Detection of Multidrug Resistant *Mycobacterium tuberculosis* in Tabuk, Saudi Arabia, using Genotype MTBDRplus

Eltayib Hassan Ahmed-Abakur¹,², Tarig Mohammed Saad Alnour¹,²

¹Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Tabuk University, Tabuk, Saudi Arabia, ²Department of Medical Microbiology and Immunology, Faculty of Medical Laboratory Sciences, Al Zaiem Al Azhari University, Khartoum, Sudan

**Abstract**

**Background:** The global increase in the rates of multidrug-resistant (MDR) tuberculosis (TB) has made the timely identification of resistant *Mycobacterium tuberculosis* complex strains an important emergence to achieve effective disease management and to prevent their spread in the community. The present study aimed to determine the MDR-TB in Tabuk province, north of the Kingdom of Saudi Arabia.

**Methods:** The GenoType MTBDRplus assay was used to determine the mutations associated with isoniazid (INH) and rifampicin (RIF) resistances. A total number of 61 confirmed *M. tuberculosis* positive-sputum samples were scanned for the mutation in the *rpoB*, *inhA*, and *katG* genes.

**Results:** The present study revealed that 67.2% of the samples were susceptible, 29.5% were monoresistant, and 3.3% were MDR.

**Conclusions:** The monoresistant showed 26.2% for INH and 3.3% for RIF. The early detection of MDR could guide the starting of appropriate regimen of treatment.

**Keywords:** Isoniazid, monoresistant, multidrug-resistant, mutations, rifampicin

**Introduction**

Tuberculosis (TB) is a major public health problem globally. It is one of the top 10 causes of death worldwide.¹² According to the WHO 2017 report, 10.4 million people had TB of which 90% were adults, 10% were children, 65% were male, and 10% were people living with HIV.³

The increasing of *Mycobacterium tuberculosis* strains resistant to at least rifampicin (RIF) and isoniazid (INH), the two most effective components of first line antituberculosis therapy, termed multidrug-resistant TB (MDR-TB). Expansion of MDR-TB significantly deteriorates the prognosis for achieving a durable cure, presages treatment failure, and extends the period of transmission potential.¹² MDR-TB needs long and expensive treatment and often results in poor clinical outcome in both low- and high-income countries.⁵ Over the past two decades, there has been a rise in the number of MDR TB and extensively drug-resistant TB cases worldwide.⁶ The growing prevalence of drug-resistant strains of *M. tuberculosis* demands new measures to develop TB infection control.⁷

According to the WHO drug-resistant TB surveillance report 2014, MDR-TB occurred in 3.5% of new TB cases and 20.5% in previously diagnosed TB cases, with the incidence of MDR-TB estimated to be 5% of the overall TB cases on a global scale.⁸ In 2016, 490,000 people worldwide showed MDR-TB, and an additional 110,000 people with RIF-resistant TB which were also newly eligible for MDR-TB treatment. In 2017, the WHO estimates that there were 5,500,000 new cases with resistance to RIF—the most effective first-line drug, of which 490,000 had MDR-TB.¹¹

As in many countries, TB is common in the Kingdom of Saudi Arabia (KSA), the disease is particularly relevant in the Kingdom because of its interesting and distinct population dynamics, it undergoes vast economic expansion which associated with an influx of great numbers of overseas Workers, most of them came...
from countries with a high burden of TB such as India, Pakistan and Bangladesh. Moreover, >2 million pilgrims visiting the holy cities of the Kingdom each year, with the majority of pilgrims coming from endemic areas, this probably has adversely affected TB control in the kingdom.\textsuperscript{[8,9,12]} The WHO stated that TB had been ranked number 11 of the top leading causes of death in KSA.\textsuperscript{[11]} Infection from \textit{M. tuberculosis} leads to death of three million people worldwide per year of which an estimated one thousand are in Saudi Arabia.\textsuperscript{[12]} Moreover, Engrström \textit{et al.} in 2013 reported that Saudi Arabia is one of the countries do not meet the target of the WHO for successful treatment of TB which has been set to be of 85\%.\textsuperscript{[7]} Although the majority of the TB-related publications in the Kingdom are retrospective or population-based analyses pointed at the epidemiology or clinical manifestations of the infection. Data on basic or fundamental research related to TB in Saudi Arabia are almost nil in the arena of scientific literature.\textsuperscript{[13]} New WHO recommendations aim to speed up detection and improve treatment outcomes for MDR-TB through the use of a novel rapid diagnostic test and a shorter, cheaper treatment regimen.\textsuperscript{[14]}

However, there is strongly need to the researches treating the real situation of TB infection in the KSA. Therefore, in order to improve the control of MDR-TB infection in Saudi Arabia; the present study was aimed to determine the MDR-TB in Tabuk city, Saudi Arabia.

