Original Article

Central Asian Journal of Medical Sciences
CAJMS

Cent Asian J Med Sci. 2025 Dec;11(4):187-201 https://doi.org/10.24079/CAJMS.2025.04.004

Molecular epidemiological analysis of Mycobacterium tuberculosis isolates from rural population of nationwide tuberculosis prevalence survey in Mongolia

Oyuntuya Tumenbayar[1,2], Tsolmon Boldoo[2], Yoshiro Murase[5], Tsetsegtuya Borolzoi[1,2], Naranzul Dambaa[2], Tugsdelger Sovd[3], Naranbat Nyamdavaa[4], Satoshi Mitarai[5,6], Buyankhishig Burneebaatar[2], Sarantuya Jav[1,7]

[1] The Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[2] The National Center for Communicable Diseases, Ulaanbaatar, Mongolia;
[3] The Ministry of Health, Ulaanbaatar, Mongolia;
[4] The Mongolian Anti-TB Coalition, Ulaanbaatar, Mongolia;
[5] The Department of Mycobacterium Reference and Research, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Tokyo, Japan;
[6] Basic Mycobacteriosis, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan;
[7] The Institute of Biomedical Sciences, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia.

Submitted date: Aug 8, 2025
Accepted date: Dec 21, 2025

Corresponding Author:
Sarantuya Jav (MD., Ph.D., Prof.)
The Department of Molecular biology and Genetics, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia
E-mail: sarantuya.j@mnums.edu.mn
ORCID ID: https://orcid.org/0000-0002-3032-4490

Naranzul Dambaa (MD., Ph.D., Assoc. Prof.)
TB Surveillance and Research Department, National Center for Communicable Diseases, Ulaanbaatar, Mongolia
E-mail: ndambaa2020@gmail.com
ORCID: https://orcid.org/0000-0002-6428-0350

Objective: Mongolia is among the 30 high-burden countries for TB and DR-TB. Whole-genome sequencing (WGS) offers precise insights into TB transmission, strain diversity, and antimicrobial resistance (AMR), which are vital for TB control strategies. This study aimed to investigate the molecular epidemiology, lineage distribution, transmission clustering, and drug resistance patterns of M.tuberculosis collected from rural areas of Mongolia using NGS.

Methods: A total of 50,194 individuals were screened in a national TB prevalence survey conducted according to WHO guidelines. From rural participants, 102 TB isolates were obtained; 100 with sufficient DNA quality were subjected to WGS. Bioinformatics analyses included lineage identification, AMR mutation profiling, and phylogenetic and clustering analyses.

Result: Among the 100 isolates, lineage 2 (Beijing genotype) accounted for 72.0% and lineage 4 (Euro-American) for 28.0%. The Beijing type was dominant across all regions, especially the central region (84.6%) and showed high clustering (56.4%). A total of 13 clusters (≤12 SNVs) were identified; 86.6% were Beijing strains. MDR-TB comprised 10% of isolates, with 100% of MDR-TB strains belonging to the Beijing genotype. Resistance to isoniazid (23%) was common. Mutations associated with resistance were mainly found in katG, inhA, rpoB, pncA, rpsL, rrs, and embB genes. No resistance was observed to new drugs such as bedaquiline, linezolid, or clofazimine. **Conclusions:** In rural of Mongolia, M. tuberculosis Lineage 2 (modern Beijing genotype) is predominant, accounting for 72% of cases, with consistent distribution across geographic locations. Beijing strains demonstrated higher drug resistance compared to Euro-American types. Importantly, no resistance was detected to newly introduced TB drugs.

Keywords: M.tuberculosis, Molecular epidemiology, TB prevalence survey, NGS, Drug resistance

Introduction

Tuberculosis (TB) is a communicable disease that is a major cause of ill health and one of the leading causes of death worldwide.[1] In particular, drug-resistant TB continues to be a

public health threat. The reported global number of people newly diagnosed with TB was 7.5 million[1] and Mongolia is among the thirty high-burden countries for drug-resistant (DR) TB and TB incidence.[2] In 2022, approximate more than 2,000 new TB cases were reported in Mongolia, of which 158 cases were rifampicin-resistant *Mycobacterium tuberculosis* (RR-TB), considered as multidrug-resistant *M. tuberculosis* (MDR-TB).[3]

Resistance to rifampicin the most effective first-line drug is of greatest concern.[4] TB that is resistant to rifampicin and isoniazid is defined as MDR-TB.[5,6] World Health Organization (WHO) estimated 410,000 people (95% UI: 370,000–450,000) developed MDR or RR-TB in 2022.[1] The emergence and wide-spread of drug-resistant tuberculosis (DR-TB), especially MDR-TB and extensively drug-resistant TB (XDR-TB), has undoubtedly become one of major stumbling blocks of TB elimination worldwide.[1,7,8]

The genome size of *M. tuberculosis* is approximately 4.4 mb[9] with low spontaneous mutation rate and no evidence of horizontal gene transfer. *M. tuberculosis* comprises of seven lineages that vary in geographical distribution around the globe; the strain-types may be specifically adapted to people of different genetic backgrounds. Four lineages are predominant in humans: lineage 1, Indo-Oceanic; lineage 2, East Asian (Beijing spoligotype families); lineage 3, Central Asian Strain (CAS/Delhi spoligotype families); and lineage 4, Euro-American (Latin American–Mediterranean (LAM), Haarlem and the 'ill-defined' T spoligotype families).[10-13]

Since the full-genome sequence of *M. tuberculosis* H37Rv was completed and published in 1998, whole-genome sequencing (WGS) has been widely used in research, clinical, and routine surveillance work, including predicting drug resistances, investigating transmission chains, identifying mixed infection, and revealing evolutionary laws of *M. tuberculosis* (MTB).[14-17] These expanding applications provide clear insights into the molecular epidemiology of MTB, which contribute significantly to the precise control and prevention of TB.[18,19]

WGS of MTB is an attractive method for the confirmation of genetically identical strains and accurate detection of clinically relevant mutations/indels for AMR predictions.[20,21] Mongolia has conducted national TB prevalence survey in 2014–2015. TB prevalence rate was much higher (560/100,000) than the estimation by the WHO for 2013.[22] The National Tuberculosis Reference Laboratory (NTRL) and the Research Institute of Tuberculosis (RIT) have conducted the molecular analysis of MTB isolates collected from this survey to examine TB transmission and analyse DR-TB details using WGS.

