

ANALYSIS OF RIFAMPICIN RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS DETECTED BY XPERT MTB/RIF ASSAY IN A TERTIARY HEALTH CARE HOSPITAL

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Abstract

Background: Tuberculosis is a potentially infectious disease that has been a major cause of death worldwide since its genesis thousands of years ago. The incidence rate of tuberculosis increased by 3.6% in 2021 relative to 2020, suggesting a reversal from the trend of nearly 2% decrease per year during the past two decades. The burden of drug-resistant tuberculosis also increased by 3% between 2020 and 2021, with 4,50,000 incident cases of Rifampicin resistant (RR) tuberculosis reported in 2021. This study was undertaken to determine the frequency of mutations in rpoB gene in Rifampicin resistant cases among total samples accepted for Xpert/MTB Rif Assay to help in determining the transmission of resistant cases across borders. **Materials and Methods:** This Study was done using GeneXpert MTB/RIF reports of suspected Tuberculosis cases during the years 2020, 2021 and 2022. The assay detects presence of Mycobacterium tuberculosis in the sample along with mutations in the 81 base pair region of rpoB gene with the help of five molecular probes A, B, C, D and E. **Result:** The results were analysed from January 2020 to December 2022. Out of 13,560 cases 326 were RR. Out of 326 total RR cases, 236 were pulmonary and 90 were extra pulmonary samples. Overall, Probe E showed maximum mutations i.e. 83.3% which was followed by Probe D, Probe A, Probe B and Probe C. In some samples delayed amplification was seen, and in some combination of two mutations was seen. **Conclusion:** The results of the study can become a useful epidemiological tool to identify dynamics of TB transmission.

INTRODUCTION

Tuberculosis is a potentially infectious disease that has been a major cause of death worldwide since its genesis thousands of years ago. The predominant organ affected is the lungs; however, the disease can affect any organ in the body. The symptoms of the primary organ are often accompanied by an evening rise of fever and significant weight loss along with the prominent chronicity of symptoms.^[1] The burden of the disease despite being preventable and treatable is due in part to the lack of diagnostic availability in remote areas and in part due to the chronic nature of treatment which leads to non-compliance. The

disease is also noted to activate from previously latent infectious states. The result of such disparity has been the development of resistance in the organism to previously effective treatment regimens.^[1]

An estimated 10.6 million people became ill with tuberculosis in 2021, compared with 10.1 million in 2020, and 1.6 million people died from tuberculosis in 2021, compared with 1.5 million in 2020. In addition, the incidence rate of tuberculosis increased by 3.6% in 2021 relative to 2020, suggesting a reversal from the trend of nearly 2% decrease per year during the past two decades. The burden of drug-resistant tuberculosis also increased by 3% between 2020 and 2021, with 4,50,000 incident cases of

rifampicin resistant tuberculosis reported in 2021.^[2] 21.4 Lakh TB cases notified in India in 2021, 18% higher than 2020.^[3]

The recent push in the diagnosis of the infection along with presence of resistance has been a step in the direction of detection of resistance further while simultaneously attempting to eliminate tuberculosis altogether. The development of resistance to the 1st line agent Rifampicin has always been the priority of various diagnostic tests in determining the initial course of treatment for the patient.^[4] The GeneXpert MTB/RIF assay system is one of the major modalities of detection of such resistance. The assay detects the absence or delay in binding of specific probes designed to cover the 81 base pairs long Rifampicin resistance determining region in the bacilli.^[4]

Data regarding the molecular epidemiology and mutational frequencies of Rifampicin resistance in rpoB gene using GeneXpert/MTB Rif Assay is sparse and may help in determining the utility of the probes. The objectives of present study were to detect percentage positivity in total samples accepted for Xpert/MTB Rif Assay and to determine prevalence of Rifampicin resistance causing mutation detected in rpoB gene using Xpert/MTB Rif Assay. Present study also aims to determine prevalence of Rifampicin resistance in different age groups, gender and different samples.

MATERIALS AND METHODS

Study Design: This Study will be done using reports of GeneXpert MTB/RIF that detects mutations in the 81 base pair region of rpoB gene with the help of five molecular probes A, B, C, D and E. The study is a Retrospective descriptive cross-sectional study, using data from the period of January 2020 to December 2022 for all samples received for Xpert MTB/RIF in the Department of Microbiology of TNMC & B.Y.L. Nair hospital in Mumbai.

