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Panton–Valentine leukocidin-positive *Staphylococcus aureus* skin and soft tissue infections among children in an emergency department in Madrid, Spain

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Abstract

Fifty-three children who attended the emergency department with community-associated (CA) *Staphylococcus aureus* skin and soft tissue infections (SSTIs) were enrolled in the study. Seven cases of infection (13.2%) were due to methicillin-resistant *S. aureus* (MRSA). Twelve of 46 available isolates (26.1%) were Panton–Valentine leukocidin (PVL)-positive. PVL-positive *S. aureus* SSTIs were more frequently associated with abscesses and cellulitis (75% vs. 38%, p 0.028), and more commonly required incision and drainage (75% vs. 21%, p 0.001). Most PVL-positive CA-MRSA isolates belonged to a single multilocus sequence type (ST8). In contrast, PVL-positive methicillin-susceptible *S. aureus* isolates belonged to four different sequence types (ST8, ST30, ST80, ST120).

Keywords: Community-associated infections, emergency department, methicillin resistance, paediatric infections, Panton–Valentine leukocidin, Spain, *Staphylococcus aureus*

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) was confined to hospitals until 1982, when it was first described among drug users [1]. In Spain, the first case of MRSA infection was reported in 1981 [2]. In 1998, community-associated (CA)-MRSA emerged as a pathogen in children when four deaths were reported [3]. CA-MRSA is predominately associated with skin and soft tissue infections (SSTIs) that are most commonly mild, but which can also develop into deep-seated lesions that require emergency care or hospitalization [4].

Although increased prevalence of CA-MRSA among children has been reported in the USA, few such studies have been documented in Europe [5–7]. In a multicentre investigation, Panton–Valentine leukocidin (PVL), a two-component pore-forming cytotoxin associated with *S. aureus* causing furuncles and necrotizing pneumonia, was detected in 98% of MRSA isolates and 42% of methicillin-susceptible *S. aureus* (MSSA) isolates from CA SSTIs [8,9]. After detecting the emergence of a single clone of CA-MRSA among children at our hospital, we decided to conduct a prospective study to determine (i) the prevalence of CA-MRSA among children with SSTIs who were treated in the emergency department, (ii) the frequency with which PVL genes are present in CA *S. aureus* SSTIs, and (iii) the epidemiological and molecular characteristics of CA *S. aureus* infections.

Materials and Methods

This study was conducted at the Hospital Universitario 12 de Octubre, a 1300-bed tertiary-care facility that comprises two separate buildings, one for children (176 beds) and the other for adults. This public hospital provides specialized healthcare to a population of approximately 550 000 residents in southern Madrid, Spain. The number of children treated at the children emergency department during 2007 was 67 861. From January to December of 2007, we conducted a prospective study in which we enrolled children <15 years of age who attended the emergency department

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of the Hospital Universitario 12 de Octubre and who were diagnosed with CA *S. aureus* SSTIs. Patient data were collected on a standardized form. Among the patients admitted to hospital, *S. aureus* isolates were considered to be community-associated if there were no predisposing factors for healthcare-acquired infection, such as underlying disease (e.g. cystic fibrosis, chronic renal failure, history of malignancy, immunodeficiency, asthma, or chronic skin illness), previous hospitalization or surgical procedures within the past year (excluding healthy newborns), or the presence of any indwelling catheter or percutaneous medical device.

Bacterial identification and antimicrobial susceptibility testing were performed with Wider panels (Soria-Melguizo, Madrid, Spain) [10], based on the broth microdilution method, and were interpreted using the CLSI criteria [11]. Susceptibility tests were carried out with penicillin, oxacillin, ciprofloxacin, gentamicin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, vancomycin, teicoplanin and linezolid. All *S. aureus* isolates underwent PCR analysis for the *mecA* gene [12]. The presence of PVL genes (*lukS-PV* and *lukF-PV)* was also determined by PCR [8]. Macrorestriction analysis of *Sma*I-digested chromosomal DNA was performed in all MRSA and PVL-positive MSSA [13]. Additionally, MRSA isolates underwent staphylococcal cassette chromosome *mec* (SCC*mec*) characterization using PCR [12]. All PVL-positive MSSA and all MRSA were characterized by multilocus sequence typing (MLST) as described previously [14]. The attending physicians remained blind to the molecular test results until the end of the study.

Univariate analysis was performed using the *t*-test for continuous variables and the χ^2 or Fisher's exact test for categorical variables. Statistical analysis of the data was performed with Epi-Info (CDC, Atlanta, GA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) software.

