Brief Original Article

Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in an Algerian hospital

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Abstract

Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of both hospital- and community-acquired infections worldwide. However, data about the molecular epidemiology of MRSA in North Africa are still scarce.

Methodology: All MRSA isolates recovered between January 2006 and July 2011 from one Algerian hospital were genetically and phenotypically characterized.

Results: The predominance of a European community-associated-MRSA (CA-MRSA) clone (ST80-SCCmec IV-PVL positive) was revealed by this analysis.

Conclusion: Our data suggest that a CA-MRSA clone recently invaded the hospital setting in Algiers and replaced a typical hospital-associated pandemic clone such as the Brazilian clone (ST239-SCCmec IIImercury-PVL negative).

Key words: MRSA; molecular epidemiology; Algeria; DLST.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of both hospital and community-acquired infections worldwide. However, there is a considerable epidemiological variation of MRSA at both regional and global levels [1]. For example, Mediterranean countries likely represent a hyperendemic region for MRSA whereas the proportion of MRSA in northern Europe is much lower [2]. In particular, the proportion of MRSA in southern Mediterranean countries (*e.g.,* Egypt, Tunisia and Algeria) showed a recent spectacular increase [2]. Nevertheless, data about the molecular epidemiology of MRSA in North Africa are still scarce compared to European countries.

Methodology

In this study, all MRSA isolates (one per patient; n = 84) recovered between January 2006 and July 2011 from the Bologhine Ibn Ziri University hospital (250 beds) located in Algiers (Algeria) were characterized. The epidemiological characteristics of these isolates are indicated in Table 1-S. Each bacterial isolate was identified with standard bacteriological procedures (*i.e.,* Gram stain, colonial/morphological appearance, tube coagulase, catalase and DNase tests); susceptibility to 12 common antimicrobial agents (Table 1) was tested using the disc-diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [3].

The genetic diversity of this MRSA population was assessed with the highly discriminatory Double Locus Sequence Typing (DLST) method as previously described [4,5]. This method is based on the analysis of partial sequences (about 500 base pairs) of the variable *clfB* and *spa* genes. Additionally, the staphylococcal cassette chromosome (SCC*mec*) element was determined in each strain using a multiplex PCR method described by Kondo *et al.*; the presence of Panton-Valentine-leukocidin (PVL) genes was tested as previously described [6,7].

Results and discussion

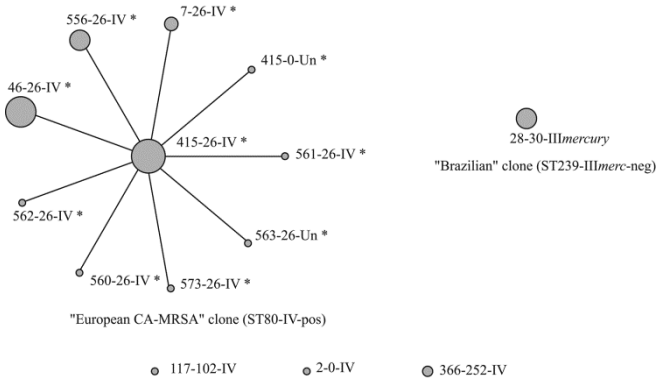
As expected, all MRSA isolates were resistant to penicillin, oxacillin and cefoxitin (Table 1). In contrast, all isolates were susceptible to rifampin, vancomycin and teicoplanin. Variable resistance rates were observed for kanamycin (77.4%), tetracycline

(58.3%), clindamycin (9.5%), co-trimoxazole (23.8%), ofloxacin (15.5%) and fusidic acid (53.6%).

A total of 13 *clfB* and 5 *spa* alleles were observed among the 84 MRSA isolates typed by DLST and these accounted for 13 different DLST types. eBURST software was used to cluster DLST types sharing at least one identical allele (http://eburst.mlst.net/). There is generally a good concordance between DLST clusters and MLST clonal complexes and it is possible to relate these clusters with international clones [8]. This analysis identified two main clusters as well as three singletons (Figure 1). First, the cluster with DLST ancestor 415-26 represents 85.7% (72/84) of all isolates. This cluster corresponds to the European CA-MRSA clone (ST80) and as expected all isolates of this clone were PVL positive [5]. In addition, most isolates of this clone carry the SCC*mec* type IV although it was not possible to identify the *ccr* type in two isolates. Second, the clone with DLST type 28-30 represents 8.3% (7/84) of all isolates. This cluster is related to the Brazilian clone (ST239) and as expected all the isolates of this clone carried the SCC*mec* III*mercury* and were PVL negative [5]. Finally, the remaining five isolates (6.0%) belonged to three single DLST types and could not be associated with international clones. All these isolates carried the SCC*mec* IV and were PVL negative.