Inclusion and exclusion criteria: All suspected pulmonary and extra-pulmonary Tuberculosis samples received for Xpert MTB/RIF at T.N.M.C and B.Y.L Nair Ch. Hospital, Mumbai, were included in the study. Exclusion criteria involve the blood, urine and stool samples for Xpert MTB/RIF testing. **Statistical analysis plan:** All the data was entered in Excel Sheet and graphs/ tables were made accordingly. The data was analysed and expressed in percentages.

RESULTS

In the current study samples were received from T.N.M.C & BYL Nair Ch. hospital and D ward and were tested by GeneXpert MTB/RIF assay. During the three-year period (2020, 2021 and 2021), total 14,325 samples were received out of which 765 samples were not included due to various reasons (Duplicates-172, Error- 450, Invalid-130, MTB detected, RIF indeterminate – 58) and 13,560

samples were analysed. Out of the 13,560 samples, 11,542 were MTB not detected and 1969 were positive for Mycobacterium tuberculosis. Among the positive samples, 1643 were found to be Rifampicin sensitive and 326 were Rifampicin resistant.

In [Table 1], Probe E showed maximum mutations across all 3 years, i.e. 82.17%, 86.89% and 79.59% in 2020, 2021 and 2022 respectively which was followed by Probe D, Probe A, Probe B and Probe C. In a few samples delayed amplification and combination of two mutations were seen.

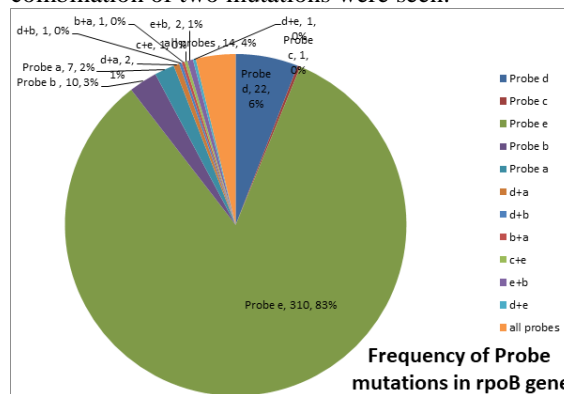


Figure 1 Frequency of Probe mutations in rpoB gene.

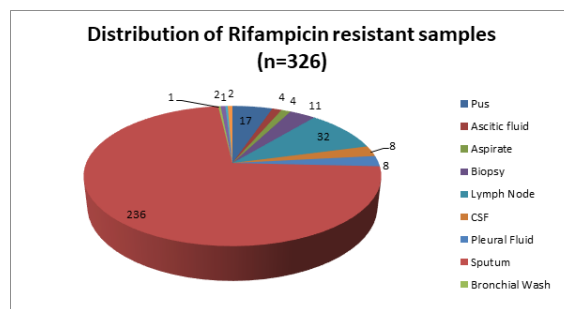


Figure 2: Distribution of Rifampicin resistant samples.

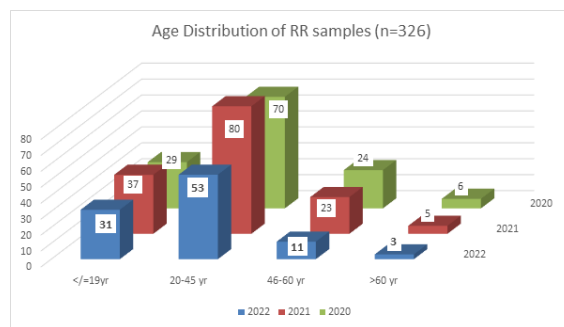


Figure 3: Age Distribution of Rifampicin Resistant samples.

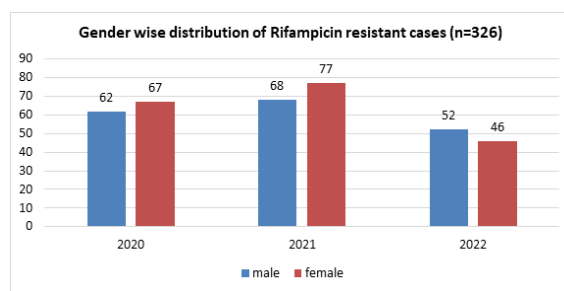


Figure 4: Gender wise distribution.

