



## Evaluation of Antimicrobial Resistance of New Cases of Pulmonary Tuberculosis, in Khorasan, Iran

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### ABSTRACT

**Background:** Multi drug-resistant *Mycobacterium tuberculosis* (MDR-TB) is an infection with a causative bacillus which is resistant to at least two drugs, isoniazid and rifampin. The purpose of this study is to evaluate the prevalence of TB resistance to first-line drugs of newly diagnosed active pulmonary tuberculosis.

**Methods:** This cross-sectional study was performed on 77 patients with newly diagnosed active pulmonary tuberculosis (according to national protocols of TB). Sputum samples were cultured and antibiogram for isoniazid, rifampin, pyrazinamide, ethambutol, and Streptomycin were performed on the positive cultures.

**Results:** From 77 patients with tuberculosis, 48 cases have positive sputum culture. Antibiogram was done by proportional standard method and all samples were found to be fully sensitive to all first-line TB drugs.

**Conclusions:** According to the results of this study, the primary resistance was low to the first-line drugs for pulmonary tuberculosis of the samples collected from Khorasan, an east province of Iran. The data showed that in all patients with active pulmonary tuberculosis who were diagnosed with Ziehl–Neelsen stain of sputum, the use of the first-line drugs for tuberculosis treatment is necessary and could be sufficient.

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## Introduction

Tuberculosis (TB) is the most common infectious disease of human, as one of the biggest killers of people known in history. Every year, 9 million people suffer from active TB, and about 1.5 million people dies from the disease (1). Ninety present of these patients belong to the developing countries. According to the World Health Organization (WHO) in 2010, the prevalence of TB is 17 per thousand person and its incidence is 13 per thousand (2). Multi-drug resistant (MDR) TB \*, is the result of poor management of TB and is a serious problem in many countries (1). Multi-drug resistant bacilli are resistant to at least two drugs, isoniazid and rifampin (3). WHO has estimated that the number of the new cases of MDR-TB are 440,000 patient in 2009 (4- 6). In patients who are initially diagnosed with MDR-TB, a short-term administration of standard therapy does not cure patients, and causes resistance to a wider range of anti-TB drugs (7). There are three types of resistance in tuberculosis, including: 1. Innate resistance † that bacilli are resistant inherently without a history of drug usage. 2. Acquired or secondary resistance caused by improper use of medications and 3. Primary resistance that patients with acquired resistance infect others (8). Tuberculosis in Iran has high incidence, especially in Khorasan province due to its proximity to countries like Afghanistan and immigration from these countries. In patients with MDR-TB, mortality rate and treatment failure and recovery time and treatment costs are high and its control is difficult. So far, many studies have been done in most cases of MDR-TB .However, fewer studies have been done in the case of tuberculosis with primary resistance. Thus we decided to examine the prevalence of primary resistance in newly diagnosed patients with active pulmonary tuberculosis who have not been treated.

## Material and method

This is a cross-sectional study which has been done at the Mashhad University of Medical Sciences from 2011 till 2012. Seventy seven patients with proven pulmonary tuberculosis were studied .The sampling method was based on “Purposive Sampling”. The inclusion criteria for confirmed TB cases were based on criteria outlined in the “The National Tuberculosis Protocol” (7) as: 1-At least two positive sputum smears.2-Sputum smear-positive plus a chest X rays compatible with pulmonary tuberculosis. Those who had taken anti TB drugs more than five days were excluded. All patients received a full explanation of the aims and research designs and a written consent was taken from them. Morning sputum samples were obtained from patients. Equal volume of sodium hydroxide 4% was added to each sputum sample for homogenization and decontamination. Then the mixture was vortexed and the resulting mixture was left for 15 minutes under the hood. Then sputum samples were centrifuged at around 3000 rpm for 15 min, then the upper liquid were discarded inside the dish which containing phenol 10%. A few drops of phenol red, were added to the sediment as a pH indicator. Then the samples are vortexed and neutralized by adding hydrochloric acid. Then the samples were again stirred with a vortex and 3 to 4 drops of the this liquid were added to the Löwenstein–Jensen culture medium and incubated at 37 ° C. After the third day onwards, we investigated the environment for fast growing mycobacteria. After that the cultivation environments were investigated to find MTB, every week (Figure1) antibiogram test by “Proprtional method” for isoniazid, rifampin, pyrazinamide, ethambutol, and Streptomycin was performed.

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\* MDR-TB

† Natural Resistance

**Table 1.** Frequency distribution according to the severity of sputum smear and culture results

The intensity of smears	Sputum culture results				Test results
	Positive		Negative		
	%	No	%	No	
1	29.2	14	21.4	6	0.806
2	31.2	15	42.9	12	
3	39.5	19	35.7	11	
Total	100.0	48	100.0	29	

*Data analysis*

Descriptive statistical analyses were used for the data (including demographics, clinical, and results related to antibiotics susceptibility test). Data were analysed using SPSS and results are given in percentages. To calculate the resistance in new cases, the number of resistant cultures with corresponding drug susceptibility tests (DST) results in these patients was divided by the total number of cultures with corresponding DST results from new cases.

**Result**

Among 77 patients with tuberculosis, 37 (48.1%) were males. Their median age was 51.18±23.54 years. Fifty-four (70.1%) were Iranian people and 23(29.9%) were Afghan. Sputum culture was positive in 48 patients. Mann-Whitney test showed that there is no correlation between the severities of sputum smear and culture results.

