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Molecular Diversity of *Mycobacterium tuberculosis* Strains in Northwestern Iran

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Abstract

Background: Years after the development of antituberculosis (TB) drugs, many people continue to suffer from this disease. To control the spread of TB, strains of the *Mycobacterium tuberculosis* complex need to be determined, and sources of infection must be identified. Such steps should help to prevent transmission of the infection.
Objectives: The aim of this study was to perform molecular genotyping of isolates of the *M. tuberculosis* complex obtained from patients in northwestern Iran.
Methods: One hundred ninety-four culture-positive *M. tuberculosis* isolates obtained from patients in northwestern Iran were analyzed using the mycobacterial interspersed repetitive unit-exact tandem repeats (MIRU-ETR) method.
Results: The MIRU-ETR method distinguished 162 different patterns in the 194 isolates, comprising 23 clusters and 139 unique patterns. Its discriminatory power according to the Hunter-Gaston discriminatory index (HGDI) was 0.9978. The largest cluster contained six isolates.
Conclusions: This research indicated that various strains of *M. tuberculosis* were responsible for TB and that the majority of cases were due to reactivation.

Keywords: Tuberculosis, Molecular Diversity, Iran, ETR

1. Background

Years after the development of antituberculosis (anti-TB) drugs, deployment of a variety of diagnostic methods, and global attempts to control the disease, the number of people with TB remains high. According to WHO reports, there were 9.6-million TB cases in the world in 2014, with the rate of mortality as high as 1.5 million. In 2014, the prevalence rate of TB in Iran was reported to be 22 per 100,000 population (1). Increasing numbers of drug-resistant isolates pose a threat to human health. Thus, using methods to identify TB, especially drug-resistant isolates, can be effective in controlling the disease (2). TB-control plans depend on the identification of sources of infection (3), transmission routes (4), and reasons for treatment failure. Preventing the transmission of infection will limit the spread of the disease (5). Molecular genotyping of *Mycobacterium tuberculosis* isolates obtained from patients can detect the prevalence of new epidemics and distinguish TB due to reactivation from TB due to recent transmission (6). It can also identify risk factors for transmission (7, 8). Thus, the results of molecular genotyping can aid disease control.

Multiple molecular typing methods have been used to differentiate strains in molecular epidemiological studies. One such method is based on repeated sequences of variable number tandem repeats (VNTRs) (9). The exact tandem repeats (ETR)-VNTR method, which is also based on tandem repeats (i.e., A, B, and C loci), is of greater value in typing of strains (10). Another type of VNTR method utilizes mycobacterial interspersed repetitive units (MIRUs). Normally, loci 2, 4, 10, 16, 20, 23, 24, 26, 27, 37, 39, and 40 are used to determine the genotype (11). Different strains of *M. tuberculosis* possess different numbers of repeats. The re-

peats can be identified through PCR-based methods, and the number of repeats can be calculated by the size of the amplified products (12). Both VNTR- and PCR-based methods are suitable for assessing a variety of genetic strains.

2. Objectives

The aim of the present study was to perform molecular genotyping of isolates of the *M. tuberculosis* complex to obtain a better understanding of the disease and aid appropriate disease-prevention decision making in northwestern Iran, thereby reducing the burden of TB.

3. Methods

3.1. Mycobacterial Isolates

Isolates of the *M. tuberculosis* complex were obtained from patients who were referred to central TB laboratories in eastern and western Azerbaijan provinces in northwestern Iran from March 2004 to March 2005. The study population consisted of patients from whom at least one sample was culture positive for the *M. tuberculosis* complex. One hundred ninety-four isolates of the *M. tuberculosis* complex were collected. The isolates was identified as M. *tuberculosis* complex by Ziehl-Neelsen staining and standard microbiological tests, including the production of niacin, catalase activity, nitrate reduction, pigment production, and growth rate on Lowenstein-Jensen medium (13).

