There is significant relationship between Beijing genotype family strains and resistance to the first-line anti-tuberculosis drugs in the Iranian population

Masoud Keikha[a,b,c]

[a] Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran

Dear Editor,

Tuberculosis is one of the most important global concerns, as it is considered as the second leading cause of death due to the infectious diseases after HIV. According to the WHO reports in 2020, it is estimated that around 10 million people have affected to TB and 1.5 million deaths occurred due to that in 2019 [1,2].

Iran is also one of the countries in the Middle-East region with a population of about 82 million; incidence of TB in Iran is estimated at about 11,000 people. Tuberculosis control and monitoring in Iran is very important due to its proximity to the countries such as Azerbaijan and Armenia (both high MDR-TB) and Afghanistan and Pakistan (with high-TB burden) [1-3]. One of the most important strategies in designing the regional programs for the control and prevention of tuberculosis is the identification and tracking of community circulating *Mycobacterium tuberculosis (Mtb)* strains by the molecular epidemiology techniques [3,4].

According to the literature review, the most common *Mtb* families in Iran are Beijing (Beijing / W), Haarlem (HaarlemI / HaarlemII and New-1), Delhi/Cas, East-Asian Indian, while TUR, Ural, LAM and bovis are less separable [5,6].

Evidence has been suggested in recent years about the relationship of drug resistance and some *Mtb* lineages [5-7]. Thus, this study was performed aiming to determine the frequency of dominant lineages in Iran to evaluate the possible relationship of each of the Beijing, Delhi/Cas, Haarlem and EAI lineages with the first-line drug resistance, especially isoniazid, rifampin and MDR-TB (Multi-drug resistant TB).

In order to fulfil the study, we attempted to investigate all the required studies of the Iranian population by searching PubMed, Scopus, Embase, Cochrane library, Google Scholar and Iranian databases Iranmedex, SID and ISC by March 2020. The search was done with the MeSH keywords including "*Mycobacterium tuberculosis*", "TB", "drug-resistance", and "genotyping". The inclusion criteria included: 1- Studies involving the prevalence of our genotypes in susceptible isolates and drug resistance, and 2- Studies in which genotyping was determined by the standard techniques of Spoligotyping, MIRU-VNTR, IS6110 RFLP, and Whole genome sequencing (WGS); and case reports,

repetitive sample studies with, and nonclinical studies were included. Finally, the probable relation between the infection and each of the Beijing, Delhi/Cas, Haarlem and EAI genotypes with first-line drug resistance was measured by the Odds Ratio (ORs) with 95% confidence intervals and the Egger weighted regression method was also determined [8].

Based on our investigation, 28 original articles including Persian and English articles were included in the present quantitative analysis (Table 1). In the present study, 6671 *Mtb* strain data were evaluated. The studies were limited to 2006–2019 and were conducted in the areas including Tehran (National Research Institute of Tuberculosis and Lung Disease), Tabriz, Golestan, Kermanshah and Mashhad.

The studied *Mtb* strains included two groups of susceptible and drug-resistant TB, with drug-resistant TB strains being resistant to the antibiotics such as isoniazid, rifampin, streptomycin, ethambutol and pyrazinamide.

In general, the frequency of Beijing (22.5%), Delhi/Cas (66.7/66.7%), Haarlem (264/60.2) and EAI (22.3/514.5%) lineages were evaluated, while the distribution of lineages in first-line drug resistant strains were evaluated as Beijing (76.2%/398%), Delhi/Cas (19 / 129.5%), Haarlem (29/17.1%) and EAI (16/81%). The distribution of *Mtb* lineages between susceptible and drug-resistant TB is demonstrated in Fig. 1.

