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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

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თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

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GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения.

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

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7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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ავტორთა საყურადღებოდ!

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2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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USE OF 15 MIRU-VNTR GENOTYPING FOR DISCRIMINATING *M. TUBERCULOSIS* CLINICAL ISOLATES FROM KAZAKHSTAN

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Tuberculosis (TB) still remains one of the important health issues in Kazakhstan. According to World Health Organization (WHO), in 2018 the estimates of TB incidence and TB mortality in Kazakhstan were 68 cases per 100,000 population and 2,6 deaths per 100,000 population, respectively. Kazakhstan is in the list of 30 countries with high multidrug-resistant tuberculosis (MDR-TB) cases in the World. The rate of MDR-TB associated with resistance to the main two first-line antituberculosis (anti-TB) drugs rifampicin and isoniazid in Kazakhstan was 26 cases per 100,000 population [1]. With widespread of drug-resistant *M. tuberculosis* strains globally, especially MDR-TB and extensively drug-resistant TB (XDR-TB), the more severe form of MDR-TB monitoring and control of the disease distribution is increasingly important [2].

Genotyping methods of *M. tuberculosis* isolates are important tools that can be used to investigate outbreaks in order to find the source of the infection; to control recurrent cases of tuberculosis; to determine laboratory mistakes. Insertion sequence (IS) 6110 restriction fragment length polymorphism (RFLP) method is the first standardized technique for *M. tuberculosis* isolates that was developed by van Embden and his collegues in 1993 [3]. IS*6110*-RFLP genotyping is based on determination of the number of IS*6110* insertion sequence copies. The number of IS*6110* fragments may vary from 0 to 26 copies per isolate. IS*6110*-RFLP provides the highest discriminatory power for differentiating isolates in comparison to other *M. tuberculosis* genotyping approaches. For this reason today the method is a 'gold standard' of *M. tuberculosis* genotyping. In cases where isolates have less than six IS*6110* fragments, a secondary genotyping technique is needed to provide sufficient discrimination [3,4]. Other limitations of IS*6110*-RFLP method include complexity of the methodology, the need to use a large amount of biomass for the analysis. The method is time-consuming as it can take about 14-21 days from receipt of a sample before IS*6110*-RFLP results are at hand for comparison. Yet, interlaboratory comparison of IS*6110*-RFLP patterns can be difficult because of different experimental conditions and data interpretation [5-8]. Spacer oligonucleotide typing (Spoligotyping) is the second widely used method in genotyping of *M. tuberculosis* isolates [9]. This PCR-based technique is based on detection the presence or absence of 43 unique spacers in the direct repeat (DR) locus in the *M. tuberculosis* genome [10]. Small quantities of DNA are sufficient for the analysis, biological samples (sputum, biopsy material) can also be used. Spoligotyping is a quick and convenient genotyping approach that is well suited for the determination of *M. tuberculosis* Beijing family strains [11] the hybridization pattern of which is characterized by the absence of signals between 1 and 34 spacer sequences. Obtained spoligotyping results can be easily analyzed in publicly available databases such as SITVIT2 [12] and MIRU-VNTR*plus* [13]. However, discriminatory power of the technique is low. Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) is another widely used method for genotyping of *M. tuberculosis*. MIRU-VNTR is based on PCR amplification of overlapping regions of MIRU-VNTR loci scattered throughout the genome and determination of obtained amplicon sizes. As the sizes of repetitive units are known, obtained sizes affect to the number of amplified MIRU-VNTR copies. The method is fast and easy to perform. It takes around 2-3 days from the delivery of a sample to obtain MIRU-VNTR profiles [14]. Digital format of the data enables to compare obtained genotyping data with the derived MIRU-VNTR results in different laboratories of the globe using MIRU-VNTRplus database. The initial scheme of VNTR typing consisted of six exact tandem repeat (ETR) loci in 1998 [15], in 2001 Supply *et.al* [14] suggested to conduct the genotyping by 12 MIRU loci, that is known as classical MIRU set today. In 2006, an extended set of 24 MIRU-VNTR loci that included 15 highly discriminative loci was proposed by Supply *et.al* [16]. Investigations reveal that 12 MIRU typing can be used for large-scale prospective studies, but still needed higher discriminatory power comparable with IS*6110* RFLP [14, 17]. However, the use of additional MIRU-VNTR loci in combination with the previously proposed 12 MIRU scheme in both 15 and 24 loci techniques increased discriminatory power of MIRU-VNTR up to that of IS*6110* RFLP [16,18,19].

In our previous work (unpublished data), MIRU-VNTR genotyping was carried out for 81 *M. tuberculosis* clinical isolates from Almaty city using 24 loci. Evaluation of different MIRU-VNTR schemes was done for this sample collection. According to the obtained results 15 MIRU-VNTR (12 MIRU+3 ETR) loci were chosen for prescreening of *M. tuberculosis* isolates to reduce labor intensity of the method.

Aim of this study: to conduct genotyping to obtain preliminary information about the genetic diversity and a dominant genotype of *M. tuberculosis* in different regions of Kazakhstan by 15 MIRU-VNTR (12 MIRU+3 ETR) analysis.

