Original Article

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Microbiology Section

A Comparative Study of Smear Microscopy with GeneXpert MTB/RIF Assay for Rapid Detection of *Mycobacterium tuberculosis* and Rifampicin Resistance in a Tertiary Care Hospital: A Cross-sectional Study

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ABSTRACT

Introduction: Tuberculosis (TB), primarily caused by *Mycobacterium tuberculosis*, remains a major global health threat, further intensified by the emergence of drug-resistant strains. Rapid molecular diagnostic tools such as the GeneXpert MTB/RIF assay have significantly improved early detection of TB and identification of rifampicin resistance, offering faster and more accurate results than conventional methods.

Aim: To compare smear microscopy with the GeneXpert MTB/RIF assay for the rapid detection of *Mycobacterium tuberculosis* and simultaneous detection of rifampicin resistance.

Materials and Methods: A cross-sectional study was conducted over one year (September 2022 to September 2023) at Geetanjali Medical College and Hospital, Udaipur, Rajasthan, India. A total of 314 clinical specimens (233 pulmonary and 81 extrapulmonary) were tested using both Ziehl–Neelsen (ZN) staining and the GeneXpert MTB/RIF assay. Specimens were processed according to standard protocols for both diagnostic methods. Demographic details such as age and gender were recorded. Statistical analysis was performed using the Chi-square test, with a p-value <0.001 was considered statistically significant.

Results: Among the 314 samples, males constituted the majority (204; 65%), and the most affected age group was 51-70 years (133; 42.36%). Using GeneXpert, 87 (27.7%) samples tested positive for *M. tuberculosis*, whereas smear microscopy detected only 60 (19.1%). Among pulmonary samples (n=233), GeneXpert detected MTB in 81 (35%) cases, while smear microscopy detected 58 (25%). Among extrapulmonary samples (n=81), GeneXpert detected MTB in 6 (7%) cases, whereas smear microscopy detected only 1 (1%). Of the total samples, 59 (19%) were positive by both methods, 28 (9%) were positive only by GeneXpert, and 1 (0.3%) sample was positive only by smear. GeneXpert demonstrated a 6% higher diagnostic yield for extrapulmonary TB compared with smear microscopy. Rifampicin resistance was identified in 10 (11%) of the 87 GeneXpert-positive samples, indicating the presence of potential multidrug-resistant TB.

Conclusion: The GeneXpert MTB/RIF assay is more sensitive than smear microscopy for detecting MTB in both pulmonary and extrapulmonary samples. It enables rapid diagnosis and simultaneous detection of rifampicin resistance, which is essential for the timely initiation of appropriate therapy.

Keywords: Antitubercular agents, Diagnosis, Multidrug-resistant acid amplification techniques, Rifampin

INTRODUCTION

The rise of drug-resistant strains has exacerbated the global public health burden posed by TB [1]. Caused by *Mycobacterium tuberculosis*, TB remains one of the leading causes of death from infectious diseases worldwide, second only to HIV/AIDS [2,3]. While pulmonary TB is the most common form, the disease can also involve other organs such as the brain, lymph nodes, kidneys, spine, and soft tissues, resulting in extrapulmonary TB [2,3]. India carries the world's highest TB burden, accounting for nearly 24% of global cases, with an incidence rate of 210 per 100,000 population [4]. According to the World Health Organisation (WHO) Global Tuberculosis Report, there were an estimated 10.6 million new TB cases and approximately 1.6 million TB-related deaths worldwide [4].

Traditional diagnostic tools such as smear microscopy have significant limitations, including low sensitivity and specificity, especially when bacterial load is below 10,000 bacilli/mL. Furthermore, smear microscopy cannot differentiate between viable and non-viable bacilli, reducing its usefulness in monitoring treatment response [5,6]. Although conventional culture methods such as Löwenstein-Jensen and Mycobacteria Growth Indicator Tube (MGIT) provide superior sensitivity and are considered the gold standard, they require two to eight weeks to yield results and necessitate biosafety facilities and trained personnel [7,8]. These limitations contribute to diagnostic delays, continuing transmission, and increased morbidity and mortality.

The GeneXpert MTB/RIF assay addresses many of these challenges [9]. With a detection limit of approximately 131 bacilli/mL, it simultaneously identifies *Mycobacterium tuberculosis* and rifampicin resistance within two hours [9]. The WHO strongly recommends the use of GeneXpert for both pulmonary and extrapulmonary TB due to its high sensitivity and ability to detect multidrug-resistant (MDR) TB [10].