Results

A total of 53 children with CA *S. aureus* SSTIs were enrolled from among the attendees of the emergency department. The median age was 42 months (range 1–174) and 37 (70%) were boys. Fifty-one percent of the children had parents of Spanish origin, whereas 49% had parents who were born abroad. Seven of the 53 infections (13%) were attributed to CA-MRSA. Infections were classified into three groups: 31 (59%) were superficial (impetigo, folliculitis), 17 (32%) were associated with cellulitis and abscesses, and five (9.4%) were deep-seated. Seventeen (32%) of the patients were admitted to the hospital and 16 (30%) required incision and drainage. No statistically significant differences were found between children with MRSA and MSSA with regard to age [median 24 months (range 12–174) vs. 44 months (range 1–174); p 0.752], male sex (86% vs. 67%, p 0.661), type of infection (abscesses and cellulitis, 57% vs. 39%, p 0.431), or admission to hospital (43% vs. 30%, p 0.667). Nonetheless, the proportion of children whose parents were from Ecuador was higher in the MRSA group (4/7, 57%) than in the MSSA group (5/46, 11%) (p 0.012).

Isolates from 46 children (seven MRSA and 39 MSSA) were available for further molecular characterization. Of these, 12 (26%) were PVL-positive. The comparison between PVL-positive and -negative *S. aureus* infections showed that both groups were similar in age, proportion of male sex and those of Ecuadorian parentage, and proportion admitted to hospital (Table 1). In contrast, PVL-positive *S. aureus* SSTIs were more frequently associated with abscesses and cellulitis (75% vs. 38%, p 0.028), more commonly required incision and drainage (75% vs. 21%, p 0.001), and more frequently were methicillin-resistant (42% vs. 5.9%, p 0.009) (Table 1). We repeated this analysis by removing the seven MRSA cases, and still found that cases of PVL-positive MSSA SSTIs more frequently required surgical intervention with incision and drainage than cases due to PVL-negative organisms (71% vs. 23%, p 0.022).

All MRSA isolates were susceptible to all the antibiotics tested, except for the β-lactams. Six of seven (86%) PVL-positive MSSA isolates were susceptible to all antibiotics tested, whereas the remaining isolate was resistant to gentamicin. Pulsed-field gel electrophoresis (PFGE) analysis of seven MRSA isolates showed that six (86%) exhibited the same PFGE pattern (Fig. 1). Results from MLST and SCC*mec* typing showed that this common MRSA pattern belonged to ST8 SCC*mec* type IV.

Five of the ST8 SCC*mec* type IV isolates were PVL-positive and one was PVL-negative. Four of the PVL-positive MRSA isolates were from children of Ecuadorian parentage

TABLE 1. Characteristics of the children with Panton–Valentine leukocidin (PVL)-positive and PVL-negative *Staphylococcus aureus* infections

Characteristic	PVL-positive (*n* = 12)	PVL-negative (*n* = 34)	p
Age (months)			
Median	28	55	0.879
Range	8–174	1–160	
Male	10 (83%)	23 (68%)	0.461
Origin of parent from Ecuador	4 (33%)	4 (13%)	0.178
Type of infection (cellulitis, abscesses)	9 (75%)	13 (38%)	0.028
MRSA	5 (42%)	2 (6%)	0.009
Hospital admission	6 (50%)	11 (32%)	0.314
Incision and drainage	9 (75%)	7 (21%)	0.001

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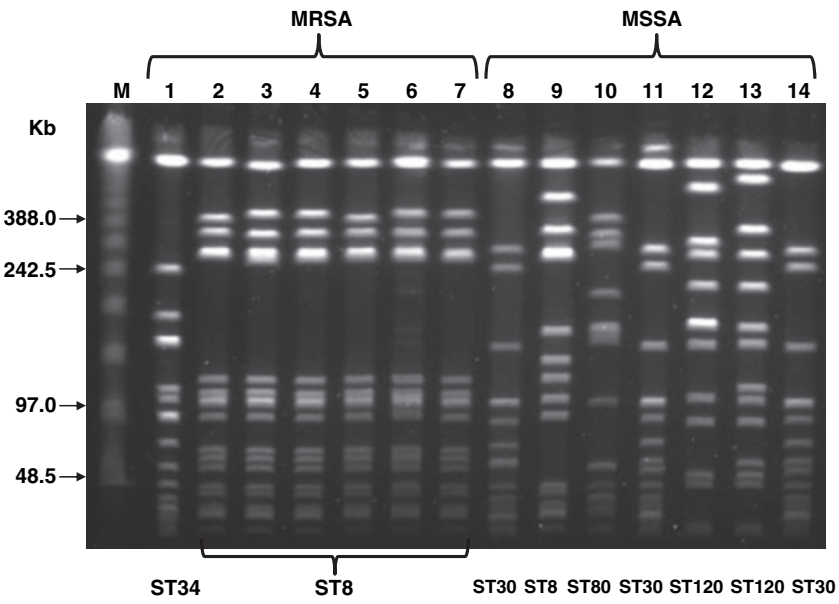

