

# A Three-Year Experience with GeneXpert MTB/RIF Assay in Tuberculosis Control Programme (RNTCP)- A Clinical Study

Santosh Kumar Nemagouda<sup>1</sup>

<sup>1</sup>Associate Professor, Department of Pulmonology, Sri B.M. Patil Medical College, BLDE University, Vijayapur, Karnataka, India.

## ABSTRACT

### BACKGROUND

MDR-TB caused an estimated 600,000 new TB cases and 240,000 deaths in 2016 and MDR-TB accounts for 4.1% of all new TB cases and 19% of previously treated cases worldwide. Globally, most MDR-TB cases occur in South America, Southern Africa, India, China, and the former Soviet Union. Rapid detection of drug resistance of *M. tuberculosis* and MDR-TB strains ensures effective treatment of TB patients and limits further development of resistance to additional drugs. The GeneXpert test is a molecular test for TB which diagnoses TB by detecting the presence of TB bacteria, as well as testing for resistance to the drug Rifampicin. In India, where this test is starting to be widely used, it is known as the CBNAAT. The full name of the CBNAAT test is the Cartridge Based Nucleic Acid Amplification Test. The objective of the study is to assess the performance of GeneXpert MTB/RIF as an initial diagnostic tool for the diagnosis of *Mycobacterium tuberculosis* and Rifampicin Resistance.

### METHODS

The RNTCP programme is being strictly followed and carried out in Shri B.M. Patil Medical college Hospital, BLDE University Vijayapur Karnataka. Samples of patients with symptoms suggestive of tuberculosis including, suspected new TB cases, old TB cases and on treatment TB cases, were received from all the Primary Healthcare Centers, and all other Healthcare Centres including two Medical College Hospitals of Vijayapur district. Samples collected under this programme were analysed by GeneXpert Test at District TB Hospital CBNAAT center Vijayapur, Karnataka. A retrospective study was conducted from 1 January 2016 to 31 December 2018. The samples were subjected to GeneXpert MTB/ RIF manufactured by Cepheid, France for the detection of *M. tuberculosis* and then rifampicin resistance in them. Data analysis was done using SPSS Version 22.0.0.0.

### RESULTS

In this study a total of 4978 patients' samples was processed for CBNAAT test. Out of these cases, MTB was detected in 1186 (23.82%), Rifampicin Resistance (RR) was detected in 165 (13.91%) cases. Among these 165 MTB-RR cases, the MTB detection level was, 50 (30.30%) High, 65 (39.39%) Medium, 26 (15.79%) Low, 16 (9.69%) Very Low & 8 (4.84%) Intermediate. Out of 4978 cases 427 (8.58%) were HIV positive, among these HIV positive cases MTB was detected in 51 (12%) & RR detected in 13 (25.50%) cases.

### CONCLUSIONS

The implementation of the GeneXpert MTB/RIF assay in programmatic management of TB & MDR-TB in RNTCP has dramatically improved the rapid diagnosis of tuberculosis & drug resistance, especially in People Living with HIV/AIDS (PLWHA).

### KEY WORDS

RNTCP, CBNAAT, GeneXpert Test, Xpert MTB/RIF, HIV/AIDS

Corresponding Author:

Dr. Santosh Kumar Nemagouda,

S/o. R. S. Nemagouda,

Sri Siddeshwar Housing Colony,

#85, North Athani Road, Vijayapur,

Karnataka, India.

E-mail: pulmonologist2007@yahoo.co.in

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## BACKGROUND

Tuberculosis (TB) is an ancient disease of mankind. TB is an infectious and contagious disease. TB is caused by *Mycobacterium tuberculosis* (MT). MT has very ancient origins; it has survived over 70,000 years and it currently infects nearly 2 billion people worldwide,<sup>[1]</sup> with around 10.4 million new cases of TB each year, almost one third of the world's population are carriers of the TB bacillus and are at risk for developing active disease.<sup>[2]</sup> TB has always been associated with a high mortality rate over the centuries, and also nowadays, it is estimated to be responsible for 1.4 million TB deaths, among infectious diseases after human immunodeficiency virus (HIV).<sup>[2]</sup> In India the prevalence of TB is 299 cases per 100 000 population or 3.4 million prevalent cases.<sup>[3]</sup>

