GENOTYPING OF *M. TUBERCULOSIS* CLINICAL ISOLATES FROM NEW CASES OF TUBERCULOSIS IN KAZAKHSTAN

A. Akhmetova [1], A. Akilzhanova [1], B. Assatova [2], N. Moldakhmetova [2], B. Toksanbaeva [3], V. Bismilda [3], L. Chingissova [2], U. Kozhamkulov [1]

[1] National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan
[2] School of Science and Technology, Nazarbayev University, Astana, Kazakhstan
[3] National Scientific Center for Phthisiopulmonology, Almaty, Kazakhstan

Introduction

- Tuberculosis (TB) remains a major health problem in Kazakhstan. Despite of that the rates of TB disease and mortality decreased from 58,5/100000 and 4,1/100000 in 2015 to 52,7/100000 and 3,4/100000 in 2016 respectively, the rate of **multidrug-resistant TB** (**MDR-TB**) increased from 12,2/100000 in 2014 to 13,5/100000 in 2015 [1];
- MDR strains associated with resistance to the main two first line anti-TB drugs – isoniazid and rifampicin are the most dangerous. **27 countries** relate to **high MDR-TB burden countries**, 15 of them in the European region, **including Kazakhstan** [2];
- Methods of genotyping of *M. tuberculosis* play important role in TB infection control. There are more than 10 methods of genotyping, **MIRU-VNTR** is one of the widely used method in the world.

