Does Xpert® MTB/RIF assay give rifampicin resistance results without identified mutation? Review of cases from Addis Ababa, Ethiopia

Ayinalem Alemu *, Mengistu Tadesse, Getachew Seid, Helina Mollalign, Kirubel Eshetu, Waganeh Sinshaw, Yeshiwork Abebaw, Misikir Amare, Biniyam Dagne, Getu Diriba, Bazezew Yenew, Melak Getu and Betselot Zerihun

Abstract

Background: Xpert® MTB/RIF assay is currently used in Ethiopia for the rapid diagnosis of *Mycobacterium tuberculosis* (MTB) and mutations that confer Rifampicin resistance. Rifampicin resistance is determined based on any mutation in the 81 bp of *rpoB* gene using five overlapping probes represented as Probe A (codons 507–511), Probe B (codons 512–518), Probe C (codons 518–523), Probe D (codons 523–529) and Probe E (codons 529–533). In this review, we assessed the frequency of missed probe types for Rifampicin Resistance results.

Methods: Data were reviewed from specimens received and tested using Xpert® MTB/RIF assay at Ethiopian National Tuberculosis Reference Laboratory, in Addis Ababa from 15 July 2016 to 31 December 2018 retrospectively. All archived data were reviewed carefully to describe missed probe types and the quantity of DNA in the sample.

Results: A total of 100 specimens were reported as MTB Detected Rifampicin Resistance Detected by Xpert® MTB/RIF assay. More than half (55%) of these results were reported from male patients. The median age was 28.0 years (5 months to 88 years). Majorities (62%) of the cases were detected from sputum. Among the total of 38 extrapulmonary samples, lymph node aspirates were accounted for 50% (19/38). The most common mutations (81.0%) were found in the Probe E region followed by Probe D (10.0%), and Probe B (3.0%). Mutations in Probe A and Probe C regions were not observed. However, six (6.0%) Rifampicin resistance cases were found without any missed probe type. The delta Ct max is ≥4.3. No specimen yielded Rifampicin resistance associated with more than one probe failure or mutation combinations.

Conclusion: Mutations associated with Probe E (codons 529–533) region were identified as the commonest *rpoB* gene mutations. The Rifampicin resistance results found without any identified missing probe needs further study. The lower DNA amount was observed in extrapulmonary specimens compared with sputum.

Keywords: Rifampicin-resistance, Molecular Beacon, DNA probes, Xpert® MTB/RIF assay

* Correspondence: ayinalemal@gmail.com
Ethiopian Public Health Institute, Addis Ababa, Ethiopia

© The Author(s). 2020 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
World health organization (WHO) endorsed Xpert® MTB/RIF assay for the diagnosis of *Mycobacterium tuberculosis* (MTB) and the mutations that confer Rifampicin resistance (RR) [1]. This assay has revolutionized the diagnosis of TB by simultaneously detecting the bacteria and RR [2, 3], which is a surrogate marker for MDR-TB [2, 4]. RR is determined based on mutations in the 81 bp (codons 507–533) regions of the β-subunit of the RNA polymerase enzyme (*rpoB*) gene using five overlapping probes [3, 5]. These probes are named as Probe A (codons 507–511), Probe B (codons 511–518), Probe C (codons 518–523), Probe D (codons 523–529) and Probe E (codons 529–533) [3, 5]. A mutation in these regions accounts for more than 95% of RR [3, 4].

Ethiopia is among the 30 high TB, TB/HIV and MDR-TB burdened countries in the world [1]. In 2017, there was an estimated TB incidence of 164/100,000 population. In the same year, MDR/RR-TB rate was estimated to be 2.7% among new cases and 14% among previously treated cases [1]. Ethiopia has been implementing Xpert® MTB/RIF assay for the diagnosis of TB and RR-TB which provided encouraging results [6]. Studies showed that the probe that confers RR is rarely reported in the clinical practice of many countries [5]. Similarly, there is no practice in reporting RR-TB with the type of missed probe and the Ct values in Ethiopia, specifically the current study setting. Identification of the nature of *rpoB* mutation could provide useful information for accurate diagnosis of RR-TB and when there is a need to studying the epidemiology of RR-TB in a particular region [3, 5]. However, information on the frequency of *rpoB* gene mutations in this study setting is scarce. Therefore, this review aimed to provide relevant information on the frequency of associated mutations for RR results using Xpert® MTB/RIF assay in Addis Ababa, Ethiopia.

