Drug-resistance profiles, population structure, genomic clustering, and temporal trends in drug resistance among *Mycobacterium tuberculosis* complex isolates in Ukraine, 2019–2023: a multicentre cohort study

Dmytro Butov,[a,b,] Tetiana Butova,[c] Valerii Miasoiedov,[d] Yurii Feshchenko,[e] Oksana Nakonechna,[f] Mykhailo Kuzhko,[e] Alex Rosenthal,[g] Alina Grinev,[g] Drew Hoppes,[g] Julia Kilmnick,[g] Daria Levandovska,[h] Anastasiia Hryhorieva,[h] Vitalii Vekshyn,[i] Liliya Abramova,[i] Stefan Niemann,[b,j,k,l,m] and Viola Dreyer,[b,j,m] Ukrainian TB Portal Collaborators*

[a]Department of Infectious Diseases, Childhood Infectious Diseases and Phthisiology, Kharkiv National Medical University, Kharkiv, Ukraine
[b]Research Center Borstel–Leibniz Lung Center, Molecular and Experimental Mycobacteriology Group, Borstel, Germany
[c]Outpatient Department, Merefa Central District Hospital, Merefa, Ukraine
[d]Department of Biology, Kharkiv National Medical University, Kharkiv, Ukraine
[e]National Scientific Center of Phthisiatry, Pulmonology and Allergology named after F. G. Yanovskyi, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
[f]Department of Biochemistry, Kharkiv National Medical University, Kharkiv, Ukraine
[g]Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA
[h]Cherkasy Regional Tuberculosis Dispensary, Cherkasy, Ukraine
[i]Central Scientific and Research Laboratory, Kharkiv National Medical University, Kharkiv, Ukraine
[j]German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany
[k]EPHE, PSL University, Paris, France
[l]Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France

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Summary

Background Ukraine has a high burden of rifampicin-resistant (RR) tuberculosis (TB). We characterised whole-genome sequencing (WGS)-inferred drug-resistance (DR) profiles, population structure, genomic clustering, and temporal trends in DR among *Mycobacterium tuberculosis* complex (Mtbc) isolates during 2019–2023, amid the COVID-19 pandemic, large-scale displacement, health-system disruptions, and changes in DR-TB-policy.

Methods In this multicentre cohort study, we analysed WGS data from pretreatment Mtbc isolates collected across 18 of 24 Ukrainian regions. WGS enabled phylogenetic classification, resistance prediction, clustering. Clinical data were prospectively collected.

Findings WGS was completed for 4162 Mtbc-isolates, of which 3112/4162 (74.8%) were at least RR. Among 3040 multidrug-resistant (MDR) isolates, 1101/3040 (36.2%) had WGS-inferred fluoroquinolone (FQ) resistance and 78/3040 (2.6%) were classified as extensively DR-TB (XDR-TB); 57 had bedaquiline resistance, 32 had linezolid resistance, and 11 had resistance to both drugs. Lineage 2 (L2) accounted for 1608/1939 (82.9%) of MDR, 891/1023 (87.1%) of pre-XDR, and 70/78 (89.7%) of XDR isolates. Overall, 1712/3112 (55.0%) of DR-isolates formed genomic clusters; the three largest comprised 671/3112 (21.6%) of DR-TB cases and consisted exclusively of L2-isolates. WGS-inferred FQ resistance declined from 276/675 (40.9%) to 216/767 (28.2%) during 2019–2023 (absolute difference −12.7 percentage points [95% CI −17.5 to −7.8]; FDR-adjusted $q = 4.1 \times 10^{-7}$).

Interpretation DR-TB in this cohort was characterised by the predominance of genomically clustered L2 strains and frequent FQ resistance. Although WGS-inferred bedaquiline resistance and XDR-TB were uncommon, combined FQ, bedaquiline, and linezolid resistance warrants continued genomic surveillance. Temporal trends may reflect concurrent epidemiological, diagnostic, and health-system changes.

*Corresponding author. Molecular and Experimental Mycobacteriology Group, Research Center Borstel–Leibniz Lung Center, Parkallee 1, 23845, Borstel, Germany.
E-mail address: dbutov@fz-borstel.de (D. Butov).
[m]These authors contributed equally as last authors.

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Keywords: Tuberculosis; Drug-resistant tuberculosis; *Mycobacterium tuberculosis*; Whole-genome sequencing; Lineage 2; mSTRs; BPaL/M; Multidrug-resistant TB; Ukraine and war

Research in context

Evidence before this study

We searched PubMed, Scopus, and the electronic resources of the Vernadsky National Library of Ukraine from database inception to August 11, 2026, without language restrictions, using combinations of the terms "tuberculosis (TB)", "*Mycobacterium tuberculosis*", "Ukraine", "whole-genome sequencing (WGS)", "drug resistance", "MDR", "XDR", "Beijing", "Lineage 2", "fluoroquinolone", "bedaquiline", "BPaL", "BPaLM", "war", "conflict", and "displacement". We also reviewed WHO reports, official documents and orders of the Ministry of Health of Ukraine, and reference lists of relevant articles. We included original studies reporting clinical, epidemiological, drug-resistance (DR), molecular, or genomic data on tuberculosis in Ukraine or among people originating from Ukraine, and excluded studies without Ukrainian data, non-tuberculous mycobacterial studies, and non-informative case reports. Previous evidence documented a high burden of multidrug- and rifampicin-resistant tuberculosis and major Lineage 2 (Beijing) outbreak clones, but was generally limited by geography, sample size, heterogeneous methods, or focus on specific clinical questions. We identified no relevant meta-analysis providing a pooled estimate, and pooling was not considered appropriate because of substantial between-study heterogeneity. We found no large multicentre Ukrainian genomic cohort spanning the periods before and after the 2022 invasion that integrated pretreatment WGS with prospectively collected clinical data.

Added value of this study

This study integrates pretreatment WGS and prospectively collected clinical data from 4162 patients across 18 of 24 Ukrainian regions between December 2019 and November 2023. DR-TB was dominated by Lineage 2 strains and extensive genomic clustering. Fluoroquinolone resistance declined over time, whereas WGS-inferred bedaquiline and linezolid resistance remained uncommon. The study also characterises resistance relevant to contemporary shorter all-oral regimens and identifies associations between Lineage 2, drug resistance, and genomic clustering.

Implications of all the available evidence

These findings support sustained genomic surveillance of DR-TB in Ukraine and neighbouring countries, particularly for dominant Lineage 2 clones and resistance to fluoroquinolones, bedaquiline, and linezolid. WGS can complement routine diagnostics and support monitoring of resistant clone circulation. Temporal declines in fluoroquinolone resistance should be interpreted cautiously because they coincided with changes in sampling, population displacement, diagnostic capacity, treatment access, tuberculosis policy, and the COVID-19 pandemic. Future surveillance should integrate genomic data with phenotypic susceptibility testing, treatment exposure, and detailed epidemiological information.

Introduction

Ukraine has one of the highest burdens of rifampicin-resistant (RR) and multidrug-resistant (MDR) tuberculosis (TB) in the World Health Organization (WHO) European Region.[1,2] Since 2014, particularly after the 2022 invasion, population displacement, damaged health infrastructure, and disrupted diagnostic and treatment services have complicated TB control.[3] A separate 2022 countrywide survey of 21 accessible regional TB centres found increased out-of-region care (1.3%–7.0%) but generally preserved access to Xpert MTB/RIF (20/21 centres) and fluoroquinolone drug-susceptibility testing (DST, 21/21), despite selected diagnostic shortages.[3] These aggregate data were not linked to participants in the present cohort.

