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Addressing Diagnostic Gaps in Paediatric Tuberculosis in the DRC: Insights from the Use of GeneXpert on Stool Specimens

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Abstract

Background: Diagnosing paediatric tuberculosis (TB) remains challenging, partly because they are often unable to produce sputum samples. The use of stool samples with the Xpert MTB/RIF Ultra test for diagnosing paediatric pulmonary TB has already been implemented in several countries; however, this method remains very limited in the Democratic Republic of the Congo (DRC). The objective of this study was to estimate the prevalence of tuberculosis among children under 10 years of age using the GeneXpert test on stool samples in Tanganyika province. **Methods:** This was a retrospective cross-sectional study including 91 children under the age of 10 who were suspected of having tuberculosis. These children underwent TB screening using the GeneXpert test on stool samples collected in healthcare facilities located in five health zones of Tanganyika province. Sociodemographic and biological characteristics were analyzed. **Results:** The median age of the children was 24 months, with no sex predominance (sex ratio 1M:1F). The prevalence of pulmonary TB detected by the GeneXpert test on stool samples was 15.4% (95% CI: 12.3 - 18.6). Among the 14 positive samples, 78.6% were drug-sensitive, 14.3% were drug-resistant, and 7.1% were classified as trace results. TB prevalence was significantly higher among girls ($p = 0.016$) and malnourished children ($p < 0.001$). **Conclusion:** This study demonstrates that the GeneXpert-Ultra test on stool samples is a viable alternative to sputum testing in children who are unable to spontaneously produce expectorated samples and would otherwise require invasive procedures to obtain specimens for TB diagnosis.

Keywords

GeneXpert-Ultra Test, Stool Samples, Children, DRC

1. Introduction

Tuberculosis (TB) is a communicable disease and a major cause of morbidity and mortality among children, particularly those under the age of 10. The World Health Organization (WHO) estimates that 1.25 million children and young adolescents (aged 0 to 14 years) contracted tuberculosis in 2022 [1].

Among these cases, an estimated 214,000 children died from tuberculosis in 2022 [1]. Most of these deaths occurred in children who were neither diagnosed nor treated. This age group is particularly vulnerable due to the challenges in diagnosing the disease, which are compounded by often non-specific symptoms and the inability of young children to produce sputum, the standard diagnostic specimen [2] [3].

Sputum examination remains the traditional method for confirming pulmonary tuberculosis. However, children—especially young children—generally struggle to spontaneously expectorate sputum for TB screening [4]. Obtaining alternative recommended samples for TB diagnosis in children requires invasive procedures such as gastric aspiration or sputum induction, trained clinical personnel, and specific consumables that are often unavailable in many resource-limited settings [5].

Since 2021, the World Health Organization (WHO) has recommended the use of the Xpert MTB/RIF Ultra test on stool samples to diagnose pulmonary tuberculosis in children under 10 years of age [6]. Among the various documented methods for stool processing, the Global Laboratory Initiative Manual provides detailed guidance on the most commonly used techniques [7]. These include the optimized sucrose flotation (OSF) method developed by the TB-Speed project [8] and the simple one-step (SOS) method developed by the KNCV Tuberculosis Foundation [5].

The emergence of GeneXpert MTB/RIF technology has significantly advanced pediatric TB diagnosis, particularly through its application to alternative specimens such as stools. These are easier to collect from young children compared to sputum or gastric aspirates and offer a practical advantage for pulmonary TB screening [9]. GeneXpert detects *Mycobacterium tuberculosis* DNA and identifies rifampicin resistance, while offering high sensitivity and specificity on stool samples, as demonstrated in several studies [2] [10].

This method is especially valuable in low-resource settings, where it can considerably reduce the time to diagnosis and improve access to treatment. WHO endorses the use of GeneXpert on stool specimens as a valid alternative for children, helping to overcome diagnostic barriers and enhance clinical outcomes [11] [12]. Despite WHO recommendations, stool-based Xpert testing remains underutilized in DRC. By leveraging this innovation, health systems can improve early detection, reduce transmission, and alleviate the burden of disease in this vulnerable population. Recent studies have shown that stool-based Xpert MTB/RIF sensitivity ranges from 32% to 83.3% [10] [11].

In the DRC, studies using GeneXpert on stool samples for TB diagnosis remain

limited. We hypothesized that stool-based Xpert could provide a feasible, non-invasive diagnostic tool for childhood in Tanganyika province, DRC. The objective of this study is to estimate the prevalence of tuberculosis among children under 10 years of age using GeneXpert on stool samples in Tanganyika province, DRC.

2. Methods

2.1. Study Population and Design

The study was conducted in five health zones (HZs) of Tanganyika province: Kabalo, Kalemie, Kongolo, Manono, and Moba. This was a retrospective cross-sectional study involving children under the age of 10 who were suspected of having TB, with stool sample tested and result available during the study period. Eligible children underwent TB screening using the GeneXpert method on stool samples collected in healthcare facilities located in these five HZs. Malnutrition was defined based on WHO growth standards (weight-for-age-score < −2). All children who provided a stool sample for TB testing and had complete results available (negative, sensitive, trace, resistant) were included. Children who had received anti-TB treatment prior to screening were excluded from the study.

