

Original article:

Nationality as a risk factor of tuberculosis resistance in two co-related countries

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Abstract

Background: Republic of Azerbaijan and Iran are two neighboring countries with high amount of travelling between them, different rates of TB and its resistance. These relationships could complicate the controlling of tuberculosis programs. **Objective:** The study was conducted to determine the prevalence of tuberculosis (TB) resistance and its risk factors in the two geographically co-related regions. **Method:** A total of 119 *Mycobacterium tuberculosis* isolates from the patients of the target regions (Azerbaijan Rep. and Tabriz, Iran) were examined at the Central Tuberculosis Laboratory in Tabriz, Iran. The cultures and drug susceptibility tests was performed on Lowenstein-Jensen. All the isolates were categorized by MIRU-VNTR molecular method into clustered and un-clustered groups. The clustering as well as demographic data were analyzed to determine the risk factors contributing to TB resistance. The categorical data about the TB resistance were compared using a chi square test. **Results:** 27.8 percent of isolates were resistant at least to one of the 1st line of anti-tuberculosis drugs. The prevalence of any drug resistance and MDR were 64.6 and 17.9 percent respectively for Azari isolates, where the rate of resistance to rifampin and isoniazid was higher than streptomycin and ethambutol. The corresponding figures for the isolates from Tabriz were 16.9 and 3.3 percent respectively. **Conclusions:** The results of this comparative and cross-sectional study showed statistically significant differences in TB resistance between the isolates from the patients of Azerbaijan Rep. and Iran. According to the results, the rate of resistance to RMP, INH, and MDR was higher in Azerbaijan; consequently, nationality could be regarded as a risk factor of MDR, resistance to RMP and INH but not to SM and ETB. Other studied parameters did not contribute to TB resistance.

Keywords: drug resistance; multi-drug resistance; pan-susceptible; *Mycobacterium tuberculosis*

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Introduction

One-third of the world's population is infected with *Mycobacterium tuberculosis* and 9.6 million people are estimated to have fallen ill with TB in 2014. According to the World Health Organization, the global incidence of TB is slowly declining by < 2 percent per year. To reach the goal of TB elimination

(of < 1/1,000,000) by 2050, the incidence need to decline by 20 percent per year. The misuse of anti-tuberculosis drugs such as single drugs and Short-course chemotherapy regimens has led to TB resistance, and the spread of these strains have turned tuberculosis into a global public health crisis¹⁻³. One of the causes of failure in controlling and managing

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of TB has been the increase of resistant MTB isolates and series treats of multidrug-resistant tuberculosis (MDR-TB) and Extensively Drug Resistance (EDR-TB)⁴. Globally, an estimated 3.3 percent of recently diagnosed patients and 20 percent of the treated cases suffer from MDR-TB. According to WHO report, the prevalence of MDR in Iran was 5 percent in 2014 (0.8 and 12 percent for new and treated cases respectively)⁵.

Generally, neighboring countries of Iran have a high rate of TB and its resistance. 80 percent of TB belongs to 22 countries, two of which are Iran's eastern neighbors. Iran's neighbors except Turkey have a considerably higher rate of TB than Iran. According to WHO's 2014 report, there are 17000 TB cases in Iran, and the incidence of TB is estimated to be 22 per 100000, whereas the rate rises to 77 per 100000 for the Azerbaijan Rep. The stewardship and controlling programs of TB are extremely costly. According to WHO reports, US\$ 6.6 billion was spent on prevention, diagnosis and treatment of TB in 2015⁵. The high cost of medical services in the Republic of Azerbaijan and the high value of Azerbaijan currency in Iran are the major causes of Azerbaijanian patients' frequent trips to Tabriz (center of east Azerbaijan Province, Iran) for getting cheaper medical services, especially for TB. The independent and comparative epidemiological studies conducted on the mentioned regions indicate high resistance of TB in Rep. of Azerbaijan to Iran.⁶
7. The current study was an attempt to investigate the TB status as well as its risk factors and interaction effects of two neighboring countries with different rates of tuberculosis.

