Original Research

DIAGNOSTIC VALUE OF CBNAAT FOR MYCOBACTERIUM TUBERCULOSIS IN A TERTIARY CARE HOSPITAL RAIPUR, CHHATTISGARH, INDIA

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ABSTRACT

Background: Tuberculosis is the ninth leading cause of death worldwide. India contributes to about one fifth of global TB burden. It is very important to diagnose early and treat Tuberculosis to cut down transmission of Tuberculosis. "Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) plays a vital role in the rapid and accurate detection of Mycobacterium tuberculosis.

Material and Methods: A retrospective, observational, record-based study conducted from March 2023 to February 2024 in Department of Microbiology. We included all patients who were subjected to CBNAAT and fluorescent microscopy for tuberculosis in the study period. Data was collected from CBNAAT centre, department of microbiology. We collected total number of samples regardless of age and tested for CBNAAT as well as smear microscopy for AFB.

Results: A total of 1299 presumptive Tuberculosis cases were included in our study. Out of these, 143 (11%) patients were diagnosed as positive for TB in GeneXpert MTB/RIF assay in all age groups whereas AFB microscopy was positive in 41 (28.67%) cases. 102(71.33%) cases were missed by AFB microscopy which were MTB detected by molecular CBNAAT.

Conclusion: CBNAAT is a highly sensitive molecular test that not only detects TB but also identifies rifampicin resistance within a short time, typically within two hours. CBNAAT is especially valuable in resource-limited settings due to its automation, minimal hands-on time, and applicability to various specimen types. By using this technique, we could detect the in the patients with the negative smear for microscopy.

Keywords: Tuberculosis, Resistance, CBNAAT, MDR, Fluorescent Microscopy, Mycobacterium Tuberculosis, GeneXpert.

INTRODUCTION

Tuberculosis (TB) is one of the oldest known human diseases, and it is still one of the most frequent infectious diseases in India today. [1] The rise of multidrug-resistant tuberculosis (MDR-TB) has made diagnosis, treatment, and control of tuberculosis (TB) more difficult, with rifampicin resistance (RR) serving as a significant surrogate measure. [2] Tuberculosis (TB) is an infectious illness of the lungs caused by the bacteria Mycobacterium tuberculosis (MTB). Traditionally, pulmonary tuberculosis was thought to account for around 85% of all TB cases, with the remaining 15% attributed to extrapulmonary tuberculosis (EPTB). However, current global data show a significant increase in the proportion of extrapulmonary tuberculosis (EPTB) cases, which range from 15% to 53% of total tuberculosis (TB) infections. [3] According to the WHO Global TB Report 2023, around 10.6 million persons worldwide were newly diagnosed with tuberculosis (TB), up from 10.3 million in 2021. In addition, the disease is estimated to have killed 1.3 million people worldwide. In 2022, about 410,000 people got multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB). [4] According to the India TB Report 2023, the case notification rate was at 172 per 100,000 people. The total number of MDR/RR patients diagnosed in 2022 is 63,801.[5] According to the RNTCP, sputum microscopy is the primary diagnostic method for TB. WHO endorsed LED Fluorescent Microscopy (LED-FM) as a preferable option, providing at least 10% higher sensitivity, equivalent accuracy, and

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cost, efficiency, and workload management benefits for laboratories. [6]

Effective TB control requires a rapid diagnostic test that is both highly specific and sensitive, enabling early and accurate detection for timely treatment. In December 2010, the World Health Organization (WHO) recommended the use of the cartridge-based nucleic acid amplification test (CBNAAT) as a breakthrough tool. This test detects both pulmonary and extrapulmonary (EP) tuberculosis while simultaneously identifying rifampicin resistance, thereby facilitating comprehensive and effective TB management. [7]

CBNAAT by GeneXpert MTB/RIF is a cost-effective molecular assay unlike others such as latest-generation liquid culture diagnostics and molecular line probe assays which are costly and need biosafety measures and specialized staff. Therefore, CBNAAT, which is highly specific, having five unique molecular probes to target the rpoB gene of MTB associated with RR, is an esteemed diagnostic tool [8].

