e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2025; 17(9); 70-83

Original Research Article

An Observational Study on Extra-Pulmonary Tuberculosis with Special Reference to Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and Assessment of Drug Resistance Pattern in a Tertiary Care Hospital

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Received: 01-06-2025 / Revised: 15-07-2025 / Accepted: 21-08-2025
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Conflict of interest: Nil

Abstract

Background: Extra-pulmonary tuberculosis (EPTB) constitutes a significant proportion of the tuberculosis burden in India, particularly among immunocompromised populations. Diagnostic delays arise from its paucibacillary nature and the limitations of conventional methods. Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) offers rapid detection of Mycobacterium tuberculosis and rifampicin resistance, yet its performance in high-burden, resource-constrained settings requires further evaluation.

Methods: In this hospital-based, cross-sectional observational study, 200 consecutive patients clinically suspected of EPTB were prospectively enrolled. Standardized inclusion and exclusion criteria ensured diagnostic purity. Specimens underwent Ziehl–Neelsen microscopy, culture on Lowenstein–Jensen medium, and CBNAAT. Diagnostic accuracy indices were calculated using culture as the reference standard. Logistic regression identified predictors of rifampicin resistance.

Results: Of 200 patients, 112 (56%) were male, with mean age 36.4 ± 14.8 years; 62% belonged to low socio-economic strata, and 14% were HIV seropositive. Lymph node TB (32%) and pleural TB (24%) predominated. Microscopy, culture, and CBNAAT positivity rates were 13%, 32%, and 46%, respectively. Relative to culture, CBNAAT demonstrated sensitivity 84.3% and specificity 98.5% ($\kappa = 0.83$). Importantly, CBNAAT detected 28 additional clinically adjudged culture-negative cases. Among CBNAAT-positive patients, rifampicin resistance was identified in 14 (15.2%), yielding an overall resistance prevalence of 7%. Prior TB treatment (aOR 3.6, p=0.008) and HIV co-infection (aOR 2.9, p=0.03) independently predicted resistance.

Conclusion: CBNAAT significantly enhances diagnostic yield in EPTB and permits timely detection of rifampicin resistance, particularly in high-risk subgroups. Its universal integration into diagnostic algorithms is imperative to strengthen India's TB elimination trajectory.

Keywords: Extra-Pulmonary Tuberculosis, CBNAAT, Rifampicin Resistance, Diagnostic Accuracy, India, HIV Co-Infection, Molecular Diagnostics.

Introduction

Tuberculosis (TB) persists as one of the most formidable challenges in global infectious disease epidemiology, exerting a disproportionate toll upon low- and middle-income nations. According to the World Health Organization's Global Tuberculosis Report 2021, an estimated 10.6 million cases and 1.6 million deaths occurred worldwide, with India contributing nearly 27% of the global burden, a staggering figure that underscores the endemicity of the disease in this subcontinent [3]. While pulmonary tuberculosis (PTB) remains the archetypal form, extra-pulmonary tuberculosis (EPTB) — involving lymph nodes, pleura, central nervous system, abdomen, skeletal system, and

other organs — constitutes an increasingly recognized facet of this burden, accounting for approximately 15–20% of all cases in immunocompetent populations and up to 50% in immunocompromised cohorts [5,6].

The diagnosis of EPTB, however, is fraught with formidable impediments. Its paucibacillary nature, protean clinical manifestations, and the difficulty in obtaining adequate specimens severely compromise the utility of conventional microbiological modalities. Ziehl-Neelsen (ZN) staining of extra-pulmonary samples yields sensitivities as low as 10–20%, while mycobacterial culture on Lowenstein–Jensen (LJ) medium, though still considered the gold standard, requires six to eight weeks for definitive results and is impractical in the exigencies of clinical decision-making [1,6]. Histopathology, though supportive, remains non-specific, while serological assays and tuberculin skin tests have been largely discredited in endemic populations owing to confounding by BCG vaccination and environmental mycobacteria [1].

The introduction of molecular diagnostic platforms, specifically the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT, commercially GeneXpert MTB/RIF), has fundamentally altered the diagnostic landscape.

CBNAAT, by simultaneously detecting Mycobacterium tuberculosis DNA and rifampicin resistance within a span of two hours, offers both enhanced sensitivity in paucibacillary specimens and rapid drug resistance profiling [2]. In a landmark multi-country study, Boehme et al. documented a sensitivity of 81.3% and specificity of 99.2% in extra-pulmonary specimens, findings corroborated by subsequent analyses that highlighted CBNAAT's superior diagnostic yield in TB meningitis, pleural TB, and lymph node TB [2,7].

In India, the Revised National Tuberculosis Control Programme (RNTCP, now NTEP) formally incorporated CBNAAT as a frontline diagnostic tool for EPTB in 2016, an acknowledgement of both its diagnostic accuracy and its potential to accelerate early initiation of treatment [4,5]. Yet, despite this integration, regional disparities in access, implementation, and utilization persist. In Eastern India, with its high TB prevalence and limited penetration of molecular diagnostics, the evaluation of CBNAAT's role assumes salience.

The present observational study was thus conceived with three interlinked objectives:(i) to delineate the prevalence and clinical spectrum of EPTB among suspected cases in a tertiary care hospital; (ii) to assess the diagnostic yield of CBNAAT in comparison with microscopy and culture; and (iii) to analyze the rifampicin resistance patterns in EPTB as a proxy for multidrug resistance, thereby contributing to both local epidemiological data and the broader discourse on molecular diagnostics in tuberculosis.

Methodology

Study Design and Setting: This hospital-based, cross-sectional, observational study was undertaken in the Department of Microbiology, Medical College and Hospital, Kolkata, over a period of three months (July–September 2025). Ethical clearance was obtained from the Institutional Ethics Committee, (MC/KOL/IEC/2791/06/2025) and informed consent was procured from all participants or their guardians, in conformity with the principles of the Declaration of Helsinki.

Study Population and Criteria: A total of 200 consecutive patients were prospectively enrolled based on clinical suspicion of extra-pulmonary tuberculosis (EPTB). Clinical suspicion was determined through a combination of symptomatology (such as chronic fever, weight loss, night sweats, and site-specific manifestations), radiological findings (ultrasound, CT, or MRI suggestive of TB involvement), and treating physician's judgment. Patient eligibility was stringently defined according to the following criteria:

Inclusion criteria

1. Patients of any age or sex presenting with clinical features suggestive of EPTB (e.g., lymphadenitis, pleural effusion, meningitis, abdominal distension, joint swelling).
2. Radiological or imaging findings supportive of extra-pulmonary disease (e.g., hypodense lesions, pleural thickening, meningeal enhancement).
3. Patients who were referred for diagnostic confirmation of EPTB to the Department of Microbiology.
4. Willingness and ability to provide written informed consent (for minors, assent along with parental/guardian consent was obtained).
5. Patients not previously on anti-tubercular therapy (ATT), or on ATT for less than 14 days at the time of enrolment, to avoid diagnostic distortion.

