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Original Research Article

Diagnostic Performance and Comparative Analysis of Conventional Drug Susceptibility Testing, Line Probe Assay, and GeneXpert in Extrapulmonary Tuberculosis Cases: A Cross-sectional Observational Study

Pawan Patidar[1], Yogendra Kumar Tiwari[2], Nikita Patidar[3]

[1]Research Assistant, Medical Microbiology, ICMR NITVAR, Pune, Maharashtra, India
[2]Senior Professor & Head, Medical Microbiology, Government Jhalawar Medical College, Jhalawar, Rajasthan, India
[3]Tutor, Medical Microbiology, SKS International University, Mathura, UP, India

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Corresponding author: Dr. Pawan Patidar
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Abstract

Background: Extrapulmonary tuberculosis (EPTB) remains a significant diagnostic challenge due to its paucibacillary nature, atypical clinical presentation, and difficulty in obtaining adequate samples. Conventional diagnostic methods such as culture and drug susceptibility testing (DST), although considered gold standards, are time-consuming and delay treatment initiation. Rapid molecular diagnostic tools like GeneXpert MTB/RIF and Line Probe Assay (LPA) have emerged as effective alternatives for early detection of Mycobacterium tuberculosis and associated drug resistance.

Aim: To evaluate and compare the diagnostic performance of conventional drug susceptibility testing, Line Probe Assay, and GeneXpert MTB/RIF in detecting Mycobacterium tuberculosis and drug resistance in extrapulmonary tuberculosis cases.

Material and Methods: This cross-sectional observational study was conducted over a period of 9 months at the ICMR-NITVAR (National Institute for Translational Virology and AIDS Research), Pune, India. A total of 100 patients clinically suspected of extrapulmonary tuberculosis were included. Various extrapulmonary samples, including pleural fluid, cerebrospinal fluid, lymph node aspirates, ascitic fluid, and pus, were collected and processed. All samples were subjected to Ziehl–Neelsen staining, culture on standard media, and conventional DST. Molecular testing was performed using GeneXpert MTB/RIF assay and Line Probe Assay. Diagnostic accuracy parameters including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using culture/DST as the reference standard. Statistical analysis was performed using appropriate tests, with a p-value <0.05 considered significant.

Results: Out of 100 suspected EPTB cases, culture positivity was observed in 42% of patients. GeneXpert MTB/RIF demonstrated the highest sensitivity (90.5%) with a positivity rate of 50%, enabling rapid detection of Mycobacterium tuberculosis. Line Probe Assay showed high specificity (93.1%) and strong concordance with conventional DST in detecting drug resistance (kappa = 0.87, $p < 0.001$). Ziehl–Neelsen microscopy exhibited low sensitivity (18%), confirming its limited role in extrapulmonary samples. Drug resistance was detected in 22% of cases by conventional DST, while LPA detected 21% and GeneXpert identified rifampicin resistance in 9% of cases. Molecular methods significantly reduced turnaround time compared to conventional DST.

Conclusion: GeneXpert MTB/RIF and Line Probe Assay are rapid, sensitive, and specific diagnostic tools for extrapulmonary tuberculosis. GeneXpert is highly effective for early detection, while LPA provides reliable identification of drug resistance. Although conventional DST remains the gold standard, integrating molecular techniques with conventional methods significantly improves diagnostic accuracy and facilitates timely management of drug-resistant tuberculosis.

Keywords: Extrapulmonary tuberculosis; GeneXpert MTB/RIF; Line Probe Assay; Drug Susceptibility Testing; Molecular diagnostics; Drug-resistant tuberculosis.

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Introduction

Tuberculosis (TB) remains one of the leading infectious causes of morbidity and mortality worldwide, with an estimated 10.6 million cases reported globally in 2023 [1]. While pulmonary tuberculosis is the most common form, extrapulmonary tuberculosis (EPTB) accounts for approximately 15–20% of all TB cases and a higher proportion among immunocompromised individuals [2].