METHODOLOGY

This was a retrospective, observational, record-based study was conducted from March 2023 to February 2024 in Department of Microbiology of tertiary healthcare institution situated in Raipur, state of Chhattisgarh, India. We included all patients who were subjected to CBNAAT and fluorescent microscopy for tuberculosis in the study period. Data was collected from CBNAAT centre. We collected total number of samples tested for CBNAAT, indication for CBNAAT, result of smear microscopy for AFB and CBNAAT.[2]

Inclusion criteria- The patients whose demographic data and CBNNAT reports along with RR and AFB microscopy reports accessible were included in the study regardless of age.

Specimen collection- pulmonary and extrapulmonary both specimens were collected and subjected for AFB microscopy and CBNAAT as per standard microbiological procedure.[3]

Sample processing-For direct sputum processing, 2 volumes of Sample Reagent (SR) were added to 1 volume of sputum (minimum 1 ml). The mixture was shaken vigorously 20 times or vortexed, then incubated at room temperature for 10 minutes. It was shaken again and incubated for an additional 5 minutes. Finally, 2 ml of the liquefied sample was loaded into the cartridge. Then the tests were run on CBNAAT machine as per procedures laid by the kit manufacturer. Data regarding demographic variables, TB-positive, and RR-TB cases were extracted from records. [8-9]

Ethical clearance: study approval obtained from the Institutional Ethics Committee with approval no: No./MC/Ethics/2025/918; Date 24/3/25.

Data analysis tools: Data were analysed using Microsoft Excel and SPSS version 20 descriptive statistics (percentage and proportion) were calculated.

Quality control: All testing procedures were performed as per the manufacturer's quality control guidelines and RNTCP/NTEP standards.

RESULTS

A total of 1299 presumptive tuberculosis cases were included in our study. A total of 143 samples tested positive by CBNAAT, while 41 samples were positive by AFB Microscopy. The age-wise distribution of positivity revealed the highest number of CBNAAT-positive cases in the 21–40 years age group (76/143, 53.15%), followed by the 41–60 years group (39/143, 27.27%). A similar trend was observed in AFB Microscopy with the 21–40 years age group showing the highest positivity (20/41, 48.77%), followed by 41–60 years (15/41, 36.59%). The age group below 20 years and above 60 years had relatively lower positivity rates in both tests.

In terms of gender distribution, male patients showed higher positivity in both CBNAAT (94/143, 65.73%) and AFB Microscopy (31/41, 75.61%) compared to females.

When evaluating sample types, pulmonary specimens accounted for the majority of positive results: 65.73% (94/143) in CBNAAT and 90.24% (37/41) in AFB Microscopy. In contrast, extrapulmonary samples showed lower positivity, with only 4 (9.76%) being positive by AFB Microscopy and 49 (34.27%) by CBNAAT.

Among the extrapulmonary specimens, CBNAAT detected the highest positivity in FNAC samples (18/49, 36.73%) followed by pus samples (15/49, 30.61%). Other samples such as CSF, fluids, and lymph node aspirates showed lower detection rates. AFB Microscopy was able to detect only 4 cases among extrapulmonary samples, with pus (2/4, 50%) and FNAC (1/4, 25%) yielding positive results.

Out of a total of 143 CBNAAT-positive tuberculosis cases, 140 cases (97.90%) were Rifampicin-sensitive, while 3 cases (2.10%) showed Rifampicin resistance. This indicates that the majority of patients were infected with drug-sensitive Mycobacterium tuberculosis strains.

DISCUSSION

The findings of this study underscore the superior sensitivity of CBNAAT over AFB Microscopy, particularly in the diagnosis of extrapulmonary tuberculosis (EPTB). CBNAAT detected 143 positive cases more than three times the number identified by AFB Microscopy (41 cases) demonstrating its robust diagnostic capability [2],[6],[18]. These results align with previous studies highlighting CBNAAT's ability to detect low bacillary loads, especially in paucibacillary and extrapulmonary forms of TB [3],[16],[17],[19]–[21].

