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COMPARISON OF DETECTION OF RIFAMPICIN RESISTANT MYCOBACTERIUM TUBERCULOSIS USING RAPID MOLECULAR TECHNIQUES

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1. *Abstract*: The study was conducted to assess the diagnostic efficacy of TrueNat and CBNAAT in detection of Mycobacterium tuberculosis in a municipal hospital. Both these molecular tests are currently being used for diagnosis of tuberculosis under National TB Elimination programme. Both the methods were sensitive in detection of TB but discordant results were found in detecting Rifampicin resistance.

2. *Index Terms*: Mycobacterium tuberculosis, TrueNat, CBNAAT, Rifampicin resistance

1. Introduction

For more than 50 years India has been engaged in Tuberculosis (TB) control activities. In spite of this TB continues to be India's greates health problem. TB kills an estimated 480,000 Indians per year and more than 1,400 per day. Another problem affecting India's TB control is that of 'missing' cases which are more than a million per year. These are cases that are not notified or remain either undiagnosed or inadequately diagnosed and treated in the private sector. This puts a patient into an unending cycle of suffering, poverty which leads to loss of life and disastarous financial consequences for the family. This cycle can be broken by concerted efforts from all the stakeholders in the health sector.

The Government of India has devised the National Strategic Plan (NSP 2017-25) as the framework for elimination of TB in India. Its four pillars are Prevent, Detect, Treat and Build. [1] This plan aims to find all Drug Sensitive TB(DS-TB) and Drug Resistant TB(DRTB) cases with special emphasis on universal testing for DRTB in TB patients. Especially amongst those who seek treatment from private sector and in high-risk populations.

For several years sputum smear microscopy (SSM) was the cornerstone of TB diagnosis in India. This had several limitations including its low sensitivity which made diagnosis of TB difficult .[2] The introduction of rapid molecular diagnostics in 2009 was a game changer which helped in rapid diagnosis of TB. By 2014 Cartridge based nucleic acid amplification technique (CBNAAT) was made available in ART centres in INDIA under RNTCP.[1] Another armament was added in the fight against TB with the introduction of TrueNat™ MTB/Rif (Molbio Diagnostics, Verna, India). This was a made in India rapid molecular test for the diagnosis of active TB. It was a portable, battery-operated, chip-based test which detects Mycobacterium tuberculosis (MTB) in less than 1 hour and rifampicin (RIF) resistance in another 60-80 minutes. TrueNat MTB has high sensitivity (91.1%) and specificity (100%) compared with a composite reference standard consisting of smear and culture results, clinical treatment and follow-up, and radiology findings.[3]There was high agreement (92.7%) between Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) and TrueNat in MTB detection.[4] TrueNat had significantly higher sensitivity than Xpert (84.1% vs. 81%; p value 0.001).[5] Unlike Xpert TrueNat doesn't require airconditioned environment and has UPS which enable it to work in areas with erratic power supply .[5] The 2020 rapid communication on molecular diagnosis of tb by WHO's , stated that the accuracy of TrueNat was comparable to that of Xpert.

In the year 2020 India conducted the following number of tests using both these rapid molecular tests.[7]

table no.1: India tb report

NAAT	No. of test conducted	MTB Detected	R Resistant
CBNAAT	2858713	779195(27.2%)	53826(7%)
TrueNAT	125923	11124 (8.8%)	340(3.1%)

Both these tests are being used in government and private sector for early diagnosis and treatment of both pulmonary and extrapulmonary TB. This study was planned to see the efficacy of thes systems in diagnosis of TB in a municipal setup.

2. Aims and Objectives

1. To study diagnostic efficacy of TrueNat and CBNAAT in detection of Mycobacterium tuberculosis (MTB)
2. To study the efficacy of TrueNat and CBNAAT in detection of Rifampicin resistance in MTB
3. To analyze epidemiology of Rifampicin resistant and indeterminant cases

3. METHODS

3.1 Study Setting

This was a Retrospective study done for samples received from 15th March 2021 to 30th April 2022 in a municipal laboratory with TrueNat and CBNAAT machine. This laboratory received samples from 6 tuberculosis units. All sputum samples were tested using TrueNat. Samples found to have Rifampicin resistance or indeterminate results were re-checked on CBNAAT. Some samples were not cross checked due to non-availability of CBNAAT cartridges and were sent to IRL for same. All samples in which Mtb was detected were sent to IRL for FLLPA and those with Rifampicin resistance for SLLPA.

