mustapha Bacha hospital in Algiers over a 20-month period. *S. aureus* nasal colonization was studied during a further 6-month period. The European clone ST80-IV was responsible for more than one-third of both community infections (35.7%) and hospital infections (35.8%). Panton–Valentine leukocidin (PVL)-positive MRSA isolated from hospital inpatients were resistant to multiple antibiotics, including fluoroquinolones in 44.9% of cases. The PVL-positive MRSA nasal carriage rate was high among patients and staff in the dermatology unit (8.7% and 18.5%, respectively), but low (2.7%) among patients attending the outpatient clinic. The European PVL-positive C-MRSA clone ST80-IV is widespread in the Algiers hospital and community settings.

Keywords: Community-acquired infections, hospital, infections, methicillin-resistant *Staphylococcus aureus*, Panton–Valentine leukocidin
Original Submission: 16 February 2010; **Revised Submission:** 29 April 2010; **Accepted:** 17 May 2010
Editor: J.-L. Mainardi
Article published online: 27 May 2010
Clin Microbiol Infect 2011; **17:** 526–532
10.1111/j.1469-0691.2010.03273.x

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Introduction

Staphylococcus aureus is the main cause of suppurative infections due to secreted toxins. Community-acquired methicillin-resistant *S. aureus* strains (C-MRSA) are spreading worldwide, in the form of a limited number of clones, including USA300 in the USA, ST80-IV in Europe, and the South-west Pacific clone in Asia and Oceania [1,2]. New C-MRSA clones are continually emerging [1], and most of them possess the Panton–Valentine leukocidin (PVL) genes. These PVL-positive C-MRSA clones are commonly responsible for skin and soft-tissue infections, but can also cause life-threatening infections such as necrotizing pneumonia [3], fasciitis [4], and bone and joint infections [5,6].

The prevalence of MRSA in the community varies considerably from one country to another. In the USA, MRSA have been isolated from up to 59% of patients with community-acquired infections [7]. The prevalence is generally low in Europe but reaches 45% in Greece [8]. In the USA and Greece, PVL-positive MRSA have also become prevalent in hospitals, accounting for, respectively, 8% and 25% of isolates [9,10], thus blurring the historical distinction between hospital- and community-acquired MRSA. Once established in the hospital environment, C-MRSA frequently becomes resistant to multiple antibiotics, including fluoroquinolones [11–13].

In Algeria, the European clone ST80-IV has been detected [14]. Most Algerian PVL-positive MRSA isolates are resistant to kanamycin, tetracycline and fusidic acid, and resistance to fluoroquinolones has also been described in hospital isolates [14]. To assess the prevalence of PVL-positive MRSA in Algeria, we conducted a prospective study at Mustapha Pacha hospital in the capital, Algiers, from April 2006 to December 2007.

Materials and Methods

Patients and MRSA isolates

From April 2006 to December 2007, a total of 700 *S. aureus* isolates (all found during the study period) were recovered

from 663 patients at Mustapha Bacha University Hospital, a 1800-bed facility located in Algiers. As defined by the Centers for Disease Control and Prevention (Atlanta, GA, USA), a case was considered to be community-acquired (by analysis of paper-based patient records) when MRSA infection was identified <48 h after admission in a patient with no history of hospitalization, surgery, catheterization, prosthesis placement or positive MRSA culture in the previous year. The characteristics recorded in an epidemiological database were: gender, date of birth, type of sample, diagnosis, and ward. Written informed consent to participate in the study was obtained from the patient or guardians, and the protocol was approved by the ethics committee of Mustapha Bacha Hospital. Two hundred and twenty-one *S. aureus* isolates were randomly selected for further analysis. *S. aureus* identification was based on colony morphology, microscopic examination, and the coagulase rabbit plasma and Staphyslide agglutination tests (bioMérieux, Marcy l'Etoile, France).