FIG. 1. Pulsed-field gel electrophoresis and multilocus sequence types of 14 methicillin-resistant (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates from children with community-associated skin and soft tissue infections. Lane M, λ molecular weight marker; lanes 1–7 represent MRSA isolates [1 and 2 Panton–Valentine leukocidin (PVL)-negative, and 3–7 PVL-positive]; lanes 8–14 represent PVL-positive MSSA isolates.

and one was from a Spanish-born child. The remaining PVL-negative MRSA isolate, obtained from a Bulgarian boy, belonged to ST34 and could not be typed by SCC*mec* analysis even when a second PCR strategy was used [15]. PFGE analysis of seven PVL-positive MSSA isolates showed at least five different macrorestriction patterns belonging to four MLST types: ST30 (three isolates), ST120 (two isolates), ST8 (one isolate), and ST80 (one isolate) (Fig. 1).

Discussion

We identified a high incidence of CA-MRSA (13%) among children with *S. aureus* SSTIs who were treated in the emergency department during 2007, despite the fact that CA-MRSA in children was reported for the first time in Madrid only in 2006 [6]. In the year prior to this study, MRSA was detected in <1% of children who attended the emergency department (data not shown). Nevertheless, the prevalence of CA-MRSA at our institution is still far below that reported elsewhere, where the proportion of CA-MRSA infections may be as high as 74% [4].

Because most of the MRSA isolates in this study belonged to a single MLST-SCC*mec* clone (ST8 SCC*mec* type IV) and are therefore likely to share a common ancestry with the USA300 strain that is widespread in the USA [16], our results may represent an early warning of the emergence of CA-MRSA in Spain. Noteworthy is that four of the MRSA isolates, all of which were of the same PFGE, MLST and SCC*mec* types, were recovered from unrelated children, all of whom have Ecuadorian parents. Other studies in Spain have detected the emergence of CA-MRSA clones in patients from Ecuador [17]. Although we have no data about the prevalent clones of MRSA in Ecuador, immigration to Spain has increased sharply in the last few years and might explain the appearance of this new clone in Madrid. An important observation was that the PVL-positive MLST lineages causing CA-MSSA infections (ST30, ST80 and ST8) were the same as those reported in other countries [18]. These MSSA clones are probably circulating in the community and could be the parental reservoir for the development of common CA-MRSA strains through the acquisition of SCC*mec* and other resistance determinants. More extensive studies are necessary to understand the respective contributions of immigration and genetic evolution to increased rates of CA-MRSA infection.

Although our study was of limited size, an important finding was that children with CA PVL-positive *S. aureus* SSTIs were more likely to have severe local disease and require incision and drainage. Even when CA-MRSA infections were removed from the analysis, and only the CA-MSSA SSTIs were included, PVL-positive MSSA SSTIs still required more frequent surgical intervention. It is important to note that the decision to treat with incision and drainage was made prior to the availability of the molecular test results to the physician. Although recent animal studies comparing the virulence and lethality of PVL-positive and isogenic PVL-negative MRSA strains have produced conflicting results [19,20], clinical studies have found the presence of PVL genes to be associated with enhanced inflammatory response and local disease [21,22]. A recent study also identified novel cytolytic peptides as key virulence determinants for CA-MRSA [23].

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Additional studies are needed to determine the involvement of PVL in CA *S. aureus* infections and whether the detection of PVL genes or other toxin genes might be useful for patient management and infection control.

Although culture and antimicrobial susceptibility testing have not been a routine component of SSTI management, the emergence of CA-MRSA indicates a need to modify this practice. It is also important to investigate all possible reservoirs and transmission pathways as a means to improve infection control. Importation of new clones by displaced populations has worldwide implications for the epidemiology of MRSA, and we believe that regular monitoring of children for CA-MRSA is critical in helping to predict and mitigate the spread of the corresponding infections.

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Transparency Declaration

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