The European CA-MRSA (ST80-IV-pos) is the most common community acquired clone in Europe [9,10]. Although this clone has been recovered from many European countries, it has often been associated with patients with Middle Eastern or North African origin [9,10]. This is probably explained by the predominance of the European CA-MRSA clone in Algeria and in surrounding North African countries

Figure 1. DLST single-locus variant clustering of 84 *S. aureus* isolates from the Bologhine Ibn Ziri University hospital in Algeria using eBURST. Each circle represents one DLST type, and the diameter of the circle reflects the frequency (i.e., the number of isolates) of that type. Linked DLST types differ at one of the two loci (*clfB* or *spa*). SCC*mec* type is indicated next to each DLST type ("Un" indicates isolates with unknown ccr type) and each DLST types including PVL-positive isolates are indicated by asterisks. Two isolates had a null allele (*i.e.* 0) at the *spa* locus. The name of the international clone associated with each of the main cluster is indicated under the cluster.



[11-15]. Even though this clone is generally associated with community-acquisitions, our data did not allow discrimination between hospital and community acquisitions. Nevertheless, the widespread occurrence of this clone in Algerian hospitals indicates it has invaded the hospital setting. Moreover, the proportion of isolates belonging to ST80 changed during our study period. Whereas a similar proportion of the Brazilian and European CA-MRSA clone was observed in 2006 (36% vs. 43%), the European CA-MRSA clone accounted for > 90% of the isolates in subsequent years (Table 2). This observation as well as similar previous observations suggest a recent

Table 1. Patterns of susceptibility of the European CA-MRSA and Brazilian clones as well as overall Algerian MRSA isolates.

	Resistant isolates (%) among:		
Antibiotic	**European CA-MRSA isolates (ST80) (n = 72)**	**Brazilian MRSA isolates (ST239) (n = 7)**	**All isolates (n = 84)**
Penicillin	100	100	100
Oxacillin	100	100	100
Cefoxitin	100	100	100
Kanamycine	69.3	100	77.4
Tetracycline	55.6	71.4	58.3
Clindamycin	8.3	0	9.5
Co-trimoxazole	20.8	57.1	23.8
Ofloxacine	8.3	71.4	15.5
Rifampin	0	0	0
Vancomycin	0	0	0
Teichoplanine	0	0	0
Fusidic acid	55.6	42.9	53.6

Table 2. Number of isolates belonging to the European CA-MRSA and Brazilian clone per year.

Year	European CA-MRSA clone	Brazilian clone	Other	Overall
2006	6	5	3	14
2007	9	0	0	9
2008	19	0	1	20
2009	26	0	0	26
2010	6	2	0	8
2011	6	0	1	7
Overall	**72**	**7**	**5**	**84**

emergence of the ST80 clone in Algerian hospitals and indicate that this community associated clone is able to quickly replace typical hospital-associated clones such as the Brazilian clone [12,14].

To compare the antibiotic susceptibility and virulence patterns of the major clones recovered in this study with international clones, we analyzed two representative Algerian isolates of the European CA-MRSA clone and one Algerian isolate of the Brazilian clone with the Alere StaphyType DNA microarray (Alere Technologies, Jena, Germany) [16]. This microarray targets approximately 170 distinct genes and it was used following protocols and procedure previously described into detail [16]. Interestingly, the presence/absence of resistance and virulence genes for each major Algerian clone was similar to other isolates of the same clone as reported by Monecke (Table 2-S) [16]. This confirms the general homogeneity among Algerian and European isolates of the ST80 clone [10].

In conclusion, our data confirmed the predominance of the PVL-positive European CA-MRSA (ST80-IV-pos) clone in Algeria. In addition, our findings suggest that this clone recently replaced other hospital associated pandemic clones in Algerian hospitals.

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Supplementary Items

Table 1-S: Characteristics of the 84 Algerian MRSA recovered in the Bologhine Ibn Ziri University hospital (Algiers, Algeria).