The highest proportion of positive cases was observed in the 21–40 years age group, consistent with epidemiological data showing that TB predominantly affects individuals in their most productive years [9],[10],[12]. This pattern has significant public health implications, as disease in this age group may contribute to increased transmission and economic burden [1],[10]. A distinct male preponderance was observed in both CBNAAT and AFB Microscopy results, which may be attributed to higher occupational exposure, lifestyle factors, and gender-based differences in healthcare access [11]–[14]. Similar gender disparities in TB incidence and diagnosis have been documented globally [10],[14]. The diagnostic yield was higher in pulmonary samples, particularly by AFB Microscopy, which is expected given the higher bacillary load in sputum [7],[18]. However, the data also highlight the limitations of smear microscopy in EPTB diagnosis—only 4 extrapulmonary samples were smear-positive compared to 49 detected by CBNAAT. Among EPTB specimens, FNAC and pus samples showed substantial diagnostic yields by CBNAAT, emphasizing its utility in resource-limited settings where tissue biopsies may not be feasible [16],[17],[19]–[21]. Overall, the study reinforces the need to integrate molecular diagnostics such as CBNAAT into routine TB diagnostic algorithms, particularly for extrapulmonary and smear-negative cases [15],[20],[25],[26]. The low detection rate by AFB Microscopy in extrapulmonary samples underscores the risk of missed diagnoses when relying solely on conventional smear techniques [7],[18]. Rifampicin resistance was detected in 2.10% of tuberculosis cases, reflecting a relatively low but significant burden of drug-resistant TB (DR-TB) in the study population [22]–[24]. As rifampicin resistance serves as a surrogate marker for multidrug-resistant TB (MDR-TB), its early identification is essential for initiating appropriate second-line therapy and preventing further transmission [2],[23],[24],[27]. The high proportion of rifampicin-sensitive cases (97.90%) suggests that most patients would likely respond well to standard first-line anti-tubercular therapy [5],[10]. Nonetheless, even a small percentage of resistant cases highlights the importance of routine drug resistance testing in all bacteriologically confirmed TB cases, especially in regions with increasing MDR-TB prevalence [4],[5],[24]. Rapid molecular diagnostics such as CBNAAT provide a distinct advantage by simultaneously detecting Mycobacterium tuberculosis and rifampicin resistance within hours, thereby enabling early treatment initiation and containment of resistant strains [6],[15],[25]. These findings support the continued use and expansion of CBNAAT-based testing, particularly in high-burden settings, to ensure prompt diagnosis, guide targeted therapy, and reduce the risk of resistance amplification [20],[25],[26].

Further evaluation through culture-based drug susceptibility testing (DST) for additional first- and second-line drugs is essential in rifampicin-resistant cases to confirm MDR-TB and guide individualized treatment plans [23],[24],[27]. Following points to be addressed for the importance of the study:

- **Public Health Relevance:** Early molecular diagnosis through CBNAAT plays a pivotal role in interrupting transmission chains by facilitating prompt initiation of treatment [6,15,25]. Rapid detection not only reduces the duration of infectiousness and hospital stay but also helps limit community spread, thereby contributing significantly to TB control efforts at both individual and population levels [4,10].
- **Comparison with Other Rapid Diagnostic Methods:** Although line probe assays and liquid culture systems are considered reference standards for TB detection and drug susceptibility testing [8], they are often time-consuming, expensive, and require advanced biosafety infrastructure. In contrast, CBNAAT offers a rapid, cost-effective, and field-deployable molecular diagnostic option with minimal technical expertise [2,6,25], making it particularly suitable for use in resource-limited healthcare settings [15,26].
- **Programmatic Implications:** The findings of this study highlight the need of expanding molecular diagnostics within the scope of the National Tuberculosis Elimination Programme (NTEP) [5,15]. CBNAAT's superior sensitivity and rapid turnaround time are consistent with the NTEP's goal of achieving universal molecular testing for all presumptive TB cases [15,20,26], thereby accelerating early detection, ensuring appropriate therapy, and supporting India's mission to eliminate tuberculosis by 2025 [4,5].

