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ORIGINAL ARTICLE

Drug Resistance Pattern of *Mycobacterium tuberculosis* Isolates From Patients Referred to TB Reference Laboratory in Ahvaz

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Abstract
Objectives: Tuberculosis remains one of the top three infectious disease killers. The prevalence of multidrug-resistant tuberculosis (MDR-TB) has increased substantially in the past 20 years. When drug resistance is not detected, MDR-TB patients cannot access life-saving treatment; this puts their communities at risk of ongoing MDR-TB transmission. We aimed to determine the patterns of resistance to antituberculosis drugs among *Mycobacterium tuberculosis* isolates from Khuzestan province in Iran.
Methods: A total of 850 clinical specimens from patients suspected of active TB were cultured in 2015. Drug susceptibility testing to the first line antiTB drugs for culture positive MTB was performed on Lowenstein–Jensen medium using the proportion method.
Results: Of 850 cultured specimens, 272 (32%) were culture positive for mycobacteria. Of 64 MTB isolates that were analyzed by the proportion method, 62 (96.8%) were pan-susceptible and two (3.1%) were MDR.
Conclusion: An important way to prevent the emergence of MDR and XDR TB, and the principles of full implementation of the strategy is directly observed treatment, short-course (DOTS). The efficient diagnosis and timely treatment of MDR-TB patients can prevent disease transmission, reduce the risk of drug resistance developing, and avoid further lung damage.

1. Introduction

Tuberculosis is a leading cause of death by an infectious disease worldwide, despite global efforts and financial investment by governments and nongovernmental organizations in disease-control programs during the past 20 years [1].

The World Health Organization (WHO) estimates that onethird of the world's population is infected with *Mycobacterium tuberculosis*, resulting in an estimated 8

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million new cases of tuberculosis and nearly 2 million deaths each year [2].

Antituberculosis (TB) drug resistance is a major public health problem that threatens progress made in TB care and control worldwide [3]. Drug resistance arises due to improper use of antibiotics in chemotherapy of drug-susceptible TB patients and because of insufficient diagnostic facilities. This improper use is a result of a number of actions including, administration of improper treatment regimens and failure to ensure that patients complete the whole course of treatment [4,5]. A patient who develops active disease with a drug-resistant TB strain can transmit this form of TB to other individuals. Drug-resistant bacteria specially multidrug-resistant tuberculosis (MDR) and extensively drug-resistant (XDR), persists as a global public health problem [6].

In 2011, WHO estimated 12 million prevalent cases of tuberculosis worldwide, of which ~630,000 (~5%) were multidrug resistance(MDR) tuberculosis. MDR-TB is caused by TB organisms that are resistant to the first-line drugs, isoniazid and rifampin, used to treat individuals with TB [7].

According to WHO, the estimated incidence rate of tuberculosis in Iran is 21 per 100,000 of the population. Therefore, control and prevention of TB in Iran is the main health concern of the national TB program [7].

Early diagnosis of TB is important for prevention of its spread and one way of reducing MDR-TB. The gold standard test for diagnosis of TB is through the culture method [8].

Culture remains the most sensitive method for the detection of *M. tuberculosis* in clinical specimens. Isolation in culture provides for all subsequent testing, including identification and antibiotic susceptibility testing. Culture is capable of detecting as few as 101−102 organisms/mL of specimen, surpassing the sensitivity of smear (104 CFU/mL) [9,10].

Accurate drug susceptibility testing (DST) for *M. tuberculosis* is highly important for both therapy guidance and surveillance of drug resistance. DST should be performed for individuals at high risk of drug resistance including patients with treatment failure and chronic TB cases (most high risk), Individuals with close contact with diagnosed MDR-TB patients, patients with treatment failure in the first treatment group, patients whose sputum smear at the end of the acute phase of treatment becomes or still remains positive, patients with pulmonary TB/HIV and in prisons [11].

The present study was designed to determine drug resistance pattern of MTB isolates from patients referred to the TB reference laboratory in Ahwaz (south western part of Iran, bordering with Iraq) during a 6-month period.

2. Materials and methods

2.1. Study population and samples

The study was approved by the Ethics Committee of the Ahwaz Jundishapor University of Medical Science in Ahwaz, Iran.

The study was conducted in 2015.The studied population composed of 850 clinical individuals who were referred to the TB reference laboratory in Ahwaz. A total of 513 samples belonged to men, 334 samples belonged to women, and 45 samples were taken from prisoners (Table 1).

2.2. Identification of mycobacteria

Smear and culture of specimen for acid fast bacilli was performed on all patients.

