

Panton-Valentine leukocidin positive sequence type 80 methicillin-resistant *Staphylococcus aureus* carrying a staphylococcal cassette chromosome *mec* type IVc is dominant in neonates and children in an Algiers hospital

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major antimicrobial drug-resistant pathogen causing serious infections. It was first detected in healthcare settings, but in recent years it has also become disseminated in the community. Children and young adults are most susceptible to infection by community-acquired (CA) MRSA strains. In this study 25 MRSA isolates implicated in infections of neonates and children admitted to an Algiers hospital during an 18 month period were characterized by molecular methods including staphylococcal cassette chromosome (SCC) *mec* typing, PCR amplification of *pvl* genes, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Fifteen out of 25 isolates were from hospital-acquired infections. Twenty-four isolates carried SCC*mec* type IVc and belonged to the sequence type (ST) 80, one isolate carried SCC*mec* type II and was ST 39. Twenty-two out of 24 ST80-MRSA-IVc isolates carried *pvl* genes. Our results suggest that the Panton-Valentine leukocidin positive ST80-MRSA-IVc is the dominant MRSA clone causing disease in neonates and children in Algiers.

KEY WORDS: Children, MRSA, PVL, ST80, SCC*mec*IV.

Received May 18, 2012

Accepted August 13, 2012

INTRODUCTION

Staphylococcus aureus is a bacterial pathogen distributed worldwide and a leading cause of morbidity and mortality. It causes a variety of infections ranging in severity from mild to severe, life-threatening conditions (Miller *et al.*, 2005). The pathogenicity of *S. aureus* is related to an exhaustive arsenal of virulence factors and toxins.

In particular, Panton-Valentine leukocidin (PVL), a cytotoxin causing leukocyte destruction and tissue necrosis, is known to be a stable marker of community-acquired MRSA strains (Vandenesch *et al.*, 2003). PVL-producing strains are typically associated with severe skin and soft tissue infections (SSTIs) and necrotizing pneumonia (Miller *et al.*, 2005; Durupt *et al.*, 2007).

Further issues of concern are the ability of *S. aureus* to acquire antibiotic resistance, and its emergence and dissemination outside of the healthcare setting. Since the first appearance of methicillin-resistant *S. aureus* (MRSA) in healthcare facilities 50 years ago, several epidemiological changes have affected this pathogen, beginning with resistance to non β -lactam antibiotics until the more recent involvement in community infections (Chambers and DeLeo, 2009). Since the

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first report of cases of MRSA infections in previously healthy young people, community-acquired (CA)-MRSA strains have been increasingly reported worldwide (Vandenesch *et al.*, 2003). In recent years, a progressive blurring of boundaries between hospital-acquired (HA)-MRSA and CA-MRSA infections was first anticipated and then described in several countries (Saiman *et al.*, 2003; Skov and Jensen, 2009). Previous studies have reported high rates of MRSA infection in Algeria both in community and healthcare facilities. The most prevalent clone is described to be ST80, carrying *pvl* genes and a SCCmec IV element (Antri *et al.*, 2011). Other clones have also been reported, but usually within the hospital setting.

The aim of this study was to describe the molecular and epidemiological features of MRSA strains involved in infections of children and newborns in an Algiers hospital.

MATERIALS AND METHODS

Patients and MRSA strains

The "Mother and Child" central laboratory of Beni-Messous University hospital in Algiers is dedicated to microbiological investigations concerning perinatal and pediatric transmissible diseases. All MRSA strains implicated in infections of neonates and children were collected during the 18 month period April 2010-September 2011. Information including gender, age, site of isolation and diagnosis was collected. For the purposes of this study, MRSA strains isolated from outpatients or, alternatively, from inpatients within 48 hours after their admission were identified as community-onset (CO)-MRSA, whilst those isolated more than 48 hours after admission were considered HA-MRSA. Strain identification was based on colony morphology on Mannitol salt agar (OXOID, Basingstoke, UK), production of coagulase by using rabbit plasma (OXOID) and Staphyslide agglutination tests (BioMérieux, Marne-La-Coquette, France). Methicillin resistance was screened by subculturing the isolates on Müller Hinton agar with 4% NaCl and 6 µg/ml of oxacillin and confirmed by testing resistance to ceftioxin by the disk diffusion method, according to the Clinical Laboratory Standards Institute (CLSI, 2011).

Antimicrobial susceptibility testing

Susceptibility of the MRSA strains was tested against 11 antimicrobial agents using the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2011). The antibiotics tested were ciprofloxacin (5 µg), clarithromycin (15 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), rifampicin (5 µg), tetracycline (30 µg), teicoplanin (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and vancomycin (30 µg).

