

## DETECTION OF RIFAMPICIN RESISTANCE MUTATIONS IN THE 81 BP RRDR OF RPOB GENE IN MYCOBACTERIUM TUBERCULOSIS USING GENE EXPERT MTB/RIF ASSAY: A STUDY FROM RURAL NORTH INDIA

Prasant Singh<sup>1</sup>, Dimpi Bhankhur<sup>2</sup>, Pratibha Mane<sup>3</sup>, Jyoti Sangwan Lathwal<sup>4</sup>

Received : 02/10/2025  
Received in revised form : 21/11/2025  
Accepted : 06/12/2025

### Keywords:

Gene Xpert MTB/RIF assay, *Mycobacterium tuberculosis*, Rapid Assay, Rifampicin Resistance, Rural Settings.

Corresponding Author:

**Dr. Dimpi Bhankhur,**

Email: [dimpibhankhur@gmail.com](mailto:dimpibhankhur@gmail.com)

DOI: 10.47009/jamp.2025.7.6.134

Source of Support: Nil,

Conflict of Interest: None declared

*Int J Acad Med Pharm*  
2025; 7 (6); 727-731



<sup>1</sup>Assistant Professor, Department of Microbiology, Venkateshwara Institute of Medical Sciences, Gajraula, Amroha. Uttar Pradesh, India.

<sup>2</sup>Assistant Professor, Department of Microbiology, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Nuh, Haryana, India.

<sup>3</sup>Professor & Head, Department of Microbiology, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Nuh, Haryana, India.

<sup>4</sup>Professor, Department of Microbiology, Shaheed Hasan Khan Mewati Government Medical College, Nalhar, Nuh, Haryana, India.

### ABSTRACT

**Background:** Tuberculosis (TB) is a major cause of morbidity and mortality all over the world. Diagnosis, treatment, and prevention of TB have become more complex because of resistance to commonly used anti-tubercular drugs. To address this issue, the WHO endorsed Xpert MTB/RIF assay, which is a point-of-care molecular assay to detect *Mycobacterium tuberculosis* and Rifampicin resistance simultaneously in approximately two hours. The assay detects mutations in *rpoB* gene (RR) using five overlapping probes (A, B, C, D, and E). **Aim & Objectives:** The present study was conducted to determine the frequency of Rifampicin resistance and the pattern of *rpoB* gene mutations among suspected tuberculosis cases in a rural tertiary center using Gene Xpert MTB/RIF assay. **Material and Methods:** A total of 4360 sputum samples from suspected TB and MDR TB were processed using Gene Xpert MTB/RIF assay in the Microbiology department from July 2019 to September 2020. **Result:** Out of 4049 valid results, *Mycobacterium tuberculosis* was detected in 1232 (30.4%) samples. Among these, Rifampicin resistance was detected in 154 (12.5 %) samples. The most common *rpoB* mutants were located in the region of Probe E in 107 cases (69.5%) followed by Probe B and D in 12 cases each (7.8%). **Conclusion:** Gene Xpert MTB/RIF assay is a useful test providing valid results and prompt diagnosis of Rifampicin Resistant Tuberculosis (RRTB) in settings with limited laboratory infrastructure.

## INTRODUCTION

Tuberculosis is one of the major causes of death worldwide. According to WHO estimates, in 2017 alone, close to ten million people developed TB worldwide. The emergence of first-line drug-resistant tuberculosis (TB), especially multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, poses formidable challenges to controlling TB in high-burden countries.<sup>[1]</sup> Rifampicin is one of the key anti-tuberculosis drugs that binds to the  $\beta$ -subunit of the bacterial DNA-dependent RNA polymerase enzyme (*rpoB*) and inhibits DNA-directed ribonucleic acid synthesis of *Mycobacterium tuberculosis* (MTB) proteins. Rifampicin resistance is a surrogate marker of multidrug-resistant tuberculosis, as it indicates isoniazid resistance of

greater than 90%.<sup>[2]</sup> Worldwide, *rpoB* mutations are reported in 95-97% of RIF-resistant MTB. Generally, these mutations are located in a region at the 507-533 amino acid residues (81bp) in the *rpoB* gene, which is usually known as Rifampicin Resistant Determinant Region (RRDR).<sup>[3,4]</sup>