In the year 1961 government of India had launched National TB Programme (NTP). The shortcomings of the NTP were highlighted by the Joint Review by the Government of India, the Swedish International Development Agency and WHO in 1992. The Revised National TB Control Programme (RNTCP) was established in 1993 and the programme is based on DOTS (Directly Observed Treatment Short course) – the internationally-recommended strategy to control TB, was launched in 1997.<sup>[4]</sup>

Worldwide, sputum is most common and easily available respiratory specimen for the diagnosis of pulmonary TB and every guideline recommends the use of sputum-based tests. Recently, the World Health Organization (WHO) endorsed a new, rapid, automated, 2-hour molecular test called Xpert MTB/RIF, based on the GeneXpert platform (Cepheid Inc, USA) which can diagnose TB with great accuracy and can also detect those with drug resistance.<sup>[5]</sup> A recent Cochrane review has shown that the Xpert MTB/RIF test has 88% sensitivity and 98% specificity when compared to culture.<sup>[6]</sup> Xpert MTB/RIF can detect rifampicin resistance with a sensitivity of 94% and specificity of 98%. Data from many countries, including India, clearly show substantially better performance of the Xpert MTB/RIF test over conventional smear microscopy.<sup>[6]</sup>

MDR-TB caused an estimated 600,000 new TB cases and 240,000 deaths in 2016 and MDR-TB accounts for 4.1% of all new TB cases and 19% of previously treated cases worldwide. Globally, most MDR-TB cases occur in South America, Southern Africa, India, China, and the former Soviet Union. Rapid detection of drug resistance of *M. tuberculosis* and MDR-TB strains ensures effective treatment of TB patients and limits further development of resistance to additional drugs. Hence, we studied the performance of CBNAAT in diagnosis TB and early detection of Rifampicin resistance in TB control programme (RNTCP).

## METHODS

The RNTCP programme is being strictly followed and carried out in Shri BM Patil Medical college Hospital, BLDE University Vijayapur Karnataka. The samples of the patients with symptoms suggestive of Tuberculosis including, suspected new TB cases, old TB cases and on treatment TB cases, were received from all the Primary Healthcare Centers, and all

other Healthcare Centres including two Medical College Hospitals of Vijayapur district. The samples were analysed by GeneXpert Test at District TB Hospital CBNAAT center Vijayapur, Karnataka. A retrospective study was conducted from 1 January 2016 to 31 December 2018. The samples were subjected to GeneXpert MTB/ RIF manufactured by Cepheid, France for the detection of *M. tuberculosis* and then rifampicin resistance in them. GeneXpert MTB/RIF is a cartridge-based nucleic acid amplification technique which includes semi-quantitative, nested real-time PCR in vitro diagnostic test for the detection of MTBC DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputum. RIF-resistance associated mutations of the *rpoB* gene in the samples from patients at risk for rifampicin resistance. As per manufacturer's guidelines, 2 ml of sample reagent is added to 1 ml of each fresh sample directly into collection container. The lid was replaced and shaken vigorously 10–20 times. It was incubated at room temperature. After 10 minutes of incubation, the specimen was shaken vigorously 10–20 times. It was incubated for 5 minutes again. The sample was perfectly fluid before being processed with no more clumps of sputum. If still it is viscous, waited for 5–10 minutes further before processing it in the cartridge. At least 2 ml of processed sample was taken with plastic transfer pipette from the collection container to single use, disposable, self-contained GeneXpert cartridge. Then, it was subjected to GeneXpert MTB/RIF to create a test. After scanning the cartridge barcode, loading was done on blinking module. The results were visualised on the attached computer and interpreted by using software. The GeneXpert MTB/RIF was repeated on the second sample if it has shown indeterminate susceptibility to RIF.

## Statistical Analysis

Data analysis was done using SPSS Version 22.

