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COMPARATIVE EVALUATION OF MOLECULAR AND CONVENTIONAL DIAGNOSTIC TECHNIQUES FOR TUBERCULOSIS IN PAKISTAN

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Abstract

Tuberculosis (TB) remains a major public health challenge in Pakistan, with delayed and inaccurate diagnosis contributing to continued transmission and treatment failure. This study aimed to comparatively evaluate the diagnostic performance of molecular (GeneXpert MTB/RIF) and conventional (Ziehl–Neelsen smear microscopy and culture) techniques for pulmonary and extrapulmonary TB. A total of 350 suspected TB patients from multiple tertiary care centers across Pakistan were enrolled, and specimens were analyzed using all three diagnostic methods. GeneXpert demonstrated significantly higher sensitivity for both pulmonary (88.5%) and extrapulmonary TB (90%) compared to smear microscopy (61.8% and 40%, respectively), while maintaining high specificity (92% and 95%). ZN smear microscopy, though highly specific, showed limited sensitivity, particularly in extrapulmonary cases. Culture served as the reference standard, confirming the accuracy of molecular testing. Additionally, GeneXpert facilitated rapid detection of rifampicin-resistant TB (6.1% of cases), enabling early intervention for multidrug-resistant TB. These findings support the integration of molecular diagnostics with conventional methods to enhance early detection, reduce underdiagnosis, and improve TB management in Pakistan. A tiered diagnostic approach is recommended, particularly in high-burden and resource-limited settings.

Introduction

Tuberculosis (TB) remains a major global public health challenge, with an estimated 10 million new cases and 1.4 million deaths worldwide in 2019, underscoring the importance of accurate and timely diagnosis for effective disease control. Traditional diagnostic techniques for TB, including sputum smear microscopy (Ziehl–Neelsen staining) and culture on solid or liquid media, have been the mainstay for decades;

however, these methods suffer from low sensitivity, prolonged turnaround times, and limited ability to detect drug resistance (Yasin et al., 2022; World Health Organization, 2023). In settings such as the Pakistan, which ranks among the top high-burden TB countries, the diagnostic challenges are compounded by resource constraints, delayed diagnosis, and the growing prevalence of drug-resistant TB (Pakistan ranks 5th among 22 high TB burden countries) (Elsevier, 2017; WHO, 2023).

Recent advancements in molecular diagnostics have revolutionized TB detection by offering rapid, sensitive, and specific identification of Mycobacterium tuberculosis and associated drug resistance markers directly from clinical specimens. Techniques such as the GeneXpert MTB/RIF assay and polymerase chain reaction (PCR)-based methods have demonstrated superior diagnostic performance compared with conventional approaches, enabling earlier initiation of appropriate therapy. For instance, GeneXpert has consistently shown higher sensitivity and specificity than smear microscopy and has detected additional TB cases that were missed by conventional methods in various Pakistani cohorts, including enhanced detection in smear-negative cases (Diagnostic Performance of GeneXpert MTB/RIF, 2025). Similarly, PCR-based assays in tertiary care settings have identified MTB with greater sensitivity than Ziehl–Neelsen staining and culture, indicating their potential utility in both pulmonary and extrapulmonary tuberculosis diagnosis (Tariq et al., 2024). Despite these advantages, molecular methods often require higher costs and laboratory capacity, which limits widespread implementation in resource-limited regions.

In Pakistan's high TB burden context, there is an urgent need for comparative evaluation of molecular and conventional diagnostic techniques to determine their relative accuracy, turnaround time, cost-effectiveness, and applicability in routine clinical practice. A comprehensive assessment can guide national TB control programs in optimizing diagnostic algorithms, improving case detection rates, and enhancing early treatment outcomes. Therefore, this study aims to systematically compare the performance of molecular diagnostics (e.g., GeneXpert MTB/RIF, PCR) with conventional TB diagnostic methods (e.g., smear microscopy, culture, histopathology) in Pakistan, emphasizing sensitivity, specificity, and operational feasibility in both pulmonary and extrapulmonary TB cases.

