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.

METHODS

The study included 127 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 [2]. 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

Based on a SIT number in the SITVIT database, 127 isolates presented 53 different profiles split into 14 clusters (*n* = 88, 69.3%, 2-29 isolates per cluster) and 39 (30.7%) unique patterns (**Fig. 1A**). Upon phylogenetic analysis, the most abundant families were CAS, T, and BEIJING, accounting for 62 (48.8%), 16 (12.6%) and 10 (7.8%) of the isolates, respectively. The remaining five lineages (MANU, EAI, LAM, H, and X) involved 13 (10.2%) isolates (**Fig. 1B**).
The discriminatory power of each VNTR locus (Hunter and Gaston discriminatory index, HGDI) is shown in **Figure 2**. The most distinguishing locus was MIRU 960 (HGDI = 0.78), whereas the lowest diversity was observed for MIRU 02 locus (HGDI = 0).
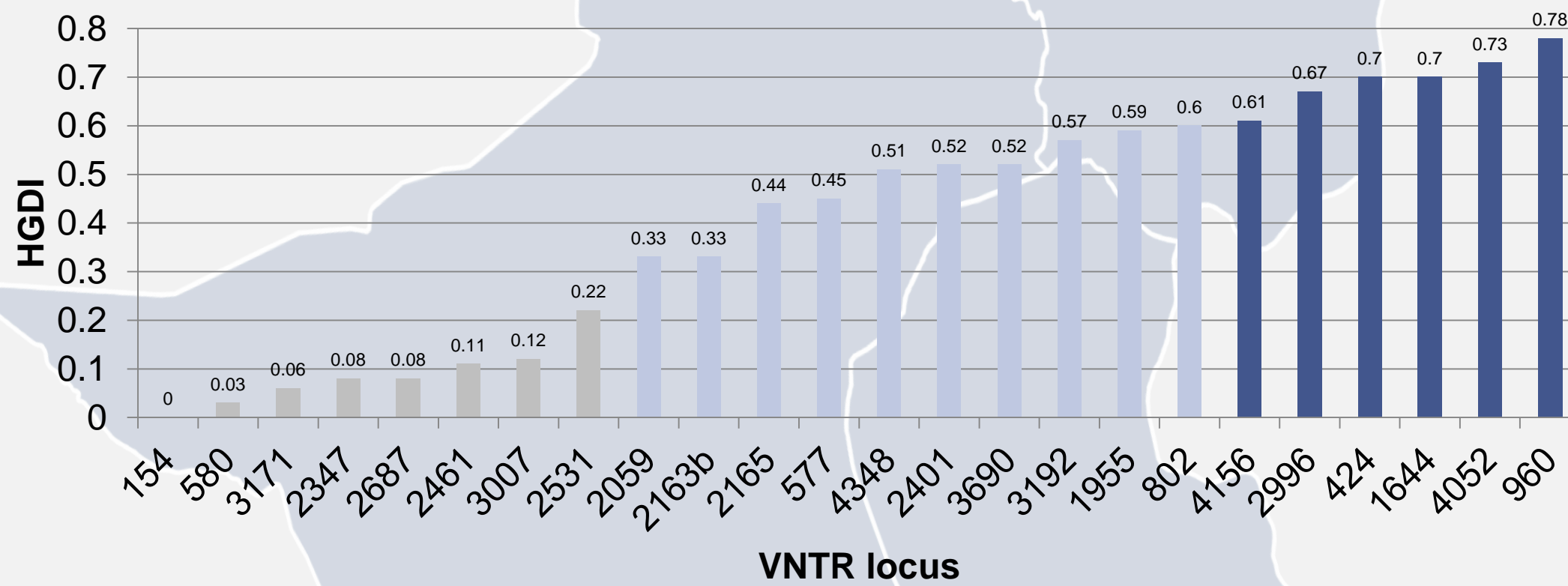

Figure 2. Discriminatory power of each MIRU-VNTR locus. Grey, sky blue and navy blue color denote poor (HGDI < 0.3), moderate (0.3 ≤ HGDI ≤ 0.6) or high (HGDI > 0.6) discriminatory power.

