Title: METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* RNA PROFILING USING NEXT-GENERATION SEQUENCING TECHNOLOGIES AFTER TREATMENT WITH VIOLACEIN

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Abstract: Violacein ([3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one]) is a purple pigment produced by environmental gram-negative bacterial species such as *Janthinobacterium lividum* and *Chromobacterium violaceum.* This compound is indole-derivative synthesized from the condensation of two L-tryptophan molecules and the synthesis is known to be induced under aerobic conditions and in response to *quorum sensing* mechanism. This pigment has shown to have several biological properties including anti-inflammatory, antimicrobial, antiprotozoal and antitumor activity. As antibiotic, Violacein has significant activity against gram-positive bacteria, especially methicillin and vancomycin resistant *Staphylococcus aureus,* MRSA and VRSA respectively, at low concentrations. In this context, the aim of this work is to investigate the transcriptome profile of MRSA treated with Violacein. The MRSA N315 strain assayed regarding the minimal inhibitory concentration MIC and sub-MIC concentration of Violacein for 15 minutes, 1 hour and 3 hours of treatment and the transcriptome was analyzed using high throughput sequencing (RNA-Seq) on the Illumina HiSeq platform. The profiles generated from the RNA sequencing provided the opportunity to investigate the expression of identified genes less or more abundant after analysis of RPKM numbers and fold changes. Overall, no results of difference in genes expression were found after 15 minutes. After 1 hour of treatment with violacein at sub-MIC, 134 genes were up expressed and 24 genes were down, at MIC 22 were up expressed and 8 down. In 3 hours, at sub-MIC, 4 genes were expressed positively and 8 genes negatively, at MIC, 180 genes up and 59 genes were down. Differences in expression of metabolism-related genes can be noted, where various enzymes and general transport genes can be related, as also some genes involved in cell surface modeling and metabolic behavior. Interestingly, many genes with differential expression were uncharacterized or 'hypothetical' genes, suggesting a possible new or not explored mechanism of action. We demonstrated that our approach provides a way to survey global transcriptional activity in MRSA treated with Violacein and enables discovery of specific areas in the genome that merit further investigation to study the mechanisms of action.

Keywords: MRSA, RNA-Seq, Violacein.

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