Original Article

DOI: 10.7860/JCDR/2022/55315.16413

Rifampicin Resistance by Xpert MTB/RIF Assay in Pulmonary Tuberculosis- Is there a Need for Confirmation by Retesting?

Microbiology Section

SWAPNA RAJESH KANADE[1], SWATI VIJAY[2], GITA NATARAJ[3]

ABSTRACT

Introduction: Xpert *Mycobacterium tuberculosis*/Resistance to Rifampicin (MTB/RIF) assay detects MTB Complex (MTBC) and rifampicin resistance simultaneously. In high prevalence countries like India, detection of rifampicin resistance in sputum specimen of a newly diagnosed case of pulmonary TB with a low pretest probability needs to be confirmed by retesting.

Aim: To evaluate the results of retesting of rifampicin resistant specimens in newly diagnosed pulmonary TB cases.

Materials and Methods: A retrospective analysis of the data of Xpert assay was performed on specimens received in Department of Microbiology, Seth G.S. Medical College and KEM Hospital, Mumbai, Maharashtra, India from 1st June 2018 to 31st May 2020. If rifampicin resistance was detected in a newly diagnosed case of Tuberculosis (TB), a second specimen was retested by Xpert assay for confirmation. Concordance of retesting was seen with results of Line Probe Assay (LPA).

Results: Total 27,429 specimens were processed by Xpert assay of which 803 specimens showed rifampicin resistance, 157 sputum specimens fulfilling criteria of Programmatic Management of Drug resistant Tuberculosis (PMDT) guidelines were retested. High, medium, low and very low bacterial load was observed in 30, 51, 34 and 42 specimens' respectively. All specimens having high or medium bacillary load showed rifampicin resistant result on retesting. On retesting 34 sputum specimens with low bacterial load, rifampicin resistance was confirmed in 30 specimens. LPA done after growing them by liquid culture confirmed rifampicin resistance in remaining four specimens.

Conclusion: Xpert assay is recommended when the bacterial load identified by Xpert assay is very low and when there is discordance between Xpert results of rifampicin resistance and the reflex LPA testing.

Keywords: Drug resistance, Molecular assay, Mycobacterium tuberculosis/Resistance to rifampicin, Repeat testing

INTRODUCTION

Drug resistant TB is an important challenge in the control and elimination of TB. World Health Organisation (WHO) endorsed Xpert MTB/RIF assay (Xpert assay) for the diagnosis of MTB and Rifampicin resistance [1]. The gene Xpert diagnostic system developed by Cepheid (Sunnyvale, CA, USA) has been in use since 2013 and has proved to be a game changer in TB diagnosis and rifampicin resistance detection especially in high burden countries. The Xpert assay uses molecular beacon technology to detect DNA sequences amplified in a heminested real-time-Polymerase Chain Reaction (PCR) assay. The test uses a cartridge-based system. All the steps required for PCR like extraction, amplification and detection of targeted sequences from the patient's samples takes place inside the cartridge [2]. The limit of detection of the assay is approximately 131 colony forming units per ml (cfu/mL) of sputum specimen [3].

Xpert MTB/RIF assay detects MTBC and rifampicin resistance simultaneously as most common mutation conferring rifampicin resistance occurs in the same 81 bp region of genome which also codes for MTBC specific Deoxyribonucleic Acid (DNA) sequence [4,5]. This assay identifies rifampicin resistance associated mutations in codon 507 to 533 of *rpoB* gene of Mycobacterial genome [6]. It detects either absence of binding of the probe affected (negative analyte result for the affected probe) or sensing a "significant" delay in amplification of the mutated segment/s compared to wildtype segments. The delay is considered as significant if it is more than four cycles. [7]. The assay also gives a semi-quantitative indication of the bacterial load in the specimen as high, medium, low and very low [2].

Rifampicin resistance is considered a surrogate marker of multidrug resistant TB [8]. Timely and accurate diagnosis of rifampicin resistant TB is important as the diagnostic algorithm under PMDT guidelines depend totally on status of rifampicin resistance [9]. In high prevalence countries like India, detection of rifampicin resistance in sputum specimen of a newly diagnosed case of pulmonary TB with a low pretest probability needs to be confirmed by retesting. Since June 2018, all such specimens were retested for confirmation of rifampicin resistance irrespective of their bacterial load [10]. As per revised guidelines of June 2021, only specimens having low (Cycle threshold (Ct) value 22-28] and very low (Ct value >28) bacterial load needed to be retested [9]. Xpert assay is an expensive test and each cartridge used for retesting creates huge burden on the National TB Elimination programme [11]. Hence, this study was undertaken with the primary objective of evaluating the results of retesting of rifampicin resistant specimens in newly diagnosed pulmonary TB cases.