Although the diagnostic performance of GeneXpert has been widely validated, region-specific assessments remain necessary to guide local health policies. This study, conducted at Geetanjali Medical College and Hospital, a tertiary care centre in Udaipur, Rajasthan, India, provides essential comparative evidence between GeneXpert and smear microscopy. Local epidemiological patterns, laboratory capabilities, and patient demographics can influence diagnostic effectiveness; therefore, regionally tailored evaluations are crucial.

The rationale for this study lies in the need to assess the operational feasibility, diagnostic accuracy, and cost-effectiveness of GeneXpert

within diverse healthcare settings. By evaluating its performance in this specific context, the study highlights its clinical relevance as a rapid and reliable diagnostic tool, particularly in the face of rising drug resistance. The findings aim to contribute to enhanced diagnostic algorithms, improved laboratory workflow, and ultimately better patient outcomes. This evidence supports informed decision-making aligned with national TB elimination goals, especially in high-burden regions such as Rajasthan, India.

Study objective: To compare the diagnostic performance of smear microscopy and the GeneXpert MTB/RIF assay for the rapid detection of *Mycobacterium tuberculosis* and simultaneous detection of rifampicin resistance.

MATERIALS AND METHODS

This was a cross-sectional study conducted for a period of one year (September 2022 to September 2023) in the Central Laboratory, Department of Microbiology, Geetanjali Medical College and Hospital, Udaipur, Rajasthan, India. Ethical clearance was obtained from the institutional ethical committee (GU/HRECIEC/2022/ 2152).

Inclusion criteria: Patients above 18 years of age, with clinical suspicion of tuberculosis (pulmonary or extrapulmonary) and patients who had previously received anti-TB treatment were included in the study.

Exclusion criteria: Samples received without relevant clinical history, patients with a history of lung malignancy, fungal infections, or any other confirmed etiology of respiratory illness were excluded from the study.

Sample size: A total of 314 clinical specimens were included in this study. The sample size was determined based on a previous study by Shetye S et al., [2], who reported a TB positivity rate of 30% among clinical specimens tested using the GeneXpert MTB/RIF assay in an Indian population. The sample size was calculated using the formula:

$$n = \frac{z^2 \times p \times (1-p)}{d^2}$$ where:

- n = required sample size
- z = Z-score for 95% confidence level (1.96)
- p = expected prevalence (0.30 or 30%)
- d = desired precision (0.05 or 5%)

Substituting the values:

$$n = \frac{(1.96)^2 \times 0.30 \times (1-0.30)}{d^2} \approx 323$$

To accommodate potential non-response or sample attrition, a final sample size of 314 specimens was selected.

Study Procedure

In accordance with the National Tuberculosis Elimination Programme (NTEP) guidelines, a variety of clinical specimens- including sputum, pus, cerebrospinal fluid (CSF), urine, bronchial aspirates, pleural fluid, and ascitic fluid- were collected from patients exhibiting symptoms suggestive of tuberculosis [11]. Each patient was assigned a unique Nikshay ID to ensure accurate tracking and documentation.

All specimens were subjected to the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) using the GeneXpert MTB/RIF assay for rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance. Additionally, each sample underwent smear microscopy using the Ziehl-Neelsen (ZN) staining technique to identify Acid-Fast Bacilli (AFB) [11].

Demographic and clinical information- including age, gender, date and time of sample collection, and presenting symptoms- were obtained from the requisition forms accompanying each sample. Laboratory processing began with ZN staining for direct microscopic examination, followed by the GeneXpert MTB/RIF assay to confirm the presence of *M. tuberculosis* and assess rifampicin resistance.

STATISTICAL ANALYSIS

All data were entered into an MS Excel spreadsheet. Upon completion of the study, statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 26.0. Inferential analysis was conducted using the Chi-square test to compare the results obtained through smear microscopy (ZN staining) and the GeneXpert MTB/RIF assay. A p-value <0.001 was considered statistically significant.

RESULTS

A total of 314 clinical samples from both indoor (IPD) and outdoor (OPD) patients were included, processed, and analysed in the Microbiology laboratory. Among the 314 samples, the majority were pulmonary in origin: 183 (58%) were sputum samples, 46 (15%) were bronchial aspirate/lavage specimens, and 4 (1%) were endotracheal (ET) secretions. The remaining samples included 6 (2%) tissue specimens, 4 (1%) pus samples, and 71 (23%) sterile body fluids- such as pleural fluid, ascitic fluid, CSF, pericardial fluid, and others- as shown in [Table/Fig-1].

