<u>ORIGINAL RESEARCH</u>

Detection of pulmonary tuberculosis by CBNAAT and its effectiveness on preventing drug resistance

[1]Dr. Sakshita Agnihotri, [2]Dr. Ritu Bhatnagar

[1]Ph.D. Scholar, Pacific Medical College and Hospital, Udaipur, Rajasthan, India
[2]Professor and Head, Department of Microbiology, Pacific Medical College and Hospital, Udaipur, Rajasthan, India

Corresponding author
Dr. Sakshita Agnihotri
Ph.D. Scholar, Pacific Medical College and Hospital, Udaipur, Rajasthan, India

ABSTRACT
Background: Early drug resistance can be identified with the CBNAAT (Cartridge-based nucleic acid amplification Test) in a short amount of time after sample submission. Patients with tuberculosis, both those who have recently been diagnosed as well as those who have relapsed, have been experiencing an exponential spread of drug-resistant TB. The main causes of the development of drug-resistant TB, beyond from the initial drug resistance seen in new cases of pulmonary tuberculosis, include patient non-compliance and unneeded and puzzling regimens prescribed by clinicians in failed instances. **Objective:** 1) To determine the usage of CBNAATas a tool for definitive diagnosis of Pulmonary Tuberculosis. 2) To access the extent of Rifampicin resistance amongst these freshly diagnosed cases of Pulmonary Tuberculosis. **Material and Methods**: A total of 275 Sputum samples were obtained from the individuals in western Rajasthan who were clinico-radiologically significant. These were handled at diagnostic facilities equipped to conduct CBNAAT tests. All samples were processed using CBNAAT while adhering to the advised aseptic techniques and PPE (Personal Protective Equipment) criteria. **Conclusion:** The CBNAAT is one of the most efficient diagnostic methods available in India right now. It could be used to concurrently diagnose medication resistance and pulmonary TB. Compared to the standard sputum smear microscopy, it is more useful. Additionally, it may take the place of traditional culture-based drug sensitivity testing techniques.
Keywords: Pulmonary TB, Rifampicin resistance, CBNAAT

INTRODUCTION

Tuberculosis is one of the earliest recognised human diseases. The illness is still widespread, though.In spite of estimates that the global incidence rate has peaked and is already beginning to drop, the absolute number of TB cases occurring each year is currently higher than it has ever been. Progress towards TB control is therefore extremely slow.[1]

Mycobacterium tuberculosis is responsible organism that causes tuberculosis. According to the Global Tuberculosis Report 2021, there were an expected 1.3 million HIV-negative deaths globally in 2020, an increase from 1.2 million in 2019. The number of deaths among HIV-positive people was also increased by 214 000, from 209 000 in 2019. According to WHO estimates, between 2000 and 2020, there would be around one billion new cases of tuberculosis, 200 million illnesses, and 35 million deaths due to the disease.[2]

There is an urgent need for technologies that can quickly detect Mycobacterium tuberculosis and identify drug-resistant infections due to a shocking rise in the frequency of drug-resistant Mycobacterium tuberculosis infection on a global scale. Drug-resistant tuberculosis (DR TB), particularly MDR and XDR TB, is difficult to diagnose and treat, which increases mortality, causes nosocomial epidemics, and makes it more difficult to treat patients with additional antituberculosis medications. However, if MDR and XDR tuberculosis is correctly detected, it can be efficiently treated.[3]

Inadequate medical systems, the amplifying of resistance patterns as a result of insufficient treatment, and transmission in communities and facilities are the main causes of the spread of resistant tuberculosis. Although treating patients with MDR and XDR strains might be extremely difficult, cures are typically possible with early resistance identification and the administration of a precisely catered regimen.