Material and methods. *Mycobacterial isolates and clinical data.* A total of 271 *M. tuberculosis* clinical isolates were obtained from newly diagnosed pulmonary tuberculosis patients from South and North Kazakhstan and Almaty city, the largest city in Kazakhstan from 2016 to 2018. TB patients with a positive result of sputum culture on *M. tuberculosis* were included in the study. Epidemiological and clinical data, including age, gender, ethnicity, susceptibility profiles of mycobacterium samples etc. were collected for each patient. Study protocol, informed consent and all types of recruitment were approved by the Ethics committee of Center for Life Sciences, National Laboratory Astana, Nazarbayev University (Protocol №20 from 22.09.2017 and Protocol №05-2020 from 24.09.2020, Nur-Sultan city). Microbiological identification and isolation of pure cultures of *M. tuberculosis* were carried out in National Reference Laboratory of National Scientific Center of Phthisiopulmonology of the Republic of Kazakhstan (Almaty city).

Microbiological methods. Solid Lowenstein–Jensen nutrient medium was used for cultivation and isolation of *M. tuberculosis*, all the tubes with cultures were incubated at 36-37°C until colonies grew. Smears were prepared for microscopic examination from all the tubes with grown colonies. These smears were stained according to Ziehl - Neelsen. *In vitro* determination of

susceptibility to anti-tuberculosis drugs was carried out on the Löwenstein–Jensen medium by absolute concentrations method [20, 21] and by using BACTEC-MGIT 960 Mycobacteria Growth Indicator Tube (BD Diagnostic Systems, USA). Drug susceptibility to first line anti-tuberculosis drugs was carried out by absolute concentration method in accordance with WHO recommendations [21]. The results of microbiological investigations were recorded 28 days after sowing the cultures. Isolates were considered resistant when more than 20 colonies had grown on media containing anti-tuberculosis drugs. A loop with the microorganisms was placed in 1 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.0) and the bacteria were lysed by boiling at 80°C for 45-60 minutes. Cells were centrifuged at 12,000 rpm for 5 min and then cooled overnight at -20°C. The supernatant containing DNA was used for MIRU-VNTR genotyping of *M. tuberculosis* clinical isolates.

MIRU-VNTR genotyping. All 271 *M. tuberculosis* clinical isolates were genotyped by PCR amplification of 12 MIRU + 3 ETR (ETRA, ETRB, ETRC) in a Thermal cycler (BioRad, USA). Primer sequences for all loci are shown in Table 1. The PCR conditions for all MIRU-VNTR loci were 30 cycles of 30 seconds at 95°C, 1 minute at 63°C, and 1 minute at 72°C. Denaturation and extension steps for all loci were done for 15 minutes. PCR products were visualized on 2% agarose gel stained with ethidium bromide. 50 and 100-bp DNA Ladders (Invitrogen Life Technologies, USA) were used as molecular markers.

Quantity 1 (BioRad) program was used to detect the PCR fragment size and calculate the number of tandem repeats in each MIRU-VNTR locus. 15 MIRU-VNTR (12 MIRU +3ETR) profiles were obtained for all 271 clinical isolates where every number corresponded to the number of tandem repeats in a locus. Reference strain *M. tuberculosis H37Rv* was used as a control. 15-digit allelic profiles of all clinical isolates then were analyzed in MIRU-VNTR*plus* web resource to identify *M. tuberculosis* lineages. MIRU-VNTR clustering was evaluated using Unweighted pair group method with arithmetic mean (UPGMA) algorithm. Index of allelic polymorphism (h) was used to assess the numerical diversity of MIRU-VNTR loci and was calculated as follows [22]:

$h=1-\sum xi2\,[n/(n_i-1)]$,

where xi is the i allele frequency at the locus, and n is the number of isolates.

Results and discussion. Among 271 patients, 179 (66.1%) were males and 92 (33.9%) were females. The mean age of TB patients was 36.8 years. Two patients were 1 and 2 years old children, the age of the rest patients varied from 18 to 77. Regarding ethnicity 182 (67.2%) TB patients were Kazakhs, 53 (19.6%) - Russians, 12 (4.4%) – Ukrainians, 6 (2.2%) – Uyghurs, 4 (1.5%) – Koreans. Azerbaijanis, Tatars, Germans and Uzbeks were represented by 2 patients (0.7%) in each group. The rest six TB patients (2.2%) belonged to other ethnic groups such as Chechen, Lithuanian, Kyrgyz, Bulgarian, Armenian and Belarusian, respectively.