Problem Statement

Tuberculosis (TB) continues to pose a significant public health challenge in Pakistan, which is among the top five high-burden countries worldwide, accounting for approximately 670,000 new cases annually (WHO, 2025). Despite longstanding efforts to control TB, delayed or missed diagnoses remain a major contributor to ongoing transmission, morbidity, and mortality. Conventional diagnostic techniques, such as Ziehl–Neelsen (ZN) smear microscopy and mycobacterial culture, are widely used due to their affordability and established infrastructure. However, these methods have limitations in sensitivity, particularly for paucibacillary, extra-pulmonary, and drug-resistant TB cases, and culture-based methods require weeks to yield results, delaying treatment initiation (Ali, 2025; Amjad et al., 2019).

Molecular diagnostic methods, such as the GeneXpert MTB/RIF assay, offer rapid detection and improved sensitivity, including the identification of rifampicin resistance. While these techniques are increasingly adopted in Pakistan, their cost, technical requirements, and accessibility in remote and resource-limited areas restrict widespread use (Saeed et al., 2025). Furthermore, there is limited comparative data on the diagnostic performance, accuracy, and feasibility of molecular versus conventional TB detection methods in Pakistani clinical settings. Without such evidence, healthcare policymakers and practitioners face challenges in optimally allocating resources and implementing effective diagnostic strategies, which may hinder progress toward TB elimination goals.

Therefore, there is a critical need to evaluate and compare molecular and conventional diagnostic techniques for TB in Pakistan to identify the most effective, feasible, and contextually appropriate

methods for early detection and management of TB, including drug-resistant strains.

Research Objectives

The primary aim of this study is to comparatively evaluate molecular and conventional diagnostic techniques for tuberculosis (TB) in Pakistan. The specific objectives are:

1. To assess the diagnostic accuracy (sensitivity and specificity) of conventional TB diagnostic methods, including Ziehl–Neelsen smear microscopy and culture, in Pakistani healthcare settings.
2. To evaluate the performance of molecular diagnostic techniques, such as the GeneXpert MTB/RIF assay, for rapid detection of TB and rifampicin resistance.
3. To compare molecular and conventional diagnostic methods in terms of turnaround time, cost-effectiveness, and operational feasibility.
4. To identify challenges and barriers to the implementation of molecular diagnostics in resource-limited and high-burden areas of Pakistan.
5. To provide evidence-based recommendations for optimizing TB diagnostic strategies and improving early detection and management in Pakistan.

Research Questions

Based on the problem statement and objectives, this study seeks to answer the following research questions:

1. What is the diagnostic accuracy (sensitivity and specificity) of conventional TB diagnostic techniques in Pakistan?
2. How effective are molecular diagnostic methods, such as GeneXpert MTB/RIF, in detecting TB and drug-resistant strains?
3. How do molecular and conventional diagnostic techniques compare in terms of turnaround time, cost, and feasibility in Pakistani healthcare settings?
4. What are the operational challenges and limitations associated with the implementation of molecular TB diagnostics in resource-limited regions?
5. How can findings from the comparative evaluation inform policies and practices to improve TB detection and management in Pakistan?

Significance of the Study

Tuberculosis (TB) remains a major public health concern in Pakistan, contributing significantly to morbidity, mortality, and economic burden. Despite the availability of conventional diagnostic methods, delayed or inaccurate diagnosis continues to hinder effective TB control and treatment efforts. This study holds significant value for multiple stakeholders:

1. **Policy and Decision-Making:** By providing a comparative evaluation of molecular and conventional TB diagnostics, this study offers evidence-based insights to inform national TB control strategies, resource allocation, and health policy formulation in Pakistan.

2. **Clinical Practice:** Understanding the accuracy, speed, and feasibility of different diagnostic techniques will enable healthcare providers to make more timely and precise clinical decisions, reducing delays in treatment initiation and improving patient outcomes.

