Vol. 15(1), pp. 10-23, January-June 2023
DOI: 10.5897/JIDI2022.0223
Article Number: 0A3EB2E70595
ISSN 2141-2375

http://www.academicjournals.org/JIDI

ACADEMIC JOURNALS
expand your knowledge

Journal of Infectious Diseases and Immunity

Full Length Research Paper

Skin diseases caused by Panton-Valentine leucocidin-producing *Staphylococcus aureus*: Profile and immunological follow-up of patients in a peri-urban area in Benin

Hachime R. B. I. MAMADOU[1,2], Bawa BOYA[1], Cyrille A. VODOUNON [2], Haziz SINA[1]*, Jean Robert KLOTOÉ[3], Théodora A. AHOYO[1] and Lamine BABA-MOUSSA[1]

[1]Laboratory of Biology and Molecular Typing in Microbiology, University of Abomey-Calavi, Cotonou 05 BP 1604, Benin.
[2]Laboratory of Biochemistry and Immunology, ENS-Natitingou, National University of Sciences, Technology, Engineering and Mathematics, BP: 72 Natitingou, Republic of Benin.
[3]Research Unit in Applied Microbiology and Pharmacology of Natural Substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Abomey-Calavi, Benin.

Received 21 November, 2022; Accepted 20 March, 2023

This study aims to highlight the role of Panton and Valentine Leucocidin (PVL) in Staphylococcus aureus isolated from skin diseases in the peri-urban area of Benin. This study, conducted from November 2014 to December 2017, include 124 wound, abscess, furuncle, osteomyelitis and pyomyositis patients with staphylococcal in the commune of Zogbodomey. The 124 patients were profiled based on sociodemographic, clinical, microbiological, hematological and immunological parameters. Then CRP, SV, NB and CD4 were evaluated in for 4 weeks. The patients were predominantly female (sex ratio = 0.8) and the age group [1; 10 years] was the most represented (68.55%). Before the medical consultation, 54.03% of the patients first used traditional medicine to treat themselves and 10.28% used probabilistic antibiotic therapy. All the isolated S. aureus strains were isolated in pure culture in the cases of pyomyositis and 29.61% of S. aureus strains are resistant to the tested antibiotics. The isolated S. aureus strains are 100% sensitive to vancomycin and ciprofloxacin. LPV was produced by 73.98% of S. aureus strains and 89.15% are sensitive to methicillin. The follow-up showed that patients with LPV+ presented at the beginning of the treatment, a hyperleukocytosis (91.18%) and a decrease of CD4 compared to those with LPV. Furthermore, a progressive improvement of the immunological parameters was noted, which became routine in almost all patients in the 4th week of follow-up. LPV-producing S. aureus is essential in staphylococcal infections in the study area. The presence of methicillin-sensitive strains suggests the loss of resistance gene by horizontal transfer..

Key words: Benin, community, LPV, *S. aureus*, staphylococcal skin infections.

INTRODUCTION

Staphylococcus aureus harmlessly colonizes the skin and mucous membranes of approximately 30% of healthy

adults (Gorwitz et al., 2008). It is the most common cause of mild skin and soft tissue infections, such as abscesses and wound infections. These infections cause many primary care visits, but rarely result in hospitalization or surgical treatment. The incidence of community-acquired invasive staphylococcal infections, such as pneumonia or osteomyelitis, is low (Hayward et al., 2008). But, *S. aureus* is a common cause of healthcare-associated diseases, with methicillin-resistant *S. aureus* (MRSA) strains reported in hospitals and healthcare facilities in most industrialized countries (Shallcross et al., 2013). These strains often produce a specific virulence factor, Panton-Valentine leucocidin (PVL) (Otto, 2014). This toxin is also responsible for a much more severe emerging pathology, staphylococcal community-acquired necrotizing pneumonia (Gillet et al., 2001).

Panton-Valentine leucocidin-producing *S. aureus* (PVL-SA) is a recognized cause of recurrent multifocal skin and soft tissue infections (MSI), necrotizing pneumonia (NP), and severe musculoskeletal infections, especially in children (Health Protection Agency, 2008; Saeed et al., 2018). It is characterized by fever, hemoptysis, multi-lobar alveolar infiltrates and leukopenia, the latter possibly related to the pro-apoptotic effect of LPV on neutrophils (Saeed et al., 2018). Furthermore, relapse after adequate treatment is common in the absence of decolonization (Saeed et al., 2018; Nurjadi et al., 2015). The biological criteria for skin infection diagnosis are most often elevated C-reactive protein, sedimentation rate, and blood count, significantly decreasing leukocytes. Suspicion may arise from certain characteristic features, but a confirmed diagnosis requires special non-routine tests. Given its increasing prevalence (Fogo et al., 2011; Vindel et al., 2014; Waldenburger et al., 2014), understanding the specific features and risk factors of PVL-SA is necessary for better clinical practice. In this work, we characterize all recorded PVL-SA infections diagnosed in the "Réservoir de Siloé" health center in Hlagba-ouassa, commune of Zogbodomey. This center received patients with staphylococcal skin diseases (wounds, abscesses, furuncles, osteomyelitis, and pyomyositis). In view of this situation, and for good sound management of these diseases, we decided to highlight the role of Panton-Valentine leucocidin in staphylococcal skin diseases through the profile and immunological follow-up of patients in peri-urban areas in Benin.

MATERIALS AND METHODS

Study setting

The "Réservoir de Siloé" health center in Hlagba-ouassa, the village of investigation, served as a setting for the reception, sampling, and treatment of patients, except for cases of complications. The microbiology and pediatrics laboratories of the Centre Hospitalier Départemental Zou Collines (CHD Z /C.) were used to process the samples. In addition, the biology and molecular typing in microbiology laboratory served as a setting for the research of Panton and Valentine leucocidin on *S. aureus* strains.

Inclusion criteria

This study includes samples from patients with abscesses, boils, myositis and/or supportive wounds. Finally, samples from patients that gave their consents were considered for analysis.

Type and period of the study

This study focused on a cohort of 124 patients with staphylococcal diseases received at the health center "Réservoir de Siloé" of Hlagba-ouassa, commune of Zogbodomey from November 2014 to December 2017. Data were collected retrospectively in 71 patients and prospectively and longitudinally in 53 patients. The profile of the 124 patients was established based on sociodemographic, clinical, microbiological, hematological, and immunological parameters. Then the immunological follow-up of the cohort of 53 patients (34 LPV+ and 19 LPV-) was performed weekly for four weeks on the following parameters: CRP, VS, white blood cell count, and T4 lymphocyte. For the retrospective study, all the data were provided by the registers of surgical reports and laboratory registers. For the prospective study, the data were collected from the patients' and accompanying persons' histories; the biological examinations were systematically requested for to diagnose of the disease and/or treatment.

Clinical diagnosis

Based on clinical diagnosis, 124 samples (from abscesses, boils, myositis and supportive wounds) are taken using sterile swabs directly inoculated in nutrient broth and in an aseptic environment. All samples are transferred to the laboratory, incubated at 37°C and processed.

Microbiological diagnosis

After fresh examination and Gram staining, each sample was culture on Mac Conkey and Chapman agar medium using a platinum loop. After 18 to 24 h, the bacteria have a characteristic morphology and suspected colonies are plated on nutrient agar and subjected to Gram staining and identification tests. Gram (+) positive Cocci colonies are subjected to catalase and free coagulase and DNase tests, Gram (-) bacilli, after reading the characteristics on the selective agar medium, are subjected to the classical identification test of Enterobacteria.

