

Anti-tuberculosis drug resistance and associated risk factors in a tertiary level TB centre in Iran: a retrospective analysis

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Abstract

Introduction: This study aimed to determine first-line anti-tuberculosis drug resistance rates in new and previously treated cases and to identify risk factors associated with multidrug resistant tuberculosis (MDR-TB) at the National Reference Tuberculosis Laboratory of Iran.

Methodology: This was a retrospective analysis of all confirmed TB patients from December 2000 to June 2005. Drug susceptibility testing to isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide was performed on Löwenstein-Jensen (LJ) medium according to the proportion method.

Results: Mycobacterium tuberculosis strains were isolated from 1,742 patients with TB, of whom 935 (53.7%) were male. The mean age of patients was 44.2 ± 17.4 years (SD). A total of 1,074 patients were native Iranians while 668 (38.3%) were immigrant patients. Out of 1,139 (65.4%) new cases, 340 (29.9%) had at least one drug resistance. Of 603 (34.6%) previously treated cases, 416 (69.0%) had resistant strains. There were 263 patients (15.1%) with MDR-TB, 72 of whom were new (6.3% of all new cases) and 191 were previously treated (31.7% of all previously treated cases). Factors associated with MDR-TB included age under 45 years, male sex, previous TB treatment, immigration, poor living conditions, and unemployment.

Conclusions: The high rate of initial resistance in MDR-TB cases and the high rate of MDR-TB in a young age group were indicators of recent transmission. Therefore, closer monitoring of transmission trends of drug resistant strains should be considered as priority, to ensure a successful TB control programme.

Key words: Mycobacterium tuberculosis; Iran; multidrug resistant tuberculosis; risk factor

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Introduction

Tuberculosis (TB) is becoming a worldwide problem due to the recurrence of the disease, the appearance of drug resistant strains, and the association of TB with the HIV pandemic [1]. The emergence of multidrug resistant TB (MDR-TB), i.e. Mycobacterium tuberculosis strains, resistant to at least isoniazid (INH) and rifampicin (RMP), is of great concern, because it requires the use of second-line drugs that are difficult to procure and are much more toxic and expensive than the first-line regimen [2]. Hence the detection and treatment of drug susceptible or single drug resistant TB are important strategies to prevent the emergence of MDR-TB [3]. Recently, reports of M. tuberculosis strains with extensively drug resistant-TB (XDR-TB), that is MDR-TB strains with resistance to at least three of the six classes of second-line drugs, have been described [3,4]. The

global increase in drug resistance, particularly MDR-TB, reflects, at least in part, inappropriate use of anti-TB drugs during the treatment course of TB patients with drug susceptible strains [2,5]. Additional factors such as immigration, age, sex, human immunodeficiency virus (HIV) infection, and socio-economic factors have been shown to be associated with the increased prevalence of MDR-TB [6]. The World Health Organization (WHO) has documented that MDR-TB is becoming extensively widespread today. According to the most recent data, in 2008, among all incident TB cases worldwide, 3.6% were estimated to have MDR-TB. Almost half of the global MDR-TB cases were reported from China and India [7].

In Iran, anti-TB drug resistance rates have been reported [8,9]; however, their associated risk factors have not been fully investigated [10]. To develop a

reasonable health-care strategy, particularly for difficult problems such as MDR-TB, current knowledge of drug resistant TB in Iran is important.

The objectives of this study were to determine first-line anti-TB drug resistance rates in new and previously treated cases at the National Reference Tuberculosis Laboratory and Lung Disease (NRITLD) of Iran. The investigation also aimed to identify certain risk factors associated with MDR-TB.

Methodology

Setting

The Iranian National Research Institute of Tuberculosis and Lung Disease (NRITLD), Masih Daneshvari Hospital, the WHO Collaborating Centre for the Middle East, is the only laboratory for Drug Susceptibility Testing (DST) of culture-positive TB specimens in the country. The NRITLD is under the technical supervision of the Supranational Reference Laboratories of the Swedish Institute (Solna, Sweden) for DST quality assurance.

Study design and patients

We reviewed retrospectively the case notes of all patients referred to our unit between December 2000 and June 2005. The inclusion criteria were all patients diagnosed with active TB with positive culture for *M. tuberculosis* complex who were tested for susceptibility to first-line anti-TB drugs. Thereafter, the patients were classified into two groups: MDR-TB and non-MDR-TB.

