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CBNAAT: Rapid Diagnostic Tool for Detection of Mycobacterium Tuberculosis in Pulmonary Samples

Priya Srivastava[1], Sarvesh Gautam[2], Dr. AnjuTyagi[3]
[1, 2]*Institute of Applied Medicines and Research, Ghaziabad*
[3]*Assistant Professor, Institute of Applied Medicines and Research, Ghaziabad*

Abstract: ***Introduction: Multi drug resistant (MDR) tuberculosis is a major problem worldwide. There is an urgent need to diagnose MDR cases and initiate treatment to interrupt the chain of transmission of Mycobacterium Tuberculosis. Rapid detection of tuberculosis and Rifampicin (RIF) resistance are vital for successful disease management. CBNAAT (Xpert MTB/ RIF) is a fully automated diagnostic test which detects tuberculosis and rifampicin drug resistance simultaneously in few hours.***
Aim of the Study: The aim of this study was to evaluate the sensitivity and specificity of GeneXpert assay in patients with smear positive and smear negative sputum samples of suspected pulmonary tuberculosis and compared with AFB smear microscopy (ZN staining)
Material and Methods: This is an observational study conducted at Community Health Centre, Muradnagar, from January, 2019 to April, 2019. Total 377 patients showing symptoms and signs of pulmonary tuberculosis or having close contact with previously diagnosed tuberculosis patients in the hospital were included in this study. All sputum samples were tested for MTB/RIF detection on GeneXpert.
Result: Out of the 377 sputum samples of the patients having signs and symptoms of pulmonary tuberculosis, 145 (38.46%) were MTB positive and 232 (61.53%) were negative. The CBNAAT test identified the agent in 109 out of 114 smear positive sputum samples, and 36 out of 263 sputum smear negative cases. 13 patients out of 377 (3.44%) suspected cases were found to be rifampicin resistant. In our study, overall sensitivity and specificity of GeneXpert were 95.6% and 86.31% respectively which is quite similar with other studies.
Conclusion: CBNAAT is a convenientmolecular tool in the identification of pulmonary TB cases because of its easiness and fast turnaround time. It is more viable as compared to AFB smear in the analysis of TB cases and should be routinely utilized for quick detection of TB along with other methods like AFB smear examination and culture DST for better results in the diagnosis of TB.
Keywords: Tuberculosis; MDR-TB; Rafimpicin Resistance; GeneXpert; CB-NAAT; diagnosis

I. INTRODUCTION

The reported cases of Tuberculosis (TB) are around 95 % till date, which relates to poverty. Out of which 98 % cases results as death in developing countries. India is one of the high tuberculosis (TB) burden nations within the world accounting for about 20% of the worldwide incidence constituting 9.4 million TB cases. India secures second position in possessing multi drug resistant (MDR) TB cases, i.e., around 99,000 cases [3]. In 2013, 5% of all TB cases over the world were evaluated to be MDR-TB, in which 3.5% cases were newly diagnosed and 20.5% were previously treated TB cases [7].According to WHO reportpublished in 2016, 3.9% of new cases and 21% of previously treated cases havingMDR-TB or rifampicin-resistant TB were estimated worldwide in 2015 [10]. The biggest threat of TB is Multi-Drug Resistance pattern. Presence of Multi drug resistance is due to mismanagement of tuberculosis patients, incorrect diagnosis, and delay in diagnosis, interrupted treatment and mistreatment of both first and second line drugs.False results and misdiagnosis of TB plays an important role in diagnosis of TB. Wrong diagnosis of TB suspects are common in emerging countries, as most TB control programmes utilizes Ziehl-Neelsen (ZN) smear microscopy, which has poor affectability and several visits are required that leads to default results. Mycobacterial culture, even though taken as the gold standard but is time taking and usually takes 2-6 week time for final result and needs proper organization and technical expert knowledge [11]. Such delays in diagnosis increases morbidity and mortality results in the spread of resistant strains. Thus, a new molecular technique, Cartridge Based Nucleic Acid Amplification test (CB-NAAT), named GeneXpert system was introduced by WHO in December, 2010.

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The GeneXpert utilizes PCR technique for simultaneous identification of *Mycobacterium tuberculosis* and mutations related to Rifampicin resistance. It is a completelyautomatic bench top cartridge based nucleic acid amplification (CB-NAAT) assay for detection of tuberculosis that involves all the general steps of PCR. It provides results within 2 hours. Diagnostic precision of GeneXpert for pulmonary TB has been found to be high [8]. Patients with high chanceof tuberculosis like possible HIV-associated TB patients and pediatric presumptive including extra pulmonary cases in whom AFB smear examination is usually negative, are the mostly benefited from GeneXpert [1].

