



## Original Research Article

## Rapid diagnosis of multidrug-resistant tuberculosis by Xpert MTB/RIF assay

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## ABSTRACT

**Introduction:** India has the highest burden of both tuberculosis (TB) and Multidrug Resistant tuberculosis (MDR-TB), based on estimates reported in Global TB Report 2016. Conventional diagnostic techniques are less sensitive & time consuming. The Xpert MTB/RIF Assay has the capacity to revolutionize the diagnosis of TB because of its speed, sensitivity and specificity. The objectives of our study are to determine the rate of positivity of Mycobacterial tuberculosis complex among the different clinical specimen and to know the rate of Rifampicin resistance among these specimens by using Xpert MTB/RIF Assay.

**Materials and Methods:** The study was conducted in Department of Microbiology, Rural Medical College, (PIMS), Loni. Clinical Samples from suspected TB cases were treated according to Manufacturer's Instructions & Standard operating procedure.

**Result:** A total of 1528 Samples received during the study period out of which 267 (17.47%) were positive for *Mycobacterium tuberculosis* complex (MTBC) and 15 (0.98%) were rifampicin resistant. Maximum positivity was noted in age group 21-30 years. Out of total 126 extrapulmonary specimens, 10 were positive for MTBC. Also out of 273 HIV infected cases 16 samples were positive for MTBC while 1 was Rifampicin resistant.

**Conclusion:** Accurate early diagnosis and treatment of TB has the potential to reduce morbidity and mortality associated with MDR TB. Xpert MTB /RIF assay has brought revolution in the earliest diagnosis (Turn around time-2.5hrs) compared to culture (which takes weeks) with simultaneous drug susceptibility testing.

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## 1. Introduction

India, second most populous country account for 23% of total global burden of tuberculosis, according to WHO.<sup>1</sup> The incidence rate of tuberculosis is 217 per one lakh population every year and approximately 4.8 lakh deaths occurred due to tuberculosis annually.<sup>2</sup> India has the highest burden of both TB and Multidrug Resistant tuberculosis (MDR-TB), based on estimates reported in Global TB Report 2016 (0.13 million of 0.48 million of cases of MDRTB).<sup>3</sup> Multi-drug resistant (MDR) tuberculosis is defined as TB disease caused by a strain of *Mycobacterium tuberculosis* that is resistant to at least isoniazid and rifampicin (RIF). In most of the cases, the disease affects

the lungs, but there are also not negligible numbers of cases (about 15%) with extrapulmonary presentation in low incidence countries. These rates are even high in high incidence nations. HIV-co infected TB patients often develop extra pulmonary tuberculosis and may progress fast unless its timely diagnosed managed appropriately.<sup>4</sup> Early diagnosis of this disease is the key for successful treatment and reduction of disease transmission. Most deaths due to TB can be prevented if patient receives timely management.

In most of the developing countries, smear microscopy remains the primary procedure for diagnosing TB. It is cheap and comparably easy to perform, but has a sensitivity ranging from (35%–80%).<sup>5</sup> In addition to that, it does not differentiate drug-sensitive strain from drug-resistant strain of *Mycobacterium tuberculosis* (MTB). Traditional solid culture method takes time around 2-6

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weeks. The Mycobacterium Growth Indicator Tube (liquid culture) was developed in recent times but still it takes 21 days for growth.<sup>6</sup> Because of the delayed diagnosis, morbidity and mortality increases which predispose patient for secondary resistance and cause transmission of resistant strains among others.<sup>7</sup>

The GeneXpert (XpertMTB/RIF Assay) technique has the capacity to revolutionize the diagnosis of TB because of its speed, sensitivity and specificity.<sup>8</sup> It is a cartridge-based nucleic acid amplification test that can, in less than 2 h, simultaneously detects *Mycobacterium tuberculosis* complex bacteria along with susceptibility result to rifampicin drug.<sup>9</sup> WHO, in 2013 had endorsed conditional recommendation for Xpert MTB/RIF as the initial diagnostic test in all adults with presumptive tuberculosis and Multidrug resistant tuberculosis.<sup>10</sup>

The objectives of our study are to determine the rate of positivity of Mycobacterial tuberculosis complex among the different clinical specimen and 'to know the rate of Rifampicin resistance among the clinical specimens from the suspected cases of pulmonary or extrapulmonary tuberculosis.

