

Xpert® MTB/RIF Ultra in stool samples for the diagnosis of pediatric pulmonary tuberculosis

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Abstract

Introduction: Diagnosing childhood tuberculosis presents challenges due to nonspecific symptoms, invasive sample collection, and limited performance of microbiological methods. Consequently, new diagnostic methodologies, notably the widespread adoption of Xpert® MTB/RIF Ultra in many countries, have emerged.

Aim: To determine the diagnostic utility of stool samples in microbiologically confirming pulmonary tuberculosis in children using Xpert® MTB/RIF Ultra.

Methodology: A cross-sectional study that included children with a presumptive diagnosis of active pulmonary tuberculosis. Initial diagnoses relied on clinical signs, symptoms, and associated risk factors. Samples of induced sputum, gastric aspirates, and stool underwent Xpert® MTB/RIF Ultra and liquid medium cultures. Reliability and diagnostic validity of stool samples were analyzed in comparison to respiratory samples.

Results: A total of 57 patients with a presumptive diagnosis of pulmonary tuberculosis were included. The median age was 5 years (IQR= 3.7-6.0), with 28 (51.9%) females, and 23 (42.6%) children presenting cough as the most frequent symptom. Active tuberculosis was diagnosed in 21 children. Sensitivity, specificity, positive predictive value, and negative predictive value of Xpert® MTB/RIF Ultra in stool samples, compared to respiratory samples,

were 60%, 86.4%, 50% y 90.5%, respectively. A Kappa index of 0.43 indicated moderate agreement.

Conclusion: Xpert® MTB/RIF Ultra in stool samples provides a complementary alternative for microbiological confirmation of childhood pulmonary tuberculosis. It reduces time, workload, invasive procedures, and costs compared to respiratory samples. However, further studies with larger sample sizes are needed to substantiate our findings.

KEYWORDS

Tuberculosis; feces; Xpert® MTB/RIF Ultra; child, preschool; molecular diagnosis techniques.

INTRODUCTION

Tuberculosis (TB) is one of the most significant infectious diseases globally and ranks among the top ten causes of death in the population; in 2022, the World Health Organization (WHO) reported 10.6 million cases of individuals developing the disease, with 12% of those affected being children aged 0-14 years and 15% (224,000) of the mortality attributed to this cause (Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO). In Colombia, 17,595 cases of TB were reported in 2022, of which 3.4% (592) were children aged 0-14 years. It is noteworthy that, for this age group, the proportion of cases in 2022 increased by 36% compared to 2021, however is under the global 12% estimation of new TB cases worldwide (Instituto Nacional de Salud. 2022. Informe de evento Tuberculosis, Colombia, 2022. [Internet].<https://www.ins.gov.co/eventos/Informesdeevento/TUBERCULOSIS%20INFORME%202022.pdf>; Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO). Despite advances in the diagnosis and treatment of the disease, the WHO emphasizes in the global TB report that the number of cases may be higher due to underreporting, i.e., the lack of notification of cases in each

country (Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO).

The underreporting of cases is largely due to the lack of awareness about the natural course of infection in the pediatric population and difficulties in diagnosis, including nonspecific clinical manifestations, the paucibacillary nature of the disease, low sensitivity in traditional diagnostic methods, and invasive sampling methods (Marais BJ, Graham SM. Childhood tuberculosis: A roadmap towards zero deaths. *Journal of Paediatrics and Child Health*. 2016 Jun 13;52(3):258–6). Consequently, TB in children has become a hidden epidemic that has received little attention (Kunkel A, Abel zur Wiesch P, Nathavitharana RR, Marx FM, Jenkins HE, Cohen T. Smear positivity in paediatric and adult tuberculosis: systematic review and meta-analysis. *BMC Infectious Diseases*. 2016 Jun 13;16(1)).

The limited understanding of TB in the pediatric population has prompted a focused effort to address this issue. This is evident in the publication of guidelines for TB control programs, emphasizing the diagnosis and management of childhood TB. Additionally, the 'Roadmap for Childhood TB: Toward Zero Deaths' recommends specific research in various diagnostic aspects. This step is deemed necessary to generate evidence that supports more robust recommendations, ultimately enhancing the management of children with TB (Ministerio de Salud y Protección Social de Colombia. ¿Qué es tuberculosis (TB)? [Internet]. Minsalud.gov.co. 2019. Available from: <https://www.minsalud.gov.co/salud/publica/PET/Paginas/Tuberculosis.aspx>; World Health Organization (2013) Roadmap for Childhood Tuberculosis towards Zero Deaths. WHO, Geneva).

