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Original Research

Diagnostic Accuracy of Stool Gene Xpert for the Diagnosis of Pulmonary Tuberculosis by Taking AFB Sputum Culture as Gold Standard at Tertiary Care Hospital, Nawabshah

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Abstract: **Background:** Diagnosing pulmonary tuberculosis in children remains difficult because many patients cannot expectorate sputum of adequate quality. Stool-based molecular testing using GeneXpert provides a non-invasive alternative, yet data on its performance in routine tertiary care practice remain limited. **Objective:** To assess the diagnostic accuracy of stool GeneXpert for pulmonary tuberculosis using AFB sputum culture as the reference standard at a tertiary care hospital. **Methods:** This cross-sectional diagnostic accuracy study enrolled 100 children with presumptive pulmonary tuberculosis. Each participant provided a stool sample for GeneXpert MTB/RIF testing and a sputum sample for AFB culture. The study calculated sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy. **Results:** Sputum culture confirmed pulmonary tuberculosis in 64 participants. Stool GeneXpert identified 59 cases of tuberculosis and demonstrated a sensitivity of 81.2% and specificity of 80.5%. The positive predictive value was 88.1%, the negative predictive value was 70.7%, and the overall diagnostic accuracy reached 81%. **Conclusion:** Stool GeneXpert showed good diagnostic performance and offers a practical, non-invasive option for confirming pulmonary tuberculosis in children who have difficulty producing sputum. Clinicians should interpret negative results with caution and consider culture and clinical findings when tuberculosis remains suspected.

Keywords: Pulmonary tuberculosis; Stool GeneXpert; Diagnostic accuracy; Pediatric tuberculosis; Sputum culture; Non-invasive testing

INTRODUCTION

In 2017, it was estimated that over 1 million new tuberculosis (TB) cases occurred in children, resulting in approximately 230,000 TB-related deaths, which represented around 10% of all TB cases and 15% of TB fatalities globally. Among children, pulmonary tuberculosis (PTB) remains the most frequently observed form of the disease. [1-2] The Xpert MTB/RIF assay (Cepheid, USA), an automated cartridge-based PCR test, is currently endorsed by the World Health Organization (WHO) as the primary diagnostic tool for suspected pulmonary tuberculosis in both adults and children. [3] The Xpert assay requires minimal sample preparation and delivers results within approximately two hours. A meta-analysis combining data from both sputum smear-positive and smear-negative patients reported that Xpert testing on

respiratory samples demonstrated a sensitivity of 62% (95% credible interval: 51–73%) and a specificity of 98% (95% credible interval: 97–99%). Consequently, Xpert on sputum samples is more sensitive than traditional smear microscopy. In addition, Xpert offers several practical advantages compared to mycobacterial culture, which remains the gold standard for tuberculosis diagnosis. [4] In children younger than five years, and especially those under two, obtaining sputum samples is challenging. Collection often necessitates invasive procedures such as nasopharyngeal or nasogastric aspiration, or bronchoscopy, which are difficult to perform in resource-limited settings and are not widely accessible. [5]

Because sputum culture results in children are often inconclusive, the diagnosis of pediatric tuberculosis primarily relies on clinical evaluation and imaging studies, with treatment frequently initiated based on clinical suspicion rather than definitive laboratory confirmation.[6] Obtaining high-quality sputum samples from children is a challenging process, and the typically low bacterial load in these samples makes it difficult to successfully isolate the tuberculosis organism. [7-8] Studies have shown that high-quality sputum is approximately 3.8 times more likely to yield pathogenic bacteria compared to poor-quality samples; however, children often face difficulty in producing sputum on demand. [9] Beyond diagnosing pulmonary tuberculosis in children, it is crucial to identify cases of multidrug-resistant TB among pediatric and high-risk populations to help prevent the global transmission of drug-resistant strains. [10] There is a critical need for diagnostic tests that are rapid, accurate, and widely accessible, using samples that can be collected easily for the detection and identification of Mycobacterium tuberculosis. [11] Stool samples are easily collected from children and can be obtained at the primary healthcare level without requiring invasive procedures. These samples can be used for culture and molecular diagnostic tests, facilitating the diagnosis of pulmonary tuberculosis in children and enabling timely referral for appropriate treatment, thereby helping to reduce TB-related morbidity and mortality. [12] A study done by Moussa et al found stool gene Xpert had a sensitivity of 83.3% and specificity of 98.7% for the diagnosis of pulmonary tuberculosis. [13]