Nasal carriage screening

From January to June 2008, 105 consenting patients and 27 volunteer staff of the dermatology unit were prospectively screened for *S. aureus* nasal carriage. During the same period, 300 consenting adults attending the outpatient clinic were also screened. None of these 432 adults was being treated for *S. aureus* infection. The nasal samples were obtained with a sterile cotton swab and processed in the bacteriology department within 4 h.

Antimicrobial susceptibility testing

Susceptibility to penicillin, oxacillin, cefoxitin, kanamycin, tobramycin, gentamicin, erythromycin, clindamycin, tetracycline, ofloxacin, fosfomycin, trimethoprim/sulfamethoxazole and rifampicin was determined by the disk diffusion method, as recommended by the CLSI [15]. Comité de l'Antibiogramme de la Société Française de Microbiologie interpretative criteria were used for pristinamycin and fusidic acid [16].

DNA extraction and *agr* allele detection

The isolates were grown on brain-heart infusion agar or in brain-heart infusion broth at 37°C overnight. Genomic DNA was extracted with a standard procedure [17]. Amplification of *gyr*A was used to confirm the quality of each DNA extract and the absence of PCR inhibitors. All PCR products were analyzed by electrophoresis on ethidium bromide-stained 1% agarose gels (Sigma, Lyon, France). The accessory gene regulator alleles (*agr* types 1–4) were detected by PCR as described previously [17].

*Mec*A gene detection and SCC*mec* typing

The *mec*A gene coding for methicillin resistance was detected by PCR as described previously [18]. The staphylococcal chromosomal cassette *mec* (SCC*mec* I-IV) was detected as described by Oliveira *et al.* [19], and the SCC*mec* type V was detected as described previously [20]. The *S. aureus* reference strains used as controls were: COL (SCC*mec* I), BK2464 (SCC*mec* II), HU106 (SCC*mec* III) and BK2529 (SCC*mec* IV).

Detection of toxin genes

PCR was used to detect 22 specific staphylococcal virulence genes, as described previously [17,21]. The isolates were screened for sequences specific for staphylococcal enterotoxin genes, as well as the toxic-shock syndrome toxin gene (*tst*), exfoliative toxin genes, PVL genes, *luk*E-*luk*D leukocidin genes, the class F l*uk*M leukocidin gene, hemolysin genes, and epidermal cell differentiation inhibitor (*edin*) genes.

PFGE fingerprinting

*Sma*I macrorestriction patterns were obtained with a contour-clamped homogeneous electric field system (DR-II; Bio-Rad, Hercules, CA, USA), as described previously [22]. Resolved macrorestriction patterns were compared as recommended by Tenover *et al.* [23]. Strains that differed by up to three fragments were considered to be subtypes of the same clonal type.

Spa typing

Spa typing was performed as described previously [24,25]. The *x* region of the *spa* gene was amplified by PCR and the *spa* type was determined with RIDOM STAPH TYPE software (Ridom GmbH, Würzburg, Germany).

Multilocus sequence typing

Multilocus sequence typing was performed on representative strains of each clonal group, as described previously [26]. The allelic profile of each strain was obtained by sequencing internal fragments of seven housekeeping genes that defined sequence types [26]. Similar sequence types were grouped together into clonal complexes.

Statistical analysis

Continuous variables are expressed as medians and interquartile ranges, and categorical variables as frequencies and percentages. Nonparametric tests were used to compare distributions (chi-square test or Fisher's exact test for categorical variables; and Student's *t*-test, after log transformation if necessary). $p < 0.05$ was considered statistically significant. All analyses were performed using EPIINFO software, version 3.4.3

(Centers of Disease Control and Prevention, Atlanta, GA, USA).