As a result, the WHO emphasizes the need for research in diagnostic, treatment, and prevention issues, as well as the recording and reporting system for childhood TB programs worldwide (Guidance for national tuberculosis programmes on the management of tuberculosis in children Second edition [Internet]. [cited 2023 Nov 25]. Available from: https://iris.who.int/bitstream/handle/10665/112360/9789241548748_eng.pdf?sequence

=1). The Xpert® MTB/RIF Ultra is a commercially endorsed test by the WHO for use as an initial diagnostic test on various samples from children with suspected TB (WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF ultra compared to Xpert MTB/RIF [Internet]. www.who.int. [cited 2023 Nov 25]. Available from: <https://www.who.int/publications/i/item/WHO-HTM-TB-2017.04>). The use of stool samples could be highly beneficial, as it allows for the collection and detection of genetic material after respiratory secretions are swallowed by children. Recent studies have demonstrated a sensitivity of 90.2% and specificity of 100% when using Xpert® MTB/RIF Ultra in stool samples from adults with pulmonary TB (Rahman SMM, Maliha UT, Ahmed S, Kabir S, Khatun R, Shah JA, et al. Evaluation of Xpert MTB/RIF assay for detection of Mycobacterium tuberculosis in stool samples of adults with pulmonary tuberculosis. Goletti D, editor. PLOS ONE. 2018 Sep 13;13(9):e0203063). In other studies, targeting the pediatric population with Xpert® MTB/RIF Ultra, a sensitivity of 100% and specificity of 99.3% were found in the diagnosis of pulmonary TB in stool samples compared to culture as the reference standard (Dubale, M., Tadesse, M., Berhane, M., Mekonnen, M., & Abebe, G. (2022). Stool-based Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children at a teaching and referral hospital in Southwest Ethiopia. Plos one, 17(5), e0267661). The development of this methodology, which improves sensitivity compared to the previous version, suggests that it could be a better tool for the diagnosis of childhood tuberculosis, and it is necessary to generate evidence through its evaluation for implementation in the country (Gaensbauer J, Broadhurst R. Recent Innovations in Diagnosis and Treatment of Pediatric Tuberculosis. Current Infectious Disease Reports. 2019 Jan;21(1)).

Despite these findings, there is a continuing need in Colombia and Latin America to further improve and explore new methods for diagnosing TB in children, as highlighted by WHO recommendations emphasizing the development of tests for the diagnosis of any form of TB in samples other than sputum (World Health Organization. 2014. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting, 28–29 April 2014, Geneva, Switzerland. Available from

<https://www.who.int/publications/i/item/WHO-HTM-TB-2019.18>). The objective of this study was to determine the diagnostic utility of fecal samples in the microbiological confirmation of pulmonary TB in children using Xpert® MTB/RIF Ultra.

METHODOLOGY

Study type and population: This was an observational, cross-sectional study assessing children under 15 years, from Medellín and Metropolitan Area, Colombia, between 2020 and 2023, in contact with pulmonary tuberculosis patients, and/or with clinical and/or radiological findings of active TB.

Participants were recruited from the Medellín, Bello and Itagui TB control programs and contacted to clinical evaluation at the Corporación para Investigaciones Biológicas (CIB) or from two high-complexity health institutions where they were admitted with clinical suspicion of active pulmonary TB. Children with severe asthma or planning to relocate quickly from the city were excluded. The study was approved by the CIB Research Ethics Committee. Written informed consent and assent was obtained from parents or legal guardians, and from children as was needed.

Data collection: Clinical information and TB suggestive symptoms provided to medical staff during consultation (fever for more than 2 weeks, cough, night sweats, weight loss, and family history of tuberculosis) were collected. Sociodemographic and clinical data, including BCG vaccination evidence, previous anti-tuberculosis treatment, tuberculin skin test, among others, were also gathered. Data were collected on Microsoft Access software (Microsoft 365).

Samples and laboratory procedures: Gastric aspirate, spontaneous or induced sputum (for children unable to produce spontaneous sputum), and stool samples were collected for microbiological confirmation of TB. Samples were evaluated for *Mycobacterium tuberculosis* (MTB) detection using direct microscopic examination with auramine-rhodamine staining, liquid culture (BACTEC™ MGIT™ system), and real-time PCR for the detection of the *Mycobacterium tuberculosis* complex using the Xpert® MTB/RIF Ultra system according to

institutional protocols and manufacturer recommendations (Corporación para Investigaciones Biológicas (CIB). Instructivo de ensayo institucional. 2022; BD BACTEC™ MGIT™ System User's Training Manual. 130 páginas. 2020).