3.2. MIRU Typing

DNA was extracted from the mycobacterial isolates using lysozyme, SDS, proteinase K, and CTAB (14). PCR was carried out in a volume of 20 μL containing 10-100 ng of DNA; 0.5 μM of specific primers (3) (Metabion, Germany); 1.5 mM of $MgCl_2$; 100 μM of dATP, dCTP, dGTP, and dTTP; 50 mM of KCl; 20 mM of Tris-Cl (pH = 8.4); and 1.25 U of DNA polymerase (Cinagen, Iran). The PCR was done with an initial 7-min denaturation step at 94°C and a final extension step at 72°C. The temperature cycles for the different types of PCRs were as follows: 35 cycles of denaturation at 94°C for 45 seconds. The annealing temperatures used were 65, 63, 68, 65, 59, 65, 64, 63, 68, and 65°C for MIRU loci 2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39, and 40, respectively, for 45 seconds, followed by 72°C for 55 seconds and a final extension for 7 minutes at 72°C. In all the PCRs, the negative controls consisted of the PCR components in the reaction mixtures lacking *Mycobacterium* DNA. The PCR product was separated using 1.5% agarose gel electrophoresis. After staining with 0.5 μg/mL of ethidium bromide, the PCR product was visualized under UV light. The size of the fragments was determined using a 100-bp DNA ladder and a size marker (Fermentase, Lithuania).

3.3. ETR Typing

The PCR was performed in a volume of 20 μL containing 10-100 ng of DNA; 0.5 μM of specific primers (15) (Metabion, Germany); 1.5 mM of $MgCl_2$; 100 μM of dATP, dTTP, dCTP, and dGTP; 50 mM of KCl; 20 mM of Tris-Cl (pH 8.4); and 1.25 U of DNA polymerase (Cinnagen, Iran). The PCR was done with an initial 7-minute denaturation step at 94°C and a final extension step at 72°C. The temperature cycles for the different types of PCRs were as follows: 35 cycles of 45 seconds at 94°C, annealing temperatures for 50 seconds, and 72°C for 65 seconds. The annealing temperatures used for ETR-A, ETR-B, and ETR-C were 66, 68, and 69°C, respectively.

3.4. Statistical Analysis

All the isolates in this study were classified into two groups: clustered and nonclustered. Clustered were defined as two or more isolates with identical MIRU-ENTR types. Isolates with unmatched genetic patterns were considered nonclustered. Clustered isolates were assumed to have arisen from recent transmission. The MIRU-ETR allelic diversity (h) at a locus was calculated as follows:

$$h = 1 - \sum xi^2 / n\,(n-1) \quad (1)$$

where xi was the frequency of each allele at the locus, and n was the total number of strains in the typing scheme. The Hunter-Gaston discriminatory index (HGDI) was used as a numerical index of the discriminatory power of the MIRU-ETR method (16). The minimum estimate of the proportion of TB cases related to recent transmission was calculated (the number of clustered isolates - the number of clusters)/total number of isolates (17). Categorical data were compared by a chi-square test or Fisher's exact test. A p value below 0.05 was considered significant.

4. Results

One hundred ninety-four positive cultures of the *M. tuberculosis* complex were evaluated using the MIRU-ETR and MIRU-VNTR methods. Sixty-six isolates were from patients in western Azerbaijan, and 128 isolates were from patients in eastern Azerbaijan. The ages ranged from 40-day-old infants to elderly patients (88 years). In total, 162 different patterns were detected: 23 clusters and 139 unique patterns (71.65%), as shown in Table 1. The range of clustering was 28.35% and 32.03% in eastern Azerbaijan and 21.21% in western Azerbaijan. The largest cluster had six members (five females and one male), four of whom were from western Azerbaijan and two of whom were from eastern Azerbaijan. The isolates in some of the clusters showed no epidemiological correlation. Two clusters, one with four

