

Dimension of the transmission of drug-resistant *Mycobacterium tuberculosis* lineage two strains in Ukraine on a nationwide level

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Background. Ukraine remains one of the highest-burden settings for rifampicin-resistant and multidrug-resistant tuberculosis (TB) in the WHO European Region. Armed conflict, internal displacement, and disruption of TB services may facilitate the spread of highly transmissible *Mycobacterium tuberculosis* complex (Mtb) Lineage 2 (L2, Beijing) strains and accelerate resistance evolution. We aimed to define the impact of the transmission of MDR L2 Mtb clones on the drug resistant TB epidemic in Ukraine based on a countrywide molecular epidemiological investigation based on whole genome sequencing (WGS).

Materials and methods. We performed a nationwide multicenter retrospective analysis of prospectively collected clinical and microbiological data from patients with TB enrolled across 18 Ukrainian oblasts between December 2019 and November 2023. Mtb genomes were sequenced using paired-end Illumina technology at the Research Center Borstel, Borstel, Germany, following previously described protocols. WGS was successfully completed for 4,162 baseline Mtb strains after exclusion of low-quality and mixed-strain samples. Phylogenetic classification and genomic resistance prediction were performed with the MTBSeq pipeline. Genomic clustering was inferred by core-genome multilocus sequence typing (cgMLST; 2,891 loci), with <5 allelic differences used to define recent transmission clusters.

Results. Among 4,162 sequenced isolates, 3,112 (74.8 %) belonged to the drug-resistant cohort, including 72 rifampicin-resistant (RR), 1,939 multidrug-resistant (MDR), 1,023 pre-extensively drug-resistant (pre-XDR), 78 extensively drug-resistant (XDR) isolates and 1,050 (25.2 %) belonged to the control group with 701 drug-susceptible and 349 – non-MDR Mtb strains. L2 strains accounted for 2,606/3,112 (83.7 %) RR/MDR strains, whereas Lineage 4 strains predominated in the control group (714/1,050; 68.0 %).

Overall, 2,015/4,162 isolates (48.4 %) formed genomic clusters. Clustering was significantly more frequent in the drug-resistant cohort than in controls (55.0 % vs 28.9 %; $p < 0.001$) and was highest among MDR and pre-XDR strains (55.7 % and 56.0 %, respectively). The three largest clusters comprised 671/3,112 (21.6 %) of all drug-resistant cases and were composed exclusively of L2 strains belonging to previously described MDR clones. Cluster 1 ($n=307$) corresponded to the Ukraine outbreak clone and was enriched for pre-XDR/XDR disease (55.7 %). Cluster 2 ($n=296$) represented the Europe/Russian W148 outbreak clone and was predominantly MDR (74.0 %). Cluster 3 ($n=76$) corresponded to the Central Asia Outbreak (CAO) clone and was also mainly MDR (76.3 %). These clusters showed highly conserved high-confidence resistance mutation patterns, consistent with long-standing transmission of successful resistant genotypes rather than repeated independent resistance acquisition. Overall, strains of the three major L2 clones (Ukraine outbreak, W148, and CAO) represent 2,079/3,112 (66.8 %) of all RR/MDR strains, pointing to longitudinal clonal expansion of highly resistant Mtb strains in the country.

Fluoroquinolone resistance remained substantial in the major clusters (258/679; 38 %), especially Cluster 1 (171/307; 55.7 %), whereas resistance to bedaquiline and linezolid was uncommon and largely restricted to Clusters 1 and 2.

Conclusions. Ukraine's drug-resistant TB epidemic is driven predominantly by recent and sustained transmission of highly clonal L2 strains, especially the Europe/Russian W148, Ukraine outbreak, and CAO clones. Genomic clustering shows that MDR and pre-XDR disease are maintained by successful transmission chains with fixed resistance-conferring variants rather than solely by *de novo* resistance emergence. Continued WGS-based surveillance is essential to detect expansion of pre-XDR/XDR clusters and to monitor emerging resistance to new core drugs.