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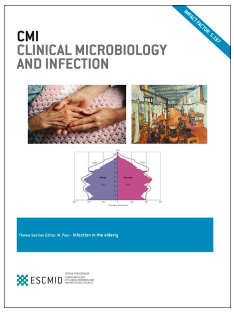

Factors Associated with Severity in Invasive Community-Acquired *Staphylococcus aureus* Infections in Children: A Prospective European Multi-Center Study

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Factors Associated with Severity in Invasive Community-Acquired *Staphylococcus aureus* Infections in Children: A Prospective European Multi-Center Study

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Keywords: *Community acquired Staphylococcus aureus; Panton valentine leukocidin; methicillin resistance; severe infections; children*

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ABSTRACT

Background: Staphylococcus aureus is the main pathogen responsible of bone and joint infections worldwide and capable of causing pneumonia and other invasive severe diseases as well. Panton-Valentine leukocidin (PVL) and methicillin resistance (MRSA) have been studied as factors related with severity in these infections. The aims of this study were to describe invasive community acquired-S. aureus (CA-SA) infections and to analyze factors related to severity of disease.

Methods: Pediatric patients (0-16 years) who had a CA-SA invasive infection were prospectively recruited from 13 centers in 7 European countries. Demographic, clinical, and microbiological data were collected. Severe infection was defined as invasive infection leading to death or admission to intensive care due to hemodynamic instability or respiratory failure.

Results: A total of 152 children (88 boys) were included. The median age was 7.2 years (IQR 1.3-11.9). Twenty-six of the patients (26/152; 17%) had a severe infection including 3 deaths (3/152; 2%). Prevalence of PVL-positive CA-SA infections was 18.6% and 7.8% of the isolates were MRSA. The multivariate analysis identified pneumonia (aOR 13.39 [4.13–43.56]; p=0.008), leukopenia on admission (<3000/mm3) (aOR 18.3 [1.3 -259.9]; p= 0.03) and PVL-positive infections (aOR 4.69 [1.39–15.81]; p= 0.01) as the only factors independently associated with severe outcome. There were no differences in MRSA prevalence between severe and non-severe cases (aOR 4.30 [0.68– 28.95]; p=0.44).

Conclusions: Our results show that, in European children, PVL is associated with more severe infections, regardless of methicillin resistance.

INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens, usually responsible for minor skin and soft-tissue infections (SSTIs). However, this pathogen is also the main cause of bone and joint infections worldwide and capable of causing complicated pneumonia and other invasive diseases. Invasive disease often occurs as a complication of a preceding SSTI or viral respiratory tract infections (particularly influenza), but also spontaneously in otherwise healthy children without recognized preceding infections or risk factors [1].

In the past 15 years there has been an increase of publications describing severe cases of CA-SA infections [2,3]. This has occurred simultaneously with the emergence of community-acquired methicillin-resistant S. aureus (CA-MRSA) in the USA [4,5].

Several risk factors associated with the increasing prevalence of severe *S. aureus* infections have been studied. Some authors have suggested that methicillin-resistance could be related to an increase of illness severity [6,7] although others have shown no relationship [8]. Other risk factors, including the interaction of the host and the pathogen have been studied. One of the potential virulence factors to be associated with severity and worse outcome has been Panton-Valentine leukocidin (PVL). PVL is a bicomponent, pore-forming toxin produced by some strains of *Staphylococcus aureus.* There is some evidence suggesting that PVL presence is indeed related to severity [3,9], and there are also data questioning the importance of PVL in pathogenesis of severe infections [10,11].

The aims of this European multi-centre prospective study were to define the clinical and microbiologic characteristics of invasive *S. aureus* infections in children and to analyse factors related to severity in this disease.

MATERIAL AND METHODS

We performed a prospective, multi-center European study during a 2-year period (1-10-2012 to 30-09-2014) using a standardized data sheet that incorporated epidemiologic (sex, age), microbiologic (site of isolation, antibiotic resistance, PVL presence), and clinical data (symptoms on admission, duration of symptoms before admission, duration of hospital stay, admission to pediatric intensive care unit [PICU], duration of PICU stay, use of inotropic drugs or mechanical ventilation in PICU). All children aged 1 day to 16 years with CA-SA invasive infections and meeting criteria were prospectively and consecutively enrolled in 13 European centers of 7 different countries (Spain, Lithuania, Israel, Italy, Germany, Greece and Romania). Data was collected by the main investigator of each site approaching the patients, parents, and files.