Efforts to manage drug-resistant (DR)-TB have increasingly incorporated novel treatment strategies, including WHO-recommended shorter and individualised regimens, which were implemented in Ukraine starting in 2020.[4,5] This scale-up coincided with the COVID-19 pandemic and the 2022 invasion, periods associated with changes in TB service delivery, diagnostic access, population mobility, and regional participation.[3,6]

Whole-genome sequencing (WGS) enables high-resolution assessment of population structure, transmission, and resistance evolution of *Mycobacterium tuberculosis* complex (Mtbc) isolates.[7] Particular attention has been given to isolates of Lineage 2 (L2, Beijing), which predominate across Eastern Europe and have been associated with enhanced virulence,

increased transmissibility, and a higher likelihood of acquiring drug resistance mutations.[8] Conversely, Lineage 4 (L4, Euro-American) isolates exhibit broader genetic diversity and lower mutation rates but may possess alternative mechanisms of drug tolerance that contribute to persistence under chemotherapy.[9]

In a previous small-scale study of Mtbc isolates collected in 2015, we showed that the MDR-TB epidemic in Ukraine, defined by resistance to at least isoniazid and rifampicin, was largely driven by L2 isolates, particularly the Europe/Russia W148 outbreak (W148), Central Asia outbreak, and the Ukraine outbreak clones.[10]

Given the convergence of ongoing war, population displacement, high baseline MDR-TB burden, dominant genomically clustered L2 clones, and the programmatic transition to modified shorter treatment regimens (mSTRs) and BPaL/M (bedaquiline [BDQ], pretomanid, and linezolid [LZD], with or without moxifloxacin), robust genomic surveillance is needed to characterise resistance patterns and transmission dynamics in Ukraine. In this multicente prospective cohort study, we combined clinical, microbiological, and WGS data from 4828 TB patients from 18/24 regions of Ukraine between 2019 and 2023. We aimed to describe WGS-inferred drug resistance, Mtbc population structure, genomic clustering, and temporal trends in DR-TB during a period of major health-system disruption and treatment–regimen transition.

Methods

Study design and geographic coverage

This was a multicentre observational genomic surveillance cohort study using routinely collected clinical and microbiological data from patients with culture-confirmed TB. The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines; a completed STROBE checklist is provided in the Supplementary Material.

At participating centres, rifampicin resistance was assessed at presentation using either Xpert MTB/RIF or Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA), together with culture-based phenotypic DST for first-line drugs. Consecutive patients with rifampicin resistance and a pretreatment culture-positive Mtbc isolate were eligible for DR-TB enrolment. Final RR, MDR, pre-extensively drug-resistant TB (pre-XDR, MDR plus further resistance against one fluoroquinolone [FQ]), and extensively DR (XDR, pre-XDR plus additional resistance against one WHO Group A drug, such as BDQ or LZD, or both) categories were assigned from WGS-inferred resistance profiles according to WHO definitions. The comparator cohort was assembled using region-specific consecutive sampling. Each participating centre was asked to contribute a prespecified number of eligible patients with non-MDR or drug-susceptible TB and enrolled consecutive eligible patients until the site-specific target was reached (Fig. 1). This strategy provided a contemporaneous genomic comparator. The study was intentionally enriched for DR-TB; neither cohort was population based, and the observed group proportions should not be interpreted as regional or national prevalence estimates. Patients with pulmonary, extrapulmonary, or combined pulmonary and extrapulmonary TB were eligible if a culture-positive Mtbc isolate obtained before treatment initiation was available for WGS. For each patient, only one baseline pre-treatment isolate from the current tuberculosis episode was included; follow-up isolates collected during or after treatment were excluded from this analysis. Baseline TB case-registration categories were defined according to WHO recording and reporting definitions and reflected previous TB treatment history. New cases were defined as patients who had never received anti-tuberculosis treatment or had received treatment for less than 1 month. Recurrent TB was defined as a new episode in a patient whose most recent previous treatment episode ended in cure or treatment completion. Treatment after failure and treatment after loss to follow-up indicated that the most recent previous treatment episode ended in failure or loss to follow-up, respectively.[11]

Patients were recruited from 18 of 24 Ukrainian regions between December 2019 and November 2023. According to official national surveillance data, treatment was initiated for 18,163 MDR/RR-TB cases between January 1, 2020, and December 31, 2023.[12] The 3112 patients in the study DR-TB cohort corresponded to approximately 17.1% of this national total. Because the recruitment period (December 2019–November 2023) did not exactly coincide with the national reporting period, this percentage represents an approximate measure of sampling coverage rather than prevalence. Participating regions changed during the study after the start of the war in Ukraine on February 24, 2022, when some TB facilities were destroyed or became inaccessible. During the initial study phase, data were collected from 15 regions: Chernihiv, Donetsk, Dnipropetrovsk, Ivano-Frankivsk, Kharkiv, Kherson, Kyiv City, Mykolaiv, Sumy, Ternopil, Vinnytsia, Volyn, Zakarpattia, Zaporizhzhia, and Zhytomyr. After February 24, 2022, Chernihiv, Donetsk, and Kherson could no longer contribute data; Cherkasy, Khmelnytskyi, and Odesa were therefore included during 2022–2023 to maintain multicentre geographic coverage (Fig. 2). To assess changes in regional sampling over time, we summarised the number and proportion of successfully sequenced Mtbc isolates by participating region and study interval (Table S1). Year-by-region denominators could not be determined because samples that failed sequencing quality control were excluded before linkage to the TB Portals

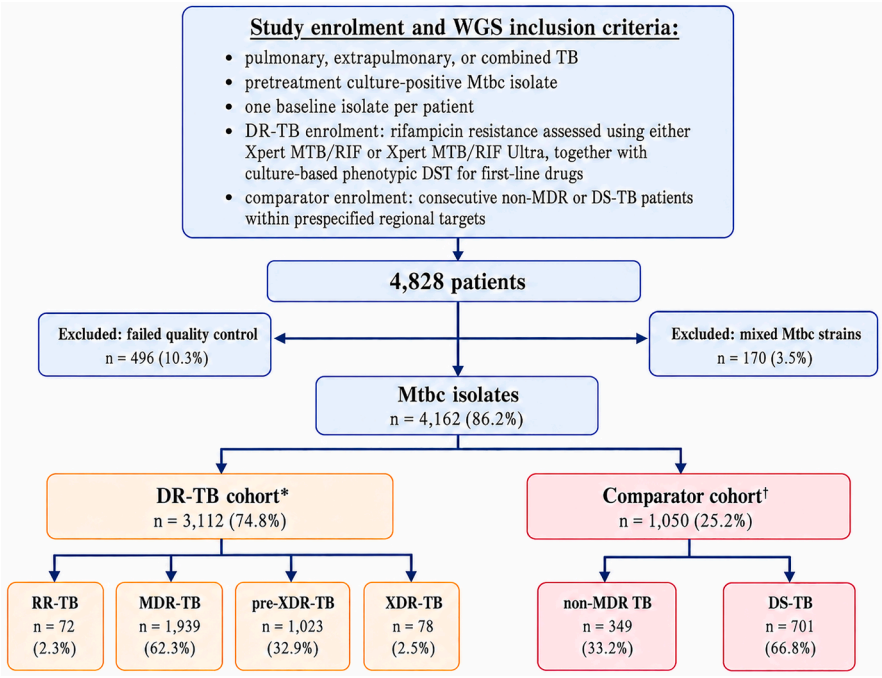


Fig. 1: **Flowchart of patient selection and group allocation based on whole-genome sequencing of *Mycobacterium tuberculosis* complex (Mtbc) isolates.** Patients were recruited from 18 regions of Ukraine between December 2019 and November 2023. The flowchart shows the number of patients screened, excluded after WGS quality control or because of mixed Mtbc infection, and included in the drug-resistant tuberculosis cohort or comparator cohort. Drug-resistance categories were assigned from WGS-inferred resistance using the WHO mutation catalogue (second edition, 2023) and a previously validated resistance-mutation catalogue. DR = drug-resistant; DS = drug-susceptible; Mtbc = *Mycobacterium tuberculosis* complex; MDR = multidrug-resistant; pre-XDR = pre–extensively drug-resistant; RR = rifampicin-resistant; TB = tuberculosis; WGS = whole-genome sequencing; XDR = extensively drug-resistant. * The DR-TB cohort comprised consecutive eligible patients with RR, MDR, pre-XDR, or XDR TB enrolled at participating centres. † Patients with non-MDR or drug-susceptible TB were consecutively enrolled within prespecified regional targets during the same study period and formed the comparator cohort (Visual layout generated with ChatGPT, OpenAI; image generation feature).

database. Regions were classified as consistently sampled if they contributed isolates across all four study intervals, as early participating if they contributed isolates during the initial study intervals but not the final interval, and as later participating if they contributed isolates only after the initial study interval. This classification was used to guide sensitivity analyses restricted to consistently sampled regions.

