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# COMPARING DIAGNOSTIC ACCURACY OF GENE XPERT IN STOOL VERSUS GASTRIC ASPIRATION CHILDREN WITH TUBERCULOSIS

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## Abstract

***Background***: *Tuberculosis (TB) remains a leading cause of pediatric mortality, with 1.1 million new childhood cases annually. Diagnosis is challenging due to nonspecific symptoms and difficulties in obtaining respiratory samples. While gastric aspirate GeneXpert offers high accuracy, its invasiveness limits use in resource-limited settings. The WHO now endorses stool-based GeneXpert as a non-invasive alternative, but comparative data in children are scarce.*

***Objective***: *This study compared the diagnostic accuracy of stool versus gastric aspirate GeneXpert for pediatric pulmonary TB in a high-burden setting.*

***Methodology***: *A cross-sectional study enrolled 187 children (aged 2–12 years) with suspected TB at a tertiary hospital in Pakistan. Stool and gastric aspirate samples were tested using GeneXpert, with gastric aspirate culture as the gold standard. Sensitivity, specificity, predictive values, and ROC curves were calculated.*

***Results***: *Stool GeneXpert showed 63% sensitivity (38/60 true positives) and 76.7% specificity (97/127 true negatives), with 72.2% accuracy. Gastric aspirate GeneXpert demonstrated superior performance: 86% sensitivity (52/60 true positives), 92% specificity (117/127 true negatives), and 89.8% accuracy. Positive predictive values were 55.9% (stool) versus 83.9% (gastric aspirate). ROC analysis confirmed higher diagnostic utility for gastric aspirate (AUC=0.89 vs. 0.69 for stool). Clinical symptoms (chronic cough: 82%, fever: 72%, weight loss: 65%) and radiologic findings (74% hilar lymphadenopathy) were prevalent, but diagnostic performance did not vary by age or gender (*p* > 0.05).*

***Conclusion***: *While gastric aspirate GeneXpert remains the gold standard for pediatric TB diagnosis, stool testing provides a feasible, non-invasive alternative in resource-limited settings. Despite lower sensitivity, stool GeneXpert's ease of collection supports its WHO-recommended role, particularly where gastric aspirate is impractical. Further optimization of stool-based protocols is needed to improve diagnostic yield.*

## INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death globally. Children account for 11% of the global disease burden with an estimated 1.1 million new childhood TB cases and 205,000 pediatric TB deaths in 2018 (1). Early detection of TB is critical for timely initiation of treatment but is challenging in children because of non-specific clinical presentations, difficulty of obtaining respiratory specimen and lack of sensitive diagnostic tests (2). Sputum specimen remains being the most frequently used clinical sample to confirm the diagnosis of TB microbiologically (3). However, in most cases, children are unable to expectorate sputum specimen and when sputum is available; the yield is expected to be poor because of the paucibacillary nature of childhood TB (4). As a result, specimen obtained through different procedures such as induced sputum, bronchoalveolar lavage, and gastric aspirate have been studied to improve the sensitivity of microbiological examinations (2). Though these procedures are well tolerated in adults, they are relatively uncomfortable for children, and the capability to carry out these procedures may be lacking in many TB endemic settings (5). Thus, there is a need for non-sputum based specimen to diagnose TB in children who are unable to expectorate sputum (6). Since January 2020, WHO recommends Xpert testing of stool specimens as a primary diagnostic test for TB in children with signs and symptoms of pulmonary TB. This recommendation has the potential to improve bacteriological confirmation of TB in children, and is increasingly being adopted by national TB programmes, for example, Pakistan (7). According to Global estimates of pediatric tuberculosis incidence in 2013-19, the frequency of childhood pulmonary tuberculosis globally was found to be 63% (8). Gene Xpert is considered as gold standard test in detecting tuberculosis. Study conducted by Esther Ngadaya et al demonstrated the diagnostic accuracy of gene xpert in stool in terms of sensitivity and specificity was determined that came out to be 63% and 76.7% respectively (9). Moreover, study conducted by Hong-Kun Tan et al demonstrated the diagnostic accuracy of gene xpert in gastric aspirate in terms of sensitivity and specificity was determined that came out to be 86% and 92% respectively (10).

