Different bioinformatics strategies to *Mycobacterium tuberculosis* genomic variants identification associated with D-cycloserine resistance.

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Abstract 2

The increase in cases of resistant tuberculosis (TB) is worrying since therapeutic options are reduced. D-cycloserine (DCS) is a bacteriostatic antibiotic widely used in the treatment of resistant TB, however, the genetic bases of the resistance mechanisms that mycobacteria develop to this drug are not yet fully described. The objective of this study was to compare two bioinformatic approaches for variants identification in the MTB genome associated with resistance to DCS.

We used MTB H37Rv_27294 strain to mutant generation in-vitro. 13 resistant mutants were selected and colonies were sequenced by Illumina Hiseq-2500. Two bioinformatics analysis strategies were applied, in the first one an in-house workflow was used doing the reads pre-processing of the reads with Trimmomatic, and the assembly was done by mapping using Bowtie2. Bam files were used to perform variant calling with BCFtools and visualization of variants across the genome was performed with the IGV program. As a second strategy, the standardized pipeline of the MTBseq was used, considering parameters of depth and allelic frequency for the detection of known variants associated with resistance to DCS.

We identified 25 mutations between two pipelines. MTBseq identified 23 mutations in 22 known genes, one of them *alr* strongly associated with resistance to DCS. With the in-house pipeline, the same 23 mutations were identified in 22 genes, and two mutations additionally were found in the genes *pknB* and *ufaA1* involved in the formation of the cell wall and peptidoglycan, which have been described as a target for DCS.

MTBseq's standardized pipeline facilitated data processing in execution and processing time but didn´t identify additional mutations in genes that were not reported in its database. With the in-house pipeline, additional mutations possibly associated with DCS resistance were identified, which is an important contribution to the study of new molecular mechanisms of DCS resistance.

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