MATERIALS AND METHODS

This retrospective analytical study was conducted in the Department of Microbiology Seth G.S. Medical College and KEM Hospital, Mumbai, Maharashtra, India. Data was collected for a period of two years, from 1st June 2018 to 31st May 2020 and was analysed in next four months (June 2020-September 2020). This study was ethically approved from the Institutional Ethical Committee (IEC) (EC/OA-174/2020) of same medical college. As this study involved analysis of results entered in laboratory register; waiver was obtained for informed consent.

Inclusion criteria: A total of 27,429 patients, either outpatient or admitted in the hospital, submitted sputum specimen to the laboratory for Xpert assay during the study period. All specimens were tested and results were entered in lab register.

Exclusion criteria: All the specimens were included in this study and there was no exclusion criterion.

Study Procedure

Clinically suspected cases of pulmonary tuberculosis is any person who presents with symptoms or signs suggestive of tuberculosis [12]. Two sputum specimens were collected from the cases which were clinically suspected cases of pulmonary tuberculosis. Same sample ID was given to both specimens with sublabel as "A" and "B". This assay provided the result as "MTB not detected" or "MTB detected" using three specific primers and five unique molecular probes to ensure a high degree of specificity. Positive result like "MTB Detected" is reported when minimum two probes combine and the difference between their Ct value is less than 2.0 [13]. If MTB is detected, a semi-quantitative estimation of bacterial load is also provided by machine as high (Ct value <16), medium (Ct value 16-22), low (Ct value 22-28) and very low (Ct value >28) [2]. Simultaneously information was obtained as "Rifampicin resistance detected' or "Rifampicin resistance not detected". If Rifampicin resistance was detected in a newly diagnosed case of TB, then second specimen was retested for confirmation as per PMDT guidelines [9]. Results of testing as well as retesting were entered in the laboratory register. Results obtained by retesting were analysed and percentage of discordant results was determined. As per PMDT guidelines, additional sputum specimen was collected from the patient having discordant results and it was tested by LPA for first line anti-TB drugs [9]. LPA technology involves the DNA extraction from MTB culture isolates or directly from clinical specimens. Using PCR, amplification and hybridisation was done to detect presence or absence of resistance to rifampicin in of MTBC [14]. LPA was performed only for specimens showing discordance between first and repeat Xpert assay.

STATISTICAL ANALYSIS

Data was analysed using Microsoft excel. Total number and percentage of concordant and discordant results were calculated. Result of LPA testing was compared with result of Xpert assay.

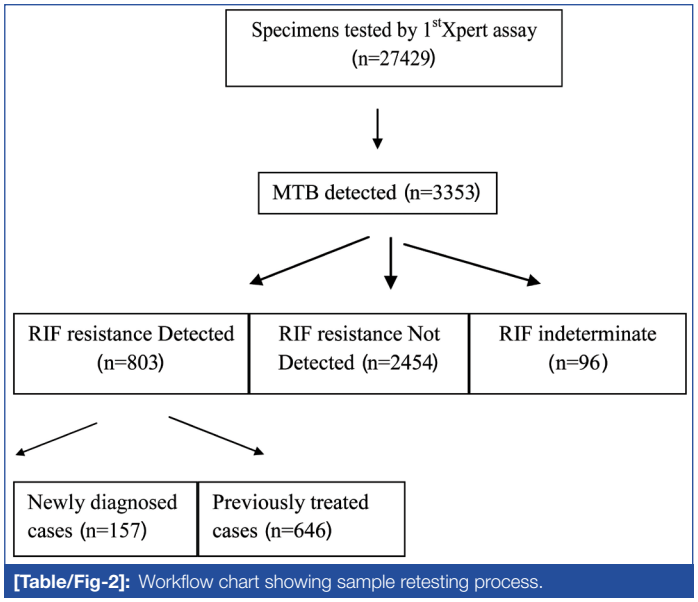
RESULTS

During the study period, total 27,429 patients visited the lab and submitted same number of specimens. The age range were from 1-60 years with mean age being 39.59±10.74 years, including both male (16404) and female (11025) [Table/Fig-1]. They were processed by Xpert MTB/RIF assay. Of these 27,429 patients, MTB was detected in 3353 (12.22%) specimens and in this category, rifampicin resistance was detected in 803 (23.95%), but was not detected in 2454 (73.19%) and indeterminate resistance was found in 96 (2.86%) specimens. Of the 803 specimens showing rifampicin resistance results, 157 sputum specimens were from newly diagnosed patients and 646 from previously treated patients [Table/Fig-2].