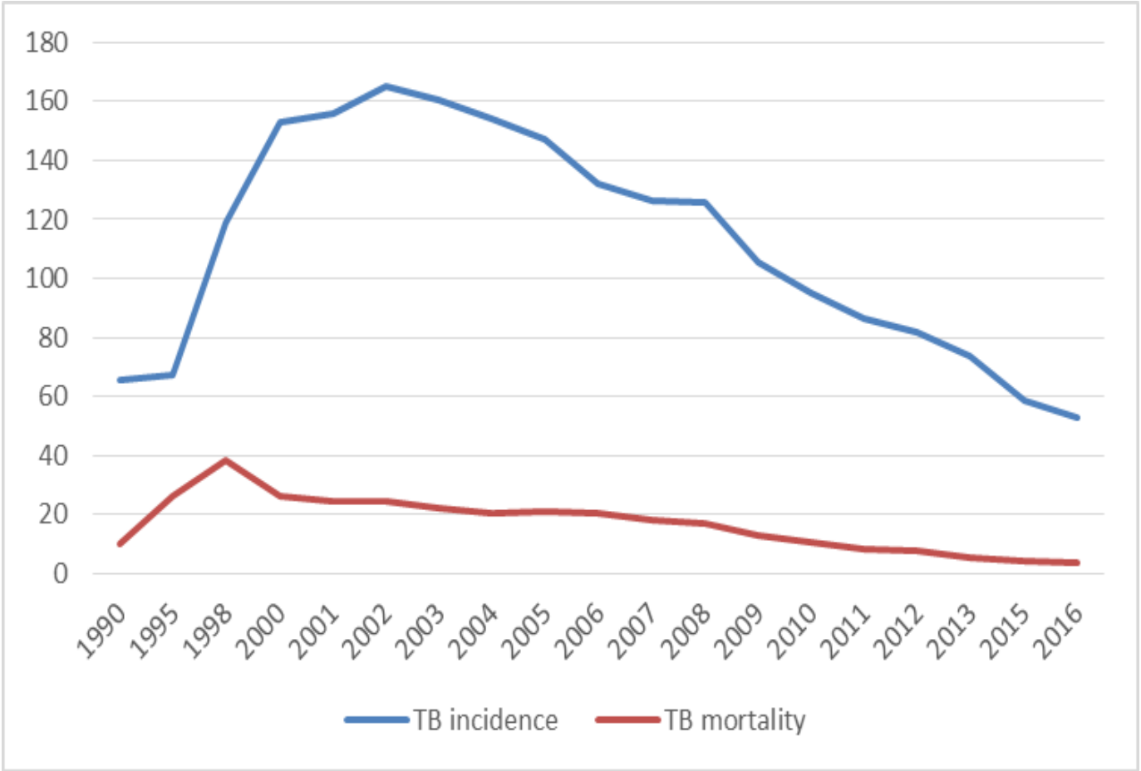


Figure 1. TB incidence and TB mortality in Kazakhstan

