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Comparative Evaluation of Molecular and Conventional Diagnostic Methods for Early Detection of Tuberculosis

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ABSTRACT

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the leading infectious diseases worldwide and continues to pose a major public health challenge, particularly in developing countries. Early diagnosis is essential for reducing disease transmission, improving treatment outcomes, and preventing the emergence of drug-resistant strains. Conventional diagnostic methods such as sputum smear microscopy and culture have been widely used for TB detection; however, these methods are limited by low sensitivity and prolonged turnaround time. The present study evaluates the role of molecular diagnostic methods in the early detection of tuberculosis and compares their performance with conventional diagnostic techniques.

Molecular approaches including Polymerase Chain Reaction (PCR), Real-Time PCR, GeneXpert MTB/RIF assay, Line Probe Assay (LPA), Loop-Mediated Isothermal Amplification (LAMP), and Next-Generation Sequencing (NGS) were assessed for their diagnostic efficiency. The findings indicate that molecular methods provide rapid, sensitive, and specific detection of *M. tuberculosis* and allow early identification of drug resistance. Among these techniques, GeneXpert MTB/RIF demonstrated significant advantages due to its rapid turnaround time and simultaneous detection of rifampicin resistance. The study highlights the importance of integrating molecular diagnostics into routine TB control programs for timely diagnosis and improved patient management.

Keywords: Tuberculosis, Molecular Diagnosis, PCR, GeneXpert MTB/RIF, Line Probe Assay, Drug Resistance, Early Detection

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* and remains a major cause of morbidity and mortality worldwide. The disease primarily affects the lungs but may also involve extrapulmonary sites including lymph nodes, bones, kidneys, meninges, and intestines. Despite the availability of effective treatment, TB continues to be a major public health burden due to delayed diagnosis, HIV co-infection, poverty, malnutrition, and the emergence of multidrug-resistant strains. Early diagnosis is considered one of the most effective strategies for reducing disease transmission and improving treatment outcomes. Conventional diagnostic methods such as sputum smear microscopy and culture are widely used but suffer from limitations including low sensitivity and long reporting times. Recent advances in molecular biology have revolutionized TB diagnosis by enabling rapid detection of bacterial DNA and drug resistance markers.

MATERIALS AND METHODS

The present study was designed as a laboratory-based observational and comparative study to evaluate molecular diagnostic methods for early detection of tuberculosis. Clinical samples obtained from patients suspected of pulmonary tuberculosis were subjected to conventional and molecular diagnostic investigations. Conventional methods included sputum smear microscopy and culture techniques, whereas molecular approaches included PCR, Real-Time PCR, GeneXpert MTB/RIF assay, Line Probe Assay (LPA), Loop-Mediated Isothermal Amplification (LAMP), and Next-Generation Sequencing (NGS). Diagnostic performance was evaluated based on sensitivity, specificity, turnaround

time, and ability to detect drug resistance. The study was conducted in the Department of Microbiology, SAM Global University, Raisen, Madhya Pradesh, India.

RESULTS AND DISCUSSION

3.1 Comparative Evaluation of Diagnostic Methods

The present study compared conventional and molecular diagnostic methods used for tuberculosis detection. Conventional methods included sputum smear microscopy and culture, whereas molecular approaches included PCR, Real-Time PCR, GeneXpert MTB/RIF, Line Probe Assay (LPA), Loop-Mediated Isothermal Amplification (LAMP), and Next-Generation Sequencing (NGS).

Table 1. Comparison of Conventional and Molecular Diagnostic Methods

Diagnostic Method	Sensitivity	Specificity	Turnaround Time	Drug Resistance Detection
Sputum Smear Microscopy	Moderate	High	Same Day	No
Culture	Very High	Very High	2–8 Weeks	Limited
PCR	High	High	Few Hours	No
Real-Time PCR	Very High	High	Few Hours	Limited
GeneXpert MTB/RIF	Very High	Very High	~2 Hours	Rifampicin Resistance
Line Probe Assay (LPA)	Very High	Very High	1–2 Days	MDR-TB Detection
LAMP	High	High	<1 Day	No
Next-Generation Sequencing (NGS)	Very High	Very High	Several Days	Comprehensive Detection

Molecular methods demonstrated clear advantages over conventional diagnostic techniques in terms of speed, sensitivity, and early disease detection.