Clinical data were obtained from the Ukrainian component of the NIAID TB Portals programme, a standardised international repository linking de-identified demographic, clinical, microbiological, treatment, and outcome data with pathogen genomic data.[13] During database finalisation, potentially missing, unknown, inconsistent, or discrepant values in variables used for the manuscript were queried with regional physicians and verified against source medical records. Potentially missing or unknown values were not coded as absence. After verification, missing data remained only for radiographic findings in 195 participants; no statistical imputation was performed, and analyses of radiographic features used available-case denominators.

DNA extraction and whole-genome sequencing

Clinical isolates were cultured on Löwenstein–Jensen or in Mycobacteria Growth Indicator Tube (MGIT) systems, and genomic mycobacterial DNA was extracted from positive cultures using the IndiSpin Pathogen Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany; cat. no. SP54106) according to the manufacturer's instructions. Whole-genome sequencing of Mtbc isolates was performed using paired-end Illumina sequencing technology at the Research Center Borstel (Borstel, Germany).[14] DNA concentration and quality were assessed at the Research Center Borstel before library preparation. Samples with insufficient DNA or sequencing quality for reliable genome analysis, contamination, or mixed Mtbc infection were excluded.

Analysis of whole-genome sequencing data

Whole-genome sequencing data were analysed using the MTBseq pipeline version 1.1.0 with default parameters. Phylogenetic lineages and major sublineages were inferred from lineage-defining SNPs based on Coll et al. and Merker et al.; previously described L2 (Beijing)

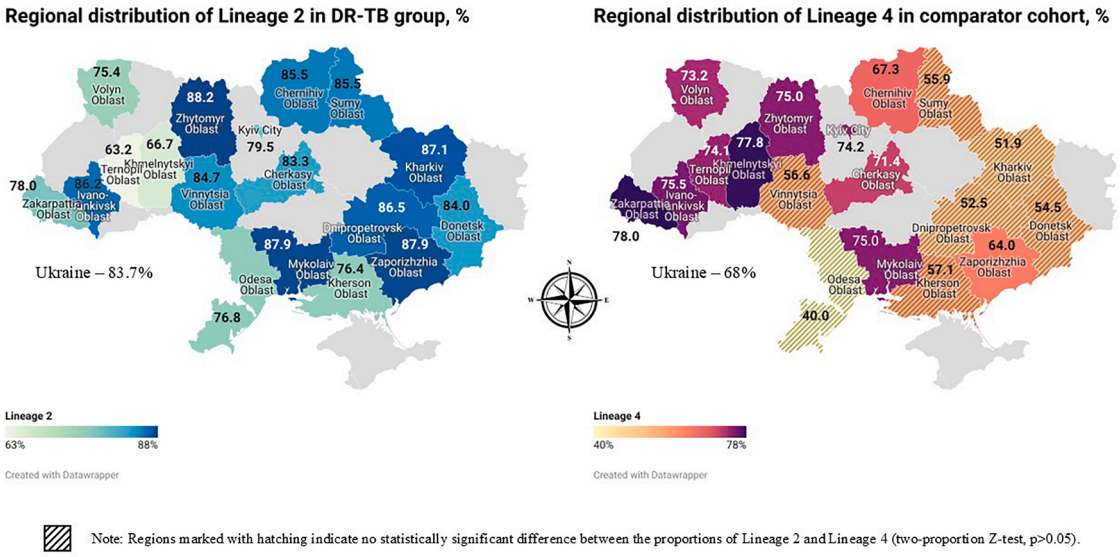


Fig. 2: **Regional dominance of *M. tuberculosis* Lineages 2 and 4 in drug-resistant and comparator cohorts across participating regions of Ukraine.** Maps show the regional distribution of statistically significant lineage dominance among 18 Ukrainian regions included in the study, based on whole-genome sequencing data collected between December 2019 and November 2023. The left panel shows the proportion of Lineage 2 among patients in the DR-TB cohort; the right panel shows the proportion of Lineage 4 among patients in the control cohort. Only regions with statistically significant lineage predominance are colour shaded. Hatched regions indicate no statistically significant difference in lineage frequency between cohorts; grey regions were not included in the study. Statistical significance was assessed with a two-proportion z-test. DR-TB = drug-resistant tuberculosis. Maps were created with Datawrapper (Datawrapper GmbH, Berlin, Germany).

clades and outbreak clones, including the Central Asia clade, Central Asia outbreak clone, W148-outbreak clone, and Ukraine outbreak clone, were classified separately.[8,15] Resistance-conferring mutations and WGS-inferred drug-resistance profiles were interpreted using the WHO catalogue of mutations in Mtbc and their association with drug resistance (second edition)[16] and a previously validated resistance-mutation catalogue.[14] Low-frequency resistance-associated variants were detected at allele frequencies ≥5%, provided that sequencing-quality criteria were met, including support by forward and reverse reads and at least one read with a Phred quality score >20. Unclassified or unknown variants (not classified resistance-associated in either the WHO catalogue or our validated resistance-mutation catalogue) were not considered evidence of drug resistance. For cluster analysis we used the core-genome multilocus sequence typing (cgMLST) approach from MBioSEQ Ridom Typer,[17] which includes 2891 loci. Isolates with ≤5 cgMLST allelic differences were considered to belong to the same genomic cluster, indicating close genetic relatedness following Meehan et al.[18]

Treatment outcomes

Treatment outcomes were classified according to WHO definitions and national TB guidelines of Ukraine. Outcomes were dichotomised as successful (cured or treatment completed) and unsuccessful (death, failure, or lost to follow-up).[13]

Statistical analysis

Continuous variables are summarised as mean (SE) and compared with Welch's t-test. Categorical variables were compared with Pearson's χ^2 test, two-proportion z-tests, Fisher's exact test, or odds ratios with 95% CIs, as appropriate. Distributions of continuous variables were inspected visually, and Welch's t-test was used because equal variances were not assumed. For categorical comparisons, expected cell counts were assessed, and Fisher's exact test was used instead of χ^2-based tests when cell counts were sparse. Multivariable logistic regression models were fitted for selected key binary outcomes and adjusted for lineage, age, sex, baseline TB case-registration category, HIV infection, homelessness, previous incarceration, study interval, and region. The prespecified binary outcomes were RR/MDR/pre-XDR/XDR TB status, WGS-inferred fluoroquinolone resistance, genomic clustering, and treatment success. RR/MDR/pre-XDR/XDR TB status and genomic clustering were modelled among all successfully sequenced L2/L4 isolates, whereas FQ resistance and treatment success were modelled among patients in the DR-TB cohort. Fluoroquinolone resistance was defined as WGS-inferred resistance to moxifloxacin or levofloxacin. Genomic clustering was defined as membership in a cgMLST cluster at a threshold of ≤5 allelic differences. Treatment success was defined as cure or treatment completion; all other recorded outcomes were coded as not successful. Region was included as a fixed effect.

BDQ and LZD resistance were not modelled because event numbers were small and adjusted estimates would have been unstable. Temporal trends were assessed with χ^2 tests for trend across four annual intervals. Regional lineage dominance was compared with two-proportion z-tests. Analyses were done with STATISTICA version 8.0, Microsoft Excel, GraphPad QuickCalcs, MedCalc, and jamovi version 2.7. For temporal trend analyses, the Benjamini–Hochberg procedure was applied separately to each family of related tests. Raw two-sided p values and FDR-adjusted q values are reported; $q \leq 0.05$ was considered statistically significant.

Calendar period was analysed descriptively because longitudinal service-delivery and individual-level regimen data were unavailable, precluding estimation of the effects of the invasion, displacement, health-system disruption, or specific regimens.

Ethics

The study protocol was approved by the Ethics Committee of Kharkiv National Medical University, Kharkiv, Ukraine (Approval No.1, February 5, 2020).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Participants

Of 4828 enrolled patients, 4162/4828 (86.2%) had one successfully sequenced Mtbc isolate after exclusion of 496 samples with low DNA concentration or contamination and 170 with mixed Mtbc infection (Fig. 1).

For further analysis, patients were divided into two cohorts: a DR-TB cohort comprising 3112/4162 (74.8%) patients with RR, MDR, pre-XDR, or XDR TB, and a comparator cohort comprising 1050/4162 (25.2%) patients with non-MDR TB (mono- or polyresistant TB without rifampicin resistance) or drug-susceptible TB, enrolled consecutively within prespecified regional targets during the same study period (Fig. 1).