Samples from each patient were decontaminated by Petroff's method and were inoculated into Lowenstein−Jensen media. The bacterial suspension (1 mL) was decontaminated with sodium hydroxide (NaOH) at a final concentration of 0.5N for 20 minutes at room temperature with intermittent agitation and then neutralized with 1N hydrochloric acid (HCl). This suspension was then centrifuged at 2,500g for 20 minutes to pellet the bacilli and then resuspended in sterile saline at a concentration of 10^7 bacilli/mL [12].

2.3. Inoculation and incubation

Glass or plastic pasture pipettes were used and each slope was inoculated with 0.2−0.4 mL (2−4 drops) of the centrifuged sediment. Cultures were incubated in a slanted position for at least 24 hours to ensure the complete distribution of inoculum. All cultures were incubated at 35−37°C until growth was observed or discarded as negative after 2 months.

Biochemical and phenotypic methods for identification of mycobacteria include observation of rate of growth, colony morphology, pigmentation, and biochemical profiles. Biochemical testing (i.e., niacin, nitrate reduction, and 68°C labile catalase tests) was used to identify the isolated mycobacteria once they were categorized into a preliminary subgroup based on their growth characteristics [13].

2.4. Drug susceptibility testing

Drug susceptibility testing for cases that were ordered by doctor were performed, for example HIV positive patients, prisoners, and patients with relapse.

The susceptibilities of the isolates which were identified as *M. tuberculosis* were determined against isoniazid, rifampicin, ethambutol, and streptomycin by standard proportional method using Lowenstein−Jensen medium as recommended by WHO. This method is considered to be a reference standard against which other routine methods should be assessed.

Table 1. The age distribution of cases.

Age (y)	%
18−50	42.3
> 50	50.7
< 18	7

Susceptibility was defined as no or < 1% growth on media containing the critical concentration of drug (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). Resistance was defined as growth in the number of colonies of > 1% in the drug containing media as compared with the nondrug-containing media [14].

3. Results

Of 850 clinical specimens received by the TB reference laboratory in Ahwaz, 35% was smear positive (Table 2). All of the 850specimen were cultured and 272 specimens of them were *M. tuberculosis* (32%).

3.1. Drug susceptibility patterns

Drug susceptibility testing was performed for 64 patients according to physician orders (Table 3). A total of 60 isolates were from male patients and four from female patients, 62 specimens (96.8%) were pan-susceptible, and two specimens (3.1%) were resistant to isoniazid and rifampin (MDR-TB).

Both isolates of drug-resistant *M. tuberculosis*, belonged to women.

Sensitivity was defined as follows: isoniazid 0.2 mg/mL, rifampin 40 mg/mL, ethambutol 2 mg/mL, and streptomycin 4 mg/mL.

The drug susceptibility pattern was interpreted according to WHO guidelines [15].

4. Discussion

Drug susceptibility testing for TB is the most effective tool of control and management of MDR-TB. The efficient diagnosis and timely treatment of MDR-TB patients can prevent disease transmission, reduce the risk of drug resistance developing, and avoid further lung damage.

Table 2. Smear test results by positivity grade.

Smear positivity score	%
<1+	9
1+	15
2+	12
3+	64

Table 3. Pattern of drug resistance by treatment exposure groups.

Drug resistance pattern	*n* (%)
Pun susceptible	62 (96.8)
Multidrug resistant	2 (3.1)
Mono-drug resistant	0

In this study, the resistance of *M. tuberculosis* to isoniazid and rifampin (MDR TB) was 3.1%. In a 2006 survey in Khuzestan, the rate of MDR-TB was reported as 8.7% by conventional Minimal Inhibitory Concentration (MIC) method and 6.2% by Polymerase Chain Reaction (PCR) technique [16]. A study from the 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 [17].

The prevalence of MDR-TB in Iran, in the year 2011, was 5% for new cases as reported by WHO [18]. A study by Alavi and Salami [19] showed that mortality rates of tuberculosis in Khuzestan was 3.15%, (2.8% in females and 3.3% in males).

The incidence of smear positive pulmonary TB in Khuzestan, south west Iran, is estimated to be 25 per 100,000 of the population [20]. In a study by Nasiri et al [21], drug resistance of *M. tuberculosis* isolates from patients of five provinces of Iran was determined and 6.3% MDR were seen. Another study by Metanat et al [22] in Zahedan showed that 16% of patients were MDR. In study by Hadizadeh Tasbiti et al [23], between 2006 and 2009, the rate of MDR TB was 2.5%.

The basic and most important way to prevent the emergence of MDR and XDR-TB, and the principles of full implementation of the strategy is directly observed treatment, short-course (DOTS).

Conflicts of interest

All authors have no conflicts of interest to declare.

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