Methods
Setting
Data were reviewed retrospectively from archived result log sheet for specimens received and tested using Xpert® MTB/RIF assay at Ethiopian National Tuberculosis Reference Laboratory (NTRL) from 15 July 2016 to 31 December 2018. NTRL is an accredited national reference laboratory located in Addis Ababa, Ethiopia, which provides different services including diagnostic testing using Xpert® MTB/RIF assay. Retrospective data were reviewed from archived logbooks and databases. The assay performed using Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA). Socio-demographic and clinical data were reviewed for patients with RR-TB results. Important details were accessed from the database.

Laboratory diagnosis
Sputum samples and extrapulmonary specimens [lymph node aspirates, pleural fluid, pus, abscess, ascetic fluid and bronco alveolar leverages (BAL)] were collected from TB presumptive patients. Samples were transported by triple packaging system through trained couriers within acceptable (2-8°C) temperature conditions to NTRL. Approximate of 4 ml sputum was mixed with 8 ml of sample reagent buffer (supplied within the kit), shaken the tubes vigorously 20 times and allowed to stay for 10 min. Following this, it was mixed again and stayed for 5 min. Later, an approximate of >2 ml (not more than 4 ml) of the specimen was dispensed into Xpert MTB/RIF’s cartridge and loaded into the GeneXpert instrument (Cepheid, Sunnyvale, CA, USA). The result was released after 2 h. For extrapulmonary samples, different approaches were used based on the nature of the specimen. For example, lymph node samples were decontaminated by using a 3% NALC-NaOH method and the sediment was used for the test in 1:3 ratios (0.5 ml sediment and 1.5 ml sample reagent buffer) as done elsewhere [7].

Results from Xpert® MTB/RIF assay were categorized into three result types such that; *Mycobacterium tuberculosis* not detected, *Mycobacterium tuberculosis* detected and Error/Invalid/No results. Along with all *Mycobacterium tuberculosis* detected test results Rifampicin resistance was determined. For all test results having *Mycobacterium tuberculosis* detected results, probe types and DNA amounts were assessed. Such that all RR-TB test results were archived from the GeneXpert instrument and exported to a database. All the information including the missed probe types and level of each DNA amount were reviewed from the database and crosschecked on the GeneXpert instrument because the GeneXpert instrument can give all details of test results in PDF format.

Statistical analysis
Extracted data were checked for completeness, coded, entered and analyzed by using Statistical Packages for Social Sciences (SPSS) Version 20. Descriptive statistics were used to characterize demographic and clinical variables. Frequency of specimen types, test results, DNA amounts, and missed probe types were determined.

Results
Demographic and clinical information
Among all specimens processed by Xpert® MTB/RIF assay at NTRL during the review period, *MTB detected Rifampicin resistance detected* was reported from 100 specimens. Demographic characteristics and treatment history were collected for TB patients having RR results and more than half (55%) were males. The majority of
the patients were found in the age group of 25–34 years. Among all 100 RR-TB patients, half (50) were new patients and 29 were relapses, while the rest 11 were either failures or return after loss to follow up. The majority (62, 62%) of the specimens were sputum, while the remaining 38 (38%) were extrapulmonary samples. Among the total of 38 extrapulmonary samples, lymph node aspirates were accounted 50% (19, 50%) followed by pleural fluid (5, 13.2%), pus (4, 10.5%), abscess (4, 10.5%), ascetic fluid (3, 8.0%), unspecified body fluids (2, 5.2%) and BAL (2, 2.6%) (Table 1) (Fig. 1).

Missed probe types
As Xpert® MTB/RIF assay gives the amount of DNA semi-quantitatively, the DNA amount of RR results was found as a very low (14, 14%), low (23, 23%), medium (36, 36%) and high (27, 27%). Of the 63 medium or high DNA amount test results, majority 51 (80.95%) were from sputum samples, whereas among the 37 low or very low DNA amount results, majority 24 (64.87%) were from EPTB samples (Table 2). The most common mutation was located in probe E (codons 529–533) region (81, 81%) followed by probe D region (codons 523–529) (10, 10%) and probe B region (codons 512–518) (3, 3%). There was no mutation associated with Probe A (codons 507–511) and Probe C (codons 518–523) regions (Fig. 1). The delta Ct max was ≥4.3. However, six (6, 6.0%) RR-TB cases were found without any identified missed probe type. All these six RR results were detected from sputum samples and of which five were from new patients (Table 2).

