Rifampin resistance among individuals with extrapulmonary tuberculosis: 4 years of experience from a reference laboratory

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Abstract

Information is limited about the drug resistance patterns in extrapulmonary tuberculosis (EPTB) in Iran. This study aimed to determine the prevalence of EPTB and to investigate the drug-resistance pattern in Mycobacterium tuberculosis strains collected from extrapulmonary samples at the Tehran regional TB reference laboratory. Extrapulmonary specimens from individuals with suspected TB referred to the TB reference laboratories in five cities of Iran were collected. Both standard conventional methods (culture and direct smear microscopy) and Xpert MTB/RIF assay were used for the identification of mycobacteria. Drug susceptibility testing was done using Xpert MTB/RIF. The proportion method on Lowenstein–Jensen medium was performed for confirmation. Between 2016 and 2020, a total of 12,050 clinical specimens from individuals with suspected TB were collected, of which 10,380 (86%) were pulmonary specimens and 1,670 (14%) were extrapulmonary. Of the extrapulmonary specimens, 85 (5.0%) were positive for M. tuberculosis, and the remaining 1,585 (95.0%) samples were negative by standard methods. Of 85 M. tuberculosis isolates, drug susceptibility testing was performed for 32 isolates, of which 1 (3.1%, 95% CI 0.0%–9.4%) was rifampin resistant and 31 (96.9%, 95% CI 90.1%–100%) were pan-susceptible. The rifampin-resistant isolate was also resistant to isoniazid, so was assigned as a multidrug-resistant TB. Our study indicated the frequency of drug-resistance among EPTB in Iran. Establishing rapid diagnostic methods for detection of drug-resistance in EPTB, performing drug susceptibility testing for all EPTB cases to provide effective treatment, and continuous monitoring of drug resistance, are suggested for prevention and control of drug resistance in EPTB in Iran.

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Introduction

Mycobacterium tuberculosis is an important public health problem because of the high mortality that it causes [1]. The main strategy to control this problem is the rapid diagnosis of M. tuberculosis infection and identification of high-risk cases [2,3]. In addition to pulmonary tuberculosis (PTB), this infectious agent can cause extrapulmonary tuberculosis (EPTB) in other organs and tissues [4,5]. There are some obstacles in the diagnosis of EPTB that cause difficulty. These include that a clinical sample requires invasive procedures, it is hard to access infrequent bacterial load, and signs and symptoms are non-specific [4,6,7]. For the diagnosis of EPTB, several conventional and molecular methods, such as smear microscopy, culture identification and Xpert MTB/RIF, have been widely used [5,8]. Similar to PTB, a major problem in EPTB is the increasing incidence of drug-resistant M. tuberculosis giving high mortality because EPTB is mostly found with a compromised immune system [2,9].
To date, the rates of multidrug-resistant TB in pulmonary specimens have been reported in several studies in Iran. However, there is limited analysis of drug-resistance in EPTB. This study aimed to determine the prevalence of EPTB and to investigate the drug-resistance pattern in M. tuberculosis strains collected from extrapulmonary samples at the Tehran regional TB reference laboratory.

Materials and methods

Setting, study population and samples
This retrospective study was conducted over 4 years (from April 2016 to October 2020) in the regional reference laboratory of TB in Tehran, Iran. The laboratory quality was supervised by the Swedish Institute for Infectious Disease Control. Clinical specimens from individuals with suspected TB from five cities—Tehran, Mashhad, Ahvaz, Shiraz and Isfahan—were included in this study. Specimens were either from new adult cases or from patients with treatment failure or relapse.

Clinical samples were collected in sterile containers from each patient for microscopy and culture tests. All specimens were held at 4°C until processed by standard laboratory procedures. The majority of specimens were processed within 24 hours at the reference laboratory. One specimen was collected from each patient.

Identification of mycobacteria
Both standard conventional methods (culture and direct smear microscopy) and Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) were used for the identification of mycobacteria.

For microscopy examination, a smear from samples was stained by the Ziehl–Neelsen method. Known negative and positive slides were prepared with every batch of the specimens.

A subsample from each patient was decontaminated by Petroff’s method and inoculated into two tubes of Lowenstein–Jensen medium (Merck, Kenilworth, NJ, USA) [10]. The slope cultures were incubated at 37°C and examined for growth once weekly up to 8 weeks. Each isolate was examined regarding morphology, pigmentation and date of growth. Bacterial isolates were identified as M. tuberculosis complex using standard biochemical tests, including the production of niacin, nitrate reduction and catalase [11].