Statistical Analysis: Data were registered into a Microsoft Excel database for analysis. For quantitative variables (age and weight), normality assumptions were checked using the Shapiro-Wilk test, and medians with interquartile ranges were calculated. For qualitative variables (sex, TB contact history, BCG vaccination, and clinical manifestations), absolute and relative frequencies were used. The McNemar test was applied to compare the proportion of positive results between Xpert® MTB/RIF Ultra in stool samples vs. Xpert® MTB/RIF Ultra in respiratory samples. Cohen's Kappa test was used to determine the concordance between the diagnostic microbiological tests. Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated for diagnostic validity. The statistical analyses were conducted using SPSS v25, Jamovi, and OpenEpi v.3.01 software.

RESULTS

Patient characteristics: A total of 57 children with presumptive diagnosis of pulmonary TB were included. Twenty (35.1%) cases of active TB were confirmed based on clinical, radiological, epidemiological, and microbiological findings (global diagnosis). Demographic and clinical characteristics of the patients are presented in Table 1. The median age of study participants was 5 years (IQR: 3.7-6.0), 28 (51.9%) were female, and 23 children (42.6%) had cough as the most frequent symptom. Notably, 32 children (59.3%) had direct contact with cohabitants with active TB.

A total of 246 samples were obtained, including 107 gastric aspirates, 82 sputum samples, and 57 fecal samples. Respiratory samples (gastric aspirates, and sputum) were grouped for statistical analysis.

Positivity on each method: A positivity rate of 4.1% (10 samples) was observed through direct examination (Auramine-Rhodamine staining), 11.3% through molecular testing (28 samples), and 3.6% through phenotypic tests (10 samples). Among the samples positively detected by Xpert® MTB/RIF Ultra, 2 (7.14%) exhibited high detection level, 5 (17.86%)

exhibited medium detection level, 3 (10.71%) exhibited low detection level, 2 (7.14%) exhibited very low detection level, and 16 (57.14%) exhibited trace detection level, with 56.25% of trace detection level observed in stool samples. *Mycobacterium tuberculosis* was detected by culture in nine samples and a non-tuberculous mycobacterium (*Mycobacterium gordonae*) in one sample.

Concordance of Xpert® MTB/RIF Ultra in stool and respiratory samples: As showed in table 2, two stool samples and seven respiratory samples were positive in both, liquid culture and Xpert® MTB/RIF. Comparing the proportion of positive samples by Xpert® MTB/RIF Ultra in stool samples with the proportion of positive respiratory results using the same methodology yielded a Z statistic of 0.3733 with a p-value of 0.7089. Additionally, there was a moderate agreement between Xpert® MTB/RIF Ultra in stool samples vs. Xpert® MTB/RIF Ultra in respiratory samples (Kappa of 0.43, 95% CI: 0.136-0.725); the sensitivity and specificity of Xpert® MTB/RIF Ultra in stool samples, using the combined result of both respiratory samples as the gold standard, were 60% (95% CI: 24.6-95.4) and 86.4% (95% CI: 75.1-97.6), respectively, the PPV was 50% (95% CI 17.5-82.5), and NPV was 90.5% (95% CI 80.4-100). Significant differences were detected when comparing results obtained using Xpert® MTB/RIF Ultra in stool versus grouped respiratory samples ($p < 0.01$) (table 3). As mentioned above, there were 20 children with active TB global diagnosis, 9 out of 20 (45%) had negative microbiological studies, 2 out of 20 (10%) had positive microbiological diagnosis in both, molecular and liquid culture tests in respiratory and stool samples, 4 out of 20 (20%) had positive microbiological diagnosis only in stool sample evaluated by Xpert® MTB/RIF Ultra and, 2 out of 20 (10%) had positive microbiological diagnosis only in respiratory samples evaluated by liquid culture.

DISCUSSION

Given the known limitations in the natural infection process in the pediatric population, difficulties in diagnosis, nonspecific clinical manifestations, the paucibacillary nature of the disease, and low sensitivity of traditional diagnostic methods, stool samples may be an effective alternative for detecting TB in children (World Health Organization. 2014. High-

priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting, 28–29 April 2014, Geneva, Switzerland. Available from <https://www.who.int/publications/i/item/WHO-HTM-TB-2014.18>). This study demonstrated that the use of Xpert® MTB/RIF Ultra in stool samples performs similarly to respiratory samples, consistent with other studies (Dubale, M., Tadesse, M., Berhane, M., Mekonnen, M., & Abebe, G. (2022). Stool-based Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children at a teaching and referral hospital in Southwest Ethiopia. *Plos one*, 17(5), e0267661; Abdella, M., Simbo, T., & Aman, H. (2020). Evaluation of gene Xpert in detecting suspected pulmonary tuberculosis from stool sample for children < 15years, Adama, East-shao zone, Ethiopia. *International Journal of Infectious Diseases*, 101, 458-459).