CONCLUSION

CBNAAT provides a very sensitive, fast, and dependable diagnostic tool for both pulmonary and extrapulmonary tuberculosis. Its implementation at all levels of healthcare can speed up TB detection, improve patient outcomes, and help India's TB elimination efforts.

Limitations: The findings of this retrospective single-center investigation may not be generalizable to all locations. Culture confirmation and follow-up therapy outcomes were not considered. Future multicentric investigations with greater sample sizes and cultural linkage are recommended.

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

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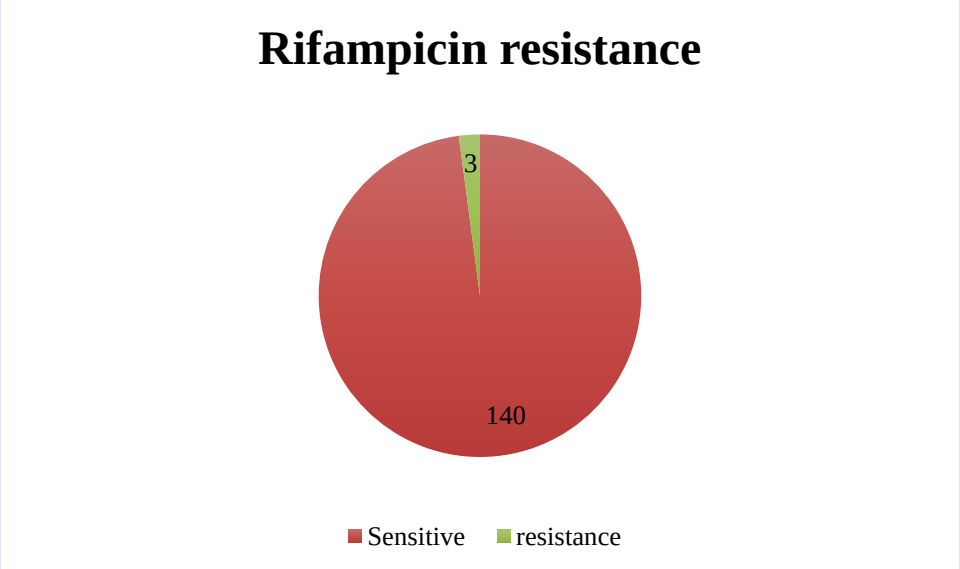
TABLES AND GRAPHS

Table 1: Sociodemographic and Clinical Characteristics of Tuberculosis Patients (N = 143)

Age group (year)	**CBNAAT positive (143) (%)**	**AFB Microscopy positive (41) (%)**
Less than 20	15 (10.49)	03 (7.32)
21-40	76 (53.15)	20 (48.77)
41-60	39 (27.27)	15 (36.59)
More than 60	13 (9.09)	03 (7.32)
Gender		
Male	94 (65.73)	31 (75.61)
Female	49 (34.27)	10 (24.39)
Type of specimen		
Pulmonary sample	94 (65.73)	37 (90.24)
Extrapulmonary	49 (34.27)	04 (9.76)
EXTRAPULMONARY TYPE	**CBNAAT (49) (%)**	**AFB Microscopy (4) (%)**
Pus	15 (30.61)	02 (50)
CSF	03 (6.12)	00
FNAC	18 (36.73)	01 (25)
Fluid	03 (6.12)	00
Lymph node	02 (4.08)	00
Cytological fluid	06 (12.24)	01 (25)
Knee fluid	01 (2.04)	00



Graph 1: Rifampicin Resistance in Tuberculosis Patients (N = 143)