\textbf{Methods}

The present study was a cross-sectional study conducted in Tabuk province, Saudi Arabia, during the period from March 2017 to August 2018. The sputum samples showing smear-positive acid-fast bacilli and confirmed as \textit{M. tuberculosis} complex (MTBC) using GeneXpert were enrolled in this study.

To determine the MDR \textit{M. tuberculosis}, Genotype MTBDR\textit{plus} version 2 assay was carried out (Hain Lifescience GmbH, Nehren, Germany). DNA was extracted directly from the sputum using HIPurA \textit{M. tuberculosis} DNA purification kit (HIMEDIA–India), from the extracted DNA, 10 µL was used directly for polymerase chain reaction (PCR) amplification. The preparation of the master mix, DNA addition, amplification, and hybridization was performed as recommended by the manufacturer. The test detects gene mutations in the \textit{rpoB}, \textit{katG}, and \textit{inhA} genes and is based on multiplex PCR followed by reverse hybridization of amplicons to respective wild-type (WT) and mutation probes.

Interpretation of the susceptibility to anti-TB drugs was defined as hybridization (presence of a band) to all the WT probes and no hybridization (absence of a band) to the mutant probes. The absence of hybridization of any WT and/or hybridization of any mutant gene indicates resistance to the respective drugs. When the resistance occurred on both genes (\textit{rpoB} gene and \textit{inhA} and/or \textit{katG} genes), it indicates MDR-TB.

\textbf{Ethical consideration}

The study was approval by the Research Ethics Committee (Reference Number: (UT-56-23-2018), University of Tabuk) and by Fahad Bin Sultan Research Chair University of Tabuk, Saudi Arabia.

\textbf{Results}

A total number of 61 sputa, which showed smear-positive acid-fast bacilli, were collected from newly suspected TB patients; all samples were confirmed as MTBC using the Genexpert. The age of the patients ranged from less than 1 year to 88 years with a mean age of 34.95 years, most of them 62.2\% were male.

In this study, 67.2\% of the samples were susceptible, 29.5\% were monoresistant, and 3.3\% were MDR. The majority of monoresistant was INH 26.2\% (mutation in \textit{katG}, \textit{inhA}, or both) [Figures 1 and 2]; 1 (50\%) of the mutation in \textit{rpoB} gene was deletion on the region 505–509 (∆\textit{WT1}), and 1 (50\%) was complete deletion of the target sequence (\textit{rpoB} gene region). 3 (75\%) of the mutation in \textit{katG} gene were deletion of the target sequence of \textit{katG} gene (∆\textit{katG}) and 1 (15\%) was deletion on codon 315 (∆\textit{katG} WT). 1 (7.1\%) of the mutation in \textit{inhA} occurred in A16G T8A (WT1+WT2 MUT2MUT3B), 3 (21.4\%) deletion at the positions -15,-16 (∆\textit{inhA-WT1}), 4 (28.6\%) showed complete deletion of the target sequence \textit{inhA} region (∆\textit{inhA} region), 2 (4.3\%) deletion in position 16 (inhAWT1 + WT2 + MUT2), and 4 (28.6\%) deletion in the positions −15;−16 and −8 (∆\textit{WT1} + WT2). The MDR strains 2 (3.3\%) showed mutation in position −16 for the \textit{inhA} gene (inhAWT1 + WT2 + MUT2) and in the position 526 for \textit{rpoB} gene (WT7 + MUT2B).

\textbf{Discussion}

MDR-TB remains a public health crisis and a health security threat.\textsuperscript{[1]} Therefore, the present study was aimed to detect the MDR-TB in Tabuk province, Saudi Arabia.

Positive-sputa samples were scanned for the presence of MDR using Genotype MTBDR\textit{plus} VER 2.0. MTBDR\textit{plus} have the capability to identify smaller proportions of resistance

\textbf{Figure 1:} The patterns of susceptibility and resistance of \textit{Mycobacteria tuberculosis} using Genotype MTBDR\textit{plus} assay
in a heterogeneous mixture than INNO-LiPA. However, Genotype MTBDR assay was used to identify such cases among TB patients of the directly observed treatment strategy center in Tashkent, Uzbekistan. Moreover, the assay can directly apply to the clinical specimens that may enhance the chance of detecting the resistance.

