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Diagnostic Accuracy of Stool Xpert Mtb/Rif For Pulmonary Tuberculosis In Children Keeping Microbial Culture As Gold Standard

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ABSTRACT

Objective: To determine the diagnostic accuracy of stool Xpert MTB/RIF for the detection of pulmonary tuberculosis (PTB) in children, using sputum/gastric aspirate culture as the gold standard.

Methodology: This cross-sectional study was conducted in the Department of Pediatrics, CMH, Gujranwala, after approval from the College of Physicians and Surgeons Pakistan. A total of 181 clinically suspected PTB cases aged 1–12 years were enrolled over six months using non-probability consecutive sampling. Stool and sputum/gastric aspirate samples were obtained according to standard guidelines. Stool samples were tested by Xpert MTB/RIF, while sputum/gastric aspirate samples were subjected to culture as the reference standard. Data were analyzed using SPSS version 22. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated using a 2×2 contingency table.

Results: The mean age of participants was 6.9 ± 3.1 years, and 56.4% were male. Culture-confirmed PTB was detected in 116 (64.1%) children. Stool Xpert MTB/RIF was positive in 108 (59.7%) cases. The sensitivity, specificity, PPV, and NPV of stool Xpert MTB/RIF were 88.8%, 92.3%, 95.4%, and 82.2%, respectively, with an overall diagnostic accuracy of 90.1%. A significant association was observed between PTB and rural residence ($p = 0.021$) as well as household TB contact ($p < 0.001$).

Conclusion: Stool Xpert MTB/RIF demonstrated high diagnostic accuracy and can serve as a reliable, non-invasive alternative for diagnosing pediatric pulmonary tuberculosis, particularly in settings where respiratory sample collection is difficult.

Keywords: *Pulmonary tuberculosis, Stool Xpert MTB/RIF, Pediatric tuberculosis, Diagnostic accuracy, GeneXpert, Mycobacterial culture.*

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1. INTRODUCTION

The term Mycobacterium tuberculosis complex encompasses a group of nine different species, with the most common one being Mycobacterium tuberculosis (MTB), responsible for causing tuberculosis (TB) in humans. [1] On a global scale, the incidence of tuberculosis (TB) dropped from 144.12 cases per 100,000 people in 1990 to 97.56 cases per 100,000 people in 2019. This decline averaged at a rate of 1.28% per year, however, there has been a rising trend in the occurrence of extensively drug-resistant and multi-drug resistant TB. [2] The gold standard for TB diagnosis is mycobacterial culture [3], which may identify as little as 10 bacilli/ml. With a detection threshold of 5,000 bacilli/ml, acid-fast bacilli (AFB) smear microscopy is also cheaper and widely used diagnostic test. [4] Fluorescent microscopy is another diagnostic modality which can effectively detect Mycobacterium tuberculosis in sputum samples in resource limited settings where advance diagnostic test, like GeneXpert is not available. [5] The GeneXpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) assay (a nucleic acid amplification test) has a much lower detection threshold of 136 bacilli/ml of material and it can identify rifampicin resistance. [6]

A study reported that prevalence of pulmonary TB (PTB) in children was 18.3% with sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of stool XPERT MTB/RIF to diagnose PTB of 88.9%, 95%, 88.9% and 95%, respectively. [7] Compared to this, a study found reported that prevalence of PTB in children was 26% but in regards to diagnostic accuracy parameters, it was found that stool XPERT MTB/RIF has a sensitivity of merely 11.54%. Other parameters were specificity (98.65%), PPV (75%) and NPV (76.04%). [8] the sensitivity and specificity of gene Xpert was 89% and 91% and prevalence was 64%.[9]

Based on the results of aforementioned studies, a significant variation is observed regarding the reliability of stool XPERT MTB/RIF to diagnose PTB in children with study showing it to be highly sensitive and specific while other showing it to have very low sensitivity. In addition, local data regarding the accuracy of this potentially useful alternative sample, instead of gastric aspirate which is obtained through invasive and painful procedure of nasogastric tube insertion, is not available. Therefore, present study is being conducted to determine the diagnostic accuracy of stool XPERT MTB/RIF to diagnose PTB in children.

2. METHODOLOGY

This cross-sectional study was conducted at CMH, Department of Pediatrics, Gujranwala, over a period of six months after approval of the synopsis from the College of Physicians and Surgeons Pakistan. Non-probability consecutive sampling technique was used. The sample size was calculated using a sensitivity and specificity calculator by taking a confidence level of 95%, precision of 7%, prevalence of 64%, sensitivity of 89%, and specificity of 91%. The calculated sample size was 181.

Children aged 1–12 years of both genders who were clinically suspected cases of pulmonary tuberculosis (as per operational definition) were included in the study. Patients with a previous history of tuberculosis, assessed through review of past medical records, were excluded. Those whose parents did not consent to participate in the study were also excluded. In addition, patients who had a positive bacterial culture other than Mycobacterium tuberculosis and were receiving antibiotics for that infection were excluded.