Type of samples (n=314)	n (%)
Pulmonary samples (n=233; 74%)	
Sputum	183 (58)
Bronchial aspirate lavage (BAL)	46 (15)
Endotracheal secretion	4 (1)
Extrapulmonary samples (n=81; 26%)	
Tissue	6 (2)
Pus	4 (1)
Sterile fluids	71 (23)

[Table/Fig-1]: Distribution of pulmonary and extrapulmonary samples (n=314).

[Table/Fig-2] presents the distribution of the study population by age and gender. The largest proportion of samples belonged to the 61–70-year age group (68 samples; 21.66%). Of the 314 samples, 204 (65%) were obtained from male patients and 110 (35%) from female patients, resulting in a male-to-female ratio of 1.8:1.

Age group (years)	Male	Female	Total (n=314)
18-30	32 (10.19%)	18 (5.73%)	50 (15.92%)
31-40	28 (8.91%)	21 (6.68%)	49 (15.60%)
41-50	40 (12.73%)	13 (4.14%)	53 (16.87%)
51-60	43 (13.69%)	22 (7.01%)	65 (20.70%)
61-70	41 (13.06%)	27 (8.60%)	68 (21.66%)
>70	20 (6.37%)	9 (2.87%)	29 (9.24%)
Total	204 (65%)	110 (35%)	314 (100%)

[Table/Fig-2]: Age and gender-wise distribution of the study population (n=314)

Among the processed samples, 87 (28%) tested positive for *M. tuberculosis* by GeneXpert, while 60 (19%) were positive by ZN staining [Table/Fig-3].

Total clinical samples	GeneXpert positive	Smear microscopy (ZN staining) positive
314	87 (28%)	60 (19%)

[Table/Fig-3]: Percentage of positive samples by GeneXpert and Smear microscopy (ZN staining) in all clinical samples (n=314).

Of the 87 GeneXpert-positive samples, 81 (93%) were pulmonary and 6 (7%) were extrapulmonary [Table/Fig-4]. Similarly, among the 60 ZN-positive samples, 58 (97%) were pulmonary and 2 (3%) were extrapulmonary [Table/Fig-5]. [Table/Fig-6] shows that out of the total 314 samples, *M. tuberculosis* was detected by GeneXpert in 87 (28%), while 227 (72%) tested negative. Of the 87 GeneXpert-positive samples, only 60 (19%) were positive for AFB on ZN staining. A total of 226 out of 314 samples (72%) were negative by both GeneXpert and smear microscopy, and 59 out of 314 (19%)

were positive by both methods. One sample (0.32%) was positive by smear microscopy but negative by GeneXpert.

Parameters	Pulmonary samples	Extrapulmonary samples	Total
GeneXpert positive	81 (93%)	6 (7%)	87 (100%)

[Table/Fig-4]: Percentage of positive samples by GeneXpert in pulmonary and extrapulmonary samples (n=87).

Parameters	Pulmonary samples	Extrapulmonary samples	Total
Smear positive	58 (97%)	2 (3%)	60 (100%)

[Table/Fig-5]: Percentage of positive samples by Smear microscopy in pulmonary and extrapulmonary (n=60).

Parameters	Acid Fast Bacilli (AFB) positive	Acid Fast Bacilli (AFB) negative	Total
GeneXpert positive	59 (19%)	28 (9%)	87 (28%)
GeneXpert negative	1 (0.32%)	226 (72%)	227 (72%)
Total	60 (19.1%)	254 (80.9%)	314 (100%)

[Table/Fig-6]: Comparison between Smear microscopy and GeneXpert MTB/RIF assay for all clinical samples (n=314).
*Statistical test used: Chi-square test; Chi-square value= 184.2; df=1, p<0.001

[Table/Fig-7] shows the distribution of rifampicin resistance among the GeneXpert-positive samples. Rifampicin resistance was detected in 10 out of 87 samples (11%), while 77 samples (89%) showed no resistance.

Rifampicin resistance	Pulmonary samples	Extrapulmonary samples	Total
Rif detected	9 (10%)	1 (1%)	10 (11%)
Rif not detected	71 (82%)	6 (7%)	77 (89%)
Total	80 (92%)	7 (8%)	87 (100%)

[Table/Fig-7]: Rifampicin (Rif) resistance detected by GeneXpert MTB/RIF assay (n=87).

DISCUSSION

The rapid and accurate diagnosis of TB remains a significant challenge in high-burden countries such as India, where laboratory-confirmed diagnosis is crucial for effective disease management and transmission control [2,4]. While traditional diagnostic methods like smear microscopy and culture have well-documented limitations [5-8], molecular assays such as the Xpert MTB/RIF have emerged as powerful alternatives [9]. This study evaluated the diagnostic utility of the GeneXpert assay in a real-world setting, providing insights that align with and expand upon existing literature.