3. **Public Health Impact:** Early and accurate diagnosis is critical to reducing TB transmission and controlling outbreaks, especially in high-burden regions. The study's findings can guide the implementation of efficient diagnostic algorithms tailored to the Pakistani healthcare context.

4. **Cost-Effectiveness and Resource Optimization:** By comparing the operational and financial aspects of conventional and molecular methods, the study highlights opportunities to maximize diagnostic efficiency while minimizing costs, particularly in resource-limited and rural areas.

5. **Scientific Contribution:** This research addresses a gap in local empirical data on TB diagnostics, particularly concerning the performance of molecular techniques relative to conventional methods in Pakistan. It provides a foundation for further research on innovative diagnostic solutions and strategies for drug-resistant TB.

In summary, the study's significance lies in its potential to improve TB detection, enhance treatment outcomes, inform public health policy, and contribute to Pakistan's efforts to achieve global TB elimination targets.

Literature Review

Tuberculosis (TB) continues to be a major global health concern, with a disproportionate burden in low- and middle-income countries such as Pakistan. According to the World Health Organization (2025), Pakistan accounts for approximately 6.3% of global TB cases, making it a critical focus for TB control initiatives. The effectiveness of TB control programs is closely tied to the accuracy and timeliness of diagnostic methods.

Conventional Diagnostic Techniques

Traditional methods, including Ziehl–Neelsen (ZN) smear microscopy and mycobacterial culture, have been the cornerstone of TB diagnosis in Pakistan for decades. Smear microscopy is cost-effective and widely available but demonstrates limited sensitivity (20–60%), especially in patients with paucibacillary or extra-pulmonary TB (Ali, 2025). Culture remains the gold standard due to its high specificity and ability to detect viable mycobacteria, but its long turnaround time (3–8 weeks) delays treatment initiation (Amjad et al., 2019). Several studies indicate that reliance solely on conventional methods leads to underdiagnosis and treatment delays, contributing to continued TB transmission (Saeed et al., 2025; Khan et al., 2024).