## 2. Materials and Methods

The study was conducted in Department of Microbiology, Rural Medical College, (PIMS), Loni, Maharashtra (India) for six month. TB CBNAAT laboratory receives clinical samples from suspected TB patients attending Pravara Rural Hospital (Loni), ART center (Loni), DOTS center (Loni), Private hospitals, and RH/PHCs from nearby talukas of Ahmednagar District. The pulmonary as well as extrapulmonary specimens from suspected cases of tuberculosis were collected & transported to the TB CBNAAT Laboratory. The collection & transport was done according to ' Technical & operational guidelines for Tuberculosis control in India (2016).<sup>11</sup>

### 2.1. Inclusion criteria

Clinical samples from suspected Tuberculosis cases of all age group.[Cases were diagnosed according to Case definitions given by Technical & operational guidelines for Tuberculosis control in India (2016).<sup>[11]</sup>

### 2.2. Exclusion criteria

1. Invalid' result
2. Error
3. No result
4. Indeterminate' result

Samples were treated according to Manufacturer's Instructions & Standard operating procedure for Pulmonary & Extrapulmonary TB.<sup>11</sup>

### 2.3. General procedure

Sample reagent was added directly into the falcon tube containing sample in the ratio 2:1. After placing the cap, the tube was shaken manually 10–20 times or vortexed and incubated at room temperature. After 10 min of incubation, the specimen was shaken again for 10–20 times or vortexed and kept for incubation for five minutes. The cap of test cartridge was opened after labelling the sample. Two milliliter of the specimen-reagent mixture was transferred with the sterile disposable transfer pipette from the falcon tube to the test cartridge, and the cap of cartridge was closed. All the necessary details were filled on the software, and the cartridge barcode was scanned. After entering the sample ID, the cartridge was loaded on the machine module and test was started. Results were documented within 2h. Patients details (like age, sex, type of specimen, HIV status, referral from private hospital, etc) were documented from requisition form. Ethical approval was taken for the study from Institutional ethical committee.

## 3. Result

The study was done for six months in the Department of Microbiology, Rural Medical college, Loni. A total of 1528 Samples received during this period out of which 267 (17.47%) were positive for *Mycobacterium tuberculosis* complex on GeneXpert. Out of 267 positive specimens, 15 (5.61%) were rifampicin resistant. (Table 1)

Out of 267 positive specimens 176 (65.92%) samples were from male patients while 91 (34.08%) were from female patients. Age wise distribution of the positive specimens is as shown in graph-1. Maximum positivity was noted in age group 21-30 years.

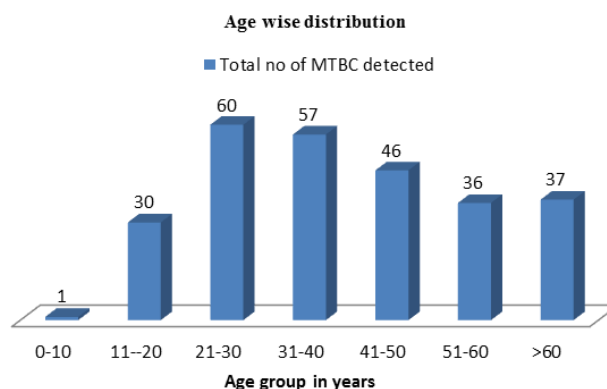


Fig. 1: Age wise distribution of positive cases

Out of total samples 126 specimens were that of extrapulmonary specimens. Total number of extrapulmonary samples positive for MTBC were 10. (Tables 2 and 3)

Out of 1528 cases, 273 cases were infected with HIV. Cases who were not infected with HIV were 1011, while

**Table 1:** Total no of Tests performed using CBNAAT

Total no of Tests performed using CBNAAT	1528
Total number of 'MTB detected & Rifampicin sensitive'	252
Total number of 'MTB detected & Rifampicin Resistant'	15

