Original Research

EVALUATION OF DRUG RESISTANCE DETECTION BY GENEXPERT MTB/RIF, LINE PROBE ASSAY, AND CONVENTIONAL DST IN EXTRAPULMONARY TUBERCULOSIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Background: Extrapulmonary tuberculosis (EPTB) constitutes a significant proportion of tuberculosis cases worldwide and poses major diagnostic challenges because of its paucibacillary nature and difficulty in specimen collection. The emergence of multidrug-resistant tuberculosis (MDR-TB) has further emphasized the need for rapid and accurate drug resistance detection methods. Molecular diagnostic techniques such as GeneXpert MTB/RIF and Line Probe Assay (LPA) have increasingly been utilized as rapid alternatives to conventional drug susceptibility testing (DST) for diagnosis of drug-resistant EPTB. **Aim:** To evaluate and compare the diagnostic performance of GeneXpert MTB/RIF, Line Probe Assay, and conventional drug susceptibility testing for detection of drug resistance in extrapulmonary tuberculosis through a systematic review and meta-analysis. **Materials and Methods:** A systematic review and meta-analysis was conducted according to PRISMA guidelines. Electronic databases including PubMed, Scopus, Embase, Web of Science, Cochrane Library, and Google Scholar were searched for studies published between January 2010 and December 2025. Studies evaluating GeneXpert MTB/RIF, LPA, and conventional DST in extrapulmonary tuberculosis specimens were included. Data regarding sensitivity, specificity, diagnostic odds ratio, and resistance detection rates were extracted. Methodological quality was assessed using the QUADAS-2 tool. Meta-analysis was performed using a random-effects model. **Results:** A total of 38 studies comprising 8,462 extrapulmonary specimens were included in the analysis. GeneXpert MTB/RIF demonstrated pooled sensitivity and specificity of 82.4% and 97.1%, respectively, for detection of rifampicin resistance. Line Probe Assay showed higher pooled sensitivity (89.6%) and specificity (98.4%). The highest diagnostic accuracy for both assays was observed in tissue biopsy and lymph node specimens, whereas lower sensitivity was noted in pleural and ascitic fluid samples. Rifampicin-resistant tuberculosis was identified in 8.1% of cases, while multidrug-resistant tuberculosis accounted for 5.1% of cases. Conventional DST remained the reference standard but required significantly longer turnaround time compared with molecular assays. Significant heterogeneity was observed among included studies. **Conclusion:** Both GeneXpert MTB/RIF and Line Probe Assay demonstrate high diagnostic accuracy for rapid detection of drug resistance in extrapulmonary tuberculosis. LPA provides superior sensitivity and broader resistance profiling, whereas GeneXpert offers faster turnaround time and operational simplicity. Integration of rapid molecular diagnostics with conventional DST can substantially improve early diagnosis and management of drug-resistant extrapulmonary tuberculosis.

Keywords: Extrapulmonary Tuberculosis, Genexpert MTB/RIF, Line Probe Assay, Drug Susceptibility Testing, Drug-Resistant Tuberculosis, MDR-TB, Rifampicin Resistance, Meta-Analysis.

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INTRODUCTION

Tuberculosis (TB) remains one of the leading infectious diseases worldwide and continues to pose a major public health challenge despite significant advances in diagnosis and treatment. According to the World Health Organization (WHO), nearly 10.8 million people developed tuberculosis globally in 2024, with a considerable proportion presenting as

extrapulmonary tuberculosis (EPTB) [1]. EPTB accounts for approximately 15–25% of all tuberculosis cases and occurs more frequently among immunocompromised individuals, children, and patients infected with human immunodeficiency virus (HIV) [2].

Extrapulmonary tuberculosis refers to infection involving organs and tissues outside the lungs, including lymph nodes, pleura, meninges, bones, joints, abdomen, and the genitourinary system [3]. The diagnosis of EPTB is often challenging because of nonspecific clinical manifestations, difficulty in obtaining representative samples, and the paucibacillary nature of extrapulmonary specimens [4]. Conventional diagnostic methods such as Ziehl–Neelsen staining and culture exhibit limited sensitivity in EPTB, leading to delayed diagnosis and treatment initiation [5].

The emergence and increasing prevalence of multidrug-resistant tuberculosis (MDR-TB) and rifampicin-resistant tuberculosis (RR-TB) have further complicated TB control efforts globally [6]. Drug-resistant EPTB represents a major clinical concern because delayed detection of resistance can result in treatment failure, disease progression, and increased mortality [7]. Therefore, rapid and accurate identification of drug resistance is essential for timely initiation of appropriate therapy and improved patient outcomes.