The objective of the study was to estimate the sensitivity and specificity of GeneXpert assay in patients with symptoms suspected of pulmonary tuberculosis and compared with AFB smear microscopy (ZN staining).

II. MATERIAL AND METHODS

This study was conducted at Community Health Centre, Muradnagar, after taking ethics committee approval and a part was done at Institute of Applied Medicines and Research, Ghaziabad from January 2019 to June 2019. Total 377 patients having symptoms and signs of pulmonary tuberculosis or having previous close contact with tuberculosis patients admitted in the hospital were included in this study. Patients with Human Immunodeficiency Virus infection were excluded from the study.

Each sputum sample received in the lab was divided into two parts; one part was tested using GeneXpert and second part used for ZN smear microscopy.

A. Collection of Specimens

A good sputum sample consists of freshly released material from the bronchial tree with low amounts of oral or nasopharyngeal material. Good quality refers to the occurrence of mucous material. Ideally, the sputum specimen should have a volume of 3-5 ml. The patient must be advised to collect the sputum sample in a sterile container (falcon tube) after thorough rinsing of the oral cavity with clean water. Sputum should be collected in the early morning.

B. Transportation of Specimens

Fresh sputum samplesmust be transferred from the DMC (District Medical Committee) to the RNTCP (Revised National Tuberculosis Control Program) – certified CBNAAT laboratory in cold chain within 72 hours. Two innovative models for specimen collection and transport using fresh samples in falcon tubes to be transported in cold chain using gel packs and their technical specifications have been developed by Gujarat and Andhra Pradesh.

1) Gujarat Model (from peripheral DMCs)

Total capacity of 4 falcon tubes

ThermacolBox: Outer Dimensions (in cm) - (18.5 x 12.5 x 13), Inner Dimensions (in cm) – (14.25 x 8.23 x 10.25)

No. of gel packs required: 2

Weight of fully packed box: 400 grams

2) Andhra Pradesh Model (from high burden TU/DTC DMCs)

Total capacity of 8 falcon tubes

Thermacol Box: Outer Dimensions (in inches) - (11 x 9 x 9), Inner Dimensions (in inches) – (8 x 6.5 x 6.5)

No. of gel packs required : 2

Weight of fully packed box : 1.5 Kg

C. Xpert MTB/RIF

GeneXpert testing was executed according to the manufacturer's instruction manual (Cepheid, Sunnyvale, CA). This technique utilizes single-use, plastic cartridges that are pre- loaded with lyophilized reagent beads and buffers necessary for the processing of sample, DNA extraction and nested real-time PCR. Clinical sputum specimens are added with Sample Reagent (SR) containing sodium hydroxide and isopropanol and stored at room temperature for about 15 min. The incubated sample is further transferred manually to the CBNAAT cartridge and loaded into the Gene Xpert instrument. Further procedure is fully automatic.

D. Ziehl Neelsen Staining

Make a thin smear of sputum sample and heat fix it. Place the slide and pour carbolfuschin over smear, heat gently and allow it to stand for 5 minutes. Rinse smears with water until no color appears in the effluent. Pour 20% sulphuric acid, wait for one minute and keep on repeating this step until the slide appears light pink in color (15-20 sec). Wash well with clean water. Cover the smear with methylene blue stain for 1–2 minutes. Wash off the stain with clean water. Examine the smear microscopically.

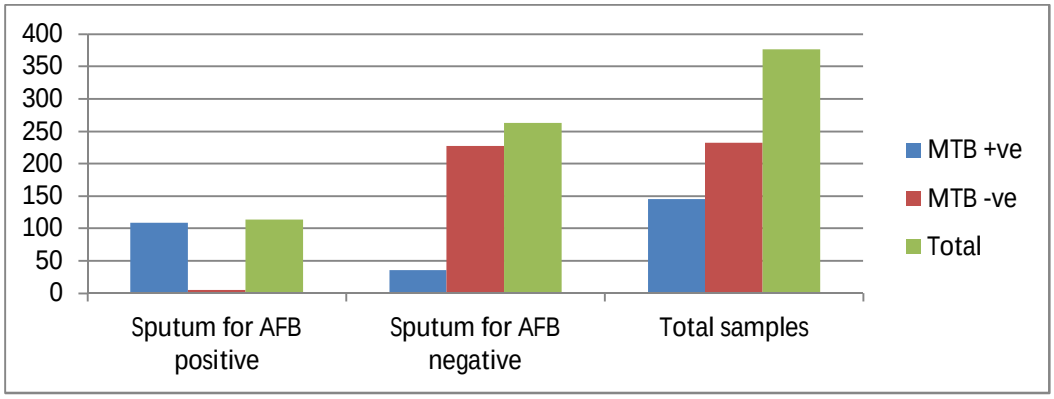
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III. RESULTS

ZN staining was performed for 377 samples of the patients who have previous history of pulmonary tuberculosis. Out of these 114 (30.23%) samples were found to be smear positive and 263 (69.49%) were negative (Table 1).All the samples were then subjected to Gene Xpert ® MTB/RIF assay. Out of the 377 samples of the patients, 145(38.46%) were MTB positive and 232 (61.53%) were negative. The GeneXpert test identified the agent in 109 out of 114smear positive cases, and 36 out of 263 smear negative cases.