Invasive infections were defined as clinical infection with isolation of *S. aureus* from a normally sterile body site such as blood, cerebrospinal fluid, pleural fluid, pericardial fluid, peritoneal fluid, synovial fluid, bone or other internal body site. For pneumonia to be included, signs and symptoms of lower respiratory tract infection (cough, expectoration, chest pain, etc.) and pulmonary lobar infiltrates on the chest radiograph not attributable to other causes were required, together with isolation of *S. aureus* as the only potential pathogen, by at least one of the following procedures: 1) puncture of a pleural effusion or lung abscess; 2) culture of bronchoalveolar lavage fluid ($\geq 10^4$ CFU/ml), or Wimberley brushing ($\geq 10^3$ CFU/ml) or protected tracheal aspiration ($\geq 10^3$ CFU/ml); 3) blood culture. Primary bacteremia was defined as not preceded by an infection in another location.

Only community-acquired infections were included. These infections were defined as a positive culture result from normally sterile site obtained at hospital admission or up to 48 hours later, or recovered after a longer interval, only if the clinical syndrome had started earlier and no other pathogen had been identified. Severe infection was defined as invasive infection leading to death or admission to PICU because of hemodynamic instability or respiratory failure.

Microbiological studies:

Clinical samples were processed in each participating centre. Identification and susceptibility testing were performed according to standard procedures. Isolates were sent to a central laboratory (Microbiology Department, Hospital Universitario 12 de Octubre, Madrid, Spain) where MRSA isolates, previously identified by phenotypic methods, underwent confirmatory PCR analysis for the *mecA* gene. The presence of PVL genes (*lukS-PV and lukF-PV)* was also determined by PCR in all isolates collected at the central laboratory, considering only one strain by patient.

Statistical analysis:

All continuous variables were expressed in mean values ± standard deviation. For univariate analysis, dichotomous variables were analysed by the X^2 test or by Fisher's exact test; continuous variables were analysed by Student's *t* test. Odds Ratios (ORs) were calculated with a 95% confidence interval (CI). Multivariable logistic regression analysis was performed to assess independent associations. The model was validated by Hosmer-Lemeshow test and simultaneous entry method was used. Independent variables included in this analysis were those observed to be statistically significant at the univariate analysis or those previously seen associated in literature. Number of independent variables was limited to 4 (6.8:1 events per independent variable). Interactions between the independent variables were tested but not found to be significative. Adjusted ORs were calculated by Exp(b) value. A *P* value <0.05 was considered to be statistically significant. Analyses were performed using SPSS v20 (SPSS Inc, Chicago, USA).

This study received the approval of the 12 de Octubre Hospital's Clinical Research Ethics Committee.

RESULTS

Demographic data

A total of 152 children (88 boys) with CA-SA invasive infection were included in the study from the following countries: Spain (68), Lithuania (56), Israel (10), Greece (5), Italy (4), Germany (5), and Romania (4). The male-to-female sex ratio was 1.4 and the median age was 7.2 years (interquartile range [IQR] 1.3-11.9).

Medical history and Microbiologic data

Risk factors for infection and/or invasive disease were found in 28% of patients (12 patients carried long term intravascular access devices, 13 had chronic or acute predisposing diseases, 11 had oncologic diseases, and 6 were neonates). Most patients presented with bone or joint symptoms (84; 55%), followed by respiratory symptoms (31; 20%) and 21 patients presented with influenza-like syndrome (fever, cough, arthralgia).

PVL-positive CA-SA infections were observed in 18.6% of children (22 of 118 strains available, 4 of them were MRSA) and methicillin-resistance was detected in 7.8% (13 children of 152) of all the isolates. Additionally, 20 isolates (13%) were resistant to erythromycin, 14 (9%) were resistant to clindamycin and 7 (5%) were resistant to gentamycin. No resistance to trimethoprim-sulfamethoxazole was found. All isolates were susceptible to glycopeptides and linezolid.