As far as the species-specific diagnosis of TB is concerned, the greatest challenge is early detection of its drug resistance pattern and availability of short-course, highly active drug treatment, lasting a few weeks. For prevention, the most desirable approach is a potent preventive vaccine. Unfortunately, both appear far as of now. Globally, eight countries accounted for two-thirds of the total TB burden, with India leading the count (26%), Indonesia (8.5%), China (8.4%), Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%), and South Africa

(3.6%). In the WHO list of 30 high-burden countries, above mentioned eight countries accounted for 79% and 22 other countries accounted for 21% of the total global burden. Drug-resistant TB continues to be a disease of public health importance. As per statistics in 2019, close to half a million people suffered from Rifampicin-resistant TB (RR-TB). Out of which 78% had developed multidrug-resistant TB (MDR-TB). The largest global burden was reported from India (27%), China (14%), and the Russian Federation (8%). In 2019, 3.3% of new TB cases and 17.7% of previously treated cases developed MDR/RR-TB globally. The highest proportions, which are >50% in previously treated cases, were reported in countries of the former Soviet Union.<sup>[5,6]</sup>

The Gene Xpert MTB/RIF assay (Cepheid, USA) is a real-time automated nucleic acid amplification system that simultaneously detects *Mycobacterium tuberculosis* and resistance to Rifampicin in less than two hours. MTB Rif resistance is detected by the presence of *rpoB* gene mutation.<sup>[2]</sup> The test procedure involves PCR amplification and detection, which happens in a closed cartridge unit. The assay utilizes 5 overlapping probes for the *rpoB* gene, which is crucial for the detection of mutations within the rifampicin resistance-determining region (RRDR). Each probe is labeled with a distinct fluorophore, which corresponds to different codons. The hybridization and emission of a positive signal by no less than two out of five probes detects the presence of MTB complex. A mutation in any codon of the 81-base-pair core region prevents the hybridization of one or more probes and is indicated by “missing probe. It is reported as rifampicin resistance.<sup>[2]</sup> Mewat region is one of the most remote and backward district of India due to poor health and education infrastructure.<sup>[7]</sup> The majority of the population resides in villages. No similar study from this part has been carried out. The present study was conducted with the aim of knowing the frequency of Rif resistance and pattern of *rpoB* gene mutations among suspected tuberculosis cases by using Gene Xpert MTB/RIF assay.

## MATERIALS AND METHODS

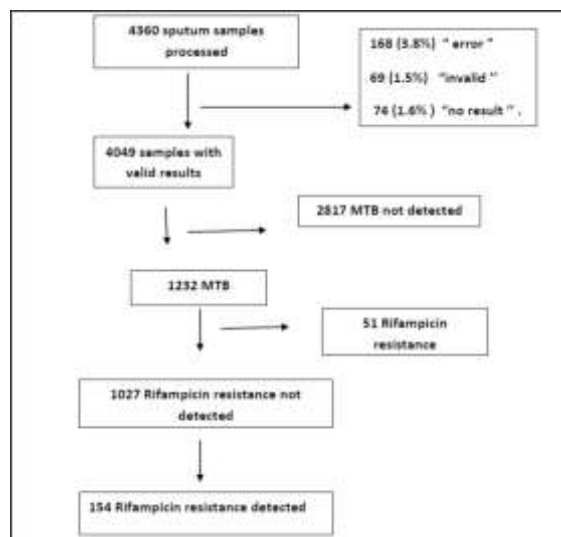
This observational cross-sectional study was carried out from July 2019 to September 2020 in the Department of Microbiology of SHKM GMC after obtaining approval from the Institutional Ethics Committee. All sputum samples received during the study period with suspected TB and MDR TB were included. A total of 4360 sputum samples were received at the CBNAAT site of the Microbiology department under RNTCP for Gene Xpert MTB/RIF assay (Cepheid, USA). Gross examination and Gram staining of the samples were done to rule out the saliva sample. Samples with a thick expectorated sputum were considered acceptable.

