ORIGINAL ARTICLE

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

S. Baghbanbashi[1], S. Mohammad J. Mousavi[1], H. Dabiri[1], M. Hakemi-Vala[1], H. Goudarzi[1], G. Hamzehloo[2], S. Amini[2] and M. J. Nasiri[1]

1) *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences and* 2) *Regional Tuberculosis Reference Laboratory, Tehran University of Medical Sciences, Tehran, Iran*

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 1670 (14%) were extrapulmonary. Of the extrapulmonary specimens, 85 (5.0%) were positive for *M. tuberculosis*, and the remaining 1585 (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.

Keywords: Drug resistance, extrapulmonary tuberculosis, Iran, *Mycobacterium tuberculosis*, *rifampin*
Original Submission: 27 October 2020; **Revised Submission:** 15 January 2021; **Accepted:** 15 January 2021
Article published online: 20 January 2021

Corresponding author: M.J. Nasiri, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail: Mj.nasiri@hotmail.com

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].

https://doi.org/10.1016/j.nmni.2021.100841

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—pleura l 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 ETPB 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.

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 positiven (%)
Urine	115 (6.9)	3	2	2	3 (3.5)
Abscess	123 (7.4)	13	9	9	21 (24.7)
Osteoarticular	187 (11.2)	3	1	2	4 (4.7)
Biopsy	349 (20.9)	21	14	12	31 (36.4)
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 (2.3)
Pleural fluid	375 (22.4)	5	3	5	8 (9.4)
Ascites	102 (6.1)	1	6	3	6 (7.4)
Total	1670 (100)	55	38	32	85 (100)

Abbreviation: MTB, Mycobacterium tuberculosis.

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].

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].

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

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-

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.

Acknowledgements

This study was financially supported by Research Department of the School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant number:22769).

References

[1] World Health Organization. Global tuberculosis report 2018. 2018. Geneva: World Health Organization; 2019.
[2] Portillo-Gomez L, Morris S, Panduro A. Rapid and efficient detection of extra-pulmonary Mycobacterium tuberculosis by PCR analysis. Int J TB Lung Dis 2000;4:361–70.
[3] Causse M, Ruiz P, Gutiérrez-Aroca JB, Casal M. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. J Clin Microbiol 2011;49:3065–7.
[4] Steingart KR, Henry M, Laal S, Hopewell PC, Ramsay A, Menzies D, et al. A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis. Postgrad Med J 2007;83:705–12.
[5] Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Microbiol 2012;66:20–36.
[6] Dusthackeer A, Sekar G, Chidambaram S, Kumar V, Mehta P, Swaminathan S. Drug resistance among extrapulmonary TB patients: six years experience from a supranational reference laboratory. Indi J Med Res 2015;142:568.
[7] Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Resp J 2012;40:442–7.
[8] Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol 2011;49:1202–5.
[9] Al-Ghafli H, Varghese B, Enani M, Alrajhi A, Al Johani S, Albarrak A, et al. Demographic risk factors for extra-pulmonary tuberculosis among adolescents and adults in Saudi Arabia. PloS One 2019;14: e0213846.
[10] Petroff S. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J Exp Med 1915;21: 38–42.
[11] Nasiri MJ, Rezaei F, Zamani S, Darban-Sarokhalil D, Fooladi AAI, Shojaei H, et al. Drug resistance pattern of *Mycobacterium tuberculosis* isolates from patients of five provinces of Iran. Asian Pac J Trop Med 2014;7:193–6.
[12] Allahyartorkaman M, Mirsaeidi M, Hamzehloo G, Amini S, Zakiloo M, Nasiri MJ. Low diagnostic accuracy of Xpert MTB/RIF assay for extrapulmonary tuberculosis: a multicenter surveillance. Sci Rep 2019;9(1):1–6.
[13] Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010;363:1005–15.
[14] Rieder HL, Chonde TM, Myking H, Urbanczik R, Laszlo A, Kim SJ, et al. The public health service national tuberculosis reference laboratory and the national laboratory network; minimum requirements, role and operation in a low-income country. Paris: International Union against Tuberculosis and Lung Disease (IUATLD); 1998.
[15] Zenebe Y, Anagaw B, Tesfay W, Debebe T, Gelaw B. Smear positive extra pulmonary tuberculosis disease at University of Gondar Hospital, Northwest Ethiopia. BMC Res Notes 2013;6:21.
[16] Butt T, Kazmi S, Ahmad R, Mahmood A, Karamat K, Anwar M. Frequency and antibiotic susceptibility pattern of mycobacterial isolates from extra-pulmonary tuberculosis cases. J Pak Med Assoc 2003;53: 328–31.
[17] Te Beek LA, Van Der Werf MJ, Richter C, Borgdorff MW. Extrapulmonary tuberculosis by nationality, The Netherlands, 1993–2001. Emerg Infect Dis 2006;12:1375.
[18] Kruijshaar ME, Abubakar I. Increase in extrapulmonary tuberculosis in England and Wales 1999–2006. Thorax 2009;64:1090–5.
[19] Forssbohm M, Zwahlen M, Loddenkemper Ra, Rieder HL. Demographic characteristics of patients with extrapulmonary tuberculosis in Germany. Eur Resp J 2008;31:99–105.
[20] Sunnetcioglu A, Sunnetcioglu M, Binici I, Baran AI, Karahocagil MK, Saydan MR. Comparative analysis of pulmonary and extrapulmonary tuberculosis of 411 cases. Ann Clin Microbiol Antimicrob 2015;14: 1–5.
[21] Ong A, Creasman J, Hopewell PC, Gonzalez LC, Wong M, Jasmer RM, et al. A molecular epidemiological assessment of extrapulmonary tuberculosis in San Francisco. Clin Infect Dis 2004;38:25–31.
[22] Boonsarngsuk V, Mangkang K, Santanirand P. Prevalence and risk factors of drug-resistant extrapulmonary tuberculosis. Clin Resp J 2018;12:2101–9.
[23] Sharma S, Hanif M, Chopra KK, Sharma M, Dwivedi KK, Sidiq Z, et al. Detection of multidrug resistance and extensively drug resistance among smear-negative extrapulmonary tuberculosis cases in a reference laboratory. Biomed Biotechnol Res J 2018;2:132.
[24] Lee HY, Lee J, Lee YS, Kim M-Y, Lee H-K, Lee Y-M, et al. Drug-resistance pattern of *Mycobacterium tuberculosis* strains from patients with pulmonary and extrapulmonary tuberculosis during 2006 to 2013 in a Korean tertiary medical center. Kor J Int Med 2015;30:325.
[25] Maurya A, Kant S, Nag V, Kushwaha R, Dhole T. Trends of antituberculosis drug resistance pattern in new cases and previously treated cases of extrapulmonary tuberculosis cases in referral hospitals in northern India. J Postgrad Med 2012;58:185.
[26] Diriba G, Kebede A, Tola HH, Yenew B, Moga S, Addise D, et al. Molecular characterization and drug resistance patterns of *Mycobacterium tuberculosis* complex in extrapulmonary tuberculosis patients in Addis Ababa, Ethiopia. PLOS One 2020;15:e0243493.
[27] Sharma SK, Ryan H, Khaparde S, Sachdeva K, Singh AD, Mohan A, et al. Index-TB guidelines: guidelines on extrapulmonary tuberculosis for India. Ind J Med Res 2017;145:448.