Age (years)	Gender	
	Male	Female
1-10	588	578
11-20	1211	1022
21-30	5126	3601
31-40	4215	3521
41-50	2951	1005
51-60	1625	968
>60	688	330
Total	16404	11025

[Table/Fig-1]: Age and gender distribution.

All 157 newly diagnosed patients were retested. High, medium, low and very low bacterial load was observed in 30, 51, 34 and 42 specimens respectively [Table/Fig-3]. All specimens having high (30, 19.11%) or medium (51, 32.48%) bacillary load showed rifampicin resistant result on retesting demonstrating 100% concordant results. However, specimens having low (34, 21.66%) or very low (42, 26.75%) bacillary load had 88.23% concordance.



[Table/Fig-2]: Workflow chart showing sample retesting process.

Bacterial load	RR by 1st Xpert assay (N=157)	Result of retesting by	MTB detected -RR	MTB detected -RS	MTB not detected
High	30	2nd Xpert assay	30	0	0
		LPA	30	0	0
Medium	51	2nd Xpert assay	51	0	0
		LPA	51	0	0
Low	34	2nd Xpert assay	30	0	4
		LPA	34	0	0
Very low	42	2nd Xpert assay	29	2	11
		LPA	38 (29+9)	4 (2+2)	0

[Table/Fig-3]: Result of retesting of specimens having rifampicin resistant result.
RR: Rifampicin resistant; RS: Rifampicin sensitive

On retesting 34 sputum specimens with low bacterial load by Xpert assay, rifampicin resistance was confirmed in 30 specimens. In remaining four specimens, MTB was not detected. These four specimens were tested by LPA, either directly or after obtaining mycobacterial isolate from Mycobacteria Growth Indicator Tube (MGIT) liquid culture. All four showed presence of rifampicin resistance.

Total 42 patients with rifampicin resistance and very low bacterial load were retested by Xpert assay. Rifampicin resistance was confirmed in 29 specimens by retesting. Remaining 13 patients were tested by LPA by using culture isolates obtained by growing them in MGIT 960 liquid culture system. Rifampicin resistance was confirmed in nine patients. Four patients showed absence of rifampicin resistance and responded to drug sensitive TB treatment.

In two specimens, rifampicin resistance was not detected which was confirmed by LPA. These two patients were started on treatment for rifampicin sensitive TB and they responded to it. In 11 specimens, Xpert assay could not detect MTB. LPA confirmed rifampicin resistance in nine patients, rifampicin resistance was detected in nine specimens. In two specimens, MTB was detected but rifampicin resistance was not detected.

Overall, in 140 patients with rifampicin resistance, concordant results were obtained on retesting by Xpert assay. In the remaining 17 patients, LPA confirmed rifampicin resistance in 13 patients. Four patients having very low bacterial load showed discordance between Xpert assay and LPA results [Table/Fig-4].

Result of 2nd Xpert assay	Result of LPA			
	RR	RS	MTB not detected	Total
RR	140	0	0	140
RS	0	2	0	2
MTB not detected	13	2	0	15
Total	153	4	0	157

[Table/Fig-4]: Comparison of result of retesting of specimens by 2nd Xpert assay and LPA (n=157).
RR: Rifampicin resistant; RS: Rifampicin sensitive

Of the 157 specimens retested, 17 specimens showed rifampicin resistance due to delay in hybridisation with probes while remaining 140 specimens showed rifampicin resistance due to dropout of one or more probe.

DISCUSSION

Molecular diagnosis of tuberculosis and rifampicin resistance by Xpert MTB/RIF assay is a game changer for tuberculosis control programme. The advantages of this test are that it can be performed directly on sample, give rapid results within two hours, provides information on rifampicin resistance when MTB is detected [2]. Getting the results on the same day helped the clinicians to decide appropriate treatment.