Exclusion criteria

1. Patients with microbiologically confirmed pulmonary TB without evidence of extra-pulmonary involvement (to maintain diagnostic purity of the EPTB cohort).
2. Patients already on ATT for more than two weeks, as prolonged therapy can reduce bacillary load and confound diagnostic yield of CBNAAT, microscopy, and culture.

3. Patients unwilling or unable to provide informed consent or whose guardians refused participation.
4. Severely ill patients for whom specimen collection was not feasible without undue risk.
5. Cases in which inadequate or insufficient sample material was obtained for diagnostic testing.

Sample Size Justification

The determination of the sample size was predicated upon the single population proportion formula:

$$N = (Z^2 * P * (1 - P)) / d^2$$

Where Z = 1.96 (95% confidence), P = 0.20 (expected prevalence of EPTB in TB cases), and d = 0.05 (precision). Substitution yields $N \approx 246$. Applying a finite population correction factor for the estimated hospital catchment (~800 suspected cases during the study period) yielded an adjusted sample size of ~190. To mitigate attrition due to inadequate samples or laboratory exclusions (~5%), the final enrolment was pragmatically fixed at 200 patients, a number sufficient to ensure statistical power for subgroup analyses while remaining operationally feasible.

Sample Collection and Processing

Specimens were procured according to site of involvement: lymph node aspirates, pleural fluid, cerebrospinal fluid (CSF), ascitic fluid, synovial fluid, pus, and other rare sites.

Processing comprised:

1. Microscopy: Ziehl–Neelsen staining for acid-fast bacilli.
2. Culture: Inoculation on LJ medium, incubated up to eight weeks.
3. CBNAAT: Performed on all specimens following WHO-endorsed protocols; results classified as MTB detected/not detected and rifampicin resistant/not resistant.

Statistical Analysis Plan (SAP): All data were entered into a secure database and subsequently analysed using the Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA).

Prior to analysis, datasets were screened for completeness, outliers, and normality of distribution. Missing values, which constituted <2% of the total dataset, were handled using pairwise deletion to preserve maximal available information without introducing systematic bias.

Descriptive statistics were computed to summarise the demographic, clinical, and laboratory characteristics of the study cohort. Continuous variables (e.g., age, duration of symptoms, time to diagnosis) were expressed as means with standard deviations (SD) when normally distributed, and as medians with interquartile ranges (IQR) for skewed distributions. Categorical variables (e.g., sex, HIV status, type of specimen, positivity rates) were summarised as frequencies and percentages.

For bivariate comparisons, Student's t-test was employed to compare mean values of continuous variables between groups (e.g., rifampicin-resistant vs rifampicin-sensitive cases) after testing for equality of variances using Levene's test. Where normality assumptions were violated, the non-parametric Mann–Whitney U test was considered. Categorical variables were compared using the Pearson Chi-square test; Fisher's exact test was applied in situations where expected cell counts were fewer than five, to ensure validity of p-values.

Diagnostic accuracy indices for CBNAAT and microscopy were computed against culture on Lowenstein–Jensen (LJ) medium, which was taken as the operational gold standard. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with corresponding 95% confidence intervals (CIs) derived using the Wilson score method. Agreement between CBNAAT and culture was assessed using Cohen's kappa statistic, interpreted according to Landis and Koch criteria, where values >0.80 indicated excellent concordance, demonstrated as a Bland–Altman style plot.

Logistic regression modelling was employed to identify independent predictors of rifampicin resistance among CBNAAT-positive cases. A univariate logistic regression was first conducted for potential covariates including age, sex, socio-economic status, HIV serostatus, history of prior TB treatment, and site of disease. Variables with $p < 0.20$ on univariate analysis were subsequently entered into a multivariate logistic regression model using a forward stepwise selection procedure. Adjusted odds ratios (aOR) with 95% CIs were calculated, and statistical significance was defined as $p < 0.05$. Model fit was evaluated using the Hosmer–Lemeshow goodness-of-fit test, while multicollinearity was assessed using variance inflation factors (VIF).

In addition, subgroup analyses were undertaken to examine diagnostic performance stratified by sample type (e.g., lymph node aspirates, pleural fluid, and cerebrospinal fluid) and HIV status. Receiver Operating Characteristic (ROC) curve analyses were planned to evaluate the discriminative capacity of CBNAAT across subgroups, with area under the curve (AUC) values interpreted as: 0.7–0.8 acceptable, 0.8–0.9 excellent, and >0.9 outstanding. All statistical tests were two-tailed, and significance thresholds were set at $\alpha = 0.05$. Results were reported in accordance with STARD (Standards for Reporting of Diagnostic Accuracy

Studies) and STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines, thereby ensuring methodological transparency and reproducibility

Results

Demographic and Clinical Profile: Of the 200 patients included in the study, 112 (56.0%) were male and 88 (44.0%) were female, giving a male-to-female ratio of 1.27:1.

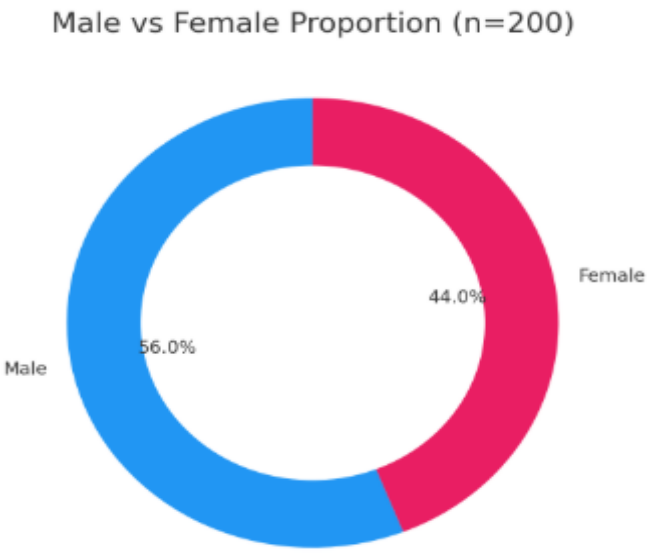

Figure 1: The donut chart shows the gender distribution of EPTB patients, with males comprising 56% and females 44% of the cohort.