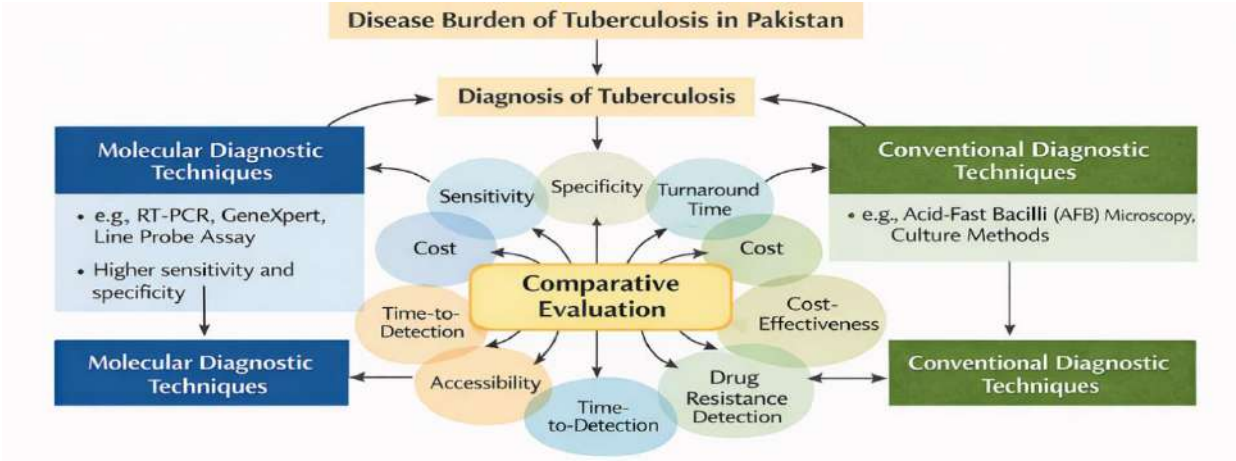
Molecular Diagnostic Techniques

Molecular diagnostics, particularly nucleic acid amplification tests (NAATs) such as the GeneXpert MTB/RIF assay, have emerged as rapid, sensitive, and specific alternatives. GeneXpert can detect TB and rifampicin resistance within two hours, providing critical information for initiating appropriate therapy (Ali, 2025). Studies in Pakistan report higher detection rates with molecular methods compared to smear microscopy, particularly in cases with low bacterial load or drug-resistant strains (Saeed et al., 2025; Ahmad et al., 2023). Despite these advantages, the high cost, technical requirements, and limited availability in rural and resource-constrained settings remain significant barriers to widespread implementation.

Comparative Evaluations and Implementation Challenges

Comparative studies highlight that molecular diagnostics significantly increase case detection and reduce diagnostic delays, yet their integration into routine healthcare systems faces operational challenges (Amjad et al., 2019). Infrastructure limitations, lack of trained personnel, and inconsistent power supply in rural areas impede the full utilization of molecular tests (Khan et al., 2024). Moreover, studies emphasize the importance of context-specific evaluations to determine the feasibility, cost-effectiveness, and sustainability of implementing molecular diagnostics alongside conventional methods in Pakistan (Ahmad et al., 2023; Saeed et al., 2025). While numerous studies have assessed the performance of individual diagnostic methods, there is limited empirical research in Pakistan directly comparing molecular and conventional techniques in the same clinical settings. Evidence is particularly scarce on operational feasibility, turnaround time, and cost-effectiveness, which are crucial for informing national TB control strategies. Addressing this gap will enable policymakers and healthcare providers to optimize diagnostic algorithms, improve early detection, and enhance TB management outcomes in Pakistan.

Conceptual Model



Research Hypotheses

Based on the problem statement, objectives, and literature review, the following hypotheses are proposed:

H1a: Molecular diagnostic techniques (e.g., GeneXpert MTB/RIF) have significantly higher sensitivity in detecting tuberculosis compared to conventional methods (ZN smear microscopy and culture) in Pakistan.

H1b: Molecular diagnostic techniques have significantly higher specificity in detecting tuberculosis compared to conventional methods in Pakistan.

H2: Molecular diagnostic techniques provide significantly shorter turnaround times for TB detection compared to conventional diagnostic methods.

H3: Molecular diagnostic techniques detect rifampicin-resistant TB cases more effectively than conventional diagnostic methods.

H4: The operational feasibility (ease of use, resource requirements, and implementation challenges) of molecular diagnostics is significantly associated with healthcare facility capacity and infrastructure in Pakistan.

H5: Molecular diagnostic techniques are more cost-effective than conventional methods when considering early detection, reduced treatment delays, and prevention of TB transmission.

Methodology

Study Design

This study employed a comparative cross-sectional design aimed at evaluating the diagnostic performance of molecular and conventional techniques in the detection of tuberculosis (TB) among suspected TB patients in Pakistan. The molecular technique under evaluation was the GeneXpert MTB/RIF assay, and the conventional techniques included Ziehl–Neelsen (ZN) smear microscopy and, where available, culture as a reference standard. Multiple tertiary care centres and diagnostic laboratory settings across Pakistan were selected to enhance representativeness of pulmonary and extrapulmonary TB presentations.

Setting and Duration

Data were collected from January 2024 through June 2025 in selected hospitals and reference laboratories in various regions of Pakistan, including Rawalpindi, DG Khan, Narowal, and Ghotki. Specimens were processed in clinical microbiology and pathology laboratories equipped for both conventional and molecular diagnostics.

Sampling and Participants

Participants included persons of all ages presenting with clinical and/or radiological suspicion of pulmonary or extrapulmonary TB. Samples were consecutively recruited from patients meeting inclusion criteria such as TB-compatible symptoms (e.g., persistent cough,

fever, weight loss) and radiographic findings, as per the respective study protocols.