Treatment outcomes can be improved through neighbourhood-based programmes that allow patients to receive care in the comfort of their own homes and address socioeconomic hurdles to adherence.[4]

Seung et al reported that there were approximately 450,000 new cases of MDR-TB and 170,000 deaths in year 2012. 20% of MDR-TB patients had a history of previous treatment and 3.8% were new patients worldwide. Eastern Europe and Central Asia were found to be with the highest rates of MDR. 35% of new patients had MDR-TB, and 75% were those who had been treated previously.[5]

The automated Gene Xpert MTB/ RIF assay is a closed cartridge device that is simple to use, easy to operate, and consistently more sensitive than sputum microscopy. It is based on a hemi-nested real-time PCR test that uses five molecular beacons, each of which is marked with a fluorophore that has a different color and spans the M. tuberculosis rpoB gene's 81-bp rifampicin resistance determining region (RRDR). The test measures MTB and rifampicin susceptibility simultaneously, which can be used as a stand-in indicator of multidrug resistance (MDR-TB).[4]As a result, it may quickly identify both TB and rifampicin resistance from untreated sputum samples.[6]

In order to diagnose TB, WHO advised using the Gene Xpert system, a novel cartridge-based nucleic acid amplification test (CB-NAAT). In accordance with the available resources, the October 2013 WHO guidelines contain a conditional recommendation for Xpert MTB/RIF as the initial diagnostic test in all persons with suspected tuberculosis and MDR. To guide policy recommendations for the programmatic use of the test in their contexts, governments will need evidence about patient, programme, and cost effectiveness outcomes. The impact of Xpert MTB/RIF will, however, depend on the system in which it is employed.[7]

As recommended by WHO in 2013 policy recommendations, the Revised National TB Control Programme (RNTCP) is also currently using Xpert MTB/RIF to diagnose pulmonary TB, pediatric TB, extrapulmonary TB, rifampicin resistance, and Multi Drug Resistance Tuberculosis in high-risk populations like HIV positive.[2,7,6]

The aim of the present study is to ascertain the usage of CBNAATas a tool for definitive diagnosis of Pulmonary Tuberculosis and to access the extent of Rifampicin resistance amongst these freshly diagnosed cases of Pulmonary Tuberculosis.

MATERIALS AND METHODS

The Department of Microbiology at the Pacific Medical College and Hospital in Udaipur, Rajasthan, India, provided the required direction and oversight for the prospective study that was carried out after receiving Ethical approval and consent from the Institutional ethical Committee. The study was carried out between 2020 and 2022.

INCLUSION CRITERIA

1. Signs and symptoms of pulmonary tuberculosis on clinical and radiographic examinations.
2. Two samples of sputum from individuals with clinical TB suspicion.
3. Patients providing written, informed consent.

EXCLUSION CRITERIA

1. Patients who are under the age of 18 or above 75.
2. Patients who have undergone antitubercular therapy in the last six months for a period longer than a month.
3. Patients with HIV.
4. Women who are lactating or pregnant
5. Potentially dangerous cases of extrapulmonary tuberculosis.
6. Patients with clotting disorders, cardiovascular events histories and respiratory failure symptoms.
7. Patients who are uncooperative.

All samples were processed while adhering to the proper aseptic protocols and PPE (Personal Protective Equipment) guidelines. The quality of the sputum sample was evaluated optically; if it comprised of more saliva, the rejection of sample was done, and a new specimen requisition was made. It was made sure that the research facility received the collected samples as shortly as possible. These samples were stored in the refrigerator at 2 to 8 °C in those situations whenever delay was anticipated.[8]

In this process, 1 ml of a sample (sputum) and 2 ml of sample reagent were added to a conical tube and vigorously stirred. The mixture was incubated at room temperature for roughly 10 to 15 minutes until being loaded into the sample cartridge chamber using a sterile graduated or ungraduated pipette. The GeneXpert (CBNAAT) device was then inserted with the cartridge. The data outcomes and interpretation were finished using the GeneXpert Dx System software, which measures fluorescent signals using an algorithm.[9,10]

RESULTS

In our study, of the 275 patients with Pulmonary tuberculosis who had clinic-radiological indicators; 262 patients (95.27%) that were a majority were tested positive by the CBNAAT approach and were promptly identified as Positive. (TABLE 1.) 13 patients or 4.73% of total samples were reported Negative by CBNAAT. (TABLE 1.)