Material and Methods

Sampling Strategy

The sampling strategy for the TB prevalence survey followed the WHO guideline.[5] Of the survey participants, 50,194 (99.8%) subjects underwent symptom screening interview, 49,521 (98.4%) subjects had CXR examination, and 749 subjects did not have CXR examination because of old age, disability, refusal, or other reasons. Through the field screening by interview and CXR, 10,359 (20.6%) of the participants were regarded as being eligible for sputum examinations, out of which 9,546 (92.1%) subjects submitted at least one sputum specimen. Two smear and culture results were available for 9,473 (91.5%) of the requested individuals. Eligibility for sputum submission was determined based on symptoms alone for 1,729 (16.9%), chest x-ray alone for 7,064 (68.2%), and both symptoms and chest x-ray for 817 (7.9%) of a total of 10,359 (20.6%) participants eligible for sputum submission. Majority of the individuals enumerated were urban (city stratum) residents accounting for 54.5% versus 45.5% of the rural (13.9% in provincial center stratum; 31.6% in soum stratum) counterparts. During the study to determine the prevalence of TB among the population of Mongolia, a total of 102 MTB were isolated in the rural population of province and soum. Due to insufficient DNA content of 2 of these strains, 100 (98%, 100/102) strains were subjected to whole genome sequencing (NGS) analysis.

Laboratory Procedures

Smear and culture examination were performed on both of the two sputum specimens per subject, according to the standard operational procedure.[23,24] NTRL performed microscopy and culture tests on the specimens. If the result of culture was positive, identification of MTB was performed using Capilia TB (TAUNS, Japan). The MTB isolates were sub-cultured and confirmed as pure culture, and the genome DNA was extracted using Isoplant (Fujifilim Wako, Japan). The DNA library was prepared using Nextera XT DNA library preparation kit (Ilumina) and subjected to MiSeq sequencer (Ilumina) following the manufacturer's instruction.

Whole Genome Sequence Analysis

MTB lineages/sublineages and potential AMRs were determined using the KvarQ script according to the manufacturer's instructions.[22] The AMR target list has been improved to detect more reliable genetic alterations for the *ahpC, embA, embB, embC, embR, ethA, ethR, gid, gyrA, gyrB, inhA, kasA, katG, pncA, rpoB, rpoC, rpsA, rpsL*, and *rrs* genes from WHO mutation catalogue[25], Most available short reads (~100-mer) can be utilized to discriminate the isolates based on the core genome phylogeny. WGS analysis solution provides a more accurate and discriminative strain typing for clinical and epidemiological investigations; NGS strain typing offers a total genotyping solution for Mtb outbreak and surveillance. The obtained fastq read data was analysed using CLC genomics workbench (Qiagen, CA, US).

MTB Lineage and Cluster Analysis

MTBseq program was used for the single nucleotide variant (SNV) analysis. For the tree construction, we employed the programme FastTree version 2 (Price, Dehal & Arkin, 2010) in the double precision built with a general time reversible substitution model, 1,000 resamples, and Gamma20 likelihood optimization. EvolView was used to visualize the phylogenetic tree.

Statistical Analysis

All data analysis was done using Stata 13/SE software package (Stata Corp, College Station, Texas, US). The first stage in the analysis focused on describing eligibility, enrolment and participants by age, sex, and strata. Subsequently outcome of screening (interview and X-ray film) and sputum testing was described, disaggregated by key characteristics namely sex, age, and type of symptoms or X-ray abnormality, setting and education level. A p value of <0.05 was considered statistically significant.

Data Availability

The WGS data generated during the current study are available as fastq files in the Sequence Read Archive of the RIT, Japan and strains following biosecurity restriction keeping at NTRL of Mongolia.

Ethical Considerations

The study protocol of the national prevalence survey and molecular epidemiology study were approved by National Ethical Committee of MoH, Mongolia. All methods were performed according to the guidelines described in the study protocol of the National prevalence survey and Molecular epidemiology study. In the context of this study, we obtained several approvals from the Medical Ethics Committee of the relevant institutions. These approvals encompass:

- Resolution No. 262, dated June 14, 2022, from the Medical Ethics Control Committee of the Ministry of Health.
- Resolution No. 49, dated March 15, 2018, from the Medical Ethics Control Committee of the Ministry of Health.
- Decision from the meeting No. 11/3/2016-11, dated April 22, 2016, of the Research Ethics Control Committee of NMUMS.
- Resolution No. 04, dated May 16, 2013, from the Medical Ethics Control Committee of the Ministry of Health (pertaining to basic research).

The Patients signed informed consent. All patients diagnosed with TB and drug-resistant TB received appropriate treatment.

Results

The average age of the participants in this study was 42.4±17.0, while it was 47.5±17.0 for the rural population of the provincial and 39.5±16.6 for the soum (t-test, p =0.023). Of all participants, 58% were male, 72% had secondary education or higher, 62% were married, and 63% were unemployed, and there was no difference between the provincial and soum population. The TB cases in the survey were found 26 in the eastern region, 39 in the central region, 25 in the highland region, and 10 in the western region (p =0.93). Among all participants, cough symptoms were found in 31 (31%), and cough lasting more than 14 days was found in 17 of them (55%). A total of 17 participants had a history of tuberculosis, 26 were TB contacts, 8 were underweight, and 36 had sputum smear-positive pulmonary tuberculosis, and no differences were observed between the study population groups (p >0.05) (Table 1).