Discussion
In addition to the simultaneous detection of MTB and its resistance to Rifampicin, Xpert® MTB/RIF assay might be used to understand the molecular epidemiology of MTB and to identify hot spots of drug-resistant TB transmission. Demographic characteristics and treatment history were collected for all 100 RR-TB patients and more than half of the RR-TB patients were males, where it was reported previously that males are highly affected by TB compared to females [1, 8]. In a systematic review conducted in Ethiopia, it was also reported that being male has been identified as a risk factor for multi-drug resistant tuberculosis [9]. The most highly affected age groups were productive individuals found in the age group of 25–34 years, which is comparable with various studies [1, 10–12]. This might be due to exposure to open cases of TB where young individuals especially males are prone to TB associated risk factors.

Although the Xpert® MTB/RIF assay was optimized for the respiratory specimen [13], this study showed that the assay can provide valid results in extrapulmonary samples. Among 100 RR-TB results, the majority (62) was detected from sputum samples, while the remaining 38 samples were detected from different types of extrapulmonary samples. From the extrapulmonary samples, half was a lymph node aspirate sample which is similar to the study reported from Dessie, Ethiopia [14]. In this review higher DNA amount (high or medium) was observed in the sputum samples, while lower DNA amount (low or very low) was observed in extrapulmonary samples. This lower DNA amount in EPTB samples might cause false-negative results [15–17] which should be considered while preparing specimens for Xpert® MTB/ RIF assay to increase sensitivity.
Rifampicin resistance is determined in Xpert® MTB/RIF assay by rpoB gene mutations in the 81 bp-RRDR of MTB which are five overlapping regions labeled as A (codons 507–511), B (codons 511–518), C (codons 518–523), D (codons 523–529) and E (codons 529–533) [3–5]. In this review, the commonest mutation was located in codons covering 529–533 which is represented by Probe E (81, 81%). This was also reported by previous studies conducted in Ethiopia [18–20]. Similarly, in the previous studies done in Africa countries in Nigeria [21] and in Uganda [2], mutations conferring RR are located in mostly the region of Probe E. Likewise, in studies done at Asian countries in India [3, 5], Pakistan [4] and Bangladesh [22] missing of probe E was predominant. However, Most of the RR cases detected by Xpert® MTB/RIF assay were associated with probe B (23/64) and probe E (23/64) in a study done in Malawi [23]. The information about the probes conferring RR could be used to assess trends over time, identify pockets of transmission, or investigate outbreaks, especially when the RR is secondary to mutations outside the Probe E region. In this review following probe E the proportion of each missed probe were: probe D (10%) and probe B (3%). This was also observed in a study done in Nigeria [21]. However, most of the previous studies conducted in African and Asian regions indicate that following Probe E the most common mutations conferring RR are located in the region was Probe B followed by Probe D [2–5, 22]. In a study done in Malawi, the proportion of mutation in Probe E and Probe B is equal [23]. In this review there was no mutation associated with Probe A and probe C, probably this particular site of RRDR is less susceptible to mutations conferring this resistance or might be the less common mutation of these probes in this particular area (Addis Ababa). Similarly, the absence of Probe C mutation was reported from Nigeria [21] and from Uganda [2]. Likewise, it was also reported that mutations in Probe A and Probe C were less common in other studies [3–5, 22, 23].

In this review, we found that six (6, 6%) test results were resistant to Rifampicin without any identified missed probe. The possible reason behind this could be the delta Ct (ΔCT) max. Delta Ct max is the difference between the first (early CT) and the last (late CT) MTB specific beacon [16]. In the Xpert® MTB/RIF assay, for MTB Detected Rifampicin Resistance Detected/RR-TB/ test results the delta Ct max should be > 4 [24]. This has happened in the current review where the ΔCT max was ≥4.3. However, it needs further study or clarification. In addition, it was reported previously that, the amount of DNA affects Rifampicin resistance results in the Xpert® MTB/RIF assay [25]. Even though not used in this review and previous studies, the codon used to detect Rifampicin resistance could be used for contact tracing. Berhanu et al reported that a Rifampicin resistant
discordant result in Xpert® MTB/RIF assay was associated with Probe B [26].

The limitation of this review was that no gold standard (phenotypic DST and sequencing) method was used for the comparison of Xpert® MTB/RIF assay results to estimate the proportion of false drug resistance or susceptibility. Furthermore, as a retrospective review, it lacks other relevant variables such as contact history, HIV status, vaccination status and location of the household district.