Table 1. Sequence of primers used in MIRU-VNTR genotyping

Locus	Sequence of primers
MIRU02F MIRU02R	5’-CAGGACACGGGTTCTACTG-3’ 5’-GGACTAGGTCGAGGTTGTGTC-3’
MIRU04F MIRU04R	5’-CAGGTCACAACGAGAGGAAGAGC-3’ 5’-GCGGATCGGCCAGCGACTCCTC-3’
MIRU10F MIRU10R	5’-GACTTCCAACAGCACCGTCTTATC-3’ 5’-TCGCACCGATCACGCTACG-3’
MIRU16F MIRU16R	5’-GTTGGAAACGGCGGTTATTGAC-3’ 5’-CGGAGTCGTCCAGCAAGACC-3’
MIRU20F MIRU20R	5’-TCGGAGAGATGCCCTTCGAGTTAG -3’ 5’-TCACGGTCTCCGCACTAACG-3’
MIRU23F MIRU23R	5’-CTCACCAGGATCGCCAAACC-3’ 5’-TCTGACTCATGGTGTCCAACC-3’
MIRU24F MIRU24R	5’-GCTTGTGCGGGAAGGCTA-3’ 5’-CGATCGCGGATCTTTGCT-3’
MIRU26F MIRU26R	5’-CCAGCAGTTGAGCACAGTTCG-3’ 5’-GGATAGGTCCGAGTTCGATTTCC-3’
MIRU27F MIRU27R	5’-CGGTGACCAACGTCAGATTC-3’ 5’-GCGATGTGAGCGTGCCACTCAA-3’
MIRU31F MIRU31R	5’-CTTCGGCGTCGAAGAGAGCCTC-3’ 5’-CGGAACGCTGGTCACCACCTAAG-3’
MIRU39F MIRU39R	5’-CATCGACAAACTGGAGCCAAAC-3’ 5’-GAAACGTCTACGCCCCACAC-3’
MIRU40F MIRU40R	5’-GCAAGAGCAAGAGCACCAAGC-3’ 5’-TGTCTAATCAGGTCTTTCTCTCACGC-3’
ETRAF ETRAR	5’-GATTGAGGGGATCGTGATTGG-3’ 5’-AAATCGGTCCCATCACCTTCTTAT-3’
ETRBF ETRBR	5’-GCGAACACCAGGACAGCATCATG-3’ 5’-GGCATGCCGGTGATCGAGTGG-3’
ETRCF ETRCR	5’-GTGAGTCGCTGCAGAACCTGCAG-3’ 5’-GGCGTCTTGACCTCCACGAGTG-3’

Table 2. M. tuberculosis families distributed among clinical isolates in Kazakhstan based on the 15 MIRU-VNTR analysis

***M. tuberculosis* families**	**Number of isolates**	**%**
Beijing	177	65.3
LAM	37	13.7
Ural	20	7.4
Cameroon	16	5.9
Haarlem	12	4.4
NEW-1	8	2.9
Delhi/CAS	1	0.4
Total:	271	100

Table 3. Allelic diversity of 15 MIRU-VNTR loci of 271 M. tuberculosis clinical isolates from Kazakhstan

MIRU-VNTR loci	**Number of repeats**											**h**
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
MIRU 2	-	30	239	2	-	-	-	-	-	-	-	0.21
MIRU 4	2	4	265	-	-	-	-	-	-	-	-	0.04
MIRU 10	-	4	22	182	33	10	6	4	2	4	4	0.52
MIRU 16	-	18	21	230	2	-	-	-	-	-	-	0.27
MIRU 20	-	11	260	-	-	-	-	-	-	-	-	0.07
MIRU 23	-	-	-	4	1	256	10	-	-	-	-	0.1
MIRU 24	-	270	1	-	-	-	-	-	-	-	-	0
MIRU 26	-	19	-	8	14	207	5	18	-	-	-	0.4
MIRU 27	-	-	3	262	6	-	-	-	-	-	-	0.06
MIRU 31	-	1	49	43	6	166	6	-	-	-	-	0.56
MIRU 39	-	2	99	169	1	-	-	-	-	-	-	0.48
MIRU 40	-	-	18	206	37	8	-	1	1	-	-	0.4
ETR A	-	1	33	54	180	3	-	-	-	-	-	0.51
VNTR 48 (ETR B)	-	9	261	1	-	-	-	-	-	-	-	0.07
VNTR 43 (ETR C)	-	-	40	13	206	12	-	-	-	-	-	0.4

MIRU-VNTR genotyping was performed for 271 *M. tuberculosis* clinical isolates using 15 MIRU-VNTR (12 MIRU + 3 ETR) loci. MIRU-VNTR analysis of 271 *M. tuberculosis* isolates from new cases of tuberculosis identified 97 genotypes, 70 (25.8%) of them were unique and were found only in one isolate among collected *M. tuberculosis* samples. The remaining 201 (74.2%) isolates were grouped into 27 clusters, each containing from 2 to 102 isolates. The biggest cluster consisted of 102 *M. tuberculosis* clinical isolates. Two clusters had 10 and 11 clinical isolates, respectively. One cluster contained 7 isolates, two clusters had 6 isolates and other two clusters 5 isolates each. Three and five clusters consisted of 4 and 3 clinical isolates, respectively. The remaining 11 clusters consisted of 2 *M. tuberculosis* clinical isolates in the cluster.

The results of the 15 MIRU-VNTR genotyping showed that 65.3% of all the isolates belonged to Beijing family strains (Table 2). The second biggest *M. tuberculosis* family that is distributed among new cases of tuberculosis in Kazakhstan is LAM, 13.7% of isolates were identified as strains of this family. In 7.4% cases strains of Ural family were determined. Cameroon and Haarlem families of *M. tuberculosis* were found in 5.9% and 4.4% cases, respectively. Other *M. tuberculosis* families such as NEW-1 and Delhi/CAS were detected in less than 3% cases.

The results of the 15 MIRU-VNTR approach revealed the most polymorphic and informative loci (Table 3). Allelic polymorphism analysis of the 15 MIRU-VNTR loci showed that MIRU 31 locus is the most discriminatory locus (h=0.56). The second polymorphic locus is MIRU 10 (h=0.52) followed by ETRA locus (h=0.51). Allelic diversity of MIRU 26, MIRU 39, MIRU 40 and ETR C loci varied from 0.4 to 0.48. MIRU 24 locus is less informative. Almost all isolates (n=270) had the same allelic variant, index of allelic polymorphism was equal to 0. MIRU 2, MIRU 4, MIRU 16, MIRU 20, MIRU 23, MIRU 27 and ETR B loci were also poor discriminant. Index of allelic polymorphism of the mentioned loci was between 0.04 and 0.27.

