www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i8.183

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Rapid detection of Mycobacterium tuberculosis and Rifampicin Resistance in extra pulmonary samples using GeneXpert MTB/RIF assay

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ABSTRACT
Background: *GeneXpert MTB/RIF assay is efficient and reliable technique for the rapid diagnosis of extra pulmonary TB (EPTB), especially in smear negative cases. For rifampicin resistance detection, it provides accurate results and can allow rapid initiation of MDR-TB treatment.*
Objectives: *The aim of the study is to evaluate Gene Xpert MTB/RIF assay for rapid detection of Mycobacterium tuberculosis and rifampicin resistance in extra pulmonary samples.*
Materials and Method: *This study was done in the department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna during the period of February to July 2016. Extra pulmonary samples were collected from clinically suspected cases of extra pulmonary tuberculosis and were subjected for Z-N staining, fluorescent microscopy and Gene Xpert MTB/RIF assay.*
Result: *123 extra pulmonary samples included 21 pus, 28 pleural fluids, 14 ascitic fluids, 7 CSF, 16 lymph nodes, 6 synovial fluid, 15 bronchoalveolar lavage (BAL) and 16 endometrial samples. Out of these, 20.3% patients were GeneXpert MTB/RIF Assay positive, 5.6% were positive on fluorescent microscopy and 4.0 % were ZN positive. Rifampicin resistance was found in pus sample, pleural fluid, and lymph node sample.*
Conclusion: *Gene Xpert MTB/RIF assay is rapid method for diagnosis of extra pulmonary tuberculosis as compared to conventional methods. Because of its simplicity, rapidity and sensitivity, this seems to be a very gorgeous tool for diagnosis of extra pulmonary tuberculosis from clinical samples.*
Keywords: *Extra pulmonary tuberculosis, Mycobacterium tuberculosis, Gene Xpert MTB/RIF assay, Rifampicin resistance.*

INTRODUCTION

India has been engaged in Tuberculosis (TB) control activities for more than 50 years. Yet TB continues to be India's severest health crisis. TB kills an estimated 480,000 Indians every year and more than 1,400 every day. India also has more than a million 'missing' cases every year that are not notified and most remain either undiagnosed or unaccountably and inadequately diagnosed and treated in the private sector[1].

Tuberculosis is typically divided into two types, pulmonary which is more widespread and extra

pulmonary which rivet 15% cases. Extra pulmonary tuberculosis (EPTB) can involve any organ in the body. Extra pulmonary infections with members of the Mycobacterium tuberculosis complex (MTBC) have high morbidity and mortality because of lack of good diagnostic methods. Diagnosis is often difficult to establish due to low number of bacteria and collection of extra pulmonary samples is not easy. A definitive diagnosis of mycobacterium infection depends on detection of the *Mycobacterium Tuberculosis* in extra pulmonary samples[2].

Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. The US CDC recommends that nucleic acid amplification tests be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB[3]. However, no recommendation exists for their use in the investigation of patients suspected of having EPTB as the evidence base is limited. The Xpert® MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics [4,5]. This multifunctional diagnostic platform is an automated, closed system that performs real-time PCR and can be used by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h. Sputum samples can be analyzed with very minimal processing, yielding positive diagnoses in 99–100% of patients with smear-positive pulmonary TB and 57–83% of patients with smear-negative pulmonary TB in clinical evaluation studies[4]. The Xpert MTB/RIF assay was rapidly endorsed by the WHO in December 2010 as a replacement for sputum smear microscopy, particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB[6]. Since Xpert MTB/RIF was specifically developed and optimized for testing sputum samples and initial large-scale evaluations were in patients with pulmonary TB, WHO endorsement specifically applied to the investigation of pulmonary TB. More recently, however, evaluations of the assay have extended to a variety of nonrespiratory 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 disease, investigation for use in EPTB is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analyses and in extraction of MTB DNA from samples, the challenge of providing a rigorous gold standard for comparison, and the range of potential ways of processing samples prior to analysis.

AIM AND OBJECTIVES

This study aims at:

- Detection of Mycobacterium Tuberculosis in extra pulmonary samples.
- Detection of resistance to Rifampicin of the bacterium present in sample.

MATERIAL AND METHOD

Specimens. All nonrespiratory specimens that were submitted to the Department of Microbiology, IGIMS, Patna during the period of February 2016 to July 2016 were included in the study. The specimens originated from patients with suspected *M. tuberculosis* or nontuberculous mycobacterial (NTM) infection on the basis of clinical criteria or to rule out these infections. 123 extra pulmonary samples included 21 pus, 28 pleural fluids, 14 ascitic fluids, 7 CSF, 16 lymph nodes, 6 synovial fluid, 15 BAL , 16 endometrial samples.