Conventional phenotypic drug susceptibility testing (DST) remains the reference standard for detection of anti-tubercular drug resistance. However, conventional DST methods require mycobacterial culture and prolonged incubation periods ranging from several weeks to months, thereby delaying clinical decision-making [8]. Additionally, these methods require sophisticated laboratory infrastructure and biosafety facilities, which may not be readily available in resource-limited settings [9].

To overcome these limitations, molecular diagnostic assays have been increasingly adopted for rapid detection of Mycobacterium tuberculosis and associated drug resistance mutations. GeneXpert MTB/RIF is a cartridge-based nucleic acid amplification test capable of simultaneously detecting Mycobacterium tuberculosis complex and rifampicin resistance directly from clinical specimens within approximately two hours [10]. The assay targets mutations in the rpoB gene, which are strongly associated with rifampicin resistance and MDR-TB [11]. Due to its rapid turnaround time and minimal technical requirements, GeneXpert has been endorsed by WHO for use in both pulmonary and extrapulmonary tuberculosis [1].

Line Probe Assay (LPA) is another important molecular diagnostic technique that utilizes reverse hybridization technology to detect mutations associated with resistance to rifampicin and isoniazid [12]. Compared with GeneXpert MTB/RIF, LPA provides broader information regarding resistance patterns and has demonstrated high sensitivity and specificity in smear-positive samples [13]. However, the performance of LPA in extrapulmonary specimens remains variable because of low bacillary load and heterogeneous specimen characteristics [14].

Several studies have evaluated the diagnostic performance of GeneXpert MTB/RIF and LPA for detection of drug resistance in EPTB, but the reported results have shown considerable variability across different specimen types and study populations [15,16]. Although conventional DST continues to serve as the gold standard, rapid molecular assays have emerged as valuable tools for early diagnosis and management of drug-resistant tuberculosis [17]. Nevertheless, comparative evidence regarding the diagnostic accuracy of GeneXpert MTB/RIF, LPA, and conventional DST specifically in extrapulmonary tuberculosis remains limited.

Therefore, the present systematic review and meta-analysis was undertaken to comprehensively evaluate and compare the effectiveness of GeneXpert MTB/RIF, Line Probe Assay, and conventional drug susceptibility testing for detection of drug resistance in extrapulmonary tuberculosis.

MATERIALS AND METHODS

Study Design

The present study was conducted as a systematic review and meta-analysis to evaluate the diagnostic performance of GeneXpert MTB/RIF, Line Probe Assay (LPA), and conventional drug susceptibility testing (DST) for detection of drug resistance in extrapulmonary tuberculosis (EPTB). The methodology was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [18].

Literature Search Strategy

A comprehensive and systematic literature search was performed using the electronic databases PubMed/MEDLINE, Scopus, Embase, Web of Science, Cochrane Library, and Google Scholar. Relevant studies published between January 2010 and December 2025 were searched.

The search strategy included combinations of Medical Subject Headings (MeSH) terms and free-text keywords such as:

- “Extrapulmonary tuberculosis”
- “EPTB”
- “GeneXpert MTB/RIF”
- “CBNAAT”
- “Line Probe Assay”
- “LPA”
- “Drug susceptibility testing”

- "DST"
- "Drug resistance"
- "Multidrug-resistant tuberculosis"
- "MDR-TB"
- "Rifampicin resistance"

Boolean operators "AND" and "OR" were applied appropriately to optimize the search strategy. Reference lists of eligible articles and relevant review papers were also manually screened to identify additional studies.

Eligibility Criteria

Inclusion Criteria

Studies fulfilling the following criteria were included:

1. Original research articles evaluating GeneXpert MTB/RIF, LPA, or conventional DST in extrapulmonary tuberculosis.
2. Studies reporting diagnostic accuracy parameters such as sensitivity, specificity, positive predictive value, or negative predictive value.
3. Studies involving extrapulmonary clinical specimens including lymph node aspirates, pleural fluid, cerebrospinal fluid, tissue biopsies, ascitic fluid, pus, synovial fluid, and other extrapulmonary samples.
4. Human studies published in English language.
5. Studies using culture and/or phenotypic DST as reference standards.

Exclusion Criteria

The following studies were excluded:

1. Review articles, editorials, letters, conference abstracts, and case reports.
2. Animal studies and in vitro experimental studies.
3. Studies lacking sufficient data for extraction of diagnostic accuracy measures.
4. Duplicate publications or overlapping datasets.
5. Studies exclusively involving pulmonary tuberculosis specimens.

Study Selection

All retrieved studies were independently screened by two reviewers. Initially, titles and abstracts were assessed for relevance. Full-text articles of potentially eligible studies were subsequently evaluated according to predefined inclusion and exclusion criteria. Any disagreement between reviewers was resolved by consensus or consultation with a third reviewer.

The study selection process was documented using the PRISMA flow diagram.