Drug susceptibility testing (DST)

Mycobacterial species identification was performed by conventional biochemical tests [11]. All isolates were identified as *M. tuberculosis* complex by production of niacin, catalase activity, nitrate reduction, pigment production and growth rate. DST was routinely performed by proportion methods on Lowenstein–Jensen (LJ) medium on isolates of *M. tuberculosis*. The following drug concentrations were used: INH (0.2 µg /ml), RMP (40.0 µg /ml), streptomycin “STM” (4.0 µg /ml), and ethambutol “EMB” (2.0 µg /ml). Susceptibility to pyrazinamide “PZA” (900 and 1200 µg /ml) was tested using a two-phase medium and if at the twenty-first reading the proportion of resistant colonies was higher than the critical proportion, the strain was reported as resistant to PZA.

Definitions

Any drug resistance was defined as resistance to one or more first-line drugs. Monoresistance was defined as resistance to only one of the five first-line drugs (INH, RMP, STM, EMB, and PZA). MDR-TB was defined as *M. tuberculosis* strains that were resistant to at least INH and RMP. Primary or initial resistance, (resistance among new cases), was defined as patients with TB resistant to one or more anti-TB drugs, but who had never been previously treated for TB. Secondary resistance, (resistance among previously treated cases), was defined as patients diagnosed with TB who started anti-TB treatment and subsequently acquired resistance to one or more of the drugs used during the treatment [12].

Statistical analysis

The results obtained were analyzed by entering the data in a binary format as a Microsoft Excel spreadsheet. Categorical data (MDR-TB and non-MDR-TB) were compared by X² test or other variables by the Fisher's exact test. A p value of less than 0.05 was considered statistically significant.

Ethics approval

The study was approved by the ethics committee of NRITLD in Tehran.

Results

Patients

Between December 2000 and June 2005 a total of 1,742 TB patients were included in the study. Of these, 1,139 (65.4%) were new cases and 603 (34.6%) were previously treated cases. The mean age of patients was 44.2 ± 17.4 yr (SD), and 935 (53.7%) were male and 807 (46.3%) were female. A total of 1,074 (61.7%) were native Iranians and 668 (38.3%) were Afghan immigrants (Afghan) patients. The characteristics of this population are summarized in Table 1.

Drug resistance of M. tuberculosis

Of the 1,742 patients with *M. Tuberculosis*, 986 (56.6%) had TB that was susceptible to all drugs, and 756 (43.4%) were resistant to at least one antimicrobial drug. With regard to at least one drug resistance, the resistance rates of *M. tuberculosis* to the tested first-line drugs were as follows: INH (414, 23.8%), RMP (307, 17.6%), PZA (165, 9.5%), EMB (207, 11.9%), and STM (478, 27.4%).

The results of the sensitivity tests in the patients with TB were classified according to the history of treatment (new versus previously treated cases), are shown in Table 2. Out of the 1,139 new cases, 799 M.

Table 1. Characteristics of the study population (no = 1742)

	Variable	no (%)
Age	Mean age	44.2 ± 17.4
Gender	Male	935 (53.7)
	Female	807 (46.3)
Type of patient	New case	1139 (65.4)
	Previous TB treatment	603 (34.6)
Region of origin	Iranian	1074 (61.7)
	Immigrant (Afghans)	668 (38.3)
Site of disease	Pulmonary	1615 (92.7)
	Extra-pulmonary	127 (7.3)
Socio-economic factors	Poor living condition	1376 (79.0)
	Un-employment	868 (49.8)

Table 2. First line anti-tuberculosis drugs resistance in *M. tuberculosis* strains (new vs previously treated cases)

DST	New Cases (no=1139) no (%)	Previously treated cases (no=603) no (%)	Total DST (no=1742) no (%)
Pan-susceptible	799 (70.1)	187 (31.0)	986 (56.6)
Any drug resistance	340 (29.9)	416 (69.0)	756 (43.4)
INH	141 (12.4)	273 (45.3)	414 (23.8)
RMP	89 (7.8)	218 (36.2)	307 (17.6)
PZA	18 (1.6)	147 (24.4)	165 (9.5)
EMB	24 (2.1)	183 (30.3)	207 (11.9)
STM	233 (20.5)	245 (40.6)	478 (27.4)
Monoresistance	222 (19.5)	157 (26.0)	379 (21.8)
INH	30 (2.6)	22 (3.6)	52 (3.0)
RMP	10 (0.9)	19 (3.2)	29 (1.7)
PZA	4 (0.4)	6 (1.0)	10 (0.6)
EMB	8 (0.7)	22 (3.6)	30 (1.7)
STM	170 (14.9)	88 (14.6)	258 (14.8)
2 drugs	41 (3.6)	49 (8.1)	90 (5.2)
> 2 drugs	5 (0.4)	19 (3.2)	24 (1.4)
MDR-TB	72 (6.3)	191 (31.7)	263 (15.1)