Based on statistical analysis by our random effects model, a significant relationship was observed between the infection with *Mtb* Beijing strains and drug resistance (ORs: 1.54 (with 95% CIs); 0.7–3.5; $Q = 35.94; I^2 = 61.04; P = 0.02$). However, no significant relationship was observed between the infection with Delhi/Cas families (0.17; 0.11–0.22; $Q = 131.4; I^2 = 84.0; P = 0.00$), Harrlem (0.18; 0.1–0.2; $Q = 126.5; I^2 = 84.9;$ EAI (0.15; 0.0–0.2; $Q = 70.3; I^2 = 81.5; P = 0.00$) and drug resistance. Moreover, there was a significant relationship between first line drugs with *Mtb* Beijing strains and resistance to isoniazid and rifampin (2.19; 1.0–4.2; $Q = 77.6; I^2 = 88.4; P = 0.027$ and 1.60; 0.75–3.41; $Q = 59.1; I^2 = 89.84; P = 0.2$ respectively). Thus, according to the present meta-analysis results, the Delhi/Cas, Haarlem, and Beijing families were the three most common lineages in the tuberculosis patients in Iran, respectively, while Beijing
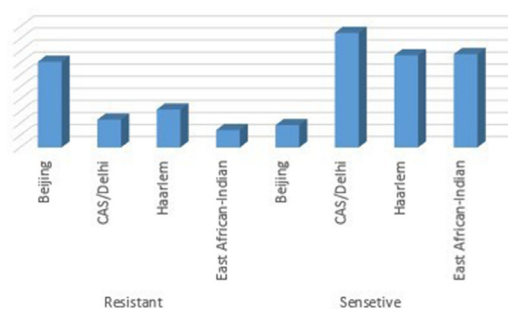
E-mail address: keikham971@mums.ac.ir.

Table 1
Characteristics of included studies.

First author	Publication year	City	Patients	MTB strains	MTB genotypes distribution								Typing method	Ref
					Beijing		CAS/Delhi		Haarlem		East African-Indian			
					DR	Total	DR	Total	DR	Total	DR	Total		
Ramazanzadeh	2006	NRITLD	345	195	195	195	0	0	0	0	0	0	Spoligotyping	9
Farnia	2006	NRITLD	3812	1074	12	12	11	11	44	44	10	10	Spoligotyping	10
Farnia	2007	NRITLD	NA	31	0	0	2	2	13	13	14	14	Spoligotyping	11
Amirmozafari	2007	NRITLD	NA	439	27	68	0	38	0	149	0	31	Spoligotyping	12
Farnia	2008	NRITLD	NA	258	1	12	0	49	8	20	0	58	Spoligotyping	13
Dousstdar	2008	NRITLD	NA	30	9	9	12	12	7	7	1	2	Spoligotyping	14
Doustdar	2008	NRITLD	NA	50	15	15	0	0	10	10	0	0	IS6110 RFLP	15
Doustdar	2009	NRITLD	NA	34	7	7	10	10	5	5	8	8	IS6110 RFLP	16
Rohani	2009	Mashhad	NA	113	5	8	0	0	0	0	0	0	Spoligotyping	17
Tajeddin	2009	NRITLD	NA	238	9	13	21	76	10	66	13	55	Spoligotyping	18
Velayati	2009	NRITLD	NA	146	5	5	4	4	9	9	5	5	MIRU-VNTR	19
Ahmadi	2009	NRITLD	238	238	9	13	21	66	10	66	13	55	Spoligotyping	20
Mozafari	2010	NRITLD	NA	105	7	20	NA	22	NA	2	0	0	Spoligotyping	21
Merza	2010	NRITLD	3812	1074	6	12	NA	181	NA	44	NA	227	Spoligotyping	22
Jafarian	2012	NRITLD	NA	96	1	3	7	7	15	36	0	0	MIRU-VNTR	23
Haeili	2013	NRITLD	NA	291	3	3	5	70	0	3	0	4	Spoligotyping	24
Sharifipour	2014	NRITLD	NA	190	5	11	8	37	8	72	0	0	Spoligotyping	25
Varahram	2014	NRITLD	151	151	10	14	4	23	12	31	14	42	Spoligotyping	26
Kardan Yamchi	2015	NRITLD	NA	31	8	8	2	2	1	1	1	1	Spoligotyping	27
Mohajeri	2016	Kermanshah	523	146	3	15	0	0	0	0	0	0	Multiplex PCR	28
Rezaei	2016	NRITLD	NA	20	3	3	3	3	10	10	0	0	Spoligotyping	29
Khanipour	2016	NRITLD	NA	723	9	9	1	1	11	11			Spoligotyping	30
Khosravi	2017	NRITLD	NA	88	9	9	0	0	3	3	0	0	IS6110 RFLP	31
Mansoori	2018	Golestan	11,807	164	5	19	3	31	0	2	0	0	MIRU-VNTR	32
Zaniani	2018	Isfahan	33	18	7	7	4	4	0	0	1	1	MIRU-VNTR	33
Vaziri	2019	NRITLD	13,892	606	14	14	7	7	0	0	0	0	WGS	34
Kazemian	2018	NRITLD	NA	34	11	11	4	4	0	0	1	1	Spoligotyping	SID
Afaghi-Gharamaleki	2019	Tabriz	125	115	3	7	0	1	0	0	0	0	MIRU-VNTR	35