The analysis of sputum samples was done as per the manufacturer’s instructions. In brief, the sputum was

diluted with lysis buffer in a ratio of 1:2 (v/v) in the sputum collection container and mixed thoroughly 10-20 times. The mixture was incubated at room temperature for 10 minutes, then the specimen was manually shaken for 10-20 times vigorously. The sample was again incubated for an additional 5 minutes. The 2 mL of this inactivated sample was transferred to the Gene Xpert test cartridge and run on the test platform. The machine was run as per the provided protocol, and the electronic results so obtained were analyzed. The results were analyzed with the sample Processing Control (SPC) and a Probe Check Control (PCC). The SPC was used to check the adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verified reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

## RESULTS

During the study period, 4360 sputum samples were processed. The assay showed valid results in 4049 (92.9%) cases. The unsuccessful results occurred in 311 (7.1%) cases. Among unsuccessful results, 168 (3.8%) samples were classified as “error”, 69 (1.5%) as “invalid”, and 74 (1.6%) as “no result”. Out of 4049 valid results, *Mycobacterium tuberculosis* was detected in 1232 (30.4%) samples. Among *Mycobacterium tuberculosis* positive samples, Rifampicin resistance was positive in 154 (12.5%) samples, negative in 1027 (83.4%) samples, and indeterminate in 51 (4.1%) as illustrated in Figure 1.



**Figure 1: Flow chart of sample results using Gene Xpert MTB/RIF assay**

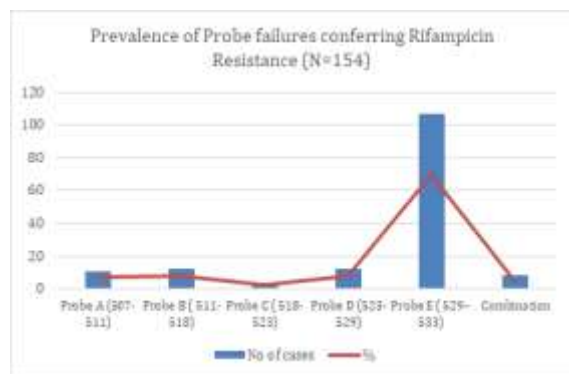
Out of 154 patients diagnosed as rifampicin resistant 54 (35 %) were females and 100 (65 %) were males. Table 1 depicts the age distribution of positive cases. Majority of cases were seen in age group of 21-40 years. The most common *rpoB* gene mutants depicted as probe failures were located in the region of Probe E (gene region 529-533) in 107 cases (69.5%)

followed by Probe B (gene region 511-518) and D (gene region 523-529) in 12 cases each (7.8%), Probe A (gene region 507-511) in 11 cases (7.1%) and Probe C (gene region 518-523) in 4 cases (2.6%).

Combination of two probes were seen in 8 cases (5.1%): A&D in four cases, A&B in one case, A&E in one case, and D&E in one case, and B&E in one case as seen in Figure 2.