Based on the clustering results (MIRU-VNTR typing + spoligotyping), the transmission rate for MDR-TB cases under the study was calculated at 3.2%, 15% and 28.3% with a tolerance of none, single or double MIRU-VNTR locus variation between two DNA fingerprints, respectively (**Figure 3**).
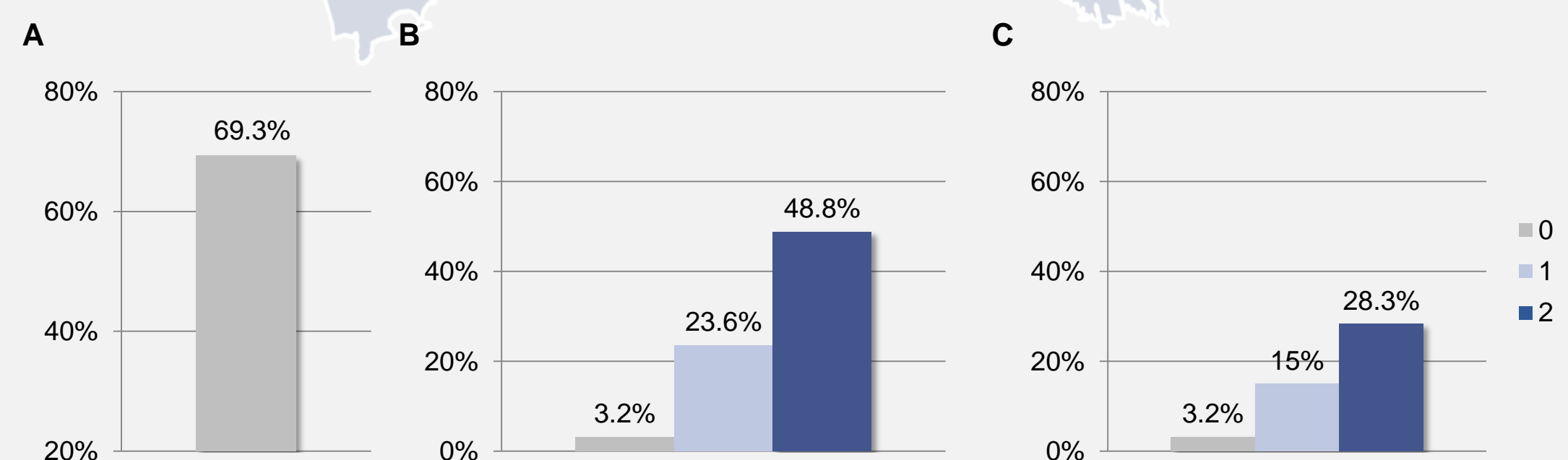