Compared with the comparator cohort, patients in the DR-TB cohort were younger and more often male; recurrent TB, treatment after failure, homelessness, drug use, previous incarceration, HIV infection, anaemia, and hepatitis B or C were also more frequently recorded (Table 1).

Drug resistance and population structure

Within the 3112 Mtbc isolates of the DR-TB group, 72/3112 (2.3%) were classified as RR, 1939/3112 (62.3%) as MDR, 1023/3112 (32.9%) as pre-XDR, and 78/3112 (2.5%) as XDR-TB (Fig. 1).

Among the 78 XDR Mtbc isolates, 57 harboured variants associated with WGS-inferred BDQ resistance and 32 harboured variants associated with LZD resistance; 11 carried variants associated with combined WGS-inferred resistance to FQs, BDQ, and LZD. Beyond the XDR subset, WGS-inferred BDQ resistance was also identified in four non-MDR, one RR, and 28 MDR isolates (90 BDQ resistant isolates overall) and was predominantly associated with loss-of-function variation in *Rv0678,* most frequently the frameshift insertions 192_ins_g (20/85; 23.5%) and 141_ins_c (19/85; 22.3%, Table S2). As *Rv0678* variants are known to confer cross-resistance to clofazimine, these findings underscore the need for genomic surveillance to monitor the evolution and transmission of BDQ resistance. Among the 57 XDR-TB isolates with WGS-inferred BDQ resistance, seven 7/57 (12.3%) carried *atpE* variants associated with BDQ resistance, including six L2 isolates and one L4 isolate. The six L2 *atpE*-mutant isolates were distributed across several L2 clades/outbreak clones, with only two belonging to the same cgMLST-defined cluster (Table S3). Comparative analysis of Ukrainian *atpE*-mutant isolates and publicly available genomes from Russia showed a minimum pairwise distance of 28 SNPs between the two datasets, providing no evidence of close clonal relatedness (Figure S1). Regional stratification showed heterogeneity in WGS-inferred resistance patterns; FQ resistance approached 50% in the DR-TB cohort in some regions and was lower in others (Figure S2).

Among the 4162 sequenced Mtbc isolates, 2940/4162 (70.6%) were assigned to L2, 1220/4162 (29.3%) to L4, and one isolate each to *M. bovis* and L3 (Delhi/CAS lineage). Within L2, 2937/2940 (99.9%) isolates were further classified as L2.2.1 and 3/2940 isolates (0.1%) as L2.2.2. Lineage 4 isolates were more diverse: 434/1220 isolates (35.6%) were assigned to the LAM clade (L4.3.3, L4.3.4.x), 310/1220 (25.4%) to H37Rv-like (L4.7, L4.8, L4.9), 222/1220 (18.2%) to the Ural (L4.2.1), 210/1220 (17.2%) to Haarlem (L4.1.2, L4.1.2.1), 27/1220 (2.2%) to Ghana (L4.1), 7/1220 (0.6%) to S-type (L4.4.1, L4.4.1.1), 2/1220 (0.2%) to X-type (L4.1.1.2, L4.1.1.3), 2/1220 (0.2%) to New1 (L4.5) and 6/1220 (0.5%) were Euro-American not further defined (Fig. 3, Table 2).

Among the DR-TB cohort, 2606/3112 (83.7%) were L2 isolates, whereas 506/1050 (16.3%) belonged to L4 (absolute difference 51.9 percentage points [95% CI 48.8–54.9]; $p < 0.001$). In contrast, in the control cohort, L4 isolates represent 714/1050 (68.0%), whereas 334/1050 (31.8%) were L2 ($p < 0.001$). In general, L2 isolates represented the majority of isolates in all DR categories: 1608/1939 (82.9%) of MDR isolates, 891/1023 (87.1%) of pre-XDR isolates, and 70/78 (89.7%) of XDR isolates (Table 2, Table S4). In multivariable logistic regression models adjusted for demographic, clinical, social, temporal, and regional factors, L2 remained significantly associated with RR/MDR/pre-XDR/XDR status, FQ resistance, and genomic clustering. Recurrent TB (aOR 1.6, 95% CI 1.4–1.9) and treatment after failure (aOR

Characteristic	Drug-resistant TB no. (%)	Control group no. (%)	Total no. (%)	p value	OR (95% CI)[b]
	3112 (100)	1050 (100)	4162 (100)		
Age: years[c]	43.5 ± 0.2	46.5 ± 0.4	44.3 ± 0.2	t(1544.68) = −6.35; p = 2.9×10^{-10}	
Distribution–no. (%):					
<15 years	6 (0.2)	3 (0.3)	9 (0.2)	0.575	0.7 (0.2–2.7)
15–24 years	89 (2.9)	34 (3.2)	123 (3.0)	0.531	0.9 (0.6–1.3)
25–34 years	581 (18.7)	157 (15.0)	738 (17.7)	0.006	1.3 (1.1–1.6)
35–44 years	1095 (35.2)	305 (29.0)	1400 (33.6)	2.7×10^{-4}	1.3 (1.1–1.5)
45–54 years	802 (25.7)	283 (27.0)	1085 (26.1)	0.451	0.9 (0.8–1.1)
55–64 years	417 (13.4)	169 (16.1)	586 (14.1)	0.03	0.8 (0.7–1.0)
≥65 years	122 (3.9)	99 (9.4)	221 (5.3)	5.9×10^{-12}	0.4 (0.3–0.5)
Male–no. (%)	2486 (79.9)	806 (76.8)	3292 (79.1)	0.031	1.2 (1.0–1.4)
Case©–no. (%)					
New	1816 (58.4)	827 (78.7)	2643 (63.5)	1.6×10^{-32}	0.4 (0.3–0.4)
Recurrent	832 (26.7)	154 (14.7)	986 (23.7)	1.8×10^{-15}	2.1 (1.8–2.6)
Treatment after failure	389 (12.5)	38 (3.6)	427 (10.3)	2.4×10^{-16}	3.8 (2.7–5.3)
Treatment after loss to follow-up	75 (2.4)	31 (3.0)	106 (2.5)	0.335	0.8 (0.5–1.2)
Tobacco use–no. (%)[a]	2329 (74.8)	646 (61.5)	2975 (71.5)	1.4×10^{-16}	1.9 (1.6–2.2)
Homelessness–no. (%)[a]	145 (4.7)	11 (1.0)	156 (3.7)	9.9×10^{-8}	4.9 (2.6–9.2)
Alcohol misuse–no. (%)[a]	1303 (41.9)	433 (41.2)	1736 (41.7)	0.719	1.0 (0.9–1.2)
Drug use–no. (%)[a]	256 (8.2)	38 (3.6)	294 (7.0)	4.7×10^{-7}	2.4 (1.7–3.4)
Previous incarceration–no. (%)[a]	268 (8.6)	30 (2.9)	298 (7.2)	4.0×10^{-10}	3.2 (2.2–4.7)
Internal migrant–no. (%)[a]	72 (2.3)	13 (1.2)	85 (2.0)	0.033	1.9 (1.0–3.4)
Comorbidities[a]–no. (%)					
HIV	587 (18.9)	107 (10.2)	694 (16.7)	7.1×10^{-11}	2.0 (1.6–2.5)
HBV	59 (1.9)	6 (0.6)	65 (1.6)	0.003	3.4 (1.5–7.8)
HCV	353 (11.3)	35 (3.3)	388 (9.3)	1.2×10^{-14}	3.7 (2.6–5.3)
COVID-19	83 (2.7)	18 (1.7)	101 (2.4)	0.083	1.6 (0.9–2.6)
Diabetes mellitus	134 (4.3)	36 (3.4)	170 (4.1)	0.214	1.3 (0.9–1.8)
Anaemia	619 (19.9)	112 (10.7)	731 (17.6)	1.1×10^{-11}	2.1 (1.7–2.6)
Localisation–no. (%)					
Pulmonary	2922 (93.9)	1010 (96.2)	3932 (94.5)	0.005	0.6 (0.4–0.9)
Extrapulmonary	21 (0.7)	6 (0.6)	27 (0.6)	0.718	1.2 (0.5–2.9)
Pulmonary and Extrapulmonary	169 (5.4)	34 (3.2)	203 (4.9)	0.004	1.7 (1.2–2.5)
Smear positive–no. (%)	2356 (75.7)	765 (72.9)	3121 (75.0)	0.065	1.2 (1.0–1.4)
Pulmonary cavitation size classification[d]–no. (%)[d]	2966 (100)	1001 (100)	3967 (100)	0.821	1.0 (0.9–1.2)
Small cavities (<2 cm)	805 (27.1)	268 (26.8)	1073 (27.0)		
Medium cavities (2–4 cm)	194 (6.5)	63 (6.3)	257 (6.5)	0.784	1.0 (0.8–1.4)
Large cavities (>4 cm)	115 (3.9)	30 (3.0)	145 (3.7)	0.199	1.3 (0.9–2.0)
Large cavity involving multiple pulmonary sextants	29 (1.0)	4 (0.4)	33 (0.8)	0.106	2.5 (0.9–7.0)
Multiple cavities of varying size	320 (10.8)	116 (11.6)	436 (11.0)	0.484	0.9 (0.7–1.2)