The mean age of the study population was 36.4 years (±14.8; range 6–72 years). Age stratification revealed that 34 patients (17.0%) were children and adolescents (<18 years), 122 patients (61.0%) were young to middle-aged adults (18–45 years), and 44 patients (22.0%) were older adults (>45 years). No statistically significant gender difference was observed across age strata (p=0.21, Chi-square).

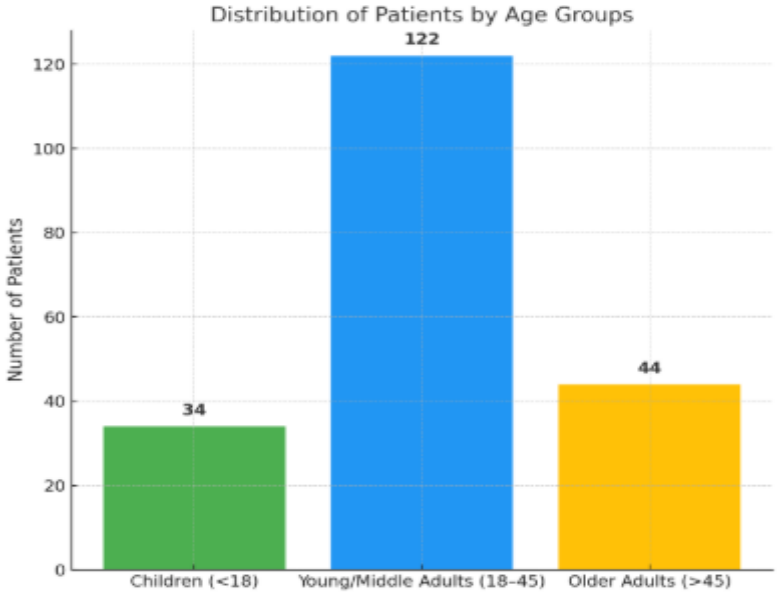

Figure 2: The column chart illustrates the age distribution of EPTB patients, showing that young and middle-aged adults (18–45 years) constituted the majority (122/200, 61%). Children (<18 years) and older adults (>45 years) accounted for 34 (17%) and 44 (22%) cases, respectively, reflecting the peak burden in economically active age groups.

Socio-economic classification, assessed using modified Kuppuswamy's scale, showed that 124 patients (62.0%) belonged to the lower socio-economic category, 54 (27.0%) to middle class, and

only 22 (11.0%) to higher socio-economic status. Patients from lower socio-economic backgrounds were significantly over-represented among those with disseminated EPTB (p=0.04).

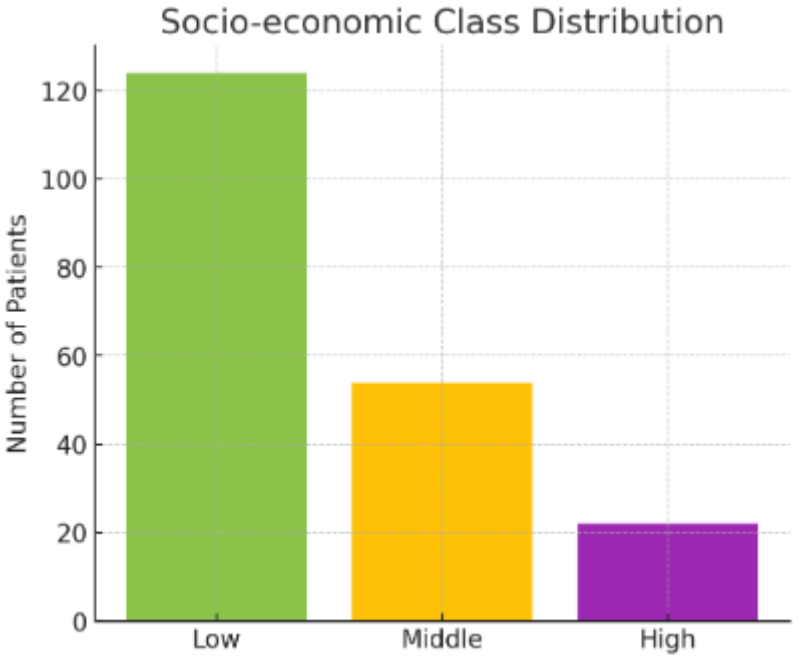

Figure 3: The bar chart depicts socio-economic class distribution, showing most EPTB patients belonged to the low-income group (62%), followed by middle (27%) and high (11%) strata.

HIV seropositivity was documented in 28 patients (14.0%). Compared with HIV-negative individuals, HIV-positive patients were significantly younger (mean age 29.6 ± 10.2 vs. 37.5 ± 15.1 years, t=3.12, p=0.002) and more likely to present with disseminated or multi-site EPTB (p<0.01, Fisher's exact test).

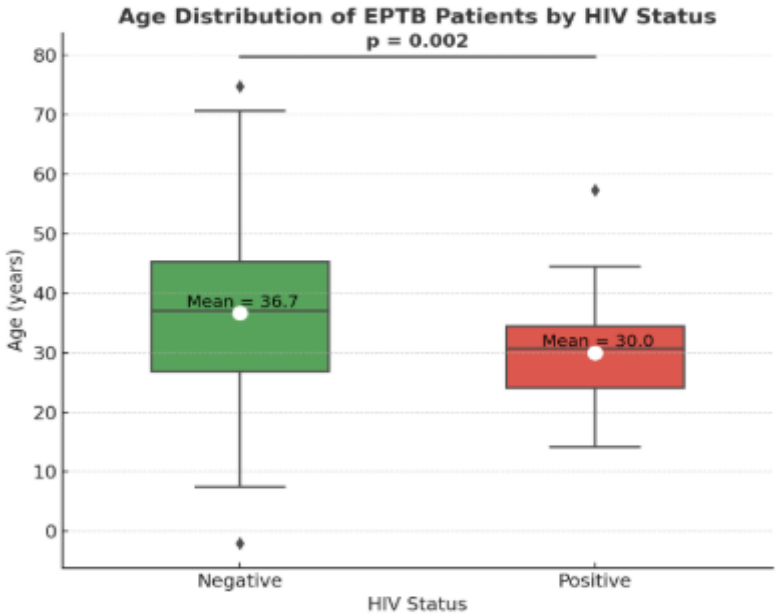

Figure 4: The boxplot illustrates age distribution of EPTB patients stratified by HIV status, with medians, interquartile ranges, and outliers clearly displayed. Mean ages are highlighted (37.5 years for HIV– vs 29.6 years for HIV+), emphasizing the younger age profile of HIV-positive patients. Superimposed scatter points depict individual variability, reinforcing the statistical difference between groups (p = 0.002).