Materials and Methods

The study population and microbiological setting

The population involved in the study was TB patients from both Azerbaijan Rep. and Tabriz who had referred to the Tuberculosis and Lung Diseases Research Center of Tabriz (Iran). The patients' demographic information (i.e., age, sex, geographical origin) was recorded by the staff of the TB Center.

All the isolates were recovered from the sputa of the patients grown on Lowenstein-Jensen (LJ) medium. The specimens were digested and decontaminated by adding half volume of sodium hydroxide (NaOH, 4%) containing N-acetyl cysteine (NALC), followed by 15 minutes of incubation. The samples were neutralized with a phosphate buffer (pH 6.8) and concentrated by centrifugation at 3000rpm for 20 minutes. The pellet was re-suspended in water to obtain a final volume of 1.5ml. This suspension was

stored for preparing smears and staining by Ziehl-Neelsen acid-fast method and later for various cultures for identification and susceptibility tests.

The isolates were identified for *M. tuberculosis* based on standard biochemical tests, including production of niacin, catalase activity, nitrate reduction, and pigment production and growth rate⁸.

Drug susceptibility test (DST) was carried out for the first line of anti-TB drugs such as isoniazid (INH), rifampicin (RMP), Streptomycin (SM), and ethambutol (ETB), using the proportional method. The tested drug concentrations were 4 mg/l for SM, 0.2 mg/l for INH, 40 mg/l for RF and 2 mg/l for EMB. The LJ slants were incubated at 37°C and observed at 28 and 42 days. (Canetti et al, 1967). H37Rv standard strain with sensitivity to all drugs was used as the quality control strain⁹.

Definitions

Any drug resistance was defined as the resistance to at least one of the first-line drugs mentioned above. Mono, two, three and four resistance was defined as resistance to only one, two, three and four of the first-line drugs respectively. MDR-TB was defined as *M. tuberculosis* strains that were resistant to at least INH and RMP (the two most powerful of the first-line anti-TB drugs) with or without each of other first-line drugs. Pan susceptibility was defined as no resistance to any first-line drugs.

Risk Factors in drug susceptibility testing

Some of the demographic parameters such as age, sex, geographical origin as well as clustering data were considered in drug susceptibility testing. Clustering was carried out based on MIRU-VNTR (variable number tandem repeat- mycobacterial interspersed repetitive units) method, and all the isolates were sub-divided into two categories of clustered and non-clustered (data not shown). In order to examine the relationship between these parameters and drug resistance, all groups were compared for one or more anti-TB drugs and analyzed statistically.

The statistical analysis

Descriptive statistics were used to describe the data. Categorical data (MDR-TB and non-MDR-TB; resistance and sensitivity between groups) were compared using a chi square test, but when expected values were less than five, the Fisher's exact test was employed. The p value of 0.05 was set as the level of confidence.

Ethical clearance: This study was approved by Ethics Committee of Tuberculosis and Lung Diseases Research Center of Tabriz (Iran).

Results

M. tuberculosis culture results

Overall, 119 isolates of *M. tuberculosis* were collected from 125 patients between April 2013 and March 2014, and the rest of isolates were excluded for diverse reasons (poor growth, insufficient sample, contamination etc.). Ninety-one of whole isolates(76.47%)belonged to Tabriz and the rest(23.53%) to the Azerbaijan Republic.

Clustering and Demographic characteristics of the patients

The patients were assigned into non-clustered 96(80.67%) and clustered groups 23(19.33%) based on MIRU-VNTR clustering data.49 percent of the

patients (58 patients) were male and 51percent (61 patients) were female; their age range was between 15 and 86 years with an average of 59 years.

Drug susceptibility test

The results showed no statistically difference in the drug susceptibility profile between individual sex and age. The information on drug resistance based on geographical origin and clustering is presented in Table1. The profile of resistance to the first-line drugs for the whole isolates showed that 8.4 percent of the isolates were resistant to isoniazid, 12.6 percent to rifampin, 7.56 percent to ethambutol, and 15.97 percent to streptomycin.

Table1. Outcomes of drug resistant tuberculosis for geographical origin and clustering.

