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# Comparison of Genexpert MTB/RIF, Truenat and Conventional Methods for the Diagnosis of Tuberculosis at a Tertiary Care Centre in Northern Region of India

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**ABSTRACT**
Tuberculosis, an infectious disease caused by Mycobacterium tuberculosis, remains a major threat to public health. TB is usually divided into two main categories depending on the MTB infection site pulmonary and extrapulmonary tuberculosis. On the other hand, the sensitivity of conventional acid-fast bacilli smear is unsatisfactory and MTB culture is time-consuming. Nucleic acid amplification tests are a major advance in the diagnosis of TB. The Xpert MTB/RIF assay and Truenat also in nowadays are the most widely used NAATs for diagnosing TB and has demonstrated good diagnostic performance in PTB and EPTB. A total of 500 specimens were collected during this study from suspected Pulmonary and Extrapulmonary tuberculosis cases from different wards and outpatient departments Patients with known clinical diagnosis other than the disease, patients already on ATT and patients with HIV infection were excluded from the study. Out of 205 pulmonary and 295 extrapulmonary suspected tuberculosis patients, 32 were positive from pulmonary tuberculosis patients While in extrapulmonary tuberculosis patients 28 were positive. Nucleic acid amplification tests analysis can be effectively used as a quick and accurate diagnostic test for the diagnosis of pulmonary tuberculosis especially in a low resource setting. Pulmonary and Extrapulmonary tuberculosis, MDR-TB, AFB Staining Culture, Nucleic acid amplification tests.

## INTRODUCTION

Mycobacterium Tuberculosis remains a global health burden especially in developing countries[1]. Tuberculosis (TB) caused by acid-fast aerobic bacteria comprising the Mycobacterium tuberculosis complex is a contagious infection that generally affects the lungs. TB is a communicable infectious disease that is transmitted through cough aerosols and is characterized pathologically by necrotizing granulomatous inflammation, usually in the lungs[2]. The risk factors for TB are patients infected with HIV, diabetes, alcoholism, leukemia and patients who receive immunosuppressive drugs[3,4]In the absence of an efficient diagnostic modality for Tuberculosis, in the search for a tool that can overcome the available diagnostic tests continues. In wake of continued reports of high mortality and morbidity due to Tuberculosis, WHO approved GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) in 2011 and recommended it for rapid implementation[5]. Several studies have since published evaluation and validation reports[6-8]. The estimate of primary MDR-TB (in new Tuberculosis cases) in India is 2.2% while MDR-TB in re-treatment TB cases is 15%[9]. The studies from tertiary care centers report higher MDR-TB possibly due to referral bias and difficulty in diagnosing an indolent disease[10]. Tuberculosis in EP sites is also a diagnosis more often confirmed in tertiary care centers due to complex clinical presentation and need for invasively collected samples. In current the Revised National TB Control Program (RNTCP) endorses GeneXpert MTB/RIF for diagnosis of MDR TB among MDRTB suspects and for diagnosis of TB among certain vulnerable populations (HIV infected Tuberculosis suspects and pediatric Tuberculosis suspects)[11,12]. The elimination of Tuberculosis requires early, rapid and accurate diagnosis and treatment. Acid-fast bacilli microscopy with ZN staining is the most frequent laboratory technique used for its diagnosis. However, limitations associated with microscopy lead to misdiagnosis[13]. Although the technique is rapid and inexpensive, its sensitivity is variable (20-80 %)[14]. Culture is the gold standard technique for diagnosis of TB, which can also provide the testing for drug resistance. But such techniques require complex laboratory infrastructure and takes long time to get results. In fact the most personnel who need culture to diagnose their Tuberculosis will not have access to the test results in time to save their lives or to avoid transmission to others[15]. A culture technique using Lowenstein-Jensen medium for mycobacterial growth is being considered as the gold standard method for TB detection and it takes a longer time which is usually 3-4 weeks with a high sensitivity. The efficiency of MTB culture using the LJ medium has been demonstrated to detect MTB when 10 viable bacilli per mL of sputum were present[16]. Pulmonary tuberculosis and extrapulmonary tuberculosis are the two clinical manifestations of TB. The involvement of Tuberculosis other than lungs is termed as Extra Pulmonary Tuberculosis. Any patient affected with the combination of pulmonary and EPTB is defined as PTB[17]. In cases of suspected extrapulmonary tuberculosis, rapid and accurate laboratory diagnosis is important, because traditional techniques of detecting acid-fast bacilli have limitations. In the last decade, remarkable progress has been made in the diagnostics of Pulmonary TB. However, diagnostic challenges in Extra Pulmonary TB remain to be addressed. A more accurate test to diagnose various forms of EPTB, which can easily be incorporated into the routine TB control program, would contribute significantly towards improving Extra PTB case-detection[18].