Data on drug resistance of 271 clinical isolates to the first-line anti-TB drugs revealed that 58.3% (158 isolates) of isolates were drug-resistant. Among 158 drug-resistant isolates 86 (54.4%) samples were MDR. 39 out of 158 isolates (24.7%) showed polyresistance, where *M. tuberculosis* samples were at least resistant to two drugs except MDR. And 33 out of 158 isolates (20.9%) were monoresistant. When analysis of drug resistance across *M. tuberculosis* families was conducted, it was noted that Beijing family isolates were more drug-resistant (121 out of 177 isolates, 68.4%), than susceptible (56 isolates, 31.6%). Among LAM family strains the number of drug-resistant isolates was higher too, more than 50% of isolates (20 isolates, 54.1%) were drug-resistant compared to the susceptible ones (17 isolates, 45.9%). MDR strains prevailed among both drug-resistant Beijing and LAM family strains. Among Ural, Cameroon, Haarlem and NEW-1 families, on the contrary, clinical isolates were more susceptible – 11 out of 20 (55%), 11 out of 16 (68.7%), 10 out of 12 (83.3%), 7 out of 8 (87.5%), respectively. One isolate that belonged to Delhi/CAS family was susceptible (Table 4).

*Table 4. Drug resistance of 271 isolates across M. tuberculosis families*e

***M. tuberculosis* families**	**Susceptible**	**Resistant**			**Number of isolates**
		Monoresistant	**Polyresistant (resistant at least to two drugs except MDR)**	**MDR**	
Beijing	56	18	32	71	177
LAM	17	7	3	10	37
Ural	11	4	3	2	20
Cameroon	11	4	-	1	16
Haarlem	10	-	1	1	12
NEW-1	7	-	-	1	8
Delhi/CAS	1	-	-	-	1
Total:	**113 (41.7%)**	33 (12.2%)	39 (14.4%)	86 (31.7%)	**271 (100%)**
		158 (58.3%)			

Today, more than ten methods of *M. tuberculosis* genotyping tools exist, MIRU-VNTR is one of the widely used method. This approach has proven to be quick and easy to conduct, and allows the comparison of obtained results between laboratories. 12 MIRU loci method has been widely used in many studies, but it is not productive for examination of clustered isolates [23, 24]. The optimized set of 24 MIRU-VNTR loci has slightly high discriminatory power compared to the 15 MIRU-VNTR loci method. However, 15 MIRU-VNTR loci approach is considered as a highly discriminatory technique for first-line *M. tuberculosis* genotyping that can replace 12 MIRU loci methodology [25,26].

Genotyping methods have been used for epidemiological studies of tuberculosis in various countries. In Kazakhstan, several studies were performed on identification of mutations in genes responsible for drug resistance [27, 28], whole genome sequencing of *M. tuberculosis* clinical isolates [29,30] and some studies on molecular genotyping of *M. tuberculosis* [28, 31, 32, 33]. In our work we performed 15 MIRU-VNTR (12 MIRU+3 ETR) analysis to obtain preliminary data about the genetic biodiversity and a prevalent genotype of *M. tuberculosis* in various regions of Kazakhstan.

Genotyping results of 271 *M. tuberculosis* clinical isolates analyzed by the 15 MIRU-VNTR in our study displayed that 7 *M. tuberculosis* families are distributed in studied regions of Kazakhstan. *M. tuberculosis* Beijing family is the largest family identified in our study. 65.3% of all isolates in the study belonged to this family. Beijing family strains were also prevalent genotype in other studies conducted in Kazakhstan. In our previous study Beijing family isolates were found in 78.4% cases among pyrazinamide-susceptible and pyrazinamide-resistant *M. tuberculosis* clinical isolates [28] and in the investigations of Ibrayeva *et.al* [32] 68.3% clinical isolates from the patients in the penitentiary system belonged to Beijing family. The genotyping results were obtained using 12 and 24 MIRU-VNTR loci, respectively in the mentioned studies. In the study of Skiba *et.al* [33] 24 MIRU-VNTR and spoligotyping techniques were used for genotyping, Beijing family strains were identified in 72.2% cases (109/151 isolates). Strains of Beijing family were found in 1990s in Beijing (China) and were responsible for several outbreaks [34, 35]. According to publications, strains of this family affect young individuals [9] and were associated with drug resistance in different countries [36, 37]. Association of Beijing genotype with drug resistance (in general) in Kazakhstan was shown by Kubica *et.al* [31]. Skiba *et.al* revealed association of MDR-TB with *M. tuberculosis* Beijing family isolates in the country [33].