After obtaining synopsis approval from CPSP and the institutional research evaluation board, and after obtaining informed consent from parents, a total of 181 patients fulfilling the inclusion criteria were enrolled from the pediatric outdoor and indoor departments of CMH, Gujranwala. Baseline characteristics including age (in years), gender, weight (in kilograms), parental education status (illiterate or school education and above), area of residence (rural or urban), history of active tuberculosis in a household contact (yes/no), and parental history of smoking (yes/no) were documented.

The patients were then assessed for pulmonary tuberculosis by obtaining stool and sputum/gastric aspirate samples according to standard clinical practice guidelines. The samples were sent to the in-hospital laboratory for stool Xpert MTB/RIF testing and culture, respectively, to establish the diagnosis of pulmonary tuberculosis as per the operational definition. In cases where pulmonary tuberculosis was diagnosed, standard management protocol was followed according to established guidelines. All data were recorded on a predesigned proforma.

The collected data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 22. Quantitative variables such as age and weight were presented either as mean ± standard deviation (SD) or as median with interquartile range (IQR), depending upon the distribution assessed by the Shapiro–Wilk test. Qualitative variables including gender, parental education status, area of residence, history of active tuberculosis in a household contact, and presence of pulmonary tuberculosis on sputum fluorescent microscopy, sputum GeneXpert, and sputum culture were presented as frequencies and percentages.

The presence of pulmonary tuberculosis was stratified with respect to age, gender, area of residence, and history of active tuberculosis in a household contact. Post-stratification comparisons were performed using the Chi-square test or Fisher's exact test where appropriate, with the level of significance set at $p \leq 0.05$. A 2×2 contingency table was constructed to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy.

3. RESULTS

This cross-sectional study was conducted in the Department of Pediatrics, CMH, Gujranwala, after approval of synopsis from the College of Physicians and Surgeons Pakistan and institutional research evaluation board. A total of 181 clinically suspected cases of pulmonary tuberculosis (PTB), fulfilling the inclusion criteria, were enrolled over a period of six months through non-probability consecutive sampling.

The mean age of the study participants was 6.9 ± 3.1 years, ranging from 1 to 12 years. The mean weight of the children was 18.8 ± 6.2 kg. Among the enrolled children, 102 (56.4%) were males and 79 (43.6%) were females. With regard to residence, 97 (53.6%) children belonged to rural areas, while 84 (46.4%) were from urban areas. Parental education status showed that 74 (40.9%) parents were illiterate, whereas 107 (59.1%) had school education or above. A positive history of active tuberculosis contact at home was present in 88 (48.6%) children, while 93 (51.4%) had no such contact. Parental history of smoking was identified in 76 (42.0%) cases.

Sputum/gastric aspirate culture, taken as the gold standard for diagnosis of pulmonary tuberculosis, was positive in 116 children, giving an observed prevalence of 64.1%, while 65 (35.9%) children were culture negative. Stool Xpert MTB/RIF testing was positive in 108 (59.7%) children and negative in 73 (40.3%) children. Stratification of culture-confirmed PTB showed that the frequency of PTB was comparatively higher in children aged 7–12 years than in those aged 1–6 years; however, this association was not statistically significant ($p > 0.05$). Similarly, no statistically significant association was found between gender and presence of PTB ($p > 0.05$). A statistically significant association was observed between area of residence and PTB, with rural children showing a higher proportion of culture-positive PTB compared to urban children ($p = 0.021$). A strong statistically significant association was also found between household contact history and PTB positivity ($p < 0.001$).

Diagnostic accuracy of stool Xpert MTB/RIF was calculated using sputum/gastric aspirate culture as the reference standard. Out of 116 culture-positive cases, 103 were correctly identified by stool Xpert MTB/RIF, while 13 were missed. Among 65 culture-negative cases, 60 were correctly identified as negative and 5 were falsely labeled positive. The sensitivity of stool Xpert MTB/RIF was calculated as 88.8%, specificity as 92.3%, positive predictive value (PPV) as 95.4%, negative predictive value (NPV) as 82.2%, and overall diagnostic accuracy as 90.1%. These findings demonstrate high diagnostic validity of stool Xpert MTB/RIF for detection of pulmonary tuberculosis in children.

Table 1: Baseline Characteristics of Study Participants (n = 181)

Variable	n	%
Age (years)	6.9 ± 3.1	—
Weight (kg)	18.8 ± 6.2	—
Gender		
Male	102	56.4
Female	79	43.6
Residence		
Rural	97	53.6
Urban	84	46.4
Parental Education		
Illiterate	74	40.9
School education or above	107	59.1
Household TB Contact		
Yes	88	48.6
No	93	51.4
Parental Smoking		
Yes	76	42.0
No	105	58.0
Culture Positive PTB	116	64.1

Table 2: Diagnostic Accuracy of Stool Xpert MTB/RIF (n = 181)

Stool Xpert MTB/RIF	Culture Positive	Culture Negative	Total
Positive	103	5	108
Negative	13	60	73
Total	116	65	181