NRITLD: National Research Institute of tuberculosis and lung disease; WGS: whole genome sequencing.

Fig. 1. Distribution of Iranian Predominant *Mycobacterium tuberculosis* lineages between susceptible and drug-resistant *Mtb* strains.

was evaluated with the most significant difference amongst DR-TB strains. We also found a significant relationship between the infection with Beijing *Mtb* strains and resistance to isoniazid and rifampin in the Iranian patients.

The Beijing tuberculosis family originated from Beijing, China; it was first introduced by Soolingen et al. (1995) as a predominant tuberculosis genotype of East Asia but has worldwide distribution and global distribution today [37,38]. The incidence of Beijing genotypes in recent years has been estimated at between 50% (East Asian countries) to 8.9% (Western countries) [6,38]. According to the review of the literature, the Beijing genotype is one of the predominant tuberculosis family in the Middle-East countries, especially in Iran [5,6,39]. Based on our analysis, the Beijing genotypes were the third most common genotype in Iran. Numerous studies have been published in recent years on the relationship between the Beijing TB genotypes and drug resistance [39-41]. Based on WGS (whole genome sequencing) data, the presence of multiple SNPs in the *katG, rpoB, embB, rpsL, rrs, eis, gyrA* and *gyrB* genes compared to other genotypes could be effective in increasing MIC relative to the anti-tuberculosis drugs [42]. There have been several studies, so far, on the relationship of the infection with

Beijing genotypes and drug resistance. However, some other studies contradict this phenomenon [30-45]. According to Ramazanzadeh et al. (2014) about 80% of drug resistant *Mtb* isolates from the Iranian patients belonged to the Beijing genotype. Also, in the study by Tarashi et al. (2017), amongst the MDR-TB isolates of Iranian patients the frequency of Beijing and Haarlem genotypes were 19.3% and 18.7%, respectively [5,6]. We also showed in this study that the Beijing genotype was the third most common genotype in the Iranian tuberculosis (TB) patients, and the infection with the Beijing family had significant relationship with the resistance to isoniazid and rifampin. Farnia et al. (2006) showed that Haarlem I and Beijing genotypes are the most common TB lineages of the Afghan population residing in Iran, who are affected to MDR-TB [10].

Other similar studies have also found that the Beijing genotype has been isolated from a significant population of TB patients in Iran neighbouring countries, especially Afghanistan and Pakistan. Hence, in order to prevent the spread of *Mtb* Beijing family infection, it seems that designing the guidelines has a significant impact on reducing the DR -TB cases in the Iranian population [5,6,39].

Ethical

Ethical Statement is not applicable for this manuscript.

References

[1] Keikha M, Esfahani BN. The relationship between tuberculosis and lung cancer. Adv Biomed Res 2018;7:8–11.
[2] World Health Organization. Global tuberculosis report 2019. World Health Organization; 2020.
[3] Zaniani FR, Moghim S, Mirhendi H, Safaei HG, Fazeli H, Salehi M, et al. Genetic lineages of mycobacterium tuberculosis isolates in Isfahan, Iran. Curr Microbiol 2017;74(1):14–21.
[4] Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol 2006;44(12):4498–510.