**Table 1: Age distribution of Rifampicin resistant TB patients**

S.No	Age group in years	No of Rif Resistant TB cases	Percentage
1	>20	34	22%
2	21-40	77	50%
3	41-60	30	19.5%
4	>60	13	8.5%



**Figure 2: Comparison of prevalence of probe mutation (failures) conferring Rifampicin resistance by Gene Xpert MTB/RIF assay**

## DISCUSSION

India has the largest number of multidrug-resistant tuberculosis cases, accounting for 27 percent of the global total. The estimated number of MDR TB cases in India is 1,30,000, which represents 27% of the world's cases. However, the biggest challenge remains early diagnosis and the availability of effective short-course treatment. Two-thirds of the Indian population lives in rural areas. In rural settings, due to a lack of infrastructure and skilled manpower, diagnosing Rif-resistant TB is difficult. Xpert MTB Rif assay can be used to diagnose RRTB in rural areas with limited laboratory infrastructure.<sup>[8]</sup> The introduction of the Xpert® MTB/RIF assay (Cepheid, USA) has transformed tuberculosis (TB) diagnosis by simultaneously detecting the bacteria and resistance to rifampicin. Rifampicin is a key first-line anti-tuberculosis drug that inhibits DNA-dependent RNA synthesis in Mycobacterium tuberculosis (MTB) by binding to the  $\beta$ -subunit of the bacterial DNA-dependent RNA polymerase (rpoB) enzyme. Generally, mutations in rpoB are found in 96.1% of rifampicin-resistant MTB strains worldwide. Rifampicin resistance serves as an important surrogate marker for multidrug-resistant (MDR) TB.

In the present study, RIF resistance was observed in 12.5% of suspected cases of MDR-TB. These findings are similar to those reported from Punjab and Rajasthan, which were 9.9% and 6.9%, respectively.<sup>[2,9]</sup> This higher figure compared to the

other studies mentioned above could be due to a lack of health facilities and poverty, which hinder timely diagnosis and treatment of tuberculosis in this region. However, a higher prevalence of MDR-TB has been reported in other Indian studies from Lucknow (27.8%), New Delhi (17.9%), and Central India (17%), likely because of better detection facilities available in these major cities.<sup>[10,11,12]</sup>

In the present study, the maximum number of cases was in the age group of 21–40 years. Other Indian studies have also reported the predominance of the younger age group with MDR-TB.<sup>[11]</sup> In the present study male preponderance was seen. This is similar to the findings of Gupta et al.<sup>[13,14]</sup> In India, young adult males are an economically productive segment, and high MDR-TB in this group can have grave socioeconomic implications.

In the present study Xpert MTB/RIF assay showed an error 3.8% while detecting TB/MDR cases. KAUR et al reported 2.1% error, Rufai et al reported 1.8% errors, whereas Mboowa et al. reported 12% errors.<sup>[2,3,15]</sup> The “Indeterminate” results of RIF resistance were detected in 51 (4.1 %) in the present study. This could be due to high Ct values corresponding to low bacterial burden.

The present study results and review of five studies performed in other developing countries from Asia and Africa indicate that the most common mutations of RR are located in the region of probe E, followed by probe B and Probe D while mutations in Probe A and Probe C were less common.[Table 2]

It was observed that the pattern of mutations in the 81 bp RRDR of MTB isolates of the present study was almost similar to that reported from other parts of India. Kaur R et al reported the most common RRDR rpoB gene mutations were in the gene region 529–533 (56%) in all the six districts of Malwa region and were recognized by probe E,<sup>[3]</sup> Mboowa et al too used Xpert MTB/RIF assay and the most common gene mutations observed were in codon 531 (58%) followed by 513 (25%), 526 (8%), and 511 (8%) designed by probes E, B, D, and A respectively. Singhal et al also reported 531(probe E) as the most commonly mutated codon in 59.0%.<sup>[3,11]</sup> Similarly, Mani et al. reported that the codons most commonly involved in these mutations were 531(probe E) (53%) and 526(probe D) being (19%).<sup>[16]</sup>

**Table 2: Comparison of prevalence of probe mutation by Gene Xpert MTB/RIF assay obtained in present study with other studies**

