

Genetic diversity of multidrug-resistant *Mycobacterium tuberculosis* isolates in Pakistan

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Background: Tuberculosis (TB) remains an inglorious leader among infectious diseases in mortality, with its annual toll of 1.7 million lives worldwide [1]. Pakistan ranks 5th among the world's highest TB burden countries and the 6th among countries with the highest burden of drug-resistant TB, including multi-drug resistant (MDR)-TB. However, very limited data are available on the genetic structure of *M. tuberculosis* strains circulating in this country.

Objectives: The objective of this study was to explore the genetic diversity of multidrug-resistant *M. tuberculosis* isolates from Pakistan with two different methodologies, i.e. spoligotyping and 24-loci MIRU-VNTR typing.

Materials and methods: The study included 130 MDR-TB isolates, recovered from as many patients from Pakistan, between January 2013 and June 2015. Conventional drug susceptibility testing was performed using the standard 1% proportion method on the Löwenstein-Jensen medium, as described elsewhere [1]. Spoligotyping was performed with a commercially available kit (Mapmygenome India Ltd., Madhapur, India) according to the manufacturer's protocol. MIRU-VNTR analysis was carried out at 24 loci, as described earlier [3]. Phylogenetic clades of *M. tuberculosis* were assigned according to signatures provided in the SITVIT database [4] online application.

Results: Spoligotypes were obtained for 127 (97.8%) isolates. Based on a SIT number in the SITVIT database, all isolates presented 53 different profiles split into 14 clusters (n = 88, 69.3%, 2-30 isolates per cluster) and 39 (30.7%) unique patterns. MIRU-VNTR typing identified 128 unique types (98.5%) and one cluster (n = 2, 1.5%). When spoligotyping and MIRU-VNTR typing was used in combination, only two, out of 130 isolates, clustered both in both methods, resulting in a clustering rate of 1.5%.

Upon phylogenetic analysis, 101 (77.7%) isolates were classified into 12 clades, with the most prevalent being CAS1_DELHI (n = 53, 41.7%) followed by T1 (n = 14, 11%) and BEIJING (n = 10, 7.8%). The remaining 9 families (CAS, MANU2, EAI5, T2, LAM10_CAM, H1, X1, H4 and CAS2) involved 24 (18.9%) isolates. Twenty-six (20.5%) isolates could not be assigned to any specific lineage.

Conclusions: This study provides a snapshot of the genetic diversity of *M. tuberculosis* strains circulating in Pakistan. The compactness of the drug resistant *M. tuberculosis* population structure was apparent, as three major lineages, i.e. CAS1_DELHI, T1, and BEIJING comprised more than half (60.6%) of the isolates studied. Furthermore, the exceptionally low clustering rate Suggest that recent transmission does not play an important role in the incidence of MDR-TB in Pakistan.

References:

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