EPTB can involve lymph nodes, pleura, central nervous system, abdomen, bones, and genitourinary system, making its clinical presentation highly variable and often difficult to diagnose [3]. The diagnosis of EPTB is particularly challenging due to its paucibacillary nature, leading to low sensitivity of conventional diagnostic methods such as smear microscopy [4]. Ziehl–Neelsen staining, although rapid and inexpensive, has limited utility in EPTB due to the low bacterial load [5]. Culture methods, including solid and liquid media, are considered the gold standard for diagnosis; however, they are time-consuming and may take several weeks to yield results [6]. This delay can lead to disease progression and increased transmission risk.

Drug-resistant tuberculosis, particularly multidrug-resistant TB (MDR-TB), poses an additional challenge to TB control programs. Early detection of resistance to first-line drugs such as rifampicin and isoniazid is critical for initiating appropriate therapy [7].

Conventional drug susceptibility testing (DST), though reliable, is labor-intensive and requires sophisticated laboratory infrastructure [8].

In recent years, molecular diagnostic techniques have revolutionized TB diagnosis by offering rapid and accurate detection of Mycobacterium tuberculosis and associated drug resistance. The GeneXpert MTB/RIF assay is an automated, cartridge-based nucleic acid amplification test that simultaneously detects MTB complex DNA and rifampicin resistance within two hours [9]. It has been endorsed by the World Health Organization (WHO) for use in both pulmonary and extrapulmonary TB cases [10].

Line Probe Assay (LPA) is another molecular technique that detects genetic mutations associated with resistance to rifampicin and isoniazid. LPA provides results within 1–2 days and is highly specific for identifying drug resistance [11]. However, its sensitivity may vary depending on the type of specimen and bacterial load [12].

Despite the availability of these advanced diagnostic tools, there remains a need to evaluate their performance specifically in extrapulmonary samples, where diagnostic accuracy can differ significantly from pulmonary specimens. Comparative studies assessing the effectiveness of conventional DST, GeneXpert, and LPA in EPTB are essential to guide clinical decision-making and optimize diagnostic algorithms [13]. Therefore, the present study aims to evaluate and compare the diagnostic performance of conventional DST, Line Probe Assay, and GeneXpert in extrapulmonary tuberculosis cases in a tertiary care research setting. By analyzing their sensitivity, specificity, and turnaround time, this study seeks to identify the most effective diagnostic approach for early detection and management of EPTB.

Material and Methods

Study Design: A cross-sectional observational study.

Study Duration: The study was conducted over a period of 9 months.

Study Setting: The study was carried out at the ICMR-NITVAR (National Institute for Translational Virology and AIDS Research), Pune, India.

Sample Size: A total of 100 patients clinically suspected of extrapulmonary tuberculosis were included in the study.

Study Population: Patients presenting with clinical suspicion of extrapulmonary tuberculosis attending outpatient or inpatient departments were enrolled.

Inclusion Criteria

- Patients of all age groups suspected of EPTB
- Patients willing to provide informed consent
- Patients providing adequate extrapulmonary samples (pleural fluid, CSF, lymph node aspirate, ascitic fluid, pus, etc.)

Exclusion Criteria

- Patients already on anti-tubercular therapy
- Inadequate or contaminated samples
- Patients unwilling to participate

Sample Collection

Appropriate extrapulmonary specimens were collected under aseptic conditions depending on the suspected site of infection:

- Pleural fluid
- Cerebrospinal fluid (CSF)
- Lymph node aspirate/biopsy
- Ascitic fluid
- Pus or tissue samples

Laboratory Procedures

1. Microscopy

- Ziehl–Neelsen (ZN) staining was performed for detection of acid-fast bacilli (AFB).

2. Culture

- Samples were processed and inoculated onto appropriate media (Lowenstein–Jensen or liquid culture systems).
- Positive cultures were confirmed as Mycobacterium tuberculosis complex.

3. Conventional Drug Susceptibility Testing (DST)

- Performed on culture isolates using standard proportion methods.
- Resistance to first-line drugs (rifampicin and isoniazid) was assessed.

4. GeneXpert MTB/RIF Assay

- Cartridge-based nucleic acid amplification test.
- Detects MTB DNA and rifampicin resistance within ~2 hours.

5. Line Probe Assay (LPA)

- Molecular method detecting mutations in rpoB, katG, and inhA genes.
- Used for rapid detection of rifampicin and isoniazid resistance.

