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CBNAAT: AN EFFICACIOUS TOOL FOR DIAGNOSING AND PREVENTING DRUG RESISTANT PULMONARY TUBERCULOSIS

Medical Microbiology

Dr. Ritu Bhatnagar Professor and Head, Department of Microbiology, Pacific Medical College and Hospital, Udaipur, Rajasthan (India).

Dr. Sakshita Agnihotri* Ph.D. Scholar, Pacific Medical College and Hospital, Udaipur, Rajasthan (India). *Corresponding Author

ABSTRACT

Background- The CBNAAT (Cartridge-based nucleic acid amplification Test) aids in the detection of early drug resistance in a brief duration of sample submission. The drug resistant TB has been exponentially spreading amongst tuberculosis patients, including all those who have just been diagnosed and also those who have relapsed. Apart from the initial drug resistance seen in fresh cases of pulmonary tuberculosis, patient non-compliance, unnecessary and inexplicable regimens advised in failed cases by doctors are the main causes for the development of drug - resistant TB.

Objective –

1) To ascertain the usage of CBNAAT as 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 processed at diagnostic centers with CBNAAT testing capabilities. Following the recommended aseptic procedures and PPE (Personal Protective Equipment) guidelines, all samples were processed by using CBNAAT. **Conclusion** CBNAAT is one of the most effective diagnostic techniques currently accessible in India. It could be utilized to diagnose Pulmonary TB and drug resistance simultaneously. It is more useful than the traditional sputum smear microscopy. This might also replace conventional culture-based drug sensitivity testing methods.

KEYWORDS

Pulmonary TB, Rifampicin resistance, CBNAAT

INTRODUCTION-

About 10.6 million individuals were anticipated to contract tuberculosis (TB) worldwide in 2021. Following COVID-19 (after HIV/AIDS), TB is the second epidemic killer disease in the world and the 13th biggest cause of death globally. All nations and age categories are affected by TB. Multidrug-resistant tuberculosis (MDR-TB) continues to be a public health emergency and a safety risk to health. When TB patients discharge countless bacteria into the air around them, this deadly pathogen spreads via aerosols (e.g. by coughing, sneezing, speaking, spitting, singing etc.). Only a few numbers of germs need to be breathed for anyone to get tuberculosis. The likelihood of transmitting *Mycobacterium tuberculosis* (MTB) is impacted by the quantity of droplet particles that spread infection in the atmosphere, the length of time being exposed to these infectious particles, and most importantly the closeness to infected individuals. [1]. However, TB is both treatable and avoidable.

Since the early 1990s, WHO has worked with nations to improve drug resistance monitoring. Drug resistance is a remarkable barrier for the treatment and for the prevention of tuberculosis (TB) worldwide, prolonging treatment duration and increasing the likelihood of adverse reports of patients. Drug-resistant tuberculosis patients deal with serious financial hardships. Using innovative technologies and researches, WHO aims to expedite the implementation of quick tests and treatments for drug-resistant TB. [2] Due to the adoption of efficient management measures along with social and economic growth, developed nations now view tuberculosis as a disease of the past. Though, the "end" of tuberculosis as a significant public health issue is still an aspiration for many low- and middle-income nations. 27% of the anticipated incidences around the world are from India.[3] Until effective treatment is started, a person with untreated Pulmonary TB (PTB) can continue to infect others. Early diagnosis, treatment commencement and completion are the best ways to stop the propagation of TB.[4]

According to estimates, 2.5% and 16%, respectively, of newly diagnosed and previously treated patients in India had multidrug-resistant (MDR-TB) including RR-TB. As per statistics, 130,000 new infections of MDR-TB and rifampicin-resistant (RR)-TB occur annually. [5] Backlogs in patients who reported their illnesses to medical facilities, in identifying them, and in beginning TB treatment are few of the factors that contribute to this infection's preponderance. Additionally, it screens for *Mycobacterium tuberculosis*. Albeit more costly than traditional microscopic examination, drug-sensitivity testing and culture are quite equal in costs. [6]

Rapid molecular diagnostics, such as "CBNAAT" are currently one of the most efficient diagnostic methods available in India. They have a far better sensitivity than the age-old sputum smear microscopy and can be utilised to concurrently diagnose Pulmonary TB and resistance to the drug Rifampicin. This has the potential to take the position of traditional culture-based drug sensitivity procedures. [7]

MATERIALAND 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.)
- 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.

DISCUSSION

The CBNAAT (CARTRIDGE-BASED NUCLEIC ACID AMPLIFICATION TEST) is an innovative highly automated device for the detection of tuberculosis. Within two hours of sample collection, this test may identify TB and RIFAMPICIN resistance directly from pulmonary specimens. It employs Nested real-time PCR for the identification of MTB-complex and RIF resistance. The technique is intended to extract, amplify, and identify the rpoB gene of *M. tuberculosis*. Greater than 95% of the genetic alterations linked to RIF (rifampicin) resistance are indeed the consequence of it. 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, which 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). Meghna Patel, Bhavsar et al. (2019) [12.] asserted that out of 333 patients, CBNAAT detected *Mycobacterium TB* (MTB) in 289 (86.2%) however, Mukherjee S et al. (2017) [13] discovered that only 111 cases out of 228 (48.68%) were positive for CBNAAT.

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. While, in other studies like that of, Mukherjee S et al (2017) claimed that Rifampicin resistance was seen in five (2.2%) cases. Similarly, Meghna Patel, Bhavsar et al found out that resistance to Rifampicin was found out amongst 15 patients (4.5%). While Bhavanarushi Sreekanth et al(2020)[14]

found resistance to Rifampicin was detected in just two (1.86%) cases. Almost all the authors believed in the speed and accuracy of CBNAAT in detection of drug-resistant tuberculosis

As known and demonstrated by researches performed like the ones by Muhammad M.Ibrahim[a] (2022) [15] and Dagnra AY et al. (2015) [16] , 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-

Since PTB accounts for around half of all tuberculosis cases, its detection is crucial. It can be difficult to identify the causative organism in such samples quickly, especially in the case of drug-resistant incidences and sputum smear-negative tuberculosis specimens by using age-old diagnostic methods. [17]

CBNAAT recognizes PTB with extraordinary specificity and sensitivity, in contrast to routinely used sputum microscopy and other liquid and solid culture medium methods. As a result, it can reliably and promptly identify MTB in less than two hours. The CBNAAT also reveals rifampicin resistance for MDR-TB screening and early treatment initiation. This might help to lower the prevalence of the new patient graph. [18]

Also, sometimes the current situation of conventional sputum smear-negative PTB is not sensitive enough to assure the diagnosis of active tuberculosis without CBNAAT. They underdiagnose people who have PTB whilst overmedicating those who don't. Since PTB accounts for around half of all tuberculosis cases, it can be difficult to identify the bacteria present in negative TB specimens using traditional methods. So as to initiate the treatment protocol of patients as early as possible and prevent the community wide spread of drug-resistant TB, CBNAAT can serve both as a wonderful screening and confirmatory choice of diagnostic test. [17.]

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

RESISTANCE TO RIFAMPICIN	Number	Percentage%
YES	31	11.27
NO	244	88.73
TOTAL	275	100.00

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