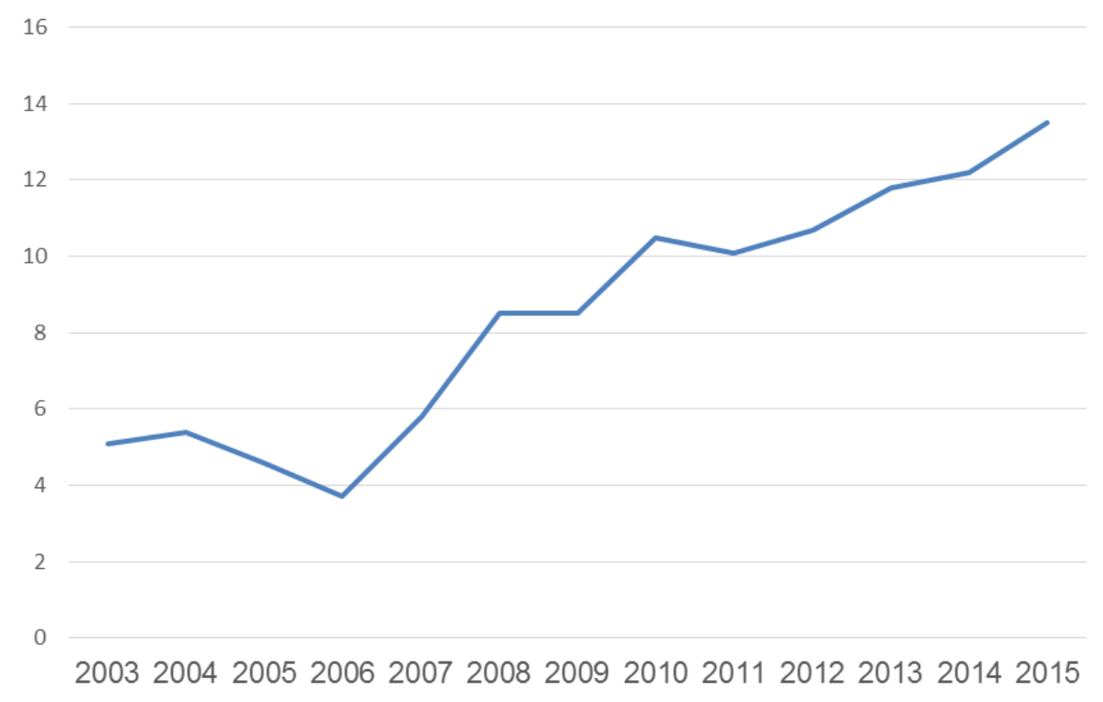
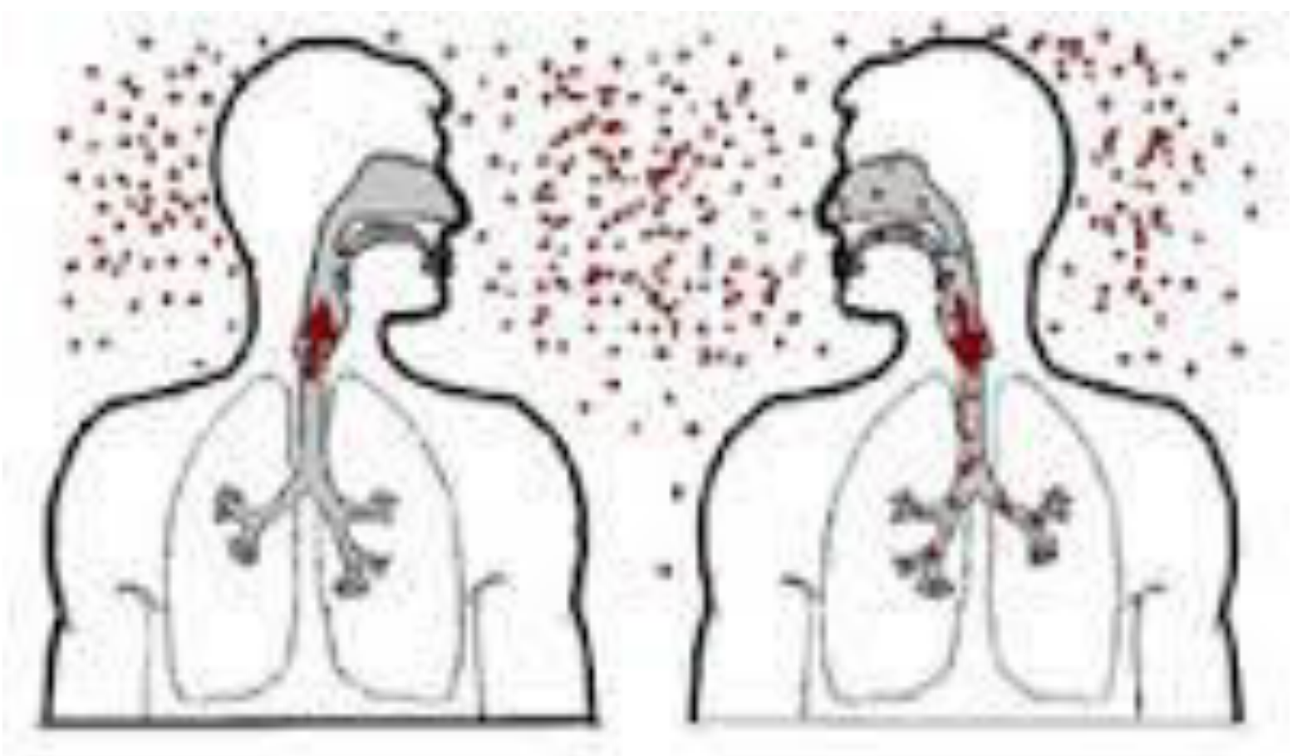


