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Recommended Citation

Ali, A., Hasan, Z., McNerney, R., Mallard, K., Cawthorne, G. H., Coll, F., Nair, M., Pain, A., Clark, T. G., Hasan, R. (2015). Whole genome sequencing based characterization of extensively drug-resistant mycobacterium tuberculosis isolates from Pakistan. *PLoS ONE*, 4(1), 11-12.

Available at: http://ecommons.aku.edu/pakistan_fhs_mc_pathol_microbiol/515

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Whole genome sequencing-based characterization of extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* from Pakistan

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ARTICLE INFO

Article history:

Received 21 October 2014

Accepted 26 October 2014

Available online 23 December 2014

Keywords:

Extensively drug resistant (XDR)

Mycobacterium tuberculosis

Pakistan

ABSTRACT

Objectives: The global increase in drug resistance in *Mycobacterium tuberculosis* (MTB) strains increases the focus on improved molecular diagnostics for MTB. Extensively drug-resistant (XDR) – TB is caused by MTB strains resistant to rifampicin, isoniazid, fluoroquinolone and aminoglycoside antibiotics. Resistance to anti-tuberculous drugs has been associated with single nucleotide polymorphisms (SNPs), in particular MTB genes. However, there is regional variation between MTB lineages and the SNPs associated with resistance. Therefore, there is a need to identify common resistance conferring SNPs so that effective molecular-based diagnostic tests for MTB can be developed. This study investigated used whole genome sequencing (WGS) to characterize 37 XDR MTB isolates from Pakistan and investigated SNPs related to drug resistance.

Methods: XDR-TB strains were selected. DNA was extracted from MTB strains, and samples underwent WGS with 76-base-paired end fragment sizes using Illumina paired end HiSeq2000 technology. Raw sequence data were mapped uniquely to H37Rv reference genome. The mappings allowed SNPs and small indels to be called using SAMtools/BCFtools.

Results: This study found that in all XDR strains, rifampicin resistance was attributable to SNPs in the *rpoB* RDR region. Isoniazid resistance-associated mutations were primarily related to *katG* codon 315 followed by *inhA* S94A. Fluoroquinolone resistance was attributable to *gyrA* 91–94 codons in most strains, while one did not have SNPs in either *gyrA* or *gyrB*. Aminoglycoside resistance was mostly associated with SNPs in *rrs*, except in 6 strains. Ethambutol resistant strains had *embB* codon 306 mutations, but many strains did not have this present. The SNPs were compared with those present in commercial assays such as LiPA Hain MDRTBsl, and the sensitivity of the assays for these strains was evaluated.

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<http://dx.doi.org/10.1016/j.ijmyco.2014.10.050>

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Conclusions: If common drug resistance associated with SNPs evaluated the concordance between phenotypic and genotypic testing, the results would be rifampicin (100%), isoniazid (89%), fluoroquinolones (95%), aminoglycoside (81%) and ethambutol (61%). This work highlights the importance of expanded targets for drug resistance detection in MTB isolates.

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