**Table 1: Distribution of cases according to CBNAAT results**

CBNAAT results	Number	Percentage %
Positive	262	95.27
Negative	13	4.73

TOTAL	275	100.00

Table 2: Distribution of cases according to Rifampicin resistance

Resistant to Rifampicin	Number	Percentage %
Yes	31	11.27
No	244	88.73
TOTAL	275	100.00

Out of total samples processed, 31 patient samples or 11.27% of the 275 cases had Rifampicin resistance (TABLE 2). The above mentioned 31 cases were specifically advised precautions to avoid spreading the disease to the community and were begun with proper Anti-tubercular treatment protocol keeping in view their drug-resistance and its probable severity.

Graph 1: Combined Distribution of cases according to Sputum for CBNAAT and Rifampicin Resistance
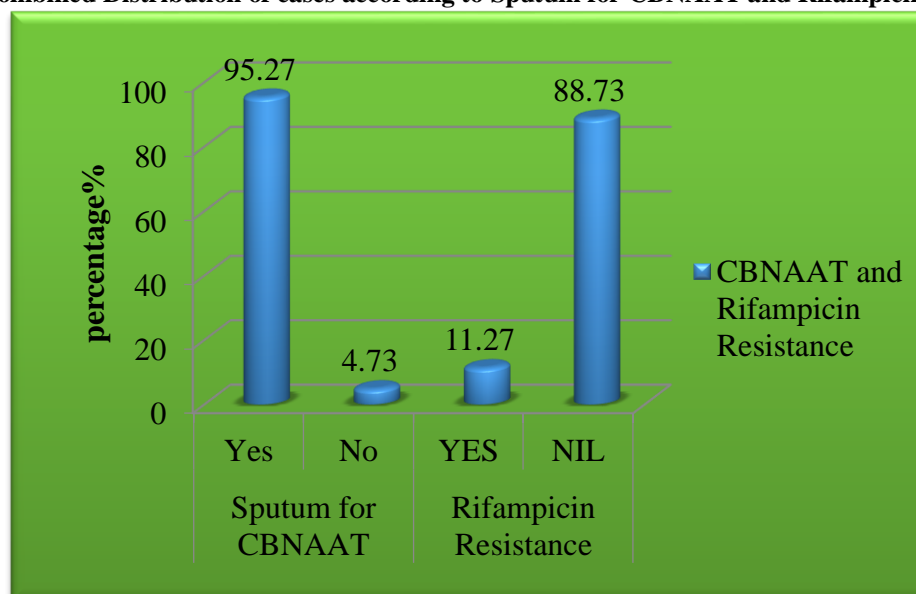
DISCUSSION

In both smear-negative and smear-positive clinical sputum samples, this study shows that the Xpert MTB/RIF assay method can quickly detect the presence of M. tuberculosis and identify the mutations most frequently linked to rifampicin resistance. The Xpert MTB/RIF assay's self-contained cartridge fluidics made it possible to develop a heminested PCR assay with a sensitivity that was comparable to culture-based diagnoses. Although PCR inhibitors may be present in sputum, the assay appears to be rather robust to them. As a result, PCR inhibitors may be to blame for the one smear-positive sample from Uganda that was Xpert MTB/ RIF negative. We were forced to rely on examinations of previously frozen sputum samples due to the retrospective character of our clinical validation investigations.[3]Utilizing three distinct primers and five different molecular probes, the GeneXpert displays a very high level of specificity.[11]

According to findings from our research, this involved processing the collected sputum sample being used to determine Rifampicin resistance using the GeneXpert MTB/RIF test (CBNAAT). From a total of 275 samples that we examined, the majority of cases, or 95.27 percent, were found to be positive by CBNAAT (n=262).

