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Primary Multidrug-Resistant Mycobacterium Tuberculosis (P-MDRTB) in Gorgan, Iran

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Abstract

Background: *Resistance to anti-tuberculosis (TB) drugs is a global problem. Previous studies showed that the prevalence of primary MDR-TB is higher and has been increasing. The purpose of this study was to determine the primary drug resistance in strains isolated from patients with smear-positive of the new cases to anti-TB drugs.*
Methods: *In this cross-sectional study, 266 smear positive pulmonary TB Iranian patients were enrolled during the April 2011 and March 2012. Drug Susceptibility Testing (DST) of M. tuberculosis isolates to the first-line drugs, isoniazid, rifampicin, ethambutol, and streptomycin, were performed by the proportion method using conventional Lowenstein-Jensen (L-J) medium.*
Results: *The average age was 48.4±21.3 years, and 56.5 percent were male. In this study, out of 266 pulmonary TB suspects studied, susceptibility testing was performed for 189 isolates. Resistance to at least one drug was present in 11.1% (21/189) of cases. Resistance among newly diagnosed patients was most common for streptomycin, and MDR prevalence rates (resistant to ISONIAZID and RIFAMPIN) among these patients were 2.1%.*
Conclusions: *Our finding suggests that the incidence of MDR tuberculosis in high TB burden settings stresses the need for drug susceptibility testing to be done for every patient who is culture positive for Mycobacterium tuberculosis.*

Keywords: Mycobacterium infections, Tuberculosis, Gram-positive bacterial infections, Multidrug-resistant tuberculosis.
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Introduction

Tuberculosis (TB) is a top infectious disease killer worldwide. In 2014, 9.6 million people fell ill with TB and 1.5 million died from the disease. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top five causes of death for women aged 15 to 44. Globally in 2014, an estimated 480,000 people worldwide developed MDR-TB. It is estimated that about 9.7% of these cases were XDR-TB.[1]

Mycobacterium tuberculosis, the bacterium that causes tuberculosis, is spread in airborne droplets when people with the disease cough or sneeze and usually infects the lungs (pulmonary tuberculosis).[2] The characteristic symptoms of tuberculosis are a persistent cough, weight loss, and night sweats.[3] Tuberculosis can be cured by taking several powerful antibiotics regularly for at least six months. The standard treatment for tuberculosis comprises an initial intensive phase lasting two months during which four antibiotics are taken daily followed by a four-month continuation phase during which two antibiotics are taken.[4]

The emergence over the past two decades of multidrug-resistant tuberculosis, or tuberculosis caused by strains of M. tuberculosis that are resistant to ISONIAZID and RIFAMPIN, with or without resistance to other agents, has greatly complicated efforts to control the global tuberculosis epidemic.[5]

Gorgan is in the center of Golestan Province in northern Iran, southeast of the Caspian Sea.[6] Due to its geographical position and vicinity to countries with a high incidence of TB and because it accepts immigrants, Golestan province at the northern part of country is one of the most infested provinces of the country. Today, drug resistance is considered as a serious problem in TB control programs in most countries. The risk of first-line treatment multi-drug-resistant tuberculosis (MDR-TB) and second-line treatment extensive-drug-resistant tuberculosis (EDR-TB) is considered a big problem in TB control. Therefore, understanding the prevalence of primary MDR-TB may help in the control and management of this disease. The current study aimed to determine the primary drug resistance strains isolated from patients with smear-positive of new cases to anti-TB drugs in Golestan in Northeast Iran.

Materials and Methods

In this cross-sectional study, which was conducted in Golestan province during 2011–2012, we determined initial drug resistance in smear-positive pulmonary TB patients (new cases) and their resistance pattern.

The population study included all new smear-positive pulmonary TB patients who came to health center of Gorgan County. Patients whose direct sputum smear microscopy test were positive (at least two positive sputum smear) and had not already received anti-TB treatment or less than one week had passed from initiation of their TB treatment entered the study. Extra pulmonary TB cases were excluded from the study.

The method of sampling was simple random. Demographic information including age, sex, place of residence, level of education, level of monthly income, patient's inmate records, and the initial sputum positivity rating were recorded on a questioner.