The second largest *M. tuberculosis* family that is determined in Kazakhstan is LAM, the frequency of distribution of LAM family is 13.7%. Strains of LAM family particularly prevalent in South America. Majority of LAM isolates (>90%) distributed in former Soviet Union countries belong to LAM-RUS sublineage and were found in 13.3-41.8% cases [38]. Ural is another *M. tuberculosis* family that is spread in Kazakhstan, strains of this family were detected for the first time in Ural area (Russian Federation) [39]. 7.4% of all isolates were detected as Ural family strains in our study. Strains of LAM and Ural families prevailed among Kazakhstani *M. tuberculosis* isolates after Beijing family strains in investigations of Skiba *et.al* as well [33]. The frequency of these families were 11.3% (17/151 isolates) and 5.3% (8/151 isolates), respectively. The rest four *M. tuberculosis* families (Cameroon, Haarlem, NEW-1 and Delhi/CAS) were identified in less than 6% cases.

When the 15 MIRU-VNTR was introduced to investigate *M. tuberculosis* isolates the most allelic diversity was noted for MIRU 31, MIRU 10 and ETRA loci (h=0.56, 0.52 and 0.51). MIRU 24 locus showed the lowest discrimination, index of allelic polymorphism was equal to 0.

In this study, more than 50% (58.3%) of collected 271 clinical isolates were drug-resistant. Among *M. tuberculosis* lineages Beijing and LAM family strains mostly consisted of drug-resistant isolates – 68.4% and 54.1%, respectively with prevalence of multi-drug resistant isolates. Skiba *et.al* [33] showed that 88.9% of MDR isolates belonged to Beijing family ($p<0.0001$) and LAM family strains were more MDR than other non-Beijing genotypes ($p=0.01$). In order to assess whether Beijing and LAM family strains are associated with drug resistance overall and specifically with MDR-TB statistically in our study, we will further conduct DNA sequencing of genes responsible for resistance to first line anti-TB drugs. In spite of some limitations of the study such as samples only from several regions of Kazakhstan, the quantity of samples were higher compared to previous published studies on genotyping of Kazakhstani *M. tuberculosis* strains [28,31,32,33].

Conclusion. The results of 15 MIRU-VNTR (12 MIRU+3 ETR) genotyping showed that Beijing genotype is a dominant genotype of *M. tuberculosis* in the studied regions of Kazakhstan. 65.3% of the clinical isolates were determined as Beijing family strains in our study. Most of Beijing family isolates (68.4% - 121/177) were drug-resistant compared to other *M. tuberculosis* families. MDR-TB prevailed among drug-resistant Beijing (58.7% - 71/121) and LAM family (50% - 10/20) strains. Whether Beijing and LAM family strains have association with

drug resistance in general and especially with MDR-TB statistically will be evaluated further after sequencing of genes responsible for the drug resistance.