Probe E (83.3%) genes were found to be the most frequent site of mutation followed by Probe D, Probe B and Probe A [Figure 1]. In a few samples delayed amplification and combination of two mutations were also seen.

Among the total positive samples, 326 were Rifampicin resistant, out of which 236 (72.39%) were pulmonary and 90 (27.60%) were extra pulmonary samples. Maximum resistance was seen in lymph node samples (n=32) followed by pus, biopsy, CSF and pleural fluid, ascetic fluid, aspirates, tracheal

aspirates, other fluids, bronchial wash and gastric lavage [Figure 2].

Among Rifampicin resistant cases, maximum number of patients belonged to the age group 20-45 years (n=203), followed by less than or equal to 19 years (n=97), 46-60 years and more than 60 years [Figure 3].

Male preponderance was seen in 2022 in contrast to 2021 and 2020, where female predominance is noted [Figure 4].

Table 1: Distribution of Probe mutations.

	Probe D (%)	Probe C (%)	Probe E (%)	Probe B (%)	Probe A (%)	D + A (%)	D + B (%)	B + A (%)	C + E (%)	E + B (%)	D + E (%)	All Probes present (%)
2020	7.75	0.77	82.17	0.77	3.1	0.77	0.77	0	0	0	0	3.87
2021	4.82	0	86.89	2.75	0	0	0	0.68	0.68	0.68	0	3.44
2022	5.1	0	79.59	5.1	3.06	1.02	0	0	0	1.02	1.02	4.08

Table 2: Prevalence of probe mutations in various studies

	Probe A (%)	Probe B (%)	Probe C (%)	Probe D (%)	Probe E (%)	Mutation combination (%)	All probes present (%)
Kaur et al, 2016 (Punjab), ^[6]	7.7	20.7	0.8	13.8	56.1	0.8	0
Reddy et al, 2017 (Andhra Pradesh), ^[7]	8.2	15.2	0.6	18.1	55	2.9	0
Kanade et al, 2018 (Mumbai), ^[8]	2.9	3.8	0.3	3.9	83.8	2	3.2
Sharma et al, 2020 (Madhya Pradesh), ^[9]	0	71.4	14.3	0	14.3	0	0
Kumar et al, 2020 (Himachal Pradesh), ^[10]	3.2	6.6	3.2	3.2	77.4	6.6	0
Sailo et al, 2022 (Mizoram), ^[11]	25.9	2.6	0.2	9.8	23.5	34.3	3.7
Jayalaxmi et al, 2022 (Navi Mumbai), ^[12]	4.24	6.02	0.22	3.79	67.63	3.57	14.95
Present study, 2023 (Mumbai)	1.8	2.7	0.3	5.9	83.3	2.2	3.8

DISCUSSION

The Gene Xpert MTB/RIF Assay is an automated in vitro diagnostic test using nested real-time PCR for the qualitative detection of MTB-complex and RIF resistance. The primers in this test amplify a portion of the rpoB gene containing the 81 base pair core region. The probes are designed to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance. The Gene Xpert MTB/RIF Assay simultaneously detects MTB-complex and RIF resistance by amplifying a MTB-complex specific sequence of the rpoB gene, which is probed with five molecular beacons (Probes A – E) for mutations within the Rifampicin resistance determining region (RRDR). Each molecular beacon is labelled with a different fluorophore. The valid maximum cycle threshold (Ct) of 39.0 for Probes A, B and C and 36.0 for Probes D and E are set for MTB/RIF data analysis.^[5]

MTB DETECTED is reported when at least two probes result in Ct values within the valid range and a delta Ct min (the smallest Ct difference between

any pair of probes) of less than 2.0. Rif Resistance DETECTED is reported if the delta Ct max is >4.0.^[5] The overall prevalence of Tuberculosis detected by Gene Xpert MTB/RIF assay as per the current study for 2020, 2021 and 2022 was found to be around 14.5% (n=1969). This average reflects the positivity in suspected tuberculosis patients brought to the centre. Mumbai is a hotspot for Mycobacterium tuberculosis due to the congested and crowded aspect of the city, which increases the likelihood of presumptive Tuberculosis cases.

