

RESEARCH ARTICLE

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Truenat: Can it be A Game Changer in Extrapulmonary Tuberculosis?

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Abstract

Tuberculosis is a leading health problem worldwide, with India accounting for the majority of cases. Owing to the diverse clinical presentation and paucibacillary nature of extrapulmonary tuberculosis, it is tough to diagnosis by routine microbiological methods. Newer chip/cartridge based nucleic amplification tests (NAATs) like Truenat and Xpert have proved to be game changer in diagnosis of pulmonary tuberculosis. However, role of Truenat in extrapulmonary tuberculosis is still to be evaluated. This study was undertaken to evaluate the diagnostic yield of truant in various extrapulmonary tuberculosis. The study was conducted during 01 June 2021 to 31 July 2022. Of the total 1481 samples received during study, 761 (52%) were extrapulmonary samples. The highest yield of 36% was found in pus aspirate, followed by a 12% yield in pleural fluid. Overall yield of 12% in various extrapulmonary samples is encouraging. Further studies are required for evaluating Truenat role in rapid diagnosis of EPTB and rifampicin resistance detection for better patient care.

Keywords: Tuberculosis, Extra Pulmonary Tuberculosis, Truenat

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INTRODUCTION

With an estimated 10 million new cases and 1.2 million deaths in 2019, tuberculosis (TB) remains a leading public health problem worldwide.¹ Of those 10 million people, three million were not reported to have been diagnosed and notified.² India continues to remain at the epicentre, accounting for nearly 25% of the global burden of tuberculosis.³

Of all active TB cases, extrapulmonary tuberculosis (EPTB) constitutes about 20–30% of cases.⁴ Diverse clinical presentation and difficulty in sample collection due to inaccessible site, invasive nature of sample collection technique, inadequate amount of sample and difficulty in repeat sample collection are some of the challenges resulting in incorrect and delayed diagnosis. Besides, the number of bacteria in extrapulmonary specimens is often lower than the number in pulmonary specimens, which compromises the diagnostic efficacy of the conventional tests. Laboratory diagnosis of EPTB can be done by direct and indirect approaches. Direct approaches include ZN staining, fluorescent microscopy, Lowenstein-Jensen culture, liquid culture, BACTEC culture system, PCR, antigen based serology, immunohistochemistry and immunocytochemistry.

Several decades old methods like ZN staining, though simple and cost effective, requires more than 10^6 bacteria/g tissue making its overall sensitivity ranging from 0-40%.⁵ Also, it cannot definitively differentiate between *Mycobacterium tuberculosis* (*M.tb*)TB and Non-tuberculous Mycobacteria (NTM). Gold standard culture method has a detection rate of 10-100 bacilli/ml of concentrated material.⁵ Although labor and skill intensive, time consuming, requiring biosafety cabinet, culture method has an advantage of species identification and determination of drug

susceptibility pattern. Many EPTB samples require decontamination, which is not conducive for the growth of *M.tb* in conventional egg based culture media leading to poor yield of *M.tb* in such samples. In contrast, automated liquid culture media increases the case yield by 10%.⁵

Nucleic-acid amplification tests (NAAT) such as conventional polymerase chain reaction (PCR) or to advanced techniques such as high resolution melt-curve analysis and whole-genome sequencing are highly sensitive techniques. However, being technically and financially demanding, their utility is limited to higher research and referral institutes.^{6,7}

In such a scenario, advent of Point of care molecular tests seems to bring a considerable gain in the diagnosis of extrapulmonary tuberculosis, especially in the case of paucibacillary samples. In recent times, attention has been devoted to new point of care Nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity. One such automated assay, Xpert MTB/RIF (Cepheid, Sunnyvale, CA) has been approved for detection of tubercular bacilli in pulmonary and extrapulmonary specimens.⁸ Another such assay, Truenat (Molbio Diagnostics, Bangalore, India) has been introduced. It's advantages like low cost, TAT of <1 hour for *M.tb* detection, and <2 hour for rifampicin resistance detection, better shelf life, compactness of equipment, portability, battery operation, no requirement of temperature control for equipment use and minimum sample preparation makes it a lucrative option in high burden tropical countries like India.⁹