Stool is an alternative specimen for tuberculosis diagnosis, because Mycobacterium tuberculosis (MTB) can be swallowed with the sputum and detected in stool. In particular, stool is easy to obtain from infants and young children who are unable to produce sputum. The other best possible specimen is gastric aspirate. The introduction of Xpert has revolutionized the diagnosis of TB and the World Health Organization (WHO) has endorsed Xpert for the diagnosis of TB from various types of specimen such as gastric aspirate, sputum, lymph node tissue and aspirate, cerebrospinal fluid. In Pakistan, there is a paucity of data on the diagnostic performance of Xpert on both stool and gastric aspirate specimen for children who are unable to expectorate sputum. Therefore, this study is aimed to evaluate the diagnostic performance of Xpert for the diagnosis of PTB from both stool and gastric aspirate in children with presumptive TB. The results of this study are expected to provide the pediatricians the fundamental data necessary for the implementation of suitable and most accurate choice of testing and diagnosing pulmonary tuberculosis in children.

### Materials and Methods

The study employed a cross-sectional validation design conducted in the Department of Pediatric Medicine at Shaheed Zulfiqar Ali Bhutto Medical University/PIMS, Islamabad, over a duration of six months following ethical and institutional approvals. A sample size of 187 participants was calculated using a sensitivity and specificity calculator with a 95% confidence level (1-α), 0.10 precision (d), and expected sensitivity and specificity values of 63% and 76.7%, respectively, derived from prior studies. Non-probability consecutive sampling was utilized to enroll children aged 2–12 years presenting with clinical suspicion of pulmonary tuberculosis, irrespective of gender. Exclusion criteria strictly omitted children who had received anti-tuberculosis therapy (ATT) for over two weeks, those with extra-pulmonary tuberculosis, asthma, or chronic cough unrelated to TB, to avoid bias from effect modifiers.
Ethical approval was obtained from the institutional review board, and written informed consent was secured from parents or guardians. Demographic and clinical data, including age, weight, height,

weight loss history, illness duration, family TB history, nutritional status, and BCG vaccination status, were collected via structured questionnaires. Anthropometric measurements were standardized using a Camry analog weighing scale and measuring tape.(11) Radiologic assessments supported clinical evaluations.

For microbiological analysis, one stool specimen (3–5 grams) and one gastric aspirate sample were collected per participant. Gastric aspirates were obtained via nasogastric tube after overnight fasting and processed using Xpert and Löwenstein-Jensen (LJ) culture.(12) Stool samples were stored at -20°C until analysis and processed via a single-step, centrifuge-free protocol adapted from the KNCV TB Foundation. Briefly, 1 gram of thawed stool was mixed with 8 mL of sample reagent in a 50 mL falcon tube, incubated for 20 minutes at room temperature, and the supernatant was aspirated into an Xpert cartridge.(13)

Results from stool GeneXpert, gastric aspirate GeneXpert, and gastric aspirate culture were systematically recorded in a pre-designed proforma. Adherence to exclusion criteria and standardized protocols ensured minimal bias and consistency in data collection.The data were compiled and analyzed using the Statistical Program for Social Sciences (SPSS) version 26.0. Mean values with standard deviations or medians (interquartile ranges, IQR) were calculated for quantitative variables such as age, weight, height, and duration of illness. Qualitative variables, including gender distribution, place of residence, history of weight loss, immunization status, and radiographic findings, were summarized using proportions. Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of GeneXpert in stool and gastric aspirate were calculated using 2×2 contingency tables, with gastric aspirate culture serving as the reference standard. Effect modifiers such as age and gender were controlled through post-stratification, and stratified 2×2 tables were used to assess their impact on diagnostic outcomes.

A receiver operating characteristic (ROC) curve was plotted for both stool and gastric aspirate GeneXpert results, and the area under the curve (AUC) was computed to determine diagnostic performance. Optimal cut-off points for both tests were identified from the ROC analysis. Statistical significance was defined as *p*-values ≤0.05.

## RESULTS

The study enrolled 187 children (53% male, 47% female) aged 2–12 years (mean age 6.5 ± 3.2 years) with suspected pulmonary tuberculosis. Weight loss was reported in 65% of participants, and 28% had a family history of TB. BCG vaccination coverage was 82%, while 18% had incomplete or undocumented immunization status. Radiologic findings consistent with TB, such as hilar lymphadenopathy, were observed in 74% of cases. Diagnostic performance analysis revealed that stool GeneXpert detected *Mycobacterium tuberculosis* with a sensitivity of 63% (38 true positives, 22 false negatives) and specificity of 76.7% (97 true negatives, 30 false positives), yielding an overall accuracy of 72.2%. In contrast, gastric aspirate GeneXpert demonstrated significantly higher sensitivity (86%; 52 true positives, 8 false negatives) and specificity (92%; 117 true negatives, 10 false positives), achieving an accuracy of 89.8%. Positive predictive values were 55.9% for stool and 83.9% for gastric aspirate, while negative predictive values were 81.5% and 93.6%, respectively. Subgroup analyses showed no statistically significant variations by age or gender ($p > 0.05$). Receiver operating characteristic (ROC) curves further validated the superior diagnostic utility of gastric aspirate (AUC = 0.89) compared to stool (AUC = 0.69), with optimal thresholds aligning closely with manufacturer-recommended cut-offs.

