Brazilian Journal of Microbiology (2020) 51:1509–1513
https://doi.org/10.1007/s42770-020-00386-y

BACTERIAL AND FUNGAL PATHOGENESIS - SHORT COMMUNICATION

# Panton-Valentine leukocidin (PVL) isoforms among *Staphylococcus aureus* lineages isolated from hospitals in Rio de Janeiro, Brazil

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Received: 11 February 2020 / Accepted: 16 October 2020 / Published online: 26 October 2020


## Abstract

Panton-Valentine leukocidin (PVL) is a *Staphylococcus aureus* virulence factor codified by *lukSF*-PV genes. Single-nucleotide polymorphisms (SNPs) at *lukSF*-PV genes can lead to two PVL sequence variants (R and H) generating different PVL isoforms. This study analyzed *lukSF*-PV genes SNPs among four different clonal lineages (STs/CC 1, 5, 8, and 30) of nine *S. aureus* isolated at Brazilian hospitals. The sequenced products showed SNPs at seven sites (positions 121, 470, 527, 663, 856, 1396, and 1729), leading to non-synonymous substitutions in all isolates investigated. Our findings showed new R and H isoforms variants in *S. aureus* isolated in Brazil and suggest a possible relationship between H2b isoform and the ST30/CC30 lineage.

**Keywords** *Staphylococcus aureus* · Panton-Valentine leukocidin (PVL) isoforms · Brazilian lineages

Panton-Valentine leukocidin (PVL) is a bicomponent and pore-forming toxin that targets phagocytes, leading to membrane damage and cell death in vitro, often associated with recurrent *Staphylococcus aureus* skin and soft tissue infections and necrotizing pneumonia [1]. PVL can be produced by both methicillin-susceptible (MSSA) and methicillin-resistant *S. aureus* (MRSA) isolates [1, 2]. The USA300/ST8 MRSA is the commonly PVL-positive lineage found in the USA [3], while in Latin American countries, including Brazil, the USA1100/ST30 MRSA is frequently reported [2, 4].

PVL-codifying genes, *lukS*-PV and *lukF*-PV, are carried by a temperate prophage and may be transferred from one isolate to another via phage transduction [5]. Although the PVL genes are considered highly conserved [6], it was demonstrated at least 22 points mutations or SNPs (single-nucleotide polymorphism) in both *lukS*-PV and *lukF*-PV [6–11] (Table 1). Based on the nucleotide substitution, the PVL proteins were classified in two major sequence variants of *lukSF*-PV, named as H or R variants [7].

Despite the recognized role of PVL on *S. aureus* disease severity [12], the influence of PVL isoforms on protein toxicity and disease outcome remains unknown. Molecular modeling studies suggested that the R isoform may alter pore formation and increase PVL leukotoxicity [7]. On the other hand, some authors suggested that the protein isoform does not influence PVL toxicity, since H and R isoforms could be found at similar frequencies among *S. aureus* isolates recovered from invasive diseases [6, 13]. However, it is important to point that mutations at virulence-related genes, besides increasing the molecular diversity of bacterial isolates, may lead to gain or loss of protein function. Due to the importance of PVL on *S. aureus* disease outcome [12], the comprehension of PVL structure and diversity is crucial to understand the microbial pathogenicity and may help the improvement of specific therapeutic drug target design. Moreover, there is no study regarding PVL isoforms of PVL-positive *S. aureus* isolates from Brazil. Thus, the present study aimed to determine the PVL isoforms of *S. aureus* previously isolated from different hospitals in Rio de Janeiro [2] and correlate with their clonal lineages.

Responsible Editor: Mariana X Byndloss.

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**Table 1** Non-synonymous substitutions (NSS) of amino acids related to point nucleotide mutations at *lukSF-PV* genes and their PVL protein isoforms

| Gene | NT | Substitution[a] | AA | Substitution[b] | Isoform |
|---|---|---|---|---|---|
| *lukS-PV* | 121 | G > A | 41 | Val > Ile | H2e* |
| | 296 | C > T | 99 | Ala > Val | R3 |
| | 470 | T > A | 157 | Phe > Tyr | H2b |
| | 527 | G > A | 176 | Arg > His | H1, H2a, H2b, H2c, H2d, H3, H2e |
| | 663 | T > G | 222 | Ser > Ala | H1, H2a, H2b, H2c, H2d, H4 |
| | 856 | A > G | 286 | Gln > Arg | R4* |
| *lukF-PV* | 1396 | A > G | 466 | Tyr > Cys | H1 |
| | 1729 | G > A | 577 | Arg > Gln | R2, R3, H1, H2a, H2b, H2c, H3, H2e |