Drug-Resistant TB = patients with rifampicin-resistant, multidrug-resistant, pre-extensively drug-resistant, or extensively drug-resistant tuberculosis; Control group–patients with non-multidrug-resistant and drug-susceptible tuberculosis; COVID-19 = Coronavirus disease; HIV = Human immunodeficiency virus; HBV = hepatitis B virus; HCV = hepatitis C virus. © Baseline TB case-registration categories were defined according to WHO recording and reporting definitions and reflected previous TB treatment history.[11] [a]Some patients presented with more than one comorbid condition and/or social risk factor. [b]Odds ratios (ORs) and 95% confidence intervals (CIs) are presented. The comparator cohort was the reference group. [c]Mean ± standard error of the mean. Between-group comparisons were performed using Welch's t-test (two-sided, assuming unequal variances). [d]Radiographic features were assessed on baseline posteroanterior chest radiographs by experienced radiologists prior to anti-TB treatment initiation. Radiographic data were unavailable for 195 participants (146 in the DR-TB cohort and 49 in the control group).

Table 1: **Baseline characteristics of patients with tuberculosis, according to drug-resistance status.**

2.6, 95% CI 2.0–3.3) were independently associated with WGS-inferred FQ resistance compared with new TB (Table S5).

Compared with patients infected with L4 isolates, those with L2 infection were younger and more likely to have a history of recurrent or treatment after failure, whereas newly diagnosed TB was more frequent among L4 cases. HIV infection, viral hepatitis, and anaemia were also more common in patients infected with L2 isolates (Table S4).

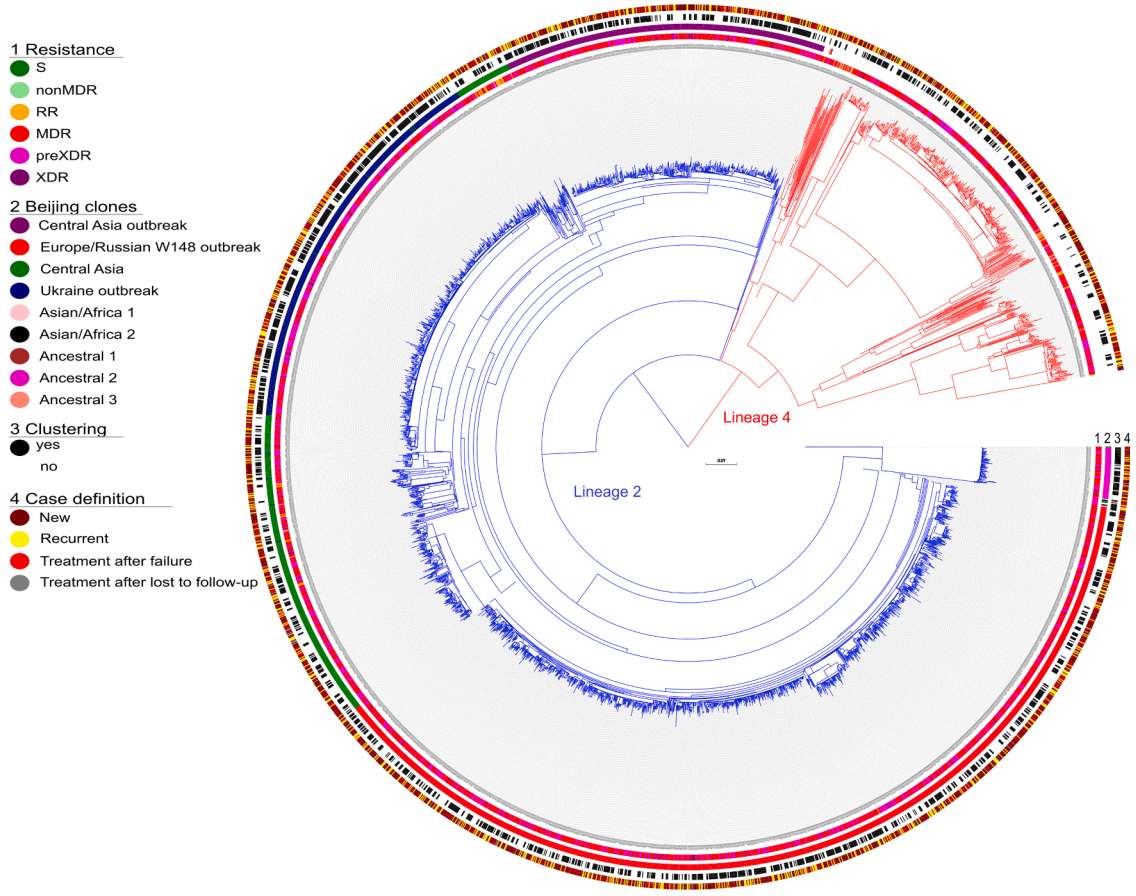


***Fig. 3:* Circular minimum spanning tree of 4161 *M. tuberculosis* complex isolates collected in 18 regions of Ukraine (December 2019-November 2023).** Circular minimum spanning tree of the Lineage 2 and Lineage 4 isolates (1 *M. bovis* excluded for visibility) based on core-genome multi-locus sequence typing (cgMLST) data generated with the Ridom Typer software and visualised with Evolview. The inner ring depicts drug-resistance categories. Subsequent rings indicate major of Lineage 2 clades/outbreak clones, cgMLST-defined genomic clustering (≤5 allelic differences), and case definitions (new, recurrent, treatment failure, and loss to follow-up). The tree highlights the extensive clonal expansion of Lineage 2 and its central role in the drug-resistant tuberculosis epidemic. L2 = Lineage 2, L4 = Lineage 4.

Furthermore, even if the L2 isolate phylogeny shows some structure, overall there is a very low L2 population diversity as the majority of the MDR/pre-XDR/XDR isolates (2497/3040 [82.1%]) belong to four Beijing clades described earlier[8,10]: Central Asia clade, Central Asia outbreak, Europe/Russian W148-outbreak and the Ukraine outbreak representing 419/3040 (13.8%), 396/3040 (13.0%), 1202/3040 (39.5%), and 480/3040 (15.8%). Lineage 2 W148-isolates represent the largest proportion of MDR 823/1939 (42.4%), pre-XDR 356/1023 (34.8%), and XDR 23/78 (29.5%) isolates, followed by Ukraine outbreak, Central Asia clade and Central Asia outbreak isolates (Fig. 3, Table 2).

Lineage 2 isolates predominated among DR-TB cases across the included regions (Fig. 2). Its proportion was modestly higher in the 11 central, southern, northern, and eastern regions than in the seven western regions (1828/2154 [84.9%] versus 778/958 [81.2%]; absolute difference 3.7 percentage points [95% CI 0.9–6.7]; Fisher's exact test p = 0.011). Overall, L2 predominated among DR-TB cases across the included regions.

In contrast, L4 isolates predominated among patients with susceptible or non-MDR TB, particularly in western regions (259/425 [60.9%] patients in central/southern/northern/eastern regions versus 455/625 [72.8%] in western regions), demonstrating a significant association with non-MDR disease (absolute difference 11.9 percentage points [95% CI 6.1–17.7]; Fisher's exact test p = 6.8×10^{-5}).