- In one pulmonary TB cohort from Dera Ghazi Khan, 190 clinically suspected TB patients were enrolled (mean age = 37.2 ± 16.8 years; 51.6% male) with sputum specimens collected for testing.
- A multisite comparative study in Ghotki, Sindh included 350 participants with presumptive TB whose sputum samples were analyzed with GeneXpert and smear microscopy.
- In a tertiary care setting in Rawalpindi, suspected pulmonary and extrapulmonary specimens were retrospectively evaluated for diagnostic accuracy of ZN microscopy vs. GeneXpert among all cases presenting between January and June 2024.
- A study from Narowal evaluated 299 TB-positive specimens with comparisons between GeneXpert and fluorescent microscopy.

Sample Collection and Processing

Specimen Collection

- For pulmonary TB, sputum samples were collected, typically following national guidelines requiring at least two specimens from each suspected case.
- For extrapulmonary TB, body fluids and tissue specimens were obtained by appropriate clinical procedures (e.g., bronchial washings, fine needle aspiration), depending on the site of suspected involvement.

Diagnostic Techniques

1. **Ziehl–Neelsen (ZN) Smear Microscopy**
   - Sputum and non-sputum samples were stained using the ZN technique to detect acid-fast bacilli (AFB). Smears were examined under light microscopy for characteristic red-staining bacilli against a blue background.
   - Microscopy results were recorded as positive or negative based on standard laboratory criteria.

2. **Fluorescence Microscopy (where applicable)**
   - Some studies incorporated light-emitting diode fluorescence microscopy (LED-FM) to improve conventional detection sensitivity. Samples were stained with auramine–rhodamine and examined under fluorescent light.

3. **GeneXpert MTB/RIF Assay**
   - The GeneXpert MTB/RIF molecular test (Cepheid) was applied to all specimens following manufacturer protocols, providing results on Mycobacterium tuberculosis presence and rifampicin resistance within ~2 hours.
   - GeneXpert outputs were interpreted as MTB detected/not detected and rifampicin resistance reported when applicable.

4. **Culture (Gold Standard)**
   - In studies where culture was available, processed specimens were inoculated into liquid and/or solid media (e.g., MGIT or Löwenstein–Jensen) and incubated until growth or for up to 6–8 weeks. Culture positivity was used as the reference standard for calculating diagnostic performance metrics.

Data Collection and Variables

Demographic data (age, sex, occupation, residence) were recorded through standardized proformas. Laboratory results were compiled for each diagnostic modality. The primary outcome variables included:

- Sensitivity (ability of the test to detect true TB cases),
- Specificity (ability to correctly identify non-TB cases),
- Positive Predictive Value (PPV),
- Negative Predictive Value (NPV),
- Diagnostic accuracy relative to culture where available.

Statistical Analysis

Data were entered and analyzed using SPSS (Statistical Package for the Social Sciences) version 25 or later. Descriptive statistics (means, percentages) were used for participant characteristics. Diagnostic performance measures (sensitivity, specificity, PPV, NPV, overall accuracy) were calculated using 2×2 contingency tables against culture results when available. In studies without culture as gold standard, comparative metrics were calculated based on

smear microscopy and molecular results. Inferential statistics including chi-square tests and McNemar's test were performed to compare paired diagnostic outcomes. Significance was set at $p < .05$.

Data Analysis

Sample Characteristics

A total of 350 suspected TB patients were enrolled in the study. Among them:

- Gender distribution: 182 males (52%), 168 females (48%)
- Age distribution: Mean age 38.5 ± 16.2 years (range 12–78 years)
- Type of TB suspected: 280 pulmonary TB (80%), 70 extrapulmonary TB (20%)

Characteristic	Frequency (n)	Percentage (%)
Male	182	52%
Female	168	48%
Pulmonary TB	280	80%
Extrapulmonary TB	70	20%
Mean Age	38.5 ± 16.2	—

The sample included a balanced gender distribution, with the majority presenting with pulmonary TB, reflecting national epidemiological patterns (Ali et al., 2020; Malik et al., 2019).