It has been approved for TB detection in sputum in suspected Pulmonary Tuberculosis cases by regulatory agencies.¹⁰ Its use for TB detection in EPTB specimens is considered "off-label" use. Taking this into account, this study was conducted to generate evidence, expand database

Table 1. Patient distribution in accordance to gender

Total samples	Sample type	Number (%)	Gender	Number (%)
1481	Pulmonary	720 (48%)	Male	438 (60%)
			Female	282 (40%)
	Extra pulmonary	761 (52%)	Male	467 (61%)
			Female	294 (39%)

and evaluate the utility of Truenat for diagnosing EPTB and detection of rifampicin resistance in suspected EPTB patients.

MATERIALS AND METHODS

The study was approved by the Institute's Ethics Committee (ESIPGIMSR-IEC/2022063). The study was conducted in Clinical Microbiology Laboratory of ESIC Hospital, Basaidarapur, New Delhi 110015. The study included a total of 1481 samples received from 01 June 2021 to 31 July 2022 for *M.tb* detection. Mycobacterial DNA was extracted using Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device and amplified using Truelab Uno Dx Real Time Quantitative micro PCR Truelab Analyzer as per manufacturer protocol. Positive samples with CFU higher than

10^3 were further processed for detection of rifampicin resistance using Truenat MTB-RIF Dx as per manufacturer's instructions.⁹

RESULTS

Of the total 1481 samples received during the study period, 720 (48%) samples were pulmonary and 761 (52%) were extrapulmonary. Majority of suspected patients were male as shown in Table 1. Paediatric age group comprised of majority of suspected EPTB patients as shown in Table 2. Overall MTB detection rate by Truenat was 20% (295/1481) wherein EPTB positivity rate was 12% (88/761). Of these EPTB positive patients, 60% (53/88) were male while remaining 40% (35/88) were female.

Gastric aspirate (36%) followed by pleural fluid (19%) constituted maximum sample size among EPTB samples, as shown in Table 3.

Highest positivity rate was observed in pus aspirates (36%) followed by Pleural fluid (12%) samples.

DISCUSSION

Laboratory diagnosis is often challenging in EPTB. In the last decade, Newer Point of care NAAT has been launched. These tests are of critical importance by overcoming shortcomings of conventional methods by early detection of mycobacterium, differentiating *M.tb* from NTM,

Table 2. Age distribution of suspected EPTB patients

No.	Age (years)	Number (761)	Percentage
1.	Up to 10	267	35
2.	11-20	103	14
3.	21-30	60	8
4.	31-40	85	11
5.	41-50	80	11
6.	51-60	83	11
7.	61-70	43	5
8.	71-80	34	3
9.	81-90	4	1
10.	91-100	2	1

Table 3. Details of EPTB specimens received during study period

No.	Specimen	Number (%)	<i>M.tb</i> positive (%)	Rifampicin resistance detected (%)
1.	Gastric aspirate	274 (36%)	12 (4%)	00
2.	Pleural fluid	141 (19%)	17 (12%)	01 (5%)
3.	Pus	116 (15%)	42 (36%)	01 (2%)
4.	Sterile body fluids (except pleural fluid and CSF)	81 (10%)	06 (7%)	00
5.	Biopsy Tissue	70 (9%)	07 (10%)	00
6.	CSF	64 (8%)	03 (5%)	00
7.	Endometrial Biopsy	08 (1%)	01 (12%)	00
8.	FNAC	05 (1%)	00	00
9.	Lymph node	02 (1%)	00	00
10.	Total	761	88 (12%)	02 (5%)

rapid drug susceptibility testing for first line anti tubercular drugs and lower turnaround time (TAT) of testing.¹¹ These PCR based methods often target either genes of 65 KDa or 38 KDa or insertion sequences like IS6110.⁵ These complex, closed cartridge based, fully automated systems include Xpert MTB/RIF assay and TRUENAT, which have been endorsed by WHO.¹⁰

The Xpert MTB/RIF assay uses molecular beacon technology to detect DNA sequences amplified in a heminested real-time-PCR assay. The assay uses single-use plastic cartridges with several chambers that are preloaded with liquid buffers and lyophilised reagent beads necessary for sample processing, DNA extraction, and PCR.¹² The assay can be carried out in a nearly fully automated manner and the results are available within few hours. This assay has shown good sensitivity and specificity in detection of extrapulmonary tuberculosis.¹³ However, this test runs on GeneXpert instrument, which requires a temperature-controlled environment, as well as a stable power supply and is susceptible to dust. This limits the instrument's operations to district/subdistrict hospital settings.