To culture samples on Lowenstein-Jensen medium, all samples were initially decontaminated using Petroff's method.[7] Also, acid-fast staining was performed using the Ziehl–Neelsen method.[4] Since the aim of this study is to determine initial drug susceptibility in patients infected with pulmonary TB induced

by M. tuberculosis strains (MTB), the species of strain isolated from sample was determined by using biochemical tests. Different tests, such as the niacin test, the nitrate reduction test, and thermostable catalase (68°C), were used to determine species.[8] The isolates obtained were subjected to the drug susceptibility test (DST) by proportion method. This method is the gold standard and is confirmed by World Health Organization (WHO) and Clinical and Laboratory Standards Institute (CLSI). The proportional method was performed according to the method of Canetti, Rist, and Grosset.[9]

This method is performed on strains for first- and second-line drugs and needs at least three to four weeks incubation.[9-11] The proportional method is based on the estimation of growth or no growth of an M. tuberculosis strain in the presence of a single "critical concentration" of one drug. The critical concentration of an antituberculous drug represents the lowest concentration of the drug in the medium that indicates clinically relevant resistance if growth is observed.[11] Susceptible wild-type strains are inhibited by this concentration. Resistance is established if over 1% of the bacterial population of a strain is able to grow. Although these techniques are widespread and have been used for a long time, a low reproducibility of the procedures due to poor standardization is sometimes reported. Different levels of reliability can be observed between the different drugs tested. Proficiency testing for DST is implemented in the Supranational Reference Laboratory Network, which was established by the WHO and IUATLD. Results from nine rounds of proficiency testing carried out between 1994 and 2002 showed higher sensitivities and specificities for INH and rifampicin (RMP) than for ethambutol (EMB) and streptomycin (SM) testing (cumulative sensitivities: 99% for INH, 97% for RMP, 91% for SM, and 89% for EMB; cumulative specificities: 98% for INH, 97% for RMP, and 94% for both EMB and SM).[11]

Obtained information was entered into version 16 of the SPSS application (SPSS Inc., Chicago, IL, USA) and was analyzed based on descriptive and analytical statistics. In this study, the confidence interval was 95%, and $P < 0.05$ was considered significant.

Results

Two-hundred sixty-six patients were eligible for the study, and an antibiogram test was performed on 214 of them. Out of these, the antibiogram test of samples from 189 patients was successful in terms of performance, such that from 214 test cases, 10 cases were non-tuberculosis mycobacterium (NTB), and 15 cases led to failure of bacteria growth in antibiogram control medium (failed). Out of 189 successful antibiogram tests, 12 patients did not come back after samples were sent, and their demographic information was not recorded, and finally these cases were announced missing (6.3% missing data). Figure 1 shows the schematic view of the study plan and the number of people omitted from the study and the reason for their omission.