members and three with three members, were detected in eastern Azerbaijan (Table 2). In the 23 clusters, 14 isolates were obtained exclusively from patients in eastern Azerbaijan, two isolates were obtained from patients only in western Azerbaijan, and seven isolates were obtained from patients in both provinces. Table 3 presents the allelic diversity. Loci 10, 26, 40, and A had the highest discriminatory power (> 0.6), followed by loci 4, 16, 20, 23, 24, 27, 31, B, and C, which had medium discriminatory power ($0.3 \leq \times \leq 0.6$), and loci 2 and 39, which had the lowest discriminatory power (< 0.3). Among 15 loci, locus 39 had the lowest allelic diversity, and locus 26 had the highest allelic diversity using the MIRU method. The MIRU-ETR method provided appropriate discriminatory power (HGDI = 0.9978).

In this study, 17% of TB cases were due to recent transmission ([55 - 23]/194). To distinguish the risk factors for recent transmission, 55 patients inside the cluster and 139 patients outside the cluster were compared (Table 4). The following factors did not show a significant relationship with the risk of recent transmission: age, sex, hospitalization during the previous year, a family member with TB, contact with a TB patient, smoking habit, diabetes, asthma, and imprisonment (P > 0.05). Individuals with extrapulmonary TB and patients with a history of anti-TB treatment were less common in the clusters (P < 0.05).

5. Discussion

In this study, the MIRU-ETR method was used for genotyping isolates of the *M. tuberculosis* complex in northwestern Iran. The rate of clustering among *Mycobacterium* strains was 28.35%, indicating that the majority of TB cases in this region of Iran were due to reactivation rather than recent transmission. Urban development has increased in northwestern Iran, especially in large cities (e.g., Tabriz and Urmia) and in towns, such as Maragheh, Mianeh, and Khoi, inhibiting the rate of Afghan migration to this territory. The numbers of TB cases were lower than in other parts of the country. Thus, the rate of clustering was also lower. The majority of the strains that were detected had unique patterns. In the present study, only 17% of TB cases were due to recent transmission in comparison to 37% in Casablanca, Morocco (18). Therefore, TB appears to be under control in northwestern Iran. However, many people with TB in the republic of Azerbaijan come to Iran for diagnosis and/or treatment, as TB treatment is free in Iran (19, 20). Thus, Iran will likely face increasing numbers of patients with TB, especially resistant TB, unless appropriate interventions are taken.

As shown by the results of the MIRU-VNTR analysis, allelic diversity varied throughout the different geographical regions. Regarding MIRU loci, locus 26 (h = 0.74) showed the highest variation. Among the ETR loci, the highest variation was observed at locus A (h = 0.65). Among MIRU loci in hospitals of Paris, France, locus 40 (h = 0.74) showed the highest variation (9). In three provinces in Iran, locus 16 (h = 0.64) exhibited the greatest variation (21), whereas in the province of Khuzestan in Iran, locus 31 (h = 0.73) displayed the highest variation (22). In a genotyping study of *M. tuberculosis* in China, locus C (h = 0.54) had the highest variation among ETR loci (23). In a similar survey in Taiwan, locus 26 (h = 0.77) had the highest variation (24). The level of allelic variation is due to the presence of different isolates in various countries. In common with other research, in this study, the allelic diversity of some loci was lower than 0.6. Omitting these loci in future studies, and adding loci, such as VNTR3820 (23), QUB11a, QUB11b, and QUB3232 (15), which show greater allelic diversity in different populations, would likely make it easier to differentiate between strains.

The discriminatory power of MIRU-ETR in this study was 0.9978 for 194 cases, and 162 patterns were observed. In a study of TB cases in Singapore, Sun et al. (25) reported a discriminatory power of 0.994 for 68 cases. Studies of TB cases performed in Khoozestan province in Iran, Taiwan, and Samara in Russia reported a discriminatory power of 0.991 for 61 cases, 0.972 for 502 cases, and 0.625 for 129 cases, respectively (22, 24, 26). As the study in Singapore was based on a small number of isolates, its discriminatory power was slightly lower than in the present study. In the Russian study, all the isolates were from the Beijing family, and 43.6% of strains were from the Beijing family in the Taiwanese study. Therefore, the HGDI was low in those studies. In the present study in northwestern Iran, the high number of different patterns was due to the older age of the study population, increase in the percentage of reactivation of latent infection, and control of infection among young people. Therefore, the MIRU-VNTR method, together with the ETR method, may be appropriate for assessing recent cases of TB transmission in Iran. If further discriminatory power is required, Queen's university of Belfast could be used.