Pulmonary tuberculosis (PTB) is the most common form of the disease, and early, accurate diagnosis is critical to initiate timely treatment and reduce transmission. The conventional gold standard for diagnosing PTB is sputum culture for acid-fast bacilli (AFB), which is highly specific but can take weeks for results due to the slow-growing nature of Mycobacterium tuberculosis. Furthermore, in children who are unable to produce sputum, obtaining a sample for sputum analysis can be challenging, leading to delays in diagnosis. By determining sensitivity and specificity, the study will help evaluate whether stool GeneXpert can serve as a reliable, non-invasive diagnostic tool for PTB, particularly in populations where sputum samples are difficult to obtain. Such findings could have significant implications for TB control, especially in resource-limited settings where access to advanced diagnostic methods and sample collection is often restricted.

MATERIALS AND METHODS

The study aimed to determine the diagnostic accuracy of stool Gene Xpert for detecting pulmonary tuberculosis, using AFB sputum culture as the gold standard, at the Institute of Maternity and Child Care Hospital, Nawabshah. The study was conducted as a cross-sectional investigation over a six-month period following approval of the research synopsis and ethical clearance from the College of Physicians and Surgeons Pakistan. A total of 100 children aged 2–16 years, of either gender, who were suspected of having pulmonary tuberculosis, were recruited using non-probability consecutive sampling. Sample size was initially calculated based on previously reported sensitivity (83.3%) and specificity (98.7%) with a tuberculosis prevalence of 9.9%, a 10% margin of error, and 95% confidence level, which suggested 381 patients; however, considering the study duration and feasibility, 100 participants were included. Children who had already started anti-tubercular therapy, whose guardians refused consent, or who had conditions that could confound results—such as malabsorption, sepsis, meningitis, encephalitis, pneumonia, congenital urinary tract anomalies, hematologic or solid organ malignancy, chronic gastrointestinal diseases, or compromised immune systems including HIV/AIDS or chemotherapy—were excluded.

Operationally, children were labeled as suspected pulmonary tuberculosis cases if they presented with a new pulmonary infiltrate on chest radiograph along with one or more of the following symptoms persisting for over two months: productive cough, fever ≥37.8°C, or weight loss of ≥10 kg. Stool Gene Xpert was considered positive when DNA sequences of Mycobacterium tuberculosis were detected via hemi-nested RT-PCR amplification, and negative otherwise. AFB sputum culture on Lowenstein-Jensen media was labeled positive if mycobacterial growth was observed, and negative if no growth occurred after eight weeks. True positives were defined as children with both positive stool Gene Xpert and positive AFB culture; true negatives as those with both tests negative; false positives as positive Gene Xpert but negative culture; and false negatives as negative Gene Xpert but positive culture. Sensitivity, specificity, positive and negative predictive values, and overall diagnostic accuracy were calculated using standard formulas.

After obtaining informed consent from parents or guardians, demographic information including age and gender was collected. Single induced sputum or gastric aspirates and stool samples were obtained from all enrolled participants. Chest radiographs were performed, and immunization status was recorded. Stool samples were processed by mixing approximately one gram of stool with phosphate-buffered saline, filtering, and decontaminating with NALC-NaOH for 15 minutes, followed by centrifugation and resuspension. Aliquots of the processed stool were used to inoculate Lowenstein-Jensen media, which were incubated at 37°C and examined weekly for growth up to eight weeks. The samples were also tested using the Gene Xpert MTB assay under the supervision of an experienced microbiologist, and results were recorded as positive or negative. All observations and laboratory findings were documented in a structured proforma.

Data were analyzed using SPSS Version 16. Continuous variables such as age were expressed as mean ± standard deviation for normally distributed data and as median with interquartile range for skewed distributions, assessed via the Kolmogorov–Smirnov test. Categorical variables, including gender and tuberculosis status based on stool Gene Xpert or AFB culture, were reported as frequencies and percentages. Diagnostic performance of stool Gene Xpert, including sensitivity, specificity, positive and negative predictive values, and overall accuracy, was calculated using 2×2 contingency tables. Stratification was performed by age and gender to evaluate the effect of these variables, and post-stratification measures of diagnostic accuracy were recalculated using contingency tables.