Notably, in seven regions (Odesa, Kharkiv, Donetsk, Kherson, Sumy, Vinnytsia, and Dnipropetrovsk), the proportions of L2 and L4 isolates in the comparator cohort were similar (Fig. 2).

Cluster analysis

2015/4162 (48.4%) Mtbc isolates formed genomic clusters at a threshold of ≤5 allelic differences. Clustering was more frequent in the DR-TB cohort

Phylogenetic group[b]		Drug-Resistant TB, 3112 (100%)				Control group, 1050 (100%)		Total, 4162 (100)	OR (95% CI)	p value[a]
		RR-TB, 72 (100%)	MDR-TB, 1939 (100%)	Pre-XDR-TB, 1023 (100%)	XDR-TB, 78 (100%)	Non-MDR TB, 349 (100%)	Susceptible TB, 701 (100%)			
Lineage 2										
Europe/Russian W148 Outbreak	Total in group, no. (%)	1203 (38.7)				27 (2.6)		1230 (29.6)	23.9 (16.2–35.2)	1.4×10^{-142}
	In the type of DR, no. (%)	1 (1.4)	823 (42.4)	356 (34.8)	23 (29.5)	25 (7.2)	2 (0.3)			
Central Asia	Total in group, no. (%)	454 (14.6)				274 (26.1)		728 (17.5)	0.5 (0.4–0.6)	2.6×10^{-16}
	In the type DR, no. (%)	35 (48.6)	264 (13.6)	143 (14.0)	12 (15.4)	124 (35.5)	150 (21.4)			
Central Asia outbreak	Total in group, no. (%)	396 (12.7)				21 (2.0)		417 (10.0)	7.1 (4.6–11.1)	4.5×10^{-30}
	In the type DR, no. (%)	0 (0.0)	270 (13.9)	112 (10.9)	14 (17.9)	21 (6.0)	0 (0.0)			
Ukraine outbreak	Total in group, no. (%)	480 (15.4)				0 (0.0)		480 (11.5)	383.5 (23.9–6145.8)	1.7×10^{-65}
	In the type DR, no. (%)	0 (0.0)	202 (10.4)	260 (25.4)	18 (23.1)	0 (0.0)	0 (0.0)			
Other	Total in group, no. (%)	73 (2.3)				12 (1.1)		85 (2.0)	2.1 (1.1–3.8)	0.0161
	In the type DR, no. (%)	1 (1.4)	49 (2.5)	20 (2.0)	3 (3.8)	2 (0.6)	10 (1.4)			
Lineage 4										
LAM	Total in group, no. (%)	324 (10.4)				110 (10.5)		434 (10.4)	1.0 (0.8–1.2)	0.953
	In the type DR, no. (%)	10 (13.9)	214 (11.0)	93 (9.1)	7 (9.0)	38 (10.9)	72 (10.3)			
H37Rv-like	Total in group, no. (%)	27 (0.9)				283 (27.0)		310 (7.4)	0.02 (0.02–0.04)	8.2×10^{-148}
	In the type DR, no. (%)	12 (16.7)	13 (0.7)	2 (0.2)	0 (0.0)	49 (14.0)	234 (33.4)			
Ural	Total in group, no. (%)	112 (3.6)				110 (10.5)		222 (5.3)	0.3 (0.2–0.4)	1.1×10^{-15}
	In the type DR, no. (%)	2 (2.8)	75 (3.9)	34 (3.3)	1 (1.3)	44 (12.6)	66 (9.4)			
Haarlem	Total in group, no. (%)	23 (0.7)				187 (17.8)		210 (5.0)	0.03 (0.02–0.05)	2.2×10^{-90}
	In the type DR, no. (%)	6 (8.3)	14 (0.7)	3 (0.3)	0 (0.0)	39 (11.2)	148 (21.1)			
Ghana	Total in group, no. (%)	18 (0.6)				9 (0.9)		27 (0.6)	0.7 (0.3–1.5)	0.373
	In the type DR, no. (%)	3 (4.2)	15 (0.8)	0 (0.0)	0 (0.0)	5 (1.4)	4 (0.6)			
Other	Total in group, no. (%)	2 (0.1)				15 (1.4)		17 (0.4)	0.2 (0.1–0.5)	7.90×10^{-8}
	In the type DR, no. (%)	2 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	14 (2.0)			

TB = tuberculosis; DR = drug-resistant; RR = rifampicin-resistant; MDR = multidrug-resistant; pre-XDR = pre–extensively drug-resistant; XDR = extensively drug-resistant. Analyses were restricted to isolates of Lineages 2 and 4; one *M. bovis* and one Lineage 3 *(Delhi/CAS)* isolate were excluded from this summary. [a]p values were calculated with Fisher's exact test (two-sided). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between clone type and drug-resistance status. [b]Phylogenetic group refers to Mtbc lineage, sublineage, clade, or previously described outbreak clone. The Europe/Russian W148 outbreak, Central Asia outbreak, and Ukraine outbreak categories refer to previously described Lineage 2 outbreak clones.

Table 2: **Distribution of *Mycobacterium tuberculosis* lineages, sublineages, clades, and outbreak clones across drug-resistance categories.**

1712/3112 (55.0%) than in the comparator cohort 303/1050 (28.9%) corresponding to an absolute difference of 26.1 percentage points [95% CI 22.8–29.3]; $p < 0.001$, indicating close genetic relatedness and sustained circulation of resistant clones. When stratified by resistance category, clustering was most frequent among MDR with 1080/1939 (55.7%) and pre-XDR with 573/1023 (56.0%) of isolates, whereas clustering was less common among non-MDR with 143/349 (41.0%), RR with 21/72 (29.2%), and drug-susceptible with 160/701 (22.8%) clustered isolates (Table S6). The composition of the ten largest genomic clusters by clade and outbreak clone is shown in Figure S3.

The three largest genomic clusters (Clusters 1–3) comprise 671/3112 (21.6%) of the DR-TB group and were exclusively composed of L2 isolates. Patients in these L2 clusters shared a less favourable clinical and epidemiological profile than those with clustered L4 isolates (Table S7). Cluster 1 included 307 isolates (maximum pairwise distance 21 alleles) and can be

assigned to the previously described L2 Ukraine Outbreak clone.[10] More than half of the isolates were at least pre-XDR 161/307 (52.4%) while the remaining isolates were classified as MDR. Cluster 2 comprised 296 isolates and was assigned to the W148 Outbreak clone, with a maximum pairwise distance of 35 alleles. Most isolates in Cluster 2 were MDR 219/296 (74.0%) or pre-XDR 67/296 (22.6%). Cluster 3 comprised 76 isolates and was exclusively composed of the Central Asia outbreak clone, with most isolates classified as MDR 58/76 (76.3%) and the remainder as pre-XDR 18/76 (23.7%, Table S6).

In all major clusters, isolates show highly similar resistance mutation patterns mainly with key high-confidence resistance mutations suggesting that these mutations have been fixed in a common ancestor and are now maintained through sustained clonal expansion and circulation. Cluster 1 strains are dominated by canonical first-line resistance mutations, including KatG S315T and RpoB S450L or S450F, together with *fabG1* –15C > T, *embA* –12C > T, GyrA D94G and A90V, consistent with long-established clonal expansion and circulation of resistant genotypes. In contrast, Cluster 2 strains displayed a distinct pattern characterised by KatG S315T and RpsL K43R with distinct mutations conferring resistance against rifampicin, ethambutol, pyrazinamide and also a few mutations against BDQ. Cluster 3 strains exhibited a highly conserved resistance profile centred on KatG S315T, RpoB S450L, RpsL K43R, and EmbB M306V, with pyrazinamide and ethionamide-resistance largely driven by PncA P69L and EthA T314I, whereas FQ resistance, when present, was mediated by more heterogeneous *gyrA* variants (Table S8).