Rifampicin resistance when detected in patients with a low pretest probability, a repeat testing with a fresh sample is advised. In the present study, an attempt was made to understand the necessity of repeat testing for confirmation of rifampicin resistance by comparing results of first and repeat test. Of the total 27,429 specimens tested by Xpert assay, MTB was detected in 3353 (12.22%) specimens, of which 803 (23.95%) showed presence of rifampicin resistance on first testing. As per guidelines of National TB Elimination Programme, specimens from 157 newly diagnosed TB cases were retested for confirmation of presence of rifampicin resistance [10].

Xpert assay detects rifampicin resistance by probing for point mutations in the 81 bp (27 codons) rifampicin resistance determining region of *rpoB* gene of MTB. It is detected by using five overlapping probes labelled as A, B, C, D and E [15-17]. The Xpert assay also offers a semi-quantitative estimation of bacterial burden in form of Ct values. Ct values have been useful to predict rifampicin resistance. The difference between the first (early Ct) and last (later Ct) MTB-specific molecular beacon (delta CT Max) is the basis of *rpoB* mutation and rifampicin resistance detection [18,19]. In Xpert assay, to detect rifampicin resistance, delta Ct max should be >4 [17]. Mutation that completely inhibits one or more probe hybridisation is defined as causing probe "dropouts" whereas *rpoB* mutation that permit partial probe hybridisation and produce a measurable Delta CT Max of >4 cycles is considered "delays" [20]. Amount of DNA in the specimen may have some impact on detection of rifampicin resistance [21].

Xpert assay also provides information about semi-quantitative load of MTBC in the given sample as per the Ct value as high, medium, low and very low. Ct values demonstrate the number of PCR cycles that the MTB DNA goes through to reach the level of detection; higher Ct values correlate with lower bacterial loads [22]. In the present study, bacterial load of rifampicin resistant specimens was found as high (30), medium (51), low (34) and very low (42) in first testing. Such variable Ct values are observed in various other studies also [23,24]. On retesting, 81 specimens having high and medium bacterial load showed 100% concordant results for MTB detection and rifampicin resistance.

On retesting of second specimen from 34 patients with low bacterial load, 30 (88.23%) specimens confirmed rifampicin resistance. In remaining four specimens, LPA testing confirmed presence of rifampicin resistance in these specimens. All of them responded to treatment and got cured. On retesting 42 specimens with very low bacterial load, rifampicin resistance was confirmed in 29 (69.04%) specimens. Of the remaining 13 patients, LPA confirmed rifampicin resistance in 11 patients. Similar finding were reported in other studies. Van Rie A et al., reported six samples as rifampicin resistant by Xpert assay. Five out of these six samples were found resistant by LPA [25]. In a study by Rufai SB et al., 64.4% of rifampicin monoresistant TB cases by LPA were correctly diagnosed by the Xpert MTB/RIF assay [26].

Detection of rifampicin resistance by Xpert assay depends on two important factors such as proportion of mutants present in the sample as well as type of mutation like "dropouts" or "delays". Some of the issues with Xpert highlighted by other studies are false rifampicin resistant results related to existence of "disputed" and silent mutations [27-32]. Blakemore R et al., evaluated the analytical performance of Xpert MTB/RIF assay in their study and tested the ability of the assay to detect the rifampicin resistant fraction of a mixed sample (MTB DNA with a wild-type *rpoB* sequence was mixed in various ratios with MTB DNA that contained *rpoB* mutations) [20]. Their study showed that the proportion of mutant DNA required for the detection of rifampicin resistance was dependent on the type of mutation. Xpert assay is capable of detecting the presence of rifampicin resistance mutations down to a concentration of 40% mutant DNA [33]. Chakravorty S et al., demonstrated that detection of rifampicin resistance in DNA mixtures with 10, 20, and 30% mutant DNA were indistinguishable from a sample containing 100% wild-type DNA [33]. Significantly higher mutant proportions were needed (65%-100% of the total bacterial population) for a positive identification compared to the minimum 1% mutant population required for clinical resistance [20,34]. Solid media-based proportion method and even liquid media based automated testing method (MGIT 960) to a certain extent, are capable of identifying such low proportions [35].

In a country like India, cost of a test is an important factor to decide its use. Cost of a single Xpert assay cartridge is approximately Rs 1500/- and hence there are strict protocols for its use under the National TB elimination programme [10]. In this study, in 140 (89.17%) specimens, same result was confirmed on retesting by Xpert assay. These patients were started on treatment for drug resistant TB and they responded well. Hence retesting of these 140 specimens did not either provide any additional information or help the clinicians to start the appropriate treatment. Instead, it delayed the report by few hours or a day and resulted in wastage of cartridges.