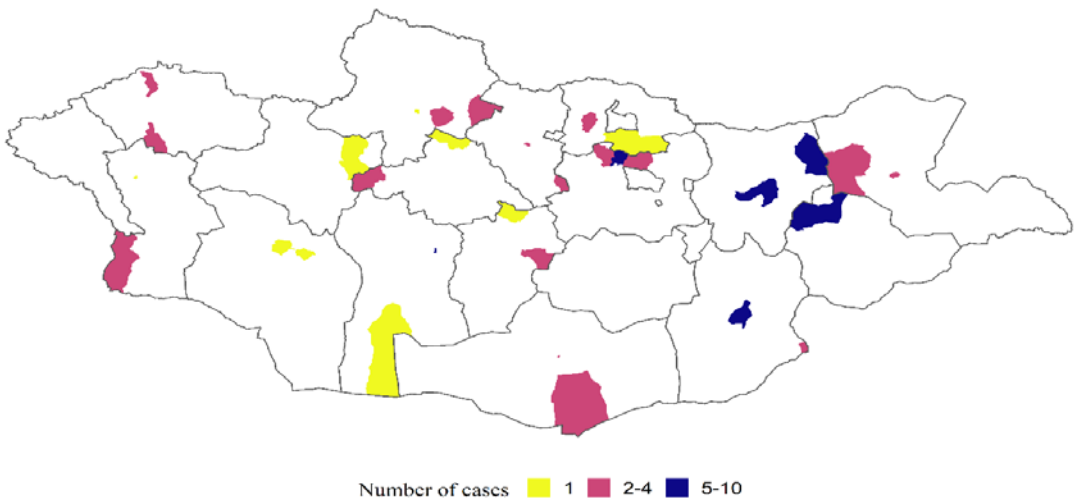
Table 1. The demographic and clinical data of patients stratified according to treatment history by stratum

Demographic and clinical		Total	Provincial center	Soum	*p* value
		N=100	N=36	N=64	
Age recorded during survey		42.4 (17.0)	47.5 (16.9)	39.5 (16.6)	0.023
Age-group	15–24	16 (16.0%)	3 (8.3%)	13 (20.3%)	0.17
	25–34	19 (19.0%)	4 (11.1%)	15 (23.4%)	
	35–44	22 (22.0%)	8 (22.2%)	14 (21.9%)	
	45–54	17 (17.0%)	8 (22.2%)	9 (14.1%)	
	55–64	18 (18.0%)	10 (27.8%)	8 (12.5%)	
	65–	8 (8.0%)	3 (8.3%)	5 (7.8%)	
Sex recorded during survey	Male	58 (58.0%)	20 (55.6%)	38 (59.4%)	0.83
	Female	42 (42.0%)	16 (44.4%)	26 (40.6%)	
Education	No formal	12 (12.0%)	7 (19.4%)	5 (7.8%)	0.13
	Primary	5 (5.0%)	3 (8.3%)	2 (3.1%)	
	Secondary	11 (11.0%)	4 (11.1%)	7 (10.9%)	
	High School	24 (24.0%)	8 (22.2%)	16 (25.0%)	
	Vocational	39 (39.0%)	9 (25.0%)	30 (46.9%)	
	Bachelor	9 (9.0%)	5 (13.9%)	4 (6.3%)	
Marital status	Married	62 (62.0%)	25 (69.4%)	37 (57.8%)	0.49
	Single	25 (25.0%)	6 (16.7%)	19 (29.7%)	
	Divorced	4 (4.0%)	1 (2.8%)	3 (4.7%)	
	Widowed	9 (9.0%)	4 (11.1%)	5 (7.8%)	
Familysize	1	7 (7%)	3 (9%)	4 (6%)	0.48
	2	19 (19%)	9 (26%)	10 (16%)	
	3	19 (19%)	5 (14%)	14 (22%)	
	4	21 (21%)	4 (11%)	17 (27%)	
	5	14 (14%)	5 (14%)	9 (14%)	
	6	15 (15%)	7 (20%)	8 (13%)	
	7	2 (2%)	1 (3%)	1 (2%)	
	8	2 (2%)	1 (3%)	1 (2%)	
Region	East	26 (26.0%)	9 (25.0%)	17 (26.6%)	0.93
	Central	39 (39.0%)	13 (36.1%)	26 (40.6%)	
	Higland	25 (25.0%)	10 (27.8%)	15 (23.4%)	
	West	10 (10.0%)	4 (11.1%)	6 (9.4%)	
Employed	No	63 (63.0%)	25 (69.4%)	38 (59.4%)	0.39
	Yes	37 (37.0%)	11 (30.6%)	26 (40.6%)	
Individual with reported cough	No	69 (69.0%)	23 (63.9%)	46 (71.9%)	0.50
	Yes	31 (31.0%)	13 (36.1%)	18 (28.1%)	
History of TB treatment in the past	No	83 (83.0%)	30 (83.3%)	53 (82.8%)	1.00
	Yes	17 (17.0%)	6 (16.7%)	11 (17.2%)	
Cough 2 weeks	No	14 (45%)	7 (54%)	7 (39%)	0.48
	Yes	17 (55%)	6 (46%)	11 (61%)	