Susceptibility of strains to antibiotics

Each strain was tested for its sensitivity to 13 antibiotics (Bio Rad®): penicillin (Benzyl penicillin) (10 IU), oxacillin (5 µg), -erythromycin (15 µg), gentamicin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), ciprofloxacin (10 µg), and vancomycin (30 µg).

*Corresponding author. E-mail: sina.haziz@gmail.com.

Table 1. Distribution of patients according to sex and age group.

Age	1-10 years	11-20 years	21-30 years	≥31 years	Total
Women	36	19	11	3	69 (55.65%)
Men	31	15	7	2	55 (44.35%)
Total	67 (54.03%)	34 (27.42%)	18(14.52)	5(4.03)	124(100)

Source: Authors 2023.

The agar disk diffusion method was used to perform the antibiogram. A bacterial suspension prepared from a 24-hour culture of *S. aureus* on agar medium and standardized to 0.5 Mc Farland was swabbed onto the surface of the Mueller-Hinton medium. After applying antibiotic discs, the plates were incubated at 37°C for 24 h. The isolates were categorized as sensitive (S) or resistant (R) to the different antibiotics according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM/EUCAST, 2019).

Hematological diagnosis

The hemogram is performed on each sample taken on an EDTA anticoagulant tube. Adapt the cannula to the pipette and aspirate the blood at 0.5 or 2 lines depending on the type of pipette for each blood cell. Wipe the outside of the pipette well, then fill with Heyem to mark 101 red blood cells and with Lazarus to score 11 for leukocytes. Shake well and mount in each chamber of the hematimeter. Allow to sediment for 3 mm and count under the microscope. For the leukocyte formula, the prepared smears are stained with May Grunwald Giemsa (MGG) stains, and then 100 leukocytes are counted by immersion.

Inflammatory anemia

During chronic inflammatory syndromes, hyposideremia and anemia rarely below 8 g/L appear. Ferritinemia increases and the total siderophilin binding capacity tends to decrease. On the contrary, in martial deficiency, the body's iron stocks are depleted. To try to compensate for this deficit, the total binding capacity of siderophilin increases. In a mixed inflammatory and deficiency situation, ferritin levels may be abnormally normal or even decreased. If an increase in the total siderophilin binding capacity is found during an inflammatory syndrome, there is a martial deficiency which the ferritin level will confirm.

Detection of Panton and Valentine Leucocidin (PVL)

Panton and Valentine leucocidin (PVL) was detected from supernatants after 18 h of culture in the YCP medium by an immunoprecipitation assay after radial diffusion (Gravet et al., 1998). The reaction was performed in a 0.6% (w/v) agarose gel in PBS buffer (10 Mm Hepes, 150 Mm NaCl, pH 7.5), containing 50 µl volume wells spaced 8 mm apart, in which were deposited affinity-purified rabbit anti-leukotoxin antibodies (85) at OD 280 nm equal to 3 that will react with the culture supernatant or bacterial lysates. After a 16-hour diffusion, the precipitation arcs were viewed directly with the naked eye or after staining with Coomassie blue.

Sedimentation rate

Our samples' sedimentation rate was determined using the previously used method (Liu et al., 2020a). Thus, samples were taken in the morning on an empty stomach. First, the blood (1.6 ml) was collected in a tube with blood anti-coagulant (0.4 ml of citrate solution). After mixing, the citrated blood was then aspirated into a Wintergreen tube up to the 0 graduations, the tube was then fixed to the stand, well upright with the base of the stand horizontal and placed in a place protected from heat for one hour. The height of the supernatant plasma was noted. The normal values are 0-15 mm (for men), 0-20 mm (for the woman), and 0-10 mm (for children).

Data analysis

Microsoft Excel spreadsheet version 2016 was used for data entry and coding. GraphPad Prism (version 8.0) was used to create the graphs and analysis of variance (ANOVA). Finally, a structuring the averages allowed us to compare and interpret the different results thanks to a t-test on data (R software). The differences are considered significant if the p-value < 0.05 and very significant if the p-value < 0.001.

RESULTS

Characteristics of the sample

During the study, 124 patients were followed up. The most infected age group was 1-10 years (54.03%) followed by 11-20 years (27.42%). The male/female sex ratio is equal to 0.80; with a female predominance. The distribution of patients according to age and sex are shown in the Table 1.

Table 2 shows the distribution of patients according to their occupation. Again, the most infected domains were without profession (56.45%) followed by housewives (19.35%) and students (15.32%).

Profile of patients with staphylococcal diseases

Table 3 shows the distribution of patients by age group and skin condition. The table shows that the age group 1 to 10 years is the most affected, regardless of the type of disease. The most common diseases were pyomyositis (42.74%), osteomyelitis (24.19%) and abscesses (20.97%).

Previous treatments

Figure 1 shows the distribution of patients according to

Table 2. Distribution of patients by occupation.

Parameter	Job							
	Craft man	Farmer	Housekeeper	Teacher	Nurse	Student	Without profession	Total
Numbers	5	2	24	3	1	19	70	124
Percentage	5.56	2,22	19.35	2.42	1.11	15.32	56.45	100

Source: Authors 2023.

Table 3. Distribution of the 124 patients according to their age group and disease.

Illness	Age				
	1-10 years	11-20 years	21-30 years	≥31 years	Total
Pyomyositis	28	12	10	3	53 (42.74%)
Osteomyelitis	25	2	3	0	30 (24.19%)
Abscess	21	05	0	0	26(20.97%)
Furuncle	8	2	0	0	10(8.06%)
Wounds	3	1	0	1	05(4.03%)
Total	**85(68.55%)**	**22(17.74%)**	**13(10.48%)**	**04(3.23%)**	124(100%)

Source: Authors 2023.

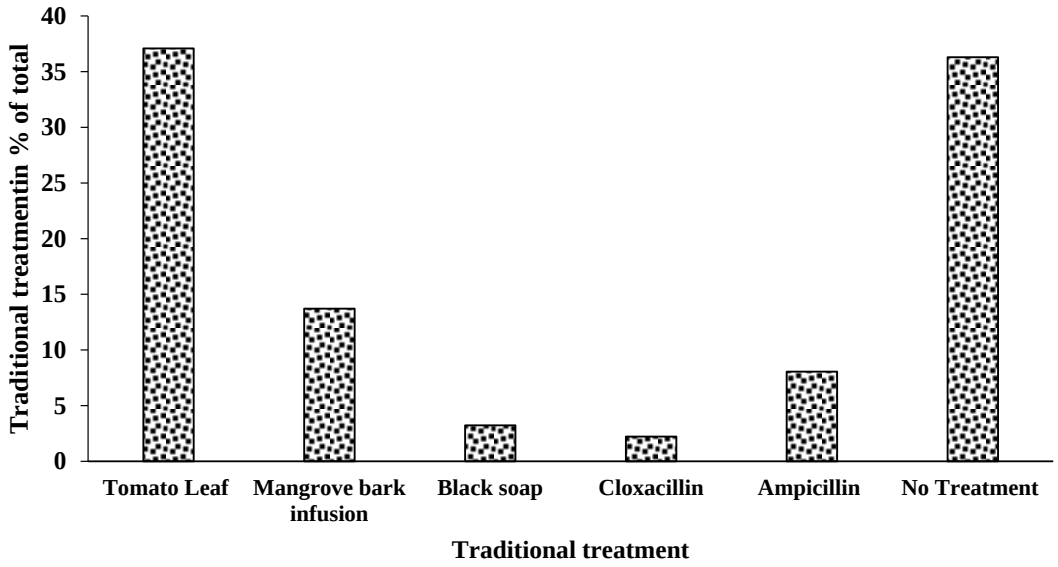


Figure 1. Distribution of patients according to the treatment followed at home.
Source: Authors 2023.

the treatment followed at home. The analysis of this figure indicates that 54.03% of the patients use traditional medicine to treat themselves and only 10.28% use probabilistic antibiotic therapy.