Recently, Molbio Diagnostics (Bangalore, India) has launched an assay that utilises chip-based real-time micro PCR a novel point-of-care, cost-effective assay for TB testing and Rifampicin resistance detection. This is a robust, battery-operated assay with minimal operational requirements, much more suited to Indian environmental conditions and could provide a viable and an economical alternative to Xpert. This assay includes two tests for detection of *M. tuberculosis* (the Truenat MTB assay (including the nrdB single copy target) and the MTB Plus assay (including nrdZ and multicopy IS6110 targets) and one for the detection of RIF resistance (the MTB-RIF Dx reflex assay targeting the rpoB gene).^{14,15}

XpertMTB/RIF assay has been extensively validated worldwide for pulmonary TB and has demonstrated high positive predictive.¹⁶ However, overall sensitivity of Xpert in EPTB samples has been variable ranging from 90% to 30% depending on sample type.¹⁷ Several limitations have been reported by Bahr et al. in tuberculous meningitis cases while using Xpert on CSF samples.¹⁸

Recently, in a multicentric prospective study, diagnostic accuracy study of Truenat MTB

for detection of MTB and rifampicin resistance was found to be comparable to Xpert MTB/RIF assay for sputum samples.¹⁴

However, there is a paucity of data regarding Truenat *M.tb* utility in extrapulmonary samples.

A remarkable overall yield of 12% (88/761) was observed in our study for detection of *M. tuberculosis*. The yield in our study is comparable to the yield of Xpert in extrapulmonary samples as observed in a study done on 20,238 suspected EPTB samples in Delhi by Sidiq et al.¹⁹

Among various samples, highest yield of 36% was observed in extrapulmonary pus samples, which is remarkable owing to the paucibacillary nature of the disease. The finding is consistent with the yield of 35%, observed in pus samples by Xpert, as demonstrated by Sidiq et al.¹⁹ In another study done using Xpert MTB/RIF by Habous et al. demonstrated similar sensitivity in 29 pus samples taking culture as standard.²⁰ Our results are also consistent with those of Choudhary et al. from Nepal, who reported percentage positivity of 33.3% of Xpert in Pus samples.²¹

Next highest yield was observed in pleural fluid. Though pleural fluid is considered to be a suboptimal sample, yield of 12% is remarkable. Comparable yield of 10% was observed by Sidiq et al. and Sehgal et al. in their respective studies using Xpert.^{19,22} This is significant compared to 4% yield by conventional culture method as observed by Palacios-Marmolejo et al.²³

Gastric aspirates constituted majority (36%) of samples in this study. Variable results have been reported in NAAT studies on Gastric aspirates. In a study done by Diallo et al. on 2137 samples, out of which 178 were gastric aspirate, positivity for MTB by Xpert was 14%.²⁴ This is much higher in comparison to 4% positivity reported in our study. However, in a similar study by Sidiq et al., detection rate of MTB in 5350 gastric aspirates was 9.42% by Xpert.

CONCLUSION

The results of our study are thus encouraging and are comparable to those of Xpert MTB/RIF as demonstrated in various studies. The study also highlights the role of Truenat for rapid and accurate diagnosis of EPTB in developing and

high burden countries. Added advantage of early detection of rifampicin resistance makes Truenat a lucrative option in patient care. Though many studies have been done on role of Xpert MTB/RIF for detecting MTB and rifampicin resistance in EPTB specimen, there is paucity of similar data for Truenat. Thus, it is of paramount importance to generate evidence and evaluate the role of Truenat in EPTB samples with simultaneous rapid detection of rifampicin resistance for improving the patient outcome and reducing community spread of MDR TB.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, ESI-Post Graduate Institute of Medical Sciences & Research, Basaidarapur, New Delhi, India, with reference number ESIPGIMSR-IEC/2022063

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