BMI category	Under-weight	8 (8%)	3 (8%)	5 (8%)	0.030
	Normal	69 (70%)	20 (56%)	49 (78%)	
	Overweight	18 (18%)	12 (33%)	6 (10%)	
	Obese class II	1 (1%)	0 (0%)	1 (2%)	
	Obese class III	3 (3%)	1 (3%)	2 (3%)	
BMI index		22.7 (4.0)	23.7 (4.6)	22.1 (3.5)	0.051
Heavysmoker	No	24 (69%)	8 (57%)	16 (76%)	0.28
	Yes	11 (31%)	6 (43%)	5 (24%)	
Final category by panel	S+ TB	36 (36.0%)	13 (36.1%)	23 (35.9%)	1.00
	S- TB	64 (64.0%)	23 (63.9%)	41 (64.1%)	
Outcome of S+ TB	Negative	64 (64.0%)	23 (63.9%)	41 (64.1%)	1.00
	Positive	36 (36.0%)	13 (36.1%)	23 (35.9%)	
Outcome of S-C+ TB	Negative	36 (36.0%)	13 (36.1%)	23 (35.9%)	1.00
	Positive	64 (64.0%)	23 (63.9%)	41 (64.1%)	
Outcome of bac-conf TB	Positive	100 (100%)	36 (100%)	64 (100%)	
Contact with TB patients	No	74 (74.0%)	26 (72.2%)	48 (75.0%)	0.81
	Yes	26 (26.0%)	10 (27.8%)	16 (25.0%)	
Currently smoking	No	62 (63%)	21 (60%)	41 (64%)	0.83
	Yes	37 (37%)	14 (40%)	23 (36%)	
Frequency of drinking alcohol last year	1	56 (56.0%)	18 (50.0%)	38 (59.4%)	0.093
	2	32 (32.0%)	10 (27.8%)	22 (34.4%)	
	3	9 (9.0%)	5 (13.9%)	4 (6.3%)	
	4	2 (2.0%)	2 (5.6%)	0 (0.0%)	
	5	1 (1.0%)		0 (0.0%)	

BMI-body mass index; S+-smear positive; S- C+- smear negative and culture positive; Bac-conf-bacteriological confirmed

Figure 1 represents the number of positive cases detected in the samples collected during the TB prevalence study. This data reveals that, on average, 5–10 cases were identified in the soums of the eastern regions, whereas an average of 2–4 cases were discovered in the western provinces (Figure 1).



As shown in Figure 2, the isolates in prevalence survey were mainly composed of lineage 2 72% (95% CI 62.1-80.5) especially East-Asian Beijing (2.2.1), and 28(28% (95% CI 19.5-37.9)) of lineage 4; Euro-American lineage subtype 4 (1%), 4.5 (4%), 4.1.2 (2%), Haarlem (1%), Latin American Mediterranean (LAM , 10%), mainly T (7%), and Ural (3%), respectively. The Beijing type of M. tuberculosis is prevalent among the population of soum in the center of the province and in rural areas. However, Beijing type is more prevalent in the center of the province, while Euro-American type is slightly higher (p =0.616). among the population of rural soum compared to the center of the province.

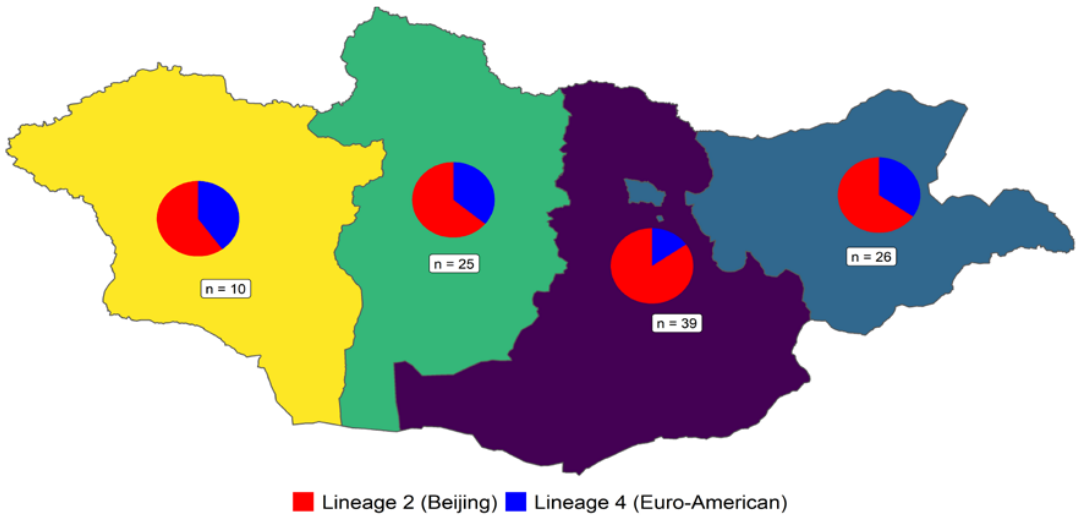


Figure 2. M.tuberculosis genotype by region

Table 2. Lineage of MTB

Lineage	Sub-lineage	Total	Provincial center	Soum	p value (chi²)
		N=100 (%, 95% CI)	N=36 (%, 95% CI)	N=64 (%, 95% CI)	
2 (Beijing)	2.2.1	72 (72.0%, [62.1-80.5])	27 (75.0%, [57.8-87.9])	45 (70.3%, [57.6-81.1])	0.616
4 (Euro-American)		28 (28.0%, [19.5-37.9])	9 (25.0%, [12.1-42.2])	19 (29.7%, [18.9-42.4])	
Lineage 4	4	1 (1.0%)	1 (2.8%)	0 (0.0%)	0.474
	4.1.2	2 (2.0%)	1 (2.8%)	1 (1.6%)	
	4.5	4 (4.0%)	1 (2.8%)	3 (4.7%)	
	Haarlem	1 (1.0%)	0 (0.0%)	1 (1.6%)	
	LAM	10 (10.0%)	3 (8.3%)	7 (10.9%)	
	Ural	3 (3.0%)	2 (5.6%)	1 (1.6%)	
	mainly T	7 (7.0%)	1 (2.8%)	6 (9.4%)	

Beijing genotype (lineage 2) was widely distributed in all regions of Mongolia, especially in the Central region. In addition to the Beijing genotype, Euro-American type and Ural subtypes were more common in the Eastern region, while Mainly T was more common in the Western region (Table 3, Figure 2). A total of 30 (30.0%) out of 100 isolates were involved in 13 clusters (Table 3).

