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Investigation of isoniazid and ethionamide cross-resistance by whole genome sequencing and association with poor treatment outcomes of multidrug-resistant tuberculosis patients in South Africa

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ABSTRACT

Objective/background: Ethionamide (ETH) and isoniazid (INH) are part of the backbone regimen used for the treatment of multidrug-resistant tuberculosis (MDR-TB). Both ETH and INH are structurally similar and are activated by *ethA* and *katG* gene products. Resistance to INH among MDR-TB patients may cause ETH to be ineffective, as both target nicotinamide adenine dinucleotide-dependent enoyl-acyl carrier protein reductase *inhA* protein and mutations within *inhA* gene may lead to their cross-resistance. Furthermore, ETH resistance is caused by mutations within *ethA* and *ethR* genes forming part of the ETH drug activation pathway. Nicotinamide adenine dinucleotide is coded by the *ndh* gene, and its overexpresion may lead cross-resistance between INH and ETH drugs. Phenotypic drug susceptibility testing of ETH is difficult and often unreliable. We used whole genome sequencing to compare *inhA*, *inhA* promoter, *ethA*, *ethR ndh*, and *katG* genetic regions in serial isolates (baseline and follow-up) with treatment outcomes.

Methods: MDR-TB strains were collected from 46 patients before and during second-line drug treatment in KwaZulu-Natal and Eastern Cape between 2005 and 2009. All patients had phenotypically determined MDR-TB at baseline and had treatment outcomes documented. Unfavorable treatment outcomes were defined as death, default, and failure, while favorable outcomes were cure and treatment completion. Each strain had baseline and at least one strain collected on follow-up. From each strain, DNA was extracted from colonies grown on Löwenstein–Jensen slants, and fragment and jumping paired-end Illumina DNA libraries were constructed and sequenced on the Illumina HiSeq 2000 (Broad Institute, Cambridge, MA, USA). Sequences were aligned to H37Rv genome and Pilon was run to generate a list of SNPs. In silico spoligotyping was performed to a database 43 unique spacer sequences. Cross-resistance was defined as the presence of both *inhA* and either *ethA* or *ethR* mutations in clinical isolates.

Results: A total of 92 sequences from 46 serial isolates of MDR-TB patients from KwaZulu-Natal (29 isolates) and Eastern Cape (17 isolates) were analyzed. Most patients (29/46; 63.0%)

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had unfavorable outcomes, 13 (28.3%) had favorable outcomes, while four (8.7%) had unknown outcomes. Phylogenetic reconstruction revealed that primary genotype differed by province. The Beijing genotype was predominant in Eastern Cape, while EuroAmerican lineage (S, T, LAM, X) was found in KwaZulu-Natal. Whole genome analysis revealed non-synonymous insertions and deletions within *katG*, *ethA*, *ethR*, *ndh*, and *inhA* and its promoter region. Among patients with treatment outcome data, mutations were detected in 92.8% in *katG*, 50% in *inhA*, 53.6% in *ethA*, 2.4% in *ethR*, and 19% in *ndh*. The majority of mutations causing ETH (20/29; 68.9%) and INH (18/29; 62.1%) resistance occurred among patients with unfavorable outcomes. Both *inhA* and either *ethA* or *ethR* mutations were detected in 16/29 (55.2%) patients with unfavorable outcomes. Cross-resistance of both INH and ETH drugs was associated with unfavorable treatment outcomes ($p = 0.021$) in 16/29 (55.2%) patients compared with favorable treatment outcomes in 2/13 (15.4%) patients.

Conclusion: Baseline ETH molecular resistance before second-line treatment is a concern. Unfavorable treatment outcomes of patients with *ethA*, *ethR*, and *inhA* mutations highlight the importance of genotypic testing before initiation of treatment containing ETH. The clinical significance of whole genome analysis for early detection of mutations predictive of treatment failure needs further investigation.

Conflicts of interest

All authors declare no conflicts of interest.

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