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REFERENCES

1. Global tuberculosis report 2019. Geneva: World Health Organization. Licence: CCBY-NC-SA3. 0IGO. 2019; 58-268.
2. Akkerman O *et.al*. Surveillance of adverse events in the treatment of drug-resistant tuberculosis: A global feasibility study. // International Journal of Infectious Diseases. 2019. 83: 72-76.
3. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B *et al.* Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. // J Clin Microbiol. 1993; 31:406–9.
4. Molina-Moya B, Lacoma A, García-Sierra N *et. al.* PyroTyping, a novel pyrosequencing-based assay for *Mycobacterium tuberculosis* genotyping. // Sci Rep . 2017; **7,** 6777. https://doi.org/10.1038/s41598-017-06760-5.
5. Jeon S, Lim N, Park S, Park M, Kim S. Comparison of PFGE, IS *6110*-RFLP, and 24-Locus MIRU-VNTR for Molecular Epidemiologic Typing of *Mycobacterium tuberculosis* Isolates with Known Epidemic Connections. // J Microbiol Biotechnol. 2018; 28(2):338-346. doi: 10.4014/jmb.1704.04042.
6. Cláudio José Augusto, Wânia da Silva Carvalho, Isabela Neves de Almeida, Lida Jouca de Assis Figueiredo, Nayanne Gama Teixeira Dantas, Philip Noel Suffys, Silvana Spíndola de Miranda. Comparative study of RFLP-IS6110 and MIRU-VNTR from *Mycobacterium tuberculosis* isolated in the state of Minas Gerais, Brazil. // Bacterial, Fungal and Virus Molecular Biology. 2018; 49(3):641-646. DOI: 10.1016/j.bjm.2017.04.015.
7. Jagielski T, Minias A, Ingen JV, Rastogi N, Brzostek A, Żaczek A and Dziadekb J. Methodological and Clinical Aspects of the Molecular Epidemiology of *Mycobacterium tuberculosis* and Other Mycobacteria. // Clin Microbiol Rev. 2016; 29(2): 239–290.
8. Ei PW, Aung WW, Lee JS, and Chulhun L. Molecular Strain Typing of *Mycobacterium tuberculosis*: a Review of Frequently Used Methods. // J Korean Med Sci. 2016; 31(11):1673–1683.
9. Erie H, Kaboosi H, Javid N, Shirzad-Aski H, Taziki M, Kuchaksaraee MB and Ghaemi EA. The high prevalence of *Mycobacterium tuberculosis* Beijing strain at an early age and extra-pulmonary tuberculosis cases. // Iran J Microbiol. 2017; 9(6): 312–317.
10. Ramazanzadeh R, Shakib P, Rouhi S, Mohammadi B, Mohajeri P, Borji S. Molecular epidemiology of Mycobacterium tuberculosis isolates in Iran using spoligotyping. // New Microbes and New Infections. 2020; 38: 100767
11. Mathuria JP, Srivastava GN, Sharma P, Mathuria BL, Ojha S, Katoch VM, Anupurba S. Prevalence of *Mycobacterium tuberculosis* Beijing genotype and its association with drug resistance in North India. // Journal of Infection and Public Health. 2017; 10 (4): 409-41.
12. Couvin D, David A, Zozio T. *et.al*. Macro-geographical specificities of the prevailing tuberculosis epidemic as seen through SITVIT2, an updated version of the *Mycobacterium tuberculosis* genotyping database. Infect.// Genet. Evol. 2019; 72: 31–43.
13. Thain N, Le C, Crossa A, Ahuja SD, Meissner JS, Mathema B, Kreiswirth B, Kurepina N, Cohen T, Chindelevitch L. Towards better prediction of Mycobacterium tuberculosis lineages from MIRU-VNTR data. Infection, // Genetics and Evolution. 2019; 72: 59-66.
14. Shi, J., Zheng, D., Zhu, Y. *et.al*. Role of MIRU-VNTR and spoligotyping in assessing the genetic diversity of *Mycobacterium tuberculosis* in Henan Province, China. // BMC Infect Dis. 2018; 18: 447.
15. Frothingham, R., and W. A. Meeker-O'Connell. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. // Microbiology. 1998; 144:1189–1196.
16. Silva C, Perdigao J, Jordao L, Portugal I. Mycobacterial interspersed repetitive unit typing and mutational profile for multidrug-resistant and extensively drug-resistant tuberculosis surveillance in Portugal: a 3-year period overview. // Int J Antimicrob Agents. 2014; 44(6):546–51.
17. Han H, Wang F, Xiao Y, Ren Y, Chao Y, Guo A *et al*. Utility of mycobacterial interspersed repetitive unit typing for differentiating *Mycobacterium tuberculosis* isolates in Wuhan, China. // J Med Microbiol 2007; 56:1219–23.
18. Weerasekera D, Pathirane H, Madegedara D, Dissanayake N, Thevanesam V & Magana-Arachchi DN. Evaluation of the 15 and 24-loci MIRU-VNTR genotyping tools with spoligotyping in the identification of Mycobacterium tuberculosis strains and their genetic diversity in molecular epidemiology studies, Infectious Diseases. 2019; 51 (3): 206-215, DOI: 10.1080/23744235.2018.1551619
19. Sann WWM, Namwat W, Faksri K, Swe TL, Swe KK, Thwin T, Sangka A. Genetic diversity of *Mycobacterium tuberculosis* using 24-locus MIRU-VNTR typing and Spoligotyping in Upper Myanmar. *J Infect Dev Ctries* 2020; 14(11):1296-1305. doi:10.3855/jidc.12998
20. World Health Organization. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. WHO. ISBN 978-92-4-151484-2. 2018. P. 13.
21. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. Ed. 2nd. Geneva. World Health Organization. 2003. P. 73.
22. Lan Y, Liu M, He R, Li N, Zhang J *et.al.* Multipleloci Variable-number Tandem Repeat Typing of Clinical *Mycobacterium tuberculosis* isolates from Zunyi, Guizhou Province of China.// J Clin Microbiol Biochem Technol. 2015. 1(1):001-006
23. Supply P, Lesjean S, Savine E, Kremer K, van Soolingen D, Locht C. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. // J Clin Microbiol. 2001; 39(10):3563–71.
24. Shabbeer A, Cowan LS, Ozcaglar C *et al.* TB-Lineage: an online tool for classification and analysis of strains of *Mycobacterium tuberculosis* complex. // Infect Genet Evol. 2012;12:789–797.
25. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rusch-Gerdes S, Willery E *et. al.* Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. // Journal of clinical microbiology. 2006; 44(12):4498–510.
26. Baghbanian M, Zandi H, Zamani S, Javadpour S, Hamzehloo GR, Feizabadi MM. MIRU-VNTR analysis of *Mycobacterium tuberculosis* from Tehran, Sistan-Baluchestan, Kermanshah and Hormozgan during 2014 and 2015. // Cellular and Molecular Biology. 2017; 14-21.
27. Kozhamkulov U., Akhmetova A., Rakhimova S *et al*. Molecular characterization of rifampicin- and isoniazid-resistant *Mycobacterium tuberculosis* strains isolated in Kazakhstan. // Japanese Journal of Infectious Disease. 2011; 64:253-255.
28. Akhmetova A., Kozhamkulov U., Bismilda V *et al*. Mutations in the *pncA* and *rpsA* genes among 77 *Mycobacterium*