Results

Clinical characteristics of community and hospital infections due to both methicillin-susceptible (MSSA) and – resistant (MRSA) *Staphylococcus aureus* strains

The 221 *S. aureus* infections were community-acquired in 38% of cases (n = 84) and hospital-acquired in 62% of cases (n = 137). The most frequent diagnoses were skin and soft-tissue infections (75% and 69.4%, respectively, of community and hospital infections), bone and joint infections (9.5% and 12.4%), bacteraemia (3.5% and 8.7%), pneumonia (6% and 4.4%) and others (6.0% and 5.1%); none of these differences was statistically significant. Most community infections were diagnosed in the emergency room or during outpatient visits (53.6%) or in medical wards (36.9%), whereas most hospital infections were diagnosed in medical (65%) or surgical (20.4%) wards (data not shown).

Prevalence and characteristics of community and hospital MRSA infections

MRSA accounted for 40.5% (n = 34) of the 84 community infections and for 47.4% (n = 65) of the 137 hospital infections (no significant difference, table 1). Patients with C-MRSA infections were significantly younger than patients with hospital MRSA infections. Skin and soft-tissue infections were significantly more frequent among patients with C-MRSA than among patients with C-MSSA (p <0.05, table 1). None of the other studied characteristics were significantly different between the four subgroups of patients. Community isolates were PVL-positive MRSA in 35.7% of cases (30/84) and PVL-positive MSSA in 14.3% of cases (12/84) (Table 2). Hospital isolates were PVL-positive MRSA in 35.8% of cases (49/137) and PVL-positive MSSA in 5.8% of cases (8/137). Thus, the proportion of PVL-positive isolates among all *S. aureus* infections was 41.6% (57/137) in the hospital and 50.0% (42/84) in the community (no significant difference; Table 2).

MRSA characteristics

All PVL-positive MRSA were *agr* type 3, and most (n = 79) were positive for the exfoliative toxin D (*etd*) and *edin* genes. The SCC*mec* cassette was always type IV. The *spa* type was determined for 46 isolates, of which 45 were *spa* t044 and one was *spa* t4143 (related to *spa* t044). The sequence type was determined for ten isolates and was always ST80. All these characteristics were consistent with those of the European clone ST80-IV (Fig. 1).

PVL-negative MRSA were *agr* type 1 (n = 3, 75%) or *agr* 3 (n = 1, 25%) in the community, and *agr* 1 (n = 8, 50%), *agr* 2 (n = 7, 43.7%) or *agr* 3 (n = 1, 6.3%) in the hospital. Nine *agr* 1 isolates were related to the Hungarian clone [27]: they possessed an SCC*mec* type III cassette, the *selk* and *selq* genes, and ST241 (a single-locus variant of clone ST239). Two *agr* 1 isolates were ST8, possessed the *sea* genes and an SCC*mec* type V cassette, and were related to the Lyon clone described by Ferry *et al.* [27]. Seven *agr* 2 isolates had characteristics of the paediatric clone [27], possessing an

TABLE 1. Baseline characteristics in 122 cases of methicillin-resistant *Staphylococcus aureus* (MRSA) and 89 cases of methicillin-susceptible *S. aureus* (MSSA) community- and hospital-acquired infections among patients admitted to Mustapha Bacha Hospital, Algiers, between April 2006 and December 2007

	Community infections				Hospital infections			
	MSSA		MRSA		MSSA		MRSA	
Characteristic	*n*	%	*n*	%	*n*	%	*n*	%
Staphylococcus aureus isolates	50	59.5	34	40.5	72	52.6	65	47.4
Demographics								
Median age (years)	39.9		34.7*		38.6		41.3*	
Sex ratio (M/F)	1.94 (33/17)		1.27 (19/15)		1.32 (41/31)		2.09 (44/21)	
Diagnosis								
Skin/soft-tissue infection	33	66**	30	88**	50	70	45	69
Bone/joint infection	7	14	1	3	8	11	9	14
Bacteraemia	3	6	0	0	7	10	4	6
Pneumonia	3	6	2	6	2	3	5	7
ENT or eye infection	3	6	0	0	3	4	1	2
Meningitis	0	0	1	3	1	1	1	2
Urinary tract infection	1	2	0	0	1	1	0	0

*p <0.05, community-acquired MRSA vs. hospital MRSA.
**p <0.05, MSSA vs. MRSA community infections.
ENT, ear, nose and throat.

