

## Rapid Detection of Mycobacterium Tuberculosis and Rifampicin Resistance in Extrapulmonary Samples Using Gene-Xpert-A 3 Year Study

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### Abstract

**Introduction:** Diagnosis of extrapulmonary tuberculosis is still challenging. Number of mycobacterium tuberculosis bacilli present in tissues is often low and it is difficult to obtain clinical specimens from deep seated organs. Depends on culture though it is mainstay of diagnosis is time consuming and patient care is compromised.

**Aims and Objective:** The routinely used methods (cytology and smear microscopy) have suboptimal sensitivity in diagnosing extrapulmonary TB. The current study is carried out to study and assess the role of Gene- expert in diagnosing and to know the resistance in extrapulmonary TB within 2 hrs.

**Methods:** It is a prospective study conducted in department of pulmonary medicine, Guntur for 3 years from September 2016 to August, 2019. We collected extrapulmonary samples (pleural fluid, csf, pus, lymphnode, urine, ascitic fluid, gastric lavage, pericardial fluid) and sent for CBNAAT. Gen -expert was performed on total of 1693 samples during a period of 3 years.

**Results:** Out of 1693 samples, 165 samples were positive for MTB with highest positivity rate for pus followed by lymphnode, urine, pleural fluid and least positivity for CSF. Rifampicin resistance was found in 9% of cases, and high resistance to CSF.

**Conclusion:** CBNAAT is useful for diagnosis and to detect Rifampicin resistance in extrapulmonary TB samples as early as 2hrs. It is not fully satisfactory test, but more sensitive than smear and can detect mycobacterium bacilli and Rifampicin resistance much earlier than culture.

**Keywords:** CBNAAT, Extrapulmonary TB, Rifampicin resistance, CSF, Pleural Fluid.

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### I. Introduction

TB remains a key challenge to global public health and our ability to tackle this disease. It has been severely hampered by inadequate diagnostic assays<sup>1</sup>. Tuberculosis (TB) affects one-third of the global population in developing countries, with annual estimates of 10 million new cases in 2018 and 1.2 million deaths in 2018<sup>2</sup>. While pulmonary TB (PTB) is the most common presentation, extra PTB (EPTB) is also an important clinical condition.

Worldwide, EPTB accounts for approximately 25% of all TB cases and even higher percentages in HIV-infected individuals and children<sup>3</sup>. EPTB other than clinical judgement, the diagnosis relies on additional laboratory support with histopathology, biochemical and cell analysis of fluid, and response to empirical anti-TB therapy. However, the collection of extrapulmonary material often requires invasive procedures, expertise, and it is not easy to obtain additional samples.

Xpert mycobacterium TB (MTB)/Rifampicin (RIF) (Xpert) (Cepheid, Sunnyvale, CA, USA), a fully automated real-time heminested PCR system implementing molecular beacon technology for the diagnosis of PTB infection<sup>4</sup>, has been recently endorsed by the Scientific and Technical Advisory Board of the WHO as the most sensitive, fast test for TB diagnosis in paucibacillary respiratory samples<sup>5</sup>. Diagnosis of extrapulmonary TB (EPTB) remains challenging due to number of tuberculous bacilli present in extra pulmonary samples is low and clinical specimens from deep-seated organs may be difficult to obtain. GeneXpert for use in EPTB is far more complex because of the diversity of clinical sample types.

Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. Since Xpert MTB/RIF was specifically developed and optimized for testing sputum samples in patients with pulmonary TB. Later on the assay have extended to a variety of non-respiratory clinical samples from patients with EPTB. The evidence base for use in the investigation of EPTB remains comparatively weak, however, and many more studies assessing a variety of clinical samples other than sputum are therefore needed. However, compared with pulmonary, lymph nodes are the most common site of involvement in extrapulmonary TB accounting for 35% of EPTB cases followed by pleural effusion and virtually every site of the body can be affected.

**AIM :**

To study and assess the role of CBNAAT in diagnosing and to know the resistance in EPTB

**II. Materials And Methods**

It is a prospective study conducted in department of pulmonary medicine, Guntur Medical College, Guntur, A.P from September 2016 to August 2019, where 1693 extrapulmonary samples are obtained during the clinical routine for 3 years . All these extrapulmonary samples(pleural fluid, csf, pus, lymphnode, urine, ascitic fluid, gastric lavage, pericardial fluid) are sent to the CBNAAT and other biochemical tests like ADA, proteins, differential count, cytology.

