SNP discovery in the *Klebsiella pneumoniae* transcriptome after polymyxin B induction in combination with abiotic stresses using RNA-Seq technology

[1]Thiago Cardoso Pereira Carneiro[1], Guilherme Loss de Morais[2], Gisele Lucchetti da Silva, Guadalupe del Rosario Quispe Saji[2], Ana Tereza Ribeiro de Vasconcelos[2], Marisa Fabiana Nicolás[2]

[1]*Universidade Estácio de Sá, [2]The National Laboratory for Scientific Computing LNCC. Av. Getúlio Vargas, 333 Quitandinha, 25651075, Petrópolis, RJ Brazil.*

The Single Nucleotide Polymorphism (SNP) can be characterized as the sequence variation observed in a individual position in the genome, and this may involve direct and relevant way in the formation of a protein. SNPs can be used to type bacterial strains and also used to guide site-specific mutation studies. *In silico* detection of SNPs are currently applied for bacterial genomics studies. *Klebsiella pneumoniae* is a Gram-negative, rod-shaped bacterium frequently associated with nosocomial and community-acquired infections. The emergence and subsequent global spread of strains producing *Klebsiella pneumoniae* carbapenemase (KPC) represents a significant threat to public health. Many KPC infections can only be treated when resorting to last-line drugs such as colistin and polymyxin B (PB). However, resistance to these antibiotics is also observed, although insufficient information is yet described on its mode of action as well as with combination of abiotic stresses. In a recent study conducted in *Pseudomonas aeruginosa* was reported that free iron in the culture medium increases mutagenesis in the presence of cationic antimicrobial peptides (cAMPs). Considering that PB is a type of cAMP and in view of this information, the goal of this study was investigate the SNPs induced by the effect of PB in combinations with abiotic stresses, such as high level concentration of Fe or low pH. The cDNA data of *K. pneumoniae subsp. pneumoniae* Kp13 were generated by *illumina platform Hiseq* (www.illumina.com) in induced conditions (PB) and high concentrations of Fe or low pH. Firstly, the RNA-seq data were trimmed and then mapped against the Kp13 genome reference (NCBI Bioproject PRJNA78291) by the *bowtie2* (bowtie-bio.sourceforge.net). Then, the result files were ordered and indexed by *Samtools* (samtools.sourceforge.net/). The SNP search was done with GATK (software.broadinstitute.org/gatk) and the IGV (software.broadinstitute.org/software/igv) allowed a better visualization and analysis of these mapped SNPs. Finally, SNPs were classified according to synonymous or non-synonymous mutations. In the case of non-synonymous mutations, it was investigated the CDS role in the bacterial response to antimicrobial stresses. Comparing PB versus PB+pH or PB+Fe we observed a higher incidence of SNPs in PB+Fe condition. An interesting gene containing three non-synonymous mutations as well as one stop loss mutation was *sodB*, which encodes for a superoxide dismutase Fe (SOD-Fe), which is a major enzyme produced by microorganisms to evade the potentially damaging reactive oxygen species (ROS) during the stress imposed by antimicrobials.