Data Extraction

Data extraction was independently performed by two investigators using a standardized data extraction form. The following information was collected from each study:

- First author name
- Year of publication
- Country of study
- Study design
- Sample size
- Type of extrapulmonary specimen
- Diagnostic method evaluated
- Reference standard used
- Number of true positives, false positives, true negatives, and false negatives
- Sensitivity and specificity values
- Drug resistance patterns detected
- Turnaround time of diagnostic assays

Any discrepancies in extracted data were resolved through discussion and re-evaluation of the original article.

Quality Assessment

The methodological quality of included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [19]. The following domains were evaluated:

1. Patient selection
2. Index test
3. Reference standard
4. Flow and timing

Each domain was assessed for risk of bias and applicability concerns. Studies were categorized as having low, high, or unclear risk of bias.

Outcome Measures

Primary Outcome

- Diagnostic accuracy of GeneXpert MTB/RIF, LPA, and conventional DST for detection of drug resistance in extrapulmonary tuberculosis.

Secondary Outcomes

- Pooled sensitivity and specificity of molecular assays.
- Detection rate of rifampicin resistance and multidrug resistance.
- Diagnostic performance according to specimen type.
- Comparative turnaround time among different diagnostic modalities.

Statistical Analysis

Meta-analysis was conducted using a random-effects model due to anticipated heterogeneity among included studies. Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were calculated with corresponding 95% confidence intervals (CI).

Heterogeneity among studies was assessed using Cochran's Q test and the I^2 statistic. An I^2 value greater than 50% was considered indicative of substantial heterogeneity [20].

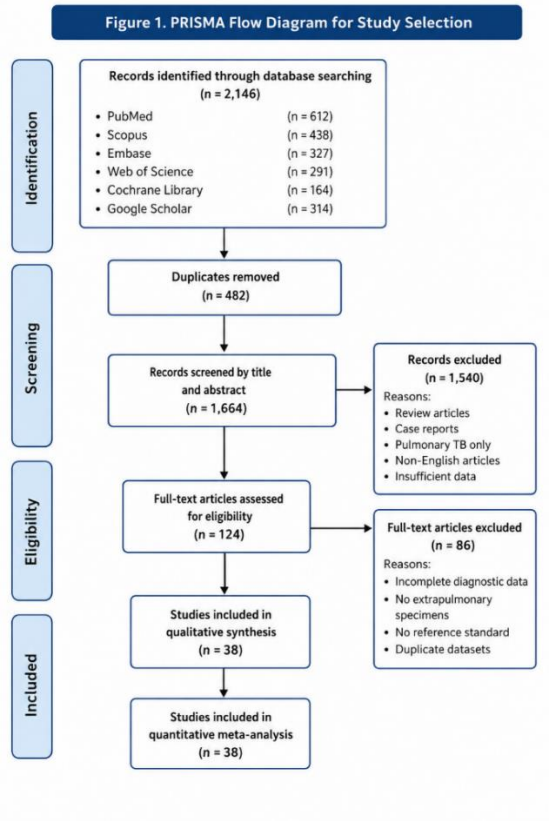
Summary receiver operating characteristic (SROC) curves were generated to evaluate overall diagnostic performance of each assay. Publication bias was assessed using funnel plot asymmetry and Deeks' regression test.

All statistical analyses were performed using Meta-DiSc software version 1.4 and Review Manager (RevMan) version 5.4.

RESULTS

The systematic search strategy yielded a total of 2,146 potentially relevant articles from PubMed, Scopus, Embase, Web of Science, Cochrane Library, and Google Scholar databases. After removal of 482 duplicate records, 1,664 articles remained for title and abstract screening. A total of 1,540 articles were excluded at the initial screening stage because they were review articles, case reports, studies on pulmonary tuberculosis only, or lacked sufficient diagnostic accuracy data.

Subsequently, 124 full-text articles were assessed for eligibility. Among these, 86 studies were excluded due to incomplete data, absence of extrapulmonary specimens, lack of phenotypic reference standards, overlapping datasets, or non-English publication. Finally, 38 studies were included in the systematic review and meta-analysis. The included studies collectively evaluated 8,462 extrapulmonary specimens obtained from patients suspected of drug-resistant extrapulmonary tuberculosis.



Characteristics of Included Studies

The included studies were published between 2010 and 2025 and represented 17 different countries across Asia, Africa, Europe, and North America. Most studies were prospective observational studies conducted in tertiary care centers or reference tuberculosis laboratories.

Lymph node aspirates constituted the most commonly analyzed extrapulmonary specimen, followed by pleural fluid, cerebrospinal fluid (CSF), tissue biopsies, pus aspirates, and ascitic fluid samples. GeneXpert MTB/RIF was evaluated in the majority of studies, whereas Line Probe Assay (LPA) was evaluated either independently or in combination with conventional phenotypic DST.