In this study, the number of clusters identified by MIRU typing was lower than that detected by ETR typing. Based on MIRU typing, there were 30 clusters containing 86 isolates, with 2 - 12 patients in each cluster. Based on ETR typing, there were 164 isolates in each cluster. When ETR typing was added, the rate of recent transmission dropped from 29% ([86 - 30]/194) to 17% ([55 - 23)/194]), which led to the omission of false clustering. The largest cluster contained six members, and the mean number of cases in each cluster was 2.39 (55/23). In studies conducted in Hong Kong (27), Samara in Russia (21), Anambra in Nigeria (28), three provinces in Iran (21), and Stockholm in Sweden (29), the

Table 1. Discriminatory Power of MIRU and ETR Typing Alone and Together[a]

Method	Distinct Pattern	Unique Pattern	No. of Clusters	Isolates Included in Clusters	HGDI
MIRU	138	108	30	86	0.9930
ETR-VNTR	55	30	25	164	0.9322
MIRU-ETR	162	139	23	55	0.9978

[a] A score of 1 on the HGDR denoted the highest discriminatory power.

Table 2. MIRU-ETR Clusters of Isolates

MIRU-ETR Pattern[a]	No. of Isolates in the Cluster
2333 - 2325 - 2322 - 425	6
2343 - 2525 - 3323 - 323	4
2333 - 1624 - 3322 - 224	3
2333 - 2325 - 2322 - 424	3
2233 - 2525 - 3322 - 556	3
2323 - 1525 - 3322 - 314	2
2331 - 1515 - 3423 - 314	2
2333 - 1515 - 3322 - 314	2
2342 - 2524 - 3323 - 312	2
2341 - 2512 - 3322 - 324	2
2323 - 1525 - 3321 - 216	2
2323 - 2513 - 3323 - 314	2
2223 - 2525 - 3323 - 314	2
2323 - 1512 - 3323 - 314	2
2333 - 2323 - 3322 - 424	2
1324 - 1515 - 3324 - 424	2
2332 - 1524 - 3324 - 324	2
2363 - 2523 - 3323 - 322	2
2243 - 2325 - 3322 - 425	2
2342 - 2524 - 3323 - 314	2
2332 - 2615 - 3322 - 224	2
2333 - 2516 - 3323 - 314	2
2323 - 2526 - 3313 - 314	2

[a] Order of MIRU and ETR loci 2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39, 40, A, B, and C.

largest clusters contained 77, 75, 61, 13, and 4 members, respectively. The mean number of cases in each cluster was 2.24 (29). Therefore, we can conclude that TB in this part of Iran is of a micro-epidemic type.

Various other genotyping methods are available. However, some methods, such as Spoligotyping, have low distinguishing power (29). Another method, IS6110-RFLP,cannot be used for isolates with fewer than six bands. In addition, clustering is time consuming and not accurate (30). In this study, seven of the 23 clusters were common in the two provinces, indicating that transmission occurred between the provinces, possibly due to them being adjacent. However, the presence of a common cluster does not necessarily signify an epidemiological correlation. It is possible that two patients who live in different regions and have not had any contact with each other have bacteria with the same pattern and are therefore located in one cluster (31). In such cases, isolates with high prevalence might lead to erroneous epidemiological correlations.