TABLE 2. Panton–Valentine leukocidin (PVL) genes in 221 *Staphylococcus aureus* isolates recovered from patients admitted to Mustapha Bacha Hospital, Algiers, between April 2006 and December 2007

	Community infections		Hospital infections		
Characteristic	***n***	**%**	***n***	**%**	**p-value**
All Staphylococcus aureus	84		137		
PVL-positive	42	50.0	57	41.6	Ns
PVL-negative	42	50.0	80	58.4	
All MSSA	50		72		
PVL-positive	12	14.3	8	5.8	0.06
PVL-negative	38	45.2	64	46.7	
All MRSA	34		65		
PVL-positive	30	35.7	49	35.8	0.13
PVL-negative	4	4.8	16	11.7	

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

SCC*mec* type IV cassette and, in most cases, the *selm* and *selo* genes. The two *agr* 3 MRSA isolates had all the characteristics of the clone ST80-IV apart from the PVL genes. Hence, except for the two MRSA isolates belonging to clone ST80-IV, the other 18 PVL-negative MRSA isolates belonged to well-known epidemic hospital clones.

Antibiotic resistance and characteristics of fluoroquinolone-resistant PVL-positive MRSA strains

Twenty-two (44.9%) of the 49 PVL-positive H-MRSA isolates exhibited resistance to fluoroquinolones, whereas no fluoroquinolone resistance was noted among the 30 PVL-positive C-MRSA isolates. The characteristics of 22 fluoroquinolone-resistant PVL-positive MRSA isolates were compared with those of 24 fluoroquinolone-susceptible PVL-positive MRSA isolates to determine whether a specific clone was spreading within the hospital. PFGE failed to segregate the two resistance phenotypes into distinct populations, with some pulsotypes being shared by resistant and susceptible isolates (Fig. 1). This indicated that the resistant strains did not correspond to a specific PFGE type. Fluoroquinolone-resistant PVL-positive MRSA strains were more resistant to kanamycin, tobramycin, gentamicin, erythromycin and fusidic acid than were fluoroquinolone-susceptible strains (Table 3). Fluoroquinolone-susceptible strains were more resistant to tetracycline than were fluoroquinolone-resistant strains.

We also compared the clinical and demographic characteristics of patients with fluoroquinolone-susceptible and -resistant PVL-positive MRSA infections. Fluoroquinolone-resistant isolates were associated with older age (median 46.6 and 35.7 years, $p < 0.05$) and with medical wards (found only in the dermatology unit), but not with a particular type of infection.

Nasal carriage

S. aureus nasal carriage was detected in 52 (50%) of 104 patients hospitalized in the dermatology ward. Twenty-three of the 52 isolates (22.1%) were MRSA, of which nine (8.7%) were PVL-positive; four of these nine patients had secondary infections due to PVL-positive MRSA. Interestingly, one patient harboured both PVL-positive and PVL-negative MRSA strains. *S. aureus* nasal carriage was associated with a higher risk of *S. aureus* infection: 12 infections on 52 colonized patients vs. no infection on 52 patients without *S. aureus* nasal carriage (infinite relative risk). Nine (33.3%) of the 27 dermatology unit staff members were *S. aureus* carriers; six isolates were MRSA (22.2%) and five were PVL-positive MRSA (18.5%), one of which was also resistant to fluoroquinolones. Seventy-five (25%) of the 300 outpatients screened for *S. aureus* carriage were positive, of whom ten (3.3%) carried MRSA and six (2.7%) carried PVL-positive MRSA. None of the PVL-positive MRSA isolated from outpatients were fluoroquinolone-resistant.