**Table 2:** EPTB samples processed

EPTB samples processed	126
MTB Not detected	116
Total number of 'MTB detected & Rifampicin sensitive'	10
Total number of 'MTB detected & Rifampicin Resistant'	0

**Table 3:** Distribution of Extrapulmonary specimens

Result	Abdominal fluid	Joint fluid	Lymph node/ LN biopsy/ FNAC	Pleural fluid	Pus	CSF	Tissue	Total
Total no of samples tested	15	2	5	66	9	27	2	126
Total number of 'MTB detected & Rifampicin sensitive'	0	0	3	3	3	1	0	10
Total number of 'MTB detected & Rifampicin Resistant'	0	0	0	0	0	0	0	0

those whose HIV status was not known were 244. Out of 273 HIV infected cases 16 samples were positive for MTBC, while 1 strain showed resistance for Rifampicin. (Table 4)

Out of total samples, five samples were positive for MTBC from paediatric patients. None of them was Rifampicin resistant. (Table 5)

Samples received from referrals from private sectors were 39, out of which 10 were showed for Rifampicin sensitive MTBC infection while 1 strain was resistant for Rifampicin. (Table 6)

#### 4. Discussion

Rapid detection of drug resistant Tuberculosis is critical for improving patients care and reducing transmission of tuberculosis. The second line drugs for treatment of MDRTB are costlier and not easily available. These drugs also have more adverse effects and requires long duration treatment.<sup>12</sup> The best method of diagnosing an infectious disease is to demonstrate the causative organism in representative samples. The MTB/RIF technique is a good and reliable proxy for the diagnosis of Multidrug-resistant tuberculosis. Its short turnaround time which enables timely initiation of treatment and hence better clinical outcome which are essential in reducing transmission of drug resistant tuberculosis infection.<sup>13</sup>

This study was done to determine the diagnostic utility of the CBNAAT (MTB/RIF assay) for the rapid diagnosis of tuberculosis and detection of rifampicin resistance from different clinical specimens. A total of 1528 samples were processed on GeneXpert out of which 267 (17.47%)

were positive for *Mycobacterium tuberculosis* complex on GeneXpert. From these positive specimens, 15/267 (5.6%) were rifampicin resistant. Ramos JM et al (2018) have reported 22.4% positivity while 1.5%(1/67) Rifampicin resistant.<sup>14</sup> Virupakshappa V et al (2018) in karnataka had reported 17.03% positivity with 8/329(2.4%) of rifampicin resistance.<sup>7</sup> Jing H et al (2017) reported positivity as 33.66%.<sup>15</sup>

In our study, 66% positivity was observed in male patients while, 34% were female patients. Okonkwo RC et al (2017) in his study, reported rate of detection in males as 65.5% while in females its 34.5%.<sup>9</sup> Similar preponderance of male cases was also noted by Guenaoui et al. (2016).<sup>16</sup> The gender difference is probably due to difference in natural as well as acquired risk factors, such as associated habits of alcoholism, smoking, tobacco use among the males which expose them & makes them susceptible to the infection.

Age wise maximum percentage of detection was observed in 21-30 years of age group followed by 31-40 years, while minimum positivity was found in below 10 years of the age. There were 4 cases below 14 years of age (Pediatric cases). Okonkwo RC et al (2017) reported maximum positivity in 31-40 years of group.<sup>9</sup> TB is a largest killer among communicable diseases in young reproductive adult human population (15-49 years age group).<sup>2</sup> Mycobacteriological diagnostic techniques used in adult cases remain the "gold standard" but demonstrate a lower sensitivity in kids. This is may be because of paucibacillary nature of the disease in children and the problem obtaining adequate pulmonary or extrapulmonary specimens. Young kids are frequently unable to voluntarily

**Table 4:** Samples processed from PLHIV

Particulars	Cases those were HIV positive	Cases those were HIV negative	Cases whose HIV status was UNKNOWN
<b>Total</b>	<b>273</b>	<b>1011</b>	<b>244</b>
Total number of 'MTB detected & Rifampicin sensitive'	16	183	53
Total number of 'MTB detected & Rifampicin Resistant'	1	11	3