[5] Tarashi S, Fateh A, Jamnani FR, Siadat SD, Vaziri F. Prevalence of Beijing and Haarlem genotypes among multidrug-resistant Mycobacterium tuberculosis in Iran: systematic review and meta-analysis. Tuberculosis 2017;107:31–7.

[6] Ramazanzadeh R, Sayhemiri K. Prevalence of Beijing family in Mycobacterium tuberculosis in world population: systematic review and meta-analysis. Int J Mycobacteriol 2014;3(1):41–5.

[7] Garzon-Chavez D, Zurita J, Mora-Pinargote C, Franco-Sotomayor G, Leon-Benitez M, Granda-Pardo JC, Trueba G, Garcia-Bereguiain MA, de Waard JH. Prevalence, drug resistance, and genotypic diversity of the Mycobacterium tuberculosis Beijing Family in Ecuador. Microbial Drug Resistance 2019;25(6):931–7.

[8] Lee J. Odds ratio or relative risk for cross-sectional data? Int J Epidemiol 1994;23(1):201–3.

[9] Ramazanzadeh R, Farnia P, Amirmozafari N, Ghazi F, Ghadertotonchi Z, Kamran J, et al. Comparison between molecular epidemiology, geographical regions and drug resistance in Mycobacterium tuberculosis strains isolated from Iranian and Afghan patients. Chemotherapy 2006;52(6):316–20.

[10] Farnia P, Masjedi MR, Mirsaeidi M, Mohammadi F, Vincent V, Bahadori M, Velayati AA. Prevalence of Haarlem I and Beijing types of Mycobacterium tuberculosis strains in Iranian and Afghan MDR-TB patients. J Infect 2006;53(5):331–6.

[11] Farnia P, Masjedi MR, Nasiri B, Mirsaedi M, Sorooch S, Kazeampour M, Velayati AA. Instability of IS6110 patterns in multidrug-resistant strains of Mycobacterium tuberculosis. Epidemiol Infect 2007;135(2):346–52.

[12] Amirmozafari N, Ramezanzadeh R, Farnia P, Ghazi F. The frequency of Beijing genotype of Mycobacterium tuberculosis isolated from tuberculosis patients. Razi J Med Sci 2006;13(52):7–17.

[13] Farnia P, Masjedi MR, Varahram M, Mirsaeidi M, Ahmadi M, Khazampour M, et al. The recent-transmission of Mycobacterium tuberculosis strains among Iranian and Afghan relapse cases: a DNA-fingerprinting using RFLP and spoligotyping. BMC Infect Dis 2008;8(1):109.

[14] Doustdar F, Khosravi AD, Farnia P, BAHREHMAND A, Masjedi MR, Velayati AA. Mutations in rpoB gene and genotypes of rifampin resistant Mycobacterium tuberculosis isolates in Iran. Tanaffos 2008;7(2):11–7.

[15] Doustdar F, Khosravi AD, Farnia P, Masjedi MR, Velayati AA. Molecular analysis of isoniazid resistance in different genotypes of Mycobacterium tuberculosis isolates from Iran. Microbial Drug Resistance 2008;14(4):273–9.

[16] Doustdar F, Khosravi AD, Farnia P. Mycobacterium tuberculosis genotypic diversity in pyrazinamide-resistant isolates of Iran. Microbial Drug Resistance 2009;15(4):251–6.

[17] Rohani M, Farnia P, Nasab MN, Moniri R, Torfeh M, Amiri MM. Beijing genotype and other predominant Mycobacterium tuberculosis spoligotypes observed in Mashhad city, Iran. Indian J Med Microbiol 2009;27(4):306.

[18] Tajeddin E, Farnia P, Kargar M, Noroozi J, Ahmadi M, Kazempour M, Masjedi MR, Velayati AA. Identification of Mycobacterium tuberculosis Beijing genotype using three different molecular methods. Koomesh 2009;11(1).