SCCmec typing and type IV SCCmec subtyping

All strains were cultured on brain heart infusion broth and incubated at 37°C overnight, and then DNA was extracted according to standard protocols (Kostman *et al.*, 1992). Multiplex PCR was performed to identify the SCCmec types I to VI with the *mecA* gene as a positive control (Milheiriço *et al.*, 2007a). PCR products were then visualized on 3% TBE-agarose gels. To distinguish between the different SCCmec IV subtypes, a further multiplex PCR was performed as previously described (Milheiriço *et al.*, 2007b).

Panton-Valentine leukocidin genes detection

The presence of the *pvl* genes, *lukS-PV* and *lukF-PV*, was detected as described previously (Said-Salim *et al.*, 2005). PCR products were resolved by electrophoresis on 1.5% agarose gel.

Multiple loci variable number tandem repeat fingerprinting (MLVF)

MLVF typing was performed according to the procedure described by Sabat *et al.* (2003). Electrophoresis was performed in 0.5X Tris-borate buffer-2% agarose gels. Banding patterns were visually compared and those differing by at least one band considered different.

Pulsed field gel electrophoresis (PFGE) fingerprinting

MRSA isolates representative of the different MLVF profiles were submitted to PFGE after *Sma*I macrorestriction of genomic DNA as described previously (Mulvey *et al.*, 2001). DNA fragments were separated by CHEF Mapper® (BioRad Laboratories, Hercules, CA, USA), and analyzed by BioNumerics software version 6.5 (Applied Math, St-Martin-Latem, Belgium).

MultiLocus sequence typing (MLST)

MLST was performed on strains representative of the different PFGE profiles as previously described (Enright *et al.*, 2000). Seven housekeeping genes were sequenced to determine the allelic profile. Strains were assigned to a sequence type (ST) using the MLST database (<http://saureus.mlst.net/>).

RESULTS

Patient characteristics

During the 18 month period of study, 25 strains of MRSA and 104 of MSSA were isolated from as many cases of infection in neonates and children. Ten MRSA strains were isolated from outpatients or inpatients within 48 hours after their admission and categorized as CO-MRSA.

The remaining 15 isolates were recovered from hospitalized patients from 48 hours after admission onwards and defined as HA-MRSA (Table 1). The median age was 74 months (ranging from 7 days to 15 years).

Eight patients were aged two years or less, nine 2-10 years, and eight 10-15 years. Male gender was more prevalent than female with an M/F ratio of 1.78 (Table 1). The clinical characteristics of the infection cases are summarized in Table 1. Most isolates - 15 out of 25 - were implicated in SSTIs and five further isolates in bone and joint infections. Six out of 10 CO-MRSA were implicated in SSTIs, whereas the three MRSA strains identified from bacteremia cases were all hospital-acquired (Table 1).

Antibiotic susceptibility patterns

Nineteen strains (76%) were resistant to tetracycline, eight to clarithromycin, seven to erythromycin, four to ciprofloxacin and three to clindamycin. No resistance was detected towards gentamicin, rifampicin, trimethoprim-sulfamethoxazole, vancomycin, teicoplanin or linezolid. Antibiotic resistance patterns are illustrated in Table 1.

Molecular characterization of isolates

Among the 25 MRSA isolates under study, SCCmec IV was found in 24 and SCCmec II in the remaining isolate (Figure 1). Subtyping of SCCmec IV disclosed the subtype IVc in all 24 iso-

TABLE 1 - Characteristics and resistance patterns of 15 Hospital-Acquired and 10 Community-Onset Methicillin-Resistant *S. aureus* recovered from patients admitted to Beni-Messous Hospital, Algiers, from April 2010 to September 2011.

MRSA strains	HA-MRSA	CO-MRSA	Total
	15	10	25
<i>Patients characteristic</i>			
Median age			74 months
7 days-2 years	5	3	8
2-10 years	6	3	9
10-15 years	4	4	8
M/F ratio			1.78
Female	6	3	9
<i>Site of infection</i>			
SSTI infections	9	6	15
Bone/joint infection	1	4	5
Bacteraemia	3	0	3
Otitis	1	0	1
Other	1	0	1
<i>Antibiotic resistance to non β-lactams</i>			
None	2	2	4
TE	8	3	11
TE, CIP	1	1	2
TE, E, CLR	3	0	3
TE, CIP, CLR	0	1	1
TE, E, CLR, CIP, DA	1	1	2
E, CLR	0	1	1
E, CLR, DA	0	1	1

CO-MRSA: community onset MRSA, HA-MRSA: hospital acquired MRSA, SSTI: skin and soft tissue infections, TE: tetracycline, CIP: ciprofloxacin, E: erythromycin, CLR: clarithromycin, DA: clindamycin.

lates, of which 22 tested positive for *pvl* genes. The two PVL negative *S. aureus* isolates carrying SCCmec IVc were CO-MRSA, the first causing osteomyelitis and the second SSTI. The isolate with SCCmec II was a CO-MRSA implicated in SSTI, and tested *pvl* negative.