Presentation and outcome

Main demographic, clinical, microbiology and laboratory characteristics of the patients are shown in **Table 1**. Twenty-six patients (17%) had a severe infection of which 3 children (2%) died. Bone and joint infections (55%), followed by bacteraemia (22% [13% primary and 9% secondary to SSTIs]) and pneumonia (17%) were the most common invasive infections. The most common antibiotics used, including empiric treatment and definitive therapy targeting *S aureus,* were betalactams (82%), 33 % of which received cloxacillin, and glycopeptides (17%), the latter being vancomycin in 14 cases. Only ten patients (6.5%) received antibiotics inhibiting toxin production (clindamycin or linezolid) during the first 48 hours from admission, only one of them with a severe infection. None of the severe cases received intravenous immunoglobulin. Concerning

interventional procedures, at least one surgical procedure was performed in 77 cases, and 25 patients needed more than one intervention.

Univariate analysis

Severe and non-severe infections were compared **(Table 2)**. No significant differences were found in gender, age, methicillin-resistance or presence of predisposing factors for a severe invasive infection. Patients with severe infection had a higher frequency of PVL-positive isolates (9/25; 36% *vs*. 13/93; 15%, *p*=0.012), were more likely to present with pneumonia (13/26; 50% *vs*. 13/126; 10.3%, *p*<0.001) and presented more typically with leukopenia (< 3000/mm^3) on admission (3/24; 12.5% *vs*. 2/125; 1.6%; *p*=0.03). Severe cases also had higher mean C-reactive protein (CRP) value on admission (17.5±11.2 vs 8.8±12.1 *p*=0.002) and longer mean hospital stay (30.4±19.5 vs15.6±10.3 days: *p*<0.001)

Comparing PVL-positive infections with PVL-negative infections (**Table 3**), patients with PVL-positive infections had longer mean hospital stay (24.3±16.1 vs 18.0±12.0 days; *p*=0.04). However, there were no differences in age, gender, methicillin-resistance or other parameters analyzed.

When comparing MRSA infections with MSSA infections (**Table 4**) no differences related to age, gender or severity were observed. Only pneumonia was more frequently associated with MRSA (6/13; 46% *vs*. 20/139; 14.5%, *p*=0.004).

Multivariate Analysis

The multivariate analysis identified pneumonia (aOR 13.39; *p*= 0.008), leukopenia on admission (<3000/mm^3) (aOR 18.3; *p*= 0.03) and PVL-positive infections (aOR 4.69; *p*= 0.01) as the only factors independently associated with severe outcome (**Table 5).**

DISCUSSION

In this European, multi-centre, prospective study the severity and impact of CA-SA invasive infections in children were evaluated. Twenty-six of the patients (17%) had a severe infection including 3 deaths (2%). Most of the children with severe infections were previously healthy, being admitted to the PICU because of hemodynamic or respiratory failure, secondary to severe pneumonia or septic shock.

CA-SA severe infections have been increasingly reported over the past 15 years, mostly in retrospective, single-center studies, each describing a few patients [5,6]. One of the first reports on severity of CA-SA infections described 4 paediatric deaths in North Dakota in 1999 [12], followed by other reports from various geographic areas in the USA, such as Houston and Chicago [5,13]. Here we describe a quite large prospective cohort of patients with invasive CA-SA infections with a relatively-high rate of severe infections in various regions in Europe caused by both CA-MSSA and CA-MRSA strains.

We have found that 18.6% of the isolates available in our study were PVL positive which seems to be in accordance with recent studies. All but one of the severe cases were tested for PVL, and most of the non-severe (93 isolates, 74%). PVL is a *S. aureus* exotoxin described almost 100 years ago as a virulence factor targeting white blood cells causing leukocyte destruction and tissue necrosis. In 1995, Prevost *et al.* reported that PVL was produced by 2% of *S. aureus* isolates in a general hospital in France [12] whilst the prevalence of PVL-positive isolates in the UK was less than 2% in 2003 [13]. More recently, an increase in the proportion of PVL-positive strains had been noted. Our group found 26% prevalence on CA-SA-SSTIs in Spain [14] and the USA-300 clone that has spread in the USA in the last two decades harbors the PVL genes in a significant proportion (98% in MRSA isolates and 42% in MSSA isolates) [15].