Table 3. Univariate analysis of association of risk factors with MDR-TB

Variable		Non-MDR-TB (no=1479) no (%)	MDR-TB (no=263) no (%)	Odd ratio (95% CI)	P-Value
Age	Age < 45 yr	888 (60.0)	185 (70.3)	1.57 (1.18-2.09)	0.002
	Age > 45 yr	591 (40.0)	78 (29.7)		
Gender	Male	751 (50.8)	184 (70.0)	2.25 (1.70-2.99)	0.000
	Female	728 (49.2)	79 (30.0)		
Type of patient	New Case	1067 (72.1)	72 (27.4)	6.87 (5.12-9.21)	0.000
	Previous TB treatment	412 (27.9)	191 (72.6)		
Region of origin	Iranian	986 (66.7)	88 (33.5)	3.97 (3.01-5.24)	0.000
	Immigrant (Afghans)	493 (33.3)	175 (66.5)		
Site of disease	Pulmonary	1375 (93.7)	240 (91.3)	1.16 (0.72-1.85)	0.5
	Extra-pulmonary	104 (7.0)	23 (8.7)		
Socio-economic factor	Poor living condition	1180 (79.8)	196 (74.5)	0.56 (0.41-0.77)	0.002
	Un-employment	756 (51.1)	112 (42.6)	0.70 (0.54-0.92)	0.01

Table 4. Multivariate analysis of risk factors associated with MDR-TB

Variable		a Odd ratio (95% CI)	P-Value
Age	Age < 45 yr	1.41 (1.02-1.93)	0.01
	Age > 45 yr		
Gender	Male	1.83 (1.28-2.57)	0.002
	Female		
Type of patient	New Case	4.43 (2.68-4.77)	0.001
	Previous TB treatment		
Region of origin	Iranian	2.52 (1.56-3.79)	0.004
	Immigrant (Afghans)		

tuberculosis isolates (70.1%) were susceptible to all first-line drugs, while 187 isolates (31.0%) of the 603 previously treated cases were susceptible to all first-line drugs. Furthermore, 141 new cases and 274 previously treated cases were resistant to INH (12.4% and 45.3%, respectively). Seventy-two cases of MDR-TB were observed among new cases (6.3%), and 191 among previously treated cases (31.7%). (Table 2)

Trends in anti-TB drug resistance

Figure 1 shows the proportion of TB cases with MDR-TB and other resistance patterns by year. From 2001 to 2005 the percentage of MDR-TB and other resistance patterns remained almost stable with a slight decrease.

Risk factors associated with MDR-TB patients

Based on DST, the patients were divided into two groups: 1,479 patients were non-MDR and 263 patients were MDR-TB. Table 3 shows the number of MDR-TB patients for each of the variables analyzed and the crude odds ratios for MDR-TB. The only variables significantly associated with MDR were under 45 years of age, male sex, previous treatment, immigrants (Afghans), poor living conditions, and unemployment

Based on the univariate logistic regression analysis, patients with MDR-TB were more likely to be under 45 years of age (70.3%) and male (70.0%) than non-MDR-TB patients (60.0%, $p = 0.002$ and 50.8%, $p < 0.05$, respectively). MDR-TB cases (72.6%) were also more likely to have received

previous treatment than non-MDR-TB (27.9%) cases ($p < 0.05$). Afghan immigrant patients (66.5%) were more likely to have MDR than non-MDR-TB (33.3%) ($p < 0.05$). In addition, patients with MDR-TB were likely to have had poor living conditions (74.5%) and to be unemployed (42.6%) in comparison to patients with non-MDR-TB (79.8% and 51.1%, $p = 0.002$ and $p = 0.01$, respectively) (Table 3). All variables that according to univariate analysis were related to MDR-TB at a p value less than 0.05 were included in the multivariate logistic regression model for real effects of variables. According to multivariate analysis, the following factors were significantly related to MDR-TB: age younger than 45 years, male sex, previous TB treatment, and immigration (Table 4).

