VOLUME - 15, ISSUE - 07, JULY - 2026 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

TO ASSESS THE UTILITY OF STOOL GENEXPERT FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS IN PAEDIATRIC POPULATION IN A TERTIARY CARE HOSPITAL

Dr Nupur Chandra* Junior Resident, Department of Microbiology. Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai.*Corresponding Author

Dr Nilma Hirani Professor, Department of Microbiology. Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai

ABSTRACT

Background-. According to global tuberculosis report 2025, a total number of 10.7 million people were detected with TB in 2024. In 2022, around 1.25 million children below 15 years of age developed TB, of which 47% were aged 5 years. Children swallow sputum, which may pass through the gastrointestinal tract. **Methods-**This study was carried out in a tertiary care hospital between October 2023 to March 2025.Gastric lavage (GL) samples were collected from children (0 to 10 years) with Pulmonary Tuberculosis (PTB). The stool samples of those patients were subjected to GeneXpert Ultra whose GL samples were found to be positive for MTB on GeneXpert using Generation IV cartridges. The GL samples were further decontaminated and subjected to liquid culture. Also, 50 GL samples found negative on GeneXpert MTB/RIF were subjected to GeneXpert Ultra to confirm the negative GeneXpert MTB/RIF results. This acted as a control group. **Results-** Of the 700 GL samples tested on GeneXpert MTB/RIF,45 samples were found to be positive for MTB. Stool sample were collected from all these 45 patients. The GL samples were subjected further to MGIT960 liquid culture. Gastric lavage samples that detected MTB both, on GeneXpert MTB/RIF and liquid culture were 40. Forty three of the 45 stool samples were detected with MTB on GeneXpert Ultra. Conclusion-stool can be recommended as an effective alternative to gastric lavage samples as it shows a good concordance and is also a non-invasive technique and easy to collect sample.

KEYWORDS : GeneXpert Ultra, gastric lavage, stool, MGIT960; pulmonary tuberculosis

INTRODUCTION

According to global tuberculosis report 2025, an estimated total number of 10.7 million people were detected with TB in 2024.[1]It was observed that the number of people who fell ill with TB decreased year-on-year since the COVID-19 pandemic, reversing the years of increases between 2020 and 2023[1]. As per global tuberculosis report 2024, a total of 10.8 million people developed TB in 2023.[2]It returned as the world's leading cause of death, subsequent to the 3 years in which it was replaced by coronavirus disease.[2] In 2022, around 1.25 million children below 15 years of age fell ill with TB, of which 47% were aged 5 years [3].It was notified that around 3.33 lakh children in the 0–14 years' age group become ill with TB each year, with a higher number of cases in males.[4] Pulmonary TB is the quite common form in children.[4]Children make up 6-7% of all the patients (0-14years) who are being treated under NTEP .[5]The difficulty to collect sputum sample and non-specific clinical features in children all the more increase the challenge in diagnosing Pulmonary tuberculosis. It has been seen that close contact with known TB cases pose an important risk factor in the case of children[6].Conditions of malnutrition and health disorders accentuate it.[6] TB and undernutrition go hand in hand. Undernutrition is one risk factor which increases the chances of latent TB which can gradually progress to active TB.[7]Inadequate nutrition can lead to childhood deaths if treatment is not provided within time[7]. WHO has recommended laboratory based rapid diagnostic tests using stool-based testing in countries with high load of childhood tuberculosis.[8] Clinicians still continue to diagnosis TB in children based on clinical and radiological findings, as children many times are unable to produce effective amount of sputum. Childhood TB is usually under-reported as it is a paucibacillary disease.[9] Alternative sample types such as induced sputum, gastric aspirate and nasopharyngeal aspirate are recommended but collection of those specimens requires technicians who are skilled, proper equipment and can lead to irritation and discomfort[10]. Recently, stool has been added to the sample types that can be used for the diagnosis of TB in children.[10] Stool can be an alternative specimen, especially in scenarios where it becomes overtly difficult to obtain good quality respiratory sample for the diagnosis of PTB, most importantly in younger children, being an invasive procedures.[11] Hence this study was undertaken to assess the utility of GeneXpert MTB/RIF Ultra assay to detect presence of Mycobacterium tuberculosis directly from stool samples in paediatric presumptive pulmonary TB cases, in a tertiary care hospital.

