



# The role of Azeri patients in transmission of tuberculosis to East Azerbaijan, Iran

Mohammad Asgharzadeh<sup>1</sup>, Hossein Samadi-Kafil<sup>\*2</sup>, Mahya Pour-Ostadi<sup>3</sup>, Sajjad Ghorghanlu<sup>4</sup>

<sup>1</sup> Professor, Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>2</sup> Assistant Professor, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>3</sup> Student of Medicine, Student Research Committee, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>4</sup> MSc Student, Infectious Disease and Tropical Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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## Abstract

**Introduction:** Human migration can transmit infectious disease like tuberculosis (TB) to recipient countries. The IS6110 is an insertion sequence used for strain differentiation of mycobacterium tuberculosis (MTB), epidemiological studies, and recent transmission. In this study, our aim was to investigate transmission TB from the Republic of Azerbaijan to Iran people.

**Methods:** Totally 119 isolates of MTB were collected from 14 patients from Republic of Azerbaijan and 105 patients from East Azerbaijan, Iran who referred to TB centers of the province. Isolates were analyzed by IS6110 restriction fragment length polymorphism (RFLP) typing. Isolates with identical RFLP patterns were considered a cluster.

**Results:** 93 distinct IS6110-RFLP patterns were revealed. 81 of these patterns were unique, and 12 were shared by 2-8 strains. 38 strains (31.9%) belonged to one of 12 clusters that were found among the total of 119 strains. One of these 12 clusters contained two Iranian patients with one Azeri patient.

**Conclusion:** Finding of the present study indicates that Azeri TB patients refer to TB centers of the province for treatment can transmit TB to East Azerbaijan people. Therefore, it needs to design a suitable program for TB control and decrease transmission of TB between these two countries.

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## Introduction

Tuberculosis (TB) among of infectious diseases is the second leading cause of death in worldwide and about a third to a half of people in the world is infected with mycobacterium tuberculosis (MTB).<sup>1</sup> The estimated rate of new TB cases in the world were 8.6 million in 2012 and 1.3 million deaths.<sup>2</sup> Human populations travel have a key role in the spread of disease. Transmission of TB in resulting of human migration has had a major effect on the spread of TB to uninfected groups.<sup>3</sup>

The important subject in the transmission of TB is travel from high incidence countries to low incidence countries.<sup>4</sup> The resurgence of TB

likely have been related to the increase of human mobility, remove of TB control programs, the appearance of drug-resistant strains of MTB, co-infection with human immunodeficiency virus (HIV), and poverty.<sup>5</sup> We need to understand about the spread of TB inside and across the borders of the country to design a suitable program for TB control. Progress in molecular biology has provided powerful methods for typing and detection MTB in epidemiological studies.<sup>6</sup>

The IS6110 is an insertion sequence that belongs to IS3 family. IS6110 commonly used for detection of MTB,<sup>7</sup> epidemiological studies<sup>8</sup> and recent transmission.<sup>9</sup> In IS6110-restriction

\* Corresponding Author: Hossein Samadi-Kafil, Email: [kafilhs@tbzmed.ac.ir](mailto:kafilhs@tbzmed.ac.ir)

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fragment length polymorphism (RFLP) method unrelated clinical isolates have polymorphic IS6110-RFLP patterns, whereas, epidemiological related strains have identical or similar (one band variation) fingerprinting patterns. Hence, MTB isolates with identical fingerprinting pattern show a probable recent transmission.<sup>10</sup>

The province of East Azerbaijan is in Northwest of Iran and neighbor with Republic of Azerbaijan. Based report of World Health Organization (WHO), Republic of Azerbaijan is one of high TB and multi drug resistance-TB (MDR-TB) burden countries.<sup>1</sup> Human migration can transmit infectious disease like TB to recipient countries.<sup>4</sup> In this study, our aim was to investigate the transmission of TB from Republic of Azerbaijan to Iran people by RFLP hybridization method to decrease transmission of TB between these two countries.