**Inclusion criteria:** All cases of presumptive extrapulmonary TB

**Exclusion criteria:** Diagnosed cases of pulmonary TB

**CBNAAT :**

TB detection was done by Xpert MTB/RIF assay, on Extrapulmonary specimens were processed according to the GeneXpert system. Our machine contains 4 cartridges, so 4 samples were processed for each run. According to standard operating procedure, the sampling reagent (containing NAOH and isopropanol) was added at 2:1 ratio to the sample and kept for 15 min at room temperature with intermittent shaking. 2 ml of this treated sample was transferred to the cartridge, and the cartridge was inserted in the module of CB-NAAT machine. An automatic process completed the remaining assay steps, and the results were displayed on the monitor of Gene Xpert after 1 h and 50 min. The test procedure is made biosafe by tuberculocidal property of the assay's sample reagent.<sup>6,7</sup>

**III. Results**

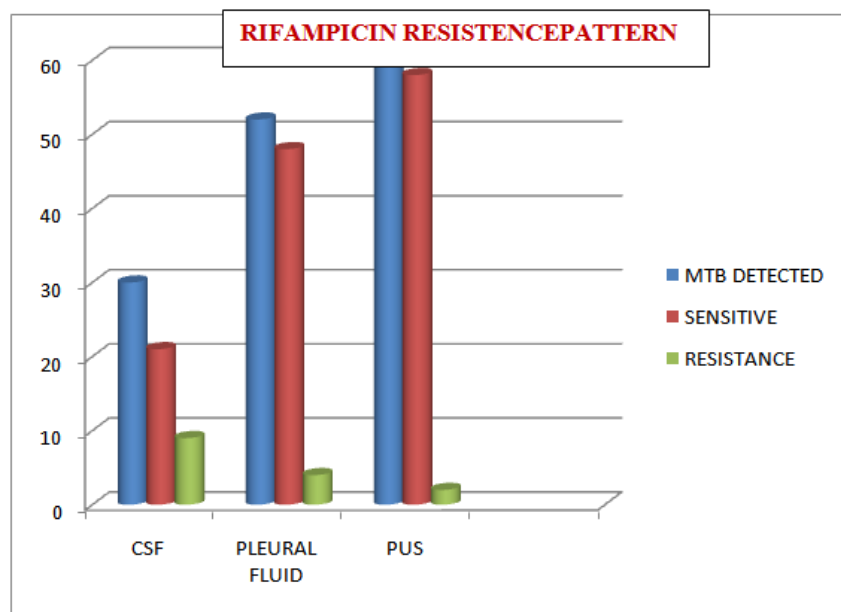
Total of 1693 extra pulmonary samples collected from various departments are included in the study. Suspected EPTB samples are collected and subjected to CBNAAT and other biochemical tests like ADA, proteins, differential count, cytology. The samples include lymph node, pleural pus, pleural fluid, CSF, gastric lavage, peritoneal fluid, ascitic fluid and urine.

Out of 1693 samples, 165 samples were positive for MTB in CBNAAT, with positivity rate more for pleural pus followed by FNAC lymphnode and pleural fluid. Rifampicin resistance was found in 9% of cases. Most of the cases are younger age group between 30 to 40 years, with 60% males and 40% females.

Out of 1693 samples 658 cases are CSF, followed by 602 pleural fluid, 147 pleural pus, 138 ascitic fluid, 51 urine, 55 lymphnode FNAC , 8 synovial and 8 pericardial fluid. MTB detected in 165 cases (9.7%), 15 cases are rifampicin resistance (9%). Out of 15 Rifampicin resistance cases, 9 samples were CSF, 4 are pleural fluid, 2 are pleural pus.

**TABLE: DETECTION IN VARIOUS SAMPLES**

EPTB sample	TOTAL SAMPLES	MTB DETECTED	RIFAMPICIN SENSITIVE	RIFAMPICIN RESISTANT
CSF	658	30	21	9
PLEURAL FLUID	602	52	47	4
PLEURAL PUS	147	60	58	2
ASCITIC FLUID	138	2	2	-
FNAC LN	55	13	13	-
URINE	51	6	6	-
GASTRIC LAVAGE	26	2	2	-
PERICARDIAL FLUID	8	0	-	-
SYNOVIAL FLUID	8	0	-	-



#### IV. Discussion

EPTB contributes to a significant burden of mortality and morbidity due to its complex and subclinical presentations, leading to a delay in diagnosis. Extra pulmonary tuberculosis constitutes 25% of burden of TB globally. EPTB is a pauci-bacillary disease as the number of bacilli are less to detect and are located deep seated in the organs.