Table 3. Genotypes and subtypes of *M. tuberculosis* by region

Indicator		East N=26	Central N=39	Highland N=25	Westren N=10	*p* value (chi²)
Genotype	Beijing	17 (65.4%)	33 (84.6%)	16 (64.0%)	6 (60.0%)	0.009
	Euro-American (EA)	3 (11.5%)	2 (5.1%)	1 (4.0%)	1 (10.0%)	
	(EA) 4	1 (3.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	(EA) 4.1.2	1 (3.8%)	1 (2.6%)	0 (0.0%)	0 (0.0%)	
	(EA) 4.5	1 (3.8%)	1 (2.6%)	1 (4.0%)	1 (10.0%)	
	Haarlem	1 (3.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	LAM	1 (3.8%)	4 (10.3%)	5 (20.0%)	0 (0.0%)	
	Ural	3 (11.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	mainly T	1 (3.8%)	0 (0.0%)	3 (12.0%)	3 (30.0%)	
Clustered	No	21 (80.8%)	17 (43.6%)	16 (64.0%)	6 (60.0%)	0.025
	Yes	5 (19.2%)	22 (56.4%)	9 (36.0%)	4 (40.0%)	

Table 4 shows the recent transmission index (RTI) according to the genotype of M. tuberculosis. The overall RTI is 17.0%, of which the RTI of genotypes of Beijing, Euro-American and the LAM were 20.8%, 7.1%, 20.0%, respectively. The clustering proportion varied across genotypes. The Beijing lineage accounted for 72.0% of all isolates and showed a higher clustering rate (36.1%) compared to the Euro-American lineage (14.3%). Statistical analysis revealed that the proportion of clustered cases was significantly higher among Beijing isolates than among Euro-American isolates ($\chi^2 = 4.57$, $p = 0.032$). Among Euro-American genotypes, clustering was detected only in the LAM lineage, in which 4 out of 10 isolates (40.0%) were clustered, while no clustering was observed among Haarlem, Ural, Mainly T, or other Euro-American sublineages. A significant difference was observed between the LAM genotype and other Euro-American lineages ($\chi^2 = 8.37$, $p = 0.0038$). These findings suggest that recent transmission was more common among isolates belonging to the Beijing and LAM genotypes.

Table 4. Recent transmission index by genotype

Genotype	Number of cases	Number of clustered cases	Number of clusters	Recent transmission index /RTI/		*p* value (chi²)
				Index, %	95%CI	
Beijing	72	26	11	20.8	12.2-32.0	0.032
Euro-American	28	4	2	7.1	0.9-23.5	
Euro-American	7	0	0	0.0		
Haarlem	1	0	0	0.0		
LAM	10	4	2	20.0	2.5-55.6	
mainly T	7	0	0	0.0		
Ural	3	0	0	0.0		
Overall	100	30	13	17.0	10.2-25.8	

Thirteen small clusters were observed in the phylogenetic mapping analysis of all isolates, and all clusters belonged to the Beijing pattern (86.6%) except for two LAM clusters.

This radial phylogenetic tree shows the genetic relationship among M. tuberculosis strains collected from different provinces and districts of Mongolia. Lineages and sublineages (e.g., 2.2.1, 2.2.2) are marked in blue, while SNP differences are indicated in red. Geographic locations and cluster sizes are marked in green boxes. A total of 13 clusters (≤12 SNVs) was identified (cluster size, 2–13). The clustering rate was 17%, and one MDR-TB clusters (size 2) were identified. Among them, a cluster of MDR-TB with 2 members of the Beijing genotype strain was identified. In terms of spatial location, one cluster with 3 members of Beijing genotypetype and one with 2 members was formed in Central province, while one cluster with 2 members of MDR-TB was registered in Dornogovi province, and other clusters were formed between provinces (Figure 3).

