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Drug resistance pattern of *Mycobacterium tuberculosis* isolates from patients of five provinces of Iran

Mohammad Javad Nasiri[1], Faranak Rezaei[1], Samin Zamani[1], Davod Darban-Sarokhalil[2], Abbas Ali Imani Fooladi[3], Hasan Shojaei[4], Mohammad Mehdi Feizabadi[1*]

[1]*Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Microbiology, School of Medicine, Alborz University of Medical Sciemces, Karaj, Iran*
[3]*Applied Microbiology Research Centre, Baqiyatallah University of Medical Sciences, Tehran*
[4]*Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences. Isfahan, Iran*

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ABSTRACT

Objective: To determine the patterns of resistance to first line anti-tuberculosis (TB) drugs among a collection of *Mycobacterium tuberculosis* (MTB) isolates from 5 provinces of Iran. **Methods:** A total of the 6 426 clinical specimens from patients suspected of active TB were collected from March 2010 to June 2012. All specimens were subjected for microscopy and culture tests in the TB centers of studies provinces. Drug susceptibility testing to the first line anti-TB drugs for culture positive MTB was performed on LÖwenstein-Jensen (LJ) medium using proportion method. **Results:** Of 6 426 clinical specimens, 261 were culture positive for mycobacteria, of which 252 were MTB and 9 were MOTT (mycobacteria other than tuberculosis). Of 252 MTB isolates, 211 (83.7%) were pan-susceptible and 41 (16.3%) were resistant to at least one drug. Resistance was most common to streptomycin, 30 isolates (12.0%), followed by isoniazid, 20 isolates (8.0%), rifampin, 15 isolates (6.0%) and ethambutol, 14 isolates (5.5%). Sixteen (6.3%) MTB isolates were MDR. A clear evidence of heterogeneity amongst the 5 provinces in the proportions with resistance to one or more drugs was observed [χ^2 = 12.209 (4 degrees of freedom), *P* values = 0.015 9]. **Conclusions:** The prevalence of drug resistance in this study area underscoring the need for further enforcement of TB control strategies in the Iran. Drug susceptibility testing for all TB cases to provide optimal treatment, establishing advanced diagnostic facilities for rapid detection of MDR-TB and continuous monitoring of drug resistance are recommended for prevention and control of drug-resistant TB.

1. Introduction

Tuberculosis (TB) still remains one of the most common infectious diseases in developing countries. According to WHO reports, there were an estimated 8.7 million incident cases of TB globally[1]. Among of these cases, 3.7% are estimated to have multidrug resistant tuberculosis (MDR-TB)[1]. Iran, one of the eastern Mediterranean countries, is located between the high MDR-TB (Azerbaijan and Armenia) and high-TB burden (Afghanistan and Pakistan) countries in the region. Since 1996, when the national TB control programs established in Iran, TB incidence has been declining from 34 per 100 000 to 21 per 100 000 cases in 2011[2]. However, in recent years, emergence and spread of MDR-TB threaten the TB control strategy. In many low- and middle-income countries, due to inadequate laboratory capacity, most of the patients with MDR-TB are not diagnosed. Treatment in these cases mostly failed and significant expenditure of health care resources is needed. The diagnosis of MDR-TB requires that TB patients are tested for susceptibility to anti-TB drugs[1]. Hence, early diagnosis of the disease and drug susceptibility testing of TB cases is essential priorities for TB care and control[1]. Though drug resistance patterns of *Mycobacterium tuberculosis* (MTB) has been previously reported from Iran, most of available information is localized and comparative

*Corresponding author: Mohammad Mehdi Feizabadi PhD, Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Tel: (9821)64053402
Fax: (9821)88955810
E-mail: mfeizabadi@tums.ac.ir
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phenotypic analyses of MTB isolates from different parts of Iran were not performed[3,4]. The present study was hence designed to determine drug resistance pattern of MTB isolates from 5 provinces of Iran including Tehran (the capital of Iran), Sistan-Baluchestan (southeast province of Iran with highest rate of TB), Kermanshah (western part of Iran, bordering with Iraq), Hormozgan (southern part of Iran and highly endemic for malaria) and Isfahan (located on the main north-south and east-west routes crossing Iran).

2. Materials and methods

2.1. Study population and samples

The study was approved by the Local Ethical Committee. The study was conducted over a 2-year of period (from March 2010 to June 2012) in the TB centers of 5 provinces of Iran that covers Eastern to the Western regions of the country. The study was cross-sectional that was performed single blinded. A total of 6 426 clinical specimens were obtained from TB suspected patients attending the 5 different TB center including Tehran (n=2 133), Sistan-Baluchestan (n=873), Kermanshah (n=377), Hormozgan (n=1 538) and Isfahan (n=1 505). Patients had clinical signs and symptoms of TB and undergoing examination for possible active TB.

The age of patients ranged from 11 to 80 years and the mean age of the patients was 45 years. Based on history and clinical examination, none of the patients had documented HIV infection.