## RESULTS

In this study total of 4978 patient's samples were processed for CBNAAT test to assess the performance of GeneXpert/RIF Assay. Out of these 4978 cases MTB was detected in 1186 (23.82%) cases and MTB was not detected in 3461 (69.52%) cases, Error in interpretation were 159(3.19%) cases, Invalid tests were 88 (1.76%) cases and no results were found in 84(1.68%) cases [Table 1]. Among 1186 MTB detected cases, 1021 (86.09%) cases were having Rifampicin sensitive TB and Rifampicin Resistance TB was detected in 165 (13.91%) cases [Table 2]. Out of these 165 MTB-RR TB cases, the MTB detection level was, 50(30.30%) High, 65(39.39%) Medium, 26 (15.79%) Low, 16(9.69%) Very Low & 8 (4.84%) Intermediate [Table 3]. The CBNAAT, MTB detection levels in 1021 Rifampicin sensitive TB cases were 300 (29.38%) High, 403 (39.47%) Medium, 230(22.52%) Low and 88(8.61%) Very Low [Table 4]. Out of 4978 cases 427 (8.58%) were HIV positive, HIV negative cases were 2855(57.35%) and HIV status was unknown in 1696 (34.06%) cases [Table 5]. Out of these 427 HIV positive cases MTB was detected in 51 (12%) cases and MTB was not detected in 376(88%) cases [Table 6]. In 51 HIV-TB case, Rifampicin Resistant TB cases were 13

(25.50%) cases and Rifampicin sensitive TB case were 38 (74.50%) [Table 7].

Test Results	Absolute Number	Percentage
MTB Detected	1186	23.82%
MTB Not Detected	3461	69.52%
Invalid Test	88	1.76%
Error during processing	159	3.19%
No Result	84	1.68%

**Table 1. Total Number of Tests (4978)**

Test Results	Absolute Number	Percentage
RR-Not Detected	1021	86.09%
RR-Detected	165	13.91%

**Table 2. MTB Detected Tests (1186)**

MTB Detection Level	Absolute Number	Percentage
High level	50	30.30%
Medium level	65	39.39%
Low level	26	15.75%
Very Low level	16	9.69%
Intermediate level	08	04.84%

**Table 3. Rifampicin Resistant MTB Detection (165)**

MTB Detection Level	Absolute Number	Percentage
High level	300	29.38%
Medium level	403	39.47%
Low level	230	22.52%
Very Low level	88	08.61%

**Table 4. Rifampicin Sensitive MTB Detection (1021)**

Test Results	Absolute number	Percentage
HIV Positive	427	8.58%
HIV Negative	2855	57.35%
Unknown	1696	34.06%

**Table 5. HIV Status (4978)**

Test Results	Absolute Number	Percentage
MTB Detected	51	12%
MTB Not Detected	376	88%

**Table 6. CBNAAT IN HIV Patients. (427)**

Test Results	Absolute Number	Percentage
RR- Detected	13	25.50%
RR-Not Detected	38	74.50%

**Table 7. Rifampicin Resistance in HIV-TB Patients (51)**

**DISCUSSION**

In this study total of 4978 patient’s samples were processed for CBNAAT test. Out of these cases MTB was detected in 1186 (23.82%) cases. Out of these 1186 MTB detected cases Rifampicin sensitive TB detected in 1021 (86.08%) cases and Rifampicin Resistance detected in 165 (13.91%) cases. Similar results were found in Dravid MN et al. study,<sup>[7]</sup> in their study out of total 475 samples MTB was detected in 193 (40.63%). Out of these 193 MTB detected cases 160 (82.90%) were sensitive to rifampicin and 33(17.10%) were resistance to rifampicin.

In Dravid MN et al. study, out of 193 MTB detected cases, 21(10.88%) were HIV positive and 172 (89.12%) were HIV negative. Out of the 21 HIV positive cases, 85.71% were rifampicin sensitive and 14.29% were Rifampicin resistant.<sup>[7]</sup> In present study, out of 4978 cases 427 (8.58%) were HIV positive and among these HIV positive cases MTB detected in 51 (12%) case. Out of HIV-TB, 38(74.50%) were Sensitive TB cases and Rifampicin Resistance detected in 13 (25.50%) cases. Sethi et al. reported HIV-TB co-infection in 20.1%

cases<sup>[8]</sup> and in the study of Arora et al., 2.5% were HIV positive.<sup>[9]</sup>

According to WHO reports on MDR, MDR-TB in India continues to be reported in between 2.5–2.8% and 14–17% amongst new TB and retreated patients, respectively.<sup>[10-13]</sup> In present study, the cases with Rifampicin resistance were 165 (13.91%) among Old and New TB cases (1186).