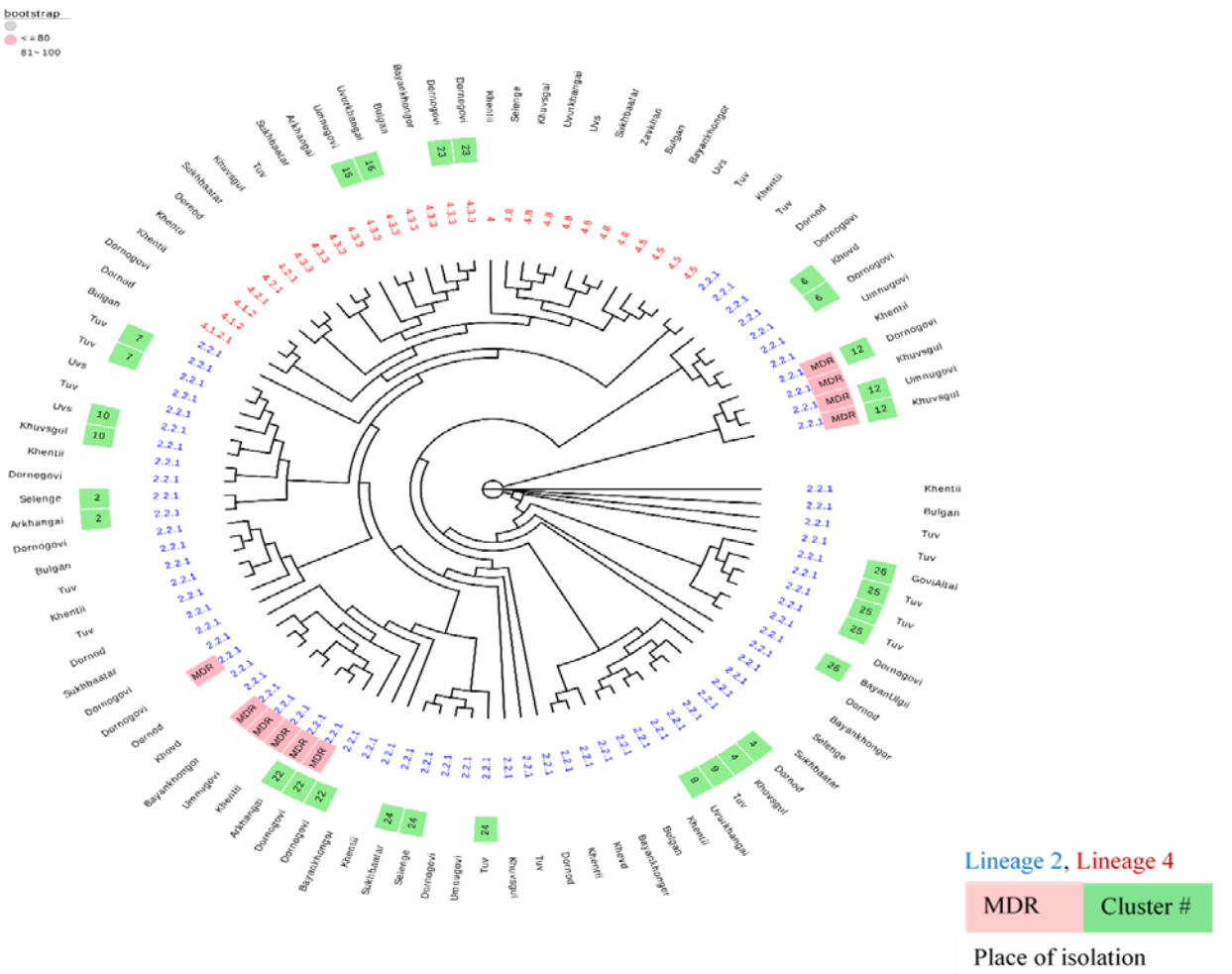


Figure 3. Phylogenetic tree of *M. tuberculosis*, N=100

In the TB prevalence study, the analysis indicated the following patterns of resistance among the TB isolates: 10% (95% CI 4.9–17.6) of the TB isolates were identified as MDR-TB. Of these cases, 2% exhibited resistance to 4 drugs (HRES), and 8% displayed resistance to 5 drugs (HRESZ). 16% (95% CI 9.4–24.7) of the TB strains demonstrated polydrug resistance. Specifically, 15.0% of these cases showed resistance to 2 drugs, namely HS, and 1.0% exhibited resistance to combinations of HE drugs. 24% (95% CI 16.0–33.6) of the TB strains were resistant to a monodrug. Among these cases, 23.0% were resistant to isoniazid, and 1% were resistant to streptomycin .

Table 5. Resistance to first and second-line anti-tuberculosis drugs by genotype

	DR pattern	Genotype						
		Total DR-T	Beijing N=72	Euro-American N=7	Haarlem N=1	LAM N=10	Ural N=3	Mainly T N=7
First line	**MDR**	10	10	-	-	-	-	-
	HRES	2	2	-	-	-	-	-
	HRESZ	8	8	-	-	-	-	-
	Poly	16	14	-	-	1	1	-
	HE	1	-	-	-	-	1	-
	HS	15	14	-	-	1	-	-
	Mono	24	22	-	-	2	-	-
	H	23	21	-	-	2	-	-
	S	1	1	-	-	-	-	-
Second line	**Total**	24	14	0	0	10	0	0
	Ethionamide	16	13			3		
	Amikacin	2	1			1		
	Kanamycin	1				1		
	Capreomycin	0						
	PAS	5				5		
	FQ	0						

Resistance to first and second line anti-tuberculosis drugs by genotype is shown in Table 5 According to this, the Beijing genotype was not only prevalent in patients with drug-resistant tuberculosis (multidrug, polydrug, and monodrug resistant tuberculosis), but also caused 100% of MDR-TB. There is also a low prevalence of LAM among patients with MDR-TB and monodrug-resistant TB.

Among second-line drugs, fluoroquinolones and injectables have shown 100% susceptibility to capreomycin, with relatively low resistance observed for kanamycin. For strains of the Beijing type, there was 18.0% resistance to ethionamide and 1.4% resistance to amikacin.

Another genotype with significant implications for drug resistance is LAM. LAM accounted for 3.7–6.2% of resistance to first-line drugs. Among second-line drug resistance, LAM subtypes exhibited 50% resistance to PAS, 30% resistance to ethionamide, 10% resistance to kanamycin, and 10% resistance to amikacin.

Among the 50 strains resistant to isoniazid, the following mutations were detected; 20 strains had mutation A187V of *mshA*, 16 strains had mutations C-15T of the *fabG1*, 7 strains had mutations only C-15T, 2 strains had substitution in codon I194T of *inhA*, 1 strain had a substitution in codon S94A of *inhA*, 3 strains had substitution in codon S94A of inhA and A187V of *mshA* and 3 strains had substitution in codon A187V of *mshA*. Thirteen strains had *katG* mutations, with one having A162T, and seven having S315T. Five strains had both c.944G>C and S315T mutation and *mshA* mutation c.560C>T. One strain had mutation in ahpC_promoter (G-48A) (Table 6).