The sensitivity and specificity of Xpert® MTB/RIF Ultra in stool samples in this study were 60% (95% CI 24.6-95.4) and 86.4% (95% CI 75.1-97.6), respectively. A recent meta-analysis reports a sensitivity of 100% and specificity of 89% in stool samples (Kay AW, Ness T, Verkuil SE, Viney K, Brands A, Masini T, et al. Xpert MTB/RIF Ultra assay for tuberculosis disease and rifampicin resistance in children. *Cochrane Database of Systematic Reviews*. 2022 Sep 6;2022(9)). However, the observed specificity was lower than previous reports which showed a specificity of 100%, possibly due to the presence of non-viable bacilli during the decontamination process of respiratory samples (Ainan S, Furia FF, Mhimbira F, Mnyambwa NP, Mgina N, Zumla A, et al. Xpert® MTB/RIF assay testing on stool for the diagnosis of paediatric pulmonary TB in Tanzania. *Public Health Action* [Internet]. 2021 Jun 21 [cited 2023 Nov 25];11(2):75–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/34159066/>).

On the other hand, the PPV and NPV of Xpert® MTB/RIF Ultra in stool samples were 50% (95% CI 17.5-82.5) and 90.5% (95% CI 80.4-100), respectively. These results differ from other studies where a PPV of 98.8% and an NPV of 37.1% were obtained (Sun L, Liu Y, Fang M, Chen Y, Zhu Y, Xia C, et al. Use of Xpert MTB/RIF Ultra assay on stool and gastric aspirate samples to diagnose pulmonary tuberculosis in children in a high-tuberculosis-burden but resource-limited area of China. *International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases* [Internet]. 2022 Jan 1 [cited

2023 Nov 25];114:236–43. Available from: <https://pubmed.ncbi.nlm.nih.gov/34774781/>). It is likely that these results are due to false positives obtained in the study, with a direct impact on the total number of true positives. This suggests that confirming the diagnosis of the disease with a single test is challenging in our context as previously reported (Pérez I, Taito-Vicenti IY, González-Xuriguera CG, Carvajal C, Franco JVA, Loézar C. How to interpret diagnostic tests. Medwave [Internet]. 2021 Aug 4;21(07)).

In conclusion, the use of Xpert® MTB/RIF Ultra in stool samples demonstrates moderate concordance with respiratory samples, suggesting its potential as a non-invasive alternative for diagnosing pulmonary TB in children. While the sensitivity was 60%, the ease of collection and handling of fecal samples may make this test an effective alternative, particularly in situations where obtaining respiratory samples from children is challenging. Further studies with larger sample sizes are needed to confirm these findings. This methodology could prove valuable in primary healthcare and clinical settings to enhance the diagnosis of TB in the pediatric population.

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CONFLICT OF INTEREST

The authors declare that they have no personal interests or relationships that could have influenced the work presented in this article.

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Table 1. Demographic and clinical characteristics of the study participants.

Characteristic n=57	n (%)
Sex	
Female	29 (50,9)
Male	28 (49,1)
Age, Me (RIQ)	5 (3,7-6,0)
TB contact history	32 (59,3)
Symptoms	
Cough	23 (40,4)
Weight loss	16 (28,1)
Night sweets	19 (33,3)
Fever	21 (36,8)
BCG vaccination	48 (84,2)
Weight Me (IQR)	13,3 (10-14,7)

Me: Median, IQR: Interquartil range, BCG: Bacilli Calmette-Guérin vaccine; TB: Tuberculosis

Table 2. Concordance between Xpert® MTB/RIF Ultra in respiratory and fecal samples using liquid culture as the reference standard.

Test/Sample	Xpert® MTB/RIF Ultra	Culture results		Total
		Positive	Negative	
Stool samples	Positive	2	10	12
	Negative	1	44	45
Grouped respiratory samples	Positive	7	8	15
	Negative	0	174	174
Total		10	236	246

Table 3. Concordance between Xpert® MTB/RIF Ultra in respiratory and stool samples.

Test/Samples		Xpert® MTB/RIF Ultra results in respiratory samples		Total	Kappa index 0,43 95% IC (0,14- 0,72)
		Positive	Negative		
Xpert® MTB/RIF Ultra results in stool samples	Positive	6	6	12	
	Negative	4	38	42	
Total		10	44	54	