### Methods

All isolates of MTB were collected from patients who referred to TB centers of the province for this descriptive study. The study population comprised all patients from whom at least one sample was positive for MTB by culture. 125 isolates of MTB were collected from 14 patients were from Republic of Azerbaijan and 111 patients were from East Azerbaijan. The species identification of the isolates was based standard biomedical tests, including the production of niacin, catalase activity, nitrate reduction, pigment production and growth rate.<sup>11</sup> Sample size of the study was calculated by G-power software ( $\alpha$  error probability = 0.05). Prevalence was calculated from preciously published studies.<sup>12</sup> Power of the study was 80.

### IS6110-RFLP

Extraction of deoxyribonucleic acid (DNA) from MTB isolates and fingerprinting was performed as described by van Soolingen et al.<sup>12</sup> A 245 bp fragment amplified by polymerase chain reaction (PCR) on purified chromosomal mycobacterium bovis Bacillus Calmette-Guérin (BCG) DNA by using used from these oligonucleotides INS-1 (5'-CGT

GAG GGC ATC GAG GTG GC) and INS-2 (5'- GCG TAG GCG TCG GTG ACA AA) TIB Molbiol, Germany,<sup>13</sup> and then this fragments purified and after labeling with digoxigenin, used as IS6110 probe. The extracted mycobacterial DNA was digested with PvuII enzyme (Cinnagen, Iran) and restriction fragments were separated in 0.8% agarose gel electrophoresis at 20 v for 18 h. The fragments were transferred from gel to positively charged nylon membranes then hybridized with IS6110 probe and insertion sequences were visualized with a colorimetric system.

A mixture of PvuII-digested supercoiled DNA ladder (Sigma, USA) and HaeIII-digested  $\Phi$ X174 DNA (Fermentas, Lithuania) was used as an internal marker. The internal marker was added to the wells together with the cleaved MTB DNA and visualized by reprobng the blots with DIG DNA labeling and detection Kit (Roche, Germany). In addition to internal size marker, PvuII-digested genomic DNA of reference strain MTB 14323 was used as external marker in each gel. The IS6110 fingerprinting patterns were compared by visual examination.<sup>12</sup> A cluster was defined as two or more isolates from different patients whose RFLP fingerprints were identical with respect to both the number and molecular size of all bands.<sup>14</sup> All strains were compared to detect probable transmission of infection between patients.

### Results

The RFLP patterns of 119 isolates from 119 patients were determined (Figure 1). The copy numbers of IS6110 in each of isolates varied from 0 to 18. 93 (78.0%) distinct IS6110-RFLP patterns were revealed. 81 of these patterns were unique and 12 were shared by 2-8 strains. 38 strains (31.9%) belonged to one of 12 clusters that were found among the total of 119 strains. The largest cluster contained 8 strains, 2 clusters included 5 and 4 strains each, and 3 clusters comprised three strains and 6 clusters comprised two strains each. The rate of the diversity of the RFLP pattern with IS6110 for

the MTB strains in this study was 78.0% (12 + 81/119). One of these 12 clusters were containing two Iranian patients' shared RFLP pattern with one strain of Azeri patient.

### Discussion

The crucial component of any TB control program is the ability to estimate transmission of infection and identify infected patients until make suitable preventing and treatment. Lack of targeted TB prevention and control policies for migrants, they make major barriers in preventing TB. East Azerbaijan is neighbor with Republic of Azerbaijan that it's one of high burden TB,<sup>1</sup> MDR-TB<sup>15</sup> and transmission in this region is high.<sup>16</sup>