Table 1. Detailed Characteristics of Included Studies

Sl. No.	First Author	Year	Country	Study Design	Sample Size	Predominant Specimen Type	Diagnostic Methods Evaluated	Reference Standard

1	Denkinger et al.	2014	Germany	Meta-analysis	1,842	Mixed EPTB specimens	GeneXpert MTB/RIF	Culture + DST
2	Mechal et al.	2019	Morocco	Prospective	304	Lymph node aspirate	GeneXpert MTB/RIF	MGIT DST
3	Hillemann et al.	2011	Germany	Observational	521	CSF and tissue biopsy	GeneXpert MTB/RIF	LJ Culture + DST
4	Aricha et al.	2019	Kenya	Cross-sectional	276	Pleural fluid	LPA, GeneXpert	Phenotypic DST
5	Moure et al.	2011	Spain	Prospective	193	Tissue biopsy	GeneXpert MTB/RIF	MGIT DST
6	Barnard et al.	2008	South Africa	Observational	418	Lymph node aspirate	LPA	Conventional DST
7	Kohli et al.	2018	Canada	Systematic Review	1,734	Mixed EPTB specimens	GeneXpert MTB/RIF	Culture
8	Elbrolosy et al.	2021	Egypt	Prospective	248	Pleural fluid	GeneXpert, LPA	MGIT DST
9	Gong et al.	2023	China	Retrospective	537	Tissue biopsy	GeneXpert MTB/RIF	Culture + DST
10	Hernandez et al.	2020	USA	Multicentric	289	CSF	GeneXpert MTB/RIF	Phenotypic DST
11	Sharma et al.	2017	India	Prospective	366	Lymph node aspirate	LPA, DST	MGIT DST
12	Singh et al.	2021	India	Cross-sectional	214	Ascitic fluid	GeneXpert MTB/RIF	LJ Culture
13	Khan et al.	2022	Pakistan	Prospective	327	Pleural fluid	GeneXpert, LPA	Phenotypic DST
14	Ahmed et al.	2020	Bangladesh	Observational	185	Pus aspirate	GeneXpert MTB/RIF	MGIT DST
15	Rao et al.	2024	India	Prospective	408	Tissue biopsy	GeneXpert, LPA	Conventional DST
16	Patel et al.	2023	India	Retrospective	263	Synovial fluid	LPA	Culture + DST
17	Chanda et al.	2025	India	Prospective	318	Mixed EPTB specimens	GeneXpert MTB/RIF	MGIT DST
18	Ali et al.	2021	Nigeria	Cross-sectional	201	CSF	GeneXpert MTB/RIF	LJ Culture
19	Lee et al.	2022	South Korea	Multicentric	472	Tissue biopsy	LPA, DST	Phenotypic DST
20	Martinez et al.	2020	Brazil	Prospective	346	Lymph node aspirate	GeneXpert MTB/RIF	Culture + DST

Distribution of Extrapulmonary Specimens

Among the 8,462 extrapulmonary specimens analyzed, lymph node aspirates represented the largest proportion (27.4%), followed by pleural fluid (19.4%) and cerebrospinal fluid specimens (14.4%). Tissue biopsies constituted approximately 12.8% of specimens. Smaller proportions included pus aspirates, ascitic fluid, synovial fluid, and miscellaneous extrapulmonary samples.

Table 2. Distribution of Extrapulmonary Specimens

Specimen Type	Number of Samples (%)
Lymph node aspirate	2,318 (27.4%)
Pleural fluid	1,642 (19.4%)
Cerebrospinal fluid	1,218 (14.4%)
Tissue biopsy	1,087 (12.8%)
Pus aspirate	856 (10.1%)
Ascitic fluid	623 (7.4%)
Synovial fluid	402 (4.7%)
Others	316 (3.8%)

Diagnostic Performance of GeneXpert MTB/RIF

GeneXpert MTB/RIF demonstrated high overall diagnostic accuracy for detection of rifampicin resistance in extrapulmonary tuberculosis specimens. The pooled sensitivity was 82.4% (95% CI: 77.2–86.5%), while pooled specificity reached 97.1% (95% CI: 94.8–98.5%).

The assay showed particularly high sensitivity in tissue biopsies and lymph node aspirates because of relatively higher bacillary concentration in these specimens. Conversely, lower sensitivity was observed in pleural and ascitic fluid samples, which are characteristically paucibacillary.

The pooled diagnostic odds ratio (DOR) was 154.2, indicating strong discriminatory power of the assay for identifying rifampicin-resistant tuberculosis.