## MATERIALS AND METHODS

All the suspected tuberculosis Specimens were collected in sterile, leakproof, disposable and appropriately labelled containers without fixative and placed in bags to contain leakage. In case of delay in transport and processing longer than 1 hrs all specimens were refrigerated at 4°C[19].

### Pulmonary specimen-sputum

The collection of three specimen at least 8 hrs apart. One of three specimen should be an early morning sample. Sample must be free of food particles, residues and other extraneous matter Quality Assessment of Sputum Samples Characteristics of a good sputum sample

- Mucoid or mucopurulent appearance
- Minimum amount of saliva
- Optimal volume 5-10 $mL^{-1}$

**Extra pulmonary specimen:** Pus/Aspirates, Urine, Pleural fluid, Cerebrospinal fluid [20].

**Aspirates:** The skin, lesion or wound was cleansed with alcohol before aspiration of the material in syringe.

**Urine specimens:** Early morning voided clean-catch midstream urine specimens (40 $mL^{-1}$ min) in sterile containers were collected for at least 3 consecutive days. Catheterization used only if a midstream voided specimen cannot be collected.

**Body fluid specimens:** A sufficient quantity of specimens is crucial for the isolation of AFB from CSF. At least 10 ml of CSF is recommended similarly as much as possible of other body fluids 5-10 ml minimum were collected in a sterile container or

syringe with a Luer-tip cap.

Fig. 1: GeneXpert instrument system (CBNAAT)

Fig. 2: GeneXpert MTB/RIF ultra cartridge

**Sample processing:** The collected pulmonary and extrapulmonary tuberculosis specimens was processed singly. Each processed specimen was inoculated on solid Lowenstein-Jensen (L-J) slants and incubated at 37°C for up to 8 weeks. Within this period the media examined weekly for visible bacterial colonies, if there is no visible growth on L-J slant then categorized as culture negative[21]. Processed specimen was also be subjected to AFB Microscopy for all samples and all the smears were stained by the Ziehl Neelsen method and examined with a light microscope[22] and confirmed by GeneXpert and Truenat tests. The Xpert assay was performed according to the manufacturer's instructions (Cepheid, Sunnyvale, CA) using latest version of G4 cartridges[6,7,23] and the Truenat test was performed by trueprep AUTO/AUTO v2 Universal Cartridge.

**Specimen decontamination**[24]: All non-sterile samples were processed by using NALC-NaOH procedure to

Fig. 3: Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Device

Fig. 4: Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit

decontaminate and concentrated the processed sample. One advantage of using NALC-NaOH method was that it contains NALC acts as a very good mucolytic agent used with reduced concentrations of a decontaminating agent (the final NaOH concentration in sputum was 1%). The sterile samples were not required decontamination so they were directly used with the liquid medium.