The patterns of the mono-resistant rate in this study (26.2% for INH and 3.3% RIF) were contradictory to that reported in West region of Saudi Arabia where the resistance patterns of INH 6.5% was less than resistant rate of RIF 15% while the MDR-TB 3.8% relatively similar to our results. The finding of mono-resistant rate 29.5% in this study was higher than that reported by Jiman-Fatani et al. in 2015 in King Abdulaziz University Hospital where it was 21.78%. same study reported 3.96% resistant rate for each one of RIF and INH, these results relatively matched to RIF resistant 3.3% in our study and greatly different than NIH resistant 26.2%. The MDR-TB in this study was higher than that reported in Eastern province of Saudi where it was found to be 0.7% of the isolates and less than than reported by Al Ammari et al. in 2018 where it was 4.4%. RIF resistance is of special concern because it is the most effective bactericidal drug against M. tuberculosis. The primary resistant rate of RIF in this study is comparable to that reported by the WHO in India. The prevalence of primary and acquired RIF resistance in India was 2.8% and 17.2%, respectively, this report gave strong alarm to the situation of MDR-TB in the KSA considering that India is one of the countries which has the highest rate of TB infection and MDR-TB. However, several studies carried in Saudi Arabia had shown different rates of TB drug resistance. The variation of MDR among the provinces of the Kingdom was also confirmed by Al-Hajoj 2010. Moreover, Al-Hajoj and Vargheese in 2015 stated that TB in Saudi Arabia is one of the infectious diseases that have not been brought under control, despite the government’s efforts and the kingdom falls behind the global targets set by the WHO for the success rate of TB. Anti-TB-resistant may be attributes to the mismanagement of TB treatment, Inappropriate or incorrect use of antimicrobial drugs or use of ineffective formulations of drugs and premature treatment interruption. Association between previous anti-TB treatment therapy and development of drug resistance was also reported by Yezli and Memish.

Mutations accountable for INH resistance have been reported to occur at several genomic loci, including inhA, katG, mabA, ndh, and ahpC. The most frequent mutations encoding INH resistance are at codon 315 of the katG gene and within the inhA promoter region. Contradictory to these results our study showed only 1 (15%) deletion on codon 315 of katG gene. Globally, katG codon 315 mutations occur in 64.2% of INH-resistant strains, whereas 19% of INH-resistant strains have inhA15 promoter mutations, together accounting for 83% of INH resistance.

Mutations in the RNA polymerase b subunit (rpoB) gene have been found in approximately 96% of RIF-resistant M. tuberculosis isolates. Mutations in codons 531 and 526 are the most frequently stated mutations. Our study showed 4 (80%) of the mutation in rpoB gene region 505–509. The mutation in codon 526 appeared only in the MDR samples. However, a statistically significant association between the geographical origin of the patients and the type of mutations observed were clearly evident.

The present study showed that the TB is more common among male (62.2%) and affecting all age group. This finding was in alignment with several reports. Males generally are known to be more susceptible to TB than females because of their relatively large social network that increases their risk of infection, in addition to the higher prevalence of smoking, which has a confirmed association with TB.

**CONCLUSIONS**

TB remains an important public health problem in the KSA affecting all age, this study revealed MDR of 3.3% in Saudi Arabia (Tabuk province). The early detection of MDR could guide the starting of an appropriate regimen of treatment.

**ACKNOWLEDGMENTS**

The authors would like to thank deanship of scientific research Tabuk University, Kingdom of Saudi Arabia for sponsoring the current research (project number S-1438-0241. We are very grateful for the Microbiology staff of King Fahad Specialist Hospital and Prince Salman Armed Forces Hospital for their valuable efforts in sample collection and smear microscopy diagnosis. Thanks are also offered to Prince Fahad Bin Sultan Research Chair and Medical laboratory Technology staff, faculty of medical applied sciences, Tabuk University for their valuable support.

