

## EPIDEMIOLOGY AND PREVENTION OF NOSOCOMIAL PNEUMONIA ASSOCIATED WITH PANTON-VALENTINE LEUKOCIDIN (PVL) PRODUCING STAPHYLOCOCCUS AUREUS IN DEPARTMENTAL HOSPITAL CENTRE OF ZOU COLLINES IN BENIN

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*Conflict of interest:* None declared

### SUMMARY

**Background:** An eight (8) months prospective study was carried out to control an outbreak of nosocomial pneumonia due to a Pantone-Valentine Leukocidin (PVL) producing *Staphylococcus aureus*, in the paediatrics' unit at the Zou/Collines Departmental Hospital (CHDZ/C), (Benin).

**Methods:** Between 1<sup>st</sup> September 2004 and 30<sup>th</sup> May 2005 an investigation was conducted that involved the screening of all patients suspected to have nosocomial pneumonia, hospital environment sampling and the follow-up of cases until the end of hospital admission period. Isolates were identified, tested for antimicrobial susceptibility and analysed for PVL production. The study period was divided into Period I, corresponding to the outbreak period and Period II, after the complete renovation of the Unit along with hand washing promotion.

**Results:** A total of 453 patients were admitted during the period of the study; (235 during Period I and 218 during Period II) in the malnourished children sector. Twenty eight (28) cases of pneumonia due to *S. aureus* were discovered and PVL-producing *S. aureus* constituted 61% (17/28) of identified cases. The mortality rate among the PVL-producing strains was 15/17 (88%) while it was 1/11 (9%) among non PVL-producing strains. Enhanced hygiene measures helped to terminate the outbreak.

**Conclusions:** This study showed that PVL was strongly linked to nosocomial pneumonia. PVL-producing *S. aureus* can be controlled in the hospital by a combination of the promotion of preventive measures, decontamination of the environment and the early use of the correct antibiotic at the appropriate dose and for an adequate duration.

**Key-words:** Necrotizing pneumonia, Pantone-Valentine Leukocidin, *S. aureus* Prevention

### INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is a major pathogenic bacterium, causing nosocomial and epidemic infections, in hospital environments.<sup>1</sup> It is the most frequent pathological agent in nosocomial pneumonia.<sup>2</sup> Since 1950, virulent strains of *S. aureus* have been described as the aetiological agents of nosocomial infections in different hospitals.<sup>3</sup> More than forty different toxins and enzymes are produced by *S. aureus* that contribute substantially to its ability to cause diseases.

Though Pantone-Valentine leukocidin (PVL) toxin is strongly presumed to be an important virulence factor for *S. aureus*, it was only in 1999, that *S. aureus* was considered as the principal bacterium associated with necrotizing pneumonia in a close community.<sup>4,5</sup> First isolates of this bacterium, reported as methicillin-sensitive and carrying bacteriophages 80/81, for unknown reasons disappeared during the seventies.<sup>5</sup>

Outbreaks due to this bacterium have been described and published in developed countries, and community spread was suggested as a mode of transmission<sup>6</sup>. Similar observations have been made in West Africa<sup>7, 8</sup>, where only few clinical cases have been reported. Evidence of hospital ward contamination, nosocomial acquisition and cross-infection have been presented<sup>9, 10</sup> containment of the spread of the infection has not been easy in these reported cases.<sup>5, 11</sup>

In this study we examined the epidemiology of PVL-producing *S. aureus* associated with pneumonia and evaluated the possibilities of limiting the spread of this bacterium by promotion of hygienic measures.

## MATERIALS AND METHODS

### *Local situation*

In August 2004, the Infection Control Programme of the paediatrics' unit at CHDZ/C was notified of a cluster of pneumonia cases due to an unusual *S. aureus* strain among hospitalized malnourished children. This paediatrics unit of 150 beds consists of four sectors one of which is the malnourished sector with 55 beds. Approximately 5000 patients are admitted to the malnourished sector per year and more than 250 pneumonia diagnostic, therapeutic and microbiology analysis are performed annually. The paediatrics' unit started a nosocomial infection control program based essentially on the promotion of hand washing, sterilization and correct disinfection of materials. This study was carried out during the monitoring of this program.

### *Clinical Investigations*

A prospective study was carried out from 1<sup>st</sup> September 2004 to 30<sup>th</sup> May 2005 in the 55-bed paediatrics unit of the CHD Z/C. The study was divided into two periods. During the first period (Period I), which lasted from 1<sup>st</sup> September to 30<sup>th</sup> December 2004, seventy four (74) episodes of severe pneumonia were observed. The month of January was used for the renovation of the Paediatrics Unit, followed by an intensive environmental disinfection of the four sectors of the paediatric unit with particular attention paid to the kitchen.

The second period of the study was then carried out from 1<sup>st</sup> February to 31<sup>st</sup> May, 2005. The medical staff members were informed of the necessity to choose appropriate antimicrobial therapy in nosocomial pneumonia and received guidance on how to achieve this. During the study period, samples were collected from all patients hospitalized for more than 48 hours in the malnourished sector and who developed nosocomial pneumonia. The samples were taken using sterile cotton tipped swabs moistened with phosphate buffered saline and transported to the laboratory within 1 hour for analysis.

Two consecutive isolations of the same bacteria from respiratory specimens (expectorations, tracheal aspirations and pleural fluids) or isolation of a single organism from blood cultures, from the same patient, confirmed a diagnosis of pneumonia. Specimens were collected weekly from the hands of nursing staff, surfaces of medical devices, various surfaces and inanimate objects such as floor areas, bed frame, chairs, lockers, and respirators.

### *Microbiological Investigations*

Different samples collected from patients at the CHDZ/C were cultured on Chapman Agar medium (Difco). Pure colonies were stained by the Gram Staining Technique, examined using the light microscope and further identified through their Catalase, Acetoin and Coagulase reactions. Environmental specimens collected were immediately inoculated in Trypticase soya broth (Difco) and incubated at 37°C for 24 H. As soon as growth was visible, a subculture was made on Chapman medium and *S. aureus* colonies were identified.

### *Antimicrobial susceptibility testing*

Antimicrobial susceptibility was carried out by the agar disk diffusion method on Mueller Hinton agar according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations.<sup>12</sup> Interpretation of antimicrobial susceptibility followed the recommendations of the AntibioGram Committee of the French Microbiology Society (CA-SFM).<sup>13</sup> The antibiotics used included **Penicillin G** (10UI), Oxacillin (5µg), Vancomycin (30 µg), Teicoplanin (30 µg), Tetracycline (30µg), Rifampicin (30 µg), Chloramphenicol (30 µg), Gentamicin (15 µg), Trimetoprim Sulfamethoxazole (1.25/23.75 µg), Ciprofloxacin (5 µg), Pristinamycin (15 µg), Erythromycin (15 UI) and Fusidic acid (30 µg) (Biorad).

Methicillin - resistance was determined by measuring the growth inhibition diameter on agar – agar medium.

### *Panton-Valentine leukocidin (PVL) identification*

All *S. aureus* isolates were investigated for the carriage of PVL. The toxin was detected from culture supernatants after 18 h of growth in YCP medium by radial gel immunodiffusion in 0.6% (w/v) agarose in 10 mM Hepes, 150 mM NaCl pH 7.5 using component-specific rabbit polyclonal but affinity-purified antibodies.<sup>14, 15</sup> For a better determination, gels were dried, stained in Coomassie blue (Sigma), and destained in 10% (v/v) acetic acid.

### *Statistical analysis*

All statistics were performed by SPSS software 11.5. Contingency table analysis was done by  $\chi^2$  test or two-tailed Fisher's exact test for categorical variables. A P-value of 0.05 was considered statistically significant.

## RESULTS

### *Clinical Results*

During Period I, samples were collected from 235 patients out of whom seventy four (74) were diagnosed with pneumonia. *S. aureus* was isolated from twenty eight (28) of the cases, 17 of these were PVL producing *S. aureus* isolates.

The history of 15 of these lasted less than two weeks because of death. Two outbreaks were observed during Period I. The first strain of *S. aureus* was isolated on 15<sup>th</sup> September 2004; the patient developed bilateral necrotizing pneumonia ten (10) days after his hospitalization for malaria and anaemia. A multi-focal treatment was administered that comprised the draining of the pleural effusion and soaking with chlorhexidine; he was treated with oral Vancomycin (4 x 500mg daily) and chloramphenicol and died three days later.

Eighteen (18) children were affected in the first outbreak of the period. The second outbreak occurred on 20<sup>th</sup> November 2004 and involved 10 patients. During Period II, 218 patients were tested; 23 cases of pneumonia were found, involving 7 cases of *S. aureus*, 2 of these were PVL producing *S. aureus* isolates. Table 1 shows the distribution of pneumonia cases.

**Table 1** Comparison of pneumonia associated with *S. aureus* growth during period I and period II after promotion of hand washing and renovation of the unit.

Admitted Patients	Total cases of Pneumonia	Pneumonia due to other bacteria	Pneumonia due to <i>S. aureus</i>	Pneumonia due to <i>S. aureus</i> PVL+	Mortality PVL+	Mortality due to other bacteria
Period I, n= 235	74/235 (31%)	46/74 (62%)	28/74 (38%)	17/28 (61%)	15/17 (88%)	4/46 (9%)
Period II, n=218	23/218 (11%)	16/23 (70%)	7/23 (30%)	2/7 (29%)	2/2 (100%)	2/16 (13%)

There was a statistically significant difference between the mortality rates of patients infected with PVL producing *S. aureus* compared to the mortality caused by other bacteria ( $P < 0.0001$ )

#### *Characteristics of S. aureus isolated during Periods I and II*

Infected patients were often male, and they had been in the malnutrition sector of paediatrics unit during the study period. The common denominator is the fact that they come from poor social groups, were undernourished after severe malaria and had received a special diet during hospitalization before the pneumonia signs appeared. The special diet comprised a specific cow-milk based preparation with local cereals and vitamins, referenced as F100 by WHO recommendation. The medium length between the admission of patients and the appearance of pneumonia signs was 12 days with a median of 8 days.

#### **Microbiology results**

##### *Isolation of S. aureus from patients' samples*

A total of 315 samples were collected; 222 in Period I and 93 in Period II through weekly collection. Of the 74 pneumonia cases in Period I, the first isolation of *S. aureus* was from 28 of the cases with 46 of the cases due to isolates of other bacteria such as *Escherichia coli* 14, *Klebsiella pneumoniae* 8, *Streptococcus pneumoniae* 4, Enterococcus 6, *Haemophilus influenzae* 3 and some undetermined microorganisms 11. In Period II these results were obtained: 7 *S. aureus* and 16 other bacteria such as: *Streptococcus pneumoniae* 10, *Mycoplasma pneumoniae* 1, *Streptococcus pyogenes* 3, and *Mycobacterium tuberculosis* 2. Table 2 shows the result.

##### *Isolation of S. aureus in the hospital environment*

A total of 410 samples from the hands of nursing staff and from different parts of the paediatric unit (environmental source) were screened for *S. aureus* in period I; the same number of samples were collected in period II. A total of 93 isolates were obtained in Period I and 22 in Period II. The distribution of isolates from various samples is presented in Table 3.

##### *Antimicrobial susceptibility*

A total of 150 *S. aureus* isolates were tested, one hundred and twenty one (121) isolates in Period I and twenty nine (29) in Period II. Out of 150 isolates tested, 135 (90%) were resistant to one or more antibiotic, while 15 (10%) were fully susceptible. All isolates from the study were susceptible to vancomycin and teicoplanin, 45 (30%) were resistant to oxacillin and this constituted Methicillin-resistant *Staphylococcus aureus* (MRSA). The distribution of the antibiotics resistance patterns of the isolates and their PVL production status are presented in Table 4.

Considering their different antibiograms, two distinct strains were thought to be involved in the outbreak in Period I (Table 4), showing different resistance patterns; one strain showing resistance to penicillin G only and another showing resistance to Penicillin G, Erythromycin and Ciprofloxacin.

**Table 2** Distribution of isolated Strains according to period and type of specimens

Periods	Specimens Type	Culture positive	Isolation of <i>S. aureus</i>	<i>S. aureus</i> PVL +	Other bacteria
I 1 <sup>st</sup> September to 30 <sup>th</sup> December	Blood	6	3	0	3
	Tracheal aspiration	15	12	10	3
	Pleural fluid	20	10	6	10
	Expectoration	33	3	1	30
	<b>Total</b>	<b>74</b>	<b>28</b>	<b>17</b>	<b>46</b>
II 1 <sup>st</sup> February to 31 <sup>st</sup> May	Blood	0	0	0	0
	Tracheal aspiration	10	5	1	5
	Pleural fluid	0	0	0	0
	Expectoration	13	2	1	11
	<b>Total</b>	<b>23</b>	<b>7</b>	<b>2</b>	<b>16</b>

**Table 3** Distribution per sampling sites of *S. aureus* strains isolated from the hospital environment during period I and II.

Sample site	Period I (93 isolates)	PVL production	Period II (22 isolates)	PVL production
Hands of nursing staff	17/93 <b>18%</b>	7	1/22 <b>4%</b>	0
Floors	20/93 <b>22%</b>	5	6/22 <b>27%</b>	3
Bed frame	12/93 <b>13%</b>	5	2/22 <b>9%</b>	0
Locker	15/93 <b>16%</b>	3	4/22 <b>18%</b>	1
Light switch	9/93 <b>9%</b>	2	1	0
Door handle	9/93 <b>9%</b>	2	4/22 <b>18%</b>	0
Bathtub	4/93 <b>4%</b>	0	2/22 <b>9%</b>	0
Remedy bottle	2/93 <b>2%</b>	1	1	0
Table at nursing room	1/93 <b>1%</b>	0	0	0
Kitchen table	4/93 <b>4%</b>	3	1	0
<b>Total</b>	93/410 ( <b>23%</b> )	<b>28/93 (30%)</b>	22/410 ( <b>5%</b> )	4/22 ( <b>4%</b> )

**Table 4** Distribution of the patterns of resistance of *S. aureus* isolates to antibiotics according to period and reservoir, and PVL production

Antibiotics susceptibility	Period I*				Period II**			
	Patients		Hospital environment		Patients		Hospital environment	
	n	<i>S. aureus</i> PVL+	n	<i>S. aureus</i> PVL-	n	<i>S. aureus</i> PVL+	n	<i>S. aureus</i> PVL-
Fully susceptible to all tested antibiotics	0	0	9	0	0		6	0
Resistant to Penicillin G and/or SXT	18	16	14	5	0	0	2	2
Resistant to Methicillin	3	0	33	0	5	0	4	0
Resistant to more than 2 antibiotics	7	1	37	23	2	2	10	2
<b>Total</b>	28	17	93	28	7	2	22	4

\*Period I 1<sup>st</sup> September to 30<sup>th</sup> December      \*\*Period II 1<sup>st</sup> February to 31<sup>st</sup> May  
n=number of sample

### Toxins production

During Period I, 17 of the 28 *S. aureus* isolates from patients' samples were producers of PVL (Table 1), and 28 of the 93 *S. aureus* isolates from the hospital environment produced PVL. Table 2 summarizes the proportions of PVL positive isolates from the different types of patients specimens investigated. During Period II the frequency of isolation of PVL producing *S. aureus* substantially decreased from 17 strains to 2 strains in patients (Table 1), and from 28 strains in the environmental specimens to 4 strains. The reduction was significant ( $p < 0, 0001$ ).

## DISCUSSION

We observed one of the most severe outbreaks of nosocomial pneumonia associated with *S. aureus* in the paediatrics unit at C H D Z/C in Benin. Of twenty eight (28) patients involved in Period I, sixteen (16) died (57%), among them fifteen (15), 94% (15/16) were infected with PVL producing *S. aureus*. The mortality was higher than the 30% to 40% observed by Stevens *et al*<sup>16</sup>, during nosocomial pneumonia infection in a similar environment.<sup>17</sup> All *S. aureus* strains isolated from the first outbreak reported in this study belonged to antibiotic profile 2 (only resistant to penicillin G) of Table 4. At this point, the production of PVL allowed the differentiation of two groups in profile 2; one isolate from patients and six (6) from environmental samples belonged to profile 2 and were non-toxigenic for PVL production. The pathogenic role of this non-toxigenic isolates was not investigated.

The mortality rate of 88% observed among patients infected with *S. aureus* producing PVL in our study is very significant compared to the rate of 9% found in other bacteria groups' ( $p < 0, 0001$ ). During the same period, pneumonia due to Broad Spectrum Beta-Lactamase-producing *Escherichia coli* was observed in 5 patients, but with an apparent better prognoses. The PVL seem therefore, to be a factor accelerating the patient's death. This result is comparable to 80% obtained by Gillet *et al.*,<sup>5</sup> and Petros S.<sup>3</sup>

Several factors may explain why malnourished children formed a high-risk group; firstly, all these patients were young, with severe anaemia and probably lowered immunity; secondly, most of the patients were confined to bed for a long time with diminishing of physiological defence, all these facilitating bacterial adhesion to epithelium<sup>18</sup> though it is known that other factors are often involved in the pathogenesis of severe pneumonia.<sup>19</sup>

To be confined to bed could be also the main reason for environmental contamination. The result presented in Period I (Table 2) showed a relatively high level of isolation of *S. aureus* from the hospital environment. The floors accounted for 20% of the total number of isolates recovered, while hand specimens of medical personnel were 17%.

The high population density of *S. aureus* in the environment may partly explain the presence of this organism on the hands of the nursing staff. The single sink in the room was not adequate for effective hand-washing practice; and there was consequently insufficient use of a hand-sanitizer (70% ethyl alcohol, water plus glycerin) to disinfect the hands.

During Period I, 46 pneumonia cases due to other bacteria were also detected; the presence of this outbreak was unnoticed as it was masked by an endemic incidence of ordinary pneumonia during this time. Most clinical isolates of *S. aureus* reported previously were resistant, or even multi resistant to antibiotics<sup>10, 18</sup> about 30% of the *S. aureus* strains isolated in this study were MRSA and resistant to more than five antimicrobials.

Period I can be considered as the basal situation, during which care practices in the paediatrics' unit had several inherent flaws. Non-invasive medical devices were used for several patients without decontamination. Nappies were changed at the same location without surface disinfection between infants. Hand hygiene compliance was poor between patient contacts. Standard precautions were neglected. All these situations are known to promote colonization of hospital environments by *S. aureus*.

The role of the environment in nosocomial spread of *S. aureus* has been established<sup>11, 19</sup> but in this study it was noticed that 30% of *S. aureus* isolated from various environmental samples are producers of PVL during the Period I (Table 3). It seems likely that this was the main path of spread; the carrying of PVL positive *S. aureus* by health care workers increases the risk that nosocomial spread may occur. Between the first and the second periods, the paediatrics unit was closed for one month for complete renovation and disinfection.

In Period II when the nosocomial contamination was reduced, the percentage of pneumonia decreased from 31% to 11% ( $p < 0.0001$ ). The strict infection control measure in the second period most definitely contributes to the significant reduction of the bacterium in this sector of the hospital. It seems, therefore, that the control of nosocomial *S. aureus* infections must rely on several simultaneous control measures; interruption of

the routes of transmission and prompt diagnosis of patients infected with PVL producing *S aureus* is essential. It is therefore critical to treat nosocomial infections appropriately by starting antimicrobial treatment early in the course of infection, using the correct agent, at the most appropriate dose, and for an adequate duration.

The PVL toxin detection associated with antimicrobial susceptibility testing revealed two dominant profiles. The dominant profile among patients' isolates comprised 64% of all isolates in Period I. Such clear predominance of one clone indicates that *S. aureus* producing PVL may be capable of permanent existence within a hospital environment.

The high isolation rates of PVL producing *S aureus* from hospital environmental sites highlight the necessity to decontaminate the environmental reservoirs more thoroughly. It is interesting to notice that the four PVL producing isolates from the environment during Period II displayed another antibiotic profile different from that in Period I, along with a significant reduction of the mortality rate. These results showed the efficacy of the program instituted to control PVL producing *S aureus* the CHDZ/C hospital, though reports in the literature have stressed the difficulty involved in decontaminating such an environment. Talon and Dancer,<sup>19,20</sup> reported no significant benefit from decontaminating the hospital environment.

## CONCLUSION

The present study suggests that *S. aureus* producing PVL is a relatively frequent colonizing bacterium in hospital environment, although the presumed density of colonization is low. It seems very probably that cross infection took place in the pneumonia outbreak observed because of the lack of hygienic measures in hospital environment. In such an environment, high infant mortality rate investigations must look out for PVL producing *Staphylococcus aureus*.

Early identification of patients contaminated by an epidemic strain, especially when an index case has been diagnosed within a ward, should allow effective preventive measures. The results obtained by our preventive measures were beneficial for the patients in curtailing the outbreak of nosocomial pneumonia caused by PVL producing *S aureus*.

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