

Rapid Detection of *Mycobacterium tuberculosis* by Cartridge-based Nucleic Acid Amplification Test in a Rural Tertiary Care Hospital in Khanpur Kalan, Sonapat Haryana

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Abstract

Introduction: Tuberculosis (TB) is a major public health problem the infection affects up to one-third of the world population. Early diagnosis is needed for early patient management and successful patient treatment. The TB situation is worsened by the emergence and spread of multidrug resistant (MDR) TB cases. Mycobacterial culture is considered as the gold standard but is slow and usually takes 2–6 weeks' time to get the final result. Cartridge-based nucleic acid amplification test (CBNAAT) can detect TB along with rifampicin resistance in <2 h. This study was done to detect MDR TB by CBNAAT machine in a rural tertiary care hospital.

Materials and Methods: In the present study, samples presumptive of TB since February 2018 to July 2019 were subjected to CBNAAT for the diagnosis of rifampicin resistance.

Results: In the present study, total number of 3281 presumptive TB samples was tested by CBNAAT. Out of 3281 presumptive TB samples, 963 (29.35%) were *Mycobacterium* positive and rifampicin sensitive and 66 (2.01%) were positive for *Mycobacterium tuberculosis* and were rifampicin resistant. *Mycobacterium* was not detected in 2252 (68.64%) cases.

Conclusion: Detection of rifampicin resistant TB by CBNAAT is done within few hours. Consequently early diagnosis of TB patients helps in early and precise treatment and prevents transmission of MDR strains of TB in the community.

Key words: Cartridge-based nucleic acid amplification test, Multidrug resistant, Rifampicin, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a major public health problem the infection affects up to one-third of the world population, and almost 2 million people are killed by TB each year.^[1] India is the highest TB burden country in the world. The global incidence of multidrug resistant (MDR) TB is 630,000 cases. India have one-tenth of the global burden with 64,000 cases.^[2]

Early diagnosis is needed for early patient management and successful patient treatment. The TB situation is worsened by the emergence and spread of MDR TB cases, defined as simultaneous resistance to at least rifampicin and isoniazid, with or without resistance to any other drug. False-negative results and misdiagnosis of TB suspects are common in developing nations, as most TB control programs use Ziehl-Neelsen smear microscopy, which has poor sensitivity and multiple visits are required that leads to higher default. Mycobacterial culture is considered as the gold standard but is slow and usually takes 2–6 weeks' time to get the final result and it requires proper infrastructure and technical expertise.^[3]

In December 2010, WHO recommended use of a new Cartridge Based Nucleic Acid Amplification test

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(CB-NAAT), named GeneXpert system. The Xpert MTB/RIF assay employs five distinct molecular nucleic acid probes, each labeled with a differentially colored fluorophore and responding to a specific nucleic acid sequence within the *rpoB* gene of *Mycobacterium tuberculosis*. It can detect TB along with rifampicin resistance in <2 h.^[4]

CBNAAT technique is not liable to cross-contamination; it requires minimal Biosafety facilities and has a high sensitivity in smear-negative pulmonary TB. The diagnosis of extrapulmonary TB (EPTB) is often difficult to establish, because number of bacteria in the specimens is often very low and collection often requires invasive procedures, and it is not easy to obtain multiple samples. GeneXpert is a useful tool for extrapulmonary specimens.^[5]

This study was done to detect MDR TB by CBNAAT machine in a rural tertiary care hospital.

MATERIALS AND METHODS

Inclusion Criteria

Patients with clinical suspicion of pulmonary TB including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, hemoptysis, and loss of appetite were included in the study.

Exclusion Criteria

The following criteria were included in the study:

1. Samples received without clinical history.
2. Patient with history of lung malignancies or fungal infections.

This study was done at rural tertiary health care hospital. The study was conducted in the department of microbiology for 1½ year. In the present study, samples presumptive of TB since February 2018 to July 2019 were subjected to CBNAAT for the diagnosis of TB and rifampicin resistant TB. Total of 3281 sputum samples of the patients with symptoms suggestive of pulmonary TB including both new cases and on treatment were received. All specimens were collected in pre-sterilized falcon tubes with three layer packing system, samples along with prescribed Performa containing details of patients such as name, address, age, and sex.

TB detection was done by Xpert MTB/RiF assay, made by Cepheid-Sunnyvale-USA. Sputum specimens were processed according to the GeneXpert Dx system operator manual given by Central TB division, Government of India, Guidance document for use of CB-NAAT under RNTCP. The assay is designed for extraction, amplification and identification of *rpoB* gene of *M. tuberculosis* as it accounts for more than 95% of mutations associated with rifampicin resistance, ensuring high degree of specificity by use of three specific primers

and five unique molecular probes. The number of positive beacons, their detection timing indicated by rise of fluorescent signal above a predetermined baseline cycle threshold and the results of sample processing controls, allows the test to distinguish among the following results: No TB; TB detected, rifampicin resistance detected; TB detected, no rifampicin resistance detected; TB detected, rifampicin resistance indeterminate; and an invalid result. Xpert MTB/RIF cartridge is a disposable, single self-enclosed test unit in which all steps of NAAT, that is, sample processing, PCR amplification, and detection are automated and integrated. The manual steps involved in the assay are adding reagent to liquefy sputum and sample loading. The test procedure is made biosafe by tuberculocidal property of the assay's sample reagent.