By MLVF typing 23 different patterns were detected. PFGE was then performed on these 23 isolates: 22 of them showed closely related patterns differing between each other by less than three bands with more than 85% similarity. The last isolate showed an unrelated pattern (Figure 1). MLST typing assigned all MRSA strains but one to ST80. The remaining PVL negative strain, carrying SCCmec II, was attributed to ST39.

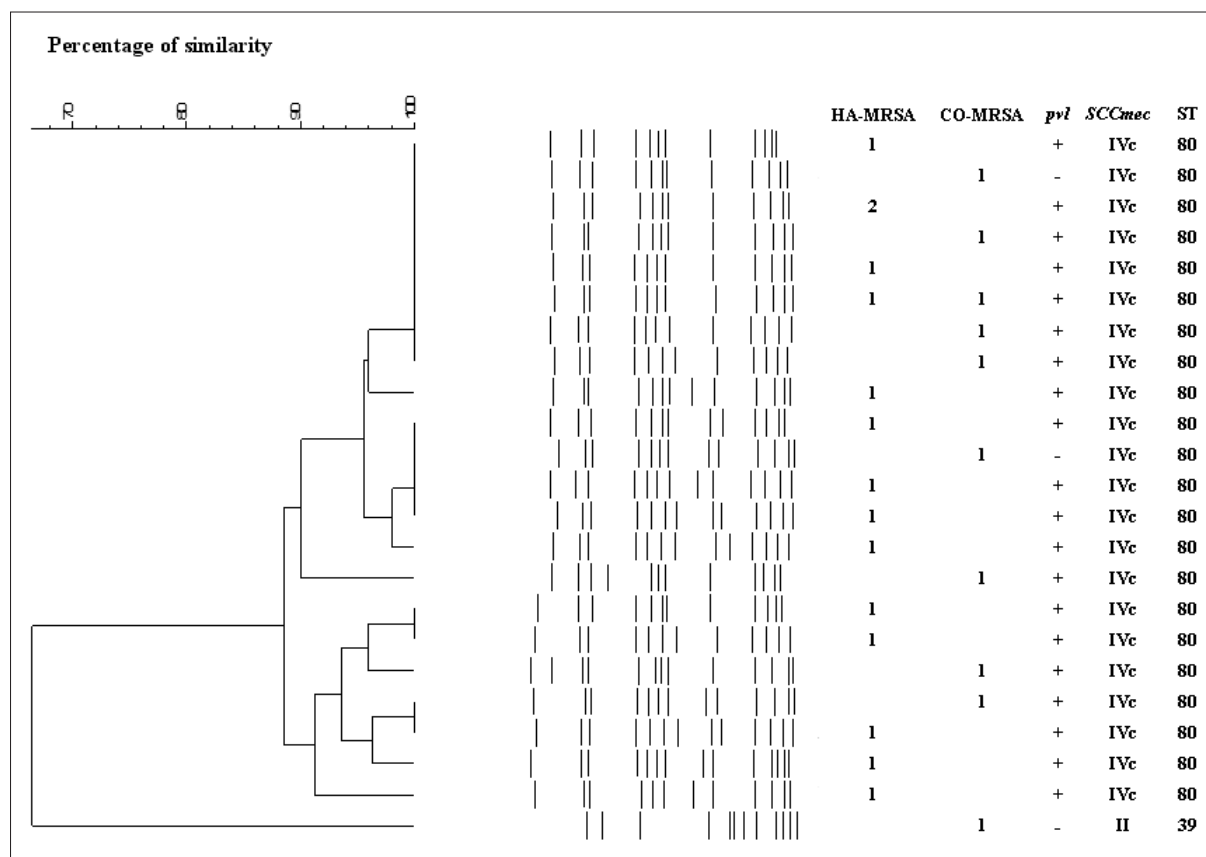


FIGURE 1 - Dendrogram of the electrophoretic patterns of DNA macrorestriction by *Sma*I of 23 MRSA isolates, Algiers 2011. CO-MRSA, community onset MRSA; HA-MRSA, hospital acquired MRSA; pvl, Panton-Valentine leucocidine sequence; ST: sequence type.

DISCUSSION

Infections by MRSA, especially by community-associated PVL-producing clones are increasingly reported worldwide (Vandenesch *et al.*, 2003). In the USA, the rapid emergence of CA-MRSA as a cause of invasive and noninvasive infections in children and young people has always reached epidemic proportions (Purcell *et al.*, 2005). MRSA infections also appear to be increasing in newborns and infants (Fortunov *et al.*, 2006).