Limitation(s)

Phenotypic DST was not performed for confirmation of rifampicin resistance could be a limitation of the present study. Long turnaround time of phenotypic test limit its use for starting the early treatment to the patient.

CONCLUSION(S)

Based on the results of the present study, it can be suggested that retesting by Xpert assay is recommended in situations (a) when the bacterial load identified by Xpert assay is very low and (b) when there is discordance between Xpert results of rifampicin resistance and the reflex LPA testing, a repeat Xpert assay with backup liquid culture DST should be considered.

Acknowledgement

Mumbai District Tuberculosis Control Society provided the consumables required for Xpert MTB/RIF assay under National Tuberculosis Elimination Programme.

REFERENCES

[1] WHO. Global Tuberculosis Report. Geneva: World Health Organization. 2018. [Accessed on: 19.8.2021].
[2] Lawn SD, Nicol MP. XpertMTB/RIF assay: Development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011; 6:1067-82.

[3] Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48:229-37.
[4] Musser JM. Antimicrobial agent resistance in mycobacteria: Molecular genetic insights. Clin Microbiol Rev. 1995;8:496-14.
[5] Sreevatsan S, Pan X, Stockbauer KE, Connell ND, Kreiswirth BN, Whittam TS, et al. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. Proc Natl Acad Sci USA. 1997;94:9869-74.
[6] Zaw MT, Emran NA, Lin Z. Mutations inside rifampicin-resistance determining region of *rpoB* gene associated with rifampicin-resistance in *Mycobacterium tuberculosis*. J Infect Public Health. 2018;11(5):605-610. Doi: 10.1016/j.jiph.2018.04.005. Epub 2018 Apr 26. PMID: 29706316.
[7] Sanker P, Kottuthodi RP, Ambika AP, Santhosh VT, Balakrishnan R, Mrithunjayan SK, et al. Predictable repeatability issues with GeneXpert-Xpert MTB/RIF (version 4) derived rifampicin resistant tuberculosis results from South India: Appreciating the limits of a technological marvel!. Biomed Biotechnol Res J. 2017;1:76-80.
[8] Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber Lung Dis. 1998;79:3-29.
[9] Guidelines for programmatic management of drug resistant tuberculosis. March 2020. National TB Elimination programme. Central TB division. Ministry of health and family welfare. Government of India. [Accessed on: 12.8.2021].
[10] Guidelines for programmatic management of drug resistant tuberculosis. June 2018. National TB Elimination programme. Central TB division. Ministry of health and family welfare. Government of India. [Accessed on 21.8.2021].
[11] Muniyandi M, Lavanya J, Karikalan N, Saravanan B, Senthil S, Selvarajuet S, et al. Estimating TB diagnostic costs incurred under the National Tuberculosis Elimination Programme: A costing study from Tamil Nadu, South India. International Health. 2021;13(6):536-544. Doi: 10.1093/inthealth/ihaa105.
[12] Treatment of Tuberculosis: Guidelines, 4th edition Geneva: World Health Organization; 2010. [Accessed on 21.10.2021].
[13] Cepheid-Xpert® MTB/RIF brochure; 301-1404, Rev. G- July 2020. [Accessed on 12.10.2021].
[14] Global Laboratory Initiative (GLI), Line probe assays for drug resistant tuberculosis detection-Interpretation and reporting guide for laboratory staff and clinicians. [Accessed on 12.10.2021].
[15] Kaur R, Jindal N, Arora S, Kataria 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;8:96-100.
[16] Ullah I, Shah AA, Basit A, Ali M, Khan A, Ullah U, et al. Rifampicin resistance mutations in the81 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.
[17] 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/https://doi.org/10.1155/2017/6738095.
[18] Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert® MTB/RIF assay. Expert Rev Anti-Infect Ther. 2012;10(6):631-35.
[19] Prakash AK, Datta B, Tripathy 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.
[20] Blakemore R, Story E, Helb D, Kop JA, Banada P, Owens MR, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol. 2010;48(7):2495-01.
[21] Ocheretina O, Byrt E, Mabou MM, Mardi GR, Merveille YM, Rouzierb V, et al. False-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):53-55.