When analyzing the risk factors associated with severity in our study, we found PVL presence to be an independent factor in the multivariate analysis (aOR 4.7; p=0.01). Other studies had found similar results. In the late 90s, the French Group from Lyon described, that PVL was associated with severe pneumonia in otherwise previously healthy children and young adults [3]. Bocchini *et al.* found that PVL presence was associated with more severe local disease and a greater systemic inflammatory response in osteomyelitis caused by *S. aureus* in children [16]. Martinez-Aguilar *et al.* showed no differences between MRSA and MSSA musculoskeletal infections in general outcome, but suggested an

association between PVL presence and the complications of these diseases [17,18]. In another retrospective study, Cunnington *et al.* recovered eleven cases of severe invasive *S aureus* infections, all of the isolates being MSSA and carrying PVL genes [6]. Dohin *et al.* found that PVL-positive joint and bone infections in children were more severe than PVL-negative infections requiring more PICU support, prolonged antibiotic treatment and repeated surgical drainage [4]. The controversy on the direct role of PVL in the pathogenesis of severe staphylococcal infections led some authors to think that even if PVL is not the primary virulence factor it could be viewed as a marker of increased pathogenicity [12].

In this study severe leukopenia (<3000 cells/mm^3) on admission was independently associated with severity in univariate and multivariate analysis (aOR 18.3, p=0.03). Our result is in accordance with the studies from the French National Reference Centre for Staphylococci. In 2007, Gillet *et al.*, analyzed different factors predicting mortality in necrotizing community-acquired pneumonia in children and adults caused by PVL-positive *S. aureus* and found that leukopenia was the main laboratory feature associated with death [19]. The same group led by Khanafer expanded the previous study and confirmed that leukopenia was a significant predictive factor of disease severity (death occurred in 76% of severe leukopenia cases compared with 16% of cases with leukocyte count >3000 cells/mm^3) [20]. As leukocyte count is available on admission at any Emergency Department they may be a useful marker to assess the severity of any *S. aureus* invasive disease.

The third independent factor associated with severity of invasive CA-SA infections in our study was pneumonia (aOR 13.39, p<0.01), as these patients are more likely to suffer a respiratory failure and be admitted to PICU. *S. aureus* invades the lung directly through the tracheobronchial tree (primary pneumonia) or via hematogenous seeding (secondary pneumonia). Both, primary and secondary pneumonia have been reported in a high proportion of children with invasive CA-SA infections [21]. *S. aureus* primary pneumonia has been recognized to be more prone to complications compared with *Streptococcus pneumoniae*, the main respiratory pathogen [18]. In the past years a specific, severe, *S. aureus* primary pneumonia in children and young adults has been described, often preceded by influenza-like symptoms and characterized by hemoptysis, pleural effusion and rapid onset of acute respiratory distress, with a high fatality rate [3,22,23]. In contrast, secondary pulmonary involvement in children with CA-SA lower limb osteomyelitis associated with pelvic venous thrombosis causing septic pulmonary emboli has been reported [24,25].

In our study MRSA caused less than 10% of the invasive *S. aureus* infections and was not a significant factor related to severity. Patients who suffered CA-MRSA invasive infections did not show a worse outcome and had similar length of hospital stay and leukopenia rates compared to CA-MSSA. Some authors,

mostly from the USA, have associated MRSA with more severe infections [7, 8]. As previously reported, MRSA is presumably only a marker of severity by itself, and it is probably not as important in USA-300 low-prevalence areas, neither a significant independent predictor of severity [9,26].

There is *in-vitro* evidence showing that the use of clindamycin, linezolid or rifampicin in PVL-positive *S. aureus* isolates inhibits the production of PVL while vancomycin and trimethoprim-sulfamethoxazole have no effect [9,27,28].Based on the severity of the disease and the available evidence, some authors [29,30] and guidelines [31] are now recommending adding antibiotics that inhibit toxin production when a PVL-producing *S. aureus* invasive infection is suspected and that the use of IVIg should be considered in the most severe cases. Our study shows that the usual clinical practice is not to use toxin inhibitor antibiotics or IVIg as only one of the severe cases received toxin inhibitor antibiotics in the first 48 hours, and none IVIg.

Our study has several limitations. The inclusion of children was performed by the participating clinician, and the cultures were taken from patients who attended the hospital, with the potential reporting of more severe cases. We consider, since the definition was based on specific microbiologic data, this bias to be minimal. Not all the isolates were studied for PVL presence, but we believe that since data were available in 118 cases (77.6%), results would not be significantly affected. The major strengths of our data are the prospective, multi-center, multi-country nature of the study.

In summary, based on previous reported studies and the results presented here we suggest that PVL may play a central role in the severity of *S. aureus* invasive infections, regardless of methicillin resistance. Future studies should assess the need to include specific PVL-directed treatment in the empirical management of these severe and potentially fatal infections in children.

Potential conflicts of interest. All authors: no conflicts.

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[1] Kaplan SL, Hulten KG, Gonzalez BE, Hammerman WA, Lamberth L, Versalovic J. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. Clin Infect Dis **2005**; 40: 1785–1791.

[2] Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M. Association between Staphylococcus aureus strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet **2002**; 359: 753-759.

[3] Dohin B, Gillet Y, Kohler R, Lina G, Vandenesch F, Vanhems P. Pediatric bone and joint infections caused by Panto-Valentine leukocidin-positive Staphylococcus aureus. Pediatr Infect Dis **2007**; 26: 1042-1048.

[4] Gonzalez BE, Martinez-Aguilar G, Hulten KG, Hammerman WA, Coss-Bu J, Avalos-Mishaan A. Severe staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant *Staphylococcus aureus*. Pediatrics **2005**; 115: 642–648.

[5] Cunnington A, Brick T, Cooper M, et al. Severe invasive Panton-Valentine leucocidin positive *Staphylococcus aureus* infections in children in London, UK. J Infec **2009**; 59: 28–36

[6] Arnold SR, Elias D, Buckingham SC, et al. Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant Staphylococcus aureus. J Pediatr Orthop **2006**; 26: 703.

[7] Ochoa TJ, Mohr J, Wanger A, Murphy JR, Heresi GP. Community-associated methicillin-resistant Staphylococcus aureus in pediatric patients. Emerg Infect Dis **2005**;11: 966.

[8] Sicot N, Khanafer N, Meyssonnier V, Dumitrescu O, Tristan A, Bes M. Methicillin resistance is not a predictor of severity in community-acquired Staphylococcus aureus necrotizing pneumonia--results of a prospective observational study. Clin Microbiol Infect **2013**;19: 142-148

[9] Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis **1999**; 29:1128-1132.

[10] Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. Lancet Infect Dis **2012**; 13: 43–54

[11] David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010; 23: 616-87

[12] Prevost G, Couppie P, Prevost P, Gayet S, Petiau P, Cribier B. Epidemiological data on Staphylococcus aureus strains producing synergohymenotropic toxins. J Med Microbiol **1995**; 42: 237–245.

[13] Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol **2005**; 43: 2384-2390.

[14] Daskalaki M, Rojo P, Marin-Ferrer M, Barrios M, Otero JR, Chaves F. Panton-Valentine leukocidin-positive Staphylococcus aureus skin and soft tissue infections among children in an emergency department in Madrid, Spain. Clin Microbiol Infect **2010**; 16: 74-77.

[15] Moran G, Krishnadasa A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB. Methicillin-resistant S. aureus infections among patients in the Emergency Department. New Engl J Med **2006**; 355: 666-674.

[16] Bocchini CE, Hulten KG, Mason EO, Gonzalez BE, Hammerman WA, Kaplan SL. Panton–Valentine leukocidin genes are associated with enhaced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomielitis in children. Pediatrics **2006**; 117: 433–440.

[17] Martinez-Aguilar G, Avalos-Mishaan A, Hulten K, Hammerman W, Mason EO Jr, Kaplan SL. Community-acquired methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* musculoskeletal infections in children. Pediatr Infect Dis J **2004**; 23: 701–706.

[18] Sdougkos G, Chini V, Papanastasiou DA, Christodoulou G, Tagaris G, Dimitracopoulos G. Methicillin-resistant Staphylococcus aureus producing Panton-Valentine leukocidin as a cause of acute osteomyelitis in children. Clin Microbiol Infect 2007 Jun; 13: 651-4

[19] Gillet Y, Vanhems P, Lina G, Bes M, Vandenesch F, Floret D. Factors predicting mortality in necrotizing community-acquired pneumonia caused by Staphylococcus aureus containing Panton-Valentine leukocidin. Clin Infect Dis **2007**; 45: 315-321.

[20] Khanafer N, Sicot N, Vanhems P, Dumitrescu O, Meyssonier V, Tristan A. Severe leukopenia in Staphylococcus aureus-necrotizing, community-acquired pneumonia: risk factors and impact on survival. BMC Infect Dis **2013**; 13:359.

[21] Gonzalez BE, Hulten KG, Dishop MK, Lamberth LB, Hammerman WA, Mason EO Jr. Pulmonary manifestations in children with invasive community-acquired Staphylococcus aureus infection. *Clin Infec Dis,* 2005; 41: 583-90.

[22] Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant Staphylococcus aureus carrying the Panton-Valentine lekocidin genes. Clin Infect Dis **2005**; 40: 100-107.

[23] Finelli L, Fiore A, Dhara R, Brammer L, Shay DK, Kamimoto L, Fry A. Influenza-associated pediatric mortality in the United States: increase of Staphylococcus aureus coinfection. Pediatrics **2008**; 122: 805-11.

[24] Gorestein A, Gross E, Houri S, Gewirts G, Katz S. The pivotal role of deep vein thrombophlebitis in the development of acute disseminated staphylococcal disease in children. Pediatrics **2000**; 106: 87.

[25] Obando I, Croche B, Madrid D, Neth O. Deep vein throbosis and septicpumonary emboli associated with acute paediatric osteomyelitis due to methicillin-sensitive Staphylococcus aureus producing Panton-Valentine leukocidin. Enferm Infecc Microbiol Clin **2011**; 29: 550-559.

[26] Barrios Lopez M, Gomez Gonzalez C, Orellana MA, Chaves F, Rojo P. Staphylococcus aureus abscesses: methicillin-resistance or Panton-Valentine leukocidin presence? Arch Dis Child **2013**; 98: 608-610.

[27] Dumitrescu O, Boisset S, Badiou C, Bes M, Benito Y, Reverdy ME. Effect of antibiotics on Staphylococcus aureus producing Panton-Valentine leukocidin. Antimicrob Agents Chemother **2007**; 51: 1515-1519.

[28] Dumitrescu O, Badiou C, Bes M, Reverdy ME, Vandenesch F, Etienne J. Effect of antibiotics, alone and in combination, on Panton-Valentine leukocidin production by a Staphylococcus aureus reference strain. Clin Microbiol Infect **2008**; 14: 384-388.

[29] Gillet Y, Dumitrescu O, Tristan A, Dauwalder O, Javouhey E, Floret D. Pragmatic Management of Panton-Valentine leukocidin-associated staphylococcal diseases. Int J Antimicrob Agents **2011**; 38: 457-464.

[30] Rojo P, Barrios M, Palacios A, Gomez C, Chaves F. Community-associated Staphylococcus aureus infections in children. Expert Rev Anti Infect Ther **2010**; 8: 541–554.

[31] Nathwani D, Morgan M, Masterton RG, Dryden M, Cookson BD, French G. Guidelines for UK practice for the diagnosis and management of methicillin-resistant *Staphylococcus aureus* (MRSA) infections presenting in the community. J Antimicrob. Chemother **2008**; 61: 976–994.

Table 1. Demographic and clinical characteristics of patients with community-acquired invasive *Staphylococcus aureus* infections.