[19] Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, ZiaZarifi AH, et al. Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest 2009;136(2):420–5.

[20] Ahmadi MO, Farnia P, Tajedin E, Tabarsi P, Baghaei PA, Masjedi MR, Velayati A. Mycobacterium Tuberculosis Complex Strains Identification with Spoligotyping Method in Patients Attending to Masih Daneshvari Hospital. J Adv Med Biomed Res 2009 Aug 10;17(67):23–32.

[21] Mozafari M, Farnia P, Jafarian M, Razavi Deligani M, Kazempour M, Masjedi M, Velayati AA. Comparison of Mycobacterium tuberculosis Beijing genotype with other Mycobacterium tuberculosis strains Using MIRU-VNTR method. ISMJ 2012;15(1):1–2.

[22] Merza MA, Farnia P, Salih AM, Masjedi MR, Velayati AA. The most predominant spoligopatterns of Mycobacterium tuberculosis isolates among Iranian, Afghan-immigrant, Pakistani and Turkish tuberculosis patients: a comparative analysis. Chemotherapy 2010;56(3):248–57.

[23] Jafarian M, Farnia P, Kargar M, Aghalimerza M, Ramazanzadeh R, Ahmadi M, et al. Study of genetic diversity of M.tuberculosis strains isolated by MIRU-VNTR technique in Masih Daneshvari Hospital, Tehran. SJKU 2010;14(4):29–39.

[24] Haeili M, Darban-Sarokhalil D, Fooladi AA, Javadpour S, Hashemi A, Siavoshi F, et al. Spoligotyping and drug resistance patterns of Mycobacterium tuberculosis isolates from five provinces of Iran. Microbiologyopen 2013;2(6):988–96.

[25] Sharifipour E, Nasiri M, Farnia P, Mozafari M, Irani S. Evaluation of molecular diversity of Mycobacterium tuberculosis strains by polymorphisms in RD Regions. J Mycobac Dis 2014;4(153). 2161-1068.

[26] Varahram M, Farnia P, Nasiri MJ, Karahrudi MA, Dizagie MK, Velayati AA. Association of Mycobacterium tuberculosis lineages with IFN-γ and TNF-α gene polymorphisms among pulmonary tuberculosis patient. Mediterr J Hematol Infect Dis 2014;6(1).

[27] Yamchi JK, Haeili M, Feyisa SG, Kazemian H, Shahraki AH, Zahednamazi F, Fooladi AA, Feizabadi MM. Evaluation of efflux pump gene expression among drug susceptible and drug resistant strains of Mycobacterium tuberculosis from Iran. Infect Genet Evol 2015;36:23–6.

[28] Mohajeri P, Moradi S, Atashi S, Farahani A. Mycobacterium tuberculosis Beijing genotype in western Iran: distribution and drug resistance. J Clin Diagnostic Res: JCDR 2016;10(10):DC05.

[29] Rezaei F, Haeili M, Mohajeri P, Shahraki AH, Fooladi AA, Zahednamazi F, Feizabadi MM. Frequency of mutational changes in the embB among the ethambutol-resistant strains of Mycobacterium tuberculosis in Iran. J Infect Dev Countries 2016;10(04):363–8.

[30] Khanipour S, Ebrahimzadeh N, Masoumi M, Sakhaei F, Alinezhad F, Safarpour E, et al. Haarlem 3 is the predominant genotype family in multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis in the capital of Iran: a 5-year survey. J Glob Antimicrob Resist 2016;5:7–10.

[31] Khosravi AD, Shahraki AH, Dezfuli SK, Hashemzadeh M, Goodarzi H, Mohajeri P. Genetic diversity of multidrug-resistant Mycobacterium tuberculosis strains isolated from tuberculosis patients in Iran using MIRU-VNTR technique. Kaohsiung J Med Sci 2017;33(11):550–7.