The primary finding of this study is the significantly enhanced diagnostic yield of the GeneXpert assay compared to conventional smear microscopy. GeneXpert detected *Mycobacterium tuberculosis* in 28% of all processed samples, whereas smear microscopy identified only 19%, resulting in a 9% absolute increase in microbiologically confirmed TB cases (p-value <0.001). This substantial improvement in case detection was consistent with numerous studies from India and other settings. For instance, Shetye S et al., [2], Rani P et al., [12], and Arora D and Dhanashree B, [13] reported similar increases in diagnostic yield, reinforcing the value of GeneXpert as a frontline diagnostic tool. The superior performance of GeneXpert is largely due to its lower limit of detection (approximately 131 CFU/mL), compared to the high bacillary load required for smear microscopy (5,000-10,000 organisms/mL) [2]. Furthermore, the assay's ability to detect DNA from both viable and non viable bacilli contributes to its heightened sensitivity, especially in paucibacillary conditions such as extrapulmonary TB (EPTB) or smear-negative pulmonary disease [9,14]. In the present study, 28 TB-positive cases missed by smear microscopy were identified by GeneXpert, underscoring its clinical value in bridging this diagnostic gap.

In the present study cohort, TB was most prevalent among males (65%) and in the older age group of 51-70 years. This demographic pattern was consistent with findings from other Indian studies by Rani P et al., [12] and Chaudhary A et al., [15]. Increased susceptibility in the elderly population can be attributed to immunosenescence—the natural age-related decline of the immune system—and a higher prevalence of co-morbidities such as diabetes, malnutrition, and HIV, all of which are known risk factors for TB reactivation [16]. The observed male predominance is likely multifactorial, arising from a combination of biological and sociocultural factors in the Indian context, including higher rates of smoking and alcohol consumption, as well as potentially better access to healthcare services among men, leading to higher diagnosis rates [17].

The distribution of specimen types in the present study, with a predominance of pulmonary samples (74%), reflects the typical epidemiological pattern of TB in the region. This finding aligns with observations from Bankar S et al., [7] and Rani P et al., [12], who reported pulmonary sample proportions of 74% and 61.88%, respectively. However, present results contrast with those of Zahoor et al., [18], who reported a majority of extrapulmonary samples (56%) in their cohort. This discrepancy may be attributable to differences in study settings; for example, their study may have been conducted at a tertiary referral centre that receives a higher proportion of complex or difficult-to-diagnose EPTB cases, whereas ours reflects a more general patient population.

An essential feature of the GeneXpert assay is its ability to simultaneously detect rifampicin resistance, a surrogate marker for multidrug-resistant TB (MDR-TB). In the current study, 11% of TB-positive samples showed rifampicin resistance. This rate was comparable to the 10.20% reported by Chaudhary A et al., [15] but differs from the rates reported by Rani P et al., (2.98%) [12] and Zahoor D et al., (5.8%) [18]. Such variation likely reflects the known geographical heterogeneity in drug-resistant TB prevalence. Differences in sample size and the proportion of new versus previously treated patients among studies may also contribute to the variation. These findings highlight the importance of local drug-resistance surveillance to inform appropriate treatment strategies.

Although GeneXpert demonstrated superior sensitivity, it is not without limitations. One case in the present study was positive by smear microscopy but negative by GeneXpert. A plausible explanation is infection with nontuberculous mycobacteria (NTM), which appear morphologically identical to *M. tuberculosis* on a smear but are not detected by the species-specific primers used in the Xpert MTB/RIF assay [15].

Limitations

This highlights a limitation of smear microscopy and reinforces the need for molecular methods for accurate species identification. It is also important to note that the GeneXpert assay does not replace the need for conventional culture and Drug Susceptibility Testing (DST), which remain essential for monitoring treatment response and detecting resistance to anti-TB drugs other than rifampicin. Another limitation of the present study was the absence of mycobacterial culture as a gold standard, which prevented calculation of precise sensitivity and specificity.

CONCLUSION

Present study supports the WHO recommendation to use the GeneXpert MTB/RIF assay as an initial diagnostic test for TB. Its ability to rapidly and accurately detect TB- particularly in smear-negative and extrapulmonary cases-along with the added advantage of identifying rifampicin resistance, makes it an invaluable tool in resource-limited settings. Future large-scale, multicentre studies incorporating culture are warranted to further define the operational performance of GeneXpert within the national TB control programme.

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