tuberculosis isolates in Kazakhstan. // International Journal of Tuberculosis and Lung Diseases. 2015; 19(2):179-184.
29. Kairov U., Kozhamkulov U., Molkenov A., Rakhimova S *et al.* Draft Genome Sequences of Two Clinical Isolates of *Mycobacterium tuberculosis* from Sputum of Kazakh Patients. // Genome announcements. 2015; 3(3):1-2.
30. Daniyarov A *et.al*. Whole genome sequence data of *Mycobacterium tuberculosis* XDR strain, isolated from patient in Kazakhstan. // Data Brief. 2020; 17;33:106416. doi: 10.1016/j.dib.2020.106416.
31. Kubica T., Agzamova R., Wright A., Aziz M.A., Rakishev G., Bismilda V., Richter E., Rüsch-Gerdes S., Niemann S. The Beijing genotype is a major cause of drug-resistant tuberculosis in Kazakhstan. // Int J Tuberc Lung Dis. 2005; 9(6):646–653.
32. Ibrayeva A., Kozhamkulov U., Raiymbek D *et al.* Molecular epidemiology of *Mycobacterium tuberculosis* strains circulating in the penitentiary system of Kazakhstan. // International Journal Tuberculosis and Lung Diseases. 2014; 18(3):298-301.
33. Skiba Y., Mokrousov I., Ismagulova G *et al*. Molecular snapshot of Mycobacterium tuberculosis population in Kazakhstan: A country-wide study. // Tuberculosis. 2015; 95(5): 538-546.
34. Reichman L.B *et.al*. The global epidemic of multi-drug-resistant tuberculosis. - New York: McGraw-Hill. 2001; 320.
35. Munsiff SS, Nivin B, Sacajiu G, Mathema B, Bifani P, Kreiswirth BN. Persistence of a highly resistant strain of tuberculosis in New York city during 1990–1999. // The Journal of Infectious Diseases. 2003; 188(3):356–363, https://doi.org/10.1086/376837
36. Zhang Z, Lu J, Liu M *et al.* Genotyping and molecular characteristics of multidrug- resistant *Mycobacterium tuberculosis* isolates from China. // J Infect. 2015; 70:335–345.
37. Kozinska M, Augustynowicz-Kopec E. Drug resistance and population structure of *Mycobacterium tuberculosis* Beijing strains isolated in Poland.// Pol J Microbiol. 2015; 64: 399–401.
38. Mokrousov I, Vyazovaya A, Narvskaya O. *Mycobacterium tuberculosis* Latin American-Mediterranean Family and Its Sublineages in the Light of Robust Evolutionary Markers. // J Bacteriol. 2014; 196(10):1833–1841. doi: 10.1128/JB.01485-13
39. Kovalev, S.Y., Kamaev, E.Y., Kravchenko, M.A., Kurepina, N.E., Skorniakov, S.N. Genetic analysis of *Mycobacterium tuberculosis* strains isolated in Ural region, Russian Federation, by MIRU-VNTR genotyping. // Int. J. Tuberc. Lung Dis. 2005; 9:746– 752.

SUMMARY

USE OF 15 MIRU-VNTR GENOTYPING FOR DISCRIMINATING *M. TUBERCULOSIS* CLINICAL ISOLATES FROM KAZAKHSTAN

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Tuberculosis is one of the main problems of medicine in Kazakhstan. Kazakhstan is on the list of 30 countries with high rates of multidrug resistant tuberculosis in the world. Aim of this study is to conduct genotyping by MIRU-VNTR method to get preliminary data on *M. tuberculosis* genotypes distributed among the clinical isolates in Kazakhstan.

271 *M. tuberculosis* clinical isolates were gathered from new cases of tuberculosis from different regions of Kazakhstan in this study. Genotyping was done using 15 MIRU-VNTR (12 MIRU+3 ETR) loci. Obtained digital profiles of the clinical isolates were analyzed using the database on miru-vntrplus.org. Phylogenetic tree was built by UPGMA method.

97 genotypes were identified, 70 (25.8%) of them were unique and were determined in one isolate in the sample collection. The rest 201 (74.2%) isolates were grouped into 27 clusters, that contained from 2 to 102 isolates. According to genotyping results *M. tuberculosis* Beijing family strains were found in 65.3% cases. 121 out of 177 Beijing isolates (68.4%) were drug-resistant. Prevalence of MDR-TB was detected among drug-resistant Beijing (58.7% - 71/121) and LAM family (50% - 10/20) isolates.

Keywords: tuberculosis, MIRU-VNTR genotyping, Kazakhstan.

РЕЗЮМЕ

ПРИМЕНЕНИЕ 15 MIRU-VNTR ГЕНОТИПИРОВАНИЯ ДЛЯ ХАРАКТЕРИСТИКИ *M. TUBERCULOSIS* КЛИНИЧЕСКИХ ИЗОЛЯТОВ В КАЗАХСТАНЕ

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Туберкулез является одной из основных проблем медицины в Казахстане. Казахстан входит в число 30 стран мира с высокими показателями туберкулеза с множественной лекарственной устойчивостью.

Цель исследования - генотипирование методом MIRU-VNTR для получения предварительных данных по генетическому разнообразию клинических изолятов *M. tuberculosis,* распространенных в Казахстане.

В исследовании собран 271 клинический изолят *M. tuberculosis* от впервые выявленных больных туберкулезом из различных регионов Казахстана. Генотипирование проведено с применением 15 MIRU-VNTR (12 MIRU+3 ETR) локусов. Полученные для всех клинических изолятов цифровые профили проанализированы с использованием базы данных miru-vntrplus.org. Филогенетическое древо построено методом UPGMA.

Идентифицировано 97 генотипов, 70 (25,8%) из которых были уникальными и обнаружены только у одного изолята в выборке. Оставшийся 201 (74,2%) изолят образовал 27 кластеров, которые включали в себя от 2 до 102 изолятов. По результатам генотипирования 177 (65,3%) изолятов идентифицированы как штаммы семейства Beijing *M. Tuberculosis*, из них 121 (68,4%) - лекарственно-устойчивый. Туберкулез с множественной лекарственной устойчивостью, в основном, встречался среди лекарственно-устойчивых изолятов семейства Beijing (58,7% - 71/121) и LAM (50% - 10/20).