The increasing problem of drug resistant tuberculosis further complicates matters in areas of high prevalence and it becomes increasingly important to trace the contacts of these patients since existing treatment modalities are more likely to fail in these cases. The usual methods for contact tracing can be supplemented by measures like checking for the type of mutation that caused the resistance and tracing their origin to a particular location.

Among the studies conducted [Table 2], it was noted that the prevalence of Probe E mutations was found to be highest in Punjab,^[6] Andhra Pradesh,^[7] Mumbai,^[8] Himachal Pradesh and Navi Mumbai regions.^[9-12] Probe B mutations were found to be

dominant in the Madhya Pradesh region,^[9] while in Mizoram there was a predominance of mixed mutations rather than individual ones.^[11]

In present study, Probe E showed maximum mutations i.e. 82.17%, 86.89% and 79.59% in 2020, 2021 and 2022 respectively which was followed by Probe D, Probe A, Probe B and Probe C. In a few samples delayed amplification and combination of two mutations were seen [Table 1]. Overall, Probe E (83.3%) genes were found to be the most frequent site of mutation followed by Probe D, Probe B and Probe A [Figure 1]. This is concordant with the study conducted by Kanade et al in 2018,^[8] in the Mumbai region and Jayalaxmi et al in Navi Mumbai in 2022 which also showed Probe E mutations to be the most frequent.^[12]

This kind of distribution of mutations can help with retrospective analysis for tracing the origin of a particular strain of drug resistant Tuberculosis. It can be used to approximately trace the origins of drug resistant tuberculosis across borders, while more definitive methods can be employed by doing whole genome sequencing.

These types of studies on prevalence of mutations can also be a useful tool for policy makers to direct preventive actions in areas of high prevalence more efficiently by adjudging the cases to the right locations. Due to the looming threat of increasing resistance, the push towards ending Tuberculosis will need more support from such epidemiological tools and contact tracing measures for a definitive blow to this deadly challenge.

Distribution of samples was analysed [Figure 2] and out of the 326 total RR cases, 236 (72.39%) were pulmonary, while 90 (27.60%) were found to be extra pulmonary. Maximum resistance was seen in lymph node samples (n=32) followed by pus, biopsy, CSF and pleural fluid. Similarly, Lymph nodes were found to be the samples with maximum resistance in other studies by Jayalakshmi et al, Nishal et al and Alemu et al as well.^[12-15]

Among Rifampicin resistant cases, maximum number of patients belonged to the age group 20-45 years (n=203), followed by less than or equal to 19 years (n=97), 46-60 years and more than 60 years [Figure 3]. Seifert M et al,^[13] Kumar et al,^[10] Sailo et al,^[11] and Sharma et al,^[9] came up with comparable findings in their studies as well.

It was found that in 2020 and 2021 female preponderance was seen. In 2020, out of 129 RR samples, 67 were females and 62 were males while in 2021, Out of 145 RR samples, 77 were females and 68 were males. In 2022, out of 98 RR samples, 52 were males and 46 were females. Male preponderance was seen in 2022 in contrast to 2021 and 2020 [Figure 4]. Seifert M et al,^[15] and, Ullah et al,^[16] conducted a studies where female predominance was observed over males,^[13] which was concordant with our study, but studies conducted by Reddy et al,^[7] Kumar et al,^[10] Sailo et al,^[11] and Alemu et al,^[15] showed male preponderance.

CONCLUSION

The Gene Xpert MTB/RIF assay provides the added advantage of detecting Rifampicin resistance apart from detecting MTB by using various probes that target a portion of the *rpoB* gene. This feature allows us to find the specific mutation which confers resistance to a particular strain of MTB. This knowledge can be used to type the strain. The current study showed high prevalence of Probe E mutations which was similar to studies in the same region while studies in other regions showed different findings. The results of the study can become a useful epidemiological tool to identify dynamics of TB transmission.