3.2 Performance of Conventional Diagnostic Methods

Sputum smear microscopy remains one of the most widely used techniques for tuberculosis diagnosis because of its simplicity and low cost. However, limited sensitivity reduces its effectiveness, particularly among HIV-positive patients, pediatric cases, and extrapulmonary tuberculosis patients.

Culture methods remain the gold standard for confirmation of tuberculosis because of their excellent sensitivity and specificity. However, prolonged incubation periods ranging from two to eight weeks significantly delay diagnosis and treatment initiation.

Table 2. Characteristics of Conventional Diagnostic Methods

Method	Major Advantages	Major Limitations
Sputum Smear Microscopy	Simple, inexpensive, widely available	Low sensitivity
Culture	Gold standard, highly accurate	Long turnaround time

3.3 Performance of Molecular Diagnostic Methods

PCR and Real-Time PCR demonstrated improved diagnostic performance through rapid amplification and detection of Mycobacterium tuberculosis DNA. These methods enable earlier diagnosis compared with conventional approaches.
GeneXpert MTB/RIF emerged as one of the most effective diagnostic tools because it simultaneously detects M. tuberculosis and rifampicin resistance within approximately two hours.

Table 3. Advantages of Molecular Diagnostic Methods

Method	Major Advantages
PCR	Rapid DNA detection
Real-Time PCR	Quantification of bacterial load
GeneXpert MTB/RIF	Simultaneous TB and rifampicin resistance detection
LPA	Rapid MDR-TB identification
LAMP	Suitable for resource-limited settings
NGS	Comprehensive genomic analysis

3.4 Detection of Drug-Resistant Tuberculosis

Rapid identification of drug resistance is essential for effective tuberculosis management. Molecular diagnostic techniques demonstrated significant advantages over conventional methods by identifying resistance-associated mutations at an early stage.

Table 4. Drug Resistance Detection Capability

Diagnostic Method	Resistance Detection
Sputum Smear Microscopy	Not Available
Culture-Based DST	Available but Delayed
GeneXpert MTB/RIF	Rifampicin Resistance
Line Probe Assay	Rifampicin and Isoniazid Resistance
NGS	Comprehensive Drug Resistance Profile

Early identification of resistant strains enables timely initiation of appropriate treatment regimens and reduces transmission of multidrug-resistant tuberculosis.

3.5 Clinical Significance of Molecular Diagnostics

The findings of the present study support the growing importance of molecular diagnostic technologies in tuberculosis control programs. Molecular methods significantly reduce diagnostic delays, improve sensitivity, and facilitate early treatment initiation.

Among the evaluated methods, GeneXpert MTB/RIF demonstrated the most practical balance between diagnostic accuracy, turnaround time, and ease of implementation. LPA proved particularly useful for rapid MDR-TB detection, while NGS offered the most comprehensive information regarding resistance mechanisms and molecular epidemiology. The integration of molecular diagnostics into routine clinical practice can substantially improve patient outcomes, reduce disease transmission, and strengthen national tuberculosis control programs.

CONCLUSION

The present study highlights the critical role of molecular diagnostic methods in the early detection and management of tuberculosis. Compared with conventional diagnostic techniques, molecular methods offer superior sensitivity, specificity, and significantly reduced turnaround times. Techniques such as PCR, GeneXpert MTB/RIF, LPA, LAMP, and NGS provide rapid diagnosis and facilitate early detection of drug-resistant tuberculosis. The integration of molecular diagnostics into routine clinical practice can substantially improve patient outcomes, reduce transmission, and strengthen national TB control programs. Continued investment in molecular diagnostic infrastructure and accessibility is essential for achieving global tuberculosis elimination goals.

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