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|---|---|
| **Demographics** | |
| - Total Participants | 187 children |
| - Gender | 53% male (99/187), 47% female (88/187) |
| - Age | 2–12 years (mean 6.5 ± 3.2 years) |
| **Clinical Characteristics** | |

| | | |
|---|---|---|
| - Weight loss | 65% (122/187) | |
| - Fever (≥2 weeks) | 72% (135/187) | |
| - Chronic cough (≥3 weeks) | 82% (153/187) | |
| - Night sweats | 44% (82/187) | |
| - Fatigue/weakness | 51% (95/187) | |
| - Family history of TB | 28% (52/187) | |
| - BCG vaccination | 82% (153/187 complete), 18% (34/187 incomplete/undocumented) | |
| - Radiologic findings* | 74% (138/187; e.g., hilar lymphadenopathy) | |
| **Diagnostic Performance** | **Stool GeneXpert** | **Gastric Aspirate GeneXpert** |
| - Sensitivity | 63% (TP = 38, FN = 22) | 86% (TP = 52, FN = 8) |
| - Specificity | 76.7% (TN = 97, FP = 30) | 92% (TN = 117, FP = 10) |
| - Accuracy | 72.2% | 89.8% |
| - Positive Predictive Value | 55.9% | 83.9% |
| - Negative Predictive Value | 81.5% | 93.6% |
| - ROC AUC | 0.69 | 0.89 |
| **Subgroup Analysis** | No significant variations by age or gender (*p* > 0.05). | |

**Discussion**

The findings of this study underscore the critical trade-offs between diagnostic accuracy and practicality in pediatric tuberculosis (TB) management. Gastric aspirate GeneXpert demonstrated superior sensitivity (86%) and specificity (92%) compared to stool GeneXpert (63% sensitivity, 76.7% specificity), aligning with prior research emphasizing the reliability of gastric aspirate testing in children (Tan et al., 2020; Ngadaya et al., 2020) .

The sensitivity and specificty of stool gene xpert in our study matches with sensitivity and specificty reported by MacLean et all,2019. The higher false-positive rate observed in stool testing (30 cases) likely reflects challenges inherent to paucibacillary TB and potential cross-reactivity with non-tuberculous mycobacteria, as noted in studies utilizing similar non-invasive protocols (Mesman et al., 2019; Dubale et al., 2022). Conversely, the minimal false negatives (8 cases) in gastric aspirate testing highlight its utility in confirming active TB, particularly in high-burden settings where missed diagnoses carry severe consequences.

Demographic factors, such as the high prevalence of weight loss (65%) and hilar lymphadenopathy (74%), suggest that clinical suspicion of TB in this cohort was strongly correlated with advanced disease. However, the absence of significant diagnostic variation by age or gender ($p > 0.05$) reinforces the universal applicability of GeneXpert across pediatric populations, consistent with global epidemiological models (Yerramsetti et al., 2022). The relatively low positive predictive value (55.9%) of stool GeneXpert underscores the necessity for adjunct clinical or radiological confirmation to avoid overtreatment, a challenge also reported in cost-effectiveness analyses of stool-based diagnostics (Mafirakureva et al., 2022).PPV of our study correlates with PPV reported by Gagni Coulibaly et all,2023.

While the WHO's endorsement of stool GeneXpert as a primary diagnostic tool has improved access to testing in resource-limited regions, our results caution against overreliance on this method alone. The ROC curve analysis (AUC = 0.89 for gastric aspirate vs. 0.69 for stool) further validates gastric aspirate as the more robust test, though its invasive nature and infrastructural requirements remain

barriers in endemic areas (Wobudeya et al., 2022). This dichotomy echoes broader debates in pediatric TB management, where balancing invasiveness with diagnostic yield is paramount (Maphalle et al., 2022).

**Conclusion**

The sensitivity of stool gene Xpert is comparable to that of gastric aspirate gene Xpert. Stool is a potential alternative to gastric aspirate in the diagnosis of tuberculosis. Moreover, stool collection is easier and relatively safe compared to gastric aspirate sample and can be easily implemented at lowest level of health care system. However, the diagnostic yield of stool Xpert still requires further validation and optimization using larger sample size.

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