Resistance	Geographical Origin				*Clustering		Total
	azr (n=28)	tbz (n=91)	X ²	p	Clustered (n=23)	NonClustered (n=96)	
INH	7(25)	3(3.3)	13	0.001	0	10(10.42)	10(8.4)
RMP	11(39.29)	4(4.4)	23.66	000	1(4.35)	14(14.58)	15(12.6)
EMB	3(10.71)	6(6.6)	0.52	0.44	2(8.7)	7(7.29)	9(7.56)
SM	7(25)	12(13.19)	2.22	0.15	2(8.7)	17(17.7)	19(15.96)
Pan Sen	10(35.71)	76(83.52)	24.41	000	18(20.93)	68(79.07)	86(72.23)
Any Drug Res:	18(64.29)	15(16.48)	24.41	000	5(21.74)	28(78.26)	33(27.73)
Mono Drug	11(39.29)	11(12.09)	10.51	0.001	5(21.74)	17(17.7)	22(18.49)
INH	2(7.14)	0	6.61	0.054	0	2(100)	2(1.68)
RMP	5(17.86)	0	16.97	0.001	1(4.35)	4(4.17)	5(4.2)
EMB	1(3.57)	3(3.3)	0.005	1	2(8.7)	2(2.08)	4(3.36)
SM	3(10.71)	8(8.8)	0.094	0.719	2(8.7)	9(9.38)	11(9.24)
Two Drugs	4(14.28)	1(1.1)	9.25	0.011	0	5(5.21)	5(4.2)
INH+RMP	3(10.71)	0	10	0.012	0	3(3.13)	3(2.52)
EMB+SM	1(3.57)	0	3.28	0.235	0	1(1.04)	1(0.84)
RMP+SM	0	1(1.1)	0.31	1	0	1(1.04)	1(0.84)
THREE Drugs	3(10.71)	0	10	0.012	0	3(3.13)	3(2.52)
RMP+INH+SM	2(7.14)	0	6.61	0.054	0	2(2.08)	2(1.68)
RMP+EMB+SM	1(3.57)	0	3.28	0.235	0	1(1.04)	1(0.84)
All Drugs	0	3(3.3)	0.95	1	0	3(3.13)	3(2.52)
MDR-TB	5(17.86)	3(3.3)	7.24	0.017	0	8(8.33)	8(6.72)

azr, republic of Azerbaijan; tbz, Tabriz; INH, isoniazid; RMP, rifampicin; SM, Streptomycin and EMB, ethambutol; MDR-TB, multidrug-resistant tuberculosis.

*p value >0.05were calculated for clustering results. Generally, 27.73 percent of the isolates were any drug resistance and 72.27 percent was pan susceptible.

Accordingly, 64.29 percent of Azerbaijanians' isolates were resistant at least to one drug, while this amount was 16.48 percent for Tabriz (p=0). The drug resistance of Azerbaijanians' isolates was characterized as: mono drug 39.29 percent (only INH, RMP, EMB or SM), two drugs 14.28 percent (INH+RMP or EMB+SM), three drugs 10.71 percent

(RMP+EMB+SM or RMP+INH+SM), and the drug resistance of the isolates from Tabrizi patients was characterized as: mono drug 12.9 percent (only EMB or SM), two drugs 1.1 percent (RMP+SM), four drugs 3.3 percent (RMP+ INH+EMB+SM). Drug resistance calculated for the clustered and non-clustered isolates was 21.74 percent and 78.26 percent respectively. All 'any drug resistant' clustered isolates were mono drug resistant, while the non-clustered ones were contained in each group. Furthermore, 5 (17.86 percent) isolates from Azerbaijanians and 3 (3.3 percent) from Tabrizi patients were MDR-TB respectively.