Study	Country	Total number	Probe A	Probe B	Probe C	Probe D	Probe E	Combination
Present study	India	154	11 (7.1%)	12 (7.8%)	4 (2.6%)	12 (7.8%)	107 (69.5%)	8(5.1%)
Kaur et al 2016 [1]	India	130	11 (8.4%)	28 (21.5%)	1(0.7%)	18 (13.8%)	73 (56%)	1(0.7%)
Mboowa et al 2014 [2]	Uganda	12	1 (8%)	3 (25%)	0	1(8%)	7 (58%)	0
Ullah et al 2016 [3]	Pakistan	408	5 (1.2%)	44 (10.8%)	6(1.5%)	34 (8.3%)	314 (77%)	5(1.2%)
Durairaj G et al 2020 [5]	India	172	17 (10%)	17 (10%)	2(1%)	36 (21%)	100 (58%)	0
Reddy R et al 2017 [8]	India	171	14 (8.2%)	26 (15.2%)	1 (0.6%)	31 (18.1%)	94 (55%)	5(2.9%)
Ochang et al 2016 [16]	Nigeria	58	2 (3.4%)	8 (13.8%)	0	10 (17.2%)	35 (60.3%)	3(5.1%)
Sharma P et al 2020 [17]	India	7	0	5 (71.42%)	1(14.28%)	0	1 (14.28%)	0
Metcalfe et al 2016 [18]	Zimbabwe	43	0	6 (14%)	5(11.6%)	4 (9.3%)	26 (60.5%)	2(4.6%)
Raheman et al 2016 [19]	Bangladesh	91	2 (2.2%)	14 (15.2%)	3(3.3%)	13 (14.1%)	59 (64.1%)	0%

A combination of mutations was observed in 8 samples in the present study. Whereas Kaur et al reported in one sample, Ullah et al and Reddy et al reported in 5 samples each and Ochang et al reported a combination of mutations in 3 samples.<sup>[3,4,9,17]</sup> Moobwa et al, Durairaj et al, Sharma P et al, and Rahman et al reported no specimen with a mutation combination.<sup>[2,6,18,19,20]</sup> This may be due to adaptation of MTB to drug exposure.

**Limitations of the study:** A Gold standard test, such as mycobacterial culture and drug susceptibility test, was not used for comparison of the Xpert MTB/RIF assay, as the facility for the same was not available because of a lack of equipment and trained staff for the same.

## CONCLUSION

Xpert MTB/RIF assay is a rapid diagnostic modality for tuberculosis diagnosis and resistance to Rifampicin. This study showed that this assay can provide valid results for prompt diagnosis of RRTB in rural settings with limited laboratory infrastructure. Our results and review of literature indicate most common mutations are located in the probe E region which resulting in Rifampicin resistance. This technique provides preliminary information regarding the mutation pattern of RIF resistance, denoting the epidemiology of the disease for the implementation of the TB control program. Despite culture remaining the gold standard for laboratory confirmation of TB, GeneXpert MTB/RIF has become standard practice for patients with suspected TB, and all clinicians and public health programs for TB control should have access to molecular testing for TB to shorten diagnosis time.

**Conflict of interest:** Nil

**Acknowledgement:** None

**Source of Support:** None

## REFERENCES

1. Feliciano CS, Namburete EI, Plaça JR, Peronni K, Dippenaar A, Warren RM, et al. Accuracy of whole genome sequencing versus phenotypic (MGIT) and commercial molecular tests for detection of drug-resistant Mycobacterium tuberculosis isolated from patients in Brazil and Mozambique. *Tuberculosis*. 2018;110:59-67.
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 infectious diseases*. 2014;14(1):1-5.
3. 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. *Journal of Laboratory Physicians*. 2016;8(02):096-100.
4. Ullah I, Shah AA, Basit A, Ali M, Ullah U, Ihtesham M, Mehreen S, Mughal A, Javaid A. 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):1-6.
5. World Health Organization. 2020. Global Tuberculosis Report. [online] Available at: <https://www.who.int/publications/i/item/9789240013131> [Accessed 15 June 2021].
6. Durairaj G, Rathinam S, Vishwanathan V, Satagopan K. Detection of Rifampicin Resistance among Patients with Tuberculosis using GeneXpert MTB/RIF Assay: A Retrospective Study. *Journal of Clinical & Diagnostic Research*. 2020;14(9):DC19-22.
7. Kumar A. Nuh at bottom of NITI Aayog's 101 most backward districts. *The Hindu* (news paper on internet)2018 April 2 available from <https://www.thehindu.com/news/cities/Delhi/nuh-at-bottom-of-niti-aayogs-101-most-backward-districts/article23409464.ece>
8. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future microbiology*. 2011;6(9):1067-82.
9. 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.