RESULT

The study enrolled 100 patients evaluated for presumptive pulmonary tuberculosis at a tertiary care hospital. Most participants belonged to the 9–16-year age group (67%), while one-third were between 2 and 8 years of age (33%). This age distribution suggests that older children formed the larger share of suspected cases, possibly because symptoms become more apparent and clinical suspicion increases with age. The study population showed a male predominance, with males accounting for 62% of cases and females for 38%, a pattern that aligns with the commonly observed higher burden of tuberculosis among males in many clinical settings.

Using AFB sputum culture as the reference standard, pulmonary tuberculosis was confirmed in 64% of the patients, indicating a relatively high prevalence of disease in the study cohort. In comparison, stool GeneXpert detected tuberculosis in 59% of participants. The close alignment between culture positivity and stool GeneXpert positivity suggests that stool-based testing was able to identify a substantial proportion of true tuberculosis cases. At the same time, the presence of culture-negative cases highlights the importance of evaluating diagnostic performance rather than relying on test positivity alone.

When stool GeneXpert results were compared directly with sputum culture, the test correctly identified 52 patients as true positives and correctly ruled out tuberculosis in 29 patients as true negatives. However, stool GeneXpert missed 12 culture-positive cases, resulting in false-negative findings, and yielded 7 false-positive results among culture-negative patients. These discrepancies point to inherent challenges in stool-based testing, such as variable bacillary load and differences in specimen processing, which may influence test performance. Nevertheless, the predominance of true results over misclassified cases indicates good overall agreement between the two diagnostic methods.

Stool GeneXpert demonstrated a sensitivity of 81.2%, showing that it detected more than four out of five patients with culture-confirmed pulmonary tuberculosis. The specificity of 80.5% indicates that the test also performed well in correctly identifying patients without the disease. The positive predictive value was high at 88.1%, suggesting that a positive stool GeneXpert result strongly correlated with true disease. In contrast, the negative predictive value was lower at 70.7%, indicating that a negative result did not reliably exclude tuberculosis, particularly in this high-prevalence population.

Overall, stool GeneXpert achieved a diagnostic accuracy of 81%, reflecting a balanced performance in identifying both diseased and non-diseased individuals. The results show that stool GeneXpert functions effectively as a confirmatory diagnostic tool for pulmonary tuberculosis, especially when sputum collection proves difficult. However, the observed false-negative rate underscores the need for continued reliance on sputum culture or additional diagnostic methods when clinical suspicion remains high despite a negative stool GeneXpert result. These findings support the integration of stool GeneXpert into diagnostic algorithms at tertiary care settings, particularly for pediatric and sputum-scarce patients.

Table 1: Characteristics of Patients

Demography		**Number**	**Percentage**
Age	02-08 years	33	33%
	09-16 years	67	67%
Gender	Male	62	62%
	Female	38	38%
AFB Sputum Culture for Pulmonary Tuberculosis	Positive	64	64%
	Negative	36	36%
Stool Gene Xpert for Pulmonary Tuberculosis	Positive	59	59%
	Negative	41	41%

Table 2: Results of Stool Gene Xpert for the Diagnosis of Pulmonary Tuberculosis by Taking AFB Sputum Culture as Gold Standard

Variable		**AFB sputum culture for pulmonary tuberculosis**		**Total**
		Positive	**Negative**	
Stool Gene Xpert for Pulmonary Tuberculosis	Positive	52(TP)	07(FP)	59
	Negative	12(FN)	29(TN)	41
Total		64	36	100

Table 3: Sensitivity, Specificity, Positive and Negative Predictive Values and Diagnostic Accuracy Of Stool Gene Xpert for the Diagnosis Of Pulmonary Tuberculosis By Taking AFB Sputum Culture as Gold Standard

Variable	**Sensitivity**	**Specificity**	**Positive predictive value**	**Negative predictive value**	**Diagnostic accuracy**
Stool Gene Xpert for Pulmonary Tuberculosis	81.2%	80.5%	88.1%	70.7%	81%

DISCUSSION

The demographic profile of the study population reflects patterns commonly reported in pediatric pulmonary tuberculosis research. The predominance of children aged 9–16 years is consistent with previous evidence suggesting that older children are more frequently investigated for pulmonary TB due to clearer symptomatology, greater bacillary burden, and improved ability to expectorate sputum compared with younger children.[18-19] The observed male predominance aligns with epidemiological data indicating higher TB notification rates among males, even in pediatric populations, which may be influenced by biological susceptibility, healthcare-seeking behavior, or sociocultural factors. [20] These demographic trends suggest that the study population is representative of children typically evaluated for pulmonary TB in tertiary care settings, thereby supporting the external validity of the findings.