Discussion

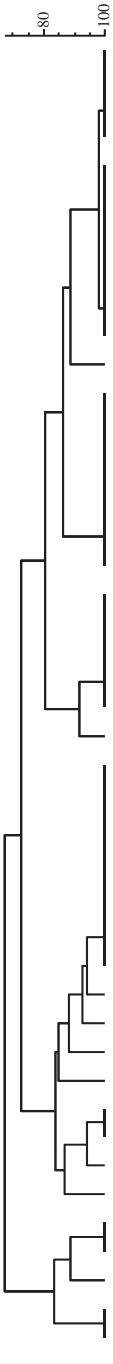
The present study shows that USA300 is not the unique C-MRSA clone to exhibit enhanced capacity to cause widespread epidemic diseases and that C-MRSA clone ST80-IV accounts for more than one-third of infections in an Algiers hospital and in the surrounding community (35.7% and 35.8%, respectively).

It has been suggested that type I ACME could contribute to the epidemic character initially unique to USA300. Because the type I ACME is not found in clone ST80-IV, other shared characteristics must explain the epidemic propensity of clones ST80-IV and USA300 [28].

Seven hundred cases of *S. aureus* infection were included in this 20-month study. Most cases (69–75%) involved skin infections. The first case of necrotizing pneumonia due to C-MRSA was observed in Algiers in 2002. In the USA, the annual number of emergency room visits for skin and soft-tissue infections nearly tripled between 1993 and 2005, coinciding with the emergence of C-MRSA. Thus, it appears that C-MRSA may be causing more disease than displacing other organisms from preexisting ecological niches [29].

As observed by Liu *et al.* [30], *S. aureus* infection tends to affect males more than females. The median age of our patients was 38–40 years overall: 46.6 years among those with fluoroquinolone-resistant PVL-positive MRSA infection, and 34.7 years among those with C-MRSA infection.

The PVL-positive MRSA isolates had characteristics of European clone ST80-IV. This clone has previously been



PFGE pattern	Strains	CA(1)/HA(2)	FQ	*agr*	Toxin genes	*spa* type
	ST20070665	2	S	3	*etd edin*	t044
	ST20070729	1	S	3	*etd edin*	t044
	ST20070668	1	S	3	*etd edin*	t044
	ST20070727	2	S	3	*etd edin*	t044
	ST20070677	2	S	3	*etd edin*	t044
	ST20070693	2	S	3	*etd edin*	t044
	ST20080238	2	S	3	*etd edin*	t044
	ST20080327	2	S	3	*etd edin*	t044
	ST20070747	2	S	3	*etd edin*	t044
	ST20071030	2	S	3	*etd edin*	t044
	ST20080343	2	S	3	*etd edin*	t044
	ST20070691	2	S	3	*etd edin*	t044
	ST20070635	1	S	3	*etd edin*	t044
	ST20070680	1	S	3	*etd edin*	t4143
	ST20070699	1	S	3	*etd edin*	t044
	ST20070736	1	S	3	*etd edin*	t044
	ST20080206	1	S	3	*etd edin*	t044
	ST20080324	1	S	3	*etd edin*	t044
	ST20070749	2	S	3	*etd edin*	t044
	ST20070641	2	R	3	*etd edin*	t044
	ST20070681	2	R	3	*etd edin*	t044
	ST20070732	2	R	3	*etd edin*	t044
	ST20070738	2	R	3	*etd edin*	t044
	ST20070751	2	R	3	*etd edin*	t044
	ST20070659	1	S	3	*etd edin*	t044
	ST20080210	2	R	3	*etd edin*	t044
	ST20080515	2	R	3	*etd edin*	t044
	ST20070753	2	R	3	*etd edin*	t044
	ST20070757	2	R	3	*etd edin*	t044
	ST20080193	2	R	3	*etd edin*	t044
	ST20080198	2	R	3	*etd edin*	t044
	ST20080199	2	R	3	*etd edin*	t044
	ST20080345	2	R	3	*etd edin*	t044
	ST20080200	2	S	3	*etd edin*	t044
	ST20080331	2	R	3	*etd edin*	t044
	ST20070717	2	S	3	*etd edin*	t044
	ST20080212	2	R	3	*etd edin*	t044
	ST20080340	2	R	3	*etd edin*	t044
	ST20070638	2	R	3	*etd edin*	t044
	ST20080239	2	R	3	*etd edin*	t044
	ST20070704	2	R	3	*etd edin*	t044
	ST20080192	1	S	3	*etd edin*	t044
	ST20070703	2	R	3	*etd edin*	t044
	ST20080363	1	S	3	*etd edin*	t044
	ST20070711	2	R	3	*etd edin*	t044
	ST20070723	2	R	3	*etd edin*	t044