Discussion

Information on anti-TB drug resistance patterns, particularly among new and previously treated cases, is crucial for planning an effective MDR-TB control programme. The objectives of this retrospective study, which included all TB patients a for 4.5-year period, were to identify first-line anti-TB drug resistance in new and previously treated cases and to determine risk factors associated with MDR-TB at the National Reference Tuberculosis Laboratory in Iran.

In the present study, we found that drug resistance to STM (478, 27.4%) was the most common, followed by INH (414, 23.8%). This is in agreement with the observations of other Iranian researchers [7,8,13]. The high prevalence of STM resistance in our study may be due to widespread use of STM in the past for

Figure 1. Anti-TB resistance trends 2001 – Jun 2005

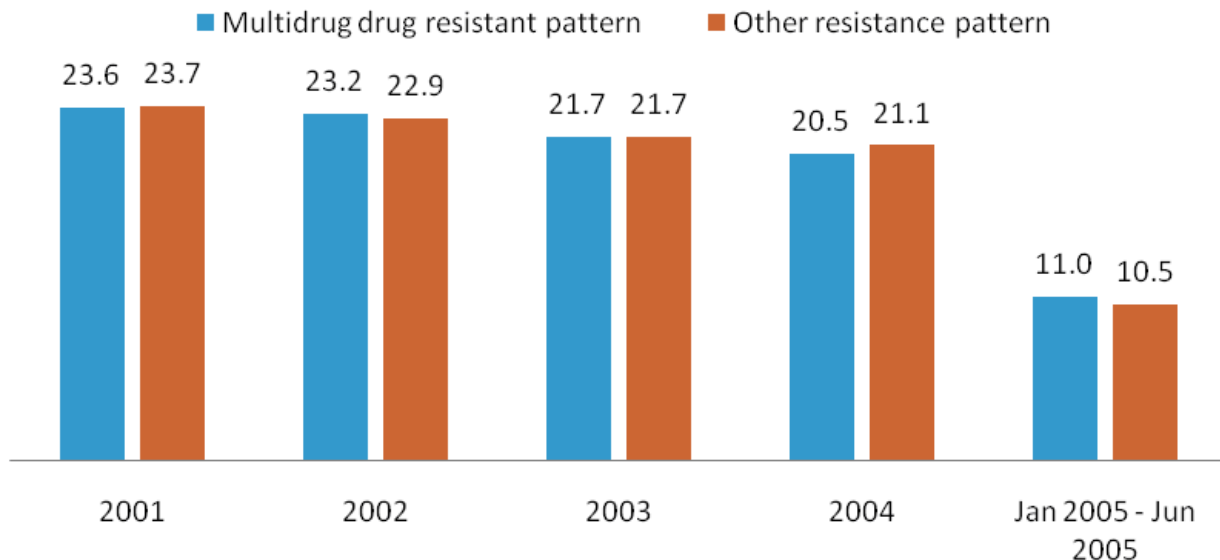
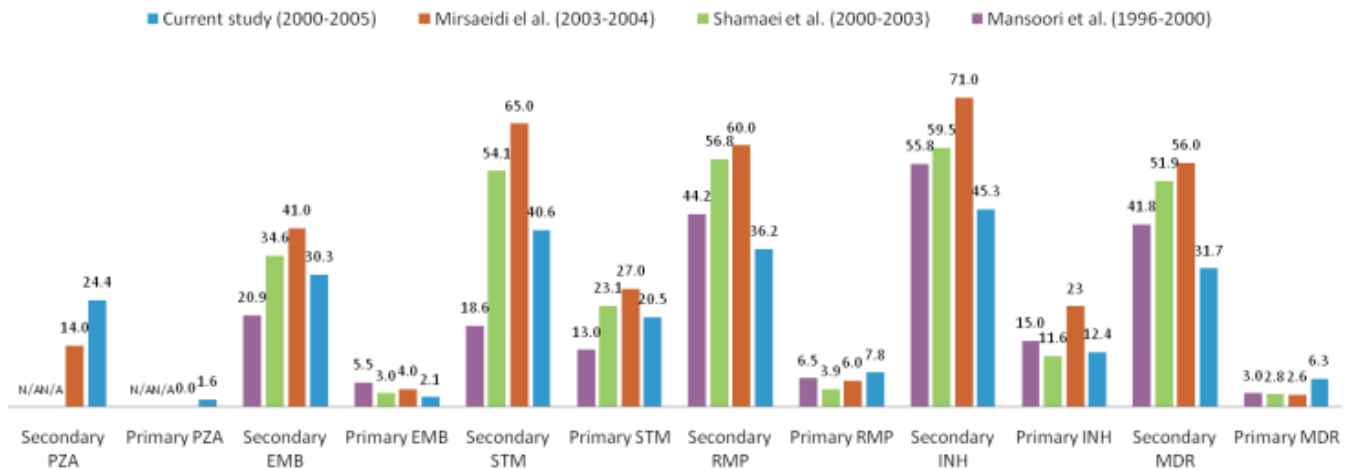


