Identification of *Mycobacterium tuberculosis* mutations related with D-cycloserine resistance using phenotypic and genomic approaches.

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Abstract

D-cycloserine (DCS) is a broad-spectrum antibiotic used in second-line treatment against *Mycobacterium tuberculosis* (MTB). Its antibacterial mechanism of action is to inhibit the cell wall mucopeptide synthesis. DCS has a great advantage in that it does not present cross-resistance with other drugs, in addition to its wide distribution and ease of absorption. However, the mechanisms of resistance of MTB to DCS are not well understood. The aim was to investigate the molecular basis of DCS resistance through the generation of MTB mutants and the identification of genomic variants associated with resistance phenotype using whole genome sequencing.

We used MTB H37Rv_27294 strain to mutant generation in Middelbrook 7H10 agar with different antibiotic concentrations. A total of 13 resistant mutants were selected. Colonies were sequenced by Illumina Hiseq-2500, the data analysis was performed with in house bioinformatic pipeline using bowtie2 and BCFtools and IGV mainly. The identified mutations associated with DCS resistance were compared and confirmed with MTBseq software.

We identified mutations in 24 genes that are possibly associated with DCS resistance. From there, we found a known resistance mutation in *alr* (rv3423c) gene but did not identify any mutation in *ddl*, *cycA*, or *ald* genes commonly associated with DCS resistance also. However, we identified 21 genes with variants only present in colonies exposed to high DCS concentrations but were not present in the H37Rv_27294 wild-type strain. The genomic variants identified in this study indicate that mechanisms of DCS resistance are complex and involve more mutations in different genes that are reported to date. For instance, we identified several genes related to the cell wall and peptidoglycan formation.

Hence the importance to validate these findings in clinical strains, relying on phenotypic susceptibility testing, molecular and genomic approaches to understand the mechanisms of resistance in MTB.

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