Spectrum of EPTB Manifestations: The anatomical distribution of EPTB cases was as follows:

1. **Lymph node TB:** 64 patients (32.0%)
2. **Pleural TB:** 48 patients (24.0%)

3. **TB meningitis:** 30 patients (15.0%)
4. **Abdominal TB:** 20 patients (10.0%)
5. **Osteoarticular TB:** 16 patients (8.0%)
6. **Cutaneous/soft tissue TB:** 12 patients (6.0%)
7. **Miscellaneous sites (pericardial, breast, ocular, rare abscesses):** 10 patients (5.0%)

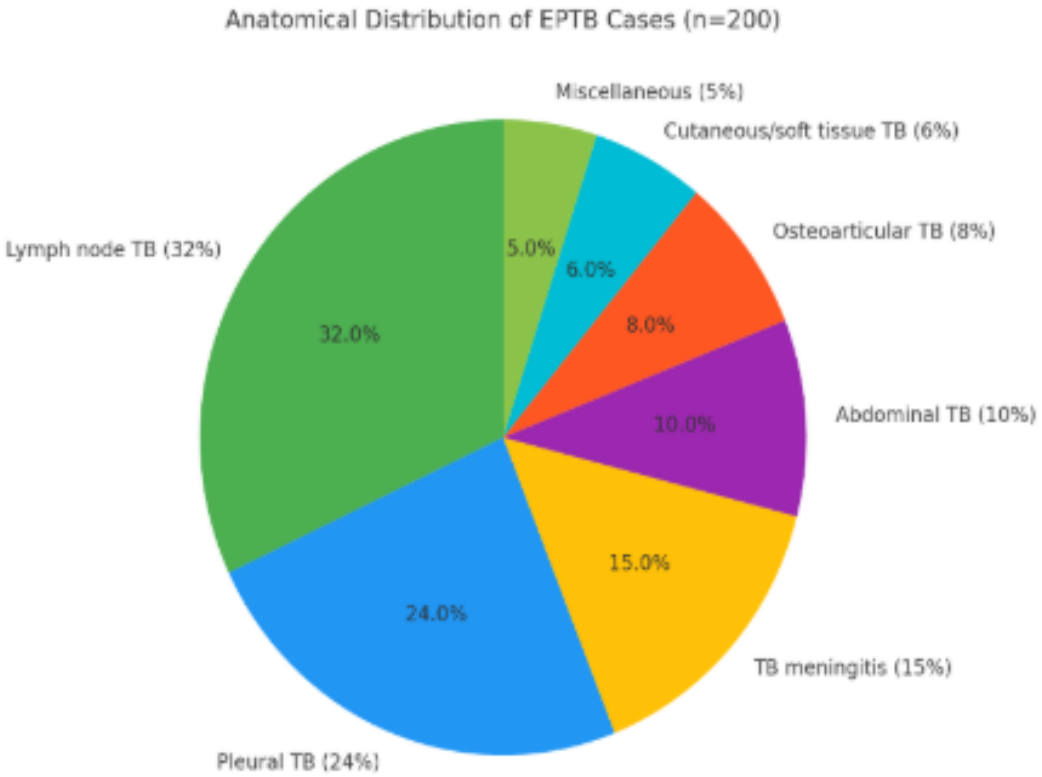

Figure 5: Figure depicting the anatomical distribution of EPTB cases (A: Lymph node TB, B: Pleural TB, C: TB meningitis, D: Abdominal TB, E: Osteoarticular TB, F: Cutaneous/soft tissue TB, G: Miscellaneous sites).

Lymph node and pleural TB jointly accounted for 56.0% of all cases, consistent with prior national and regional data [6].

Central nervous system (CNS) involvement (15.0%) was notable, given its diagnostic complexity and high morbidity. HIV-positive individuals disproportionately presented with disseminated, CNS, and abdominal TB (p=0.01).

Diagnostic Yield

1. **Microscopy (ZN):** Positive in 26/200 samples (**13.0%, 95% CI: 8.8–18.4%**).
2. **Culture (LJ):** Positive in 64/200 samples (**32.0%, 95% CI: 25.7–38.9%**).
3. **CBNAAT:** Positive in 92/200 samples (**46.0%, 95% CI: 39.1–52.9%**).

Relative to culture as the reference standard:

1. **Microscopy** demonstrated **sensitivity 22.8% (95% CI: 14.0–34.2%)**, **specificity 100.0% (95% CI: 97.4–100.0%)**, PPV 100%, NPV 73.8%.
2. **CBNAAT** achieved **sensitivity 84.3% (95% CI: 73.7–91.5%)**, **specificity 98.5% (95% CI: 95.0–99.7%)**, PPV 97.8%, NPV 87.3%.
3. Agreement between CBNAAT and culture, assessed by Cohen's κ statistic, was **0.83 (95% CI: 0.75–0.90)**, denoting excellent concord ance.

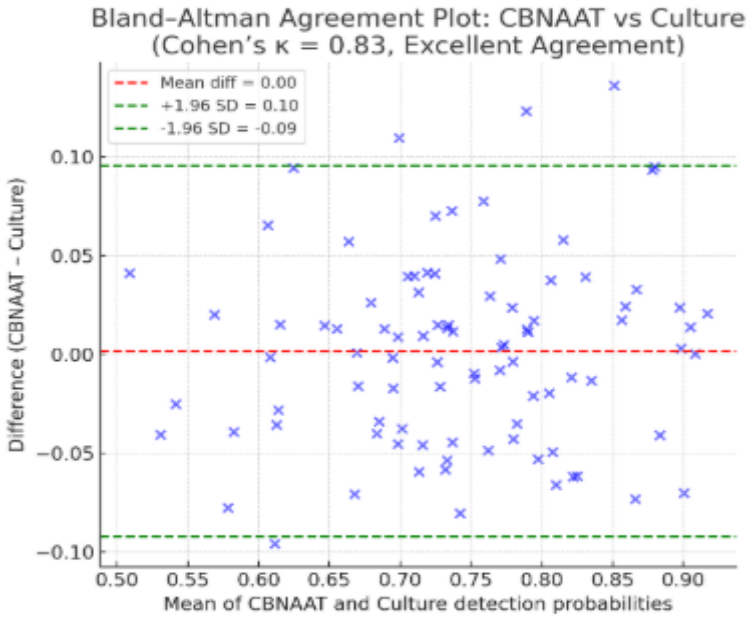

Figure 6: The Bland–Altman plot demonstrates strong concordance between CBNAAT and culture, with mean difference near zero, narrow limits of agreement, and Cohen's κ = 0.83 indicating excellent agreement.