Figure 2. Incidence of MDR tuberculosis in Kazakhstan



Aim

- Estimation of biological diversity of *M. tuberculosis* clinical isolates among new TB cases in Kazakhstan by MIRU-VNTR analysis.

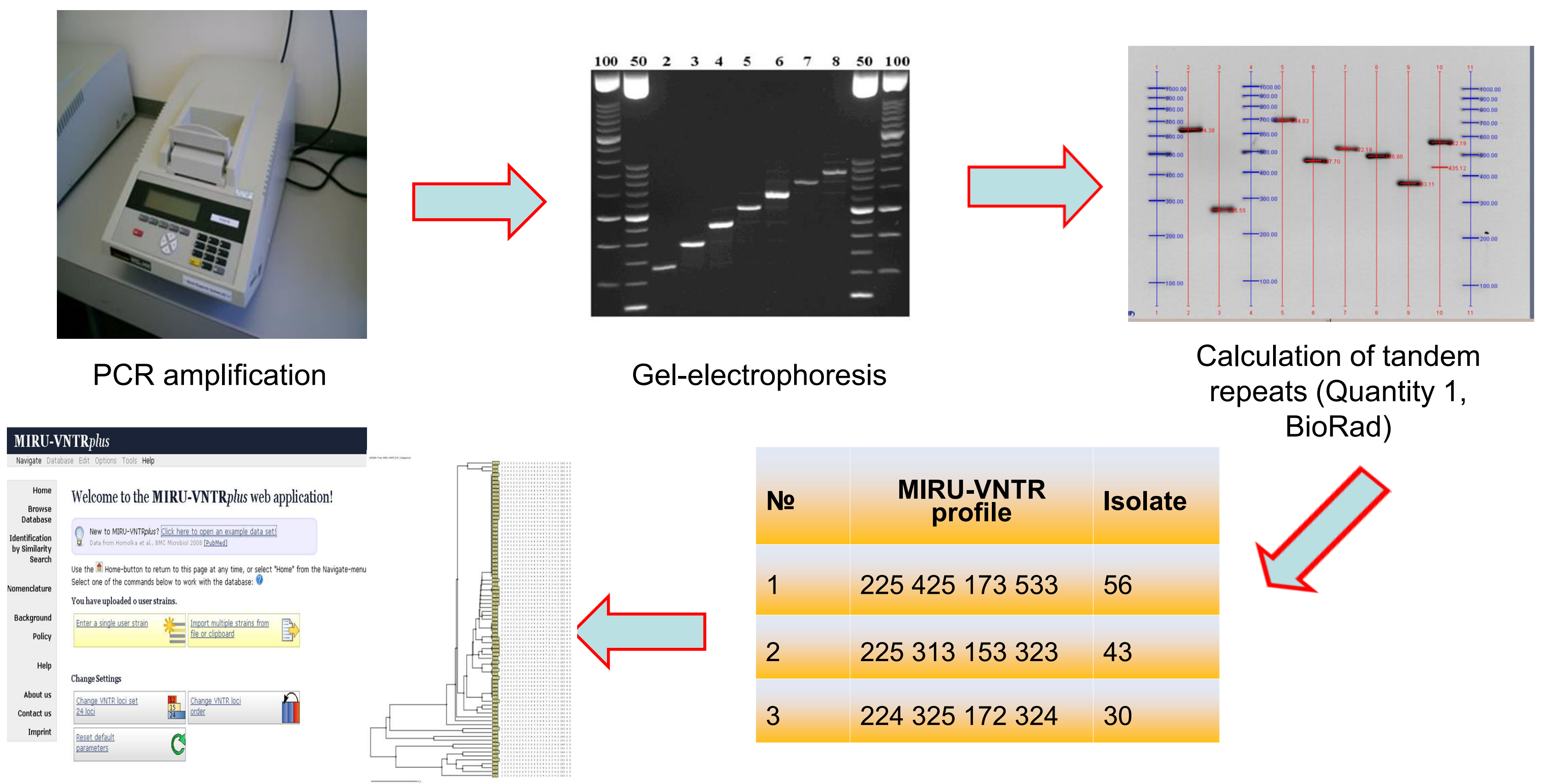
Materials and Methods

Materials:

- 385 samples of *M. tuberculosis* from new TB cases were collected from different regions of Kazakhstan.

Methods:

- Clinical isolates of *M. tuberculosis* were collected from regional TB centers and deposited at the **Reference laboratory of the National Scientific Center for Tuberculosis Problems (Almaty)**;
- After primary isolation mycobacteria were subcultured on solid **Lowenstein-Jensen medium**;
- **Drug susceptibility testing for *M. tuberculosis*** was performed using absolute concentration method;
- Heat inactivated mycobacterial colonies that were incubated at 95^0C during 45 minutes were used for MIRU-VNTR typing;
- **Genotyping of clinical isolates of *M. tuberculosis*** was carried out by 15 MIRU-VNTR method (Figure 3);
- ***M. tuberculosis* H37Rv** NC_000962.3 reference sequence was used as a control.



PCR amplification

Gel-electrophoresis

Calculation of tandem repeats (Quantity 1, BioRad)

Welcome to the MIRU-VNTRplus web application!

New to MIRU-VNTRplus? Click here to open an example data set!
Data from Homolka et al., BMC Microbiol 2008 [PubMed]

Use the Home-button to return to this page at any time, or select "Home" from the Navigate-menu
Select one of the commands below to work with the database:

You have uploaded 0 user strains.

№	MIRU-VNTR profile	Isolate
1	225 425 173 533	56
2	225 313 153 323	43
3	224 325 172 324	30

Obtaining MIRU-VNTR digital profile

Phylogenetic tree construction by UPGMA method

Figure 3. MIRU-VNTR typing

Results

- Phylogenic tree for 385 *M. tuberculosis* clinical isolates was built using www.miru-vntr.org web-resource based on the results of MIRU-VNTR analysis using UPGMA (Unweighted pair-group method using arithmetic averages) method (Figure 4).
- Beijing family strains associated with drug resistance to anti-TB drugs prevail among new TB cases in Kazakhstan. Strains of **Beijing genotype** were found in 63,1% cases (Figure 5 and Table 1).
- The second meaningful genotype that is spread in the territory of Kazakhstan among new TB cases is **LAM**, the frequency of distribution is 10,6%.
- Strains of **URAL and X families** were detected almost with the same frequency – 8.6% and 8.1%, respectively.
- Other genotypes (CAS, Cameroon, Uganda, NEW-1, Ghana) were identified in less than 2.1% cases.

Figure 4. Genotyping of MDR-TB by 15 MIRU-VNTR

M. tuberculosis genotypes	Number of isolates	%
Beijing	243	63.1
LAM	41	10.6
URAL	33	8.6
X	31	8.1
Haarlem	25	6.5
NEW-1	8	2.1
CAS	1	0.25
Cameroon	1	0.25
Uganda	1	0.25
Ghana	1	0.25
Total:	385	100

