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Remitting infections due to community-acquired Panton–Valentine leukocidin-producing *Staphylococcus aureus* in the Milan area

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ABSTRACT

One of the most important *Staphylococcus aureus* virulence factors is Panton–Valentine leukocidin (PVL). We describe an outbreak of recurrent cutaneous PVL infections in different members of three family clusters. Molecular investigations were performed to confirm the presence of the *mec*A and PVL genes and to assign the SCC*mec* type, sequence type (ST) and clonal relatedness. A strain of PVL-producing methicillin-resistant *S. aureus* (MRSA) was responsible for infection in two related families (A and B), and a third family (C) was infected with PVL-producing methicillin-sensitive *S. aureus* (MSSA). Molecular investigations revealed the same clone of community-acquired (CA)-MRSA, PVL positive ST8, and SCC*mec* IV in families A and B and CA-MSSA PVL positive ST15 in family C. *S. aureus* PVL may give rise to recurrent uncontrolled infections that are difficult to eradicate, and close family contacts are at high risk for transmission.

Introduction

Staphylococcus aureus may colonize the human body as a part of the normal flora. Approximately 30% of healthy people are inhabited by *S. aureus*, mostly in the anterior nostrils [1]. Methicillin-resistant *S. aureus* (MRSA) is a clonally evolving nosocomial pathogen, but in recent decades it has increasingly become the cause of severe community-acquired (CA) infections. Specific strains with various combinations of sequence type (ST) and staphylococcal cassette chromosome *mec* (SCC*mec*) type have been isolated. On the basis of the differences in SCC*mec* genomic islands, it is possible to define differences between CA-MRSA and hospital-acquired MRSA.

The increased virulence potential of CA-MRSA strains remains controversial [2,3]. Different virulence factors, such as Panton–Valentine leukocidin (PVL), phenol-soluble modulins, alpha toxin, arginine catabolic mobile element and protein A, contribute to the severity, persistence and increased transmission of the bacterium [4].

PVL is a pore-forming cytotoxin produced by some clones of *S. aureus* that causes leukocyte destruction and tissue necrosis. It is associated with infections ranging from uncomplicated skin and soft tissue infections to life-threatening necrotizing pneumonia. PVL is an important virulence factor that is mainly found in CA-MRSA, but it is also associated with CA-methicillin-susceptible *S. aureus* (MSSA) [5]. Epidemiological, historical and biochemical data all point towards a role for PVL in pathogenesis, but whether the toxin affects clinical presentation, disease severity or outcome is unclear [6].

Several different clones of CA-MRSA are spread worldwide. In the United States, the USA300 clone ST8 is responsible for the majority of CA skin and soft-tissue infections and for outbreaks in the community and hospitals [7]. In Europe, CA-MRSA infections are less frequent than in the United States and are characterized by higher clonal diversity [8]. The USA300 clone has spread sporadically across the Atlantic to Europe in recent years via returning travelers [9]. The greatest risk of travelers importing CA-MRSA was among those from North Africa and the Middle East,

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sub-Saharan Africa, Oceania, East Asia, South America, the north-east Mediterranean and North America. Data on the intercontinental exchange of ST8, ST30, ST59 and ST80 CA-MRSA clones demonstrated that the main areas of spread have been between the North and the South Americas, and the European, North African and East Asian/Australian regions [10]. In Italy, a few case reports or small studies have been published [11–15], but a more complete overview of CA-MRSA is lacking.

PVL-positive community-acquired *S. aureus* strains have been rarely reported in Italy, but intrafamilial transmission of an epidemic PVL-producing CA-MRSA lineage has been described [16]. This is the first report of two separate outbreaks of recurrent skin infections in different family members in Lombardy with both intrafamilial and interfamilial transmission.

Strains and methods

Beginning in December 2014, recurrent cutaneous abscesses occurred in different members of three family clusters. From June to November 2015, they were admitted to our Infectious Diseases Ambulatory. The family cluster composition was as follows: Family A: parents and two children aged 7 months and 5 years; Family B: parents and two children aged 1 month and 2 years; and Family C: parents, 27-year-old daughter, 22-year-old son and daughter's partner. Families A and B attended the same playground in Milan with direct contact between the mothers and children.

Bacterial strains

From June to November 2015, wound swabs from available active lesions (n = 3), nasal swabs (n = 13) and rectal swabs (n = 3) of the three families were processed at the Laboratory of Clinical Microbiology, Virology and Bioemergencies of the University Hospital "L. Sacco" in Milan, Italy. Nasal swabs from five relatives were also investigated. All the clinical samples were collected before starting any antibiotic therapy.