Table 1 and 2 show Frequency distribution of demographic factors, clinical characteristics, severity of positive sputum smear and other factors affecting tuberculosis in two groups. The group with positive sputum culture and the group negative sputum culture were evaluated. In patients with negative sputum cultures, 55.2% were males and in patients with positive sputum culture 43.8% were males. All patients with positive cultures were fully sensitive to all 5 first TB drugs including isoniazid, rifampin, ethambutol, streptomycin and pyrazinamide.

**Discussion**

The authors identified quantitative levels and genetic mechanisms of resistance in cases who were drug-resistant clinically. They found that some strains categorized as resistant to isoniazid, ethambutol or streptomycin by standard laboratory procedures of in vitro drug susceptibility testing may still respond to a treatment regimen that includes these agents (10). In Iran, treatment will start initially in patients with positive TB smear. If at the end of the second phase of treatment the smears were still positive, sputum samples were cultured and antibiotic susceptibility testing will be perform. In a study in Iran, of 260 patients with newly diagnosed TB, only two patients were smear positive at the end of treatment. When culture results were prepared, it was found that only one patient was actually resistant to isoniazid and rifampin. Similar to our study, the prevalence of drug resistance was low in their study (11). In a Moldova research, it was concluded that because initial sputum samples are taken after initiation of therapy, their usefulness for case management or surveillance is compromising, and inappropriate treatment can lead to increased antimicrobial resistance (12).

**Table 2.** Frequency distribution of demographic characteristics and influencing factors of TB by sputum culture results

Variables	Sputum culture results		Test results
	Positive	Negative	
Sex (male)	21(43.8%)	16(55.2%)	0.331
Age (years)	51.94±23.60	49.93±23.79	0.720
Weight (Kilo Grams)	52.31±6.18	51.93± 7.73	0.808
Iranian nationality	35(72.9)	19(65.5%)	0.492
Marital status (single)	16(33.3%)	8(27.6%)	0.598
HIV-positive	0(0.0%)	0(0.0%)	-
Prison	5(10.4%)	3(10.3%)	1
Diabetes	6(12.5%)	1(3.4%)	0.244
Renal failure	2(4.2%)	2(6.9%)	0.629
Steroid	4(8.3%)	1(3.4%)	0.645
History of contact with tuberculosis	17(35.4%)	17(58.6%)	0.047
Exposed to sunlight	36(75.0%)	19(65.5%)	0.32
Total	48(100.0%)	29(100.0%)	

In a study from Tehran, a new case of resistant tuberculosis bacilli has been introduced which was resistant against all drugs (TDR) and showed that, there is some risk of spreading of TDR-TB in Asia (13). In a study of Kenya (Nairobi), the resistance pattern of pulmonary tuberculosis was studied. They concluded that resistance to isoniazid, which is a first line drug, can lead to MDR-TB unless control programs carried out carefully (14).

In another study from Iran, the resistance of mycobacteria to drugs and the risk factors for resistance were investigated. They concluded that the young age of patients and the onset of high rates of resistance to MDR-TB maybe a sign of a new transmission. Therefore, close observation is necessary for successful control of tuberculosis (15). In India, the rate of resistance was higher in those who received appropriate treatment, whereas in patients who did not receive any medication, the resistance rate was low (16). In all studies which reviewed above, the secondary resistance, were investigated.

‡ totally drug-resistant [TDR] or super extensively drug-resistant [XDR]

As mentioned in the Moldova study, most studies regarding drug resistance were after initiation of therapy. In our study, unlike above studies, patients were studied before beginning of treatment. Our goal was to find cases of drug resistance resulting from contact with TB patients who had MDR-TB. Thirty-four patients had a history of contact with tuberculosis (Table 2). Our results showed that primary resistant TB, is low. (N = 0).

**Table 3:** Frequency distribution of clinical findings by sputum culture results

signs	Sputum culture results		Test result
	Positive	Negative	
Coughing for more than two weeks	47(97.9%)	26(89.7%)	0.147
Productive cough	34(70.8%)	23(79.3%)	0.411
Chest pain	11(22.9%)	4(13.8%)	0.327
Respiratory distress	22(45.8%)	15(51.7%)	0.616
Hemoptysis	4(8.3%)	5(17.2%)	0.285
Fever	25(52.1%)	21(72.4%)	0.075
Weight loss	42(87.5%)	26(89.7%)	1
Weakness	36(75.0%)	21(72.4%)	0.733
Night sweat	34(70.8%)	19(65.5%)	0.626
Total	48(100.0%)	29(100.0%)	

The reasons for the low prevalence of primary resistance in our study could be as following: 1- Careful monitoring of patients and appropriate management of patients, by provincial health care system through Directly Observed Treatment Short- course strategy (DOTS) that has resulted in decreased incidence of tuberculosis in Iran (17). Patients with MDR-TB were referred to more equipped centers. Very low incidence of secondary resistance, as in the other studies carried out in Iran (13). The limitations of our study were the small number of patients and the fact that, patients were not followed after starting of treatment.

**Conclusion**

Our study shows that given the low incidence of primary resistant TB, short-term treatment strategy under direct observation is essential to prevent secondary resistance. Additionally starting treatment with first-line drugs (i.e., rifampin, isoniazid, ethambutol, pyrazinamide) is still appropriate for newly diagnosed cases of pulmonary tuberculosis.



**Figure1.** Sputum culture in Lovnshtayn-Johnson medium for M-TB

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## Conflict of interest

None declared conflicts of interest.

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