Figure 2. Anti-TB drug resistance pattern reported in current and previous studies in Iran



treatment of different infectious diseases. Furthermore, its high resistance rate guides the decision maker to limit STM use in the TB treatment regimen for susceptible and MDR-TB strains. Hence EMB is recommended. In this study, we found that the rate of any drug resistance and MDR-TB was 756 (43.4%) and 263 (15.1%), respectively. The rate is high compared to figures from some studies in the region [14-16]. The estimated primary and acquired resistance to any drugs (29.9%, and 69.0%, respectively) were similar to the results of previous studies in Iran with minor differences in the precise proportions for resistance to specific first-line anti-TB drugs [9,13,17] (Figure 2).

The estimated proportion of MDR-TB (4.1%) compared with that for secondary MDR-TB (11%) in our study is higher than that shown in the earlier reports [9,13,17] in the same centre. The discrepancy in findings between the present study and those of previous studies can be explained by the differences in the nature of the populations included in the different studies. The previous studies were conducted on hospitalized TB patients with a considerable proportion of treatment failures and relapses at the National Research Institute of Tuberculosis and Lung Diseases (NRITLD). The present study, which was conducted at NRITLD, included patients both hospitalized in the same centre and those referred from other hospitals and health centres for investigation and reporting. Based on the MDR-TB findings, therefore, the rate of prevalence of acquired MDR-TB was five times higher than that of primary MDR-TB in this

study. The WHO and International Union Against Tuberculosis and Lung Disease (IUATLD) working group on global surveillance for anti-TB drug resistance reported a prevalence of primary MDR of 1.4%, and acquired resistance of 13% in previously treated cases [18]. Thus prevalence of MDR-TB is 10 times higher in previously treated cases.

The high prevalence of initial MDR-TB in our study is assumed to be due to recent transmission of drug resistant strains. Overall, the rate of drug resistance in our study was high, which is in line with findings of some high-burden countries. He et al. reported the prevalence of any drug resistance in 10 provinces of China as 24.3% (range 14.8% – 42.1%) among new cases and 51.8% (range 27.5% – 67.5%) among previously treated cases. In contrast, the prevalence of MDR-TB was 5.4% (range 2.1% – 10.4%) among new cases and 25.6% (range 11.7%– 36.9%) among previously treated cases [19]. In comparison to our study, lower MDR-TB rates, specifically 2% for new cases and 29.4% for previously treated cases, were reported from the WHO Eastern Mediterranean Region [20]. Strikingly, the high MDR-TB rate in this study is a matter of great concern and may indicate increasing trends of MDR-TB. Therefore, a community-based study is recommended to gain a better understanding of the trends of drug resistance within the country. The anti-TB drug resistance trends are presented in a longitudinal graph, which shows no significant trends by year (Figure 1).

In this study, univariate analysis showed six relevant risk factors of MDR-TB. Age under 45 years,

Table 5. Significant risk factors associated with MDR-TB reported in current and previous studies in Iran

Reference	Time/place	Design	Population studies No.	No. of MDR-TB	Factor
Current study	Dec 2000 - Jun 2005, NRL, NRITLD	Retrospective laboratory notes based study	1742	263	Age < 45 Male sex Previous treatment Immigrant (Afghan) Poor living conditions Un-employment
Shamaei <i>et al.</i>	Mar 2000 - Feb 2003, TB department, NRITLD	Retrospective hospital notes based study	548	106	Age > 65 Immigrant (Afghan) Previous treatment
Mirsaeidi <i>et al.</i>	Jun 2003 to Sep 2004, TB department, NRITLD	Anterograde patient interview study	264	45	Previous treatment
Baghaei <i>et al.</i>	2002 - 2005, TB department, NRITLD	Retrospective hospital based study	282	48	Previous treatment Immigrant (Afghan) Positive sputum smear

male sex, previous TB treatment, immigration, poor living conditions, and unemployment were independent risk factors for MDR-TB. To address possible cofounders in the present study, all variables analyzed in the univariate analysis were included in the multivariate logistic regression method. After the analysis, four variables remained in the multivariate model, namely age under 45 years, male sex, previous TB treatment, and immigration. These figures are nearly consistent with findings from previous studies [9,10,13] in the same institute with the exception of the age (Table 5).

