TITLE: DETECTION OF PANTON-VALENTINE LEUKOCIDIN (PVL) ISOFORMS AND *pvl* EXPRESSION AMONG *Staphylococcus aureus* FROM DIFFERENT LINEAGES

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ABSTRACT

Most community-associated S*taphylococcus aureus* can carry the Panton-Valentine leukocidin (PVL) gene (*lukSF-PV*), characterized as a pore-forming toxin, carried by several different encoding phages. Different single-nucleotide polymorphisms (SNPs) have been identified and PVL protein isoforms carrying non-synonymous substitutions (NSS) have been described. Two *lukSF-PV* sequence variants (R and H variant) have been associated to these mutations. Besides, recent reports have shown differences on *pvl* expression among isolates belonging to specific clonal lineages. In order to detect SNPs in *pvl* genes from different clonal lineages (USA300/*agr* type I/SCC*mec* IV, USA400/*agr* III/MSSA, USA800/*agr* II/SCC*mec* IV and USA1100/*agr* III/SCC*mec* IV) three primer pairs to amplify fragments of *lukSF-PV* genes were used. All the products of PCR were sequenced and analyzed by comparison with the phage phiSLT. Besides, the isoforms found were compared with published types: H1, H2, H3, R1 and R2. The relative expression of *pvl* and *gyrB* (endogenous control) genes were accessed by quantitative real time PCR (qPCR) using the ΔΔCt method, with the USA300 as the reference isolate for *pvl* expression. The *lukSF-PV* sequenced products showed SNPs at six sites (positions 470, 527, 663, 856, 1396 and 1729), all of them causing NSS. The USA800 and USA1100 isolates shared the same isoform, differing from the H2 in one position (470), resulting in a change from tyrosine to phenylalanine (Y156F), being called H2b. The USA400 isolate presented the type H1 isoform, and the USA300 isolate,when compared with the R1 isoform differed only at the position 856, resulting in a change from arginine to glutamine (R286G), being called as R1b type, described for the first time in this study. The *pvl* expression was found up to 4 times more in USA1100 isolate than the USA300 one. However, the USA800 isolate, which presented the same isoform of USA1100, expressed 10% less the gene than USA300, while the USA400 expressed 15% more. This is the first Brazilian study to analyze SNPs among *pvl*-positive isolates and their isoforms associated to different genetic backgrounds. However, the type of PVL variant (H or R) was not associated to the expression level of *pvl*, since the USA1100 isolate showed higher expression but a H2b variant, a less virulent type. Further analysis is needed to better understand the impact of PVL isoforms in the infection outcome caused by *S. aureus pvl*-positive isolates.

Keywords: *S. aureus;* PVL; SNPs

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