Strain	Year of isolation	gender	Child/Adult	Service	Specimen	PVL	clfB	spa	mecA	SCCmec	International Clone	Antibiotic resistance profile [a]
22579	2006	F	Adult	Out	Furuncle	pos	563	26	pos	ND [b]	European CA-MRSA (ST80)	P,OXA,K,
22573	2006	M	NA	NA	Wound	pos	562	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,
22576	2006	M	Adult	Medicine	Pus	pos	560	26	pos	IV	European CA-MRSA (ST80)	P, OX, K, TE, FA
22582	2006	F	Adult	Medicine	Furuncle	pos	561	26	pos	IV	European CA-MRSA (ST80)	P, OX, K, FA, TE,
22581	2006	M	Adult	Surgery	NA	pos	46	26	pos	IV	European CA-MRSA (ST80)	P, OX, TE, K, FA
22574	2006	M	Adult	Medicine	Pus	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P, OX, K, GM, OFX
22571	2006	M	Adult	Emergency	Wound	neg	366	252	pos	IV	Other	P, OX, K, TE
22583	2006	M	Adult	Emergency	Wound	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P, OX, K, TE
22584	2006	NA	NA	NA	NA	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P,OXA,K,T,FA,E,SXT,OFX
22578	2006	M	Adult	Emergency	Wound	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P, OX,, K, TE,,FA, OFX, SXT
22575	2006	M	Adult	Emergency	Wound	neg	366	252	pos	IV	Other	P, OX,, K, TE,FA, OFX, SXT
22577	2006	M	Adult	Emergency	Wound	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P, OX,, K, TE,FA, OFX, SXT
22572	2006	NA	NA	NA	NA	neg	366	252	pos	IV	Other	P,OXA,K,
22580	2006	M	Adult	Out	Wound	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,
22593	2007	F	Adult	Out	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE
22599	2007	F	Adult	Surgery	Blood	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE
22594	2007	F	Adult	NA	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,T,K,FA
22595	2007	F	Adult	Out	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,OFX-CM-SXT
22598	2007	F	Child	Pediatrics	Pus	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K
22596	2007	F	NA	NA	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,AF-K
22597	2007	F	NA	Out	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE-FA
22600	2007	M	Child	Pediatrics	Blood	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA
22601	2007	F	Child	Pediatrics	Blood	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA-SXT
22618	2008	NA	NA	Surgery	NA	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA,SXT
22613	2008	F	Child	Pediatrics	NA	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
22603	2008	F	Adult	Out	Nose	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-FA
22615	2008	NA	NA	Surgery	NA	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA
22604	2008	F	Adult	Out	Furuncle	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K
22617	2008	M	Child	Out	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K
22612	2008	NA	NA	NA	NA	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA,SXT
22620	2008	M	Adult	Surgery	Blood	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,SXT
22616	2008	NA	NA	NA	NA	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE

Table 1-S: continued

Strain	Year of isolation	gender	Child/Adult	Service	Specimen	PVL	clfB	spa	mecA	SCCmec	International Clone	Antibiotic resistance profile [a]
22614	2008	M	Adult	Surgery	NA	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE-FA-OFX
22605	2008	NA	NA	NA	NA	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
22619	2008	M	Child	Pediatrics	Blood	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K
22606	2008	F	Adult	Intensive care	NA	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K
22607	2008	M	Adult	Intensive care	Pus	pos	415	0	pos	ND [b]	European CA-MRSA (ST80)	P,OXA,K,CM
22608	2008	F	Adult	Gynecology	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-SXT-OFX-FA
22609	2008	F	Adult	Surgery	Furuncle	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,TE,FA
22621	2008	F	Adult	Out	Urine	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA
22610	2008	F	Adult	Intensive care	Pus	neg	117	102	pos	II	Other	P,OXA,K,CM
22611	2008	F	Adult	NA	Pus	pos	573	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE,K,CM
22602	2008	F	Adult	Out	Groin	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K
22631	2009	F	Adult	Out	Nose	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE,K,FA
22632	2009	M	Adult	Out	Groin	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE,FA
22633	2009	F	Adult	Surgery	Groin	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE-SXT
22634	2009	H	Adult	Out	NA	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-OFX-FA
22635	2009	F	Child	Pediatrics	Furuncle	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
22636	2009	F	Adult	Out	NA	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T
22637	2009	F	Adult	Out	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,CM
22655	2009	H	Child	Pediatrics	Blood	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,TE-FA-SXT
22652	2009	H	Adult	Medicine	Blood	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA
22639	2009	F	NA	NA	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE-FA
22656	2009	NA	NA	NA	NA	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA
22641	2009	H	NA	NA	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,FA-SXT
22642	2009	H	Child	Pediatrics	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE-K
22643	2009	F	Adult	Intensive care	Pus	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,FA,SXT
22644	2009	H	Adult	Out	Wound	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K.T,FA
22653	2009	F	Adult	NA	Blood	pos	46	26	pos	IV	European CA-MRSA (ST80)	FA-SXT
22629	2009	F	Adult	Gynecology	NA	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE
22645	2009	F	Adult	Gynecology	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE-FA
22646	2009	H	Adult	Gynecology	Pus	pos	559	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE-FA,SXT
22647	2009	H	Child	Pediatrics	Rectal	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-T,FA
22648	2009	F	Adult	Out	Nose	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,TE-FA