Microbiological testing

Wound, nasal and rectal swabs were cultured on selective agar plates for *S. aureus* (mannitol salt 2 agar; BioMérieux, Marcy L'Etoile, France). The isolates were identified at the species level and were tested for antimicrobial susceptibility with the automated analyzer Vitek 2 (BioMérieux). Interpretation of the susceptibility patterns was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.

DNA extraction and quantification

Preparation of genomic DNA from overnight cultures on tryptic soy agar plates was performed using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA).

PVL gene and SCC*mec* typing

To confirm the presence of the *mec*A gene and to assess the presence of the PVL gene, DNA extracted from bacterial isolates was tested with the RealCycler SAMAPV real-time polymerase chain reaction (PCR) (Progenie Molecular, Valencia, Spain). For assignment of *mec* element type, a multiplex PCR strategy was used as previously described [17]. DNA was extracted from bacterial isolates, 500 ng of DNA was amplified, and the SCC*mec* type was assigned according to the amplification pattern obtained.

Multilocus sequence typing (MLST) analysis

To characterize the isolates of *S. aureus*, we used a method based on the sequence of internal fragments of seven housekeeping genes. Each gene fragment was amplified and sequenced according to the MLST.net protocol (http://saureus.beta.mlst.net/) [18], and an allelic profile was assigned according to the sequences obtained for each locus.

Repetitive extragenic palindromic (rep)-PCR

Six samples were genotyped using an automated rep-PCR (DiversiLab, BioMérieux). Band patterns were compared by means of modified Kullback–Leibler methods using DiversiLab web-based software. The isolates with band patterns that were at least 95% similar were considered to be genetically related.

Results

Case reports

In October 2015, a 31-year-old man was admitted to our day hospital because of a 1-month history of recurrent cutaneous abscesses. A swab of an abscess was positive for MRSA. From the anamnesis, it emerged that during 2015, initially his wife (32 years old) and later their daughter (19 months old) had similar manifestations, while their elder son presented with only a single episode (Fig. 1a). We collected nasal, rectal and axillary swabs along with swabs of ongoing lesions when they were present from all the members of Family A. The nasal swabs from the father, daughter and son were positive for MRSA as was the swab of the cutaneous abscess of the wife.

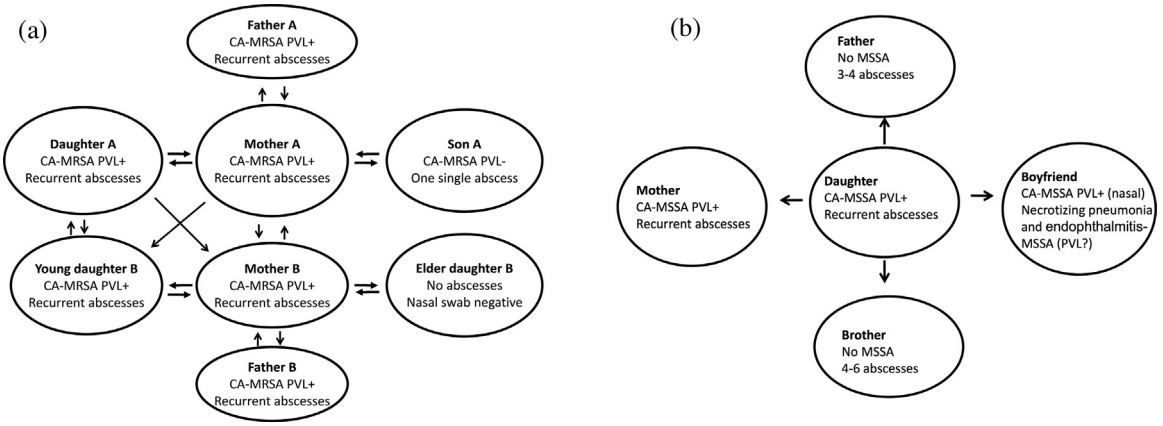

Fig. 1. Diagrams representing the single family members, their SA isolates, clinical manifestations and lines of transmission. (a) Families A and B. (b) Family C.

Two months later, we observed a second family (B) of four members who presented with a similar clinical history. Nasal swabs of the mother (30 years old), father (32 years old) and daughter (12 months old) were positive for MRSA as well, but a swab from the elder daughter was negative (Fig. 1a).

Considering the high virulence of the infection, we performed real-time PCR to assess for the presence of PVL toxin. In Family A, the father, mother and younger daughter carried MRSA PVL$^+$, while the elder son was positive for MRSA PVL$^-$. In Family B, the father, mother and younger daughter were positive for MRSA PVL$^+$. A medical review revealed that in both families, the first episode started as cutaneous abscesses in the mothers and subsequently manifested as recurrent soft tissue infections in other family members. Contact between the two families resulted from attendance at the same playground in Milan, and direct contact occurred between the mothers and the children. Given the presence of resistance to mupirocin, we attempted decolonization in all family members (except the older daughter of Family B who was negative) with 0.1% chlorhexidine dihydrochloride and 0.5% neomycin sulfate nasal ointment intranasally for 14 days, full-body washing with chlorhexidine soap for 5 days, and high-temperature (100 °C) cleaning of personal items. However, all members of both families presented again with cutaneous abscesses over the following months.