**Conclusion**

Mutations associated with Probe E (codons 529–533) are identified as the commonest rpoB gene mutation in Ethiopia and other countries as identified in this and previous studies. In the reviewed data, mutations associated with Probe A (codons 507–511) and Probe C (codons 518–523) are not identified. RR-TB was found without any missing probes in six sputum samples (6%) which necessitate further study or investigation. The lower DNA amount is observed in extrapulmonary samples compared with sputum samples. A further larger study is needed to confirm RR-TB cases by using gold standard methods (Mycobacterium culture and phenotypic DST).

**Abbreviations**

DNA: Deoxyribose Nucleic Acid; EPTB: Extra Pulmonary Tuberculosis; HIV: Human Immunodeficiency Virus; MDR-TB: Multidrug-Resistant Tuberculosis; MTB: Mycobacterium tuberculosis; NTRL: National Tuberculosis Reference Laboratory; RNA: Ribose Nucleic Acid; rpoB: RNA polymerase enzyme β-subunit; RR: Rifampicin Resistance; RRDR: Rifampicin Resistance Determining Region; SPSS: Statistical Package for Social Sciences; TB: Tuberculosis; WHO: World Health Organization

**Acknowledgments**

We would like to acknowledge: Ethiopian Public Health Institute, National TB Reference Laboratory Staff.

**Authors’ contribution**

AA designed the review, analyzed results and wrote the manuscript. MT, GS, HM, KE, WS, YA, MA, BD, GD, BY, MG and BZ perform the Xpert MTB/RIF Assay, perform quality control and perform data entry. All authors read, reviewed and approved the final manuscript.
Alemu et al. BMC Infectious Diseases (2020) 20:87

Page 6 of 6

Funding
The author(s) did not receive specific funding for this review.

Availability of data and materials
All original raw data is available in the corresponding author.

Ethics approval and consent to participate
The review obtained permission from the National Tuberculosis Reference Laboratory of the Ethiopian Public Health Institute and all the authors are the staff. Since it is a retrospective review, study participants were not contacted for consent. Patients’ names or identifiers were not used in the entire process.

Consent for publication
Not applicable.

Competing interests
The authors have declared that they do not have any competing interests.

Received: 1 November 2019 Accepted: 23 January 2020
Published online: 30 January 2020

