

Deciphering “DAP and Glycopeptide mechanism of action-related” transcriptome profiling by RNA-seq in *S.aureus*

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Daptomycin-Resistance (DAP-R) and reduced vancomycin susceptibility represent a public health issue as DAP is the last-resort treatment for heterogeneous Vancomycin-Intermediate-*Staphylococcus aureus* (hVISA) and Vancomycin-Intermediate-*S.aureus* (VISA). NGS technology applied to transcriptomics has outlined transcriptional networks distinguishing MDR microorganisms.

To have new insight into the mechanisms of DAP-R, the small-RNA (sRNA) and mRNA comparative transcriptomes of two DAP-R (1C-3B) clinical isogenic isolates vs their DAP-S (1A-3A) counterparts were investigated by Illumina RNA-seq, the Rockhopper tool, computational filtering analyses and bioinformatics.

Statistically significant filtering analysis of the RNA-seq data and bioinformatics provided evidence on differential expression changes - up or down regulation - in both DAP-R when compared with DAP-S strains in different antisense sRNAs and coding-mRNAs involved in diverse biological processes including the cell-wall and cell-membrane organization, metabolism, nucleic acid metabolic process, stress response, heat and cold stress responses, and transporter and host adhesion.

However, among these transcriptional profiles, a down-regulation of the biosynthesis of the peptidoglycan precursors and cell division related genes, an over-expression of *cidB* autolysin, a down-regulation of different membrane protein coding genes, different dysfunctions in TCA genes, an increased oxidative stress response, and finally down-regulated and up-regulated "cis-regulator" sRNAs putative regulators for the described functions were stand out as emerging traits related to the daptomycin and glycopeptide mechanism of action in DAP-R *S.aureus*. In order to validate RNA-seq data, real time qPCR was performed to quantify the transcription of the randomly genes among the daptomycin and glycopeptide mechanism of action related transcriptomic traits. In conclusion, our analysis defined the transcriptional key traits related with the DAP and glycopeptide mechanism of action in DAP-R *S.aureus*.

Keywords: Daptomycin resistance, MRSA, Comparative transcriptome, RNA-seq