In Algeria, high MRSA rates in community and healthcare setting have been reported with ST80-MRSA-IV being the most prevalent clone (Ramdani-Bougoussa *et al.*, 2006; Antri *et al.*, 2011). However, no study has been carried out on children and infants. Hence, our main interest was to assess the molecular characteristics of MRSA strains implicated in infections of these

subjects. During the 18 month period of study, 10 of the 25 MRSA strains were epidemiologically attributable to CO-MRSA. Similar results have been reported by previous studies in Algeria (Ramdani-Bougoussa *et al.*, 2006; Antri *et al.*, 2011) and throughout the world, confirming the upward trend of infections caused by CA-MRSA strains in hospital (Herold *et al.*, 1998). In other studies, CA-MRSA infection cases have already exceeded hospital-acquired infections (Liu *et al.*, 2008). Indeed, a clinical onset in the hospital setting does not rule out a community source, CA-MRSA most likely being carried in the upper respiratory tract or various cutaneous and mucosal sites and brought into the hospital by the nursing staff, the patients themselves or their caregivers (Saiman *et al.*, 2003; Skov & Jensen, 2009). Eight of the 25 patients under study were less than two years old. In a three-year study at Texas

Children's Hospital, about half the children with CA-MRSA infections were aged 2 years old or less (Kaplan *et al.*, 2005). Similarly, according to previous reports, the male gender was more prevalent (Fortunov *et al.*, 2006; Liu *et al.*, 2008; Shallcross *et al.*, 2010; Edelstein *et al.*, 2011). In this regard it has been speculated that some gender-specific physical factors might predispose to infection, but this has not been confirmed in younger subjects (Shallcross *et al.*, 2010). Moreover, in agreement with the findings of other authors, SSTIs are the most frequent type of infection.

The MRSA strains exhibited a low prevalence of resistance to non beta-lactam antibiotics, consistent with the characteristics described for the ST80-MRSA clone (Chua *et al.*, 2011). However, spilling of this community clone into the healthcare setting might constitute an important step towards the emergence and spread of more resistant strains, as is the case for the clindamycin and ciprofloxacin-resistant strains identified in this study and reported by other authors (Antri *et al.*, 2011; Vergison *et al.*, 2011).

Of the 25 MRSA isolates 22 harbored PVL genes. In previous studies on the general population in Algeria, rates of PVL-producing strains were 29.7% (Ouchenane *et al.*, 2011), 35.7% (Antri *et al.*, 2011) up to 67.2% (Ramdani-Bouguessa *et al.*, 2006). High rates of PVL-producing MRSA were also reported in other countries such as the US and Tunisia (Fortunov *et al.*, 2006; Ben Nejma *et al.*, 2008). On the contrary, lower rates or no PVL-positive MRSA were reported in other countries: 15% in the Netherlands (Adedeji *et al.*, 2007), 0.7% in a nationwide study in Japan (Yanagihara *et al.*, 2012), near to zero in some English hospitals (Shallcross *et al.*, 2010).

ST80-MRSA-IV is the most widespread clone in Algeria (Ramdani-Bouguessa *et al.*, 2006; Antri *et al.*, 2011). However, our report first detected it as the predominant strain among pediatric cases of infection, with a similar prevalence to that described for the USA300 clone in the USA. ST80-MRSA is also frequently detected in North African and Middle East countries such as Tunisia (Ben Nejma *et al.*, 2008) and Kuwait (Udo & Sarkhoo, 2010). In Egypt, this clone was not dominant and appeared to be largely susceptible to antibiotics (Enany *et al.*, 2010). In many European countries, such as Denmark

(Larsen *et al.* 2008), Belgium (Denis *et al.*, 2005) and Switzerland (Francois *et al.*, 2008), ST80-MRSA seems to be quite sporadic and many cases are associated with travel histories in Mediterranean and Middle East countries (Larsen *et al.* 2008). The PVL negative ST39-MRSA-II isolate, belonging to CC30, is attributable to EMRSA-16. Emergence and epidemic dissemination of this clone, along with ST22-MRSA-IV (EMRSA-15), was correlated with the rise in some European countries of MRSA in the 1990s and early 2000s. However, the proportion of EMRSA-16 was declining from the late 1990s (Ellington *et al.*, 2010). Other members of CC30 have been reported in Algeria (Ramdani-Bouguessa *et al.*, 2006).

We here report that PVL positive ST80-MRSA-IVc is the leading MRSA clone among children and neonates in Algiers. Moreover, it appears not only to cause community-onset infections, but is also as an emerging cause of healthcare-associated infections. Special attention is required in diagnosing and treating MRSA infections in pediatric patients in order to prescribe appropriate antibiotic therapeutic regimens, adequately control the possible severe complications and prevent the dissemination of this pathogen.

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