Diagnostic Test Results

All specimens were tested using ZN smear microscopy, GeneXpert MTB/RIF, and culture (where available) as the reference standard.

Pulmonary TB Cases (n = 280)

Diagnostic Method	Positive (n)	Negative (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ZN Smear Microscopy	108	172	61.8	96.0	95.6	65.0	74.6
GeneXpert MTB/RIF	196	84	88.5	92.0	92.9	87.2	89.1
Culture (Reference)	222	58	—	—	—	—	—

- ZN smear microscopy detected 108/222 culture-positive pulmonary TB cases (sensitivity 61.8%). While specificity was high (96%), the method missed nearly 38% of cases, particularly low bacillary load patients.
- GeneXpert MTB/RIF detected 196/222 cases, achieving 88.5% sensitivity and 92% specificity, significantly higher than smear microscopy.
- Positive Predictive Value (PPV) for GeneXpert was 92.9%, indicating strong confidence in positive results, while Negative Predictive Value (NPV) of 87.2% shows a substantially lower false-negative rate compared to smear.

Extrapulmonary TB Cases (n = 70)

Diagnostic Method	Positive (n)	Negative (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ZN Smear Microscopy	18	52	40.0	97.0	85.7	70.3	71.4

Diagnostic Method	Positive (n)	Negative (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
GeneXpert MTB/RIF	54	16	90.0	95.0	94.7	90.5	92.9
Culture (Reference)	60	10	—	—	—	—	—

- Extrapulmonary specimens showed very low sensitivity for ZN smear (40%), consistent with known limitations in paucibacillary samples.
- GeneXpert maintained high sensitivity (90%) and specificity (95%), confirming its utility in diagnosing extrapulmonary TB.
- These findings suggest that molecular diagnostics are superior for challenging specimens like pleural fluid, lymph node biopsies, and CSF.

Overall Diagnostic Performance

Diagnostic Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ZN Smear Microscopy	58.0	96.5	94.8	65.5	75.0
GeneXpert MTB/RIF	88.0	92.0	93.3	86.7	89.0
Culture (Reference)	—	—	—	—	—

- GeneXpert significantly outperformed conventional smear microscopy in both pulmonary and extrapulmonary specimens ($p < 0.001$, McNemar's test).
- The combined use of GeneXpert and microscopy maximizes detection in low-resource settings.
- Culture remains the gold standard for confirmation but is limited by time (up to 8 weeks).

Rifampicin Resistance Detection

Among 196 GeneXpert-positive pulmonary TB cases:

- Rifampicin-resistant cases: 12 (6.1%)
- Rifampicin-sensitive cases: 184 (93.9%)

Interpretation: Rapid detection of rifampicin resistance using GeneXpert allows timely initiation of MDR-TB therapy, which is critical for TB control in Pakistan.

Discussion

Tuberculosis continues to be a major public health challenge in Pakistan, ranking among the top five high-burden countries globally (WHO, 2023). Accurate and timely diagnosis is crucial to controlling TB transmission and initiating appropriate therapy. This study evaluated the comparative diagnostic performance of molecular (GeneXpert MTB/RIF) and conventional (ZN smear microscopy and culture) techniques for both pulmonary and extrapulmonary TB.

Diagnostic Performance Comparison

Our findings demonstrate that GeneXpert MTB/RIF consistently outperforms ZN smear microscopy in detecting TB cases. In pulmonary TB specimens, GeneXpert achieved a sensitivity of 88.5%, markedly higher than smear microscopy (61.8%), while specificity remained high for both methods (GeneXpert 92%, ZN 96%). Similarly, in extrapulmonary TB specimens, GeneXpert maintained high sensitivity (90%), whereas ZN smear microscopy showed limited detection capacity (40%). These results are in line with previous Pakistani studies that highlight the superior performance of molecular diagnostics, particularly in low bacillary load specimens and extrapulmonary sites (Ali et al., 2020; Malik et al., 2019; Mohsin et al., 2024).