Characteristic	
Male gender (%) (n = 152)	88 (58%)
Age (years) (n = 152)	7.2 ± 5.4
Underlying disease (n = 152)	42 (28%)
Hospital stay (days) (n = 152)	19.1±13.6
Severe infection (n = 152)	26 (17%)
Mortality (n=152)	3 (2%)
PVL+ (n=118)	22 (18.6%)
MRSA (n=152)	12 (7.8%)
Fever on admission (°C) (n=124)	38.6 ± 1.1
CRP on admission (mg/dl) (n=147)	10.1 ± 12.4
WCC on admission (n=149)	12885 ± 702
Isolate in blood° (n=152)	83 (54.6%)
Interventional procedures (n=152)	77 (49%)

Underlying disease: long term intravascular access devices carriers (12), chronic or acute predisposing diseases (13), oncologic diseases (11), neonates (6); WCC = White-cell count (cells/mm^3); PCR = C - reactive protein; °primary bacteremia and secondary bacteremia to a superficial (soft tissue infections or other superficial seated infection) or deep-sited infections (pneumonia, osteomyelitis, pyomyositis).

Table 2. Characteristics of severe versus non-severe community-acquired invasive *Staphylococcus aureus* infections.

	Severe (n=26)	Non severe (n=126)	p	OR (IC 95%)
Male /female	13/13	75/51	0.37	0.68 (0.29 – 1.58)
Age (years)	6.6±5.9	7.3±5.4	0.66	
Underlying disease	8/26 (30.8%)	34/125 (27.2%)	0.71	1.18 (0.47 – 2.96)
Mean hospital stay (days)	**30.4±19.5**	**15.6±10.3**	**<0.01**	
WCC on admission	11716±6598	13041±7080	0.39	
PVL(+)	**9/25 (36%)**	**13/93 (15%)**	**0.012**	**3.46 (1.27 – 9.43)**
MRSA	3/26 (11.5%)	10/125 (8%)	0.44	1.50 (0.38 – 5.88)
CRP on admission (mg/dl)	**17.5±11.2**	**8.8±12.1**	**0.002**	
Leucopenia on admission (<4000)	3/24 (12.5%)	4/125 (3.2%)	0.08	4.32 (0.98 – 20.71)
Leucopenia on admission (<3000)	**3/24 (12.5%)**	**2/125 (1.6%)**	**0.03**	**8.79 (1.38 – 55.77**)
Fever on admission (^{0}C)	38.6±0.8	38.6±1.2	0.69	
Pneumonia	**13/26 (50.0%)**	**13/126 (10.3%)**	**<0.001**	**8.7 (3.33 - 22.7)**
Bone and joint infections	11/26 (42.3%)	74/126 (58.7%)	0.16	0.55 (0.23 – 1.28)
Primary bacteremia	3/26 (11.5%)	22/126 (17.5%)	0.46	0.61 (0.17 – 2.23)
Secondary to SSTI bacteremia°	0/26(0%)	13/126 (10.3%)	0.13	0.16 (0.01 – 2.75)
Isolate in blood^{oo}	17/26 (65.4%)	66/126 (52.4%)	0.18	1.8 (0.75 – 4.35)

Note: PVL presence available in 118 samples.

Underlying disease: WCC = white-cell count (cells/mm^3); ° Bacteremia secondary to soft tissue infections or other superficial seated infection; ^{oo} primary bacteremia and secondary bacteremia to a superficial (soft tissue infections or other superficial seated infection) or deep-sited infections (pneumonia, osteomyelitis, pyomyositis).

Table 3. Characteristics of PVL-positive versus PVL-negative Infections.