In contrast to our findings, Shamaei *et al.* [9] reported that age older than 65 years was significantly associated with MDR-TB. However, in a study done by Baghaei *et al.* in the same centre, the mean age of MDR-TB patients was 43.51 + 18.4 yrs, but the finding was not statistically significant [10]. In the present study, the association of MDR strains with a young age group might be explained by the inclusion of a larger sample size from the whole country. Several reports found that younger age and male gender were predominantly associated with drug resistance TB [14,21,22]. A review by Faustini *et al.* [6] reported that MDR-TB was more common in patients under 65 years of age, but the association was weak and more heterogeneous in patients under 45 years. The high frequency of drug resistance among a young age group in the present study may indicate the occurrence of recent transmission, whereas in the older age group, the infection has been acquired in the past.

Therefore, careful monitoring of transmission trends of drug resistant strains should be considered a priority to ensure a successful TB control programme. This should be done through increased awareness, early case detection, rapid drug susceptibility testing, a full course of effective anti-TB treatment, continuous community-based surveillance of anti-TB drug resistance, and control of drug resistant TB at the border entry points with neighboring TB high-burden countries.

The higher prevalence of MDR-TB in men over women may be explained by the fact that women are more compliant with treatment and therefore less likely to receive inadequate treatment than men. Furthermore, men are almost always outdoors and therefore more susceptible to community-acquired resistant strains.

Previous treatment has been well documented to be associated with MDR-TB in many studies [5,9,10,13,16,21,23,24]. It may potentiate drug resistance through improper drug regimens, inadequate or irregular drug supply, unsatisfactory compliance by patients or clinicians, lack of supervision of treatment, and absence of infection control measures in hospitals. Immigration has been suggested as one factor leading to increased prevalence of MDR-TB in many countries [2,6,9,10]. In our study, a possible explanation for the high rate of MDR-TB in Afghan cases may be the poorer access to health-care facilities and higher risk of transmission of MDR strains for immigrants. It has been shown that Afghan immigrants play an important

role in maintaining recurrent tuberculosis. Our previous study at the same NRITLD showed that the intra-community transmissions between Iranian and Afghan patients were 41%. It has also been shown that more than 90% of active cases of TB in Iran resulted from reactivation of infection, and that recently transmitted diseases had a minor role. Recently, however, this picture has changed and a higher rate of reactivation (74.8%) versus recent transmission (25.2%) occurred in Iran [25]. Hence the proportion of endogenous versus imported TB changed over time. Therefore, further fingerprinting studies are recommended to gain a better understanding of change over time.

Our study revealed that Afghan immigrants were younger than Iranian (data not shown). This finding, which agrees with the observations in our previous study, [26] suggests that younger persons and persons who have emigrated from Afghanistan and from other TB high-burden settings may be at higher risk for transmitting MDR-TB strains. Furthermore, nomadic populations such as the Afghan immigrant patients contribute to the circulation of MDR strains, as opposed to the settled Iranian MDR-TB patients. This finding supports the proposal for a policy for screening all immigrants to Iran for TB, especially those from TB high-burden countries. The new strategy of the Iranian National TB Programme (NTP) is to screen all immigrants at border entry points from countries with high levels of drug resistance such as Afghanistan, Pakistan, and Azerbaijan. Positive patients would be subjected to anti-TB treatment before being allowed into the country.

The study's main limitation is that we could not include other important variables associated with MDR-TB such as HIV infection, diabetes mellitus, cavitory chest lesion, and drug abuse, as sufficient information on these parameters were missing in the case notes. Furthermore, the different sample sizes and the different time periods of different Iranian studies reduce the validity of the comparative findings.

In conclusion, this study reflects the rising trend of resistance in the community. DST is routinely recommended to provide optimal treatment to culture positive TB cases with high risk of drug resistance, especially in immigrants from TB high-burden countries. The high rate of initial resistance in MDR-TB cases and the high rate of MDR-TB among a young age group were indicators of recent transmission, which suggests potential trends. Therefore, closer monitoring of transmission trends of drug resistant strains should be considered as priority,

to ensure a successful TB control programme. This should be done through increased awareness, early case detection, rapid drug susceptibility testing, a full course of effective anti-TB treatment, continuous surveillance of anti-TB drug resistance, and control of drug resistant TB at border entry points with neighboring TB-high burden countries.

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