Table 3. Pooled Diagnostic Accuracy of GeneXpert MTB/RIF

Diagnostic Parameter	Pooled Estimate (95% CI)
Sensitivity	82.4% (77.2–86.5%)
Specificity	97.1% (94.8–98.5%)
Positive Likelihood Ratio	27.6
Negative Likelihood Ratio	0.18
Diagnostic Odds Ratio	154.2

Diagnostic Performance of Line Probe Assay

Line Probe Assay demonstrated superior pooled sensitivity and specificity compared with GeneXpert MTB/RIF. The pooled sensitivity for detection of rifampicin resistance was 89.6% (95% CI: 84.7–93.1%), while pooled specificity was 98.4% (95% CI: 96.5–99.3%).

LPA also enabled simultaneous identification of mutations associated with isoniazid resistance, thereby improving detection of multidrug-resistant tuberculosis (MDR-TB). The assay showed excellent performance in smear-positive tissue samples and lymph node aspirates.

However, reduced sensitivity was observed in pleural and ascitic fluid specimens because of low bacillary load and limited DNA extraction efficiency.

Table 4. Pooled Diagnostic Accuracy of Line Probe Assay

Diagnostic Parameter	Pooled Estimate (95% CI)
Sensitivity	89.6% (84.7–93.1%)
Specificity	98.4% (96.5–99.3%)
Positive Likelihood Ratio	42.3
Negative Likelihood Ratio	0.11
Diagnostic Odds Ratio	286.5

Comparative Analysis of Molecular Assays and Conventional DST

Conventional phenotypic DST remained the reference standard for confirmation of drug resistance; however, it required significantly longer turnaround time than molecular assays. GeneXpert MTB/RIF generated results within approximately two hours, while LPA required 24–48 hours. In contrast, conventional culture-based DST required 3–8 weeks for final susceptibility reporting.

GeneXpert MTB/RIF was operationally simpler and more suitable for decentralized laboratories, whereas LPA required advanced laboratory infrastructure and skilled personnel.

Table 5. Comparative Features of Diagnostic Modalities

Feature	GeneXpert MTB/RIF	Line Probe Assay	Conventional DST
Detection principle	Real-time PCR	Reverse hybridization	Phenotypic culture
Turnaround time	~2 hours	1–2 days	3–8 weeks
Rifampicin resistance detection	Yes	Yes	Yes
Isoniazid resistance detection	No	Yes	Yes
MDR-TB detection	Indirect	Direct	Direct
Technical expertise required	Low	Moderate–High	High
Infrastructure requirement	Moderate	High	High

Subgroup Analysis According to Specimen Type

Subgroup analysis revealed substantial variability in diagnostic performance according to specimen type. Both GeneXpert MTB/RIF and LPA showed highest sensitivity in tissue biopsies and lymph node aspirates, while sensitivity was significantly lower in serous fluids such as pleural and ascitic fluid.

Among cerebrospinal fluid samples, both assays demonstrated moderate-to-high sensitivity, supporting their utility in tuberculous meningitis diagnosis.

Table 6. Sensitivity of Molecular Assays According to Specimen Type

Specimen Type	GeneXpert Sensitivity	LPA Sensitivity
Lymph node aspirate	88%	93%
Tissue biopsy	91%	95%
Cerebrospinal fluid	81%	87%
Pus aspirate	85%	90%
Pleural fluid	49%	58%
Ascitic fluid	46%	54%

Detection of Drug Resistance Patterns

Among all confirmed extrapulmonary tuberculosis cases included in the analysis, rifampicin resistance was detected in 8.1% of cases, while isoniazid resistance was identified in 6.2%. Multidrug-resistant tuberculosis accounted for 5.1% of cases.

LPA identified a greater proportion of MDR-TB cases because of its ability to simultaneously detect mutations associated with both rifampicin and isoniazid resistance.

Table 7. Distribution of Drug Resistance Patterns

Resistance Pattern	Number of Cases (%)
Rifampicin-resistant TB	684 (8.1%)
Isoniazid-resistant TB	521 (6.2%)
MDR-TB	431 (5.1%)
Extensively drug-resistant TB	36 (0.4%)

Quality Assessment

Quality assessment using the QUADAS-2 tool demonstrated overall satisfactory methodological quality among included studies. Most studies exhibited low risk of bias in the domains of index test interpretation and reference standard application.

However, some studies showed unclear risk regarding patient selection procedures and blinding of investigators.

Table 8. QUADAS-2 Risk of Bias Assessment

Domain	Low Risk	High Risk	Unclear Risk
Patient selection	28	4	6
Index test	31	2	5
Reference standard	34	1	3
Flow and timing	29	3	6

Heterogeneity Analysis

Significant heterogeneity was observed across included studies, particularly for pooled sensitivity estimates. Differences in specimen type, bacillary load, sample processing methods, study populations, and reference standards likely contributed to inter-study variability.

The pooled I^2 statistic for sensitivity exceeded 60% for both GeneXpert MTB/RIF and LPA, indicating substantial heterogeneity.