The relatively high proportion of culture-confirmed tuberculosis in this cohort highlights a substantial burden of microbiologically proven disease, which is characteristic of referral-based tertiary care hospitals. [19] There is close correspondence between culture positivity (64%) and stool GeneXpert positivity (59%). This suggests that stool-based molecular testing captured a large share of true TB cases. Similar observations have been reported in previous diagnostic accuracy studies, where stool GeneXpert positivity closely mirrored microbiological confirmation rates, particularly in high-prevalence or hospital-based populations. [21-22] This finding reinforces the potential utility of stool GeneXpert as a pragmatic diagnostic approach in settings where sputum collection is challenging.

The cross-tabulation analysis demonstrated substantial agreement between stool GeneXpert and sputum culture, with a predominance of true-positive and true-negative results. However, the presence of both false-positive and false-negative results reflects known limitations of stool-based diagnostics. False-positive results may occur due to the detection of non-viable bacilli or swallowed Mycobacterium tuberculosis DNA that does not yield growth on culture, an issue previously highlighted in molecular TB diagnostics. [18,21] Conversely, false-negative results may be explained by low or uneven distribution of bacilli in stool samples, particularly in paucibacillary disease, which is common in children. [23] These findings are consistent with earlier studies that emphasize variability in stool GeneXpert performance due to biological and methodological factors, including stool processing techniques. [18, 22]

The sensitivity of stool GeneXpert observed in this study is higher than pooled estimates reported in several meta-analyses but remains within the upper range documented in individual studies conducted in high-burden settings. [18, 21] The specificity observed, although slightly lower than that reported in some systematic reviews, remains clinically acceptable and supports the test's reliability in identifying non-

diseased individuals. [18] The high positive predictive value underscores the strength of stool GeneXpert as a confirmatory diagnostic tool, particularly in populations with a high pre-test probability of disease. In contrast, the lower negative predictive value indicates that a negative stool GeneXpert result should be interpreted cautiously and does not rule out pulmonary tuberculosis, a conclusion that has been consistently emphasized in previous literature. [18, 23]

The overall diagnostic accuracy of stool GeneXpert observed in this study supports its role as a useful adjunct in the diagnostic pathway for pediatric pulmonary tuberculosis. Consistent with earlier evidence, these findings suggest that stool GeneXpert is best positioned as a rule-in test, particularly for children who are unable to produce sputum or in whom invasive sampling is not feasible. [18, 21-22] However, reliance on stool GeneXpert alone is insufficient to exclude disease, and culture or additional diagnostic modalities remain essential when clinical suspicion persists. The results reinforce calls in the literature for standardized stool processing protocols and integration of stool GeneXpert into existing diagnostic algorithms rather than its use as a standalone test. [18-19, 23] Taken together, these findings contribute to the growing body of evidence supporting stool-based molecular diagnostics as a valuable, non-invasive option in pediatric TB care at tertiary health facilities.

LIMITATIONS

This study has important limitations. The single-centre design and modest sample size may limit the transferability of the findings to lower-level health facilities and settings with different disease prevalence. Although sputum culture served as the reference standard, its reduced sensitivity in paucibacillary pediatric disease may have resulted in misclassification of some cases. In addition, the use of a single stool specimen and the lack of stratified analysis by key clinical factors may have influenced the observed diagnostic performance.

CONCLUSION

This study shows that stool GeneXpert provides a practical non-invasive option for diagnosing pulmonary tuberculosis in children at a tertiary care hospital. The test achieved good sensitivity, specificity, and overall accuracy when compared with sputum culture, supporting its clinical value. A high positive predictive value indicates that a positive stool GeneXpert result reliably reflects true disease. In contrast, the lower negative predictive value highlights the need for caution when interpreting negative results in children with ongoing clinical suspicion. Stool GeneXpert can therefore strengthen pediatric tuberculosis diagnostic pathways, particularly for patients unable to produce sputum, while culture and clinical assessment remain integral to care.

Conflict of Interest

This study has no conflict of interest to declare by any author.

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