METHOD

This study was a prospective study which was done in the Department of Microbiology at a tertiary care hospital. A total of 700 children of 0 to 10 years age group presenting with features of presumptive pulmonary tuberculosis were studied from October 2023 to March 2025. We included GL specimens of the children in the age group of 0-10 years who presented with clinical features suggesting of pulmonary tuberculosis, history of contact, past history, BCG vaccination status and abnormal radiological findings suggestive of pulmonary TB. We excluded samples other than GL from children between 0-10 years of age. We also excluded cases of NTM and atypical tuberculosis and the parents or guardians who did not give consent for the study.

Study Procedures

For every presumptive TB case identified, gastric lavage sample collected from paediatric population of 0-10 years was subjected to GeneXpert using generation IV cartridges according to the manufacturer instructions (Cepheid, Sunnyvale, California, United States). If the results came positive, the stool sample of the same patient was subjected to GeneXpert Ultra and the results were obtained. The GL samples were further subjected to decontamination using NALC NAOH method and the concentrates subjected to liquid culture using MGIT960 system (Becton Dickinson & Company USA).

Procedure of stool GeneXpert ultra[12]

Approximately 1 gram of stool was emptied in a conical tube.10 ml of phosphate buffer solution (7.4) was added to it. It was mixed by vigorous vortexing. The tube was placed undisturbed minimum 10 minutes for stool particles to settle down. From the supernatant formed above, we extracted 2ml and transferred it into a new 50 ml conical tube. This content after mixing with 4ml of the GeneXpert MTB/RIF buffer given in the GeneXpert kit was vortexed and kept undisturbed for 15 min. Using the sterile transfer pipette, the liquefied sample

was aspirated and at least 2ml transferred into the open port of the GeneXpert MTB/RIF Ultra cartridge. We proceeded with loading of the content into the GeneXpert Dx instrument and the test was started within 5 hours of preparing the cartridges. The results were available on the GeneXpert instrument.

Statistical analysis of data-

Clinical findings and demographic details of the patients and the GeneXpert MTB/RIF using Generation IV cartridges and GeneXpert-Ultra testing results of the samples were analysed. The data which was captured was collected and saved in an excel sheet. It was analysed using SPSS (version 20) software. The study was approved by Institutional Ethics committee. IEC NO-IEC/PG/222/Decs/2023

RESULTS-

Out of total 700 GL samples tested on GeneXpert MTB/RIF using generation IV Cartridges,45 samples were found to be positive for MTB while 655 were found to be negative. The GL samples were subjected further to MGIT960, liquid culture. Total number of gastric lavage samples showing MTB both, on GeneXpert MTB/RIF and liquid culture were 40.

Table 1. Results of GL detected with MTB on GeneXpert MTB/RIF and stool detected with MTB on GeneXpert Ultra. (n=700)

Tests	MTB detected	MTB not detected	Total
GL GeneXpert MTB/RIF	45	00	45
Stool GeneXpert Ultra	43	02	45

There were 43 out of 45 stool samples which detected MTB on GeneXpert Ultra while 45 GL samples were detected with MTB on GeneXpert MTB/RIF using Generation IV cartridges.

Table 2. Results of MTB detection using GL on GeneXpert MTB/RIF and GeneXpert Ultra. (n=50).

Tests	GL GeneXpert MTB/RIF	GL GeneXpert Ultra
MTB not detected	50	50
MTB detected	00	00

A subset of 50 GL samples found negative on GeneXpert MTB/RIF using generation IV cartridges were subjected to GeneXpert MTB/RIF Ultra testing acting as a control group. They were found to be negative for MTB on GeneXpert Ultra and hence showed 100% concordance. This was the control group.

Table 3. Interpretation of results of MTB detection using GeneXpert.

MTB detected	GL GeneXpert MTB/RIF	Stool GeneXpert ultra
Traces	00	05
Very low	14	15
Low	15	10
Medium	12	09
High	04	04
Total	45	43

Out of the 43 stool samples detected with MTB on GeneXpert Ultra,15 detected with very low MTB load,10 detected low MTB load,09 detected medium MTB load ,04 detected high MTB load and 05 showed trace detection of MTB. There were 02 GL samples which were detected as MTB on GeneXpert MTB/RIF using generation IV cartridges but the stool samples of those patients did not detect MTB when subjected to GeneXpert Ultra.