Table 6. Detection of mutations in genes contributing resistance to anti-TB drugs

Drugs	Genes	Genotype						
		Beijing	EAI	Haarlem	LAM	mainly T	Ural	Total
Isoniazid	*ahpC*_promoter (G-48A)	1						1
	*fabG1*_promoter (C-15T)	6			1			7
	*fabG1*_promoter (C-15T)inhA (I194T)	0			2			2
	*fabG1*_promoter (C-15T)inhA (S94A)	1						1
	*fabG1*_promoter (C-15T)inhA (S94A)mshA (c.560C>T, A187V)	3						3
	*fabG1*_promoter (C-15T)mshA (c.560C>T, A187V)	3						3
	katG (c.484G>A, A162T)	1						1
	katG (c.944G>C, S315T)	6					1	7
	katG (c.944G>C, S315T)mshA (c.560C>T, A187V)	5						5
	mshA (c.560C>T, A187V)	20						20
	Total	46	0	0	3	0	1	50
Rifampicin	rpoB(c.1349C>T, S450L)	10						10
	Total	10	0	0	0	0	0	10
Ethambutol	embA_promoter (C-12T)embB (c.916A>G, M306V)	2						2
	embA_promoter (C-16T)						1	1
	embB(c.916A>G, M306V)	9						9
	Total	11	0	0	0	0	1	12
Streptomycin	rpsL(c.128A>G, K43R)	19						19
	rpsL(c.263A>G, K88R)	6						6
	rrs (c.514A>C)	1						2
	Total	26	0	0	1	0	0	27
Pyrazinamide	pncA (c.14T>C, I5T)	1						1
	pncA(c.307T>G,Y103D)	1						1
	pncA (c.36C>G, D12E)	4						4
	pncA(c.512C>A,A171E)	1						1
	pncA_promoter (A-11G)	1						1
	Total	8	0	0	0	0	0	8

Table 7. Comparing the results of previous studies in collaboration with other researchers using *M.tuberculosis* strains isolated in Mongolia

Name of researchers	Year	Method	Number of strains	Genotype	
				Beijing lineage	Other lineage
Dick van Soolingen etc.,	1992	RFLP	20	Dominant	-
N.Naranbat etc.,	2004-2005	Spoligotyping	232	64.2%	35.8%
Krilova etc.,	2009	LSP	120	58%	42%
B.Buyankhishig etc.,	2009	MIRU-VNTR	46	97.8%	2.3%
Ulziijargal etc.,	2012	MIRU-VNTR	67	88%	12%
Svetlana Zhdanova etc.,	2012	MIRU-VNTR	291	74%	26%
Our study		WGS	100	72%	28%

For the *rpoB* gene, which confers resistance to rifampicin, all 10 strains exhibited S450L mutation. Eight strains resistant to pyrazinamide had *pncA* mutations, including c.14T>C, I5T, c.307T>G, Y103D, D12E, and c.512C>A. Additionally, one strain each had *pncA* promoter A-11G mutation and c.36C>G mutation, while four strains had other mutations. Among the 26 streptomycin-resistant strains, 25 had *rpsL* mutations, with 19 having K43R mutation, 6 having K88R, and 1 having c.514A>C mutation of *rrs*. Twelve ethambutol-resistant strains exhibited mutations in the *embA* promoter (C-12T), *embB* (M306V), and *embA* promoter (C-16T), as well as the *embB* (M306V) (Table 6). Notably, there were no mutations or resistance identified in the recent use of new tuberculosis treatment drugs, such as bedaquiline (Rv*0678*), linezolid (*rrl* and *rplC*), and clofazimine (Rv*0678*).[25]

Discussion

Mongolia is among the 30 countries with a high TB burden and is also categorized as one of the countries with the highest MDR-TB burden.[1,3]

Prevalence of M. Tuberculosis in Mongolia by Whole Genome Analysis

Our study conducted a comprehensive regional analysis in Mongolia, revealing that the Beijing genotype is widespread across all regions, particularly dominant in the central region compared to others. Conversely, the Euro-American type exhibited higher distribution in the western and Khangai regions, with relatively lower prevalence in the central region. One hundred isolates were analysed by WGS and 13 small clusters among 100 patients, with 86.6% of them being of the Beijing genotype. Of the 10 MDR-TB isolates, 100% were classified as lineage 2. Beijing genotype and Latin American Mediterranean (LAM) is relatively high in the recent transmission index.

Our research revealed that among the Beijing genotype, lineage 2.2.1 or the "modern" Beijing genotype accounted for 100%. Additionally, Euro-American subtypes 4 (1%), 4.5 (4%), 4.1.2 (2%), Haarlem (1%), Latin American Mediterranean (LAM, 10%), mainly T (7%), and Ural (3%) were identified.

In terms of non-Beijing genotypes, 25–42% were observed, with Euro-American genotype at 7.5–10%, LAM at 7.5–10%, Haarlem at 3%, T at 10%, Orphan at 3%, and NEW at 11.5%. However, our study showed different proportions: LAM at 9.3% (20/216), mainly (L4) at 6.9%, Euro-American (L4) at 5.6%, Haarlem (L4) at 0.9%, and Ural (L4) at 2.3%, which were previously unidentified.[26-30]

TB Prevalence in Mongolia and the Beijing Genotype

This study presents the inaugural whole-genome sequencing analysis of TB strains in Mongolia. Out of the strains analyzed, 72% (95% CI 62.1–80.5) belonged to lineage-2 or the Beijing genotype, while 28% (95% CI 19.5–37.9) were lineage-4 or Euro-American genotype. The prevalence of the Beijing genotype is noteworthy, given its frequent occurrence in both drug-susceptible and resistant TB strains found in neighboring regions such as Inner Mongolia of China and southern Russia.[31-33] This genotype's high transmissibility, rapid evolution toward drug resistance in northern China, and potential for global dissemination are well-documented.[34,35]

Whole-genome sequencing (WGS) analysis identified 13 molecular clusters, each containing 2 to 3 closely related strains, indicating recent transmission events. The clustering rate of 17% suggests ongoing community transmission, reinforcing the need for targeted public health interventions and stronger TB control programs.