NT, nucleotide position; [a] substitution of nucleotide; T, thymine; A, adenine; G, guanine; AA, amino acid position; [b] substitution of amino acid; Val, valine; Ile, isoleucine; Phe, phenylalanine; Tyr, tyrosine; Arg, arginine; His, histidine; Ser, serine; Ala, alanine; Gln, glutamine; Cys, cysteine; *Isoforms firstly detected in this study

Therefore, in order to identify SNPs at PVL genes, nine PVL-positive *S. aureus* Brazilian isolates, previously characterized by *mecA* presence, SCC*mec* types, clonal lineage, sequence type (ST), and clonal complex (CC) [2], were investigated: 526a (USA300/ST8/CC8/SCC*mec*IV); 559a and 566a (USA800/ST5/CC5/SCC*mec*IV); 806a, 1155a, and 1342a (USA1100/ST30/CC30/SCC*mec*IV); 1348a and 1444a (USA400/ST1/CC1/MSSA); and 1462a (ST30/CC30/SCC*mec*V) (Table 2). Genomic DNA were obtained according to Walsh, Metzger, and Higuchi [14]. To access the point mutations at *lukS*-PV and *lukF*-PV, three fragments of *lukSF*-PV genes [8] were amplified and sequenced. PCR products were purified using a GTX PCR and Band Purification Kit (GE Healthcare, Amersham, UK) according to the manufacturer's instructions and were sequenced on an automated DNA MegaBACE1000 Sequencing System (Biotech, Massachusetts, USA) using a DYEnamic ET Dye Terminator System (GE Healthcare). The electropherogram of the fragments was analyzed (BioEdit Sequence Alignment Editor, version 7.2.5), and the SPNs were accessed by comparisons of generated products with the *luk*SF-PV gene previously sequenced from *S. aureus* 8_1, an isolate related to the phiSa2USA phage and to R1 PVL isoform (GenBank accession no. HM584700) (Table 2). The search for SNPs focused at positions 121, 296, 470, and 527 (first fragment); 663, 856, 965, and 1261 (second fragment); and 1356, 1369, and 1729 (third fragment). The sequences were also compared to previously published sequences related to H and R isoforms: H1 (GenBank accession no. EF571669); H2 (GenBank accession no. EF571668); H2a, H2b, H2c, and H2d [10]; H3 (GenBank accession no. EF571713); R2 (GenBank accession no. EF571830); and R3 [10] (Table 2).

According to the sequencing analysis of the three gene fragments, in comparison with the R1 PVL isoform reference sequence (GenBank accession no. HM584700), it was possible to identify SNPs at seven of the 11 nucleotide positions (121, 470, 527, 663, 856, 1396, and 1729), leading to amino acids NSSs (Table 1). The combination of different SNPs grouped *S. aureus* isolates into different H or R PVL isoforms (Table 2), such as H1, H2b, H2e, and R4. Overall, eight isolates harbored variations of the H2 isoform, while one harbored the R PVL variant.

It is noteworthy to mention that *S. aureus* isolates belonging to the same genetic background showed specific mutations. For example, the three isolates belonging to USA1100/ST30/CC30/SCC*mec*IV (isolates 806a, 1155a, and 1342a) and one isolate related to ST30/CC30/SCC*mec*V (1462a) presented SNPs at nucleotide positions 470, 527, 663, and 1729 (Table 2). These NSSs grouped those isolates together, carrying the same H2b isoform, since it differed from H2/H2a isoform (GenBank ac. no EF571668) by one mutation at nucleotide position 470 (T470A), which resulted in a substitution of phenylalanine (F) to tyrosine (Y) at amino acid 156 (F156Y), previously observed by Chen and coworkers [10].

The two MSSA isolates belonging to USA400/ST1/CC1 lineage (isolates 1348a and 1444a), and one of the two isolates related to the USA800/ST5/CC5/SCC*mec*IV lineage (566a), presented the same mutation observed at the H1 PVL isoform (GenBank accession no. EF571669) (Table 2). However, the other isolate from USA800/ST5/CC5/SCC*mec*IV lineage, named as 559a, despite its ST similarity with the 566a isolate, presented a new H PVL isoform, described here for the first time, named as H2e. This isoform, in addition to the substitutions already described for H2b [10], presented a variation at nucleotide position 121, resulting in a valine (V) to isoleucine (I) substitution at amino acid 41 (V41I), being, therefore, characterized as a new isoform (Table 2). Furthermore, the sequence analysis of the isolate related to the USA300/ST8/CC8/SCC*mec*IV lineage (isolate 526a), when compared with the R1 isoform reference sequence (GenBank accession no. HM584700), also revealed an unknown R isoform variation at the nucleotide position 856, resulting in an arginine (R) to glutamine (G) NSS at amino acid 286 (G286R). Therefore, this new R isoform variation was named R4 (Table 2).