Hematology profile

Hematological examinations were performed on blood samples taken from 96 of the 124 patients in our study. The results of the hematological investigations are shown in Table 4. It appears from this table that the patients are often anemic (85.42%). Hyperleukocytosis was also noted in 86.46% of patients.

Bacterial species isolated according to the type of infection

Figure 2 shows that *S. aureus* was isolated in 83.11% of

Table 4. Distribution of haematological test results according to the age group of the patients.

Age (years)	Anemia (%)		Hyperleukocytosis (%)	
	Yes	No	Yes	No
1 - 10	38.54	4.17	42.71	3.13
11 - 20	28.13	3.13	23.96	3.13
21 - 30	13.54	4.17	15.63	4.17
≥ 30	5.21	3.13	4.17	3.13
Total (%)	85.42	14.58	86.46	13.54

Source: Authors 2023.

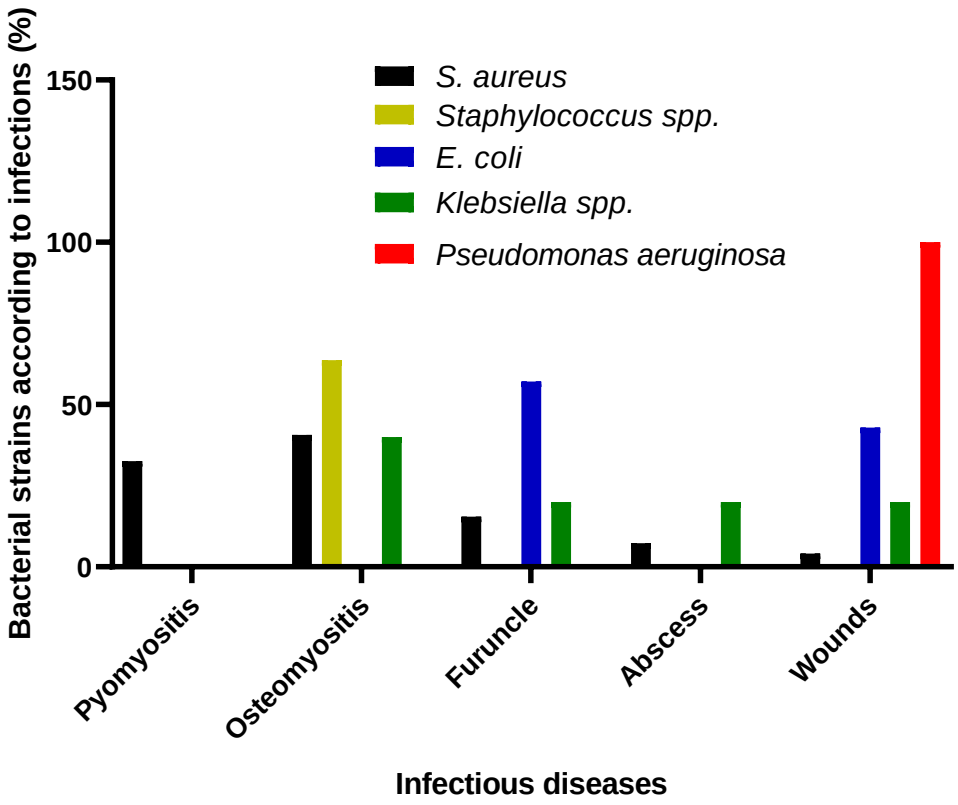


Figure 2. Distribution of bacterial strains according to infections.
Source: Authors 2023.

the cases studied. Most cases of *S. aureus* were isolated in pure culture in 100% of the cases of pyomyositis. There was no significant difference between the bacterial strains according to the infections (p >0.9999).

Sensitivity of *S. aureus* strains to antibiotics

Figure 3 shows that 29.61% of *S. aureus* strains are resistant to the antibiotics tested, including 100% to penicillin. In addition, 70.39% of *S. aureus* strains are sensitive to the antibiotics tested, including 100% to vancomycin, gentamycin and ciprofloxacin.

Panton-Valentine leucocidin (LPV) result

Ninety-one strains of *S. aureus* out of 123 isolated produced Panton-Valentine leucocidin (PVL), representing 73.98% of the strains tested (Table 5).

Figure 4 shows the susceptibility of LPV-producing *S. aureus* strains to methicillin. This figure, that all LPV-producing *S. aureus* strains are sensitive to methicillin at the same time, 40.63% of non-LPV-producing *S. aureus*

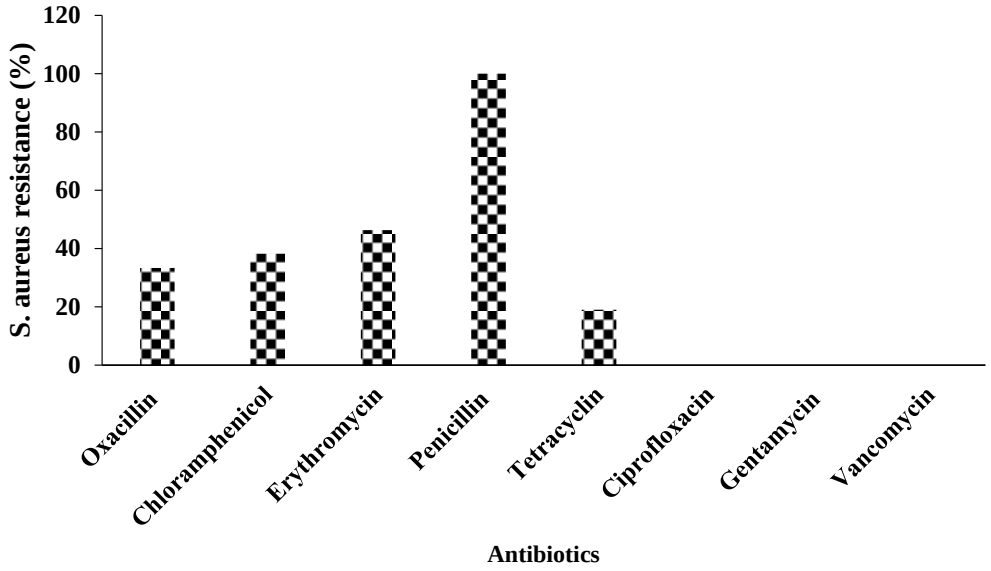


Figure 3. Susceptibility of *S. aureus* strains to antibiotics.
Source: Authors 2023.

Table 5. Distribution of *S. aureus* strains according to LPV production and conditions.

Disease	LPV (+)	LPV (-)	Total
Pyomyositis	25	15	**40**
Osteomyelitis	40	10	**50**
Abscess	15	4	**19**
Furuncle	09	0	**09**
Wounds	2	3	**5**
Total	**91**	**32**	**123**

Source: Authors 2023.