A 1-mL unconcentrated specimen was used (without centrifuge) for Xpert MTB/RIF assay [12].

A specimen was considered positive for M. tuberculosis when culture, and/or Ziehl staining, and/or Xpert MTB/RIF assay was positive.

Drug susceptibility testing
Drug susceptibility testing of isolates to rifampicin was determined using the Xpert MTB/RIF assay [13]. Briefly, Xpert sample reagent was added to 1 mL of specimens in the ratio 1:2, and then the mixture was transferred to the Xpert test cartridge. Cartridges were inserted into the Xpert machine, and the automatically generated results were read after 90 minutes.

The proportion method on the Lowenstein–Jensen medium was performed for confirmation [14]. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drug (40 μg/mL for rifampicin, 0.2 μg/mL for isoniazid). The interpretation was made according to the usual criteria for resistance, i.e. 1% for all drugs. Mycobacterium tuberculosis H37Rv strain (ATCC 27294) was used for quality control testing in drug susceptibility testing.

Statistical analysis
Statistical analysis was carried out using SPSS version 22 (SPSS Inc., Armonk, NY, USA). The frequency was reported with 95% CI.

Results

Microbiological findings
A total of 12 050 clinical specimens from individuals with suspected TB were collected, of which 10 380 (86%) were pulmonary specimens and 1670 (14%) were extrapulmonary—pleural fluid 375 (22.4%), biopsy 349 (20.9%), gastric lavage 248 (14.8%), osteoarticular 187 (11.2%), abscess 123 (7.4%), urine 115 (6.9%), cerebrospinal fluid 114 (6.8%), ascites 102 (6.1%) and blood 57 (3.4%). Specimens were either from new cases or from patients with treatment failure or relapse.

Of the extrapulmonary specimens, 85 (5.0%) were positive for M. tuberculosis and the remaining 1585 (95.0%) samples were negative by standard methods (Table 1).

Biopsy was the most common specimen among confirmed EPTB cases (36.4%) followed by abscess (24.7%), gastric lavage (10.5%), pleural fluid (9.4%), ascites (7.4%), osteoarticular (4.7%), urine (3.5%), blood (2.3%) and cerebrospinal fluid (1.1%), respectively.

Drug susceptibility testing
Of 85 M. tuberculosis isolates, drug susceptibility testing was performed for 32 isolates, of which one (3.1%, 95% CI 0.0%–9.4%) was rifampin resistant and 31 (96.9%, 95% CI 90.1%–100%) were pan-susceptible. Resistant and eight susceptible isolates (randomly selected) were confirmed by the proportion method. As shown in Table 2, the rifampin-resistant isolate was also resistant to isoniazid, and so was assigned as a multi-drug-resistant TB.
Another study conducted on 363 culture-proven EPTB cases in the USA showed that lymphatic TB was the most frequent form (45.1%) followed by bone and joint TB (15.6%) [21]. In a previous study conducted in Turkey, lymph nodes (39.4%), and pleura and pleural TB (14.3%) [21].

Our study demonstrated that the rate of drug resistance among EPTB was relatively low. Out of 85 M. tuberculosis isolates included in this study, one (3.1%) was multidrug resistant. This proportion is lower than the earlier results reported from Thailand, India and South Korea [22–24]. In a study conducted by Maurya et al., multidrug-resistant TB was observed in 13.4% of EPTB cases in India [25]. Another study from Ethiopia indicated that of 151 M. tuberculosis isolates from EPTB, 9% of isolates were multidrug-resistant TB [26].

As mentioned before, a major problem in the management of EPTB is the increasing rate of drug-resistant M. tuberculosis, because most patients are in an immunocompromised condition [2,9]. As a result, using rapid first-line diagnostic methods for drug resistance, such as Xpert MTB/RIF, is important for starting sufficient treatment and to reduce the death rate. However, according to the previous studies, an acceptable accuracy for EPTB has not been established. Previous investigators reported that the accuracy of Xpert MTB/RIF in non-respiratory specimens for the diagnosis of various forms of EPTB varies considerably with specimen type and bacillary load [27]. For example, Xpert MTB/RIF is highly sensitive in lymph node samples, moderately sensitive in meningitis, and shows low sensitivity for testing pleural fluid [27]. Therefore, the sensitivity and specificity of Xpert MTB/RIF for EPTB specimens is variable, and Xpert MTB/RIF cannot be recommended to replace standard conventional tests for diagnosis of EPTB [12]. Reflecting the needs of healthcare providers, there is a requirement for future research.