Table 1. Genotypes of *M. tuberculosis* clinical isolates

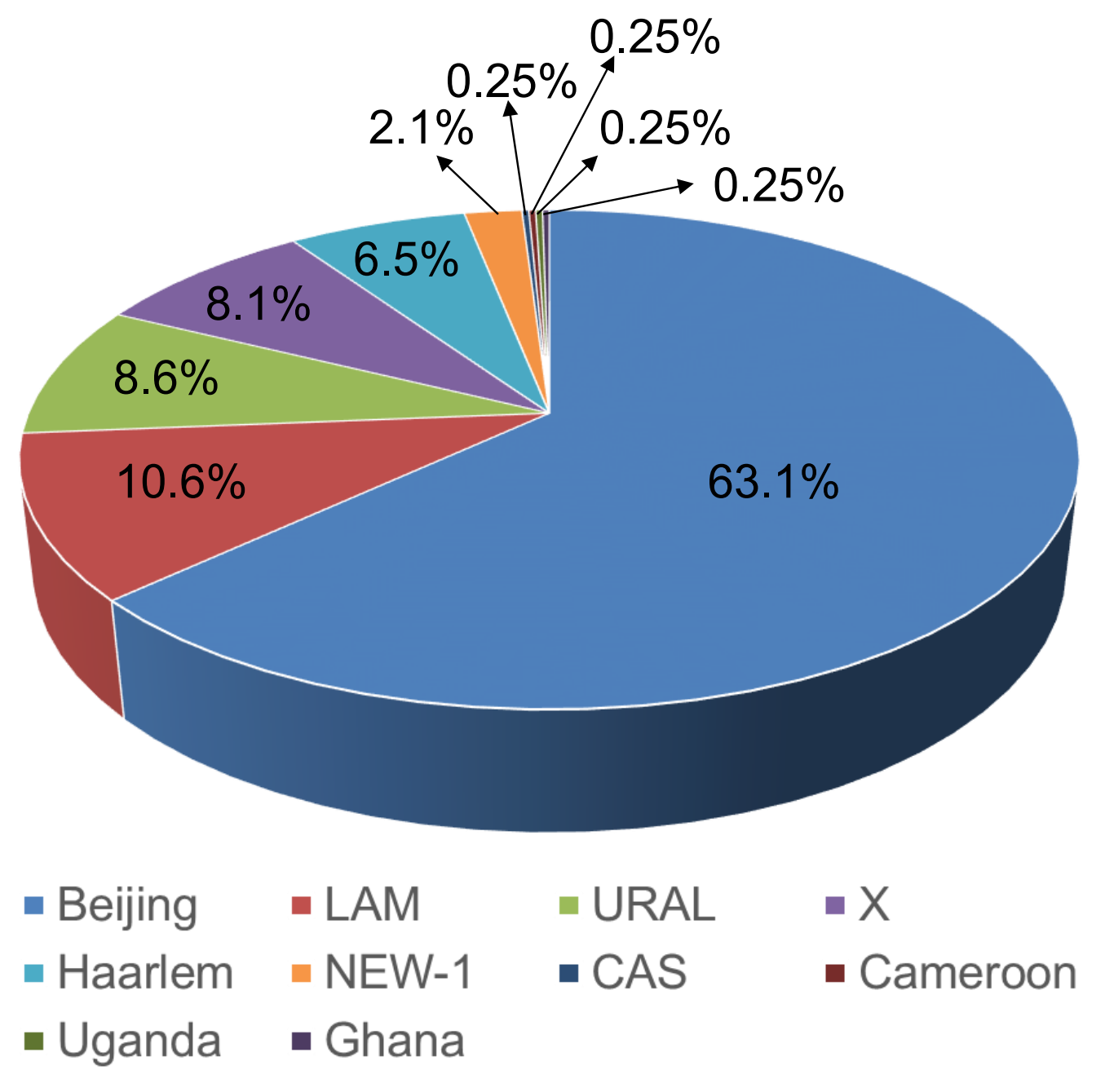


Figure 5. Genotypes of clinical isolates of *M. tuberculosis* among new TB cases

Conclusion

- Prevalence of Beijing family strains among new TB cases in Kazakhstan was shown (83,1%).
- We identified that Beijing genotype is responsible for ongoing TB transmission in Kazakhstan in the present time.
- This genotype is associated with drug resistance and currently distributed in the former USSR countries and countries of Eastern Europe.
- According to publications [3-5] the genotype is more virulent, that is why investigation of epidemiology of Beijing genotype plays crucial role in TB monitoring.

References

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Ainur Akhmetova, MSc, National Laboratory Astana, Nazarbayev University, 53 Kabanbay Batyr ave., 010000, Astana, Republic of Kazakhstan,
Tel: +7(717)2709318, e-mail: ainur.akhmetova2@nu.edu.kz, http://www.nu.edu.kz