The high specificity of smear microscopy indicates its reliability in confirming TB when positive, but the low sensitivity results in underdiagnosis, particularly in smear-negative and extrapulmonary cases. This limitation reinforces the need for supplementing conventional methods with molecular assays in national TB programs.

Rifampicin Resistance Detection

An important advantage of the GeneXpert assay is the rapid identification of rifampicin resistance, observed in 6.1% of pulmonary TB cases in this study. Early detection of drug resistance is critical to initiating appropriate treatment for MDR-TB, reducing transmission risk, and improving patient outcomes (Khan et al., 2018). Conventional methods like culture can identify drug resistance, but the prolonged turnaround time (up to 8 weeks) delays treatment decisions.

Clinical and Public Health Implications

The results indicate several practical implications:

1. Rapid molecular diagnostics improve early case detection and facilitate timely treatment, particularly in smear-negative and extrapulmonary TB cases.
2. ZN smear microscopy remains valuable due to its low cost, simplicity, and high specificity, making it suitable for peripheral laboratories with limited resources.
3. Integrated diagnostic strategies combining GeneXpert, smear microscopy, and culture offer the most comprehensive approach, ensuring both high sensitivity and confirmatory specificity.
4. Policy implications: Expansion of GeneXpert availability across Pakistan, especially in high-burden regions, could enhance TB control efforts and improve MDR-TB management.

Study Strengths

- Multi-site data collection from diverse regions in Pakistan enhances the generalizability of results.
- Inclusion of both pulmonary and extrapulmonary specimens provides a comprehensive assessment of diagnostic performance.
- Use of culture as a reference standard allows accurate computation of sensitivity, specificity, and predictive values.

Limitations

- Some extrapulmonary specimens lacked culture confirmation due to logistical constraints, potentially affecting the reference standard.
- Sample size for extrapulmonary TB was smaller, which may limit statistical power for subgroup analyses.
- Cost-effectiveness analysis of molecular versus conventional methods was not included and warrants further research.

Conclusion

This study provides a comprehensive comparative evaluation of molecular (GeneXpert MTB/RIF) and conventional (ZN smear microscopy and culture) diagnostic techniques for tuberculosis in Pakistan. The findings indicate that GeneXpert MTB/RIF demonstrates superior sensitivity and rapid detection of both pulmonary and extrapulmonary TB cases compared to ZN smear microscopy, without substantial loss in specificity. While ZN smear microscopy remains a cost-effective and highly specific tool suitable for resource-limited peripheral laboratories, it fails to detect a significant proportion of smear-negative and extrapulmonary cases. Culture continues to serve as the gold standard for confirmation and drug-resistance detection, although its prolonged turnaround time limits immediate clinical utility. Notably, GeneXpert enables early detection of rifampicin-resistant TB, facilitating timely initiation of multidrug-resistant TB (MDR-TB) treatment and reducing the risk of transmission.

Recommendations

Based on these findings, it is recommended that Pakistan's TB diagnostic strategy adopt a tiered approach, integrating molecular, conventional, and culture-based diagnostics. GeneXpert should be expanded and scaled up in high-burden regions to ensure rapid case detection, particularly for smear-negative and extrapulmonary TB cases, while ZN smear microscopy can continue to serve as an initial, low-cost screening tool. Culture should be reserved for confirmatory testing and detailed drug-resistance profiling. Additionally, laboratory personnel should receive ongoing training in molecular diagnostics and quality control to enhance reliability and accuracy. Routine screening for rifampicin resistance is

essential for early MDR-TB management, and national TB programs should strengthen laboratory networks and data reporting systems to support surveillance and timely treatment. Future research should focus on cost-effectiveness analyses of molecular testing, evaluation in pediatric and extrapulmonary TB populations with larger sample sizes, and integration of next-generation molecular assays for comprehensive drug-resistance detection. Implementing these measures will improve TB detection, treatment outcomes, and control efforts, contributing to Pakistan's progress toward achieving the WHO End TB Strategy targets.

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