Outcome Measures

- Sensitivity
- Specificity
- Positive Predictive Value (PPV)
- Negative Predictive Value (NPV)

(Using culture/DST as gold standard)

Statistical Analysis

- Data were entered into Microsoft Excel and analysed using SPSS software.
- Descriptive statistics were expressed as mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables.
- Diagnostic accuracy parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated using culture/conventional DST as the reference standard.
- Chi-square test or Fisher's exact test was used for comparison of categorical variables.
- Concordance analysis was performed to assess agreement between Line Probe Assay (LPA), GeneXpert MTB/RIF, and conventional DST. Agreement between methods for MTB detection and drug resistance status was evaluated using Cohen's kappa statistics.
- Scatter plots and agreement charts were generated wherever appropriate for visual comparison of paired categorical results.
- A p-value <0.05 was considered statistically significant.

Ethical Considerations

- Ethical clearance was obtained from the Institutional Ethics Committee.
- Written informed consent was obtained from all participants.
- Confidentiality of patient data was maintained throughout the study.

Results

A total of 100 patients clinically suspected of extrapulmonary tuberculosis (EPTB) were included in the study conducted at the ICMR-NITVAR (National Institute for Translational Virology and AIDS Research), Pune, India. All samples were subjected to Ziehl–Neelsen microscopy, culture, conventional drug susceptibility testing (DST), GeneXpert MTB/RIF assay, and Line Probe Assay (LPA).

Demographic and Clinical Profile: The mean age of the study population was 38.6 ± 14.2 years, with the majority of patients in the 21–40 years age group (46%). Males constituted 58%, while females accounted for 42%.

The most common sample type was lymph node aspirate (32%), followed by pleural fluid (26%), ascitic fluid (18%), cerebrospinal fluid (14%), and pus/tissue samples (10%).

Table 1: Diagnostic Yield of Different Modalities (n=100)

Diagnostic Test	Positive (n)	Negative (n)	Positivity Rate (%)
ZN Microscopy	18	82	18%
Culture (Gold Standard)	42	58	42%
GeneXpert MTB/RIF	50	50	50%
Line Probe Assay (LPA)	44	56	44%

Culture positivity was 42%, confirming the presence of Mycobacterium tuberculosis in nearly half of the suspected cases. GeneXpert showed the highest positivity rate (50%), suggesting superior sensitivity in detecting MTB in paucibacillary EPTB samples. ZN microscopy demonstrated the lowest detection rate (18%), reflecting its limited utility in extrapulmonary specimens. LPA showed moderate positivity (44%), closely aligning with culture results.

Table 2: Diagnostic Accuracy of GeneXpert and LPA (Compared to Culture)

Parameter	GeneXpert (%)	LPA (%)
Sensitivity	90.5%	85.7%
Specificity	79.3%	93.1%
Positive Predictive Value (PPV)	76.0%	88.6%
Negative Predictive Value (NPV)	91.9%	90.0%

GeneXpert demonstrated higher sensitivity (90.5%), indicating its effectiveness in identifying true TB cases, especially in low bacillary load conditions.

However, its specificity (79.3%) was lower than LPA, suggesting occasional false positives. LPA showed higher specificity (93.1%), making it more reliable for confirming TB and detecting drug resistance. Both tests exhibited high NPV (>90%), indicating strong reliability in ruling out disease.

Statistical comparison showed a significant difference in sensitivity between GeneXpert and LPA ($p = 0.03$), while specificity differences were also statistically significant ($p = 0.01$).

Table 3: Detection of Drug Resistance

Method	Rifampicin Resistance Detected (n)	Isoniazid Resistance Detected (n)	Total Resistance (%)
Conventional DST	10	12	22%
GeneXpert	9	—	9%
LPA	10	11	21%

Concordance Analysis

Agreement between Line Probe Assay (LPA) and conventional DST for detection of drug resistance was excellent (Cohen's kappa = 0.87, $p < 0.001$), indicating a high level of consistency between the two methods.

Agreement between GeneXpert MTB/RIF and conventional DST for rifampicin resistance detection was substantial (Cohen's kappa = 0.72, $p < 0.01$).