Conventional methods like histopathology and smear microscopy are never diagnostic and diagnostic methods like culture methods are time consuming. Hence there is a need for newer and faster diagnostic methods like nucleic acid amplification techniques. CBNAAT is a semi-quantitative nucleic acid amplification test based on molecular detection of mutated gene. It is simple, rapid, cost effective and doesn't require technical expertise.

This study conducted under RNTCP programme for 3 years with large number of EPTB samples. Collected 1693 EPTB samples from various departments and subjected to CBNAAT and other biochemical tests like protein, ADA, cytology.

Most of the cases are younger age group between 30 to 40 years, with 60% males and 40% females. Out of 1693 samples 658 cases are CSF, with highest sample size but lowest sensitivity (4.5) compared to other samples, followed by pleural fluid, pleural pus, ascitic fluid, urine, lymph node FNAC, synovial and pericardial fluid.

MTB detected in 165 cases (9.7%), 15 (9%) cases are rifampicin resistant. Out of 15 cases detected with Rifampicin resistance 9 samples are CSF, 4 are pleural fluid and 2 are pleural pus. Even though lowest sensitivity in CSF, but highest rifampicin resistance is present in CSF.

Among 602 cases of pleural fluid which were sent to CBNAAT, about 151 cases were diagnosed to be malignancy and other etiologies in follow up. 52 cases are AFB positive in CBNAAT recovered well with ATT and 5 cases which were with borderline biochemical tests were diagnosed with CBNAAT. Total 451 pleural fluid samples were analysed, out of which 52 (11.5%) were diagnosed with CBNAAT. The pleural fluid sensitivity is low in our study (11.5%) compared to Denkinger et al, (46%) and Penz et al (37%) studies. This may be due to isolated pleural effusions without much parenchymal lesions in our study. Extensive parenchymal lesions with pleural effusions may increase the sensitivity.

Out of 147 cases of pleural pus samples collected, on follow up 30 cases were found to be of bacterial etiology and recovered with antibiotics. Totally 117 cases were subjected to CBNAAT and 60 cases were detected with Mycobacterium tuberculosis, showing highest detection rate in pleural pus of 51.2%. Detection rate for pleural pus is higher when compared to other studies which is 21% in a study by Tortolli et al and 12% in study by Cause et al. This highest positivity in our study may be due to associated parenchymal TB.

Sensitivity of CBNAAT in lymph node is relatively low in our study. This is probably due to stage of lymph node (non purulent) when FNAC is done and samples which are further sent to excision biopsy to department of surgery were only sent for HPE, not subjected to CBNAAT. The sensitivity pattern of various samples are, 51.2% in pleural pus with highest sensitivity followed by 23.6% for FNAC lymph node, 11.5% pleural fluid, 11.7% urine, 5.5% gastric lavage, with 4.5% of least sensitivity in CSF.

The sensitivities of pleural fluid microscopy and culture are about 10% and 20%, respectively<sup>8,9</sup>. The previous studies have reported much lower sensitivities between 15% and 48%<sup>10</sup>. In this study also, pleural fluid sensitivity was found to be low at 11.5% comparable to that of other studies. Rufai *et al.* showed that the Xpert MTB/RIF assay test has very low diagnostic sensitivity of 14.2% in pleural fluid, even in culture proven cases.<sup>11</sup>

Not only MTB detection but also rapidly determining the patient's multidrug-resistant tuberculosis (MDR-TB) status in such cases is of prime importance in bringing to an end the spread of MDR-TB and decreasing mortality. Treatment under guidelines on programmatic management of drug-resistant TB (PMDT) could be started in fifteen (9%) patients with RIF resistance detected by Xpert RIF/MTB in the present study.

## V. Conclusion

CBNAAT is a useful test to confirm presence of MTB, with simultaneous detection of rifampicin resistance in EPTB samples. It has highest sensitivity for pleural pus and rifampicin resistance in (9%) of cases, to start early DR-TB treatment according to PMDT guidelines. CBNAAT can be used as adjunctive to culture in extrapulmonary samples.

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