# Rifampicin resistance in Mycobacterium tuberculosis in Iran: a two-centre study

#### F. Bahraminia<sup>1</sup>, M. Zangiabadian<sup>2</sup>, M. J. Nasiri<sup>3</sup>, M. Fattahi<sup>4</sup>, M. Goudarzi<sup>3</sup>, R. Ranjbar<sup>5</sup> and A. A. Imani Fooladi<sup>1</sup>

1) Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, 2) Student Research Committee, 3) Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, 4) Regional Tuberculosis Reference Laboratory, Tehran University of Medical Sciences and 5) Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

## Abstract

Multidrug-resistant tuberculosis remains a challenge. In this study, we investigated the incidence of rifampicin (RIF) resistance in *Mycobacterium tuberculosis* in a large number of pulmonary specimens.

A two-center study in Tehran, the capital of Iran, was performed with 6624 pulmonary samples of patients with tuberculosis (TB) who were subjected to detection of RIF-resistant TB by GeneXpert MTB/RIF assay between May 2014 and July 2018. Conventional drug susceptibility testing was performed to confirm the results.

Xpert MTB/RIF identified a total of 96 positives for *M. tuberculosis*, of which 5 (5.3%) samples were found to be RIF-resistant TB. All RIF-resistant and sensitive isolates detected by GeneXpert were phenotypically confirmed by drug susceptibility testing.

These results indicated that the Xpert MTB/RIF test can be used as a rapid diagnostic method and can potentially decrease the morbidity associated with diagnostic delay and mistreatment.

© 2021 The Authors. Published by Elsevier Ltd.

Keywords: Drug resistance, rifampicin, tuberculosis, Xpert MTB/RIF
Original Submission: 14 October 2020; Revised Submission: 30 May 2021; Accepted: 14 June 2021
Article published online: 23 June 2021

**Corresponding author**: A.A. Imani Fooladi, Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran **E-mail:** imanifouladi.a@gmail.com

## Introduction

Tuberculosis (TB) is one of the most serious public health problems worldwide. Globally, an estimated 10.0 million people fell ill with TB in 2019 [1]. The emergence of multidrugresistant TB (MDR-TB) that does not respond to at least isoniazid and rifampicin (RIF) has had a significant negative effect on TB control strategies. In 2019, close to half a million people developed RIF-resistant TB, of which 78% had MDR-TB [1]. According to the latest report released by the World Health Organization, the incidence rate of TB in Iran was 13 cases per

100,000 people [1]. Moreover, it was reported that multidrugresistant (MDR)/RIF-resistant TB accounted for 1% of new TB cases and 12% of previously treated TB cases [1]. MDR/RIFresistant TB has been associated with worse treatment outcomes compared with drug-susceptible TB [2,3]. RIF is used as a surrogate marker for MDR-TB, and patients with RIF were given MDR-TB treatment. Early detection of MDR/RIF-resistant TB and initiating appropriate treatment is extremely important to reduce the risk of mortality [4-9]. Conventional drug susceptibility testing (DST) is the reference standard to diagnose MDR/RIF-resistant TB but requires 3-8 weeks before the results are available [10-12]. Molecular methods can play an important role in the rapid detection and control of MDR/RIFresistant TB [13-15]. The Xpert MTB/RIF system has the advantage of being more rapid than the proportional drug susceptibility testing method for detection of RIF resistance [16,17]. To date, the use of Xpert MTB/RIF system for detection of RIF resistance has been reported from different countries [18-20]. In Iran, only some regional TB laboratories (i.e.

Tehran, Mashhad, Isfahan) use Xpert MTB/RIF for the rapid diagnosis of TB and detection of RIF-resistant TB. However, limited data are available from Iran. Thus, the present study was aimed to investigate the incidence of RIF in *Mycobacterium tuberculosis* in a large sample size using Xpert MTB/RIF assay.

# **Materials and methods**

#### Setting and samples

In this cross-sectional study, respiratory specimens were collected from two TB laboratories in Tehran (Tehran regional TB reference laboratory [TRTB-RL] and Baqiyatallah Hospital) from May 2014 to July 2018. Specimens were either from new cases or from patients with treatment failure or relapse. TRTB-RL and Baqiyatallah Hospital are well equipped and are able to perform DST. TRTB-RL is supervised by the Swedish Institute for Infectious Disease Control. A total of 6624 pulmonary samples from the same number of TB suspected cases were included in this study. Adult cases with clinical signs and symptoms suggestive of TB were included. Those with disease other than the *M. tuberculosis* were excluded.

The Ethics Committee of Baqiyatallah University of Medical Sciences approved the study, and all the patients have signed an informed consent form.

# Microscopy examination and identification of *M. tuberculosis*

Pulmonary specimens (bronchoalveolar lavage fluid and sputum) were processed by the standard sodium hydroxide method, and smears were prepared by the Ziehl-Neelsen staining method [21]. After decontamination, specimens were inoculated to Lowenstein-Jensen solid medium. For identification of mycobacteria, the slope cultures were incubated at  $37^{\circ}$  C and examined for growth once weekly up to 6 weeks. Bacterial isolates were identified as *M. tuberculosis* using standard biochemical tests (i.e. production of niacin, nitrate reduction, catalase) and a molecular method (*IS6110* based PCR assay) [21,22]. Only one culture isolated per study subject was considered for further analysis.