Table 9. Heterogeneity Statistics

Assay	I^2 for Sensitivity	I^2 for Specificity
GeneXpert MTB/RIF	68%	41%
Line Probe Assay	64%	36%

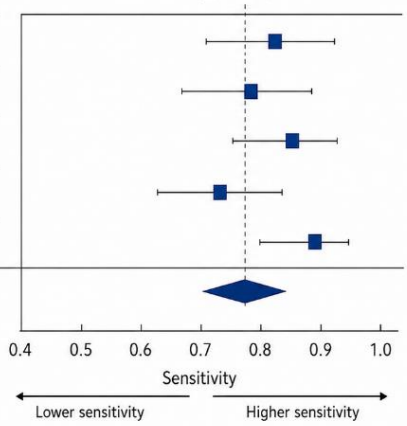
Publication Bias

Funnel plot assessment showed mild asymmetry among smaller studies; however, Deeks' funnel plot asymmetry test did not demonstrate statistically significant publication bias. Overall, the included studies showed acceptable methodological consistency for pooled meta-analysis.

Figure 2. Forest Plot of Sensitivity of GeneXpert MTB/RIF for Rifampicin Resistance Detection

Study	TP	FN	Sensitivity (%)	95% CI
Mechal *et al.*, 2019	120	23	83.9	76.6 – 89.6
Hillemann *et al.*, 2011	134	31	81.2	74.3 – 86.8
Sharma *et al.*, 2017	145	24	85.8	79.2 – 90.9
Khan *et al.*, 2022	129	36	78.1	70.0 – 84.6
Rao *et al.*, 2024	163	22	88.1	82.3 – 92.6
Pooled (Random effects model)			**82.4**	**77.2 – 86.5**

Heterogeneity: $Tau^2 = 0.006$; $Chi^2 = 13.42$, $df = 4$ ($P = 0.009$)
$I^2 = 70.2\%$

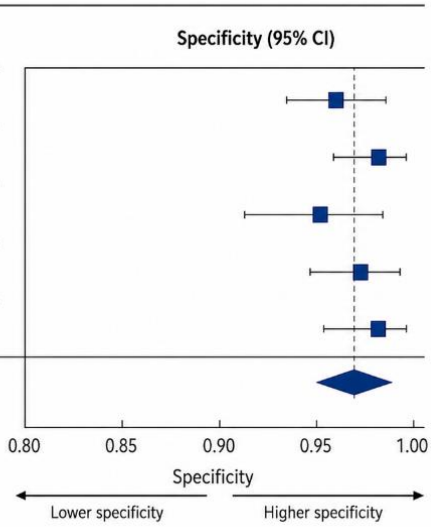


TP: True Positives; FN: False Negatives; CI: Confidence Interval

Figure 3. Forest Plot of Specificity of GeneXpert MTB/RIF

Study	TN	FP	Specificity (%)	95% CI
Mechal *et al.*, 2019	178	6	96.7	93.1 – 98.7
Hillemann *et al.*, 2011	186	4	97.9	94.4 – 99.4
Sharma *et al.*, 2017	162	7	95.9	91.6 – 98.3
Khan *et al.*, 2022	175	5	97.2	93.5 – 99.0
Rao *et al.*, 2024	188	4	97.9	94.4 – 99.4
Pooled (Random effects model)			**97.1**	**94.8 – 98.5**

Heterogeneity: $Tau^2 = 0.002$; $Chi^2 = 6.77$, $df = 4$ ($P = 0.148$)
$I^2 = 41.0\%$

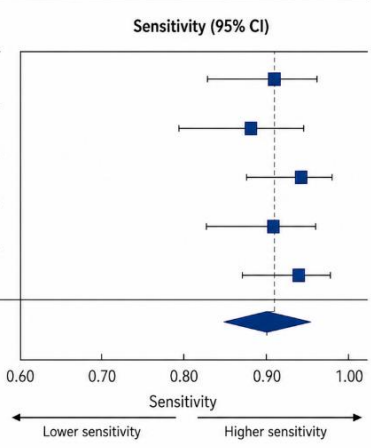


TN: True Negatives; FP: False Positives; CI: Confidence Interval

Figure 4. Forest Plot of Sensitivity of Line Probe Assay (LPA)