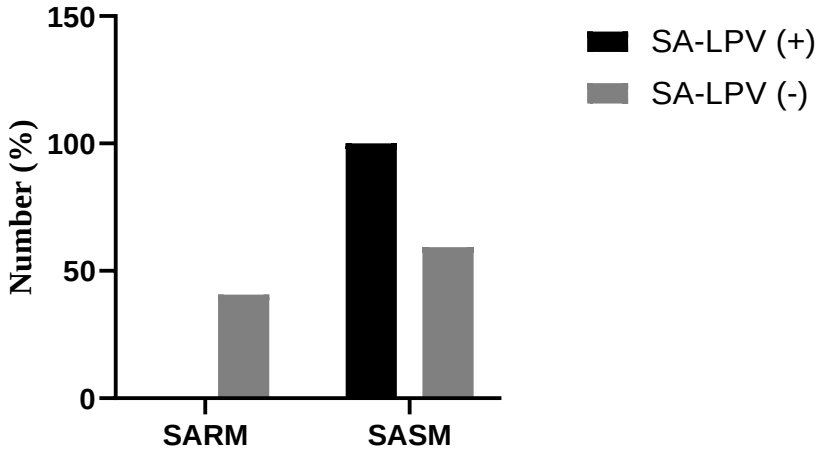
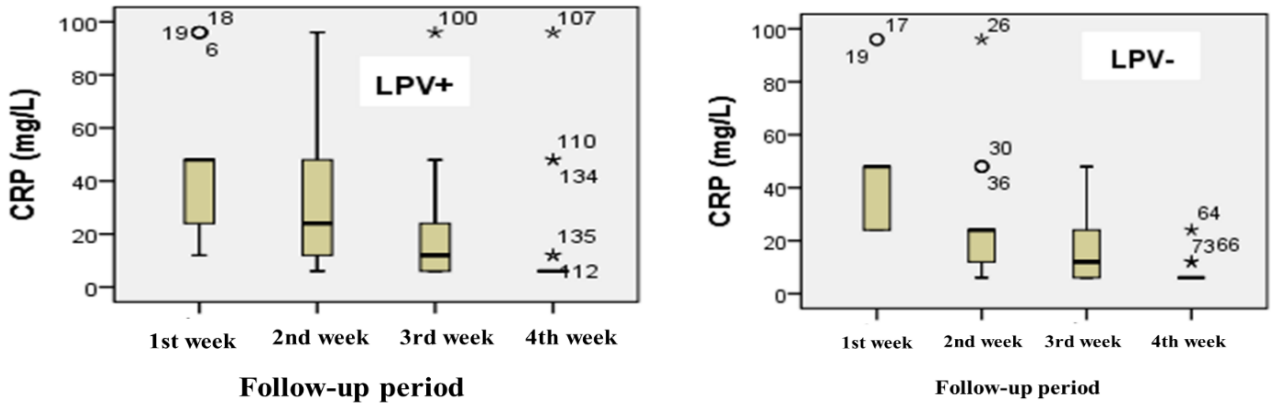


Figure 4. Susceptibility of LPV-producing *S. aureus* strains to meticillin. MSSA: Methicillin-sensitive *S. aureus* ; MRSA: Methicillin-resistant *S. aureus.*
Source: Authors 2023.

Table 6. Distribution of serology results according to the age group of LVP+ patients.

Age	HIV (+)	HIV (-)	CRP (+)	CRP (-)
	00	**51**	**38**	**07**
[1; 10 years]	02	20	23	06
[11; 20 years]	02	10	06	06
[21; 30 years]	00	05	2	3
Total	04	87	69	22

Source: Authors 2023.



(A) Evolution of CRP in LPV+ patients **(B) Evolution of CRP in LPV- patients**

Figure 5. Distribution of CRP values during patient follow-up. Source: Authors 2023.

strains are resistant to methicillin. There was no significant difference between methicillin-susceptible and methicillin-resistant *S. aureus* strains (p >0.9999) according to the production of LPV.

Immunological profile of patients

Retroviral serology and C-reactive protein (CRP) which is an inflammatory response protein were performed on the patient samples obtained and are summarized in Table 6. According to the table, the majority of the patients are seronegative with a CRP level higher than 6 mg/l.

Evolution of CRP during the follow-up of patients with SA-LPV

Figure 5 shows SV values in the patients at each week of treatment. It was noted that at the beginning of the treatment, CRP was positive in 100% of the LPV+ patients and the values vary globally between 12 and 48 mg/ml, with a median of 48 mg/ml (Figure 5A). After that, a progressive decrease in SV was observed between the first and fourth week, when most patients had a negative CRP value of 6 mg/ml. Finally, in the 4th week, the CRP was negative in 31/34 patients, that is, 91.18% of the negativity rate.

In LPV- patients, CRP positivity was also 100% at the beginning of the follow-up. However, the values were lower from the second week onwards than in LPV+ patients. In the 4th week, almost all patients became CRP-negative.

The curve showing the variation of CRP during treatment shows a decreasing trend in both LPV+ and LPV- patients. At the beginning of the follow-up, the average CRP of LPV+ patients (45.18 mg/L) were higher than that of LPV- patients (40.59 mg/L). But from the second week onwards, the averages are lower in LPV- patients (Figure 6). Moreover, the t-test on paired data shows that from the 3rd week of follow-up, statistically significant differences are observed with the CRP means of the 1st week.

Evolution of SV during follow-up

Figure 7 shows the distribution of SV values at each week of treatment. At week 1, the SV ranged from 30 to 120 mm with a median of 55 mm. At the 2nd week the

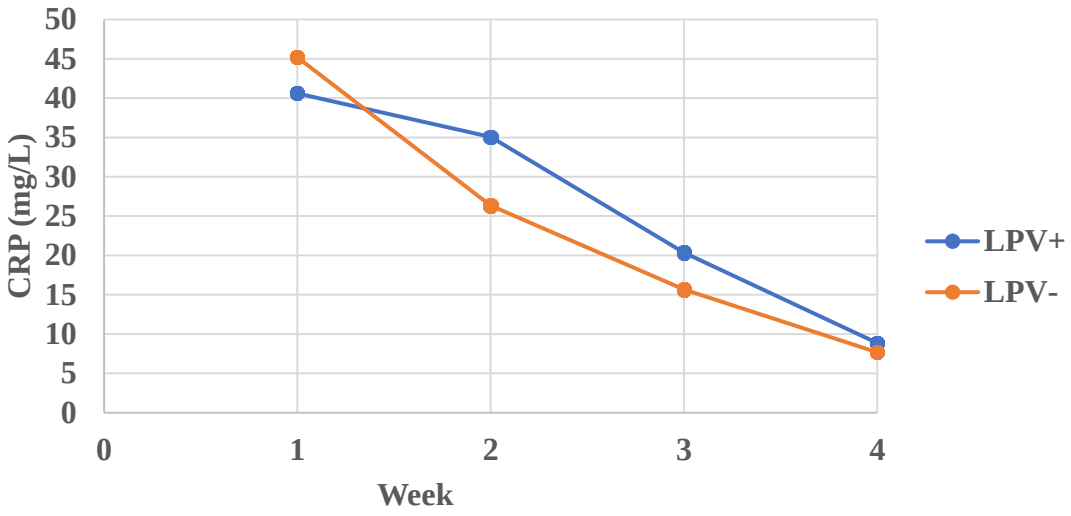
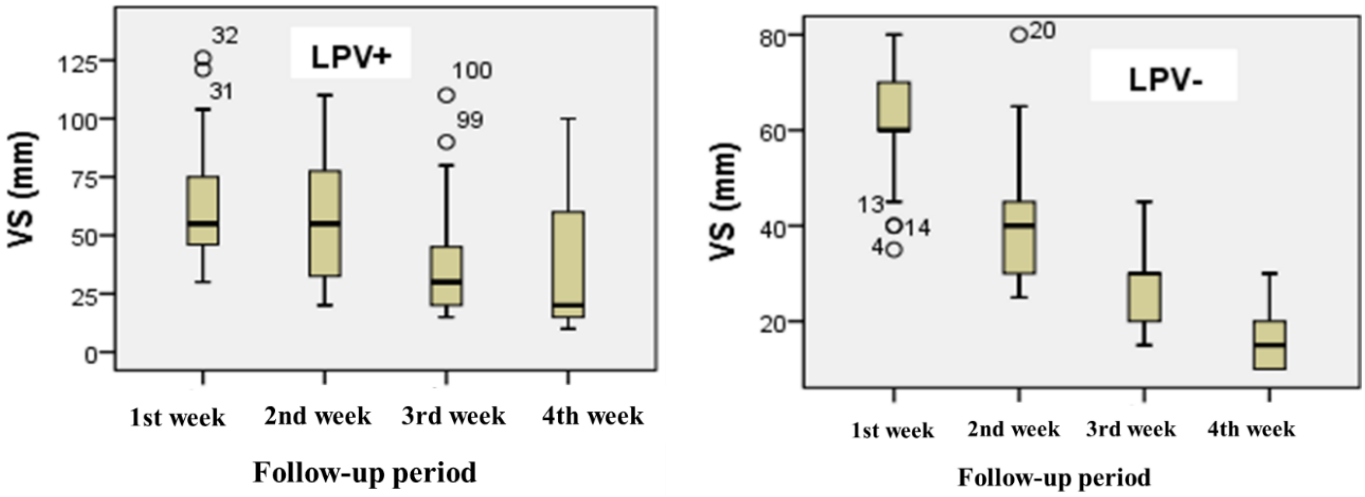