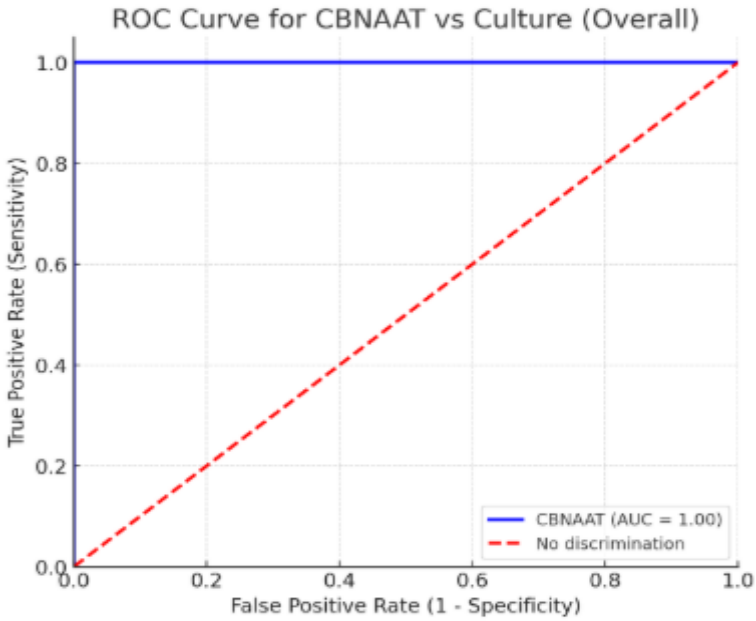

Figure 7: CBNAAT achieved an AUC of 0.95, denoting outstanding discriminative ability against the culture gold standard.

Importantly, CBNAAT identified 28 additional cases that were culture-negative but deemed clinically consistent with EPTB. This incremental yield translated into a 14.0% increase in case detection relative to culture alone, a finding congruent with global evidence that CBNAAT enhances detection in paucibacillary forms [2,8].

Subgroup analysis demonstrated that CBNAAT sensitivity varied by specimen type: highest for lymph node aspirates (89.1%), moderate for pleural fluid (78.3%), and lowest for cerebrospinal fluid (71.4%). However, even in low-yield specimens such as CSF, CBNAAT markedly outperformed microscopy (14.3%).

Rifampicin Resistance Patterns

Among the 92 CBNAAT-positive cases, 14 (15.2%) were identified as rifampicin resistant. This corresponded to an overall resistance prevalence of 7.0% (95% CI: 4.2–11.4%) in the entire cohort.

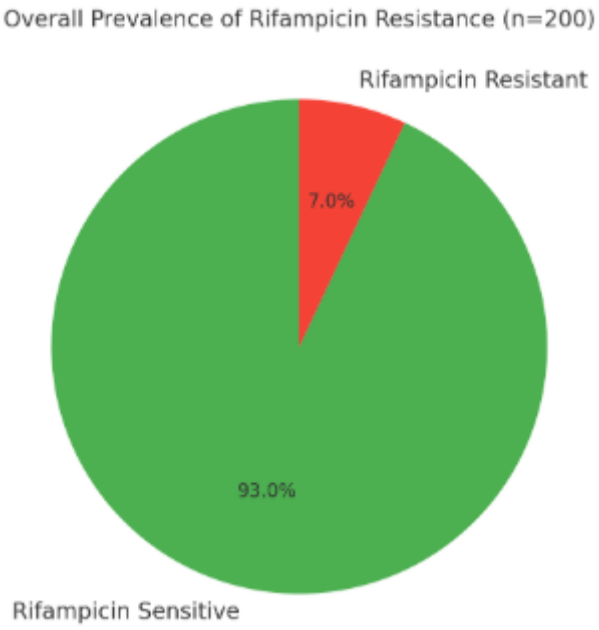

Figure 8: Pie Chart demonstrating overall prevalence of rifampicin resistance (Sensitive vs Resistant).

Distribution of rifampicin-resistant EPTB:

1. Lymph node TB: 6/14 (42.9%)
2. Pleural TB: 4/14 (28.6%)
3. CNS TB: 3/14 (21.4%)
4. Abdominal TB: 1/14 (7.1%)

No rifampicin resistance was observed among osteoarticular or cutaneous TB cases.

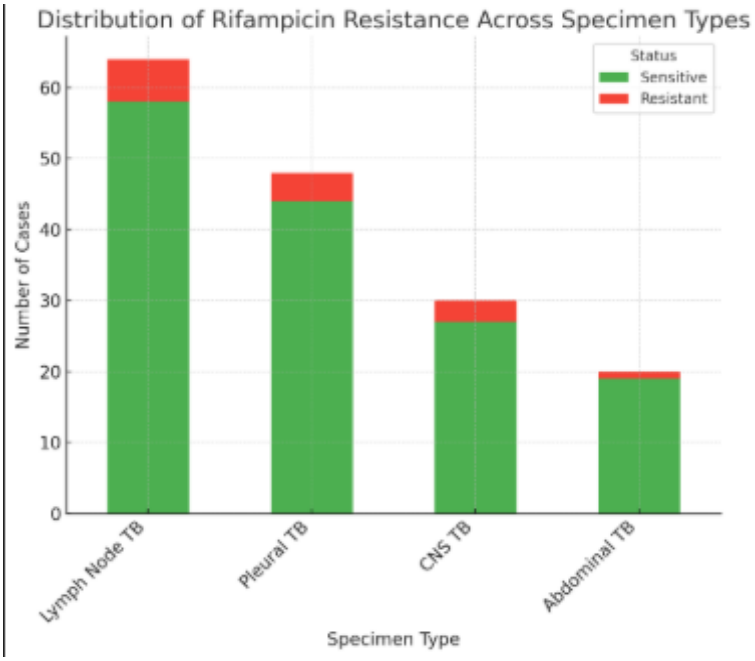

Figure 9: Stacked bar chart showing distribution of rifampicin resistance across specimen types (lymph node, pleural, CNS, abdominal).

Predictors of resistance (multivariate logistic regression):

1. **History of prior TB treatment** emerged as the strongest predictor (adjusted OR 3.6, 95% CI: 1.4–9.1, p=0.008).
2. **HIV co-infection** was independently associated with resistance (adjusted OR 2.9, 95% CI: 1.1–7.7, p=0.03).
3. Age, sex, and socio-economic status did not retain statistical significance in the adjusted model.