In Family C, the first MSSA PVL$^+$ strain was isolated from a 30-year-old woman (daughter) with a >1 year duration of recurrent cutaneous abscesses (>15 episodes) resolving after different antibiotic treatments, including amoxicillin/clavulanic acid, cotrimoxazole and doxycycline. We decided to swab all the family members and a boyfriend, and the boyfriend and mother were positive for the same MSSA-PVL$^+$ strain in the rectum, while the brother and father were negative.

All family members were previously healthy and had no immune deficits, with normal CD4 T-cell counts and immunoglobulin levels. A medical review revealed that the daughter first had abscesses after a scalp infection approximately 18 months earlier. Six months later her boyfriend was admitted to a different hospital because of severe endophthalmitis and subsequent necrotizing pneumonia. MSSA was isolated from the eye and bronchoalveolar fluid but it was not further typed. After this episode, the boyfriend did not develop any further infection but was colonized in the rectum by the MSSA-PVL$^+$ strain. On the contrary, the mother developed several episodes, while the father and brother had three or four episodes and afterwards did not show any further signs of infection (Fig. 1b). Given the recurrent infections of the daughter and mother and the colonization of the boyfriend, 14 days of treatment with doxycycline was simultaneously given to all family members in addition to 3 consecutive days of daily bathing with an antimicrobial skin cleanser containing 4% w/v chlorhexidine digluconate and daily intranasal and anal administration of 2% mupirocin nasal ointment. However, the daughter and mother again presented with cutaneous abscesses at 24 and 27 days after the end of treatment, respectively.

Microbiological isolates

Thirteen of 24 clinical samples tested positive for *S. aureus*. The PCR targeting the *mec*A gene and the gene encoding PVL revealed that eight *S. aureus* isolates from Families A and B were MRSA-PVL$^+$ and one was MRSA-PVL$^-$, and four isolates from Family C were MSSA-PVL$^+$ positive (Table 1). Subsequently, we tested these isolates to determine which SCC*mec* type and ST they belonged to. Isolates of families A and B were *S. aureus* SCC*mec* type IV and ST8. In Family C, *S. aureus* isolates were methicillin sensitive (SCC*mec* not tested) and ST15. A fingerprinting analysis conducted on rep-PCR fragments confirmed that the MRSA-PVL$^+$ isolates from Families A and B belonged to the same cluster (Fig. 2).

Discussion

As a result of its virulence, CA-MRSA represents a serious public health problem. The presence of PVL is an important virulence marker, and the clinical sequelae of PVL-positive infections are often described as more severe than those of PVL-negative infections [19].

To the best of our knowledge, this is the first report on both intrafamilial and interfamilial transmission of CA PVL-producing *S. aureus* in the Milan area. Currently, worldwide reports of CA-MRSA are associated with >20 distinct genetic lineages, five of which are globally predominant, including ST1-IV (WA-1, USA400), ST8-IV (USA300), ST30-IV (South West Pacific [SWP] clone), ST59-V (Taiwan clone), and ST80-IV (European clone) [20]. Furthermore, CC22-MRSA-IV isolates harboring the genes encoding PVL have frequently been observed in the Middle East [21–23], Iran [24], and India [25,26], and also in sporadic cases and localized outbreaks in other regions, such as Western Europe [27,28]. CC1/ST772-V is

Table 1
Microbiological data collected from June to November 2015 for three families.

No. of clinical samples	Family	Sex/age (y)	Clinical sample	Samples cultured	Real-time PCR RealCycler SAMAPV	SCC*mec*	ST
1	A	F/32	Nasal swab	MRSA	MRSA-PVL POS	IV	8
2	A		Cutaneous abscesses	MRSA	MRSA-PVL POS	IV	8
3	A	M/31	Nasal swab	MRSA	MRSA-PVL POS	IV	8
4	A		Cutaneous abscesses	MRSA	MRSA-PVL POS	IV	8
5	A	F/1	Nasal swab	MRSA	MRSA-PVL POS	IV	8
6	A	M/6	Nasal swab	MSSA	MRSA-PVL NEG	IV	8
7	B	F/30	Nasal swab	MRSA	MRSA-PVL POS	IV	8
8	B	M/32	Nasal swab	MRSA	MRSA-PVL POS	IV	8
9	B	F/3	Nasal swab	NEG	NT		
10	B	F/1	Nasal swab	MRSA	MRSA-PVL POS	IV	8
11	C	M/53	Rectal swab	NEG	NT		
12	C		Nasal swab	NEG	NT		
13	C	F/51	Nasal swab	NEG	NT		
14	C		Rectal swab	MSSA	MSSA-PVL POS		15
15	C	F/27	Scalp lesion	MSSA	MSSA-PVL POS		15
16	C		Nasal swab	MSSA	MSSA-PVL POS		15
17	C	M/22	Nasal swab	NEG	NT		
18	C	M/29	Nasal swab	NEG	NT		
19	C		Rectal swab	MSSA	MSSA-PVL POS		15

NT, not tested.

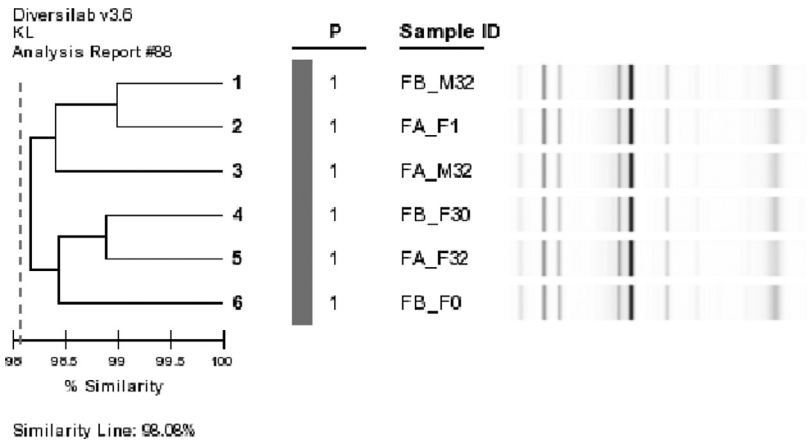

Fig. 2. The dendrogram includes six representative MRSA PVL producers from Family A (sample ID FA_F1, FA_M32, FA_F32) and Family B (sample ID FB_M32, FB_F30, FB_F0). Isolates with ≥95% similarity were considered related.

known to mainly occur in India and Bangladesh, and cases in Europe are usually linked to these countries.

ST8-IV and ST30-IV may be considered pandemic, as they have been isolated repeatedly worldwide.

Recent reports have described the situation in Italy with regard to CA-MRSA distribution. In Northern Italy, SWP was recently described in unrelated infectious episodes [29]. In Southern Italy, recent reports indicated that different CA-MRSA lineages are well represented in Palermo [30,31]. To date, the presence of PVL does not appear to be common in Italy, but the distribution of this peculiar virulence factor in the community should be investigated.

Two different STs of PVL$^+$ *S. aureus* were found in our investigation: ST8 and ST15. Strains genotyped as ST8-SCC*mec* IV are common clones with a worldwide distribution [32,33]. We also found a PVL-positive ST15 MSSA, which has been reported in the literature [34,35] as being abundant in Africa [36], although members of Family C had neither an immigration background from Africa nor a travel history in that country.

We found the same strain within the families, confirming that intrafamilial transmission is common. In Families A and B, the events began in the mothers and only the fathers and younger children (≤1 year) had MRSA-PVL$^+$, while in Family C the event began in the daughter and only the mother and her partner were positive. This path of transmission highlights that close contact and sharing bath towels and/or bed linen among family members, especially with children, may be risk factors for horizontal transmission [37].

Our findings also showed two epidemiologically linked families (A and B) sharing the same CA-MRSA ST strain, and a fingerprinting analysis confirmed interfamilial transmission. The presence of PVL-producing *S. aureus* in colonization sites in eight of the 13 members of the three families suggests that it was able to persist in these niches and cause recurrent infections in a large number of family members, despite local and systemic antibiotic treatment and hygiene measures with chlorhexidine and heat treatment. In one case, the infection probably spread systemically and induced severe and life-threatening infections of the eyes and lungs.

Most colonization was nasal, as this is the typical site of carriage; however, in Family C we found rectal colonization. In a study conducted on a population of children with *S. aureus* skin and soft-tissue abscesses caused by MRSA (60% of cases) and MSSA (40% of cases), *S. aureus* was recovered from rectal cultures in 28 patients (47%) and from nasal cultures in 16 patients (27%) [38]. More recently, it has become evident that the patterns of carriage of CA-MRSA may differ from those previously recognized for healthcare-associated MRSA: one study found that 23% of patients colonized with CA-MRSA were colonized at non-nasal sites (predominantly inguinal regions) [39].

Conclusions

Given the high recurrence and difficulty to eradicate colonization by both PVL$^+$ CA-MSSA and CA-MRSA, and given the risk for susceptible individuals to develop a potentially severe and life-threatening infection, a detailed epidemiological study and molecular analysis of *S. aureus* should be carried out in cases of recurrent infections to evaluate the incidence and spread of these *S. aureus* strains.

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