Table 1: Comparative results of GeneXpert and AFB Microscopy

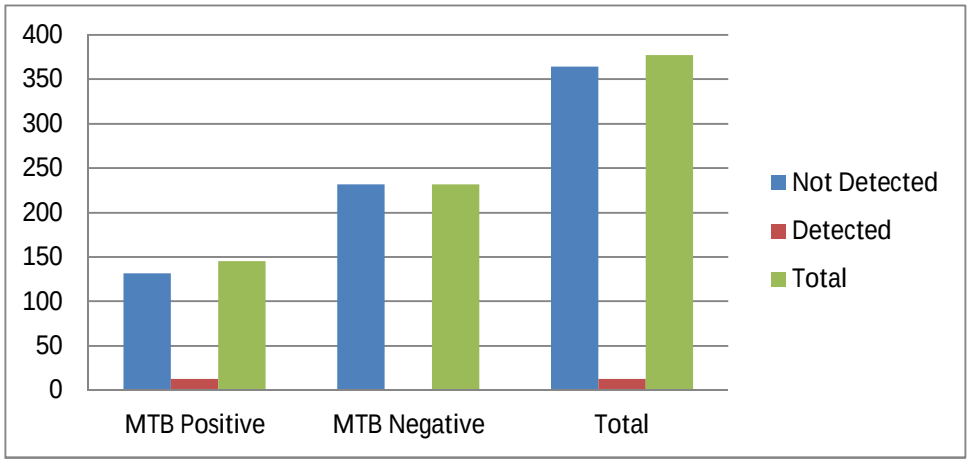
GeneXpert	Sputum for AFB positive	Sputum for AFB negative	Total samples
MTB +ve	109	36	145
MTB -ve	5	227	232
Total	114	263	377



The results of ZN staining are compared with GeneXpert in our study. It is concluded that Gene Xpert MTB/RIF is more convenient than ZN staining. As compared to ZN staining, MTB can be detected even in 1ml sputum sample using GeneXpert test. The advantage of Gene Xpert is that it detects Rifampicin (RIF) resistance associated mutations and helps us to identify MDR TB. In table 2, 13 patients out of 377 (3.44%) suspected cases were found resistant to rifampicin.

Table 2: Results of Rifampicin Resistance Detection from GeneXpert

Rifampicin Resistance	MTB Positive	MTB Negative	Total
Not Detected	132	232	364
Detected	13	0	13
Total	145	232	377



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IV. DISCUSSION

In this study, we evaluate the diagnostic yield of Gene Xpert to detect Mycobacterium tuberculosisin sputum samples and compared it with ZN Staining. GeneXpert is a simple bench top diagnostic technique that can be done with minimum training. The results are available in about 2 hours that is much prior than the culture which take few days to come positive.

In our study, the MTB/RIF test identified the causative agent in 145 out of 377 pulmonary specimens whereas sputum for AFB detects only 114 out of 377 pulmonary specimens. Out of 114 smear positive cases, MTB was identified in 109 cases, by MTB/RIF test and out of 263smears negative cases 36 cases were identified. A past research reveal that the MTB/RIF assay had a calculated detection limit of 131colony-forming units(CFU)/ml of sputum and was able to identify as few as 10 CFU/ml of sputum in 35% testsrelated with approximately 10,000 CFU/ml with traditional smear microscopy.

Variousother studies verified the efficacy of GeneXpert in diagnosing pulmonary tuberculosis. In this study, overall sensitivity and specificity of GeneXpert were found to be 95.6% and 86.31% respectively which is similar to other studies. In other studies, GeneXpert sensitivity and specificity for sputum sample was from 81%-92% and 71%- 100% which is related with our study as shown in Table 3.

Table 3: Various studies showing sensitivity and specificity of GeneXpert

Study	Sensitivity	Specificity
Pierrae et al. [6]	80%	98.6%
HY Lee et al. [5]	81.6%	100%
Barnard et al. [2]	92.3%	87.7%
Khalil KF et al. [4]	91.86%	71.42%
Sharma SK et al. [9]	90%	100%

The GeneXpert test is simple to execute and is less reliant on the operator'sexpertise. This test can be performed by routine staff with minimum training. It usually takes 1-2 days to instruct the technicians.Addition of buffer and sample are done manually and the further processing are automated. The results are available within 2 hours. Each module can process 4 samples in single time and because it is a closed system, contamination chances are less. Although the GeneXpert test is a useful tool for fast detection of rifampicin-resistant M. tuberculosis, the test results always needs to be confirmed by culture and Drug Susceptibility Testing.