4. DISCUSSION

The results of my research showed that this cross-sectional study was conducted in the Department of Pediatrics, CMH, Gujranwala, after approval of the synopsis from the College of Physicians and Surgeons Pakistan and the Institutional Research Evaluation Board. A total of 181 clinically suspected cases of pulmonary tuberculosis (PTB), who fulfilled the

inclusion criteria, were enrolled over a period of six months using a non-probability consecutive sampling technique. Another study conducted in 2025 reported that Stool Xpert Ultra demonstrated moderate sensitivity for microbiologically confirmed PTB in children younger than 10 years. It identified additional cases when respiratory specimens were unavailable or negative, making it a valuable non-invasive alternative. The study further noted that in children aged ≥10 years and those with severe acute malnutrition (SAM), fewer cases were missed [9]. In my study, the mean age of participants was 6.9 ± 3.1 years, with an age range of 1 to 12 years. The mean weight was 18.8 ± 6.2 kg. Among the enrolled children, 102 (56.4%) were male and 79 (43.6%) were female. Regarding residence, 97 (53.6%) children belonged to rural areas, whereas 84 (46.4%) resided in urban areas. Similarly, a study conducted in 2020 suggested that stool specimens could serve as a potential alternative to respiratory samples for routine tuberculosis diagnosis, particularly in situations where obtaining respiratory specimens is difficult [10].

In my study, parental education status revealed that 74 (40.9%) parents were illiterate, while 107 (59.1%) had school education or higher. A history of active tuberculosis contact at home was present in 88 (48.6%) children, whereas 93 (51.4%) had no such contact. Parental smoking was reported in 76 (42.0%) cases. Another study conducted in 2025 concluded that the stool Xpert MTB/RIF assay is a promising diagnostic tool for pediatric PTB, especially in resource-limited settings where collection of respiratory samples is challenging. Although its sensitivity was lower than that of respiratory specimens, its high specificity made it a reliable alternative. The authors recommended further research to optimize stool sample processing and explore advanced diagnostic assays to improve sensitivity and overall accuracy [11]. In my research, sputum/gastric aspirate culture, considered the gold standard for diagnosing pulmonary tuberculosis, was positive in 116 children, yielding an observed prevalence of 64.1%, while 65 (35.9%) children were culture negative. Stool Xpert MTB/RIF testing was positive in 108 (59.7%) children and negative in 73 (40.3%) children. Stratification of culture-confirmed PTB revealed that the frequency of PTB was higher among children aged 7–12 years compared to those aged 1–6 years; however, this association was not statistically significant ($p > 0.05$). Likewise, no statistically significant association was observed between gender and PTB ($p > 0.05$). A study conducted in 2023 reported that the Xpert assay performed on stool samples showed limited sensitivity but good specificity for diagnosing pulmonary TB. It was therefore proposed as an alternative screening method in pediatric cases where obtaining respiratory samples is extremely difficult. However, the authors emphasized the need for further studies with larger sample sizes and multiple baseline variables. They also recommended optimization strategies to enhance the sensitivity of stool Xpert assays for detecting Mycobacterium tuberculosis in children [12].

In my study, a statistically significant association was found between area of residence and PTB, with rural children showing a higher proportion of culture-positive PTB compared to urban children ($p = 0.021$). A strong statistically significant association was also observed between household TB contact and PTB positivity ($p < 0.001$). Another study conducted in 2022 demonstrated that, in children, stool samples can serve as an alternative to gastric aspirates (GAs) for Ultra testing. Both stool-based and GA-based Ultra tests were considered appropriate for bacteriological confirmation of tuberculosis [13]. In my research, the diagnostic accuracy of stool Xpert MTB/RIF was evaluated using sputum/gastric aspirate culture as the reference standard. Of the 116 culture-positive cases, 103 were correctly identified by stool Xpert MTB/RIF, while 13 were missed. Among the 65 culture-negative cases, 60 were correctly identified as negative and 5 were falsely reported as positive. A study conducted in 2018 reported that Gene Xpert MTB/RIF was helpful in diagnosing childhood tuberculosis, demonstrating 81% specificity but variable sensitivity depending on the sample site. The highest sensitivity was observed with lymph node aspirates [14].

In my study, the sensitivity of stool Xpert MTB/RIF was 88.8%, specificity was 92.3%, positive predictive value (PPV) was 95.4%, negative predictive value (NPV) was 82.2%, and overall diagnostic accuracy was 90.1%. These findings indicate a high diagnostic validity of stool Xpert MTB/RIF for detecting pulmonary tuberculosis in children. Similarly, a study conducted in 2019 suggested that molecular stool tests have potential as rule-in diagnostic tests; however, optimizing sensitivity remains challenging due to variations in study methodologies and testing procedures. The authors recommended future research with rigorous study designs and standardized reporting of results [15].

5. CONCLUSION

The findings of this study demonstrate that stool Xpert MTB/RIF is a reliable and non-invasive diagnostic tool for detecting pulmonary tuberculosis in children. It showed high sensitivity and specificity when compared with sputum/gastric aspirate culture, supporting its diagnostic validity. Stool-based testing may serve as a practical alternative in pediatric patients where obtaining respiratory samples is difficult or invasive. Therefore, stool Xpert MTB/RIF can be considered a valuable diagnostic option, particularly in resource-limited settings.

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