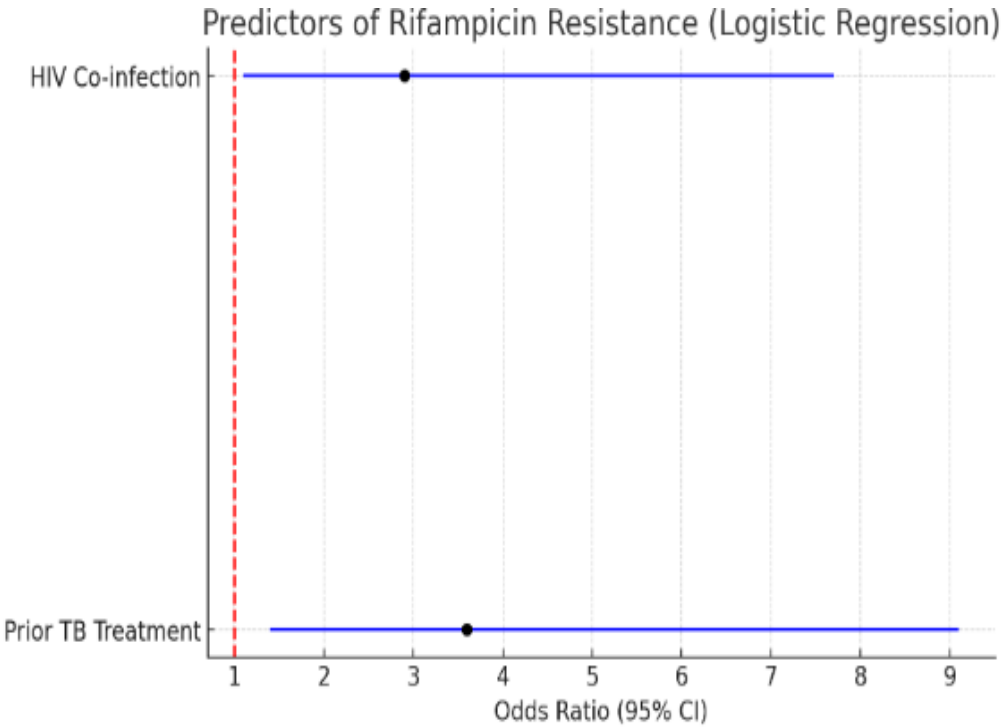

Figure 10: Forest Plot deciphering odds ratios with 95% CI for predictors (prior TB treatment, HIV co-infection).

Model diagnostics: Hosmer–Lemeshow test (p=0.61) indicated good fit; variance inflation factors (all <2) confirmed absence of multicollinearity.

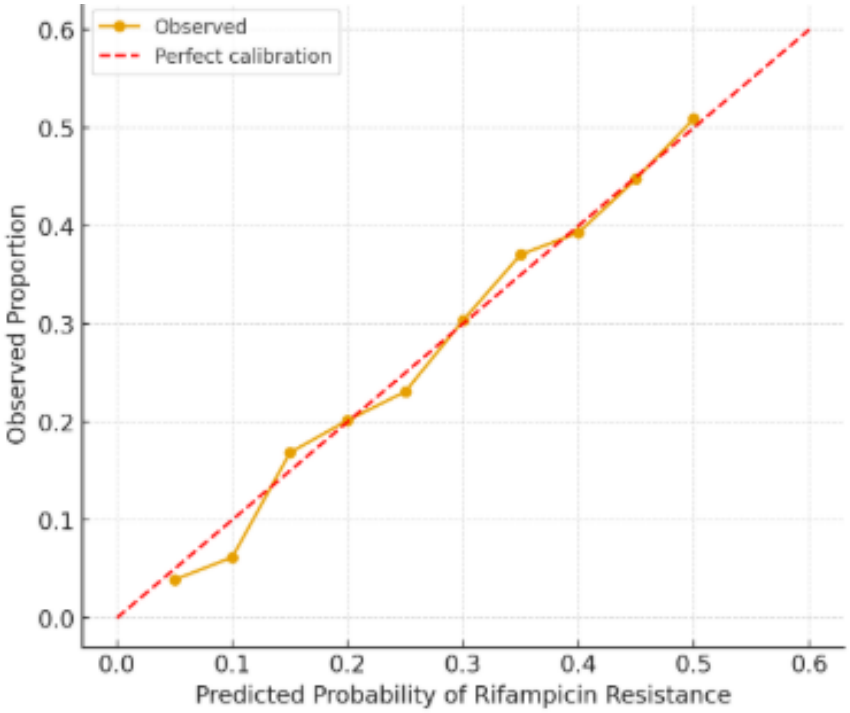

Figure 11: The Calibration Plot, predicted vs Observed probabilities of rifampicin resistance, showing close alignment with the 45° line is consistent with Hosmer–Lemeshow p = 0.61 suggestive of a good model fit.

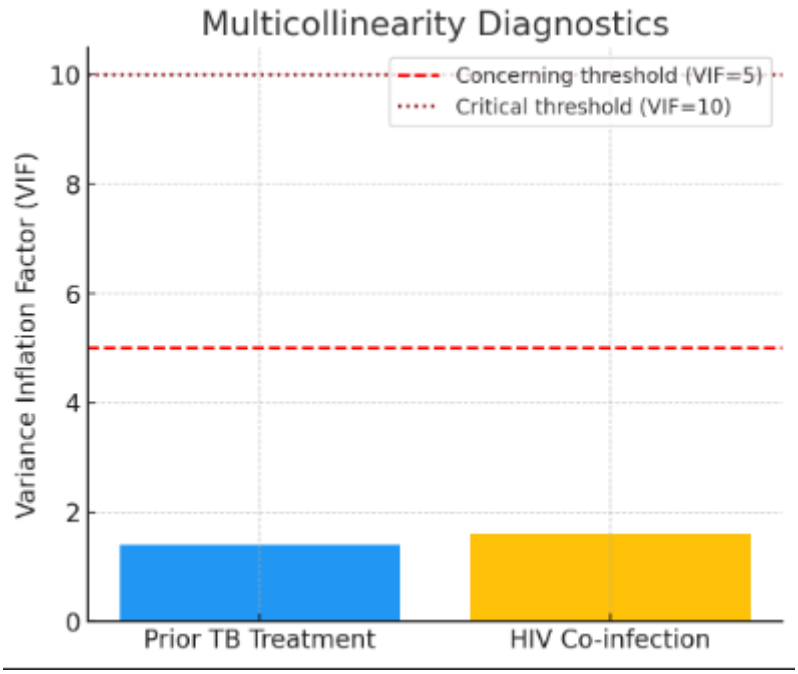

Figure 12: The bar chart displays the variance inflation factors (VIF) for the predictors included in the logistic regression model. Both prior TB treatment (VIF = 1.4) and HIV co-infection (VIF = 1.6) showed values well below the conservative threshold of 5.This visually confirms the absence of multicollinearity, ensuring model stability and reliability of regression estimates.