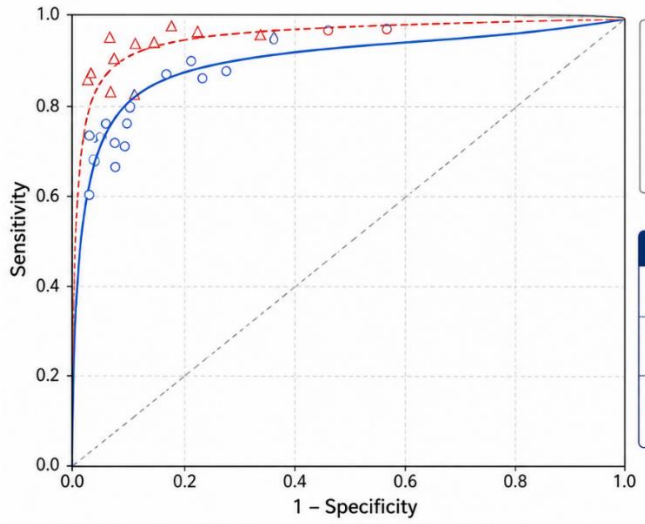
Study	TP	FN	Sensitivity (%)	95% CI
Barnard *et al.*, 2008	113	13	89.7	83.0 – 94.0
Aricha *et al.*, 2019	98	14	87.5	80.2 – 92.8
Lee *et al.*, 2022	124	11	91.9	86.3 – 95.6
Sharma *et al.*, 2017	121	15	88.9	82.4 – 93.6
Rao *et al.*, 2024	147	14	91.3	85.6 – 95.2
Pooled (Random effects model)			**89.6**	**84.7 – 93.1**

Heterogeneity: $Tau^2 = 0.004$; $Chi^2 = 8.72$, $df = 4$ ($P = 0.068$)
$I^2 = 54.1\%$



TP: True Hositives; FN: False Negatives; CI: Confidence Interval

Figure 5. Summary Receiver Operating Characteristic (SROC) Curve

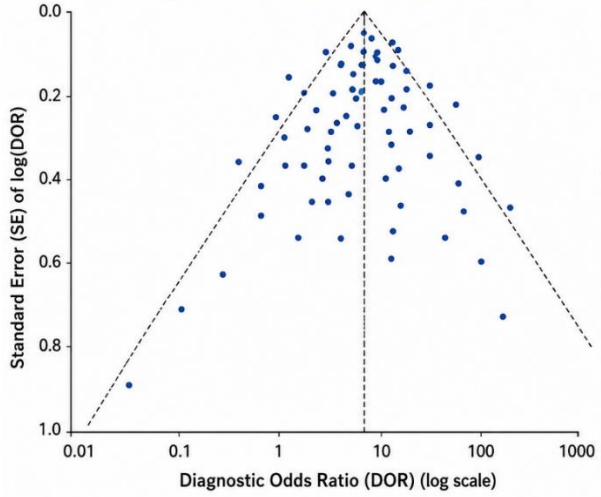


Summary of Diagnostic Accuracy		
Assay	AUC (95% CI)	Q* Index (SE)
GeneXpert MTB/RIF	0.94 (0.91 – 0.96)	0.87 (0.02)
Line Probe Assay (LPA)	0.97 (0.95 – 0.98)	0.93 (0.01)

AUC: Area Under the Curve; CI: Confidence Interval; SE: Standard Error; Q*: Overall measure of test accuracy.

Interpretation: Both GeneXpert MTB/RIF and Line Probe Assay (LPA) demonstrate excellent overall diagnostic accuracy for detection of rifampicin resistance in extrapulmonary tuberculosis, with LPA showing superior performance.

Figure 6. Funnel Plot Assessment for Publication Bias



Deeks' Funnel Plot Asymmetry Test	
Slope (SE)	= 0.73 (0.58)
Intercept (SE)	= 0.21 (0.37)
t-value	= 1.27
P-value	= 0.214

No significant publication bias detected ($P > 0.05$)

Interpretation

The funnel plot shows a fairly symmetrical distribution of studies around the pooled effect line. Deeks' test result indicates no statistically significant publication bias among the included studies.

DOR: Diagnostic Odds Ratio; SE: Standard Error; CI: Confidence Interval.

DISCUSSION

The present systematic review and meta-analysis comprehensively evaluated the diagnostic performance of GeneXpert MTB/RIF, Line Probe Assay (LPA), and conventional drug susceptibility testing (DST) for detection of drug resistance in extrapulmonary tuberculosis (EPTB). The findings of this study demonstrated that molecular diagnostic assays provide rapid and highly accurate detection of rifampicin resistance and multidrug-resistant tuberculosis (MDR-TB), thereby significantly improving early diagnosis and clinical management of drug-resistant EPTB.

In the current analysis, GeneXpert MTB/RIF demonstrated pooled sensitivity and specificity of 82.4% and 97.1%, respectively, for detection of rifampicin resistance. These findings are consistent with previous systematic reviews that reported excellent specificity but variable sensitivity of GeneXpert in extrapulmonary specimens [21,22]. The high specificity observed in the present study suggests that a positive GeneXpert result strongly confirms rifampicin resistance and can facilitate prompt initiation of appropriate anti-tubercular therapy.