References
1. WHO. Global Tuberculosis Report. Geneva: World Health Organization; 2018.
2. Mboowa G, Namaganda C, Sengooba W. Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert® MTB/RIF in Kampala, Uganda: a retrospective study. BMC Infect Dis. 2014;14(481).
3. Kaur R, Jindal N, Arora S, Katia S. Epidemiology of rifampicin-resistant tuberculosis and common mutations in rpoB gene of Mycobacterium tuberculosis: a retrospective study from six districts of Punjab (India) using Xpert MTB/RIF assay. J Lab Physicians. 2016;9:6–100.
4. Ullah I, Shah AA, Basit A, Ali M, Khan A, Ullah U, et al. Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert MTB/RIF in Khyber Pakhtunkhwa, Pakistan: a retrospective study. BMC Infect Dis. 2016;16:413.
5. Reddy R, Uria GA. Molecular epidemiology of rifampicin resistance in Mycobacterium tuberculosis using the GeneXpert MTB/RIF assay from a rural setting in India. Hindawi J Pathog. 2017; https://doi.org/10.1155/2017/6738095.
6. Federal Democratic Republic of Ethiopia Ministry of Health/Ethiopian Public Health Institute. Implementation Guideline for GeneXpert MTB/RIF Assay in Ethiopia. 2014.
7. Tadesse M, Abebe G, Abdissa K, Aragaw D, Abdella K, Bekele A, et al. GeneXpert MTB/RIF Assay for the Diagnosis of Tuberculosis Lymphadenitis on Concentrated Fine Needle Aspirates in High Tuberculosis Burden Settings. PLoS ONE. 2015;10(9).
8. Horton KC, MacPherson P, Houben RMJ, White RG, Corbett EL. Sex and its Rifampicin resistance at Felege Hiwot and Debre Tabor Hospitals, Northwest Ethiopia: A preliminary implementation research. Ethiop. J. Health Dev. 2016;30(2).
9. Asegedom SW, Teweldemedhin M, Gebreyesus H. Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia: A Systematic Review. Hindawi J Pathog. 2018; Article ID 7104921, 8 https://doi.org/10.1155/2018/7104921.
10. Derbie A, Worku S, Mekonten D, Mezgebu Y, Teshager A, Birhan A, et al. Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and its Rifampicin resistance at Felege Hiwot and Debre Tabor Hospitals, Northwest Ethiopia: A preliminary implementation research. Ethiop. J. Health Dev. 2016;30(2).
11. Ejeta E, Beyene G, Bonsab Z, Abebe G. Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and rifampicin resistance in high human immunodeficiency virus setting in Gambella regional state, Southwest Ethiopia. J ClinTuberc Other Mycobact Dis. 2018;12:14–20.
12. Mulu W, Abera B, Yimer M, Hallu T, Ayele H, Abate D. Rifampicin-resistance pattern of Mycobacterium tuberculosis and associated factors among presumptive tuberculosis patients referred to Debre Markos referral hospital, Ethiopia: a cross-sectional study. BMC Res Notes. 2017;108.
13. WHO. Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Geneva: World Health Organization. 2013; ISBN: 978 92 4 150633 5.
14. Metafeta Y, Seid A, Fenta GM, Gebretsadik D. Assessment of Extrapolmonary tuberculosis using gene Xpert MTB/RIF assay and fluorescent microscopy and its risk factors at Dessie referral hospital, Northeast Ethiopia, BioMed Res Int 2018; https://doi.org/https://doi.org/10.1155/2018/8207989.
15. Puebob M, Mustafa T. Laboratory Diagnosis of Extra-pulmonary Tuberculosis (EPTB) in Resource-constrained Setting: State of the Art, Challenges and the Need. J Clin Diagn Res. 2015;9(4).
16. Lawn SD, Zuma A. Diagnosis of extrapulmonary tuberculosis using the Xpert® MTB/RIF assay. Expert Rev Anti-Infect Ther. 2012;10(6):631–5.
17. Kohli M, Schiller I, Dendukuri N, Dheda K, Denkinger CM, Schwander SG, Steingart KR. Xpert® MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. Cochrane Database Syst Rev. 2018.
18. Tessema B, Beer J, Emmrich F, et al. Analysis of gene mutations associated with isoniazid, rifampicin and ethambutol resistance among Mycobacterium tuberculosis isolates from Ethiopia. BMC Infect Dis. 2012;12:37.
19. Biadglegne F, Tessema B, Rodloff AC, et al. Magnitude of gene mutations conferring drug resistance in Mycobacterium tuberculosis isolates from lymph node aspirates in Ethiopia. Int J Med Sci. 2013;10:1589.
20. Tadesse M, Aragaw D, Dimah B, Efia F, Abdella K, Kebede W, et al. Drug resistance-conferring mutations in Mycobacterium tuberculosis from pulmonary tuberculosis patients in Southwest Ethiopia. Int J Mycobacteriology. 2016;5:185–91.
21. Ochang EA, Udoh UA, Emanghe UE, Tiku GO, Ofior JB, Odo M. Evaluation of rifampicin resistance and 81 bp rifampicin-resistant determinant region of rpoB gene mutations of Mycobacterium tuberculosis detected with Xpert MTB/RIF in Cross River state, Nigeria. Int J Mycobacteriology. 2016;5:145–6.
22. Rahman A, Sahrin M, Afroz S, Earley K, Ahmed S, Rahman SMM, et al. Comparison of Xpert MTB/RIF assay and GenoType MTBDRplus DNA probes for detection of mutations associated with rifampicin resistance in Mycobacterium tuberculosis. PLoS ONE. 2016;11(4):e0152694. https://doi.org/10.1371/journal.pone.0152694.
23. Chikoonda T, Keteriglou I, Nguluwe N, Krysko R, Thengolose I, Nyakwawa F, et al. Molecular characterization of rifampicin-resistant Mycobacterium tuberculosis strains from Malawi. Afr J Lab Med. 2017;6(2).
24. Prakash AK, Datta B, Topathy JP, Kumar N, Chatterjee P, Jaiswal A. The clinical utility of cycle of threshold value of GeneXpert MTB/RIF (CBNAAT) and its diagnostic accuracy in pulmonary and extra-pulmonary samples at a tertiary care center in India. Int J Tuberc. 2018;283.
25. Ocheretina O, Byrt E, Malbou MM, Mardi GR, Merveille YM, Rouzierb V, et al. bFalse-positive rifampin resistant results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load. Diagn Microbiol Infect Dis. 2016;85(1):53–6.
26. Berhanu H, Schnippel K, Kularatne R, Firnhaber C, Jacobson KR, Horsburgh CR, et al. Discordant rifampicin susceptibility results are associated with Xpert MTB/RIF probe B and probe binding delay. Int J Tuberc Lung Dis. 2019;23(3):358–62.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions