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Severe invasive pediatric infections caused by Panton-Valentine leukocidin-positive *Staphylococcus* *aureus* in La Réunion

Rosalie Lacoste

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**THESE POUR LE DIPLÔME D'ETAT
DE DOCTEUR EN MÉDECINE**

**Les infections invasives sévères à *Staphylococcus aureus*
sécréteur de la toxine de Panton et Valentine à La Réunion.**

**Severe invasive pediatric infections caused by Panton-Valentine
leukocidin-positive *Staphylococcus aureus* in La Réunion.**

Présentée et soutenue en visioconférence
Le 20/04/2020 à 16h à La Réunion
Par Rosalie Lacoste

JURY

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Madame la Professeure Silvia Iacobelli

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LISTE DES ABREVIATIONS

ARDS : acute respiratory distress syndrome

CRP : C-reactive protein

DIVC : disseminated intravascular coagulopathy

ICH : intracranial hypertension

ICU : intensive care unit

LOS : length of hospital stay

MRSA : methicillin-resistant *Staphylococcus aureus*

MSSA : methicillin-sensitive *Staphylococcus aureus*

NP : necrotizing pneumonia

PE : pericardial

PL : pleural

PNO : pneumothorax

PVL : Panton-Valentine leukocidin

PVL-SA : Panton-Valentine leukocidin *Staphylococcus aureus*

SA : *Staphylococcus aureus*

SBI : secondary bacterial infection

SSTI : skin and soft tissue infections

VAP : ventilator-acquired pneumonia

WBC : white blood cells

THESE ARTICLE

Severe invasive pediatric infections caused by Panton-Valentine leukocidin-positive *Staphylococcus aureus* in La Réunion.

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ABSTRACT

Bicentric retrospective study (2014 - 2019) describing 24 children presenting severe Panton-Valentine Leukocidin-producing *Staphylococcus aureus* infections in La Réunion. Necrotizing pneumonia (54%), skin and soft tissue (25%) and osteoarticular (13%) infections were the main primary clinical diagnoses. Community-acquired strains were methicillin-sensitive. Surgical procedures were performed in 19 patients (79%). Ten patients (42%) presented complications, and two patients (8%) died.

BACKGROUND

Staphylococcus aureus (SA) pathogenicity is related to a number of virulence factors including the Panton-Valentine Leukocidin (PVL) exotoxin. PVL genes can be expressed by both methicillin-resistant SA and methicillin-sensitive SA (MSSA) strains [1].

Most commonly involved in community acquired skin and soft tissue infections (SSTI), *Panton-Valentine leukocidin Staphylococcus aureus* (PVL-SA) infections are characterized by multiple and recurrent furuncles, skin abscesses and peri orbital infections. However, PVL-SA strains have been increasingly recognized as responsible for severe invasive infections in previously healthy children [2-5].

In the Indian Ocean, including the French overseas department of La Réunion, strains of PVL-SA are responsible for a growing number of SSTI and serious illnesses [6]. The aim of our study was to describe the features of severe invasive PVL-SA infections in children in La Réunion between 2014 and 2019.

METHODS

We conducted a bicentric retrospective observational study (2014 – 2019) in the two pediatric departments of the University Hospital of La Réunion. Patients positive for PVL-SA infection and aged less than 18 years were identified through the local microbiology laboratories.

PVL infections were considered as invasive if the leukocidin was isolated from tracheobronchial secretions, pleural fluid, blood culture, joint fluid, cutaneous samples in severe SSTI or from any other normally sterile biological sample. PVL-encoding genes were detected by the French National Reference Center for Staphylococci by an in-house polymerase chain reaction. Clonal complexes were determined by DNA microarray analysis (Alere Identibac *S. aureus* Genotyping[®], Abbott Chicago, Illinois, US).

Necrotizing pneumonia (NP) was defined as a clinical case of pneumonia with chest imaging studies showing small thin-walled cavities within areas of pulmonary consolidation. Patients with minor SSTI (defined as furunculosis, folliculitis, abscesses < 5cm, and absence of cellulitis) were excluded.

We collected demographic, clinical and biological data from the electronic medical records. According to French law, written consent was not mandatory for this retrospective non-interventional study. Therefore, a written non-opposition form was sent to parents via postal mail.

Categorical variables were presented as number and frequencies, and continuous variables as medians and ranges.

RESULTS

The main characteristics of the 24 patients (13 boys, 54%) treated for invasive PVL-SA infections are reported in Table 1. Median age was 1.4 years (range, 0.1-17.7). Eleven patients (46%) originated from the neighbouring islands of Mayotte or Madagascar. Only two patients (8%) presented with pre-existing immunosuppressive conditions. Primary clinical diagnoses included NP (n=13, 54%), SSTI (n=6, 25%), osteoarticular infections (n=3, 13%), cervical mediastinitis (n=1) and a catheter-related infection with bacteremia (n=1). The median length of hospital stay was 19 days (range, 1-76), and 18 patients (75%) were admitted to an intensive care unit (ICU). Ten patients (42%) presented diverse complications. Complementary surgical procedures were performed in 19 patients (79%). *The median duration of antimicrobial therapy was 28 days (range, 1-70), and 19 patients (79%) received antitoxin antibiotherapy. Two patients (8%) died.*

All the documented strains (n=20) were methicillin-sensitive, except for one which was associated with a presumably hospital-acquired infection. The tested MSSA strains were mostly CC152 clones (n=14/18, 78%).

Necrotizing pneumonia

Of the 13 patients primarily diagnosed with NP (median age of 0.5 years), none was immunosuppressed. Four patients (31%) came from Mayotte. Eleven (85%) had preceding symptoms of upper respiratory tract infection. All patients presented with fever, 11 (85%) had patent signs of respiratory distress, and four children (31%) presented with septic shock on admission. Hemoptysis was reported in three patients.

On admission, the median WBC count was 15.8 G/L (range, 0.82-44.2) while the median CRP level was 226 mg/L (range, 6-595). Leukopenia (peripheral neutrophil cell count \leq 3 G/L) was observed in four patients (31%).

The average duration of antistaphylococcal antibiotherapy (mainly third generation cephalosporins, n=11) was 33.4 days (range, 1-70). Twelve children (92%) were treated with antitoxic antibiotics. Two patients received intravenous immunoglobulins.

Six patients (46%) developed diverse complications (Table 1). All were admitted in an ICU. Eight patients (62%) were mechanically ventilated, and three patients (23%) required noninvasive ventilation. *Extracorporeal membrane oxygenation* was deployed in three patients. Complementary surgical procedures performed on 10 patients (77%) included: drainage of pneumothorax (n=6, three patients developing secondary ventilator-acquired pneumothorax), pleural drainage (n=9), pericardial drainage for associated cardiac tamponade (n=2), and two blank laparotomies (one for suspected peritonitis, and the other for suspected mesenteric ischemia). Two patients (15%) died.

Complicated skin and soft tissue infections

Of the six patients primarily diagnosed with complicated SSTI (median age of 3.6 years), two (33%) *had pre-existing immunosuppressive conditions*. Family history of SSTI was reported in five patients (83%). Three children (50%) originated from Mayotte or Madagascar. A *skin injury preceded* infection in four patients (67%). The clinical presentations included periorbital cellulitis (patients 14 and 17), abscess of the forearm with dermocellulitis (patient 15), perineal abscess (patient 16), temporal abscess with dermocellulitis (patient 18), and inguinal adenophlegmon (patient 19). *None required ICU* admission, and no associated complications were observed.

On admission, the median WBC count was 13.0 G/L (range, 6.7-28.5) while the median CRP level was 68 mg/L (range, 7-169).

The average duration of antistaphylococcal antibiotherapy (mainly amoxicillin-clavulanic acid, n=4) was 13.7 days (range 8-28). Two children received antitoxinic antibiotics. Complementary cutaneous surgical drainage was performed on five patients (83%), all with a favorable outcome.

Osteoarticular infections

Of the three patients primarily diagnosed with osteoarticular infections (median age of 5.2 years), two came from Mayotte or Madagascar. None presented pre-existing conditions. A history of prior trauma was reported in all patients. All presented high grade fever, limping and/or articular pain on admission. Patient 20 presented unifocal arthritis of the knee with pyomyositis, patient 21 presented multifocal osteomyelitis with pyomyositis, and patient 22 presented multifocal osteoarthritis, respectively. *All three children required admission to the ICU.*

On admission, the median WBC count was 22.1 G/L (range, 21.7-28.2) while the median CRP level was 258 mg/L (range, 255-348).

The mean duration of antistaphylococcal antibiotherapy was 42 days (range, 28-56). They all received antitoxinic antibiotherapy.

Two patients (67%) developed diverse complications (Table 1). Articular drainage and/or surgical debridement were performed in all children, and patient 21 needed multiple drainages. Patient 20 required salvage amputation, and patient 21 developed an upper cervical spine subluxation secondary to a cervical adenophlegmon.