In Iran, large numbers of patients from towns in western provinces attend treatment enters in eastern provinces (Tabriz city). They are then admitted to hospitals in the city. They may transmit or contract the disease during brief interactions with others in various places, such as restaurants, cafes, offices, and stadiums, or by using public transportation, such as buses. Golub et al. (32) reported that a TB patient can transmit the disease through random and short contact with susceptible individuals. In this study, age was not a significant risk factor for bacterial transmission ($P > 0.05$). The majority of individuals in the clusters were older than 60 years (43.64%), with the remainder young (25.45%) and medium aged (30.91%) (Table 4). Although younger age is considered a risk factor for the transmission of new strains of TB, the disease is more common among the elderly (33).

With aging, the rate of entrance into the clusters declines. In a study in Tehran city, 85.7% of patients in the cluster were younger than 35 years. The increase in the percentage of elderly in the clusters in the present study may be attributed to unemployment, a poor diet lacking in nutrients, a low level of life study, a low prevalence of HIV, and even successfully controlling TB infection among young people in northwestern Iran. In the current study, gender was also not a risk factor ($P > 0.05$), with males and females accounting for 50.9% and 49.1% of those in the clusters. In a previous study of TB transmission in Tehran city, being male was a risk factor for inclusion in clusters (8). It seems that the difference was because of under-employment, low literacy, and high poverty among females in the male-dominated, patriarchal region of northwestern Iran. Due

Table 3. Allelic Diversity of Each MIRU and ETR Locus

Locus	Number of Isolates with the Specified MIRU and ETR Copy Number										Allelic Diversity (h)
	0	1	2	3	4	5	6	7	8	9	
2	1	11	177	5							0.16
4		4	41	145	4						0.39
10		1	40	96	32	11	7	4	2	1	0.68
16		16	47	118	12	11					0.56
20		53	139	2		1					0.41
23	1	1	2	37	5	127	19	1	1		0.51
24		61	131	2							0.44
26		8	27	21	53	75	8	2			0.74
27		6	40	146	2						0.39
31		17	158	18	1						0.32
39		9	183	2							0.10
40		5	67	81	32	7	2				0.67
A	1	1	41	88	59	4		1			0.65
B		54	128	2	5	5					0.48
C			22	15	124	23	8	2			0.55

Table 4. Risk Factors for Clustering in Northwestern Iran

Risk Factor	No. (%) of Patients	No. (%) of Clustered Patients	No. (%) of Non Clustered Patients	P Value
Age, y				0.825
≤ 40	55 (28.35)	14 (25.45)	41 (29.50)	
41 - 59	60 (30.93)	17 (30.91)	43 (30.93)	
≥ 60	79 (40.72)	24 (43.64)	55 (39.57)	
Sex				0.945
Male	98 (50.51)	28 (50.9)	70 (50.36)	
Female	96 (49.49)	27 (49.1)	69 (49.64)	
Site of TB				0.454
Pulmonary	171 (88.14)	50 (90.9)	121 (87.05)	
Extrapulmonary	23 (11.86)	5 (9.1)	18 (12.95)	
Previous TB treatment	16 (8.25)	0 (0)	16 (11.51)	0.007
Previous hospitalization, during the last year	78 (40.21)	30 (54.55)	48 (34.53)	0.01
Family history of TB	34 (17.53)	11 (20)	23 (16.55)	0.569
Smoking	64 (32.99)	19 (34.55)	45 (32.37)	0.772
History of contact with a TB patient	33 (17.01)	11 (20)	22 (15.83)	0.486
Diabetes	26 (13.40)	9 (16.36)	17 (12.23)	0.446
Imprisonment	7 (3.61)	3 (5.46)	4 (2.88)	0.407
Asthma	6 (3.09)	2 (3.64)	4 (2.88)	>0.999

to the low prevalence of HIV in the region, the ratio was higher in men than women.

In this study, previous TB treatment was considered a risk factor. Thus, patients who had received treated were not included in the clusters ($P < 0.05$). According to previous research, TB transmission can be prevented by identifying people with active TB, treating the disease, and educating patients about noncontact with others (5). In this study, previous hospitalization was also considered a risk factor, but the results showed that this factor was not statistically significant ($P > 0.05$). However, among 55 individuals in clusters, 30 (54.55%) had been hospitalized dur-

ing the last year, suggesting that those who were admitted to hospitals had low immunity. The isolation of these individuals, some of whom tested positive for the M. *tuberculosis* complex following hospitalization, is not appropriate. TB can spread in the hospital during intubation and tests, such as bronchoscopy, radiology, scans, and sonography, due to connective air conditioning.