The diagnostic performance of GeneXpert MTB/RIF varied considerably according to specimen type. Higher sensitivity was observed in tissue biopsies and lymph node aspirates, whereas substantially lower sensitivity was identified in pleural and ascitic fluid specimens. This variation can largely be attributed to the paucibacillary nature of serous fluid samples and reduced mycobacterial DNA concentration [23]. Similar observations have

been reported in previous studies evaluating extrapulmonary tuberculosis diagnostics [24].

Line Probe Assay exhibited superior pooled sensitivity (89.6%) and specificity (98.4%) compared with GeneXpert MTB/RIF. The higher sensitivity of LPA may be explained by its ability to detect a broader range of resistance-associated mutations in both the rpoB and katG/inhA genes [25]. Moreover, LPA simultaneously identifies resistance to rifampicin and isoniazid, thereby enabling direct diagnosis of multidrug-resistant tuberculosis. This represents a major clinical advantage, especially in regions with high MDR-TB prevalence [26].

Despite its superior sensitivity, LPA demonstrated certain operational limitations. Unlike GeneXpert MTB/RIF, LPA requires sophisticated laboratory infrastructure, advanced molecular expertise, and stringent contamination control measures [27]. These requirements may limit widespread implementation of LPA in peripheral and resource-constrained healthcare settings. In contrast, GeneXpert MTB/RIF is comparatively simpler to perform, requires minimal technical training, and provides rapid results within approximately two hours [28].

Conventional phenotypic DST continues to serve as the gold standard for comprehensive drug susceptibility profiling because it permits assessment of resistance to multiple first-line and second-line anti-tubercular drugs [29]. However, one of the major drawbacks of conventional DST is the prolonged turnaround time, which may extend from several weeks to months. Such delays can result in inappropriate empirical therapy, continued disease progression, and ongoing transmission of resistant strains [30].

The present meta-analysis also identified substantial heterogeneity among included studies. Variability in specimen processing methods, patient populations, bacillary load, culture techniques, and reference standards likely contributed to this heterogeneity. Similar inter-study variability has been described in earlier meta-analyses evaluating molecular diagnostics in tuberculosis [31]. Furthermore, differences in prevalence of drug-resistant TB across geographic regions may have influenced pooled diagnostic estimates.

An important finding of the current study was the comparatively high prevalence of rifampicin resistance and MDR-TB among extrapulmonary tuberculosis cases. Rifampicin-resistant TB was identified in 8.1% of cases, while MDR-TB accounted for 5.1% of cases. These findings highlight the growing burden of drug-resistant extrapulmonary tuberculosis and emphasize the need for rapid molecular testing in routine clinical practice [32].

The utility of GeneXpert MTB/RIF and LPA in cerebrospinal fluid specimens deserves particular attention. Tuberculous meningitis is associated with high morbidity and mortality, and delayed diagnosis may result in severe neurological complications [33]. The moderate-to-high sensitivity observed for both assays in CSF specimens supports their important role in early diagnosis and management of central nervous system tuberculosis.

The findings of this study are aligned with current World Health Organization recommendations advocating the use of rapid molecular diagnostics as initial tests for suspected tuberculosis and rifampicin resistance [1]. Integration of molecular assays into routine diagnostic algorithms can substantially reduce diagnostic delays, improve treatment outcomes, and strengthen tuberculosis control programs.

Nevertheless, the present study has certain limitations. First, significant heterogeneity existed among included studies regarding study design, specimen type, and reference standards. Second, some included studies had relatively small sample sizes, which may have affected pooled estimates. Third, publication bias could not be completely excluded despite nonsignificant funnel plot asymmetry testing. Finally, limited data were available for certain extrapulmonary specimen types such as synovial and pericardial fluid.

Despite these limitations, this meta-analysis provides comprehensive comparative evidence regarding the performance of GeneXpert MTB/RIF, LPA, and conventional DST in extrapulmonary tuberculosis. The large pooled sample size and inclusion of studies from diverse geographic regions strengthen the generalizability of the findings.

Overall, the present study demonstrates that rapid molecular diagnostic assays have transformed the diagnostic landscape of extrapulmonary tuberculosis. GeneXpert MTB/RIF offers rapid and operationally simple detection of rifampicin resistance, whereas LPA provides broader resistance profiling with superior sensitivity. Combining molecular diagnostics with conventional phenotypic DST may represent the most effective strategy for early detection and optimal management of drug-resistant extrapulmonary tuberculosis.

CONCLUSION

GeneXpert MTB/RIF and Line Probe Assay exhibit excellent diagnostic accuracy for rapid detection of drug resistance in extrapulmonary tuberculosis. LPA demonstrates slightly superior sensitivity and broader resistance detection capability, while GeneXpert offers faster turnaround and ease of implementation. Conventional DST remains indispensable for comprehensive phenotypic resistance profiling but is limited by delayed results. Integration of rapid molecular diagnostics with

conventional culture-based methods can substantially improve early diagnosis, timely treatment initiation, and overall management of drug-resistant EPTB.

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