| Patient | Age/sex/preexisting condition | Primary diagnosis | <i>S. aureus</i> bacteremia | <i>S. aureus</i> strain/allele | Surgical treatment | ICU/LOS (days) | Antimicrobial duration (days)/Antitoxin treatment | Complications | Death |
|---------|-------------------------------|--------------------------------------|-----------------------------|--------------------------------|---|----------------|---|--|-------|
| 1 | 5,2yr/F/No | Necrotizing pneumonia | Yes | CC152 MSSA/ agr1 | PNO, PL and PE drainage | Yes/30 | 30/Yes | ARDS, PNO, VAP, DIVC, tamponade, ICH, renal failure, SBI | Yes |
| 2 | 4mo/M/No | Necrotizing pneumonia | Yes | NA | PE and articular drainage | Yes/39 | 42/Yes | Tamponade, septic arthritis, SBI | No |
| 3 | 2,4yr/M/No | Necrotizing pneumonia | No | CC398 MSSA/ agr1 | PL drainage | Yes/20 | 35/Yes | | No |
| 4 | 1mo/M/No | Necrotizing pneumonia | Yes | CC152 MSSA/ agr1 | None | Yes/12 | 21/Yes | | No |
| 5 | 13,9yr/F/No | Necrotizing pneumonia | No | NA/ agr2 | PNO and PL drainage, laparotomy | Yes/42 | 42/Yes | ARDS, PNO, DIVC | No |
| 6 | 2,9yr/F/No | Necrotizing pneumonia | Yes | CC152 MSSA/ agr1 | PL drainage, laparotomy | Yes/2 | 1/No | Heart failure, DIVC | Yes |
| 7 | 3mo/M/No | Necrotizing pneumonia | No | CC152 MSSA/ agr1 | PL drainage | Yes/29 | 21/Yes | | No |
| 8 | 6mo/M/No | Necrotizing pneumonia | No | CC15 MSSA/ agr2 | PNO and PL drainage | Yes/18 | 42/Yes | | No |
| 9 | 7mo/M/No | Necrotizing pneumonia | Yes | CC152 MSSA/ agr1 | None | Yes/16 | 42/Yes | | No |
| 10 | 1mo/F/No | Necrotizing pneumonia | Yes | CC152 MSSA/ agr1 | None | Yes/16 | 28/Yes | | No |
| 11 | 2mo/F/No | Necrotizing pneumonia | Yes | CC152 MSSA/ agr1 | PNO and PL drainage | Yes/14 | 18/Yes | | No |
| 12 | 3mo/F/No | Necrotizing pneumonia | Yes | CC88 MSSA/ agr3 | PNO and PL drainage | Yes/23 | 42/Yes | PNO, thrombosis, SBI | No |
| 13 | 17,7yr/F/No | Necrotizing pneumonia | No | CC152 MSSA/ agr1 | PNO and PL drainage | Yes/76 | 70/Yes | ARDS, DIVC, seizures, SBI | No |
| 14 | 5,3yr/M/No | Skin and soft tissue infection | Yes | NA | None | No/7 | 14/No | | No |
| 15 | 10mo/M/Yes | Skin and soft tissue infection | Yes | CC152 MSSA/ agr1 | Abscess drainage | No/41 | 28/Yes | | No |
| 16 | 6,2yr/F/Yes | Skin and soft tissue infection | No | CC152 MSSA/ agr1 | Abscess drainage | No/4 | 10/No | | No |
| 17 | 23mo/F/No | Skin and soft tissue infection | No | CC152 MSSA/ agr1 | Abscess drainage | No/5 | 14/Yes | | No |
| 18 | 13,9yr/M/No | Skin and soft tissue infection | NA | CC152 MSSA/ agr1 | Abscess drainage | No/1 | 8/No | | No |
| 19 | 1mo/M/No | Skin and soft tissue infection | NA | NA | Abscess drainage | No/2 | 8/No | | No |
| 20 | 5,2yr/M/No | Osteoarticular infection | Yes | NA/ MSSA / NA | PL and articular drainage, amputation | Yes/26 | 42/Yes | NP, SSTI, SBI | No |
| 21 | 6,2yr/F/No | Osteoarticular infection | Yes | CC15 MSSA/ agr2 | PL and articular drainage | Yes/50 | 56/Yes | NP, SSTI, SBI, cervical subluxation | No |
| 22 | 3,2yr/M/No | Osteoarticular infection | No | CC152 MSSA/ agr1 | Articular drainage | Yes/12 | 28/Yes | | No |
| 23 | 6mo/M/No | Cervicomedastinitis and pericarditis | Yes | CC152 MSSA/ agr1 | Cervico-mediastinal, PL and PE drainage | Yes/42 | 28/Yes | ARDS, VAP | No |
| 24 | 5mo/F/No | Catheter infection with bacteremia | Yes | CC88 MRSA/ agr3 | None | Yes/22 | 14/Yes | NP | No |

ICU = intensive care unit ; LOS = length of hospital stay ; yr = year ; F = female ; MSSA = methicillin sensitive staphylococcus aureus ; PNO = pneumothorax ; PL = pleural ; PE = pericardial ; ARDS = acute respiratory distress syndrome ; VAP = ventilator-acquired pneumonia; DIVC = disseminated intravascular coagulopathy ; ICH = intracranial hypertension ; mo = month ; M = male ; NA : non available ; NP = necrotizing pneumonia ; SSTI = skin and soft tissue infection ; MRSA = methicillin resistant staphylococcus aureus, SBI= secondary bacterial infection

Table : Clinical and bacteriological characteristics and outcome of the 24 patients with invasive PVL-SA infections.