Figure 6. Variation in mean CRP during patient follow-up.
Source: Authors 2023.



A: Evolution of SV during follow-up in LPV+ patients. **B:** Evolution of SV during follow-up in LPV- patients

Figure 7. Distribution of CRP values during the follow-up of patients.
Source: Authors 2023.

median is maintained at 55 mm, but a more significant variability of results is noted. At the 3rd week of follow-up, the median is 30 mm. It decreases to 20 mm at the 4th week.

Similarly, the analysis of Figure 8 shows that the mean of the SV progressively decreases from the 1st to the 4th week of follow-up with respective values of 61.74 and 34.05 mm for LPV+ patients against 61.05 and 16.11 mm for LPV. In addition, a comparison of the mean by t-test reveals statistically significant differences between the SVs at the beginning of treatment and those at the 3rd and 4th week.

Evolution of T4 lymphocytes during the follow-up of SA-LPV patients

Figure 9 shows the distribution of T4 lymphocyte values between the 1st and 4th week of follow-up. In LPV- patients, the C values are all above 400. These values are atypical, so this parameter does not need to be monitored in LPV- patients. On the other hand, in LPV+ subjects, it was noted that in the first week of follow-up, the T4 lymphocyte values are between 63 and 380. The median is equal to 190. But in the 4th week the CD4 values increased significantly with a median distribution

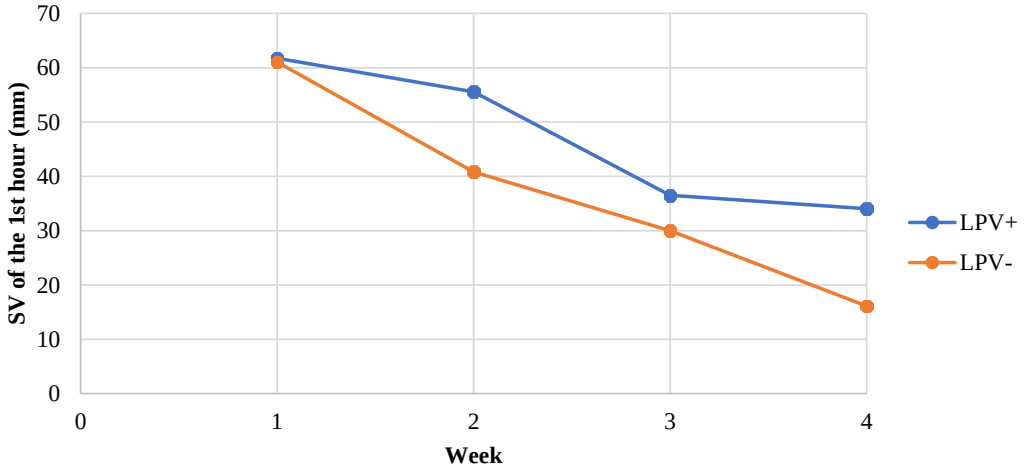
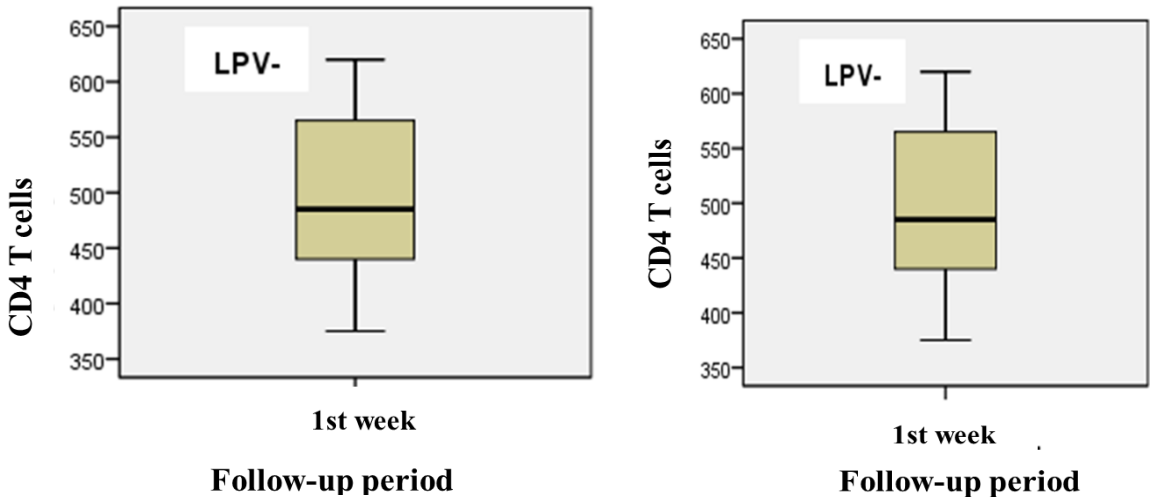


Figure 8. Variation in mean SV during patient follow-up.
Source: Authors 2023.



A: Evolution of T4 cells during the follow-up of patients with SA-LPV.

B: Evolution of T4 cells during the follow-up of SA-LPV+ patients.

Figure 9. Distribution of CD4 T cell values between 1st and 4th week of follow-up.
Source: Authors 2023.

of 317.

The average 187.15 at the beginning of the treatment increased to 313.23 at the 4th week of follow-up, that is, an increase of 67.37% (Figure 10). This difference is statistically significant (p=0.000).

Evolution of the white blood cell count during the follow-up of LPV+ patients

The white blood cell count was higher in the first week (Figure 11). The values vary from 2500 to 14000 mm^{-3}, and hyperleukocytosis is present in 91.18% of the subjects (Table 6). In the 4th week, leukocyte values were very little scattered (Table 7). The values were normal in 67.65% of cases, and there was no more hyperleukocytosis (Table 7).

In addition, there was a statistically significant decrease (p=0.000) in the mean leukocyte count from baseline to week 4 in LPV+ patients with 10041 and 4226 NB/mm^3, respectively (Figure 12).