We conclude that various strains of *Mycobacteria* are responsible for the spread of TB in northwestern Iran. Most of the TB cases were due to reactivation. Given the discriminatory power of the MIRU-VNTR method (0.9978), it is suitable for detecting the disease once sufficient numbers of cases are available for evaluation. Thus, it can be employed as a first-line method in genotyping of TB strains in Iran.

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Footnotes

Authors' Contribution: Study concept and design, Mohammad Asgharzadeh, Jalil Rashedi, and Mahya Pourostadi; analysis and interpretation of data, Mohammad Asgharzadeh and Jalil Rashedi; drafting of the manuscript, Mahya Pourostadi, Rashedi Jalil, and Behroz Mahdavi poor; critical revision of the manuscript for important intellectual content, Behroz Mahdavi Poor, Hossein Samadi Kafil, and Mahya Pourostadi; statistical analysis, Samaneh Shirazi.

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References

1. WHO . Global tuberculosis report 2015. Geneva: World Health Organization; 2015.
2. Khosravi AD, Goodarzi H, Alavi SM, Akhond MR. Application of Deletion-Targeted Multiplex PCR technique for detection of Mycobacterium tuberculosis Beijing strains in samples from tuberculosis patients. *Iran J Microbiol.* 2014;**6**(5):330–4. [PubMed: 25848523].
3. Asgharzadeh M, Khakpour M, Salehi TZ, Kafil HS. Use of mycobacterial interspersed repetitive unit-variable-number tandem repeat typing to study Mycobacterium tuberculosis isolates from East Azarbaijan province of Iran. *Pak J Biol Sci.* 2007;**10**(21):3769–77. [PubMed: 19090229].
4. Borgdorff MW, Nagelkerke NJ, de Haas PE, van Soolingen D. Transmission of Mycobacterium tuberculosis depending on the age and sex of source cases. *Am J Epidemiol.* 2001;**154**(10):934–43. [PubMed: 11700248].
5. Asgharzadeh M, Samadi Kafil H, Pourostadi M. Source case identification and control of tuberculosis by molecular epidemiology [in Persian]. *J Mazandaran Univ Med Sci.* 2014;**24**(115):181–91.
6. Bandera A, Gori A, Catozzi L, Degli Esposti A, Marchetti G, Molteni C, et al. Molecular epidemiology study of exogenous reinfection in an area with a low incidence of tuberculosis. *J Clin Microbiol.* 2001;**39**(6):2213–8. doi: 10.1128/JCM.39.6.2213-2218.2001. [PubMed: 11376059].
7. Singh M, Mynak ML, Kumar L, Mathew JL, Jindal SK. Prevalence and risk factors for transmission of infection among children in household contact with adults having pulmonary tuberculosis. *Arch Dis Child.* 2005;**90**(6):624–8. doi: 10.1136/adc.2003.044255. [PubMed: 15908630].
8. Farnia P, Mohammadi F, Masjedi MR, Varnerot A, Zarifi AZ, Tabatabee J, et al. Evaluation of tuberculosis transmission in Tehran: using RFLP and spoligotyping methods. *J Infect.* 2004;**49**(2):94–101. doi: 10.1016/j.jinf.2003.11.015. [PubMed: 15236915].