DISCUSSION

To the best of our knowledge, the present series is the first pediatric series describing severe invasive infections caused by PVL-SA in one population-based study in the Indian Ocean region.

As in other pediatric studies, NP, severe SSTI and osteoarticular infections were the most common clinical presentations of severe PVL-SA infections, and most cases occurred in previously healthy children [2-4]. Nearly half of our patients originated from the neighbouring islands of Mayotte or Madagascar which are resource-poorer countries, where neglected skin infections and close and crowded community lifestyle, are common. Those factors have been recognized as risk factors for SSTI [4].

Although the role of PVL as a direct contributor to clinical severity and outcome remains controversial, severe pediatric PVL-SA infections carry a high burden [1, 3-5,7]. Nearly half of our patients presented associated complications, 75% were admitted to an ICU, and complementary surgical procedures were needed in most children. The mortality rate observed in our cohort was 8 % and seems to be decreasing according to recent pediatric reports [1, 3-5].

NP was the most common primary clinical diagnosis, was observed in the lowest age group, and was the only instance in which death was observed. Children with NP and osteoarticular infections needed the highest levels in clinical management and presented the highest levels of inflammatory parameters. They also developed the highest rate of associated short-term complications, which were

either specific or non-specific to SA infections [2-4, 7]. Conversely, none of the patients with severe SSTI required ICU care, and all had a favorable outcome.

Unlike PVL positive community-acquired methicillin-resistant SA endemicity in the United States, PVL was associated with MSSA strains in our region, like in most European countries [4]. This information is very important in deciding the best empiric treatment in community-acquired SA infections in our setting.

In the absence of comparative studies, recommendations for the treatment of severe infections due to PVL-SA are based on expert opinions [8]. Protein synthesis inhibitors such as clindamycin, rifampicin, fusidic acid and linezolid down-regulate PVL production. In addition to antibiotics, surgical drainage should be attempted where possible to reduce the bacterial and toxin load. Intravenous immunoglobulin may be used in life-threatening conditions, particularly in severe NP [6, 8, 10]. Our study showed that local physicians adhered to the French recommendations: antitoxinic antibiotics were systematically used in NP and osteoarticular infections, surgical drainage was readily performed, and the use of intravenous immunoglobulins was exceptional [8].

The limitations of this study are the small number of patients and the retrospective study design. Also, we were unable to know how many other severe cases occurred, which were not referred for PVL testing.

CONCLUSION

Our case series illustrates the emergence of severe pediatric PVL-MSSA infections in the Indian Ocean. Given the high risk of complications, prompt evidence of the presence of PVL, multidisciplinary management, and aggressive and effective treatment are warranted.

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SERMENT D'HIPPOCRATE

Au moment d'être admis à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences. Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me le demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçu à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission.

Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonoré et méprisé si j'y manque.

Les infections pédiatriques invasives sévères causées par le *Staphylococcus aureus* sécréteur de la leucocidine de Panton-Valentine à La Réunion.

Résumé

Étude rétrospective bicentrique (2014-2019) décrivant 24 cas d'infections pédiatriques sévères à *Staphylococcus aureus* sécréteur de la toxine de Panton-Valentine à La Réunion. Pneumonie nécrosante (54%), infections cutanées et des tissus mous (25%) et infections ostéoarticulaires (13%) étaient les présentations cliniques principales. Les souches communautaires étaient sensibles à la méticilline. Une intervention chirurgicale avait été réalisée chez 19 patients (79%). Des complications étaient survenues chez 10 enfants (42%), et 2 d'entre eux étaient décédés (8%).

Mots-Clés : Leucocidine de Panton-Valentine, sepsis, pédiatrie, *Staphylococcus aureus*, pneumonie nécrosante

Severe invasive pediatric infections caused by Panton-Valentine leukocidin-positive *Staphylococcus aureus* in La Réunion.

Abstract

Bicentric retrospective study (2014 - 2019) describing 24 children presenting severe Panton-Valentine leukocidin-producing *Staphylococcus aureus* infections in La Réunion. Necrotizing pneumonia (54%), skin and soft tissue (25%) and osteoarticular (13%) infections were the main primary clinical diagnoses. Community-acquired strains were methicillin-sensitive. Surgical procedures were performed in 19 patients (79%). Ten patients (42%) presented complications, and two patients (8%) died.

Keywords: Panton-Valentine leukocidin, pediatric sepsis, *Staphylococcus aureus*, necrotizing pneumonia, skin tissue infection