DISCUSSION

The involvement of PVL in *S. aureus* virulence has been

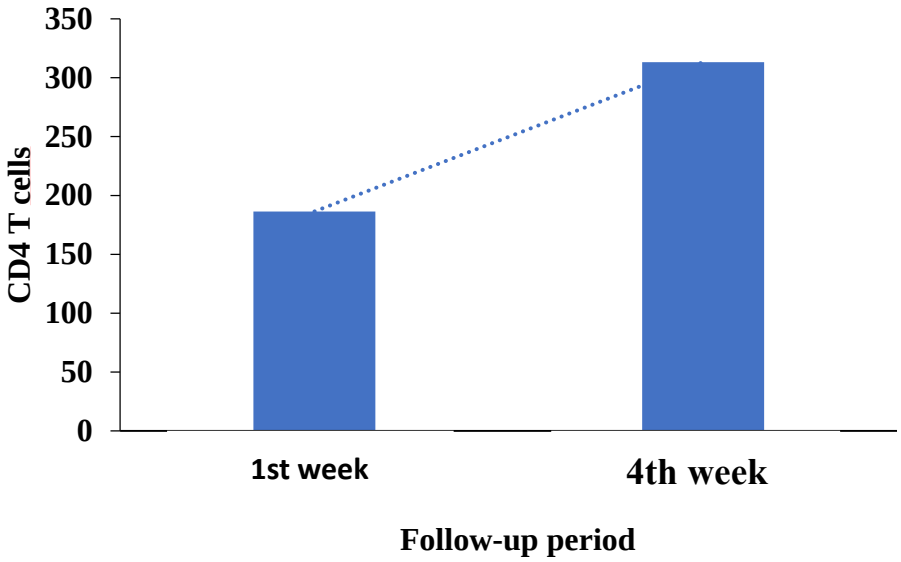


Figure 10. Change in mean CD4 T cell count between 1st and 4th week of follow-up in LPV+ patients.
Source: Authors 2023.

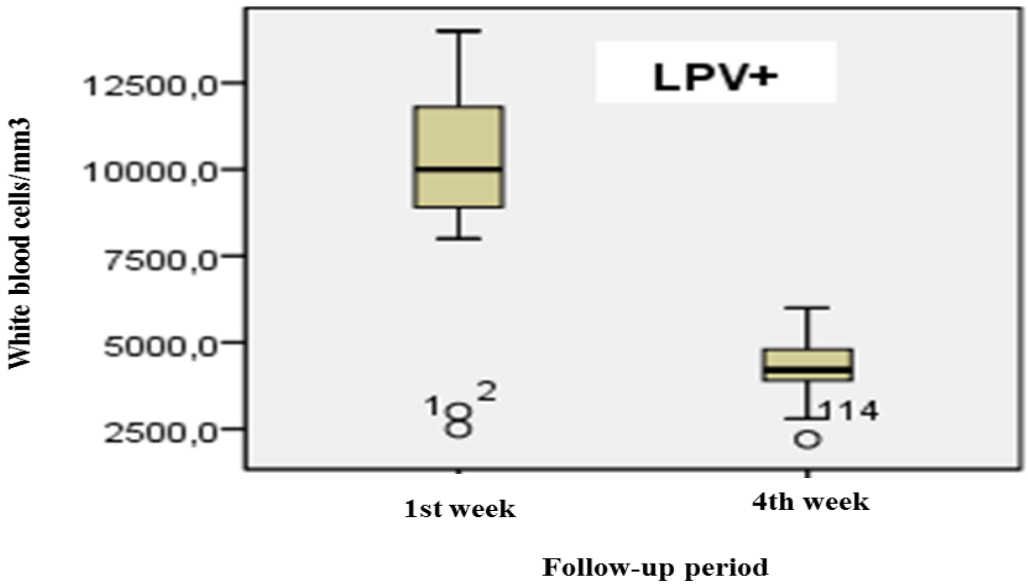


Figure 11. Distribution of T4 lymphocyte values between the 1st and 4th week of follow-up.
Source: Authors 2023.

a controversial topic for many scientists in search of factors responsible for the high pathogenicity of community-associated *S. aureus* strains. In this study, the role of Panton-Valentine leucocidin-producing *S. aureus* in Staphylococcal skin diseases was examined by studying a cohort of patients with community-acquired *S. aureus* in a context of pyomyositis and osteomyelitis in a peri-urban area of Benin to contribute to its eradication. The majority of the cases followed were children (54.03%). These results are superior to those obtained by Vieu (2014), who showed that 42% of the patients in their cohort were children under 15 years of age. The size of the cohort could explain this difference. Their cohort size was 37 patients, whereas ours was 124 patients. In addition, the epidemiological association between children and PVL+ *S. aureus* disease is well described in the

Table 7. Leukocyte abnormalities observed between the 1st and 4th week of follow-up.

Follow-up period	Parameter followed	Percentage
1st week	Leukopenia	2.88
	Hyperleukocytosis	91.18
	Standard value	2.94
4th week	Leukopenia	32.35
	Hyperleukocytosis	67.65
	Standard value	0

Source: Authors 2023.

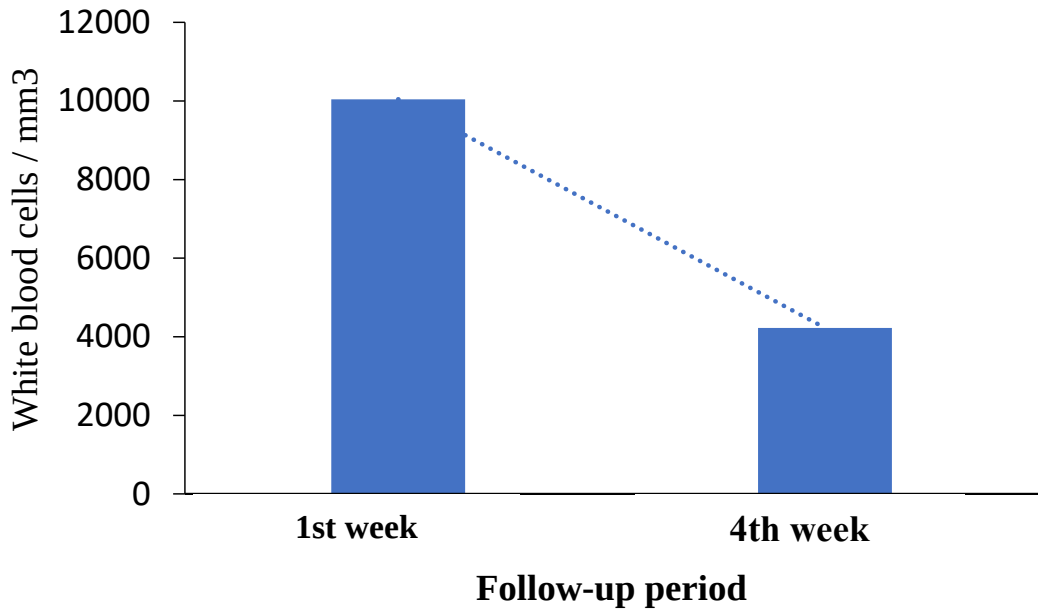


Figure 12. Variation of the average leukocytes between the 1st and the 4th week of follow-up.
Source: Authors 2023.

literature (Carré, 2010). However, our cases involve community-acquired skin conditions. Children have been described as a group at risk of developing staphylococcal disease regardless of PVL status (Gillet et al., 2002). The communal life can explain this observation in schools, which favors contact and transmission, particularly through the carriage of *S. aureus*.

According to Vieu (2014), management is marked by the need for antibiotic therapy or surgery as soon as a pus focus is present. Clinical improvement after drainage of the abscess is immediate, but effective antibiotic treatment is still necessary to avoid a relapse. In our cohort, 10% of patients resorted to traditional medicine and probabilistic antibiotic therapy for treatment. This observation can be explained by their culture and may have led to the death of 8 patients.