The GeneXpert is an automated system, there is error in processing the samples, hence the cause of error may be too viscous sample (In this error code is 2008). The other causes of error could be probe check failure; incorrect volume of sample (Code error 5006, 5007) and heater failure can also cause error. In our study, error was detected in 159 (3.19%) cases. In Bajrami et al. study the error was seen in 2.6% of cases.<sup>[14]</sup>

In present study out of 165 cases of rifampicin resistant TB, the MTB detection levels were 50 (30.30%) High, 65 (39.39%) Medium, 26 (15.79%) Low, 16 (9.69%) Very Low & 8 (4.84%) Intermediate. Out of 1021 Rifampicin sensitive TB cases the MTB detection levels were 300 (29.38%) High, 403 (39.47%) Medium, 230(22.52%) Low and 88 (8.61%) Very Low.

The Singh et al. study observations were, in a patient care setting in a high burden region, from a large collection of pulmonary and extra-pulmonary samples and puts to rest questions regarding sensitivity, specificity, detection of infrequent mutations and mutations responsible for low-level Rif resistance by GeneXpert. Improvements in the assay could offer further improvement in sensitivity of detection in different patient samples; nevertheless, it may be difficult to improve sensitivity of Rif resistance detection if only one gene is targeted. Assay specificity was high both for TB detection and Rif resistance detection. Despite a few misses, the assay offers major boost to early diagnosis of TB and MDR-TB, in difficult to diagnose pauci-bacillary TB.<sup>[15]</sup>

The Xpert MTB/RIF assay demonstrated a high capability to detect MTBC DNA in AFB microscopy- negative samples of pulmonary and extrapulmonary origin. With the exception of a specimen harbouring a mixed MTBC population, the assay correctly retrieved information from the rpoB hot spot region regarding RMP resistance. Accumulating published data, including those presented in Panayotis. Ioannidis et al., indicate that this rapid and easy-to-perform fully automated CBNAAT could prove to be an extremely helpful diagnostic tool in the fight against tuberculosis.<sup>[16]</sup>

García-Basteiro, A. L. et al. study Opined that the GeneXpert seems to be a reliable method for TB post-mortem diagnosis, more rapid and easy to perform than conventional molecular methods. And also shows that in high TB and HIV endemic countries, TB disease may be significantly underdiagnosed and often misdiagnosed. For individual and public health benefits, there is a need to develop and more widely apply new methods to reliably estimate TB mortality.<sup>[17]</sup>

Since children below 6 years are not able to expectorate the sputum, specimens like gastric lavage aspirate (GLA), induced sputum (IS), bronchoalveolar lavage (BAL) are used and each one will have its own advantages and disadvantages. In a study by Pang et al., the authors highlight limitations of their study and emphasize that Xpert MTB/RIF assay is not ideal for GA samples due to its low negative predictive value, which means overall low (70.1%)

specificity. They also emphasize that NaHCO<sub>3</sub> neutralization of GA samples is not advisable if samples have to be stored and then cultured in MGIT960 system, though these samples may be used for DNA-based tests, as the DNA of dead bacilli can be amplified by later methods. They also showed that 11.6% Xpert MTB/Rif samples yielded indeterminate Rifampicin-resistance results, which is an important cost implication.<sup>[18]</sup>

Over the last decade, the RNTCP in India has expanded its TB services in phase wise manner to cover over 1.1 billion population. The RNTCP has met the WHO objectives for case detection and treatment success, and is close to fulfilling the TB-related Millennium Development Goal indicators and the Stop TB Partnership targets by 2015. The RNTCP has also proved that ensuring free TB services, like diagnosis, treatment, health awareness campaigns and research related activities via both the public and private sector is a cost-effective and sound investment, providing a significant economic return to the country, and to the world. The RNTCP is doing well in the country; even though we have very poorly organised public health infrastructure and manpower by its commitment, careful planning, thorough implementation, stable funding. The use of an innovative network of technical consultants, has added in achieving the targets. In order to implement new activities related to the programme and to maintain the current programme activities, an annual amount of more than US\$ 100 million is required.

This level of expenditure on TB control activities is unprecedented in India; however the achievements of the RNTCP, with emphasis on the cost effectiveness of the programme, should help to convince both the Government of India and donors to increase their investment in the country with the world's greatest TB burden, both in terms of drug-susceptible and drug-resistant disease.

## CONCLUSIONS

The implementation of the GeneXpert MTB/RIF assay in programmatic management of TB & MDR-TB in RNTCP has dramatically improved the rapid diagnosis of tuberculosis & drug resistance, especially in People Living with HIV/AIDS (PLWHA).

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