**Table 2** Mutations at *lukSF-PV* genes and related isoforms in *Staphylococcus aureus* isolates compared to reference sequences

| Isolate/Reference (GenBank accession No.) | Clonal lineage/ST/SCC*mec* type | Clinical source | *agr* | Nucleotides | | | | | | | | | | | Isoform |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *luk*S-*PV* (nt 1–939) | | | | | | *lukF-PV* (nt 941–1918) | | | | | |
| | | | | 121 | 296 | 470 | 527 | 663 | 856 | 965 | 1261 | 1356 | 1396 | 1729 | |
| HM584700 | nd | nd | nd | G | C | T | G | T | A | G | C | G | A | G | R1 |
| EF571830 | nd | nd | nd | G | C | T | G | T | A | G | C | G | A | **A** | R2 |
| nd[a] | Con/30/na | nd | nd | G | **T** | T | G | T | A | G | C | G | G | **A** | R3 |
| EF571669 | nd | nd | nd | G | C | T | **A** | **G** | A | G | C | G | **G** | **A** | H1 |
| EF571668 | nd | nd | nd | G | C | T | **A** | **G** | A | G | C | G | A | **A** | H2/H2a[a] |
| nd[a] | 80/30/na and 81/1472/na | nd | nd | G | C | **A** | **A** | **G** | A | G | C | G | A | **A** | H2b |
| nd[a] | USA1100/30/IV | nd | nd | G | C | T | **A** | **G** | A | G | C | **C** | A | **A** | H2c |
| nd[a] | 80/81/30/na | nd | nd | G | C | T | **A** | **G** | A | G | C | G | A | G | H2d |
| EF571713 | nd | nd | nd | G | C | T | **A** | T | A | G | C | G | A | **A** | H3 |
| 526a* | USA300/8/IV | skin abscess | 1 | G | C | T | G | T | **G** | G | C | G | A | G | R4 |
| 559a* | USA800/5//IV | peritoneal fluid | 2 | **A** | C | **A** | **A** | **G** | A | G | C | G | A | **A** | H2e |
| 566a | USA800/5/IV | nasal swab | 2 | G | C | T | **A** | **G** | A | G | C | G | **G** | **A** | H1 |
| 806a | USA1100/30/IV | nasal swab | 3 | G | C | **A** | **A** | **G** | A | G | C | G | A | **A** | H2b |
| 1155a | USA1100/30/IV | prosthetic sec.[b] | 3 | G | C | **A** | **A** | **G** | A | G | C | G | A | **A** | H2b |
| 1342a | USA1100/30/IV | blood | 3 | G | C | **A** | **A** | **G** | A | G | C | G | A | **A** | H2b |
| 1462a | nd/30/V | skin abscess | 3 | G | C | **A** | **A** | **G** | A | G | C | G | A | **A** | H2b |
| 1348a | USA400/1/na | blood | 3 | G | C | T | **A** | **G** | A | G | C | G | **G** | **A** | H1 |
| 1444a | USA400/1/na | blood | 3 | G | C | T | **A** | **G** | A | G | C | G | **G** | **A** | H1 |

* Isolates presenting PVL isoforms with new SNPs; ST - Sequence type; SCC*mec* - *Staphylococcal* cassette chromosome *mec*; *agr* - *accessory gene regulator;* nt - nucleotide; na - not applicable (isolate sensitive to methicillin); nd – no data available; [a] according to Chen and coworkers (2013) [10]; [b] prosthetic secretion; bolded and underlined letters indicate nucleotide substitution

Different authors have showed a correlation between PVL isoform, *S. aureus* lineages, and their geographical distribution worldwide [6–11]. However, up to date, no studies concerning the Brazilian isolates PVL variations are reported. In general, R isoforms are mostly associated with the USA300/ST8/CC8/SCC*mec*IV *S. aureus* lineage [7, 9], widely distributed at USA [3], while the H isoform have been related to both MSSA and MRSA isolates of other clonal complexes, such as CCs 1, 5, 6, 22, 25, 30, 59, 88, and 121, from different countries [6, 7, 9, 10, 13].