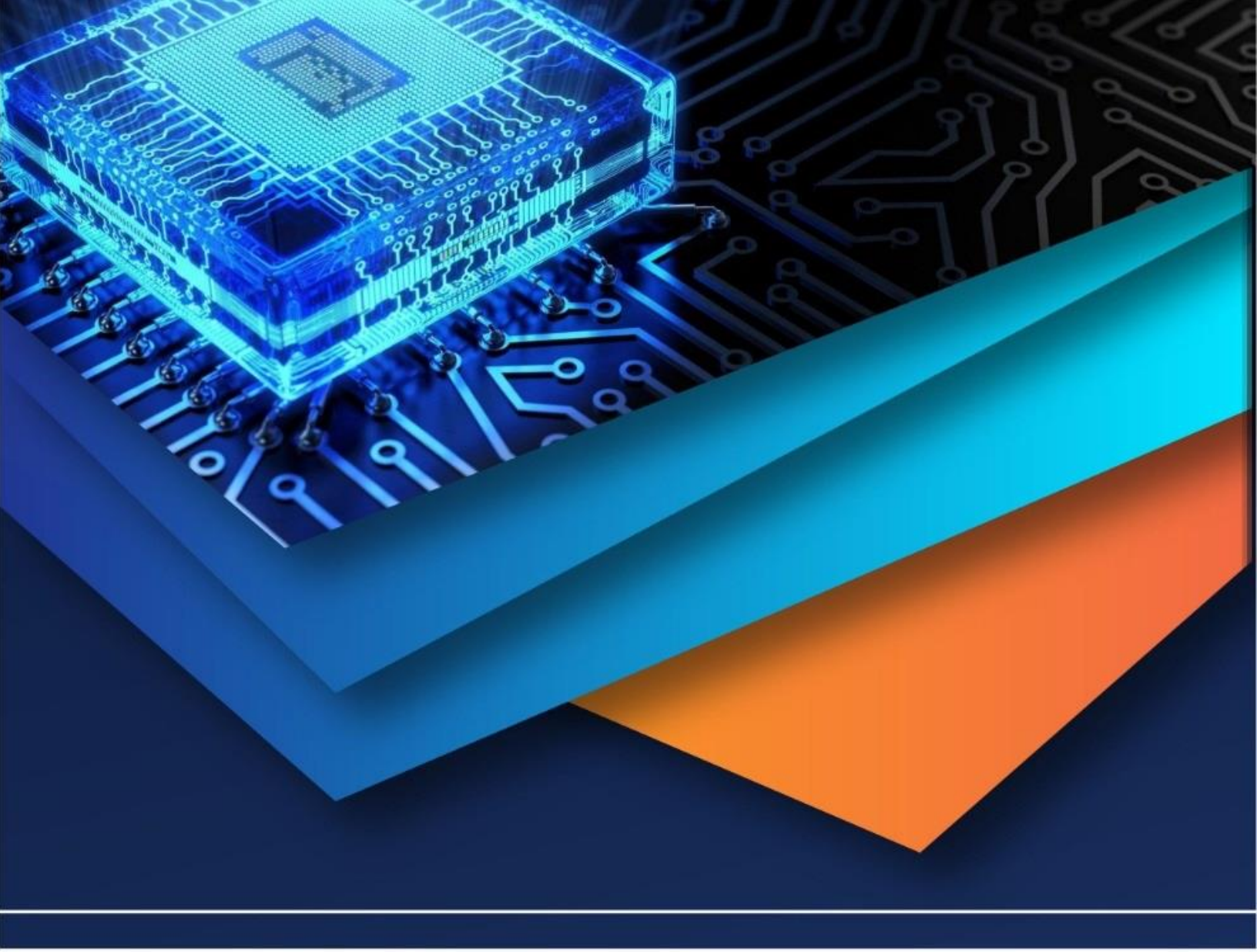
V. CONCLUSION

Specificity of GeneXpert and AFB smear microscopy is almost found to be same but sensitivity of GeneXpert isfound higher than AFB smear microscopy in sputum samples. Culture is usually taken as a standard method but it usually took days to give positive result and identification of Rifampicin resistance is not possible with it. On the other hand,GeneXpert is a convenient method in diagnosing patients of pulmonary tuberculosis due to its fast and simultaneous detection of Rifampicin resistance particularlyuseful in patients with MDR-Tuberculosis.

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