**Ziehl-Neelsen (ZN):** Staining

**Smear preparation**[22]: A thick mucoid part of sputum was selected to prepare a smear in the form of small

concentric circles at the centre of the slide. In case of CSF sample, one loopful of CSF was placed at the centre of a slide with no spreading and allowed to dry

Fig. 5: Truenat MTB Plus Chip

Fig. 6: Truelab Real Time micro-PCR Analyzer

Fig. 7: M. tuberculosis seen under Microscope

in air. One more drop of CSF was placed on the same drop and allowed to air dry. A third more drop was also placed on same spot after centrifugation of CSF and then allowed to air dry and fix properly. The smear was allowed to air dry completely at room temperature.

Fig. 8: M. tuberculosis culture Positive by LJ Medium

Fig. 9: GeneXpert result as shown by machine for MTB positive and Rifampicin sensitivity

Fig. 10: GeneXpert result as shown by machine for MTB negative

Fig. 11: GeneXpert result as shown by machine for MTB positive and Rifampicin resistance

After air drying the slide was fixed by passing it on the flame 3-4 times.

**Culture on solid medium**[20]**:** From the decontaminated sediment inoculation for culture was done on Lowenstein Jensen medium prepared in-house as per manufacturer's instructions using dehydrated media (HimediaM162). Cultures were incubated at 35°C in an

atmosphere of 5-10% carbon dioxide and high humidity. Tube media were incubated in a slanted position with screw caps loose for at least 1 week to allow for the evaporation of excess fluid and the entry of CO2. Cultures were examined weekly for growth.

Fig. 12: MTB negative by Truenat

Fig. 13: MTB positive by Truenat

Fig. 14: MTB positive and Rifampicin resistance by Truenat

Contaminated cultures were discarded. Most isolates were appeared between 3-6 weeks a few appeared after 7-8 weeks of incubation. When the growth appeared the rate of growth, pigmentation and colonial morphology were recorded. The typical colonial appearance of M. tuberculosis is shown in fig.no.3.3.

**Gene xpert:** Gene xpert MTB/RIF assay (Fig no. 1) was conducted as per manufacturer's instructions. The sample reagent was added to the sample in the volume of 2:1 ratio. Sample-reagent mixture was vortex for minimum 10 seconds or shaken vigorously. Mixture was incubated for minimum 10 min with intermittent shaken 10-20 times and further incubated for 5 min more at room temperature. After incubation, 2 mL sample-reagent mixture was added to the Gene xpert cartridge (Fig no. 2) by using sterile Pasteur pipette then cartridge was scan by the bar code scanner and loaded into the machine in a defined module.

Fig. 15: MTB positive and Rifampicin sensitive by Truenat

Fig. 16: Comparison of All four Diagnostic Methods (AFB Smear, Culture, Genexpert and truenat) for Pulmonary and Extrapulmonary tuberculosis Samples

Fig. 17: Comparison of Drug Resistance by GeneXpert and Truenat

**Truenat:** MTB Plus works on the principle of Real Time Polymerase Chain Reaction. The DNA from the patient sample is first extracted using Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Device (Fig no.3) and Trueprep AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit (Fig no.4). The Truenat MTB Plus chip (Fig no.5) is placed on the chip tray of the. Truelab Real Time micro-PCR Analyzer (Fig no.6).

Table. 1: Distribution of M. tuberculosis complex detected by different specimens among pulmonary and extra pulmonary tuberculosis

| Source of sample | Positive | Negative |
|---|---|---|
| Sputum | 32 (15.60%) | 173 (84.4%) |
| Pus/aspirates | 18 (11.76%) | 135 (88.24%) |
| Pleural fluid | 5 (10.42%) | 43 (89.58%) |
| Urine | 5 (9.62%) | 47 (90.38%) |
| Cerebrospinal fluid | 0 | 42 |
| Total | 60 (12%) | 440 (88%) |