In our study, of the 275 instances, 31 patient samples—or 11.27% of the total samples processed—were resistant to rifampicin. These drug-resistant instances benefited from CBNAAT's early and accurate identification abilities.

Other studies found findings that were comparable. According to the Global TB Report 2021, 132,222 (6.29%) of the 2.1 million individuals with bacteriologically confirmed pulmonary TB in the year 2020 were rifampicin resistant.[3] India TB Report states that 2,858,713 tests on CBNAAT and 125923 tests on Truenat were performed, out of which 53,826 (7%) and 340 (3.1%) samples were rifampicin-resistant, respectively. Out of 116 MTB positive individuals, 6.9% were rifampicin resistant, according to Okonkwo et al.[12]While Bhavanarushi Sreekanth et al (2020) discovered that just two (1.86%) cases of rifampicin resistance were reported. Nearly all of the writers supported CBNAAT's efficiency and precision in identifying drug-resistant TB.[13]

The rates of rifampicin resistance described in studies other than ours, however, ranged from 1.8% to 3.1%. Rifampicin resistance was only found 3.1% in a study by Ndubuisi et al. (2016), and 1.9% in a study by

Sowjanya et al. (2014). Seven (3.7%) of the 18 (6.54%) samples that were found to be resistant to rifampicin in the current research came from newly diagnosed cases of tuberculosis, while 11 (12.7%) came from cases that had already been treated. Similar results were observed in a study by Shrestha et al. (2018), where 3.7% rates of rifampicin resistance were discovered in new cases while 19.04% resistance was discovered in cases that had already been treated.[14, 15, 16]

As known and demonstrated by researches performed like the ones by a Muhammad M. Ibrahim (2022)[17] and Dagnra AY et al. (2015)[18], genetic mutation is the primary cause of drug resistance to rifampicin. The RNA polymerase (rpoB) enzyme's beta-subunit gene is in charge of this and is a highly significant "surrogate marker" for multidrug-resistant tuberculosis (MDR-TB). The CBNAAT device as used in our study makes the usage of the Real-Time polymerase chain reaction (PCR) so as to detect the specific sequence for Mycobacterium tuberculosis bacteria (MTB) as well as for resistance to Rifampicin.

CONCLUSION

It is essential to detect PTB because it causes around half of all tuberculosis cases. When dealing with drug-resistant cases and tuberculosis samples with negative sputum smear results, it might be challenging to quickly identify the causal bacterium in such samples. In addition, we discovered that the Xpert MTB/RIF assay is the best technique for quickly diagnosing MTB from tuberculosis specimens. Although culture is regarded as the gold standard method, simultaneous detection of rifampicin resistance is not feasible with it because it takes days to weeks to come positive. On the other hand, individuals with suspected pulmonary tuberculosis may find GeneXpert to be a helpful diagnostic tool, particularly in smear-negative instances. It would be very beneficial for the anti-TB therapy in MDR patients due to its speed and simultaneous detection of rifampicin.

Additionally, there are situations when the existing paradigm of traditional sputum smear-negative PTB is insufficiently sensitive to guarantee the diagnosis of active tuberculosis in the absence of CBNAAT. They overmedicate those without PTB while underdiagnosing those who do. Since PTB makes up around half of all cases of tuberculosis, it might be challenging to identify the bacterium in negative TB tests using conventional techniques. CBNAAT can be a great screening and confirmatory option of diagnostic test so that the treatment protocol of patients can be started as soon as feasible and prevent the community-wide spread of drug-resistant TB.

The successful management of mycobacterial infection depends on an accurate and prompt diagnosis. The Xpert MTB/RIF assay was shown to be a useful tool in the current research for MTBC diagnosis. Given that it takes little time and is inexpensive, it may be very helpful in developing nations for regular rapid diagnosis.

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