Therefore, TB patients from Republic of Azerbaijan referred to hospitals, and TB centers of Iran can transmit TB and MDR-TB to people of Iran. Our study showed that transmission of infection is occurring from one patient of Republic of Azerbaijan to two Iranian patients. In similar, transmission of TB occurred from Greenland patients to Denmark patients,<sup>17</sup> from Afghan patients to Iranian,<sup>18</sup> a United Kingdom (UK) patient transmit TB to 37 persons (Netherland; 28, UK; 7, Suriname; 1, Morocco; 1),<sup>19</sup> a traveling salesman transmit a New York strains to

three patients of Son Francisco,<sup>20</sup> transmission of TB between Somali and Denmark patients (from Somalia to Denmark: 0.9% and Denmark to Somalia: 1.8%).<sup>21</sup>

Staying time of patients in the recipient country, migration of people from high incidence country to low incidence country and active TB patients have an important role in TB transmission. The prevalence of TB among close contact is 2.5%.<sup>22</sup> Annually nearly 54 active TB were distinguished from Republic of Azerbaijan patients that referred to Tabriz, Iran. If we assume that each of these patients has three close contacts with Iranian, they can infect at least four persons in Iran.

Transmission of TB to Iranian people from Republic of Azerbaijan can be high with regard to; Azerbaijan is one of high TB<sup>1</sup> and MDR-TB burden countries<sup>15</sup> and TB treatment is free in the Iran<sup>23</sup> so patients come from Republic of Azerbaijan to Iran for treatment of TB. High TB and MDR-TB burden also cause that the number of individuals who referred to Iran is more and therefore transmission is high. Other effective factor, in this case, is similar culture between this country and East Azerbaijan province that this subject have an increase of contact between two country populations.

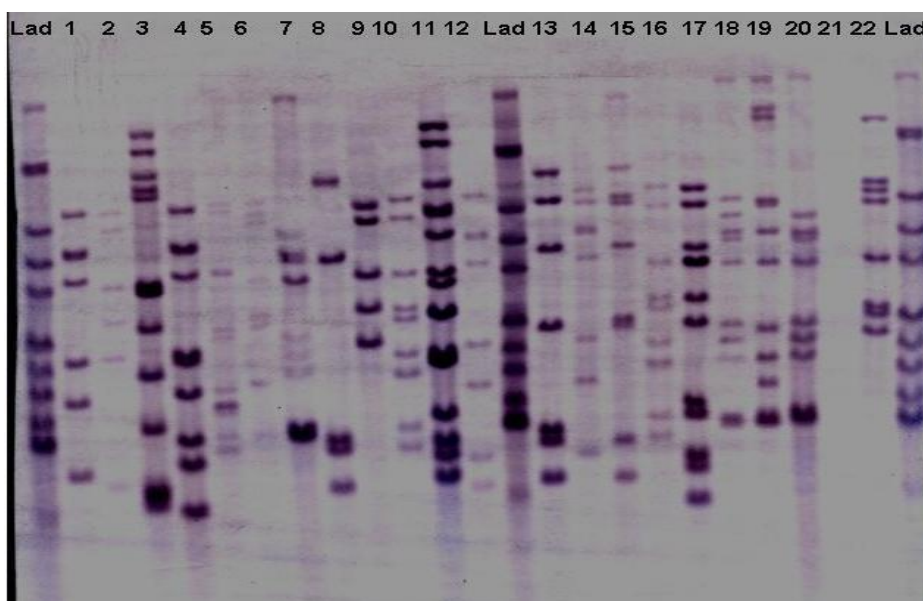


Figure 1. Hybridization with IS6110 probe and visualized insertion sequences of isolates collected from East Azerbaijan (Reference strain MTB 14323 was used as Ladder in each southern blot experiment)

Transfer MDR-TB to Iranian makes that services and health organizations paying more cost for treatment. Thus for preventing of TB transmission and its complications, we offer this suggestion: 1- The government established TB centers in cross-border or inside of Republic of Azerbaijan, 2- TB patients that came to Iran for treatment should be refer special places so that decrease contacts with people, 3- Management of patients travel from Azerbaijan to Iran. 4- More molecular epidemiology studies are necessary to perform among migrants and Iran population for better programming for TB control.

### Conclusion

Azeri TB patients refer to TB centers of the

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