Specimen processing. All specimens were processsed by the standard *N*-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) method with a final NaOH concentration of 1%. After the centrifugation step, the sediment was resuspended in 1.0 to 1.5 ml of sterile phosphate buffer (Ph 6.8).

AFB smears. After processing of the specimens, smears were prepared from all samples other than

urine and were examined for the presence of AFB. All smears were stained by Z-N staining and fluorescent staining.

Xpert procedure. The Xpert assay was performed according to policy recommendations issued by WHO.

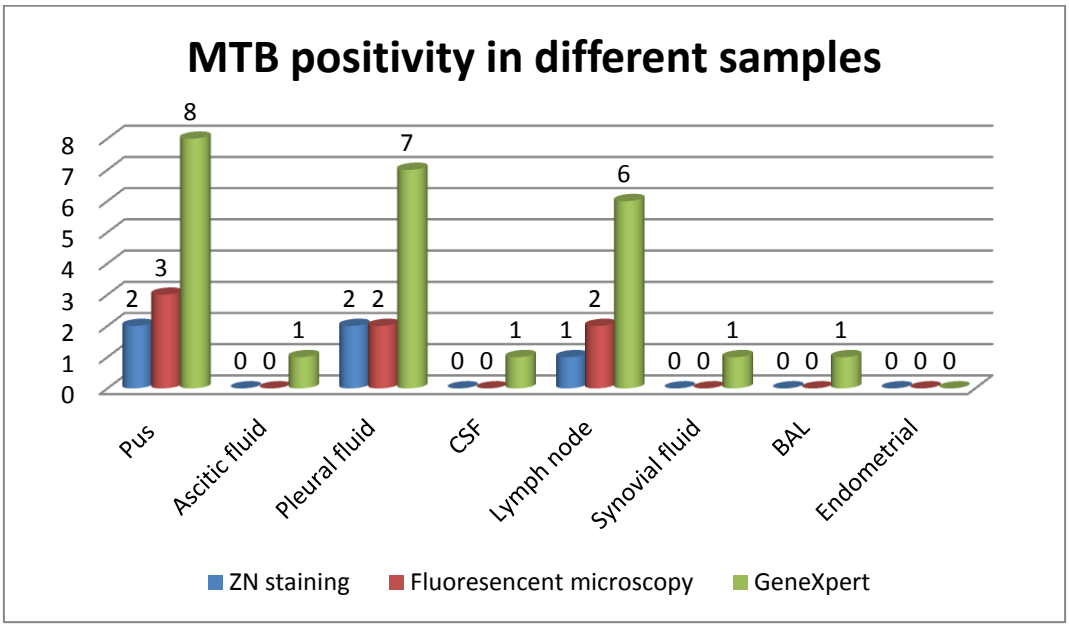
RESULT

123 extra pulmonary samples included 21 pus, 28 pleural fluids, 14 ascitic fluids, 7 CSF, 16 lymph nodes, 6 synovial fluid, 15 BAL , 16 endometrial samples.

Table 1: Distribution of samples

Sample	Number	Percent
Pleural fluid	28	22.8
Pus	21	17.0
Endometrial	16	13.0
Lymph node	16	13.0
BAL	15	12.2
Ascitic fluid	14	11.4
CSF	07	5.7
Synovial fluid	06	4.9
TOTAL	123	100.0

20.3% patients were Gene Xpert MTB/RIF Assay positive, 5.6% were positive on fluorescent microscopy and 4.0 % were ZN positive. MTB was detected in 8 out of 21 (38.1%) Pus samples, 7 out of 28 (25.0%) Pleural fluid samples, 1 out of 14 (7.1%) Ascitic fluid samples and 1 out of 7 (14.3%) CSF samples, 6 out of 16 (37.5%) lymphnodes, 1 out of 6 (16.7%) synovial fluid, 1 out of 15(6.7%) BAL, and 0 out of 16 (0.0%) endometrial samples.