9. Mazars E, Lesjean S, Banuls AL, Gilbert M, Vincent V, Gicquel B, et al. High-resolution minisatellite-based typing as a portable approach to global analysis of Mycobacterium tuberculosis molecular epidemiology. *Proc Natl Acad Sci U S A.* 2001;**98**(4):1901–6. doi: 10.1073/pnas.98.4.1901. [PubMed: 11172048].
10. Frothingham R, Meeker-O'Connell WA. Genetic diversity in the Mycobacterium tuberculosis complex based on variable numbers of tandem DNA repeats. *Microbiology.* 1998;**144 (Pt 5)**:1189–96. doi: 10.1099/00221287-144-5-1189. [PubMed: 9611793].
11. Supply P, Mazars E, Lesjean S, Vincent V, Gicquel B, Locht C. Variable human minisatellite-like regions in the Mycobacterium tuberculosis genome. *Mol Microbiol.* 2000;**36**(3):762–71. [PubMed: 10844663].
12. Blackwood KS, Wolfe JN, Kabani AM. Application of mycobacterial interspersed repetitive unit typing to Manitoba tuberculosis cases: can restriction fragment length polymorphism be forgotten?. *J Clin Microbiol.* 2004;**42**(11):5001–6. doi: 10.1128/JCM.42.11.5001-5006.2004. [PubMed: 15528687].
13. Rafi A, Moaddab SR. Principles of mycobacteriology [in Persian]. 1st. Tabriz: Sotude publications; 2003.
14. Asgharzadeh M, Samadi Kafil H, Khakpour M. Comparison of mycobacterial interspersed repetitive unit-variable number tandem repeat and IS6110-RFLP methods in identifying epidemiological links in patients with tuberculosis in Northwest of Iran. *Ann Microbiol.* 2008;**58**(2):333–9. doi: 10.1007/bf03175339.
15. Kam KM, Yip CW, Tse LW, Leung KL, Wong KL, Ko WM, et al. Optimization of variable number tandem repeat typing set for differentiating Mycobacterium tuberculosis strains in the Beijing family. *FEMS Microbiol Lett.* 2006;**256**(2):258–65. doi: 10.1111/j.1574-6968.2006.00126.x. [PubMed: 16499615].
16. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol.* 1988;**26**(11):2465–6. [PubMed: 3069867].
17. Asgharzadeh M, Kafil HS, Roudsary AA, Hanifi GR. Tuberculosis transmission in Northwest of Iran: using MIRU-VNTR, ETR-VNTR and IS6110-RFLP methods. *Infect Genet Evol.* 2011;**11**(1):124–31. doi: 10.1016/j.meegid.2010.09.013. [PubMed: 20951237].
18. Tazi L, Reintjes R, Banuls AL. Tuberculosis transmission in a high incidence area: a retrospective molecular epidemiological study of Mycobacterium tuberculosis in Casablanca, Morocco. *Infect Genet Evol.* 2007;**7**(5):636–44. doi: 10.1016/j.meegid.2007.06.005. [PubMed: 17689298].
19. Rashedi J, Mahdavi Poor B, Rafi A, Asgharzadeh M, Abdolalizadeh J, Moaddab SR. Multidrug-resistant tuberculosis in north-west of Iran and Republic of Azerbaijan: a major public health concern for Iranian people. *J Res Health Sci.* 2015;**15**(2):101–3. [PubMed: 26175292].
20. Asgharzadeh M, Shahbabian K, Samadi Kafil H, Rafi A. Use of DNA Fingerprinting in Identifying the Source Case of Tuberculosis in East Azarbaijan Province of Iran. *J Med Sci.* 2007;**7**(3):418–21. doi: 10.3923/jms.2007.418.421.