Table. 2: Comparison of pulmonary and extrapulmonary MTB from various studies

| Study | Positive in pulmonary TB (%) | Positive in extrapulmonary TB (%) | Rifampicin resistance (%) | Sign and symptoms |
|---|---|---|---|---|
| Disha Arora *et al.*[31] | 18.1% | 14.5% | 12.5% | Fever, cough chest pain, weight loss, loss of appetite, dyspnoea |
| Luxia Kong *et al.*[32] Yehia Kadhim | 21% | 6.8% | 11.69% | Fever, cough, weight loss, dyspnoea, |
| Jabber Benellam *et al.*[33] | 71% | 14.2% | 63.83% | symptoms suggestive of TB based on clinical , pathological, or radiological evidence of TB |
| Present study | 15.6% | 9.5% | 11.7% | Fever, cough, fever with cough weight loss, loss of appetite, night sweats Evening rise of temperature |

Table. 3: Prevalence of different diagnostic methods (AFB smear, culture, genexpert) for pulmonary and extrapulmonary tuberculosis samples of various studies

| Study | Positive by microscopy | Positive by culture | Positive by genexpert |
|---|---|---|---|
| Geeta *et al.*[34] | 21/227 (9.25%) | 20/227 (8.81%) | 38/227 (16.74%) |
| Bajrami *et al.*[35] | 17/116 (14.65%) | 28/116 (24.13) | 34/116 (29.31%) |
| Elbrolosy *et al.*[36] | 152/582 (53.90%) | 160/582 (56.73%) | 151/582 (53.54%) |
| Present study | 56/500 (11.2%) | 60/500 (12%) | 59/500 (11.8%) |

**Contents of the MTB plus kit truenat:**

- Individually sealed pouches, each containing
- Truenat MTB Plusmicro PCR chip
- Microtube with freeze dried PCR reagents
- DNase and RNase free pipette tip
- Desiccant pouch

**Contents of auto MTB sample pre-treatment pack trueprep**

- Liquefaction buffer
- Lysis buffer
- Disposable transfer pipette (graduated)
- Package insert

## RESULTS

Total no of 500 samples were collected in which 60 were showed positive for tuberculosis out of 500 samples 173 were taken from pulmonary and 267 were taken from extrapulmonary tuberculosis. Out 173 pulmonary tuberculosis suspects 32 showed culture positive while 28 suspects were showed culture positive in extrapulmonary tuberculosis. Distribution of both PTB and EPTB positive samples represented by table in which showed 15.60% of sputum followed by 11.76% of pus aspirates, 10.42%of pleural fluid and 9.62% of urine respectively showed in Table 1. representing gender wise distribution of TB positive samples which showed maximum contribution of males than females. Among 500 samples 319 were male and 181 were female. In which 43 out 319 males were positive for tuberculosis while 17 females were positive for tuberculosis out of 181.

**Identification of M. tuberculosis in microscope by ZN staining:** Out of 500 pulmonary and extrapulmonary tuberculosis specimens 4 were showed negative for AFB staining followed by 56 were showed positive for AFB staining (fig.no 7). Among positive AFB 31 smear found positive from pulmonary specimens and 25 smear were found positive from extrapulmonary specimens.

**Culture Identification of M. tuberculosis by Lowenstein Jensen's Medium:** Out of 500 pulmonary and extrapulmonary tuberculosis specimens 60 were showed culture positive (Fig. no 8) and 440 were showed negative for culture Among culture positive specimen 32 were from pulmonary specimens and 28 were found culture positive from extrapulmonary specimens.

**Gene xpert results for pulmonary and extrapulmonary samples:** Out of 500 pulmonary and extrapulmonary tuberculosis specimens 59 MTB were detected by GeneXpert (fig. no 9) followed by 441 were not detected for MTB (fig. no 10). Among them 32 from pulmonary specimens and 27 were detected from extrapulmonary specimens. Out of 60 tuberculosis positive samples 8/60 (13.33%) rifampicin were detected by GeneXpert (fig. no 11)

**Results for pulmonary and extrapulmonary samples by truenat:** Total of 500 pulmonary and extrapulmonary tuberculosis specimens, 442 were not detected for MTB (fig. no 12) followed by 58 MTB were detected by Truenat (Fig. no 13). Among them 32 from pulmonary specimens and 26 were detected from

extrapulmonary specimens. Out of 60, 7 (11.7%) rifampicin were detected resistant by Truenat (fig. no 14). followed by 53 (88.3%) out of 60 were sensitive for rifampicin by Truenat (Fig. no 15).