2.2. Data Collection

Children under the age of 10 were consecutively recruited from the Diagnostic and Treatment Centers (DTCs) of the five HZs from January 2021 to December 2022. TB screening was carried out in accordance with the standard of care: cough for less than two weeks (or any duration in HIV-positive children), unexplained fever lasting less than two weeks, poor growth or weight loss over the past three months. Additionally, children were included if they had a history of contact with a TB case, regardless of symptoms, or if their chest X-ray suggested tuberculosis. Children were enrolled regardless of their ability to produce sputum. Data were extracted from medical records and GeneXpert laboratory reports for children screened during the study period. Variables collected included demographic characteristics (age, sex, origin) and screening outcomes (negative, sensitive, trace, resistant).

2.3. GeneXpert Technique on Stool Samples

Stool samples were processed following the WHO 2022 Practical Manual for Stool Sample processing [7], by adding approximately 10 ml of distilled water to 2 cm^3 of stool sample, followed by vortex mixing to homogenize. The mixture was then incubated for 15 minutes at room temperature to allow particles to settle. The supernatant from each processed sample was collected in a separate container and mixed with the sample reagent according to the manufacturer's instructions (a 2:1 ratio of Xpert reagent to sample). Each mixture was vortexed and incubated for an additional 15 minutes at room temperature; 2 ml were then transferred to a GeneXpert cartridge and analyzed using the Xpert platform. Results from the stool-

based Xpert test were interpreted similarly to sputum-based Xpert results. In cases of invalid results, samples were reanalyzed using Xpert, and final reports were based on the conclusive results.

2.4. Statistical Analysis

Data was coded and entered into Microsoft Excel 2016 and then transferred to the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp, Armonk, NY, USA) for analysis. Basic demographic characteristics were analyzed using percentages for categorical variables with their 95% confidence intervals, and medians and interquartile ranges (IQRs) for non-normally distributed continuous variables. The Pearson chi-square test or Fisher's exact test was used to compare proportions. A p-value of < 0.05 was considered statistically significant.

3. Results

The median age of the children was 24.0 months (IQR: 12.0 - 48.8), with an age range from 3 to 84 months. Most children were in the 24 - 59 months age group (42.8%). Both sexes were equally represented, with a sex ratio of 1:1. A large proportion of the children came from the Kalemie Health Zone (36.3%) and Kongolo (34%). More than three-quarters of the stool samples were collected at General Referral Hospitals (91.2%), and 4.4% of the children were malnourished at the time of care (Table 1).

Table 1. General characteristics of the study population.

Variables	Sample size (n = 91)	Percentage
Age		
<24 months	33	36.3
24 - 59	39	42.8
≥60 months	19	20.9
Sex		
Male	46	50.5
Female	45	49.5
Provenance		
Kalemie	33	36.3
Kongolo	31	34.0
Moba	10	11.0
Manono	9	9.9
Kabalo	8	8.8
Admission place		
HGR	83	91.2
LPR	8	8.8
Nutritional status		
Good	87	95.6
Malnutrition	4	4.4

Among the stool samples analyzed using the GeneXpert method, 15.4% (95% CI: 12.3 - 18.6) tested positive, representing the overall prevalence of pulmonary TB among children in this province (Figure 1).

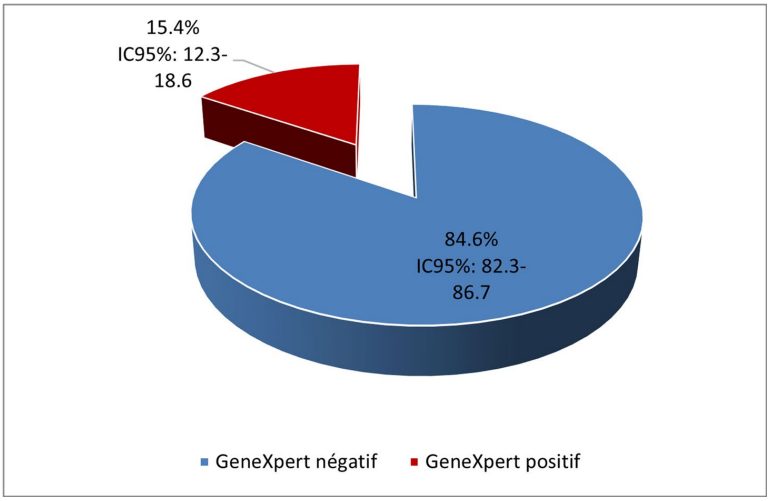

Figure 1. Overall prevalence of TB by GeneXpert on stool samples.

Among the 14 samples that tested positive with GeneXpert, 78.6% were drug-sensitive, 14.3% were drug-resistant, and 7.1% showed a trace result (Table 2).

Table 2. Severity of GeneXpert results.