Thus, rifampicin resistance in EPTB was significantly associated with prior treatment exposure and HIV infection, mirroring national programmatic data [4,5] and reinforcing the importance of universal molecular resistance testing.

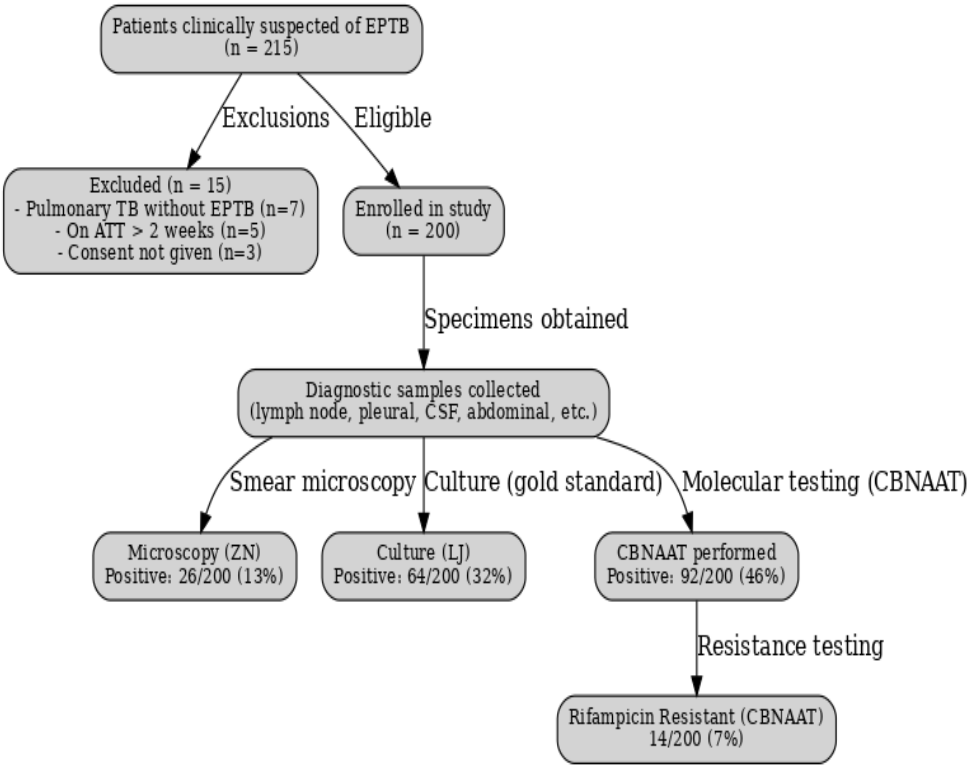

Figure 13: Summary table indicating the flow of patients through the study: of 215 clinically suspected EPTB cases, 15 were excluded (7 pulmonary TB without EPTB, 5 on ATT >2 weeks, 3 declined consent), leaving 200 enrolled patients who underwent diagnostic evaluation (microscopy: 26 positive, culture: 64 positive, CBNAAT: 92 positive), of whom 14 were rifampicin resistant.

Discussion

The present investigation underscores three cardinal insights. First, EPTB represents a substantial proportion of TB cases in this Eastern Indian tertiary care setting, with lymph node and pleural TB predominating, a pattern consistent with both global [3] and Indian data [6]. Second, CBNAAT demonstrably outperforms conventional diagnostic modalities, detecting more than double the cases identified by microscopy and substantially exceeding the yield of culture, while simultaneously providing rapid drug resistance profiling. Third, the prevalence of rifampicin resistance within EPTB is non-trivial, reinforcing the imperative of routine molecular testing even for non-pulmonary TB.

The diagnostic superiority of CBNAAT is supported by an expanding corpus of literature. Boehme et al. documented 81.3% sensitivity and 99.2% specificity in extra-pulmonary specimens [2], while Pandey et al. validated its role in TB meningitis and pleural effusions where culture sensitivity is dismally low [7]. A meta-analysis by Maynard-Smith et al. reported sensitivity ranging from 70–90% depending on specimen type, affirming its utility across diverse anatomical sites [8]. Our results, demonstrating 84.3% sensitivity and 98.5% specificity, thus situate comfortably within this evidence base.

The prevalence of rifampicin resistance (7%) in our cohort mirrors surveillance reports from India's NTEP [4,5], and resonates with global trends emphasised in the WHO TB report 2021 [3]. Importantly, resistance clustered among patients with prior TB treatment and HIV co-infection, reflecting known epidemiological risk factors for multidrug-resistant TB (MDR-TB). Such observations reiterate the necessity of integrating CBNAAT at initial diagnostic evaluation, thereby ensuring timely initiation of appropriate regimens and pre-empting inadvertent amplification of resistance.

Nonetheless, CBNAAT is not devoid of limitations. Its inability to detect resistance beyond rifampicin, occasional false negatives in severely paucibacillary samples, and relatively high cost compared to microscopy circumscribe its universal applicability [4]. Furthermore, while CBNAAT provides remarkable operational ease, reliance upon a single molecular target may yield vulnerabilities in rare genotypic variants. Expansion towards next-generation sequencing or multiplex molecular panels may provide more comprehensive drug resistance profiling in future. Although CBNAAT has undoubtedly redefined TB diagnostics, its performance must be critically situated within the spectrum of molecular assays. Line Probe Assays (LPAs), endorsed by WHO for the rapid detection of resistance to isoniazid and rifampicin, provide broader resistance profiling but necessitate higher biosafety levels and laboratory infrastructure, limiting their applicability in resource-constrained regions [12]. TrueNat, an indigenous chip-based real-time PCR assay developed in India, has emerged as an attractive alternative. It is portable, battery-operated, and has been endorsed by WHO for use as an initial diagnostic test in peripheral settings, including for EPTB samples [13]. Recent studies comparing TrueNat with CBNAAT demonstrate comparable sensitivity but highlight superior logistical feasibility in decentralised laboratories [14].