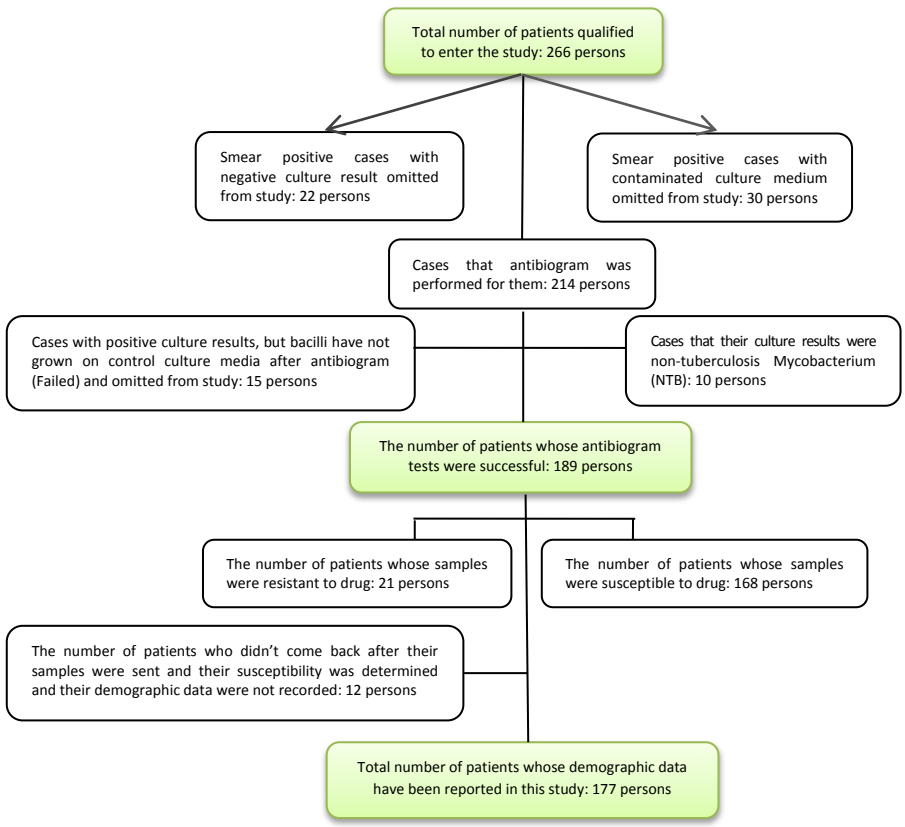

Figure: Schematic view of study plan.

Table 1: Frequency and percentage of demographic variables in initial drug-resistance smear positive pulmonary TB patients (new case), factors affecting it, and their antibiotic resistance in Golestan province in 2011–2012.

Variable		Frequency	Percentage
Gender		100	56.5
-	Male	77	43.5
-	Female		
Education			
-	Elementary	62	35
-	Middle school	24	13.6
-	High school	12	6.8
-	Postgraduate	9	5.1
-	other	70	39.5
Marriage status			
-	Single	43	24.3
-	Married	90	50.8
-	Widow	35	19.8
-	Divorcee	7	4
-	other	2	1.1
Occupation			
-	Worker	46	26
-	Retired	10	5.6
-	Technical/Self-employed	7	4
-	Unemployed/Housekeeper	96	54.2
-	0ther	18	10.6
Income status			
-	Sufficient	42	23.7
-	Partially sufficient	47	26.6
-	Insufficient	88	49.7
Ethnicity			
-	Fars	54	30.5
-	Sistanian	71	40.1
-	Turkoman	21	11.9
-	Other	31	17.6

The mean age of the patients was 48.4±21.3. All of the patients were new cases, and 56.5% of the patients were male. Only 2.8% of patients were non-Iranian and 21.5% of patients had a previous history of imprisonment. Other demographic information of patients has been given in Table 1.

Of the 189 strains, 168 (88.9%) were sensitive to all drugs tested, and 21 (11.1%) were resistant to one or more drugs, as shown in Table 2. Among 189 isolates, resistance to a single drug was observed in 14 (7.4%) isolates, two drugs in two (1.05%) isolates, three drugs in three (1.56%) isolates, and four drugs in two (1.05%) isolates. MDR (resistance to both RIF and INH) was observed in four (2.1%) isolates.

The association between age (P = 0.71), sex (P = 0.59) and drug-resistance were (chi square test and independent samples t-test) found to be insignificant.

Discussion

The present study showed that, out of 189 patients, 21 patients (11.1%) were resistant to one or more drugs and this resistance can be against one, two, or more drugs simultaneously, while the rest of the patients (88.9%) were susceptible to antibiotics. It is to mention that four patients (2.1%) were resistant to rifampin and isoniazid (MDR) at the same time. The prevalence of primary drug resistance observed in different studies from India was found to be about 18.8% (7.9%–27.1%).[12]

Abbassi's study in Golestan province, showed that, in 260 patients infected with TB, 31, 9, and 2 patients were still sputum smear-positive, respectively, after the end of second month of treatment, at the beginning of fifth month of treatment, and after the implementation of second phase of treatment, which after antibiogram it was determined that only one patient was resistant to rifampin and isoniazid, and another patient was resistant to all drugs, which translates into very low prevalence of drug-resistance (less than 0.5%). Prevalence of initial anti-TB drug-resistance is increasing, and this can have undesirable effects on the treatment of TB patients.[13]