Severity of GeneXpert results	Sample size	% (IC95%)
Sensible	11	78.6 (74.2 - 80.4)
Resistant	2	14.3 (12.4 - 17.6)
Trace	1	7.1 (5.7 - 9.8)
Total	14	100.0

Although not statistically significant, the prevalence of pulmonary TB was higher among children over 60 months of age (21.1%), those from Kalemie (22.6%), and those whose samples came from LPR facilities (25%). In contrast, the prevalence of pulmonary TB was significantly higher among female children (22.2% vs. 8.7% in males, p = 0.016) and among malnourished children (100%) (Table 3).

Table 3. General characteristics according to GeneXpert results.

Variable	Positive to GeneXpert		Negative to GeneXpert		p
	n	% (IC95%)	n	% (IC95%)	
Age					0.669
<24 months	5	15.2 (12.3 - 19.6)	28	84.8 (80.5 - 88.1)	
24 - 59 months	5	12.8 (9.2 - 14.8)	34	87.2 (83.9 - 90.6)	
≥60 months	4	21.1 (18.9 - 24.5)	15	78.9 (73.7 - 81.1)	

Continued

Sex					0.016
Male	4	8.7 (5.6 - 11.4)	42	91.3 (90.7 - 96.8)	
Female	10	22.2 (17.7 - 25.9)	35	77.8 (74.6 - 80.1)	
Provenance					0.660
Kalemie	7	22.6 (18.1 - 26.6)	24	77.4 (70.5 - 80.7)	
Kongolo	5	15.2 (12.6 - 18.8)	28	84.8 (79.8 - 89.6)	
Moba	1	12.5 (9.2 - 14.3)	7	87.5 (85.9 - 90.3)	
Manono	0	0.0	9	100.0	
Kabalo	1	10.0 (7.8 - 12.4)	9	90.0 (86.5 - 93.1)	
Admission place					0.356
HGR	12	14.5 (12.3 - 16.7)	71	85.5 (80.6 - 89.1)	
LPR	2	25.0 (22.1 - 27.4)	6	75.5 (73.2 - 77.9)	
Nutritional status					<0.001
Good	10	11.5 (9.6 - 14.7)	77	88.5 (83.7 - 91.4)	
Malnutrition	4	100.0	0	0.0	

4. Discussion

The results of this study highlight several important aspects regarding pulmonary tuberculosis (TB) screening in children under 10 using the GeneXpert method on stool samples. Although not new in the DRC, this technique offers important insights and presents diagnostic challenges for TB programs. It has already been evaluated in several countries, demonstrating sensitivities ranging from 78% to 99% and specificities between 90% and 100% [10] [13]. In 2022, the National TB Control Program (PNLT) of the DRC piloted stool testing using the GeneXpert platform in 25 health facilities in Kinshasa, showing promising results for TB diagnosis from stool samples [14].

While stool-based testing with GeneXpert and the newer GeneXpert® MTB/RIF Ultra has shown promise in pediatric TB diagnosis, current evidence does not support replacing sputum testing. Rather, stool testing should be considered a complementary approach.

In this study, the prevalence of pulmonary TB detected by GeneXpert on stool samples was 15.4% (95% CI: 12.3 - 18.6%). This aligns with findings from the PNLT and USAID study, which reported a 17.3% prevalence in 25 Kinshasa health facilities [7], and with Bhadra *et al*., who found 18% prevalence [13]. Among the positive cases, 78.6% were drug-sensitive, 14.3% drug-resistant, and 7.1% trace results.

Children under 2 and over 5 years, females, and malnourished children were more exposed to TB infection, which is harder to diagnose in these groups than in older children or adults [13]. Due to the small number of children under 2 in this study, the performance of stool-based GeneXpert in this age group could not be fully assessed. In these cases, respiratory sample collection is often invasive, and

TB progresses more rapidly to severe forms such as TB meningitis, making stool testing a valuable diagnostic alternative. Malnourished children are especially vulnerable due to weakened immunity [15]. Regarding girls, hormonal factors may increase susceptibility to infections, including TB [16], and in rural settings, girls may face higher risks due to socioeconomic disadvantages and limited healthcare access.

5. Limitations

Firstly, the small number of confirmed cases reduced statistical power and prevented subgroup analyses, such as multivariate analysis in HIV-positive children, as HIV status was not available in the dataset. We were unable to account for HIV status, an important confounder in TB prevalence, as this information was not consistently available. Second, results from fresh versus preserved stool samples were not analyzed separately, which could have provided more insight into processing methods.

6. Conclusion

This study demonstrates that the use of GeneXpert MTB/RIF Ultra on stool samples is a feasible and non-invasive approach for diagnosing pulmonary tuberculosis in children under 10 years of age in Tanganyika province, DRC. The observed prevalence of TB, higher among girls and malnourished children, underscores the vulnerability of these groups. While limited by small sample size and retrospective design, our findings support stool-based GeneXpert as a complementary tool to sputum testing, particularly in resource-limited pediatric settings. Most importantly, it facilitates access to non-invasive TB testing closer to children's homes, particularly in settings with limited resources. This approach offers a promising strategy to expand early and equitable TB diagnosis and treatment among pediatric populations who are unable to expectorate. Further operational research is recommended to optimize and scale up the implementation of stool-based GeneXpert testing for the diagnosis of pediatric pulmonary TB within the local healthcare context.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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