Figure 3. Clustering results for: **A**, spoligotyping, **B**, MIRU-VNTR typing and **C**, MIRU-VNTR typing + spoligotyping, with a tolerance of of 0, 1 or 2 locus differences between the MIRU-VNTR patterns.
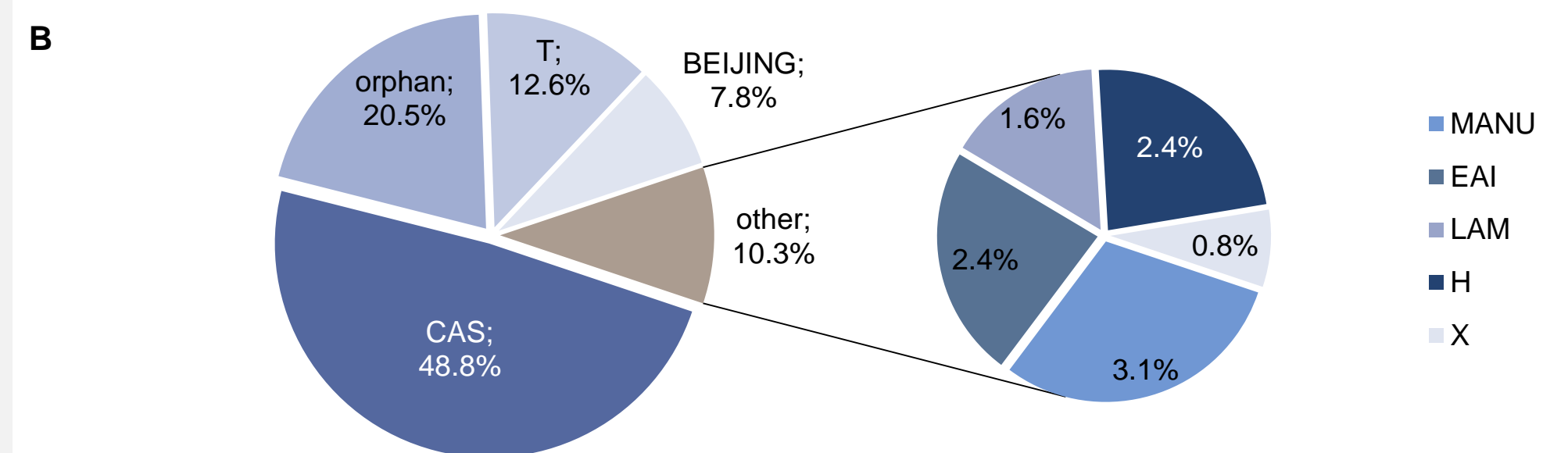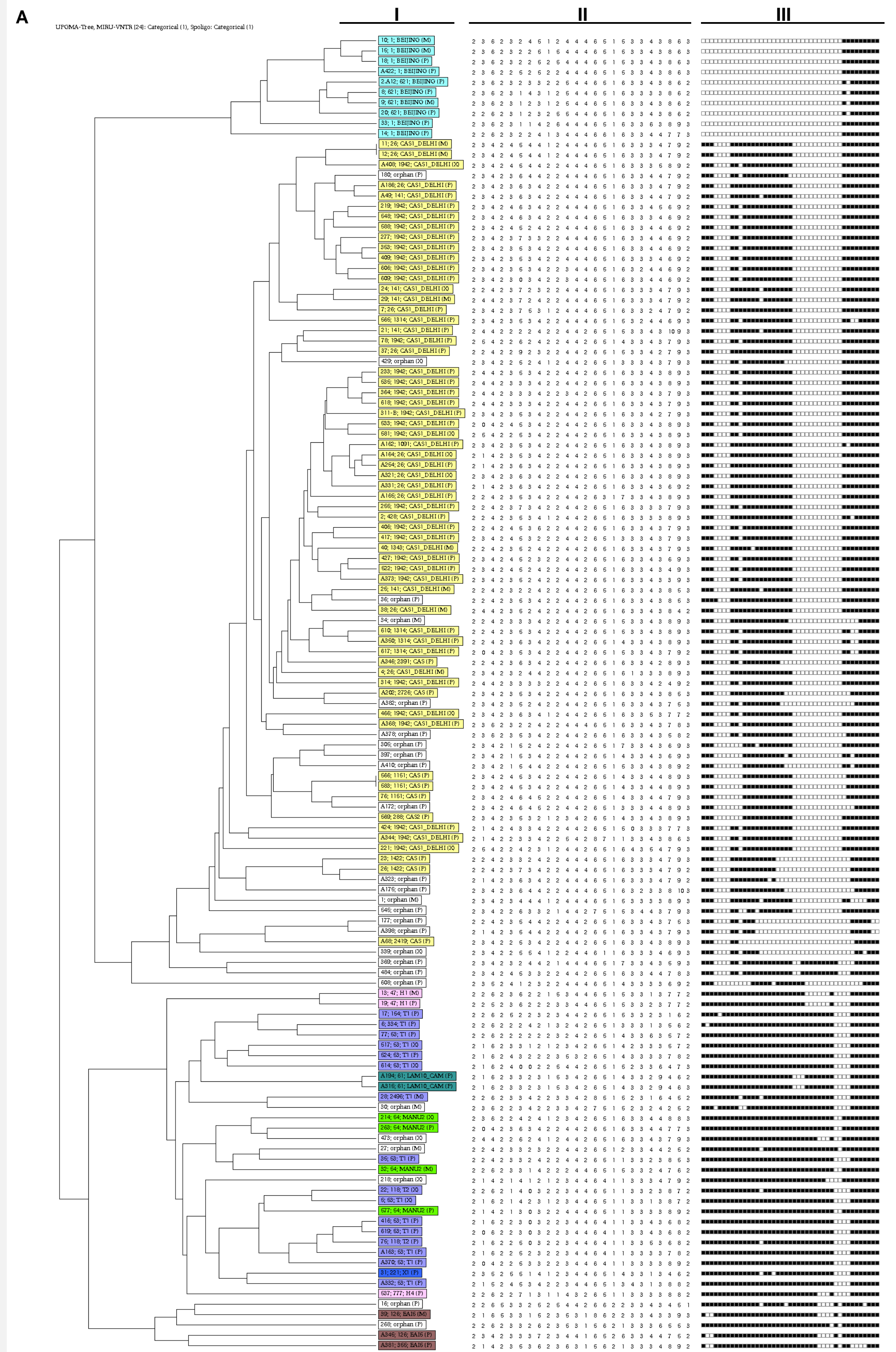

Figure 1. A. Genotyping results of the 127 *M. tuberculosis* isolates retrieved from as many TB patients in Pakistan. I. isolate ID; ST; subfamily; (susceptibility profile, i.e. M, MDR; P, pre-XDR; X, XDR) **II.** MIRU-VNTR typing pattern; **III.** spoligotyping pattern. Colors in the tree denote isolate families. **B. Prevalences of the families.**

CONCLUSIONS

- CAS, T, and BEIJING clades account for the majority of MDR-TB cases in Pakistan
- Up to a third of MDR-TB cases in Pakistan may originate from an ongoing transmission, according to the currently accepted molecular typing approach, involving spoligotyping and 24-locus MIRU-VNTR typing
- Using MIRU-VNTR typing 6 most polymorphic loci (i.e. 424, 960, 1644, 2996, 4052 and 4156) may be of particular importance for estimating relatedness of TB cases in Pakistan

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