11. Mathur RB, Shukla US, Bindal HK. Role of cartridge-based nucleic acid amplification test to diagnose tuberculosis at tertiary care teaching hospital in Rajasthan, India. *Int J Res Med Sci.* 2019;7: 4243-8.
12. Jain A, Diwakar P, Singh U. Declining trend of resistance to first-line anti-tubercular drugs in clinical isolates of *Mycobacterium tuberculosis* in a tertiary care north Indian hospital after implementation of Revised National Tuberculosis Control Programme. *Indian J Med Microbiol.* 2014;32:430-3.
13. Singhal R, Myneedu VP, Arora J, Singh N, Bhalla M, Verma A, Sarin R. Early detection of multi-drug resistance and common mutations in *Mycobacterium tuberculosis* isolates from Delhi using GenoType MTBDRplus assay. *Indian journal of medical microbiology.* 2015;33:S46-52.
14. Desikan P, Chauhan DS, Sharma P, Panwalkar N, Yadav P, Ohri BS. Clonal diversity and drug resistance in *Mycobacterium tuberculosis* isolated from extra-pulmonary samples in central India – A pilot study. *Indian J Med Microbiol* 2014;32:434-7.
15. Gupta A, Nagaraja MR, Kumari P, Singh G, Raman R, Singh SK, et al. Association of MDR-TB isolates with clinical characteristics of patients from Northern region of India. *Indian J Med Microbiol.* 2014;32:270-6.
16. Rufai SB, Singh A, Kumar P, Singh J, Singh S. Performance of Xpert MTB/RIF assay in diagnosis of pleural tuberculosis by use of pleural fluid samples. *J Clin Microbiol.* 2015;53:3636-8.
17. Mani C, Selvakumar N, Narayanan S, Narayanan PR. Mutations in the rpoB gene of multidrug-resistant *Mycobacterium tuberculosis* clinical isolates from India. *J Clin Microbiol.* 2001;39:2987-90.
18. Ochang EA, Udoh UA, Emanghe UE, Tiku GO, Ofori JB, Odo M, Nkombe E, Owuna OE, Obeten SM, Meremikwu MM. Evaluation of rifampicin resistance and 81-bp rifampicin resistant determinant region of rpoB gene mutations of *Mycobacterium tuberculosis* detected with XpertMTB/Rif in Cross River State, Nigeria. *International Journal of Mycobacteriology.* 2016;5(5):145.
19. 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. *Journal of Clinical & Diagnostic Research.* 2020;14(9):LM01-LM03.
20. Metcalfe JZ, Makumbirofa S, Makamure B, Sandy C, Bara W, Mason P, Hopewell PC. Xpert® MTB/RIF detection of rifampin resistance and time to treatment initiation in Harare, Zimbabwe. *The International Journal of Tuberculosis and Lung Disease.* 2016;20(7):882-9.
21. Rahman A, Sahrin M, Afrin S, Earley K, Ahmed S, Rahman SM, Banu S. Comparison of Xpert MTB/RIF assay and GenoType MTBDR plus DNA probes for detection of mutations associated with rifampicin resistance in *Mycobacterium tuberculosis*. *PLoS One.* 2016;11(4):e0152694.