რეზიუმე

15 MIRU-VNTR გენოტიპირების გამოყენება *M. TUBERCULOSIS*-ის კლინიკური იზოლატების დახასიათებისათვის ყაზახეთში

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ტუბერკულოზი რჩება ერთ-ერთ ძირითად სამედიცინო პრობლემად ყაზახეთში. ყაზახეთი მსოფლიოს 30 ქვეყანას შორისაა მრავლობითი სამკურნალწამლო რეზისტენტობის ტუბერკულოზის მაღალი მაჩვენებლებით.

კვლევის მიზანს წარმოადგენდა გენოტიპირება 15 MIRU-VNTR-მეთოდით წინასწარი მონაცემების მიღების მიზნით *M. tuberculosis* ყაზახეთში გავრცელებული კლინიკური იზოლატების გენეტიკური მრავალფეროვნების შესახებ.

კვლევისათვის ყაზახეთის სხვადასხვა რეგიონიდან ტუბერკულოზის პირველად დაავადებულთაგან შეგროვილია *M. tuberculosis* 271 კლინიკური იზოლატი. გენოტიპირება ჩატარდა 15 MIRU-VNTR (12 MIRU+3 ETR) ლოკუსების გამოყენებით. ყველა კლინიკური იზოლატისათვის მიღებული ციფრობრივი პროფილები გაანალიზებულია მონაცემთა ბაზის miru-vntrplus.org გამოყენებით. ფილოგენეზური ხე აგებულია UPGMA მეთოდით.

იდენტიფიცირებულია 97 ფენოტიპი, რომელთაგან 70 (25,8%) უნიკალურია და დაფიქსირდა მხოლოდ ერთ იზოლატში. დანარჩენმა 201 (74,2%) იზოლატმა შექმნა 27 კლასტერი, რომელიც მოიცავდა 2-დან 102-მდე იზოლატს. გენოტიპირების შედეგების მიხედვით, იზოლატების 65,3% იდენტიფიცირდა, როგორც *M. tuberculosis*-ის Beijing ოჯახის შტამები. Beijing-ის 177 იზოლატიდან 121 (68,4%) იყო წამალრეზისტენტული.

ტუბერკულოზი მრავლობითი სამკურნალწამლო რეზისტენტობით, ძირითადად, აღინიშნა Beijing ოჯახის წამალრეზისტენტულ იზოლატებს (58,7% - 71/121) და LAM-ს (50% - 10/20) შორის.

МЕТАБОЛИЧЕСКИЕ ОСОБЕННОСТИ ЖИРОВОЙ ТКАНИ И КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ АДИПОКИНОВ У БОЛЬНЫХ НЕАЛКОГОЛЬНОЙ ЖИРОВОЙ БОЛЕЗНЬЮ ПЕЧЕНИ (ОБЗОР)

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Жировая ткань — это не только энергетическое депо, но и эндо- и паракринный орган, который способен влиять на другие органы и системы. При избыточной массе тела пролиферация и гипертрофия адипоцитов сопровождается инфильтрацией макрофагами с последующим развитием воспалительных реакций, в результате чего изменяется метаболическая активность жировой ткани. Именно поэтому ряд ученых считают патологическое ожирение хроническим системным воспалительным процессом [4,30].

Жировая ткань является физиологическим резервуаром жирных кислот [17]. В случаях, когда способность к накоплению перегружена, эндокринные функции жировой ткани меняются, и дальнейшее накопление эктопического жира приводит к липотоксичности, которая способствует развитию воспаления и инсулинорезистентности (ИР) в печени [11]. В настоящее время липотоксичнисть рассматривается как движущая сила в механизме, который лежит в основе прогрессирования заболевания от простого стеатоза до неалкогольного стеатогепатита (НАСГ). Стеатоз печени может генерироваться такими механизмами, как: увеличение свободных жирных кислот увеличение потребления жира; повышенный липогенез de novo; уменьшение свободного окисления жиров и; снижение секреции печеночных триглицеридов.

Ожирение считается главной проблемой здравоохранения в двадцать первом веке. Это хроническое провоспалительное заболевание, которое системно влияет на нормальную физиологию и обмен веществ, вызывая множественные связанные заболевания, такие как сердечно-сосудистые заболевания, диабет, неалкогольная жировая болезнь печени (НАЖБП) и некоторые виды рака [30]. Наличие ожирения и, особенно, абдоминальный тип распределения жировой ткани способствуют формированию НАЖБП. Абдоминальное ожирение играет ведущую роль в развитии и прогрессировании ИР. Для диагностики висцерального ожирения используют индекс ОТ/ОБ (окружность талии/окружность бедра), что позволяет оценить приоритетное скопления жира в абдоминальном жировом депо.

По данным литературы [20], индекс массы тела (ИМТ) является независимым предиктором развития жировой инфильтрации печени. Как показали исследования распространенность НАЖБП линейно возрастает с увеличением ИМТ, достигая в 14 раз более высокого риска при ИМТ 37,5–40 кг/м² в сравнении с населением с нормальным весом. Как и ожидалось, абсолютный риск выше у пациентов с диабетом при любом ИМТ. В частности, что касается риска НАЖБП, наличие диабета в популяции с нормальной массой тела эк-