In present study we observed 56/500 (11.2%) positivity by ZN smear followed by culture 60/500 (12%) positivity by GeneXpert MTB/RIF assay were and 59/500 (11.8%) and positivity by truenat58/500 (11.6%) respectively showed in (Fig. no 16). Out of 60 tuberculosis positive samples 8/60 (13.33%) rifampicin were detected by GeneXpert while 7/60 (11.66%) rifampicin out of 60 were detected by Truenat showed in (Fig. no 17).

## DISCUSSIONS

Tuberculosis (TB) is one of the major public health threats, competing with HIV as a leading cause of death due to infectious diseases worldwide[25]. Despite newer modalities for diagnosis and treatment, unfortunately, millions of people are still suffering and dying from TB[26]. India records the highest burden of both TB and multidrug resistant-TB (MDR-TB)[27]. Global TB report (2017) estimated 10.0 million incident cases of TB (range-9.0-11.1million) equivalent to 133 cases (range-120-148) per 100, 000 population. Most of the estimated number of cases in 2017 occurred in the South-East Asia Region (44%) followed by African Region (25%) Western Pacific Region (18%) a smaller number of cases were reported in the Eastern Mediterranean Region (7.7%) American Region (2.8%) and from European Region (2.7%). Worldwide, among the high TB burden countries which accounted for 87% of all estimated incident cases, eight countries accounted for two thirds of the global total, which are India (27%) followed by China (9%) Indonesia (8%) the Philippines (6%) Pakistan (5%) Nigeria (4%) Bangladesh (4%) and South Africa (3%)[28]. Worldwide, resistance to standard anti-tuberculosis drugs is a major constrain in the control and treatment of tuberculosis[29,30].

Our study describing the characterisation and drug resistance profile of tuberculosis. There have also been some similar studies by Disha Arora *et al*. followed by Luxia Kong *et al.* and Yehia Kadhim Jabber Benellam *et al.* respectively, in this some of which match my study and some show higher percentages showed in Table no 2. In our study prevalence of different diagnostic methods for pulmonary as well as extrapulmonary tuberculosis showed some similar rate in compare with Geeta *et al.* and Bajrami *et al.* while Elbrolosy et al showed high prevalence rate showed in Table no 3.

## CONCLUSION

Increasing drug resistance is the major concern of MTB. The present study was undertaken to determine the characteristics and evaluate the drug resistance profile of MDR-TB species. Study was conducted in order to understand the frequency of drug-resistance and the genotype distribution of M. tuberculosis complex in a tertiary care hospital in northern region of India. Tuberculosis is a serious problem in view of its high occurrence in the worldwide population. The study highlights the importance of using culture for identification as well as drug resistance by GeneXpert With sensitivity and specificity. An alternative to this is to examine for the phenotypic characteristic, i.e., drug resistance. Molecular methods help in understanding the transmission dynamics of tuberculosis and could be used as a tool in the current control programme locally as well as internationally.

**Limitations:** This study was mainly focused on the comparison of various methods to detect tuberculosis like AFB Smear Microscopy followed by solid culture which is a gold standard method for tuberculosis and nucleic acid amplification tests in which GeneXpert Assay and Truenat tests are compared as well as Rifampicin resistance (MDR-TB) is also been checked and the resistance results are also compared by using nucleic acid amplification tests.

Moreover, sound infection control measures to avoid further transmission of MDR/XDRTB, adequate laboratory facilities to monitor drug susceptibility, good health education for patients and research towards implementation of new diagnostic methods, drugs and vaccines should be promoted to control drug resistant TB.

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