Peymani's study, which was conducted on TB patients in Tabriz-Iran in 2011, showed that only one incidence of multi-drug-resistance had been determined, and that the level of resistance was low in Tabriz province;[14] therefore, the rate of negative-sputum increase in patients is within the expected level. The scourge of drug resistance in the world and possibility of diagnosis in our country (Iran) show the necessity of screening and proper treatment to improve the status-quo.[15] Also, findings of a very large research program conducted in 36 countries to examine anti-TB drug-resistance showed that American countries, such as the Dominican Republic, have high level of resistance; the cause of this prevalence can be attributed to poorness of the TB control system, although immigration can also be a reason.[16] Also, in African countries with very high level of HIV infection and poorness of TB control system, the level of resistance was unexpectedly very low; the reason why the resistance prevalence is low could be attributed to the treatment implemented under supervision of the health system. In western European countries, prevalence of multi-drug-resistance is very low and has been reported around 1%, but in some countries, such as Span, 28% of TB patients have HIV infection simultaneously.[17] In Asian countries, in which TB is an endemic disease, resistance prevalence is different; in some countries, like Korea, prevalence is very low, and in some countries, like India, multi-drug-resistance has been reported 13.3%.[18]

A study was conducted to determine percentage of drug-resistance in pulmonary TB patients in Istanbul and was compared to research conducted in Iran and other countries showed that 91 tuberculosis mycobacterium strains were isolated from sputum of patients suspected to have pulmonary TB as determined by standard the method. Susceptibility of isolated strains to four main anti-TB drugs, that is isoniazid, rifampin, ethambutol, and streptomycin, was examined by the indirect proportional method using Lowenstein-Jensen culture medium. The general resistance level was 44%. The highest level of initial resistance was 21.1% to streptomycin and after that was 15.8% to isoniazid, 5.3% to rifampin, and 2.6% to ethambutol. The level of initial MDR (multi-drug resistance) was 2.6%. Maximum secondary resistance was 33.3% to isoniazid and after that was 28.6% to streptomycin, 23.8% to rifampin, and 14.3% to ethambutol. The level of secondary MDR was 11.9%.[15]

In another study, the goal was to demonstrate the tuberculosis mycobacterium resistance pattern to four anti-TB

Table 2: Frequency and percentage of drug-resistance patterns in smear positive pulmonary TB patients from 2011–2012.

Resistance pattern	Drug	Frequency	Percentage among total number of Resistant to at least one drug (21)	Percentage among total number of isolates tested (189)
Sensitive to all drugs	Rifampin, Izoniazid, Ethambutol, Streptomycin	168	0	88.94
Resistant to 4 drugs	Rifampin, Izoniazid, Ethambutol, Streptomycin	2	9.52	1.05
Resistant to 2 drugs	Izoniazid, Streptomycin	2	9.52	1.05
Resistant to 3 drugs	Rifampin, Izoniazid, Streptomycin	1	4.76	0.52
Resistant to 3 drugs	Rifampin, Izoniazid, Ethambutol	1	4.76	0.52
Resistant to 3 drugs	Izoniazid, Ethambutol, Streptomycin	1	4.76	0.52
Resistant to one drug	Streptomycin	14	66.68	7.4
Total		21	100	100

drugs in children infected with pulmonary TB. Among 350 children (0–15 years of age) with confirmed TB, seven patients had resistance to at least one of the anti-TB drugs. Among these seven children, six patients were Afghan refugees and one person was Iranian. In this group, 85.7% showed resistance to RMP, 4.71% to INH, 1.57% to SM, and 28.6% to EMB. Also, 28.5% showed resistance to all four anti-TB drugs, RMP, EMB, SM, and INH, 2.14% to three drugs, INH, SM, and RMP, 5.28% to two drugs, RMP and INH, and 2.14% to RMP and 14.2% to SM. In this study, it was demonstrated that 14% of patients with Fars ethnicity, 9% of patients with Sistanian ethnicity, and 8.4% of patients with Turkoman ethnicity were resistant to TB. Also, 21% of individuals with inmate records had one resistance type.[19]

The point of this study was to estimate the incidence of MDR tuberculosis in patients with smear-positive of the new cases. There are several limitations in our study. The study was limited exclusively to patients of one heath center of Golestan province and not the whole community. More studies are needed to investigate the incidence of MDR tuberculosis in other studies population.

The incidence of MDR tuberculosis in high TB burden setting stresses the need for drug susceptibility testing to be done for every patient who is culture positive for M. tuberculosis. In addition, early diagnosis may prevent the therapy with inappropriate regimens and improve prognosis from causing MDR tuberculosis strains to develop additional resistance, reduce transmission, and manage drug-resistant TB.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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