Isolates in Clusters 1–3 were also enriched for resistance profiles likely to compromise both mSTRs and BPaL/M. Overall, 413/679 (60.8%) isolates were MDR and 246/679 (36.2%) were pre-XDR. Cluster 1 was enriched for pre-XDR/XDR isolates (171/307 [55.7%]), whereas Clusters 2 and 3 were predominantly MDR (277/372 [74.5%]). WGS-inferred FQ resistance was highest in Cluster 1 isolates (moxifloxacin/levofloxacin, 170/307 [55.4%] and 171/307 [55.7%]), compared with Clusters 2 (69/296 [23.3%] for both drugs) and Cluster 3 (18/76 [23.7%] for both drugs). By contrast, resistance to core BPaL/M and mSTRs agents remained uncommon and was confined to Clusters 1 and 2 (BDQ: 9/307 [2.9%] and 4/296 [1.4%], respectively; LZD: 4/307 [1.3%] and 1/296 [0.3%], respectively), with no BDQ or LZD resistance detected in Cluster 3.

Temporal trends in drug resistance and treatment outcomes

Temporal trends in WGS-inferred resistance were assessed among patients in the DR-TB cohort across four consecutive 12-month study intervals. After FDR correction across the 16 drug-specific trend tests, significant declines were observed for moxifloxacin, from 274/675 (40.6%) in 2019–2020 to 214/767 (27.9%) in 2022–2023 (absolute difference –12.7 percentage points [95% CI –17.5 to –7.8]; $q = 4.1 \times 10^{-7}$), levofloxacin, from 276/675 (40.9%) to 216/767 (28.2%) (–12.7 percentage points [95% CI –17.5 to –7.8]; $q = 4.1 \times 10^{-7}$) and pyrazinamide, from 446/675 (66.1%) to 461/767 (60.1%) (–6.0 percentage points [95% CI –10.9 to –1.0]; q = 0.045). Amikacin resistance declined from 165/675 (24.4%) to 161/767 (21.0%) (–3.4 percentage points [95% CI –7.7 to 0.9]; q = 0.140; Table 3). In the analysis restricted to the 12 consistently sampled regions, FQ resistance decline from 226/563 (40.1%) in 2019–2020 to 191/677 (28.2%) in 2022–2023 (absolute difference –11.9 percentage points [95% CI –17.1 to –6.6]; χ^2 for trend = 22.0; $p = 2.5 \times 10^{-6}$), whereas the trends for pyrazinamide (q = 0.085) and amikacin (q = 0.420) were not significant (Table S9). Exploratory lineage- and mutation-specific analyses suggested similar patterns among L2 isolates (Tables S10-S11).

In parallel, the proportion of patients with a recorded successful treatment outcome increased from 402/675 (59.6%) to 563/767 (73.4%) across the study periods (absolute difference 13.8 percentage points [95% CI 8.9–18.6]; χ^2 for trend = 46.1; $p = 1.1 \times 10^{-11}$; Table S12, Figure S4). This increase in recorded treatment success coincided with declines in WGS-inferred FQ and pyrazinamide resistance. In a pooled analysis of DR-TB isolates belonging to Clusters 1–3, FQ resistance declined in both the full DR-TB cohort (72/157 [46.8%]–44/153 [28.0%]; absolute difference –18.0 percentage points [95% CI –28.9 to –8.0]; χ^2 for trend = 11.8, $p = 6.0 \times 10^{-4}$) and the 12 consistently sampled regions (61/130 [46.9%]–39/132 [29.5%]; absolute difference –17.4 percentage points [95% CI –28.5 to –5.6]; χ^2 for trend = 8.0, p = 0.005).

Discussion

We conducted the first large-scale prospective observational cohort study combining clinical, microbiological, and WGS data to characterise drug-resistant TB in Ukraine during a period marked by COVID-19, the war, population displacement, and transition to shorter all-oral treatment regimens. Our findings show that DR-TB in this multicentre Ukrainian cohort was characterised by the predominance of genomically clustered L2 clones, particularly W148, Ukraine outbreak, and Central Asia outbreak clones. WGS-inferred FQ resistance was common, whereas XDR-TB and BDQ resistance remained comparatively uncommon. However, the detection of isolates with combined resistance to FQ, BDQ, and LZD highlights a potential threat to current and future all-oral regimens and underscores the need for continued genomic surveillance.

The epidemiological pattern observed in our study aligns with prior regional findings,[10,19] reinforcing the

Name of drug	2019–2020—no. (%)	2020–2021—no. (%)	2021–2022—no. (%)	2022–2023—no. (%)	Total, n = 3112	χ^2 for trend	p for trend	FDR-adjusted q value
	675 (100)	1040 (100)	630 (100)	767 (100)				
INH—no. (%)	659 (97.6)	1010 (97.1)	615 (97.6)	756 (98.6)	3040 (97.7)	2.2	0.139	0.223
SM—no. (%)	630 (93.3)	979 (94.1)	588 (93.3)	725 (94.5)	2922 (93.9)	0.5	0.494	0.608
EMB—no. (%)	569 (84.3)	898 (86.3)	540 (85.7)	671 (87.5)	2678 (86.1)	2.3	0.131	0.223
PZA—no. (%)	446 (66.1)	657 (63.2)	374 (59.4)	461 (60.1)	1938 (62.3)	6.9	0.008	0.045
MFX—no. (%)	274 (40.6)	395 (38.0)	213 (33.8)	214 (27.9)	1096 (35.2)	29.7	5.2×10^{-8}	4.1×10^{-7}
LFX—no. (%)	276 (40.9)	396 (38.1)	213 (33.8)	216 (28.2)	1101 (35.4)	29.7	4.9×10^{-8}	4.1×10^{-7}
CFZ—no. (%)	20 (3.0)	22 (2.1)	20 (3.2)	18 (2.3)	80 (2.6)	0.1	0.812	0.829
KAN—no. (%)	298 (44.1)	450 (43.3)	236 (37.5)	320 (41.7)	1304 (41.9)	2.4	0.124	0.223
AMK—no. (%)	165 (24.4)	240 (23.1)	117 (18.6)	161 (21.0)	683 (21.9)	4.4	0.035	0.140
CPR—no. (%)	157 (23.3)	248 (23.8)	121 (19.2)	164 (21.4)	690 (22.2)	2.3	0.129	0.223
ETH/PTH—no. (%)	469 (69.5)	710 (68.3)	437 (69.4)	499 (65.1)	2115 (68.0)	2.6	0.104	0.223
LZD—no. (%)	10 (1.5)	9 (0.9)	8 (1.3)	6 (0.8)	33 (1.1)	0.9	0.350	0.467
BDQ—no. (%)	20 (3.0)	25 (2.4)	23 (3.7)	18 (2.3)	86 (2.8)	0.1	0.829	0.829
CS—no. (%)	11 (1.6)	15 (1.4)	6 (1.0)	8 (1.0)	40 (1.3)	1.4	0.232	0.338
PAS—no. (%)	111 (16.4)	166 (16.0)	100 (15.9)	97 (12.6)	474 (15.2)	4.1	0.044	0.141
DLM—no. (%)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.0)	0.2	0.631	0.721

INH = isoniazid; SM = streptomycin; EMB = ethambutol; PZA = pyrazinamide; MFX = moxifloxacin; LFX = levofloxacin; CFZ = clofazimine; KAN = kanamycin; AMK = amikacin; CPR = capreomycin; ETH/PTH = ethionamide/prothionamide; LZD = linezolid; BDQ = bedaquiline; CS = cycloserine; PAS = para-aminosalicylic acid; and DLM = delamanid. Resistance was inferred from whole-genome sequencing (WGS) data for 3112 *Mycobacterium tuberculosis* isolates collected across four consecutive 12-month periods using drug-specific classifications from the WHO mutation catalogue and a previously validated resistance-mutation catalogue; phenotypic DST was not used for these estimates. Values are no. (%). Raw two-sided p values were calculated using the χ^2 test for linear trend. The Benjamini–Hochberg procedure was applied across the 16 drug-specific trend tests to control the false discovery rate. FDR-adjusted q values of 0.05 or lower were considered statistically significant.