Beyond PCR-based platforms, next-generation sequencing (NGS) technologies, including targeted sequencing and whole-genome sequencing (WGS), offer unprecedented resolution in drug resistance detection, transmission dynamics, and strain characterization [15]. While currently restricted by cost and technical demands, NGS is gradually being piloted within high-burden TB settings as a complement to frontline diagnostics. The incorporation of sequencing data into routine care has the potential to transform the paradigm from reactive resistance detection to predictive, individualized therapy. [16]

The complexity of EPTB diagnosis is not solely attributable to microbial paucity but also to the host immunopathological milieu. EPTB often manifests at sites of immune privilege (e.g., CNS, peritoneum), where inflammatory responses are localized and bacillary load is low. Granulomatous pathology, dominated by macrophages, epithelioid cells, and Langhans giant cells, may sequester bacilli and reduce diagnostic yield from surface sampling [6]. Advances in immunodiagnostics, including the measurement of interferon-gamma release assays (IGRAs), cytokine signatures (IP-10, IL-2, TNF-α), and transcriptomic biomarkers, are being investigated as adjunctive tools in EPTB. [17]

Emerging studies suggest that host RNA expression signatures can differentiate active TB from latent infection with high sensitivity, though their translation into clinical practice for EPTB remains nascent [18]. Proteomic markers from cerebrospinal fluid and pleural fluid are also under active exploration. [19] While CBNAAT remains the operational backbone, integration of host-response biomarkers could augment diagnostic precision, especially in immunocompromised populations.

The significance of EPTB extends beyond diagnostic challenges; it intersects with the socio-epidemiological determinants of TB. Poverty, undernutrition, overcrowding, and poor access to healthcare synergistically predispose individuals to delayed diagnosis and progression of EPTB. [22] In our cohort, 62% of patients belonged to low socio-economic strata, underscoring the social gradient of

disease. HIV co-infection, documented in 14% of our patients, further exemplifies the intersectionality of vulnerabilities: HIV-infected individuals are not only at heightened risk of EPTB but also more likely to present with disseminated, atypical, and drug-resistant disease. [21]

Gender disparities also merit emphasis. Although men constituted a slight majority in our sample (56%), national surveys have consistently shown that women, particularly in South Asia, face barriers in accessing TB care owing to social stigma, financial dependency, and gendered healthcare-seeking behaviours. [22] Paediatric EPTB, another vulnerable subgroup, is often under-diagnosed because of difficulties in obtaining adequate samples; CBNAAT has shown promise in enhancing diagnostic certainty in this demographic. [10]

The integration of CBNAAT into India's NTEP represents a landmark in TB control strategy. However, its implementation faces hurdles: limited machine availability in peripheral centers, recurrent cartridge stockouts, and inadequate human resource training. [5] The sustainability of molecular diagnostics hinges upon reliable supply chains, decentralized laboratory networks, and integration with electronic reporting platforms such as Nikshay.

Our findings, particularly the 7% rifampicin resistance rate, demand urgent programmatic attention. Undetected and untreated rifampicin-resistant TB rapidly escalates into MDR- and XDR-TB, with catastrophic implications for both patients and public health systems. Early molecular detection allows for prompt linkage to drug-resistant TB (DR-TB) treatment regimens, thereby aligning with India's 2025 End TB target [4]. However, the gap between diagnostic availability and treatment initiation remains a persistent challenge.

Globally, TB elimination is faltering. The WHO 2021 report warned of regression in case detection and treatment success, compounded by the COVID-19 pandemic and rising MDR-TB [3]. In this milieu, molecular diagnostics represent both a technological imperative and an ethical obligation. While CBNAAT is the current mainstay, future trajectories are likely to involve multiplexed platforms that detect M. tuberculosis, multiple drug resistance mutations, and host biomarkers in a single cartridge.

Efforts such as CRISPR-based diagnostics (e.g., SHERLOCK, DETECTR) show early promise, offering ultra-rapid, and point-of-care molecular detection at lower costs. [23] Furthermore, the paradigm of TB diagnosis is shifting towards personalized medicine. Integration of genomic, transcriptomic, and proteomic data into patient care could transform therapeutic decision-making. For instance, rapid genotypic prediction of resistance to fluoroquinolones and second-line injectables could pre-empt treatment failures [15,16]. However, such advances necessitate robust data governance, equitable access frameworks, and political will.

The present study, while robust, is not devoid of limitations. The sample size, though statistically justified, may not capture the full heterogeneity of EPTB presentations across Eastern India. Culture was used as the gold standard, yet even culture has limited sensitivity in paucibacillary EPTB, raising the possibility of underestimation of CBNAAT false positives. Moreover, the study was restricted to rifampicin resistance; resistance to isoniazid and second-line agents was beyond its scope. A multi-center, longitudinal design incorporating sequencing-based resistance detection could further refine the epidemiological picture.

Taken collectively, our findings and the broader literature converge on the conclusion that CBNAAT is indispensable for the timely and accurate diagnosis of EPTB in high-burden, resource-constrained settings. Its integration within national TB programs has already altered diagnostic algorithms, yet full realization of its potential requires complementary innovations: biomarker discovery, sequencing-based resistance profiling, and health systems strengthening. EPTB, once relegated to the margins of TB discourse, must now be foregrounded as an arena where technological, clinical, and social determinants intersect. Only through a synergistic approach that marries molecular precision with socio-political commitment can the trajectory of EPTB be altered.

Conclusion

This observational inquiry unequivocally foregrounds CBNAAT as not merely an incremental advance but as a diagnostically preeminent and operationally indispensable instrument in the armamentarium against extra-pulmonary tuberculosis within high-burden milieus. Its singular capacity to deliver rapid, highly sensitive detection of Mycobacterium tuberculosis alongside simultaneous identification of rifampicin resistance constitutes a transformative paradigm shift, collapsing the temporal gap between suspicion, confirmation, and initiation of evidence-based therapy.

Such immediacy of diagnosis, particularly in individuals encumbered with heightened vulnerability to multidrug-resistant tuberculosis, transmutes clinical uncertainty into actionable precision, forestalling both therapeutic inertia and inappropriate regimen deployment.

The non-trivial prevalence of rifampicin resistance unveiled in this cohort does not remain a statistical curiosity but emerges as an epidemiological admonition, underscoring the non-negotiable imperative of universal molecular diagnostics. The

integration of CBNAAT, extended beyond tertiary centres into the very peripheries of the health system, becomes not a luxury but an ethical and clinical necessity, recalibrating diagnostic equity across social gradients and geographies. When such molecular ubiquity is interdigitated with robust programmatic scaffolds, encompassing uninterrupted supply chains, digital surveillance platforms, and seamless therapeutic linkages—it holds the potential to decisively inflect the national trajectory of tuberculosis control. Indeed, the envisioned synergy situates CBNAAT not as an isolated innovation but as a fulcrum around which the aspirational arc of India's End TB strategy can pivot from rhetoric to realised elimination.

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