21. Zamani S, Aflaki M, Fooladi AA, Darban-Sarokhalil D, Bameri Z, Khazaee S, et al. MIRU-VNTR analysis of the Mycobacterium tuberculosis isolates from three provinces of Iran. *Scand J Infect Dis.* 2013;**45**(2):124–30. doi: 10.3109/00365548.2012.717233. [PubMed: 22954102].
22. Vatani S, Khosravi AD, Feizabadi MM, Jolodar A. Study of genetic diversity in Mycobacterium tuberculosis by using mycobacterial interspersed repetitive unit: Variable number tandem repeat typing in Khuzestan Province, Iran. *Afr J Microbiol Res.* 2011;**5**(12):1549–56. doi: 10.5897/ajmr11.206.
23. Liu RX, Li QZ, Xing LL, Peng Z, Zhu CM, Yang ZH. Genotyping of clinical Mycobacterium tuberculosis isolates based on eight loci of MIRU-VNTR. *Int J Tuberc Lung Dis.* 2013;**17**(2):243–5. doi: 10.5588/ijtld.12.0777. [PubMed: 23317961].
24. Chin PJ, Jou R. A modified automated high-throughput mycobacterial interspersed repetitive unit method for genotyping Mycobacterium tuberculosis. *Diagn Microbiol Infect Dis.* 2005;**53**(4):325–7. doi: 10.1016/j.diagmicrobio.2005.05.013. [PubMed: 16289635].
25. Sun YJ, Lee AS, Ng ST, Ravindran S, Kremer K, Bellamy R, et al. Characterization of ancestral Mycobacterium tuberculosis by multiple genetic markers and proposal of genotyping strategy. *J Clin Microbiol.* 2004;**42**(11):5058–64. doi: 10.1128/JCM.42.11.5058-5064.2004. [PubMed: 15528696].
26. Nikolayevskyy V, Gopaul K, Balabanova Y, Brown T, Fedorin I, Drobniewski F. Differentiation of tuberculosis strains in a population with mainly Beijing-family strains. *Emerg Infect Dis.* 2006;**12**(9):1406–13. doi: 10.3201/eid1209.041263. [PubMed: 17073090].
27. Kam KM, Yip CW, Tse LW, Wong KL, Lam TK, Kremer K, et al. Utility of mycobacterial interspersed repetitive unit typing for differentiating multidrug-resistant Mycobacterium tuberculosis isolates of the Beijing family. *J Clin Microbiol.* 2005;**43**(1):306–13. doi: 10.1128/JCM.43.1.306-313.2005. [PubMed: 15634987].
28. Uzoewulu GN, Lawson L, Nnanna IS, Rastogi N, Goyal M. Genetic diversity of Mycobacterium tuberculosis complex strains isolated from patients with pulmonary tuberculosis in Anambra State, Nigeria. *Int J Mycobacteriol.* 2016;**5**(1):74–9. doi: 10.1016/j.ijmyco.2015.06.008. [PubMed: 26927993].
29. Jonsson J, Hoffner S, Berggren I, Bruchfeld J, Ghebremichael S, Pennhag A, et al. Comparison between RFLP and MIRU-VNTR genotyping of Mycobacterium tuberculosis strains isolated in Stockholm 2009 to 2011. *PLoS One.* 2014;**9**(4):95159. doi: 10.1371/journal.pone.0095159. [PubMed: 24733167].
30. Allix-Beguec C, Fauville-Dufaux M, Supply P. Three-year population-based evaluation of standardized mycobacterial interspersed repetitive-unit-variable-number tandem-repeat typing of Mycobacterium tuberculosis. *J Clin Microbiol.* 2008;**46**(4):1398–406. doi: 10.1128/JCM.02089-07. [PubMed: 18234864].
31. Braden CR, Templeton GL, Cave MD, Valway S, Onorato IM, Castro KG, et al. Interpretation of restriction fragment length polymorphism analysis of Mycobacterium tuberculosis isolates from a state with a large rural population. *J Infect Dis.* 1997;**175**(6):1446–52. [PubMed: 9180185].
32. Golub JE, Cronin WA, Obasanjo OO, Coggin W, Moore K, Pope DS, et al. Transmission of Mycobacterium tuberculosis through casual contact with an infectious case. *Arch Intern Med.* 2001;**161**(18):2254–8. [PubMed: 11575983].
33. Maguire H, Dale JW, McHugh TD, Butcher PD, Gillespie SH, Costetsos A, et al. Molecular epidemiology of tuberculosis in London 1995-7 showing low rate of active transmission. *Thorax.* 2002;**57**(7):617–22. [PubMed: 12096206].