RESULTS

This study was done at rural tertiary care hospital. The study was conducted in the department of microbiology for 1½ year. In the present study, samples presumptive of TB since February 2018 to July 2019 were subjected to CBNAAT for the diagnosis of TB and rifampicin resistant TB.

In the present study, total number of 3281 presumptive TB samples was tested by CBNAAT. In 3581 presumptive TB samples, *Mycobacterium* was not detected in 2252 (68.64%) cases. Out of 3281 presumptive TB samples, 963 (29.35%) were *Mycobacterium* positive and rifampicin sensitive [Figure 1]. Sixty-six (2.01%) were positive for *M. tuberculosis* and were rifampicin resistant.

DISCUSSION

In the present study, total number of 3281 presumptive TB samples was tested by CBNAAT. In 3581 presumptive TB samples, *Mycobacterium* was not detected in 2252 (68.64%) cases.

Out of 3281 presumptive TB samples, 963 (29.35%) were *Mycobacterium* positive and rifampicin sensitive. Sixty-six (2.01%) were positive for *M. tuberculosis* and were rifampicin resistant.

In the study done by Tripathi, *et al.* Status of drug resistance detected by CBNAAT, rifampicin sensitive TB was detected in 35.8%, and rifampicin resistant TB was in 53% samples by CBNAAT. In 10.7% cases, TB was not detected.^[2]

In a study done by Agrawal *et al.* a total of 170 respiratory specimens (149 BAL and 21 Sputum samples) were tested. Among 170 samples, 42 samples (24.7%) were GeneXpert TB positive.^[3]

In the study done by Arora *et al.* 84.21% samples were rifampicin sensitive and 15.78% samples were rifampicin resistant.^[4]

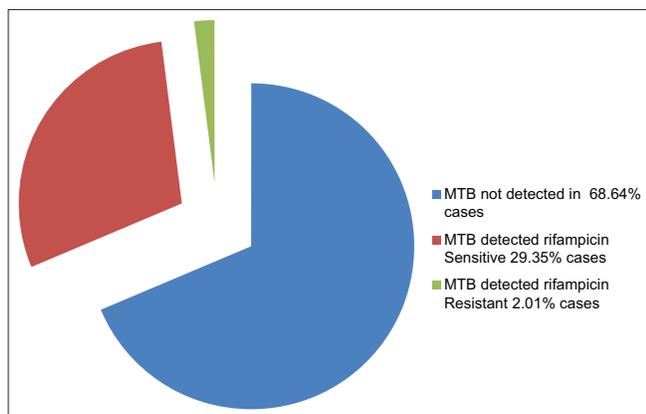


Figure 1: Detection of *Mycobacterium tuberculosis* by cartridge-based nucleic acid amplification test

According to RNTCP report 2018, in the year 2017 total 10,77,377 number of test were performed by 628 CBNAAT machines out of which 37,488 (3.48%) were rifampicin resistant TB.^[6]

In the study done by Chikaonda *et al.* the Xpert MTB/RIF assay detected rifampicin resistance in 64/995 (6.4%) specimens.^[7]

In a study done by Metcalfe *et al.* 28% samples were rifampicin sensitive and 20% samples were rifampicin resistant.^[8]

In the study done by Iram *et al.* out of total 245 sample MTB was detected in 111 (45.3%) cases.^[5]

In the study done by Sasikumar *et al.* the study enrolled 257 presumptive TB cases which included 132 pulmonary and 125 extrapulmonary presumptive TB cases Out of a total of 104 pulmonary TB cases, 73 were rifampicin-sensitive and 31 were rifampicin-resistant cases. 103 EPTB cases included 66 rifampicin-sensitive and 37 rifampicin-resistant cases.^[9]

In the study done by Youngs *et al.* MTB was detected in 60 of 100 (60%) of CBNAATs and *rpoB* mutations that is rifampicin resistance was in 3 of 60 (5%) of MTB-D samples.^[10]

In the study done by Sachdeva and Shrivastava samples subjected to CBNAAT, 58.3% were found to be positive for TB.^[11]

In the study done by Chakraborty *et al.* in Pleural fluid samples CBNAAT were positive for MTB in (32%)

subjects. Out of these patients, rifampicin resistance was detected in 8.3% individuals. In sputum, CBNAAT MTB was detected in 10.6% subjects. Among them, 12.5% had rifampicin resistance.^[12]

CONCLUSION

Inability to rapidly diagnose and treat the affected patients leads to increased morbidity and mortality and development of secondary resistance and ongoing transmission of the disease. Diagnosis of drug resistance by conventional methods takes 6–8 weeks in detection. Detection of rifampicin resistant TB by CBNAAT is done within few hours. Consequently early diagnosis of TB patients helps in early and precise treatment and prevents transmission of MDR strains of TB in the community.

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