**Table 5:** Samples processed from Paediatric patients

Description	Number
Samples processed from Paediatric patients	172
Total number of 'MTB detected & Rifampicin sensitive'	4
Total number of 'MTB detected & Rifampicin Resistant'	0

**Table 6:** Samples from private sectors

Description	Number
Samples processed from referrals from private sectors	39
Total number of 'MTB detected & Rifampicin sensitive'	10
Total number of 'MTB detected & Rifampicin Resistant'	1

expectorate sputum.<sup>17</sup>

Out of total samples 126 specimens were that from extrapulmonary site. Total number of extrapulmonary samples positive for MTBC were 10 (7.9%). None of it showed Rifampicin resistant. Virupakshappa V et al (2018) observed 11.97% detection rate among extrapulmonary samples with no rifampicin resistance. In his study, maximum positivity was found in pus followed by Pleural fluid and CSF.<sup>7</sup> Similarly Jing H et al (2017) found tubercular bacilli in 14.4 % of the samples, while Kandi et al (2017) reported positivity as 28 (31%) out of 90 extrapulmonary samples.<sup>15 18</sup> Bankar et al (2018) in her study on EPTB has found maximum percentage of detection of tubercular bacilli by GeneXpert in pus, lymph nodes & biopsy combined followed by pleural fluid which is a similar observation made in our study.<sup>19</sup>

The laboratory diagnosis of Extrapulmonary TB is challenging due to the paucibacillary quantity in the specimens, difficulty in getting specimens from deep seated organs and inability to obtain an additional sample. Therefore, failure in timely diagnosing and treating affected patients leads to increased morbidity and mortality, and hence development of secondary resistance.<sup>19</sup> Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF samples from suspected patients TB meningitis. It may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing extrapulmonary specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB.<sup>20</sup>

Out of 273 HIV infected cases 16 (6.22 %) samples were positive for MTBC while 1 (0.36%) was positive

for Rifampicin resistant MTBC strain. Virupakshappa V et al (2018) has observed 10.53% rate of detection of tubercular bacilli in specimens of PLHIV & Rifampicin resistance rate as (0.25%), while Dewan et al.(2015) in Delhi found rifampicin resistance rate as 10%.<sup>7 21</sup> This high resistance to rifampicin may be due to higher prevalence of Multidrug resistant tuberculosis in northern parts India and also referral from multiple states. Low rate in our study may be due to large no of incompletely filled requisition forms which led to higher number of specimens with 'UNKNOWN status for HIV'

Tuberculosis is the leading cause of death among PLHIV. In 2016, around 374,000 deaths occurred due to HIV-TB co-infection in India. Along with diagnostic difficulties due to absence of caseous necrosis and poor compliance of the long duration treatment for drug resistant TB, there is rising prevalence of Multidrug resistant tuberculosis globally.

Samples processed from referrals from private sectors were 39 out of which 11 (27.5%) were positive for MTBC while 1(2.56%) strain was resistant for Rifampicin. Another study from south India has reported 17.26% positivity and 0.59% Rifampicin resistance.<sup>7</sup> There is very little information about the TB patient from the private sector available to the RNTCP program and little is known about their quality of treatment, including treatment outcomes. Engaging the private sector effectively is the single most important intervention required for India to achieve the overall goal of universal access to quality TB care.

## 5. Conclusion

As reflected in our study, MTB/RIF (GeneXpert) is proving to be Game changer in tribal, rural & urban population.

Before MTB/RIF the only available methods to diagnose TB in this region were Smear microscopy & rarely culture. There was no facility for drug susceptibility in many peripheral diagnostic centers. Now patients are getting benefitted with this molecular test which is available free of cost under RNTCP. The test which also shows drug susceptibility for rifampicin that too with greater accuracy. Multiple studies have consistently shown that Xpert MTB/RIF can identify a considerable quantity of smear-negative Tuberculosis and Extrapulmonary TB. Accurate diagnosis and early treatment of TB has the potential to reduce morbidity and mortality associated with MDR TB.

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## 7. Source of funding

None.

## 8. Conflict of interest

None.

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