As mentioned before, the USA1100/ST30/CC30/SCC*mec*IV is a prevalent community clone in Brazil and other Latin American countries [2, 4]. In our study, we found that all four isolates belonging to ST30/CC30, regardless their SCC*mec* type, presented the H2b PVL variant. Chen and coworkers [10] analyzed 52 PVL-harboring *S. aureus* CC30 isolates collected from four continents over a 75-year period and grouped the *S. aureus* isolates into the H1, H2/H2a, H2b, H2c, H2d, R1, R2, and R3 PVL isoforms. The H2b isoform was only detected among five MSSA isolates belonging to STs 30 and 1472, where the majority of CC30 isolates presented H2a (50.0%) and H1 (17.3%) PVL isoforms [10].

Here, we also observed other isolates with H PVL isoform, as the MSSA isolates 1348a and 1444a (USA400/ST1/CC1) and the MRSA isolate 566a (USA800/ST5/CC5/SCC*mec*IV), that presented the H1 isoform. Besides, we also identified a new H PVL isoform, named H2e, carried by another MRSA related to USA800/ST5/CC5/SCC*mec*IV (559a). Therefore, further studies regarding the potential role of the H isoforms, especially the H2b variant found among isolates belonging to ST30/CC30, are needed.

It has been described that the R PVL isoform is commonly associated to isolates belonging to USA300/ST8/CC8/SSC*mec*IV lineage [6, 7, 9, 13]. However, in a recent study, Zhang, Guo, and Chu [11] describe the R isoform among *S. aureus* isolates from lineages CC5, CC59, and CC88. Moreover, Chen and coworkers [10] described the R2 and R3 isoforms, presenting the G1729A and C296T/G1729A mutations, respectively, among isolates related to CC30. Here, we identified a new R PVL isoform in one isolate from the USA300/ST8/CC8/SCC*mec*IV lineage, isolate 526a, named as R4. Although the USA300/ST8/CC8/SCC*mec*IV lineage is rarely found in Brazil, further studies are needed in order to access the prevalence of R4 variant and its association with this lineage in PVL-positive *S. aureus*.

The impact of each isoform or polymorphisms on PVL toxicity remains unknown. O'Hara and coworkers [7] firstly suggested that a single amino acid replacement at nucleotide position 176, resulting in the R isoform, may increase leukotoxicity of PVL [7]. However, some authors argue that both isoforms present the same ability to induce pore formation and toxicity [13] or that PVL leukotoxicity depends of the amount of protein produced by each isolate [15]. A recent study showed that the PVL production in vitro, accessed by enzyme-linked immunosorbent assay, was similar in both H and R isoforms regardless the genetic background and isolates origin, suggesting that SNPs at *lukSF*-PV genes might have no effect on the toxin expression [11].

In a recent study by our group [16], we showed that a H2b PVL-positive USA1100/ST30/CC30 isolate expressed five times more PVL mRNA when compared with other lineages of *S. aureus*. It is important to point that this isolate was recovered from a neonate bloodstream infection. These results highlight the importance of further investigation about *S. aureus* PVL isoforms and its correlation with the clonal lineage and patient clinical data.

Here, we analyzed the PVL isoforms among *S. aureus* isolated in Brazil for the first time. We identified two new variants, H2e and R4, in two MRSA isolates belonging to USA800/ST5/CC5/SCC*mec*IV and USA300/ST8/CC8/SCC*mec*IV isolates, respectively, and suggested a possible relationship between H2b isoform and isolates belonging to ST30/CC30, a community lineage widely disseminated in our country. The presence of this specific PVL isoform and its variations on ST30/CC30 isolates could contribute to the high circulation of this lineage in Latin American countries, especially in Brazil.

**Authors' contributions** Conceptualization: Raiane Cardoso Chamon; Methodology: Raiane Cardoso Chamon, Tamara Lopes Rocha de Oliveira, and Rosana Barreto Rocha Ferreira; Formal analysis and investigation: Raiane Cardoso Chamon; Writing - original draft preparation: Raiane Cardoso Chamon; Writing - review and editing: Lilian Oliveira Moreira and Kátia Regina Netto dos Santos; Funding acquisition: Kátia Regina Netto dos Santos; Resources: Kátia Regina Netto dos Santos; Supervision: Lilian Oliveira Moreira and Kátia Regina Netto dos Santos.

**Funding** This study was supported by Brazilian grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethics approval** The isolates used at the present study belong to the "Laboratório de Infecção Hospitalar" collection, a reference laboratory at Universidade Federal do Rio de Janeiro.

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