

Genetic diversity of multidrug-resistant and drug-susceptible *Mycobacterium tuberculosis* isolates in Lithuania

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Background: Lithuania is among 27 high multidrug-resistant tuberculosis (MDR-TB) burden countries in the world. Importantly, multidrug resistance is detected in every tenth and every second TB cases among newly and previously treated patients, respectively.

The objective of this study was to explore the genetic diversity of MDR and drug-susceptible (DS) *M. tuberculosis* isolates from Lithuania with a combination of spoligotyping and 24-loci MIRU-VNTR-typing.

Methods: The study included 201 TB isolates. Included in this number were 101 MDR and 100 DS isolates, recovered from as many patients from Lithuania, between 2018 and 2021. The patients were mostly men ($n=154$; 76.2%) with the mean age of 49 ± 14.3 years. Conventional drug susceptibility testing was performed using BACTEC MGIT 960 System (BD, USA). Spoligotyping was carried out with a commercially available kit (Mapmygenome India Ltd., India) as per the manufacturer's instructions. MIRU-VNTR analysis was done at 24 loci, according to a standardized protocol. Phylogenetic clades of *M. tuberculosis* were assigned according to signatures provided in the SITVIT2 database (<http://www.pasteur-guadeloupe.fr:8081/SITVIT2/>).

Results: Spoligotyping yielded a total of 42 different profiles split into 21 clusters ($n=180$, 89.5%, 2-61 isolates per cluster) and 21 (10.4%) unique patterns. Most isolates belonged to the Beijing family ($n=66$; 32.8%), followed by T ($n=42$; 20.9%), Ural ($n=38$; 18.9%), LAM ($n=18$; 9%), and H ($n=17$; 8.5%) clades. Twenty (9.9%) isolates were designated as Unknown/Not defined. Among MDR *M. tuberculosis* isolates, the most abundant were Beijing ($n=57$; 56.4%), Ural ($n=25$; 24.7%) and T ($n=10$; 9.9%) lineages. Ninety-seven profiles were produced with MIRU-VNTR typing. These were 33 clusters ($n=137$, 68.2%, 2-27 isolates per cluster) and 64 singletons. A combined analysis based on spoligotyping and MIRU-VNTR typing revealed 31 clusters ($n=127$, 63.1%, 2-27 isolates per cluster).

The overall recent transmission rate was calculated at 63% and 30% for MDR and DS isolates, respectively.

Conclusions: Whereas the Beijing family was the most prevalent (56.4%) among MDR TB cases, it accounted for only 9% of DS isolates. Over half of MDR-TB cases, twice as much as in case of DS-TB in Lithuania might have originated from an ongoing transmission.