	PVL + (n=22)	PVL - (n=96)	p	OR (IC 95%)
Male / female	13/9	60/36	0.76	0.86 (0.34 – 2.23)
Age (years)	7.9±4.9	7.1±5.4	0.61	
Underlying disease	4/22 (18.2%)	28/96 (29.5%)	0.43	1.87 (0.58 – 6.06)
Mean hospital stay	**24.3±16.1**	**18.0±12.0**	**0.042**	
WCC on admission	11723±6715	13185±70508	0.38	
Severe infection	**9/22 (40.9%)**	**16/96 (16.7%)**	**0.012**	**3.46 (1.27 – 9.43)**
MRSA*	4/22 (18.2%)	6/96 (6.25%)	0.07	3.33 (0.85 – 13.02)
CRP on admission (mg/dl)	14.4±13.3	9.8±13.5	0.16	
Leucopenia (<3000)	2/22 (9.5%)	2/96 (2.1%)	0.15	4.84 (0.64 – 36.55)
Fever on admission (^{0}C)	**38.9±0.9**	**38.4±1.2**	**0.05**	
Pneumonia	7/22 (31.8%)	17/96 (17.7%)	0.15	2.17 (0.76 – 6.13)
Bone and joint infections	12/22 (54.5%)	58/96 (60.4%)	0.61	1.27 (0.50 – 3.24)
Primary Bacteremia	4/22 (18.2%)	10/96 (10.4%)	0.31	1.91 (0.54 – 6.78)
Secondary to SSTI bacteremia°	2/22(9.1%)	6/96 (6.2%)	0.64	1.50 (0.28 – 7.99)
Isolate in blood^{oo}	14/22 (63.6%)	50/96 (52.1%)	0.33	1.6 (0.62 – 4.19)

*Note: 3 MRSA isolates were not tested for PVL genes.

WCC = white-cell count (cells/mm^3); CRP = C – Reactive Proteine; ° Bacteremia secondary to soft tissue infections or other superficial seated infections; ^{oo} primary bacteremia and secondary bacteremia to a superficial (soft tissue infections or other superficial seated infection) or deep-sited infections (pneumonia, osteomyelitis, pyomyositis).

Table 4. Characteristics of MRSA versus MSSA Infections.

	MRSA (n=13)	MSSA (n=139)	p	OR (IC 95%)
Male/female	6/4	79/60	0.141	2.64 (0.70 – 10.0)
Age (years)	6.6±6.0	7.2±5.4	0.76	
Underlying disease	5/13 (38.5%)	37/139 (27.0%)	0.38	1.69 (0.52 – 5.49)
Mean hospital stay (days)	21.2±10.6	17.8±13.6	0.31	
WCC on admission	15167±11828	12633±6416	0.46	
PVL(+)	4/10 (40.0%)	18/108 (16.7%)	0.07	3.33 (0.85 – 13.02)
Severe infection	3/13 (23.1%)	23/139 (16.7%)	0.70	1.5 (0.38 – 5.9)
CRP on admission (mg/dl)	15.3±10.9	9.7±12.4	0.12	
Leucopenia (<3000)	1/13 (7.7%)	4/139 (3%)	0.37	2.73 (0.28 – 26.32)
Fever on admission (^{0}C)	39.0±1.1	38.6±1.1	0.20	
Pneumonia	**6/13 (46.2%)**	**20/139 (14.5%)**	**0.004**	**5.06 (1.54 – 16.61)**
Bone and joint infections	5/13 (38.5%)	79/139 (57.3%)	0.19	0.47 (0.15 – 1.50)
Primary bacteremia	2/13 (15.4%)	23/139 (16.7%)	0.9	0.90 (0.19 – 4.38)
Secondary to SSTI bacteremia°	1/13 (7.7%)	12/138 (91.4%)	1.0	0.88 (0.11 – 7.32)
Isolate in blood^{oo}	7/13 (53.8%)	76/139 (55.1%)	0.93	0.95 (0.30 – 2.98)

WCC = white-cell count (cells/mm^3) ; ° Bacteremia secondary to soft tissue infections or other superficial seated infections; ^{oo} primary bacteremia and secondary bacteremia to a superficial (soft tissue infections or other superficial seated infection) or deep-sited infections (pneumonia, osteomyelitis, pyomyositis).

Table 5. Multivariate analysis: Factors associated with severity.

	Adjusted OR (95% CI)	p
PVL(+)	**4.69 (1.39 – 15.81)**	**0.01**
MRSA	4.30 (0.68– 28.95)	0.13
Pneumonia	**13.39 (4.11 – 43.56)**	**0.00**
Leucopenia <3000 on admission	**18.3 (1.3 -259.9)**	**0.03**