Prevalence of Drug-Resistant TB and Characteristics of the Beijing Genotype

Three drug resistance surveillance studies conducted in Mongolia over 8-9 year intervals revealed the following percentages of multidrug-resistant tuberculosis among new smear-positive cases: 1.0% in 1999, 1.4% in 2007, and 5.6% in 2016. This represents a 3.8-fold increase from the previous study, indicating a rising trend of multidrug-resistant tuberculosis in the community.[36] Researchers have hypothesized that the Beijing genotype may be highly transmittable, increasing the risk of developing drug resistance.[11,37,38] Approximately half of TB infections in Russia are attributed to the Beijing genotype, contributing to the escalation of MDR-TB cases.[39]

In our study of 100 strains, 10% (95% CI 4.9–17.6) were found to be multidrug-resistant, The proportion of MDR-TB among the Beijing genotype was 13.9% (95% CI: 5.7%–22.1%). Two large clusters with 2-3 representatives of MDR-TB were identified, indicating a high level of transmission. No specific strain was predominant in the country, but approximately half of the strains were considered new transmissions (Table 5).

Based on previous research conducted in Mongolia, it was found that the Beijing genotypes 94% (N. Naranbat, et al.)[40], 88% (G. Ulziijargal, et al.)[29], and 97.8% (B. Buyankhishig, et al.)[30] are associated with MDR-TB. These findings indicate a high prevalence of the Beijing genotype in MDR-TB cases and suggest a potential role of the Beijing genotype in causing MDR-TB. Comparing the results of previous studies in collaboration with foreign scientists using *M. tuberculosis* strains in Mongolia.

Based on the aforementioned research, 58-75% of the total strains were dominated by the Beijing genotype (Table 6). The prevalence of the Beijing genotype in MDR-TB is consistent with findings ranging from 88.0% to 97.8%.[26-30]

Limitations and Implications

This study provides valuable insights into the molecular epidemiology of M. tuberculosis circulating among rural populations in Mongolia, highlighting lineage-specific patterns, clustered transmission and drug resistance. However, as the isolates analyzed were obtained only from rural participants of the National prevalence survey, the findings may not fully represent the diversity and transmission dynamics of *M. tuberculosis* strains circulating in urban areas. Moreover, future nationwide investigations, including whole-genome sequencing of isolates from urban strata, are warranted to provide a more comprehensive understanding of the transmission dynamics, lineage distribution, drug resistance, and the underlying gene mutations responsible for resistance in *M. tuberculosis* in Mongolia. Strengthening early case detection, diagnostic capacity, treatment management and infection control particularly in rural settings will be crucial to reduce clustered transmission and prevent the emergence of drug-resistant tuberculosis.

Conclusion

In rural and provincial regions of Mongolia, *M. tuberculosis* Lineage 2 (modern Beijing genotype) is predominant, accounting for 72% of cases, with consistent distribution across geographic locations. In the central region, this lineage reaches 84.6% prevalence and exhibits a high clustering rate (56.4%), suggesting recent transmission. A total of 13 small genomic clusters were identified, 86.6% of which involved Beijing strains. The overall recent transmission index was 17%, with comparable rates observed among Beijing and Euro-American LAM subtypes. Beijing strains demonstrated higher drug resistance compared to Euro-American types. Importantly, no resistance was detected to newly introduced TB drugs.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Science and Technology Foundation in Mongolia. We further extend our sincere appreciation to the Department of Mycobacteriology at the Research Institute of Tuberculosis, JATA, Japan, for their invaluable technical support. Our profound gratitude is also extended to the National Center for Communicable Diseases and Mongolian National University of Medical Science for the contributions to this study. and substantial contributions to this study.

Declaration of Interest

We declare no conflict of interests.

Authors Contribution

Oyuntuya Tumenbayar: ORCID ID https://orcid.org/0009-0000-4230-7463, Conceptualization, data curation, formal analysis, funding acquisition, methodology, project administration, writing-original draft, writing-review and editing

Tsolmon Boldoo: ORCID ID https://orcid.org/0009-0001-5498-1698, Writing-review and editing

Yoshiro Murase: ORCID ID https://orcid.org/0000-0003-0240-8352, Project administration, supervision

Tsetsegtuya Borolzoi: ORCID ID https://orcid.org/0009-0009-3080-5532, Formal analysis, methodology

Naranzul Dambaa: ORCID ID https://orcid.org/0000-0002-6428-0350, Conceptualization, investigation, project administration

Tugsdelger Sovd: ORCID ID https://orcid.org/0009-0008-9229-0776, Resources, software

Naranbat Nyamdavaa: ORCID ID https://orcid.org/0000-0002-1761-1655, Conceptualization, software

Satoshi Mitarai: ORCID ID https://orcid.org/0000-0002-2288-077X, Validation, visualization

Buyankhishig Burneebaatar: ORCID ID https://orcid.org/0009-0008-0253-2859, Conceptualization, methodology, project administration

Sarantuya Jav: ORCID ID https://orcid.org/0000-0002-3032-4490, Supervision and approved by all authors.

All authors have accessed and verified the data and were responsible for the decision to submit the manuscript.

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