Table 1-S: continued

Strain	Year of isolation	gender	Child/Adult	Service	Specimen	PVL	clfB	spa	mecA	SCCmec	International Clone	Antibiotic resistance profile [a]
22649	2009	H	Adult	Out	Nose	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
22654	2009	F	Adult	NA	Blood	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA,CM
22630	2009	M	Adult	Out	Groin	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K-TE-FA
22650	2009	F	Adult	Gynecology	Nose	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,FA-CM-OFX-SXT
22651	2009	F	Adult	Gynecology	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA-SXT
22585	2010	F	Adult	Out	Nose	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
22590	2010	H	Adult	NA	Pus	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P,OXA,K-TE-OFX
22587	2010	H	Adult	Out	Wound	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE
22588	2010	F	Child	Out	Wound	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,TE
22592	2010	H	Adult	Intensive care	Blood	neg	28	30	pos	III*mercury*	Brazilian Clone (ST239)	P,OXA,K-OFX
22591	2010	F	Adult	Gynecology	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,FA-SXT
22628	2010	H	Adult	Intensive care	Pus	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,FA
22589	2010	F	Adult	NA	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,FA,CM
23878	2011	M	Adult	Medicine	Blood	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
23872	2011	M	Adult	Medicine	Blood	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
23879	2011	M	Adult	Intensive care	Blood	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
23873	2011	F	Adult	NA	Pus	pos	415	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
23874	2011	M	Adult	Medicine	Pus	pos	7	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
23876	2011	F	Adult	Gynecology	Pus	pos	46	26	pos	IV	European CA-MRSA (ST80)	P,OXA,K,T,FA
23877	2011	F	NA	Out	Groin	neg	2	0	pos	IV	Other	P,OXA,K,T,FA,SXT

[a] P = penicillin, OXA= oxacillin, K= kanamycine, TE = tetracycline, CM = clindamycin, SXT = Co-trimoxazole, OFX = ofloxacin, FA = fusidic acid ; [b] Not determined, unusual SCC*mec* element (no amplification of the ccr type with the Kondo *et al.* 2006 primers)

Table 2-S: Presence/absence of resistance- (A) and virulence- (B) associated genes as detected with the Alere StaphyType DNA microarray (Monecke *et al*. 2011, PLoS One 6(4): e17936).

(A)	European CA-MRSA (22599)	European CA-MRSA (22654)	Brazilian Clone (22590)	(B)	European CA-MRSA (22599)	European CA-MRSA (22654)	Brazilian Clone (22590)
blaZ	neg	**pos**	**pos**	*PVL genes*	**pos**	**pos**	neg
erm(A)	neg	neg	**pos**	*ACME*	neg	neg	neg
erm(B)	neg	neg	neg	*sak*	**pos**	**pos**	**pos**
erm(C)	neg	neg	neg	*chp*	neg	neg	**pos**
lnu(A)	neg	neg	neg	*scn*	**pos**	**pos**	neg
msr(A)	neg	neg	neg	*sea*	neg	neg	neg
mph(C)	neg	neg	neg	*seb*	neg	neg	neg
vga(A)	neg	neg	neg	*sek & seq*	neg	neg	**pos**
aacA-aphD	neg	**pos**	**pos**	*sec & sel*	neg	neg	neg
aadD	neg	neg	neg	*tst1*	neg	neg	neg
aphA3 &sat	**pos**	**pos**	**pos**	*sed*	neg	neg	neg
dfrA	neg	neg	neg	*sej & ser*	neg	neg	neg
far1	neg	neg	neg	*egc-genes*	neg	neg	neg
Q6GD50	neg	neg	neg	*seh*	neg	neg	neg
mupA	neg	neg	neg	*etA*	neg	neg	neg
tet(K)	neg	neg	neg	*etD*	**pos**	**pos**	neg
tet(M)	neg	neg	**pos**	*edinA*	neg	neg	neg
cat	neg	neg	neg	*edinB*	**pos**	**pos**	neg
cfr	neg	neg	neg	*splA & splB*	**pos**	**pos**	neg
fexA	neg	neg	neg	*hld*	neg	neg	**pos**
qacA	neg	neg	neg	*aur*	neg	neg	**pos**
qacC	neg	neg	neg				
mercA & merB	neg	neg	neg				
fosB	neg	neg	**pos**				