**Financial support and sponsorship**

Deanship of Scientific Research (DSR) - University of Tabuk.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. World Health Organization. Tuberculosis, Key Facts. Available from: http://www.who.int/en/news-room/fact-sheets/detail/tuberculosis. [Last
2. Pang Y, Dong H, Tan Y, Deng Y, Cai X, Jing H, et al. Rapid diagnosis of MDR and XDR tuberculosis with the MeltPro TB assay in China. Sci Rep 2016;6:25330.
3. Faye B, Jessika I, Seck MC, Ndour CT, Gueye PA, Fatoumata B, et al. Molecular evaluation of resistance to rifampicin and isoniazid of tuberculosis patients by test “Genotype® MTBDR plus” in Senegal. J Trop Dis 2018;6:5.
4. Bang H, Park S, Hwang J, Jin H, Cho E, Kim DY, et al. Improved rapid molecular diagnosis of multidrug-resistant tuberculosis using a new reverse hybridization assay, REBA MTB-MDR. J Med Microbiol 2011;60:1447-54.
5. Cabibbe AM, Miotto P, Moure R, Alcaide F, Feuerriegel S, Pozzi G, et al. Lab-on-chip-based platform for fast molecular diagnosis of multdrug-resistant tuberculosis. J Clin Microbiol 2015;53:3876-80.
6. Yezli S, Memish ZA. Tuberculosis in Saudi Arabia: Prevalence and antimicrobial resistance. J Chemother 2012;24:1-5.
7. Engström A, Hoffner S, Jurén P. Detection of heteroresistant Mycobacterium tuberculosis by pyrosequencing. J Clin Microbiol 2013;51:4210-2.
8. Poudel A, Maharjan B, Nakajima C, Fukushima Y, Pandey BD, Beneke A, et al. Characterization of extensively drug-resistant Mycobacterium tuberculosis in Nepal. Tuberculosis (Edinb) 2013;93:84-8.
9. Al-Orainey I, Alhedaithy MA, Alanazi AR, Barry MA, Almajid FM. Tuberculosis incidence trends in Saudi Arabia over 20 years: 1991-2010. Ann Thorac Med 2013;8:148-52.
10. Abouzeid MS, Zumla AI, Felemban S, Alotaibi B, O’Grady J, Memish ZA, et al. Tuberculosis trends in Saudis and non-Saudis in the Kingdom of Saudi Arabia – A 10 year retrospective study (2000-2009). PLoS One 2012;7:e39478.
11. Al Ammari M, Al Turuiki A, Al Essa M, Kashkary AM, Eloitiagi SA, Ahmed AE, et al. Drug resistant tuberculosis in Saudi Arabia: An analysis of surveillance data 2014-2015. Antimicrob Resist Infect Control 2018;7:12.
12. Al-Hajoj SA. Tuberculosis in Saudi Arabia: Can we change the way we deal with the disease? J Infect Public Health 2010;3:17-24.
13. Al-Hajoj S, Varghese B. Tuberculosis in Saudi Arabia: The journey across time. J Infect Dev Ctries 2015;9:222-31.
14. World Health Organization. What is multidrug-Resistant Tuberculosis (MDR-TB) and how do we Control it? Available from: http://www.who.int/features/qa/79/en/. [Last accessed on 2018 Nov 01].
15. Hofmann-Thiel S, van Ingen J, Feldmann K, Turaev L, Uzakova GT, Murmusaeva G, et al. Mechanisms of heteroresistance to isoniazid and rifampin of Mycobacterium tuberculosis in Tashkent, Uzbekistan. Eur Respir J 2009;33:568-74.
16. Folkvardsen DB, Thomsen VO, Rigouts L, Rasmussen EM, Bang D, Bernaerts G, et al. Rifampin heteroresistance in Mycobacterium tuberculosis cultures as detected by phenotypic and genotypic drug susceptibility test methods. J Clin Microbiol 2013;51:4220-2.
17. Jiman-Fatani AJ, El-Hossary D, Eltahlaw RA. Mycobacterium tuberculosis complex: Detection and patterns of resistance to the first line anti-TB drugs at the King Abdulaziz University hospital, Saudi Arabia. Int Arab J Antimicrob Agents 2015;5:5-2.
18. Patra SK, Jain A. Molecular diagnosis of multi drug resistant tuberculosis. Int J Biomed Adv Res 2012;3:273-80.
19. Huyen MN, Cobelens FG, Buu TN, Lan NT, Dung NH, Kremer K, et al. Epidemiology of isoniazid resistance mutations and their effect on tuberculosis treatment outcomes. Antimicrob Agents Chemother 2013;57:3620-7.
20. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: A systematic review. PLoS One 2015;10:e0119628.
21. Mekonnen D, Admassu A, Mulu W, Amor A, Benito A, Gelaye W, et al. Multidrug-resistant and heteroresistant Mycobacterium tuberculosis and associated gene mutations in Ethiopia. Int J Infect Dis 2015;39:34-8.