REFERENCES

1. Natarajan A, Beena PM, Devnikar AV, Mali S. A systemic review on tuberculosis. *Indian J Tuberc.* 2020; 67(3):295–311. Available from: <http://dx.doi.org/10.1016/j.ijtb.2020.02.005>
2. Ministry of Health, Family Welfare-Government of India. India TB Report 2022. Gov.in. [cited 2023 Jun 13]. Available from: <https://tbcindia.gov.in/index1.php?lang=1&level=1&sublinkid=5613&lid=3658>
3. TheLancet.com. [cited 2023 Jun 13]. Available from: [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(19\)30308-3/fulltext#seccesstitle130](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(19)30308-3/fulltext#seccesstitle130)
4. Nguyen TNA, Anton-Le Berre V, Bañals A-L, Nguyen TVA. Molecular diagnosis of drug-resistant tuberculosis; A literature review. *Front Microbiol* [Internet]. 2019;10:794. Available from: <http://dx.doi.org/10.3389/fmicb.2019.00794>
5. Xpert® MTB/RIF. Cepheid.com. [cited 2023 Jun 20]. Available from: <https://www.cephheid.com/en-US/tests/tb-emerging-infectious-diseases/xpert-mtb-rif.html>
6. 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 Phys* 2016;8(2):96–100.
7. Reddy R, Alvarez-Uria G. Molecular epidemiology of rifampicin resistance in mycobacterium tuberculosis using the GeneXpert MTB/RIF assay from a rural setting in India. *J Pathog* 2017;2017:6738095.
8. Kanade S, Nataraj G, Mehta P, Shah D. Pattern of missing probes in rifampicin resistant TB by Xpert MTB/RIF assay at a tertiary care centre in Mumbai. *Indian J Tuberc* 2019;66(1):139–43.
9. Sharma P, Singh R. GeneXpert MTB/RIF based detection of rifampicin resistance and common mutations in *rpoB* Gene of mycobacterium tuberculosis in tribal population of district Anuppur, Madhya Pradesh, India. *J Clin Diagnostic Res.* 2020 Sep 1;14:LM01-3.
10. Kumar V, Bala M, Kanga A, Gautam N. Identification of Missing Probes in *rpoB* gene in Rifampicin Resistant PTB and EPTB Cases Using Xpert MTB/RIF Assay in Sirmour, Himachal Pradesh. *The Journal of the Association of Physicians of India.* 2020 Nov 1;68(11):33-6.
11. Sailo CV, Lalremruata R, Sanga Z, Fela V, Kharkongor F, Chhakchhuak Z, Chhakchhuak L, Nemi L, Zothanzama J, Kumar NS. Distribution and frequency of common mutations in *rpoB* gene of Mycobacterium tuberculosis detected by Xpert MTB/RIF and identification of residential areas of rifampicin resistant-TB cases: A first retrospective study from Mizoram, Northeast India. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases.* 2022 Dec 1;29:100342
12. Jayalakshmi S, Kar H, Hodiwala AV, Wagh S, Patil MS, Wagh Sr SS. Evaluation of *RpoB* Gene and Its Various Mutants in Multidrug-Resistant Tuberculosis Cases by GeneXpert Method. *Cureus.* 2022 Nov 8;14(11).

13. Nishal N, Arjun P, Arjun R, Ameer KA, Nair S, Mohan A. Diagnostic yield of CBNAAT in the diagnosis of extrapulmonary tuberculosis: A prospective observational study. *Lung India* [Internet]. 2022;39(5):443–8. Available from: http://dx.doi.org/10.4103/lungindia.lungindia_165_22
14. Alemu A, Tadesse M, Seid G, Mollalign H, Eshetu K, Sinshaw W, et al. Does Xpert® MTB/RIF assay give rifampicin resistance results without identified mutation? Review of cases from Addis Ababa, Ethiopia. *BMC Infect Dis* [Internet]. 2020;20(1):87. Available from: <http://dx.doi.org/10.1186/s12879-020-4817-2>
15. Seifert M, Aung HT, Besler N, Harris V, Mar TT, Colman RE, Rodwell TC, Aung ST. Age and sex distribution of *Mycobacterium tuberculosis* infection and rifampicin resistance in Myanmar as detected by Xpert MTB/RIF. *BMC infectious diseases*. 2021 Dec;21:1-8.
16. 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 Infectious Diseases*. 2016;16(1). doi:10.1186/s12879-016-1745-2.