Table 3: **Trends in WGS-Inferred resistance to first- and second-line anti-tuberculosis drugs among patients in the drug-resistant tuberculosis cohort in Ukraine, December 2019–November 2023.**

association between L2 infection, MDR/pre-XDR phenotypes, and extensive genomic clustering and regional persistence in Eastern Europe. These findings highlight the need for close resistance monitoring in high-burden MDR-L2 settings, particularly during the scale-up of shorter all-oral regimens. In our cohort, L2 isolates were more frequently associated with pre-XDR and XDR-TB than L4 isolates. Within this cohort, L2 infection was also associated with documented adverse clinical and social profiles, including higher proportions of recurrent TB, treatment after failure, HIV co-infection, viral hepatitis, anaemia, homelessness, drug use, and previous incarceration.[20]

Geospatial analysis revealed that L2 isolates were dominant among DR-TB cases in almost all regions (Fig. 2), with the highest proportions in the participating central, southern, northern, and eastern regions of Ukraine and comparatively lower representation in the west, where L4 remains more common, particularly among drug-susceptible TB cases. This distribution may be consistent with historical transmission patterns–associated with large-scale outbreaks during the late Soviet era, population movements across eastern Ukraine, and amplification of L2 transmission in institutional settings.[21]

WGS-based resistance profiling demonstrated that a substantial proportion of DR-TB in Ukraine is driven by specific L2 clones with a limited set of high-confidence resistance mutations, such as KatG S315T and RpoB S450L, which have previously been associated with low fitness costs.[10] FQ resistance was primarily associated with GyrA D94G and A90V mutations, while BDQ and CFZ resistance was largely associated with *Rv0678* variants, consistent with previous reports that *Rv0678* is a major mechanism of resistance to these drugs in clinical Mtbc isolates.[22,23] In contrast to prior reports, seven BDQ resistant XDR Mtbc isolates carried *atpE* variants, including six L2 isolates and one L4 isolate. The six L2 *atpE*-mutant isolates were distributed across several L2 clades/outbreak clones rather than forming single monophyletic cluster, pointing towards independent acquisition of *atpE*-mediated BDQ resistance. Because systematic phenotypic BDQ minimum inhibitory concentration (MIC) testing, longitudinal sampling, and experimental fitness analyses were not performed, we cannot infer lineage-specific tolerance, clinical fitness, or transmission fitness of these *atpE*-mutant isolates. Similar *atpE* substitutions have recently been reported among BDQ resistant L2 isolates from Russia.[24] However, since a direct comparative phylogenetic analysis was conducted, we can rule out that the Ukrainian and Russian *atpE*-mutant isolates belong to the same clone. Together, these data suggest that *atpE*-mediated BDQ resistance may emerge more rapidly in L2 isolates, pointing to a distinct epidemiological scenario in Eastern Europe, where the DR-TB epidemic is largely driven by L2 strains, compared with other regions of the world. This concerning possibility warrants urgent investigation through phenotypic validation, systematic surveillance of BDQ

resistance, and large-scale molecular epidemiological studies across Eastern European countries.

Persistently high FQ resistance among patients with RR/MDR-TB remains clinically important because FQs are core components of several current shorter DR-TB regimens[5,25] and some regimens under development.[26] WGS-inferred resistance to moxifloxacin, levofloxacin, and pyrazinamide decreased in the full cohort, although only the fluoroquinolone trends remained significant in the consistently sampled-region analysis after false-discovery-rate correction. Sensitivity analyses restricted to the 12 consistently sampled regions and a pooled analysis of isolates belonging to the three largest genomic clusters showed similar FQ resistance trends. These contemporaneous patterns should not be interpreted as effects of implementation of mSTRs or BPaL/M,[5,25] the 2022 invasion, population displacement, or changes in TB service delivery. Individual-level regimen data, treatment interruptions, time to diagnosis, and longitudinal measures of diagnostic coverage were unavailable. The observed patterns could therefore reflect changes in case mix, regional participation, diagnostic practices, treatment policies, access to care, or other unmeasured secular factors.

In contrast, previous studies reported increasing FQ resistance in some Ukrainian regions,[27] based on phenotypic drug-susceptibility testing conducted under resource-constrained conditions.[28] In our cohort, drug-specific WGS-inferred resistance calls for levofloxacin and moxifloxacin showed 99.8% agreement (35.4% versus 35.2%), with seven discordant isolates. These differences arose from catalogue-based genotypic interpretation and were not based on phenotypic DST.[16] This high concordance is consistent with previously reported fluoroquinolone cross-resistance patterns.[29,30] A separate countrywide survey conducted in August 2022 provided quantitative context regarding TB service delivery during the 2022 invasion.[3] Among 21 accessible regional TB centres, the mean proportion of patients receiving care outside their region of permanent residence increased from 1.3% before the invasion to 7.0% in August 2022. Xpert MTB/RIF was available in 20 of 21 participating centres and FQ drug-susceptibility testing in all 21, although selected shortages of molecular diagnostic consumables and second-line drug-susceptibility testing capacity were reported. These aggregate service-level data were not linkable to participants in the present cohort and were therefore not included as covariates in the temporal analyses.

This study has several limitations. First, this observational genomic surveillance cohort was not designed for causal inference: war-related disruption was not measured at the individual level, and no geographically or temporally unaffected comparator was available. Calendar period therefore could not be used as a proxy for exposure to the invasion, displacement, or health-system disruption. Second, although the cohort covered 18 of 24 Ukrainian regions, regional participation changed after some TB facilities were destroyed or became inaccessible, which may have affected the composition of the study population and the interpretation of temporal trends. Third, the comparator cohort combined non-MDR and drug-susceptible TB, reflecting the rifampicin-based enrolment strategy but limiting subgroup-specific interpretation because these clinically heterogeneous groups were not analysed separately. Fourth, longitudinal service-delivery indicators–including diagnostic coverage, time to diagnosis, and treatment interruptions–and complete individual-level regimen data were unavailable, precluding causal assessment of war-related disruption and regimen-specific effectiveness or resistance dynamics. Fifth, systematic phenotypic drug susceptibility testing or MIC data were not available for all isolates; therefore, formal genotype–phenotype concordance analyses could not be performed, and WGS-inferred resistance should be interpreted cautiously. Finally, the observed temporal trends coincided with the COVID-19 pandemic, war-related displacement, changes in diagnostic capacity, treatment access, evolving national treatment guidelines, and regional sampling differences; these findings should therefore be interpreted as descriptive cohort-level trends. Race and ethnicity were not systematically recorded and were unavailable for analysis. Nevertheless, the broad multicentre coverage and integration of WGS with clinical metadata support the value of this cohort for genomic surveillance.

In conclusion, this large-scale multicentre genomic analysis shows that DR-TB in this Ukrainian cohort is characterised by dominant L2 clones, extensive genomic clustering, and high levels of MDR and pre-XDR TB. WGS-inferred BDQ and LZD resistance remained comparably uncommon, but the detection of isolates with combined resistance to FQs, BDQ, and LZD represents an important warning signal for settings using or scaling up shorter all-oral regimens. Resistance to several key drugs declined between 2019 and 2023 alongside improved treatment outcomes; however, these trends should be interpreted cautiously given the observational design and concurrent COVID-19, war-related displacement, changes in diagnostic access, changing regional participation, and evolving treatment guidelines. Continued genomic surveillance, systematic FQ testing before use of short all-oral regimens, targeted BDQ/LZD phenotypic confirmation where available, WGS integration into TB care, and cross-border data-sharing for displaced, migrant, and refugee populations will be essential for TB control in Ukraine and across Europe.

Contributors

DB conceived and designed the study, with input from SN and VD. SN and VD supervised the study and provided statistical guidance. VV, LA, DL, and AH were responsible for patient recruitment and data

acquisition. VD, AR, AG, JK, and DH coordinated site participation and provided access to clinical and laboratory data. DB, TB, AR, AG, JK, and DH curated and managed the dataset. DB did statistical analyses with input from TB, YF, MK, VM, ON, SN, and VD. DB drafted the first version of the manuscript. DB is the chief investigator for this study in Ukraine. DB, TB, AR, AG, JK, DH, SN, and VD had full access to and verified all the data; no author was precluded from accessing any data. All authors contributed to the interpretation of the findings, critically revised the manuscript for important intellectual content, and approved the final version for submission. DB, SN, and VD had final responsibility for the decision to submit the manuscript for publication.

Data sharing statement

Clinical and genomic data are publicly available through the NIAID TB Portals programme - https://tbportals.niaid.nih.gov. Raw sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProjects PRJNA766649 and PRJNA825524.

Editor note

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Declaration of interests

We declare no competing interests.

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During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with editing the English language, improving readability of the manuscript, and checking consistency of terminology and formatting. After using this tool, the authors reviewed and edited all content as necessary and take full responsibility for the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanepe.2026.101865.

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