3.2 Sample Processing

Patient with suspected TB submitted 2 early morning samples to our lab. Sample were received in a falcon tube which were given lab ID. Both the samples were mixed and one part of sample was processed for testing using Truenat. Manufacturer instructions were followed for this. This required DNA extraction using Universal cartridge-based sample prep kit on Trueprep Auto v2. Mtb was then detected using TrueNat MTB chip on Truelab Real time

micro–PCR Analyzer. If MTB was detected the extract was used to test for Rifampicin resistance using Truenat MTB-RIF Dx according to manufacturer instructions. If the result was Rifampicin resistant or indeterminate the part of sample was processed for Mtb detection using Xpert MTB/Rif according to manufacturer instructions. Remaining sample was sent for FLLPA to IRL and its results were obtained from them.

4.RESULTS

Sputum samples processed in our laboratory using TrueNat and CBNAAT respectively were as shown in table no.2

table no.2: total number of sputum samples processed from 15-03-2021 to 30-04-2022

	Test done	M tb Detected	Rifampicin Sensitive	Rifampicin Resistant	Rifampicin Indeterminate
TrueNat	1845	720	648	74	62
CBNAAT	701	192	173	19	0

108 samples of patients were found to be either rifampicin resistant or indeterminate by TrueNat. Amongst these patients 70 were males and 38 were females. In both sexes age group wise distribution was as shown in the table no. 3

table no.3: age wise sex distribution of patients with rifampicin resistance or indeterminant result by truenat

Age Groups	Males	Females
11-20	10	5
21-30	14	18
31-40	12	7
41-50	13	5
51-60	15	2
61-70	5	1
71-80	1	0

These 108 samples that were Rifampicin resistant or indeterminate were crosschecked with CBNAAT. Of these 108,50 samples showed Rifampicin resistance. These when retested using CBNAAT showed 88.46% MTB detection. Only 38.46 %samples had Rifampicin resistance when tested by CBNAAT when Truenat had shown them as Rifampicin resistant. These results are shown in Table No.4.

table no.4: rifampicin resistant samples by truenat on testing by cbnaat

	Truenat	Cbnaat
Mtb detected	50	44
Rifampicin Resistant	50	17
Rifampicin Sensitive	0	27

Rifampicin Indeterminate	0	0
Mtb not detected	0	6

The remaining 58 samples were indeterminant by Truenat. These when tested by CBNAAT showed 85.2% MTB detection. In 9 samples MTB was not detected. 2 samples showed Rifampicin resistance. These results are shown in table no.5.

table no.5: rifampicin indeterminate samples by truenat on testing by cbnaat

	Truenat	Cbnaat
Mtb detected	58	49
Rifampicin Resistant	0	2
Rifampicin Sensitive	0	46
Rifampicin Indeterminate	58	0
Mtb not Detected	0	9

25 samples could be sent to reference lab for Line Probe Assay (LPA). 8 samples were those that were Rifampicin resistant by truenat. In these 8 samples CBNAAT could detect MTB in 6 samples only. Whereas LPA detected MTB in all 8. The results were as shown in table no.6.

table no.6: rifampicin resistant samples by truenat on testing with cbnaat and lpa

	Truenat	CBNAAT	LPA
Mtb Detected	8	6/8	8/8
Rifampicin Resistant	8	2	4
Rifampicin Sensitive	0	4	4

Amongst these 25,17 samples had shown Rifampicin indeterminate result by Truenat. CBNAAT could detect MTB in only 14 of these. LPA detected MTB in 13 of these and 2 samples had possible Non tubercular mycobacteria (NTM). LPA found 2 out of these 13 samples to be Rifampicin resistant. The results obtained are tabulated in table no.7.

table no.7 :rifampicin indeterminant by truenat on testing with cbnaat and lpa

	Truenat	CBNAAT	LPA	Comments
Mtb detected	17	14/17	13/17	2 samples possible NTM
Rifampicin Resistant	0	0	2	