There were some limitations to this study. First, the potential influence of age, sex, previous treatment and human immunodeficiency virus on drug resistance could not be analysed because of the limited information obtained from the clinical records of the patients. Second, although, clinical samples were collected from five cities of Iran, it cannot fully represent the prevalence of drug resistance in EPTB, because the magnitude of drug resistance is not yet reported in several regions of the country. Finally, the sample size for positive M. tuberculosis isolates was low and further studies with larger sample sizes from more cities are recommended.

In conclusion, our study indicated the frequency of drug-resistance among EPTB in Iran. Our results suggest that establishing rapid diagnostic methods for detection of drug-resistant M. tuberculosis is essential for effective management of EPTB. Further research is needed to better understand the drug resistance patterns and to improve the diagnostic accuracy for EPTB.

### TABLE 1. Mycobacterial identification from extrapulmonary specimens

| Specimens          | Total number, n (%) | No. of smear positives | No. of culture positives | No. of GeneXpert positives | Total MTB positive (%) |
|--------------------|---------------------|------------------------|--------------------------|-----------------------------|------------------------|
| Urine              | 115 (6.9)           | 3                      | 2                        | 2                           | 3 (3.5)                |
| Abscess            | 123 (7.4)           | 13                     | 9                        | 9                           | 21 (47)                |
| Osteoarticular     | 187 (11.2)          | 3                      | 1                        | 2                           | 4 (4.7)                |
| Biopsy             | 349 (20.9)          | 21                     | 14                       | 12                          | 31 (24.4)              |
| Cerebrospinal fluid| 114 (6.8)           | 0                      | 1                        | 1                           | 1 (1.1)                |
| Gastric lavage     | 248 (14.8)          | 7                      | 2                        | 2                           | 9 (10.5)               |
| Blood              | 57 (3.4)            | 2                      | 1                        | 0                           | 2 (3.3)                |
| Pleural fluid      | 375 (22.4)          | 5                      | 3                        | 5                           | 8 (9.4)                |
| Abscess            | 102 (6.1)           | 1                      | 6                        | 3                           | 6 (74)                 |
| Ascites            | 55 (32)             |                        | 38                       | 32                          | 85 (100)               |

Abbreviation: MTB, Mycobacterium tuberculosis.

### TABLE 2. The drug-resistance pattern of the extrapulmonary tuberculosis isolates

| Type of resistance | No. of resistant isolates |
|--------------------|---------------------------|
| Pan-susceptible    | 31                        |
| Mono-resistance    | 0                         |
| Multidrug resistance | 1                        |

Discussion

According to the current data, the prevalence of EPTB among clinical samples collected at the Tehran regional TB reference laboratory was 85/1670 (5%). The proportion of EPTB among EPTB clinical samples in other countries varies, being 9.9% in Ethiopia, 19% in Pakistan, 36.7% in Netherlands, 53% in England and Wales, and 59% in Germany [15–19]. The discrepancy between the current study and previous reports might be the result of methodological differences, such as the difference in the inclusion criteria.

In the current study population, biopsy and abscess specimens were commonly seen EPTB types, comprising 36.4% and 24.7% of the EPTB samples, respectively. A similar observation was reported by an earlier study in Iran, in which biopsy (26.5%), abscess (20.4%) and pleural fluid (14.2%) were the most commonly involved samples for EPTB [12]. In a previous study conducted in Turkey, lymph nodes (39.4%), and pleura (23.6 %) were the most common sites of EPTB involvement [20]. Another study conducted on 363 culture-proven EPTB cases in the USA showed that lymphatic TB was the most frequent form (45.1%) followed by bone and joint TB (15.6%) and pleural TB (14.3%) [21].

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resistance in EPTB, performing DST for all EPTB cases to provide effective treatment, and continuous monitoring of drug resistance are required for prevention and control of drug resistance in EPTB in Iran.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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