### Conventional DST of M. tuberculosis

DST for rifampicin was performed with the proportional method on Lowenstein-Jensen solid medium with a standard critical concentration of 40  $\mu$ g/ml for RIF as previously described [21]. *M. tuberculosis* H37Rv strain (ATCC 27294) was used for quality control testing in DST.

#### **Xpert MTB/RIF** assay

Xpert MTB/RIF assay was performed for collected samples according to the manufacturer's instructions [16]. Briefly, Xpert sample reagent was added to 1 ml of specimens in the ratio 1:2, and 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 min.

## Statistical analysis

All analyses were performed using the statistical software package SPSS, version 22 (SPSS, Chicago, Illinois).

# **Results**

# Xpert MTB/RIF assay for detection of RIF resistance

Of 6624 samples of patients with suspected TB, 96 (1.4%) were positive for *M. tuberculosis*. Of positive *M. tuberculosis* isolates, 5 (5.3%) were found to be RIF-resistant TB (Fig. 1).

## Culture and DST

All RIF-resistant and RIF-sensitive isolates detected by GeneXpert were phenotypically confirmed by DST.

## Performance of Xpert MTB/RIF

Sensitivity, specificity, positive predictive value, and negative predictive value of Xpert MTB/RIF to detect RIF resistance in comparison with DST were found equal to the rates of 100%, 100%, 100%, and 100%, respectively.

## Discussion

Rapid diagnosis of MDR/RIF TB can potentially decrease the mortality associated with diagnostic delay and mistreatment. Several methods have recently been described for the rapid diagnosis of MDR/RIF TB [23]. The Xpert MTB/RIF assay tested in our study targets the RIF resistance—associated *rpoB* gene region by nested PCR with three specific primers [16]. Accordingly, the incidence of RIF resistance was found to be 5.3% among clinical isolates of *M. tuberculosis*. During 2010–2012, Nasiri et al. [21] performed DST on 252 strains of *M. tuberculosis* which were isolated from new patients with TB. They reported that 15 (6%) isolates were RIF-resistant TB [21]. Similarly, in a subsequent investigation in Iran, a total of 334 clinical isolates of *M. tuberculosis* from the same number of patients with either new or retreatment TB were included for DST [12]. They

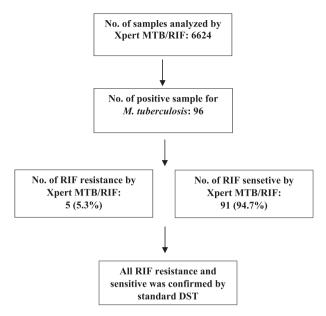


FIG. 1. Incidence of RIF using Xpert MTB/RIF assay. RIF, rifampicin.

indicated that 3.6% of TB cases showed resistance to RIF [12]. In these studies, conventional DST was used to report the drugresistant TB in Iran [21,12]. However, routine DST of *M. tuberculosis* is difficult and time consuming. Consequently, delay in diagnosis and start of treatment has a negative impact on TB control programs. The yield of Xpert MTB/RIF for the diagnosis of RIF in *M. tuberculosis* was studied previously, and the sensitivity of the Xpert MTB/RIF test for detecting RIF resistance was reported to be 94.4–100%, with a specificity of 98.3–100% [24–27]. Similarly, in this study, the Xpert MTB/RIF correctly identified RIF resistance (100% sensitive) and RIF sensitive isolates (100% specific).

The results of the current research showed that all RIF resistance statuses detected by Xpert MTB/RIF were phenotypically confirmed by DST. These data suggest that the test can be used in various settings for rapid screening of RIF-resistant TB. Although the Xpert MTB/RIF is a rapid, reliable and simple method for detection of RIF resistance, its inability to detect mutations outside the RIF-resistant determining region raises a concern [28]. Xpert MTB/RIF assay may cause false-negative and/or falsepositive RIF resistance results [29]. Therefore, the detection of RIF resistance by the Xpert MTB/RIF assay may need to be used in concert with conventional diagnostic methods.

The main limitation of the present study was that it cannot fully represent the incidence of RIF-resistant TB in Iran because the scale of drug resistance is not yet investigated in some areas of the country.

In conclusions, these results indicated that the Xpert MTB/ RIF test can effectively be used as a rapid diagnostic method and can potentially decrease the morbidity associated with diagnostic delay and mistreatment.

## Ethics approval and consent to participate

The Ethics Committee of Baqiyatallah University of Medical Sciences approved the study, and all the patients have signed an informed consent form.

# **Transparency declaration**

Research reported in this publication was supported by Elite Researcher Grant Committee under award number 976978 from the National Institute for Medical Research Development (NIMAD), Tehran, Iran.

# Author contributions

The authors contributed equally to this manuscript.

# **Acknowledgements**

This study was jointly supported by the Baqiyatallah University of Medical Sciences and Shahid Beheshti University of Medical Sciences, Tehran, Iran.