Rifampicin Sensitive	0	14	11	
Rifampicin Indeterminant	17	0	0	

5.Discussion

India accounts for around one-fourth of the world's TB cases. (8) Detection of AFB in sputum smear is a simple, rapid, inexpensive and very specific method for diagnosing pulmonary TB but has a limitation of low sensitivity. (9,10) Sputum culture for Mycobacterium tuberculosis is more sensitive and specific and considered the current gold standard. Culture requires specialized and controlled lab facility and highly skilled manpower but it takes 2-8 weeks' time depending on the method. (11,12) Chest x-ray is neither sensitive nor specific for diagnosis of pulmonary TB.(12) Molecular techniques such as Polymerase chain reaction(PCR) or Real Time PCR tests are much more sensitive than both microscopy and culture. Rapid molecular diagnostics introduced since 2009 and scaled up from 2012 onwards has ensured that Line Probe Assay and CBNAAT testing is available throughout the country. In 2016, 520,000 patients were tested and 35,000 Rifampicin resistant/MDR-TB patients were diagnosed. Second line DST using Liquid culture systems are in place and are being scaled up to cover the entire country.

WHO has endorsed the use of CBNAAT and Truenat as a rapid diagnostic test for diagnosis of tuberculosis and drug-resistant tuberculosis. (6) In cases of pulmonary TB CBNAAT's overall specificity was 98% in all specimens (smear-positive and smear-negative). Overall sensitivity of the Truenat MTB assay was 83% and that of the MTBPlus assay 89%. Specificity was 99% for the MTB and 98% for the MTBPlus assay. (6)

The Truenat MTB assay detects Mtb by amplifying the nrdB gene and has a limit of detection of approximately 100 CFU/mL as reported by the manufacturer. (13) The primers in the Xpert MTB/RIF assay amplify portions of the rpoB gene and it's limit of detection of approximately 131 CFU/mL (14). Xpert MTB/RIF is known to have a limited capacity to detect RIF-R-associated mutations. (14,15) It is also known to have a decreased capacity of detecting *rpoB* C533G mutations responsible for some cases of RIF-R (16). It may also give occasional false-positive RIF-R for paucibacillary samples due to delays in the real-time signal generated by assay probes D and E (17). It's also known to give RIF-R due to false recognition of a nonfunctional *rpoB* F514F silent mutation. (18). In a prospective multicentric study to test the diagnostic accuracy of Truenat, it showed higher diagnostic compared to Xpert MTB/RIF assay. (19)

In this study MTB detection by TrueNat was more as compared to Xpert. In a study by Nikam etal also concordance was seen between results of both devices in MTB detection. (4) However in a study by Meaza A etal diagnostic accuracy of Truenat in detecting MTB was found to be more than that of Xpert. (20) Truenat and CBNAAT results showed concordance when tested for rifampicin resistant samples in our study though discordance in results were seen when samples with indeterminant results were compared. Truenat manufacturers recommends repeat testing in case of indeterminant result. But, in a resource limited setting where chip supply is an issue using another chip was not feasible and not done.

Only 25 samples could be tested using LPA. MTB detection by LPA and Truenat were comparable in both RIF-R and indeterminant samples. Yadav, etal had found Xpert MTB/RIF to be superior to LPA in detecting MTB in smear negative pulmonary samples and comparable in smear positive samples. (21) In a study by Syed Rufai, etal LPA and MGIT960 results of indeterminant samples showed 100% agreement with LPA results but only 64.4% agreement with Xpert MTB/RIF results. Sequencing analysis of discrepant samples showed 91.3% concordance with LPA but only 8.7% concordance with the Xpert MTB/RIF assay. (22) No study comparing Truenat and LPA could be found

There are several limitations to this study. Indeterminant test in Truenat were not repeated due to paucity of chip. Gold standard culture was not performed as laboratory facility for it was not available. LPA was performed on very

few samples and discordant results were not resolved. Demographic details of patients like their occupation, HIV status,etc were not available as it was a retrospective study.

In conclusion, Truenat and CBNAAT are both sensitive for Mtb detection. However, discordant results are seen in case of Rifampicin resistance. So, for starting treatment for drug resistant tb drug sensitivity testing should be done.

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