[32] Mansoori N, Yaseri M, Vaziri F, Douraghi M. Genetic diversity of Mycobacterium tuberculosis complex isolates circulating in an area with high tuberculosis incidence: using 24-locus MIRU-VNTR method. Tuberculosis 2018;112:89–97.

[33] Zaniani FR, Moghim S, Esfahani BN. Genetic diversity of drug-resistant Mycobacterium tuberculosis isolates in Isfahan province of Iran. Adv Biomed Res 2018:7.

[34] Vaziri F, Kohl TA, Ghajavand H, Kamakoli MK, Merker M, Hadifar S, et al. Genetic diversity of multi-and extensively drug-resistant Mycobacterium tuberculosis isolates in the capital of Iran, revealed by whole-genome sequencing. J Clin Microbiol 2019;57(1). e01477-18.

[35] Afaghi GA, Moaddab SR, Darbouy M, Ansarin K, Hanifian S. Genotypic diversity of resistant Mycobacterium tuberculosis strains isolated from tuberculosis patients in East Azerbaijan center by MIRU-VNTR. J Microbial World. 2009;11(4):320–31.

[36] Van Soolingen D, Qian L, De Haas PE, Douglas JT, Traore H, Portaels F, et al. Predominance of a single genotype of Mycobacterium tuberculosis in countries of east Asia. J Clin Microbiol 1995;33(12):3234–8.

[37] Toungoussova OS, Sandven P, Mariandyshev AO, Nizovtseva NI, Bjune G, Caugant DA. Spread of drug-resistant Mycobacterium tuberculosis strains of the Beijing genotype in the Archangel Oblast, Russia. J Clin Microbiol 2002;40(6):1930–7.

[38] Tanveer M, Hasan Z, Siddiqui AR, Ali A, Kanji A, Ghebremicheal S, et al. Genotyping and drug resistance patterns of M. tuberculosis strains in Pakistan. BMC Infect Dis 2008;8(1):171.

[39] Hoffner S, Sahebi L, Ansarin K, Sabour S, Mohajeri P. Mycobacterium tuberculosis of the Beijing genotype in Iran and the World Health Organization Eastern Mediterranean Region: a meta-analysis. Microbial Drug Resistance 2018;24(6):693–8.

[40] Sun H, Zhang C, Xiang L, Pi R, Guo Z, Zheng C, Li S, Zhao Y, Tang K, Luo M, Rastogi N. Characterization of mutations in streptomycin-resistant Mycobacterium tuberculosis isolates in Sichuan, China and the association between Beijing-lineage and dual-mutation in gidB. Tuberculosis 2016;96:102–6.

[41] Nhu NT, Lan NT, Phuong NT, van V, Chau N, Farrar J, Caws M. Association of streptomycin resistance mutations with level of drug resistance and Mycobacterium tuberculosis genotypes. Int. J. Tuberculosis Lung Dis 2012;16(4):527–31.

[42] Alvarez N, Haft D, Hurtado UA, Robledo J, Rouzaud F. Whole-genome sequence of a Beijing extensively drug-resistant Mycobacterium tuberculosis clinical isolate from Buenaventura, Colombia. Genome Announc 2016;4(1). e01549-15.

[43] Yuan L, Huang Y, Mi LG, Li YX, Liu PZ, Zhang J, Liang HY, Li F, Li H, Zhang SQ, Li WJ. There is no correlation between sublineages and drug resistance of Mycobacterium tuberculosis Beijing/W lineage clinical isolates in Xinjiang, China. Epidemiol Infect 2015;143(1):141–9.

[44] Li Y, Cao X, Li S, Wang H, Wei J, Liu P, et al. Characterization of Mycobacterium tuberculosis isolates from Hebei, China: genotypes and drug susceptibility phenotypes. BMC Infect Dis 2016;16(1):107.

[45] Liu Y, Jiang X, Li W, Zhang X, Wang W, Li C. The study on the association between Beijing genotype family and drug susceptibility phenotypes of Mycobacterium tuberculosis in Beijing. Sci Rep 2017;7(1):1–7.