In the each TB centers, 3 samples of sputum and other specimens (Pleural fluid, wound, urine and CSF) were collected on sterile containers from each patient for microscopy and culture tests. All specimens were held at 4 ℃ until processed by standard laboratory procedures[5]. The majority of specimens were processed within 24 h at TB laboratory.

2.2. Identification of mycobacteria

For microscopy examination, a smear from samples using a wire loop was made on clean slide. The smear was stained by Ziehl Neelsen method. Known negative and positive slide were prepared with every batch of the specimens. Samples from each patient were decontaminated by Petroff's method and were inoculated into Lowenstein-Jensen (LJ) media (Merck)[6]. The slope cultures were incubated at 37 ℃ and examined for growth once weekly up to 6 weeks. Each isolate was examined regarding morphology, pigmentation and date of growth. Bacterial isolates identified as MTB complex using standard biochemical tests, including production of niacin, nitrate reduction, catalase and inhibition by thiophene-2-carboxylic acid hydrazide[7,8]. Isolates of MTB cultured from patients of each province were sent to mycobacteriology center, Baqiyatallah hospital, Tehran, for confirmation and susceptibility testing.

2.3. Drug susceptibility testing

Drug susceptibility of isolates to first-line anti-TB drugs was determined using the proportion method on Lowenstein-Jensen medium[7]. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drugs: 0.2 μg/mL for isoniazid (INH), 2.0 μg/mL for ethambutol (EMB), 4 μg/mL for streptomycin (STM) and 40 μg/mL for rifampicin (RMP). Interpretation was made according to the usual criteria for resistance, *ie.*, 1% for all drugs. We used the protocols of quality control in our laboratories which are mentioned in different sources[7,9].

2.4. Definition

Any drug resistance was defined as resistance to one or more first-line anti-TB drugs. Monoresistance was defined as resistance to only one of the four first-line anti-TB drugs (INH, RMP, EMB and STM). MDR was defined as MTB strains that were resistant to at least INH and RMP.

Primary resistance (resistance among new TB cases) was defined as *in vitro* resistance in patients who did not have a history of anti-TB treatment, while secondary resistance (resistance among previously treated cases) was defined as *in vitro* resistance in patients previously treated with anti-TB drugs.

2.5. Statistical analysis

Statistical analysis was carried out using SPSS version 18. Prevalence was reported by 95% confidence intervals (*CI*). Comprasion of propotion was done by *Chi* squere test. *P* values less than 0.05 were considered statistically significant.

3. Results

Of 6 426 clinical specimens, 261 culture were mycobacteria positive, of which 252 were MTB [Tehran (n=85), Sistan-Baluchestan (n=59), Kermanshah (n=15), Hormozgan (n=48) and Isfahan (n=45)] and 9 were MOTT (mycobacteria other than tuberculosis). Of 252 MTB isolates, two hundred and thirty-three were from pulmonary sites [Sputum (n= 136) and bronchoalveolar lavage (n=97)] while 19 isolates were from extra-pulmonary sites [Pleural fluid (n=5), wound (n=6), urine (n=5) and CSF (n=3)]. Also based on patients information, all MTB cultured was from new cases.

3.1. Drug susceptibility patterns

Of 252 MTB isolates for whom drug susceptibility testing was performed, 211 (83.7%, 95% *CI* = 79.1%-88.2%) were pan-susceptible and 41 (16.3%, 95% *CI* = 11.7%-20.8%) were resistant to at least one drug. The majority of drug resistant isolates showed resistance to STM (12.0%, n=30, 95% *CI*=16%-8%) followed by INH (8.0%, n=20, 95% *CI*=4.6%-

11.3%), RMP (6.0%, n=15, 95% CI=3%-9%) and EMB (5.5%, n=14, 95% CI=2.6%-8.3%) (Table 1).

Among the investigated isolates from different parts of Iran, 16 (6.3%, 95% CI=3.3%-9.2%) MTB isolates were MDR. A clear evidence of heterogeneity amongst the 5 provinces in the proportions with resistance to one or more drugs was observed [χ^2 = 12.209 (4 degrees of freedom), P values=0.0159]. Complete drug resistance patterns of MTB isolates are shown in Table 1.

4. Discussion

In the last decades, by running the national TB control program in Iran, TB incidence has been decreased. However, emergence and spread of MDR-TB has threaten the national TB control programs. Drug susceptibility testing for TB patients is one of the most effective tools of control and management of MDR-TB.

In the present study, 41 (16.3%) of 252 MTB isolates collected from patients of different parts of Iran were resistant to at least one or more anti-TB drugs. This observation indicating lower any drug resistance than studies conducted in Turkey, Iraq and Pakistan with any drug resistance rates of 21.1%, 29.7% and 64% respectively[10-12]. According to WHO reports in the year 2011, Iran has a lower incidence of TB (21 per 100 000 population) than the all of neighboring countries (Turkey: 24 per 100 000, Iraq: 45 per 100 000, Armenia: 55 per 100 000, Azerbaijan: 113 per 100 000, Afghanistan: 189 per 100 000 and Pakistan: 231 per 100 000)[2].