FIG. 1. Dendrogram constructed from the schematic representation of the pulsed-field gel electrophoresis types of methicillin-resistant *Staphylococcus aureus* isolates with their genetic characteristics, such as agr allele group, toxin gene content and spa type. Fluoroquinolone (FQ) susceptibility is given: R, resistant; S, susceptible. Strains were community-acquired (CA = 1) or hospital-acquired (HA = 2).

detected in Algeria [31] and Tunisia, and in the Middle East [32]. Sporadic cases of ST80-IV infection reported in many European countries often involve travelers from these countries. For example, clone ST80-IV has been introduced into Denmark from the Middle East on more than one occasion [33].

Hospital-acquired PVL-positive MRSA infections in our study were due to fluoroquinolone-resistant strains in 44.9% of cases. These isolates did not correspond to a specific PFGE type or subtype. Multidrug-resistant PVL-positive C-MRSA isolates usually emerge when they had become responsible for hospital infections. Diep *et al.* [34] also described a USA300 clone isolate that had accumulated multiple resistance genes making it resistant to β-lactams, fluoroquinolones, tetracycline, macrolides, clindamycin and mupirocin [34]. In San Francisco and Boston, multidrug-resistant USA300 strains were found to spread rapidly among men who have sex with men [34]. In the present study,

TABLE 3. Antibiotic resistance profiles of 27 fluoroquinolone-susceptible (FQS) and 22 fluoroquinolone resistant (FQR) Panton–Valentine leukocidin-positive MRSA strains

	FQS strains		FQR strains		
Antibiotic resistance	***n***	**%**	***n***	**%**	**p-value**
Kanamycin	27	100.0	2	9.1	<0.05
Kanamycin-Tobramycin	0	0	10	45.4	<0.05
Kanamycin-Tobramycin-Gentamicin	0	0	10	45.4	<0.05
Tetracycline	18	66.7	7	31.8	<0.05
Erythromycin	6	22.2	19	86.4	<0.05
Cotrimoxazol	0	0	0	0	
Rifampicin	0	0	0	0	
Fosfomycin	0	0	0	0	
Fusidic acid	19	70.4	22	100.0	<0.05

fluoroquinolone-resistant PVL-positive MRSA were solely detected in the hospital setting (mainly in the dermatology unit), suggesting that they were transmitted by healthcare workers or by environmental contamination. We found a very high rate of nasal carriage of PVL-positive MRSA in the hospital setting, especially among patients and staff of the dermatology unit. The risk that multidrug-resistant ST80-IV strains will spread to the community in Algeria is a worrying prospect.

Recent data suggest that nasal colonization may play a less important role than skin–skin and skin–fomite contact in the pathogenesis of C-MRSA infection. A common feature of outbreak and endemic C-MRSA infections is the lack of an identifiable endogenous source of MRSA, such as asymptomatic carriage in the anterior nares [35]. C-MRSA might thus be acquired preferentially from other body sites or from contaminated environments. The relatively low rate of nasal carriage observed in the Algiers community is similar to that reported elsewhere [35].

In conclusion, PVL-positive MRSA are endemic in both the community and hospital settings in Algiers, the capital city of Algeria. If the PVL-positive USA300 clone has spread all over the USA, the ST80-IV is also a highly epidemic clone which has already spread in Algeria.

Acknowledgements

The authors thank David Young for editorial assistance. This work was partly supported by a grant from Nabi Biopharmaceuticals.

Transparency Declaration

We declare that we have no conflicts of interest.

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