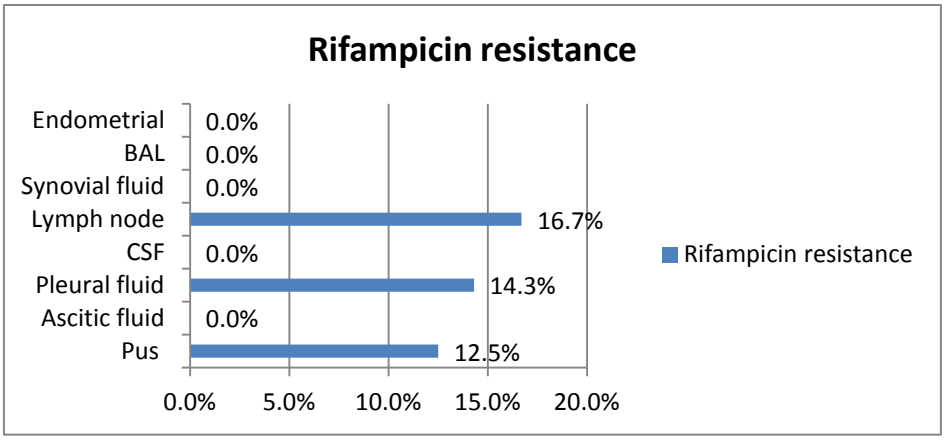
Out of 123 cases, 7 cases of Gene Xpert MTB/RIF assay positive were fluorescent microscopy positive, 18 cases of Gene Xpert MTB/RIF assay positive were fluorescent microscopy negative, 98 cases of both Gene Xpert MTB/RIF assay and fluorescent microscopy were negative.

Out of 123 cases, 5 cases of Gene Xpert MTB/RIF assay positive were Zn smear positive, 20 cases of Gene xpert MTB/RIF assay positive were Zn smear negative, 98 cases of both Gene Xpert MTB/RIF assay and Zn smear were negative. It is noted that none of the Zn smear positive and fluorescent microscopy positive samples gave negative results by Gene Xpert. On the other hand all the Zn negative and fluorescent microscopy negative samples were also negative on Gene Xpert indicating Xpert MTB/RIF assay is highly sensitive and specific technique.

Table 2: Comparison of Gene Xpert assay with ZN smear and fluorescent microscopy results

Gene Xpert and MTB/RIF Assay	Diagnosis on Ziehl-Nelsen		Diagnosis on Fluorescent microscopy	
	Positive	Negative	Positive	Negative
Positive Results of Gene Xpert	5	20	7	18
Negative Results of Gene Xpert	0	98	0	98
Total count	5	118	7	116

Rifampicin resistance was seen in 1(12.5%) pus sample, 1(14.3%) pleural fluid, and 1(16.7%) lymph node sample.



DISCUSSION

Conventional laboratory techniques like direct microscopy for the diagnosis of tuberculosis are far from being sensitive. Moreover, cultures are time-consuming, require biosafety measures, and need trained laboratory personnel.

Molecular techniques have substantially changed the field of tuberculosis diagnosis and have been proven to yield rapid results while being highly sensitive[7]. Numerous PCR assays employing a number of different *M. tuberculosis* targets have recently been described[8.9]. The new Xpert assay tested in our study targets the rifampin resistance-associated *rpoB* gene region by heminested PCR with three specific primers and combines the sensitive detection of *M. tuberculosis* DNA and determination of RMP resistance. Furthermore, the hands-on time is short due to automation of bacterial lysis, DNA extraction, real-time PCR amplification, and amplicon detection in a single system.

In this study, a total of 123 extra pulmonary samples were processed. 20.3% patients were Gene Xpert MTB/RIF Assay positive, 5.6% were positive on fluorescent microscopy and 4.0 % were ZN positive. In a study done by Scott et al in 2014, *M. tuberculosis* positivity determined by Xpert MTB/RIF in extra pulmonary samples was 22% (260/1,175). Stephen D Lawn[10] in 2012 used same technique Xpert MTB/RIF for diagnosis of extra pulmonary Tuberculosis. Out of the total of 268 samples the positivity rate observed for tissue biopsies or fine-needle aspirates (35%), gastric aspirates (23%), pus (21%), urine (6%), CSF (5%) and other body fluids i.e. peritoneal, synovial and pericardial (4%). In another study done by Sajed et al[11] in 2014, MTB was detected in 31 out of 60 (51.7%) pus samples, 3 out of 19 (15.8%) pleural fluid samples, 1 out of 16 (6.3%) ascitic fluid samples and 2 out of 5 (40.0%) cerebrospinal fluid samples.

The study revealed that the Xpert test has true diagnostic potential with good sensitivity for specimens such as pus which is difficult to diagnose by other laboratory techniques.

In a study by Hilleman et al[2] in 2011, out of 26 samples, 25 were found to be susceptible and 1 was found to be resistant. In a study done by Scott

et al[12] in 2014, by Xpert MTB/RIF, which provided an early diagnosis of RIF resistance (9.6%) in 25/260 cases (with 100% specificity). In this study, only 3 samples were found to be rifampicin resistant out of 123 samples.

CONCLUSION

Gene Xpert MTB/RIF assay is efficient and reliable technique for the rapid diagnosis of extra pulmonary TB, especially in smear negative cases. Its simplicity, sensitivity, speed and automation, make this technique a very attractive tool for diagnosis of *Mycobacterium tuberculosis* from extra pulmonary samples in MDR cases and smear negative cases of TB suspects.

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