The lower number of new cases in Iran and subsequently lower amount of previous anti-TB drug taking may explain the lower any drug resistance in this study.

Drug resistance to STM (12.0%) was found to be the highest proportion among anti-TB drugs, followed by INH (8.0%) which is consistent with previous reports from Iran[13-16]. The cause of the high rates of primary drug resistance to STM and INH in MTB isolates from new cases is difficult to explain. The high prevalence of STM resistance isolates may be due to previous usage of other aminoglycosidal drugs for treatment of different infectious diseases like brucellosis that resulting in cross-resistance[14,17]. Also, widespread use of STM in the past for treatment of the brucellosis, a disease that is endemic in Iran may explain another reason of high rate of STM resistance. This high rate of STM resistance has very important implications for TB control strategies as it would make treatment regimes less effective. Definitive studies have not been performed to determine the best treatment for different patterns of drug resistance. However, to prevent treatment failure, recommended regimes that suggested by WHO should be given to TB patients[18].

As reported by other studies, we found that Monoresistance RMP was rare and most of the RMP resistant strains were also INH resistant. Thus RMP resistance would be a good marker for the identification of MDR-TB[13].

As reported by the WHO International Project in 79 countries worldwide over 1999-2002, MDR-TB was found in 1.1% of new cases in Iran[19]. A recent study from National Research Institute of Tuberculosis and Lung Disease in Iran, that presented drug resistance patterns of TB from 2003 to 2008, has shown that 2% of MTB isolates from new cases were MDR[20]. Prevalence of MDR-TB in Iran, in the year 2011, was 5% for new cases as reported by WHO[2]. Our result shows a little higher percentage of MDR-TB (6.3%), compared to WHO data and other studies of Iran[14,16,20]. This supports the idea of inadequacy in TB infection control programs and irregular treatments during recent years. Drug susceptibility testing for all new cases to provide optimal treatment is strongly recommended to avoid the emergence of MDR-TB. The rate of drug resistance in Kermanshah and Sistan-Baluchestan was in comparable with the studies conducted in other provinces of Iran[4,21]. Bordering with the high levels of drug resistance countries (Iraq and Pakistan) and possible transmission of MDR-TB may explain the rate of drug resistance in these provinces. Screening all immigrants at border of entry from these counties could contribute to national TB control program in Iran.

Hormozgan, a province on the Persian Gulf coast, is highly endemic for malaria[22]. The rate of MDR-TB in this province was relatively higher than what was reported in other studies[4]. Interactions between TB and malaria have been demonstrated previously by authors. Malaria infection affects severely ill TB patients and exacerbates mycobacterial infection[23-26]. Moreover, the respiratory distress that is frequent during acute malaria can worsen the respiratory effort related to TB[27]. Therefore, given the multiple interactions between malaria and TB, public health strategies for prevention and management of these infectious

Table 1
Resistance to first-line drugs among MTB isolates from different parts of Iran.

Type	Tehran No (%)	Sistan-Baluchestan No (%)	Kermanshah No (%)	Hormozgan No (%)	Isfahan No (%)	Total No (%)
Strains	85 (33.7)	59 (23.4)	15 (5.4)	48 (19.0)	45 (17.8)	252 (100.0)
Pan-susceptible	68 (80.0)	49 (83.0)	9 (60.0)	42 (87.5)	43 (20.0)	211 (83.7)
Any drug resistance	17 (20.0)	10 (17.0)	6 (40.0)	6 (12.5)	2 (3.2)	41 (16.3)
INH	6 (7.0)	5 (8.4)	4 (26.6)	3 (6.2)	2 (7.1)	20 (8.0)
RMP	7 (8.2)	3 (5.0)	3 (20.0)	2 (4.1)	2 (8.6)	15 (6.0)
EMB	6 (7.0)	3 (5.0)	3 (20.0)	2 (4.1)	0 (0.0)	14 (5.5)
STM	14 (16.4)	8 (13.5)	3 (20.0)	4 (8.3)	1 (2.2)	30 (12.0)
Monoresistance	11 (13.0)	7 (11.8)	2 (13.3)	4 (8.3)	0 (0.0)	24 (9.5)
Multidrug resistance	6 (7.0)	3 (5.0)	3 (20.0)	2 (4.1)	2 (11.7)	16 (6.3)

diseases should be investigated.

In conclusion, the prevalence of drug resistance in this study area was relatively high comparable to WHO data and previous studies, underscoring the need for greater enforcement of TB control strategies in the country. Drug susceptibility testing for all TB cases to provide optimal treatment, establishing advanced diagnostic facilities for rapid detection of MDR-TB, continuous monitoring of drug resistance and control of drug resistant TB at border entry points with high-TB burden countries are recommended for prevention and control of drug-resistant TB.

Conflict of interest statement

The authors declare that there are no conflicts of interest, financial or otherwise in the publication of this manuscript

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