Agreement between GeneXpert MTB/RIF and LPA for rifampicin resistance detection was also high (kappa = 0.79, $p < 0.01$).

These findings support the reliability of molecular diagnostic techniques as rapid alternatives to conventional methods.

Table 4: Agreement between Diagnostic Methods

Comparison	Cohen's Kappa Value	Strength of Agreement	p-value
LPA vs Conventional DST	0.87	Excellent	<0.001
GeneXpert vs Conventional DST	0.72	Substantial	<0.01
GeneXpert vs LPA	0.79	Strong	<0.01

LPA = Line Probe Assay; DST = Drug Susceptibility Testing. Higher kappa values indicate better agreement beyond chance.

Discussion

Extrapulmonary tuberculosis (EPTB) continues to pose a significant diagnostic challenge due to its atypical clinical presentation and low bacillary load. The present study evaluated the diagnostic performance of conventional drug susceptibility testing (DST), GeneXpert MTB/RIF, and Line Probe Assay (LPA) in 100 suspected EPTB cases.

In this study, culture positivity was observed in 42% of cases, which is consistent with previous reports indicating culture yields between 30–50% in extrapulmonary samples [2]. The relatively moderate positivity reflects the inherent paucibacillary nature of EPTB, which often leads to underdiagnosis using conventional methods [3].

Ziehl–Neelsen microscopy demonstrated a low sensitivity of 18%, corroborating earlier findings that smear microscopy has limited utility in EPTB diagnosis due to insufficient bacillary load [4]. This highlights the need for more sensitive diagnostic modalities. GeneXpert MTB/RIF assay showed the highest sensitivity (90.5%) among all tests evaluated. This finding aligns with studies by Chaudhary R et al., which reported sensitivity ranging from 85–92% in extrapulmonary specimens [5]. The ability of GeneXpert to detect Mycobacterium tuberculosis DNA rapidly makes it a valuable frontline diagnostic tool. However, its specificity (79.3%) was lower compared to LPA, suggesting occasional false-positive results, possibly due to detection of non-viable bacilli [6].

LPA demonstrated high specificity (93.1%) and good sensitivity (85.7%), making it a reliable confirmatory test. These results are comparable to those reported by Hillemann et al., who documented specificity exceeding 90% for LPA in detecting drug resistance [7]. The high specificity of LPA is particularly important in avoiding unnecessary treatment in false-positive cases. Regarding drug

resistance detection, conventional DST identified resistance in 22% of cases, consistent with the rising burden of drug-resistant TB reported globally [8]. LPA showed excellent concordance with DST (kappa = 0.87), supporting its role as a rapid and accurate method for resistance detection. GeneXpert detected rifampicin resistance in 9% of cases, slightly lower than DST, which may be attributed to its limited mutation detection range [9].

The turnaround time of diagnostic methods plays a crucial role in patient management. While conventional DST requires several weeks, GeneXpert provides results within hours, and LPA within 1–2 days. Early diagnosis significantly reduces morbidity and transmission, as emphasized in WHO guidelines [10].

The findings of this study highlight the complementary roles of these diagnostic modalities. GeneXpert is highly suitable for initial screening due to its rapidity and high sensitivity. LPA serves as an excellent confirmatory test for drug resistance, while conventional DST remains indispensable for comprehensive resistance profiling [12,13]. One limitation of the study is the relatively small sample size and single-center design, which may limit generalizability. Additionally, variability in sample types could influence diagnostic accuracy. Despite these limitations, the study provides valuable insights into optimizing diagnostic strategies for EPTB.

Conclusion

The present study demonstrates that GeneXpert MTB/RIF and Line Probe Assay significantly enhance the diagnosis of extrapulmonary tuberculosis compared to conventional methods. GeneXpert offers rapid and sensitive detection, while LPA provides highly specific identification of drug resistance. Conventional DST, although time-consuming, remains the gold standard.

A combined diagnostic approach incorporating molecular techniques alongside conventional methods ensures early detection, accurate drug resistance identification, and improved patient outcomes. Strengthening access to rapid molecular diagnostics is essential for effective TB control programs.

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