[22] Bodmer T, Ströhle A. Diagnosing pulmonary tuberculosis with the Xpert MTB/RIF. J Vis Exp. 2012;(62):e3547. https://doi.org/10.3791/3547.
[23] Namugenyi J, Musaazi J, Katamba A, Kalyango J, Sendaula E, Kambugu A, et al. Baseline Xpert MTB/RIF ct values predict sputum conversion during the intensive phase of anti-TB treatment in HIV infected patients in Kampala, Uganda: A retrospective study. BMC Infect Dis. 2021;21:513. https://doi.org/10.1186/s12879-021-06220-6.
[24] Najjingo I, Muttamba W, Kirenga BJ, Nalunjogi J, Bakesiima R, Olweny F, et al. Comparison of GeneXpert cycle threshold values with smear microscopy and culture as a measure of mycobacterial burden in five regional referral hospitals of Uganda- A cross-sectional study. PLoS ONE. 2019;14(5):e0216901. https://doi.org/10.1371/journal.pone.0216901.
[25] Van Rie A, Mellet K, John MA, Scott L, Page-Shipp L, Dansey H, et al. False-positive rifampicin resistance on Xpert MTB/RIF: Case report and clinical implications. Int J Tuberc Lung Dis. 2012;16(2):206-08.
[26] Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant *Mycobacterium tuberculosis*. J Clin Microbiol. 2014;52(6):1846-52. Doi: 10.1128/JCM.03005-13.
[27] Alonso M, Palacios JJ, Herranz M, Penedo A, Menéndez A, Bouza E, et al. Isolation of *Mycobacterium tuberculosis* strains with a silent mutation in *rpoB* leading to potential misassignment of resistance category. J Clin Microbiol. 2011;49:2688-90.
[28] Moure R, Martín R, Alcaide F. Silent mutation in rpoB detected from clinical samples with rifampin-susceptible *Mycobacterium tuberculosis*. J Clin Microbiol. 2011;49:3722.
[29] Yang B, Koga H, Ohno H, Ogawa K, Fukuda M, Hirakata Y, et al. Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and *rpoB* mutations of *Mycobacterium tuberculosis*. J Antimicrob Chemother. 1998;42:621-28.
[30] Jamieson FB, Guthrie JL, Neemuchwala A, Lastovetska O, Melano RG, Mehaffy C. Profiling of *rpoB* mutations and MICs for rifampin and rifabutin in *Mycobacterium tuberculosis*. J Clin Microbiol. 2014;52:2157-62.
[31] Taniguchi H, Aramaki H, Nikaido Y, Mizuguchi Y, Nakamura M, Koga T, et al. Rifampicin resistance and mutation of the *rpoB* gene in *Mycobacterium tuberculosis*. FEMS Microbiol Lett. 1996;144:103-08.
[32] Sanker P, Ambika AP, Santhosh VT, Kottuthodi RP, Balakrishnan R, Mrithunjayan SK, et al. Are WHO approved nucleic acid amplification tests causing large-scale "false identification" of rifampicin-resistant tuberculosis? Programmatic experience from South India. Int J Mycobacteriol. 2017;6:21-26.
[33] Chakravorty S, Kothari H, Aladegbami B, Jin Cho E, Lee JS, Roh SS, et al. Rapid, high-throughput detection of rifampicin resistance and heteroresistance in *Mycobacterium tuberculosis* by use of sloppy molecular beacon melting temperature coding. J Clin Microbiol. 2012;50(7):2194-202.
[34] Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, et al. Advances in tuberculosis diagnostics: The Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis. 2013;13:349-61.
[35] Canetti G, Froman S, Grosset J, Hauduroy P, Langerova M, Mahler HT, et al. Mycobacteria: Laboratory methods for testing drug sensitivity and resistance. Bull World Health Organ. 1963;29:565-78.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, Seth G.S. Medical College and KEM Hospital, Parel, Mumbai, Maharashtra, India.
2. Assistant Professor, Department of Microbiology, Seth G.S. Medical College and KEM Hospital, Parel, Mumbai, Maharashtra, India.
3. Professor and Head, Department of Microbiology, Seth G.S. Medical College and KEM Hospital, Parel, Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Gita Nataraj,
Professor and Head, Department of Microbiology, 7th Floor, MSB, Seth G.S. Medical College and KEM Hospital, Parel, Mumbai-400012, Maharashtra, India.
E-mail: gitanataraj@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 05, 2022
- Manual Googling: Apr 09, 2022
- iThenticate Software: Apr 16, 2022 (18%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 29, 2022**
Date of Peer Review: **Feb 18, 2022**
Date of Acceptance: **Apr 18, 2022**
Date of Publishing: **May 01, 2022**