Regarding the microbiological quality of the conditions, more than half (61%) of *S. aureus* was isolated in pure culture. *Staphylococcus* is the germ most frequently involved in pilosebaceous follicle infections. Many factors favor the occurrence of this type of infection, whether local (friction, tight clothing, maceration, hyper sudation, scratching, shaving, depilation, irritating topicals, oil applications, dermo corticoids, isotretinoin) or general (obesity, diabetes, congenital or acquired immune deficiency, dialysis renal failure) (Larquey and Mahé, 2018). These strains of community-acquired *S. aureus* have the potential to produce a toxin associated with severe skin infections, the Panton-Valentine (PVL) leucocidin toxin. SA-PVL strains represent approximately

93% of the *S. aureus* strains in our cohort. This value is similar to that of Le Monnier (2002) but slightly higher than that of Vandenesch et al. (2003) which is 72.22%. This precariousness can explain this difference in results and lack of hygiene observed in the study area. SA-PVL strains are virulent and spread very rapidly. Human-to-human transmission occurs through direct or indirect (hand-to-mouth) contact with a purulent lesion, a carrier or contaminated surface, linen or objects (Castellazzi et al., 2021). Staphylococci-producing Panton-Valentine leucocidin is all sensitive to methicillin. This result is unexpected given the community origin of these isolates, where 10% of our patients use probabilistic antibiotic therapy. However, it cannot be extrapolated to all community-acquired SAMS-LPV+ because of treatment selection bias. From studies by Khatib et al. (2013), SAMS-LPV+ is still the leading cause of severe Panton and Valentine leucocidin-associated infections in many countries. It is thus the most plausible reservoir of community-acquired methicillin-resistant *S. aureus* (CA-MRSA). In the United States, most reported cases are caused by community-acquired methicillin-resistant strains (CA-MRSA). Although an increase in cases of PVL + CA-MRSA has been reported, the expression of PVL remains predominantly associated with MSSA in Europe (Moran et al., 2006; Witte et al., 2007; Rasigade et al., 2010; Shore et al., 2014). Furthermore, according to national epidemiology data from France in 2008, 43% of SA-LPV+ were susceptible to methicillin (Nizou, 2014). Like other staphylococci, they almost systematically express a penicillinase responsible for penicillin resistance. Antibiotic treatment of these infections does not pose major problems, as these germs are mainly sensitive to vancomycin and ciprofloxacin.

Furthermore, the hematological examinations showed that most of our patients are anemic followed by a neutrophilic hyperleukocytosis as soon as they are admitted for treatment, that is, about 65% of our SA-LPV+ patients. This result is in agreement with the assertion of Le Monnier (2002) that it is a cytotoxin destroying leukocytes and inducing tissue necrosis. It is a synergohymenotropic toxin, that is, a toxin that destroys leukocytes by causing major ionic disorders after the release of cytokines (class F and S proteins) that act synergistically and then activate proteases at the intracellular level that in turn induce apoptosis and cell death.

Most patients are seronegative with a CRP level higher than 6 mg/l on admission. Despite this seronegative result, a depletion of T4 lymphocytes in most patients was observed. When the infectious agent is eliminated, the effector cells will die (T4 lymphocytes) and only the antigen-specific memory cells will persist. The frequency of precursor T cells in the immune repertoire before antigen exposure determines the magnitude of the immune response in humans (Su and Davis, 2013; Nelson et al., 2015; Su et al., 2013). The smaller the number of self-peptides homologous to a given antigen, the greater the frequency of CD4 T cells naïve to that same antigen in the immune repertoire. Follow-up of patients for four weeks showed a clear improvement of immunological parameters in most patients. Although the literature does not say much about this case, this indicates the impact of LPV on the immune system of LPV+ patients.

CRP is a biomarker of the inflammatory response, which can predict the severity and prognosis of an infection (Zhang et al., 2020; Liu et al., 2020b). It activates the classical complement pathway to stimulate bacterial phagocytosis during bacterial infection. When bacterial inflammatory factors are eliminated, CRP levels decrease rapidly (Zhang et al., 2022). In this study, CRP was positive in 100% of LPV+ patients, and values ranged globally from 12 to 48 mg/ml with a median of 48 mg/ml (Figure 5A). The higher the CRP level, the more severe the infection or inflammation. Those patients with a CRP level >41.8 mg/ml are likely to develop severe disease. This was the case in a retrospective study from China that found that patients with CRP >41.8 mg/l in COVID-19 were more likely to develop the severe disease (Liu et al., 2020a).

Conclusion

The objective of this study was to highlight the role of Panton-Valentine leucocidin (PVL) in staphylococcal skin diseases through the profile and immunological follow-up of patients in peri-urban areas in Benin. The data analysis shows that in the study area (Hlagba-Ouassa), staphylococcal diseases are mainly characterized by myositis and abscesses. The patients, mostly children, are 85.42% anemic with hyperleukocytosis. More than half of them resort to traditional treatments before a medical consultation. *S. aureus* is a bacterial species found in almost all patients (123/124). These *S. aureus* strains are 70.39% sensitive to the antibiotics tested, including vancomycin and ciprofloxacin. LPV-producing *S. aureus* (SA-LPV+) is the leading cause of staphylococcal infections (73.98%). SA-LPV+ strains are all sensitive to methicillin. The presence of SA-LPV+ leads to immunological disorders characterized by hyperleukocytosis, a decrease T4 lymphocytes, CRP positivity, and a prolongation of the SV. All these parameters progressively decrease with treatment and generally return to normal after 4 weeks of follow-up. To date, the presence of LPV toxin is an aggravating factor in the staphylococcal disease. The combination of methicillin sensitivity and a virulence factor (LPV) represents a potential risk of severe complications in cutaneous localization of *S. aureus*. It is therefore essential, to set up specific studies to better characterize them and identify adequate control and prevention measures. It should also be noted that good hygiene is

necessary to avoid staphylococcal diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Carré N (2010). Épidémie d'infections cutanées à *Staphylococcus aureus* porteur des gènes codant la leucocidine de Panton-Valentine en milieu scolaire et familial, Val-d'Oise, 2006-2008. Cellule de l'Institut de veille sanitaire (InVS) en région (Cire) Ile-de-France, 2010.

CASFM/EUCAST (2019). Recommandations du Comité d'Antibiotique de la Société Française de Microbiologie. Société Française de Microbiologie Ed; 2019. [Accessed on: 20 November 2020]. Available at: https://www.sfm-microbiologie.org/2019/01/07/casfm-eucast-2019/

Castellazzi ML, Bosis S, Borzani I, Tagliabue C, Pinzani R, Marchisio P, di Pietro GM Castellazzi ML, Bosis S, Borzani I, Tagliabue C, Pinzani R., Marchisio P, di Pietro GM (2021). Panton-valentine leukocidin Staphylococcus aureus severe infection in an infant: a case report and a review of the literature. Italian Journal of Pediatrics 47(1)1-9. Italian Journal of Pediatrics 47(1):158. https://doi.org/10.1186/s13052-021-01105-5

Fogo A, Kemp N, Morris-Jones R (2011). PVL positive Staphylococcus aureus skin infections. BMJ 343:d5343-d5343. doi:10.1136/bmj.d5343

Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piémont Y, Brousse N, Floret D, Etienne J (2002). Association between Staphylococcus aureus strafins carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 359(9308):753-759. https://doi.org/10.1016/S0140-6736(02)07877-7

Gillet Y, Issartel B, Vanhems P, Lina G, Vandenesch F, Etienne J, Floret D (2001). Pneumonies staphylococciques graves de l'enfant. Archives de Pédiatrie 8(4):742-746. https://doi.org/10.1016/S0929-693X(01)80190-1

Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, Jensen BJ, Killgore G, Tenover FC, Kuehnert MJ (2008). Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004. The Journal of Infectious Diseases 197(9):1226-1234. doi: 10.1086/533494

Gravet A, Colin DA, Keller D, Giradot R, Monteil H, Prevost GGravet A, Colin D.A., Keller D, Girardot R, Monteil H, Prévost G (1998). Characterization of a novel structural member, LukE-LukD, of the bi-component staphylococcal leucotoxins family. FEBS Letters 436(2):202-208.

Hayward A, Knott F, Petersen I, Livermore DM, Duckworth G, Islam A, Johnson AM (2008). Increasing hospitalizations and general practice prescriptions for community-onset staphylococcal disease, England. Emerging Infectious Diseases 14(5):720-726. doi:10.3201/eid1405.070153.

Health Protection Agency (2008). Guidance on the diagnosis and management of PVL-associated Staphylococcus aureus infections (PVL-SA) in England. (2nd ed.) (2008 November). http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1218699411960

Khatib R, Sharma M, Iyer S, Fakih MG, Obeid KM, Venugopal A, Fishbain J, Johnson LB, Segireddy M, Jose J, Riederer K (2013). Decreasing incidence of Staphylococcus aureus bacteremia over 9 years: Greatest decline in community-associated methicillin-susceptible and hospital-acquired methicillin-resistant isolates. American Journal of Infection Control 41(3):210-213. doi:10.1016/j.ajic.2012.03.038

Larquey M, Mahé E (2018). Infections cutanées à staphylocoque et streptocoque chez l'enfant. Perfectionnement en Pédiatrie 1(1):25-31. https://doi.org/10.1016/j.perped.2018.01.015

Le Monnier A (2002). Leucocidine de Panton Valentine. Service de Microbiologie Hôpital Necker 2002. https://www.slideserve.com/macon/leucocidine-de-panton-valentine

Liu F, Li L, Xu M-D, Wu J, Luo D, Zhu Y, Li B, Song XY, Zhou X (2020a). Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. Journal of Clinical Virology 127:104370.

Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, Wang Z, Li J, Li J, Feng C, Zhang Z, Wang L, Peng L, Chen L, Qin Y, Zhao D, Tan S, Yin L, Xu J, Zhou C, Jiang C, Liu L (2020b). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China Life Sciences 63:364-74.

Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA (2006). Methicillin-resistant S. aureus infections among patients in the emergency department. The New England Journal of Medicine. 355(7):666-674.

Nelson RW, Beisang D, Tubo NJ, Dileepan T, Wiesner DL, Nielsen K, Wuthrich M, Klein BS, Kotov DI, Spanier JA, Fife BT, Moon JJ, Jenkins MK (2015). T cell receptor cross-reactivity between similar foreign and self peptides influences naive cell population size and autoimmunity. Immunity 42(1):95-107. http://dx.doi.org/10.1016/j.immuni.2014.12.022

Nizou JY (2014). Une observation de pneumonie neucrosante Infections à Staphylococcus aureus-PVL. Laboratoire biomis Yvry sur seine. 2014. www.biomis.com.

Nurjadi D, Friedrich-Jänicke B, Schäfer J, Van Genderen PJJ, Goorhuis A, Perignon A, Neumayr A, Mueller A, Kantele A, Schunk M, Gascon J, Stich A, Hatz C, Caumes E, Grobusch MP, Fleck R, Mockenhaupt FP, Zanger P (2015). Skin and soft tissue infections in intercontinental travellers and the import of multi-resistant Staphylococcus aureus to Europe. Clinical Microbiology and Infection 21(6):567.e1-567.e10. https://doi.org/10.1016/j.cmi.2015.01.016

Otto M (2014). Staphylococcus aureus toxins. Current Opinion in Microbiology 17:32-37.

Rasigade J-P, Laurent F, Lina G, Meugnier H, Bes M, Vandenesch F, Etienne J, Tristan A (2010). Global distribution and evolution of Panton-valentine leukocidin-positive methicillin-susceptible Staphylococcus aureus, 1981-2007. The Journal of Infectious Diseases 201(10):1589-97. https://doi.org/10.1086/652008

Saeed K, Gould I, Esposito S, Ahmad-Saeed N, Ahmed SS, Alp E, Bal AM, Bassetti M, Bonnet E, Chan M, Coombs G, Dancer SJ, David M. Z., De Simone G, Dryden M, Guardabassi L, Hanitsch LG, Hijazi K, Kruger R, Lee A, Leistner R, Pagliano P, Righi E, Schneider-Burrus S, Skov RL, Tattevin P, van Wamel W, Vos MC, Voss A (2018). Panton-Valentine leukocidin-positive Staphylococcus aureus: A position statement from the International Society of Chemotherapy. International Journal of Antimicrobial Agents 51:16-25.

Shallcross LJ, Fragaszy E, Johnson AM, Hayward AC (2013). The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. The Lancet Infectious Diseases 13(1):43-54. http://dx.doi.org/10.1016/S1473-3099(12)70238-4

Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC (2014). Panton-valentine leukocidin-positive Staphylococcus aureus in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. Aureus clones, and increasing multiresistance. Journal of Clinical Microbiology 52(3):859-870.

Su LF, Davis MM (2013). Antiviral memory phenotype T cells in unexposed adults. Immunological Reviews 255(1):95-109.

Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM (2013). Virus-specific CD4(+) memory- phenotype T cells are abundant in unexposed adults. Immunity 38(2):373-383. http://dx.doi.org/10.1016/j.immuni.2012.10.021

Vandenesch F, Naimi T, Lina G, Nimmo G, Hefferman H, Liassine N, Bes M, Greenland T, Reverdy M, Etienne J (2003). Community-Acquired Methicillin-Resistant Staphylococcus aureus Carrying Panton-Valentine Leukocidin Genes: Worldwide Emergence. Emergent Infectious Disease 9(8): 978-984.

Vieu G (2014). Diversité génétique des isolats de Staphylococcus aureusproducteurs de toxine de Panton-Valentine isolés au CHU de Toulouse. Etude de 37 cas de patients à l'hôpital des enfants. Universite Toulouse Iii –Paul Sabatier Faculte Des Sciences Pharmaceutiques Thèse 2014-TOU3-2073.

Vindel A, Trincado P, Cuevas O, Ballesteros C, Bouza E, Cercenado E (2014). Molecular epidemiology of community-associated methicillin-resistant Staphylococcus aureus in Spain: 2004-12. Journal of Antimicrobial Chemotherapy 69(11):2913-2919, https://doi.org/10.1093/jac/dku232.

Waldenburger S, Vogel U, Goebeler M, Kolb-Mäurer A (2014). Community-acquired skin infections caused by Staphylococcus aureus: What is the role of the Panton-Valentine leukocidin toxin? Journal der Deutschen Dermatologischen Gesellschaft 12(1):59-66. doi:10.1111/ddg.12228.

Witte W, Strommenger B, Cuny C, Heuck D, Nuebel U (2007). Methicillin-resistant Staphylococcus aureus containing the Panton-valentine leucocidin gene in Germany in 2005 and 2006. Journal of Antimicrobial Chemotherapy 60(6):1258-1263. https://doi.org/10.1093/jac/dkm384

Zhang K, Xie K, Zhang C, Liang Y, Chen Z, Wang H (2022). C-reactive protein testing to reduce antibiotic prescribing for acute respiratory infections in adults: a systematic review and meta-analysis. Journal of Thoracic Disease 14(1):123-134. doi: 10.21037/jtd-21-705.

Zhang T, Huang WS, Guan W, Hong Z, Gao J, Gao G, Wu G, Qin YY (2020). Risk factors and predictors associated with the severity of COVID-19 in China: a systematic review, meta-analysis, and meta-regression. Journal of Thoracic Disease 12(12):7429-7441. doi: 10.21037/jtd-20-1743.