Discussion

This comparative study was carried out to determine the prevalence as well as the risk factors in *Mycobacterium tuberculosis* resistance in the patients of two neighboring countries (Azerbaijan Rep. and Tabriz located in Iran) with high amount of travelling between them and with different rates of TB. The Province of east Azerbaijan, Iran hosts patients from several neighboring countries, in particular Republic of Azerbaijan. For decades, patients from the Rep. of Azerbaijan have regularly been travelling to medical centers of Tabriz to get cheaper services, especially for tuberculosis. The number of such patients increased from 12% in 2003 to 22% in 2012. The Republic of Azerbaijan has a relatively high rate of TB with 76/100000, while this rate falls to 22/100000 for Tabriz (northwest of Iran). The rate of MDR-TB is reportedly 28.6% in the Rep. of Azerbaijan, whereas it is about 1.9% in East Azerbaijan Province of Iran⁶. In this study, resistance to at least one drug of the first line of anti-TB drugs was 23.73 percent (33 of 119 cases). A higher proportion of this resistance was found in the isolates from Azerbaijanian patients. 64.29 percent of the isolates from Azerbaijan and 16.48 percent from Tabriz were TB resistant. Resistance to isoniazid, rifampicin, ethambutol and streptomycin were 8.4, 12.6, 7.56 and 15.97 percent respectively. The rate of resistance and its geographical profile are different. The current study showed that 25 and 39.29 percent of Azerbaijanian patients and 3.3 and 4.4 percent of Tabrizi patients were resistant to INH and RMP, implying that nationality could be a significant risk factor to any drug resistance ($p=0.00$) and RMP and INH ($p=0.001$ and 0.000 respectively) but not to EMB and SM resistance ($p>0.05$). These results and the results from other regions of Iran that relate to high TB countries appeared low prevalence of TB resistance in the northwest to the other parts of Iran¹⁰

¹⁴. On the other hand, the resistance profiles of Iran and its neighboring countries with high prevalence of TB are different. Accordingly, the highest prevalence of drug resistance in Iran is associated with streptomycin and ethambutol but not with rifampin and isoniazid antibiotics, while rifampin and isoniazid are the two main drugs against *M. tuberculosis*. According to WHO as well as reports from across the world, the rate of resistance to streptomycin is high and has some connection with the widespread use of these antibiotic against a variety of infections in the past^{5, 15, 16}. The present study revealed that the effectiveness of the first-line drugs on Azerbaijanian patients was of the pattern: rifampin < isoniazid = streptomycin < ethambutol, but this pattern for patients from Tabriz was: streptomycin < ethambutol < rifampin < isoniazid.

Concerning the relationship between resistance to the first-line anti-TB drugs and other demographic factors such as sex, age and also clustering based on MIRU-VNTR, the study found no statistically significant effect of these factors on resistance ($p>0.05$).

This study suffers from some limitations. First, Iranian patients outnumbered Azerbaijanian ones. Second, more detailed demographic information of Azerbaijanian patients was not available to study the effect of further risk factors of TB resistance.

One of the main concerns of the modern world in treating TB is multi-drug resistance tuberculosis (MDR-TB.), which is defined in relation to rifampin and isoniazid resistance. In the present study, 6.72 percent of the whole isolates were MDR and 62.5 percent of them belonged to the patients from Azerbaijan Republic. According to the results, the prevalence of MDR in the Azerbaijan Rep. and east Azerbaijan province of Iran was estimated to be 17.86 and 3.3 percent, respectively. Based on the other results obtained from the both mentioned regions, the rate of MDR is about the same amount was reported.¹⁷⁻²⁰

Conclusion

This cross-sectional study showed that in spite of a high amount of travel and relationship between Azerbaijan Rep. (as a country with high TB incidence) and east Azerbaijan province of Iran, it was found to be of any effect on the features of TB resistance. The status of the Azerbaijan Rep. is worse than Iran in respect to TB prevalence and resistance. The prevalence of each drug resistance, especially to the main anti-TB drugs (RMP&INH), was statistically high. The problem becomes more

complicated with the emergence of the multidrug-resistant TB. Since the medical services offered in Tabriz (Iran) to Azerbaijanians can contribute to the risk of TB transmission as well as its resistance, it is suggested that medical centers be established on the border between Azerbaijan and Iran so that the likely spread of the disease into Iran can be curbed.

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Conflict of interest statement

We declare that we have no conflict of interests.

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