## References

- Organization WH. Global tuberculosis report. In: Global tuberculosis report 2020; 2020.
- [2] Migliori GB, et al. MDR-TB and XDR-TB: drug resistance and treatment outcomes. Eur Resp J 2009;34(3):778-9.
- [3] Varahram M, et al. A retrospective analysis of isoniazid-monoresistant tuberculosis: among Iranian pulmonary tuberculosis patients. Open Microbio J 2014;8:1.
- [4] Mai NT, Thwaites GE. Recent advances in the diagnosis and management of tuberculous meningitis. Curr Opin Infect Dis 2017;30(1):123-8.
- [5] Gupta PS, et al. MDR-TB and XDR-TB surveillance highlights need for rapid detection of drug-resistance in Mycobacterium tuberculosis infections. Int J Curr Microbiol App Sci 2017;6(1):539–42.
- [6] Weyer K, et al. Drug-resistant tuberculosis: what is the situation, what are the needs to roll it back. AMR Cont 2017.
- [7] Ahmad N, et al. Management and treatment outcomes of MDR-TB: results from a setting with high rates of drug resistance. The International Journal of Tuberculosis and Lung Disease 2015;19(9): 1109-14.
- [8] Khurram M, Khaar HTB, Fahim M. Multidrug-resistant tuberculosis in Rawalpindi, Pakistan. J Infect Develop Count 2012;6(1):29–32.

- [9] Gebrehiwet GB, et al. Rifampicin resistant tuberculosis in presumptive pulmonary tuberculosis cases in Dubti Hospital, Afar, Ethiopia. J Infect Develop Count 2019;13(1):21–7.
- [10] Jacobson KR, et al. Implementation of GenoType MTBDR plus reduces time to multidrug-resistant tuberculosis therapy initiation in South Africa. Clin Infect Dis 2012;56(4):503–8.
- [11] Chen Y, et al. Rapid diagnosis of pulmonary tuberculosis and detection of drug resistance by combined simultaneous amplification testing and reverse dot blot. J Clin Pathol 2018;71(6):498-503.
- [12] Amini S, et al. Direct drug susceptibility testing of Mycobacterium tuberculosis using the proportional method: a multicenter study. | Glob Antimicrob Resist 2019;17:242-4.
- [13] Sachdeva KS, et al. The potential impact of up-front drug sensitivity testing on India's epidemic of multi-drug resistant tuberculosis. PLoS One 2015;10(7):e0131438.
- [14] Campbell PJ, et al. Molecular detection of mutations associated with first-and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrob Agent Chemother 2011;55(5):2032–41.
- [15] Barnard M, et al. Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Resp Crit Care Med 2008;177(7):787–92.
- [16] Boehme CC, et al. Rapid molecular detection of tuberculosis and rifampin resistance. New Engl J Med 2010;363(11):1005-15.
- [17] Kabasakalyan E, et al. Change in TB diagnostic profile after introduction of GeneXpert MTB/RIF assay in National TB Program of Armenia, 2013-2017. J Infect Develop Count 2019;13(5.1):225–75.
- [18] Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for the rapid diagnosis of tuberculosis and detection of RIF-resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol 2011:4138–41. JCM.
- [19] Rufai SB, et al. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampicin monoresistant Mycobacterium tuberculosis. Journal of Clinical Microbiology 2014:3005–13. JCM.

- [20] Williamson DA, et al. An evaluation of the Xpert MTB/RIF assay and detection of false-positive rifampicin resistance in Mycobacterium tuberculosis. Diagn Microbiol Infect Dis 2012;74(2):207–9.
- [21] Nasiri MJ, et al. Drug resistance pattern of Mycobacterium tuberculosis isolates from patients of five provinces of Iran. Asian Pacific Journal of Tropical Medicine 2014;7(3):193–6.
- [22] Nasiri MJ, et al. High rates of nontuberculous mycobacteria isolation from patients with presumptive tuberculosis in Iran. New Microb New Infect 2018;21:12–7.
- [23] Galarza M, et al. High-resolution melting analysis for molecular detection of multidrug resistance tuberculosis in Peruvian isolates. BMC Infect Dis 2016;16(1):260.
- [24] Boehme CC, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011;377(9776):1495–505.
- [25] Moure R, et al. Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J Clin Microbiol 2011;49(3): 1137-9.
- [26] Helb D, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. Journal of Clinical Microbiology 2010;48(1):229–37.
- [27] Guenaoui K, et al. Use of GeneXpert Mycobacterium tuberculosis/ rifampicin for rapid detection of rifampicin resistant Mycobacterium tuberculosis strains of clinically suspected multi-drug resistance tuberculosis cases. Ann Transl Med 2016;4(9).
- [28] Sanchez-Padilla E, et al. Detection of drug-resistant tuberculosis by Xpert MTB/RIF in Swaziland. New Engl J Med 2015;372(12): 1181-2.
- [29] Rufai SB, et al. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant Mycobacterium tuberculosis. J Clin Microbiol 2014;52(6):1846–52.