Table 4. Comparison of results of GL GeneXpert MTB/RIF and GL liquid culture(N=700)

GL GeneXpert MTB/RIF	GL MGIT culture	
	MTB Grown	MTB Not Grown
MTB detected	40	5
MTB not detected	2	653
Total	42	658

Taking liquid culture MGIT 960 as reference, the sensitivity of GeneXpert MTB/RIF using gastric lavage samples was found out to be 95.2% and specificity was found out to be 99.2%.

Table 5. Results of stool using GeneXpert Ultra assay and Gastric lavage liquid culture MGIT960 (N=45)

Stool GeneXpert MTB/RIF	GL liquid culture	
	MTB Grown	MTB Not Grown
MTB detected	43	0
MTB not detected	1	1
Total	44	1

As stool sample is not considered a good sample for culture due to high risk of culture contamination17, we took liquid culture, MGIT 960 of gastric lavage, as reference and found that the sensitivity of GeneXpert Ultra using stool samples as 97.7% and specificity as 100%.

Table 6. Results of the Rifampicin resistance pattern

Rifampicin resistance pattern	GL GeneXpert MTB/RIF	Stool GeneXpert Ultra
Rifampicin resistance not detected	32	25
Rifampicin resistance detected	13	13
Rifampicin resistance indeterminate	00	05
Total	45	43

There were 25 stool samples which showed Rifampicin resistance not detected and 13 stool samples showed rifampicin resistance detected on GeneXpert Ultra. The stool samples of 02 patients did not detect MTB. Trace calls identified in 05 stool samples on GeneXpert Ultra showed Rifampicin Indeterminate status

Table 7. Comparison of GeneXpert results based on demographic findings

Age group	GL positive GeneXpert MTB/RIF	Stool-positive GeneXpert Ultra
0-5 years	35	35
6-10 years	10	08
Total	45	43

Out of 45 MTB positive patients 35 GL and stool samples belonged to 0 to 5 years age group. There were a smaller number of MTB detection in 6 to 10 years age group.

DISCUSSION

TB diagnosis in children is challenging in low-resource areas. An important reason for difficult diagnosis of childhood pulmonary TB is its paucibacillary nature.[9] Gastric lavage is an invasive procedure. Hence non -invasive TB diagnostic test which gives a fast and reliable result needs to be developed for young children.[10]

As per Table 1, we found a good concordance of 95.5% between the results of GL processed on GeneXpert MTB/RIF using generation IV cartridges and the stool GeneXpert Ultra, suggesting stool as a potential alternative sample in comparison to gastric lavage. Of the 45 GL samples which showed MTB detected on GeneXpert MTB/RIF, stool samples of the same patients showed MTB detected in 43 cases on GeneXpert Ultra. The 2 stool samples which did not detect MTB could be due to initiation of antitubercular treatment

(ATT) to the patients and this could have been a response to treatment as there was a time lapse of 7 days in stool sample collection.

As per table 2, we found that all gastric lavage samples(n=50) which were found out to be negative for TB detection on GeneXpert MTB/RIF also tested negative when subjected to GeneXpert ultra, showing a good sensitivity of GeneXpert Ultra. This was a control group in our study. This matched with a study done by John Osei Sekyere et al[13]. In their study, they did the comparative analysis of results of the GeneXpert Ultra assay and GeneXpert generation IV assay using pulmonary and extra pulmonary samples. Their results showed that GeneXpert Ultra assay gives a higher sensitivity of 88% for detection of pulmonary TB cases as compared to GeneXpert using generation IV cartridges.

As per Table 3, it was observed in this study that there was detection of Trace calls (Trace call=05) in stool GeneXpert Ultra in those patients who were detected as low and very low MTB on GL GeneXpert MTB/RIF. This could due to the paucibacillary nature of MTB in children and intermittent shedding of bacilli in stool. Our results showed an agreement with a study done by Kabir et al al[14], where, out of total 72 bacteriologically confirmed children, GeneXpert Ultra on stool was positive in 60, of whom 48 had "trace call." One study done by Yohannes Babo et al[15], they found detecting MTB from swallowed sputum in the stool (low, very low, and trace call) can be because of the paucibacillary nature of TB in children. MTB gets more diluted in intestines as sputum gets mixed with food, PCR is inhibited due to substances in stool. Our study shows similar results.

In Table 4. taking liquid MGIT 960 as reference, the sensitivity of GeneXpert MTB/RIF generation IV, using GL sample was found to be 95.2% and specificity was found to be 99.2%. One study done by Madhurima et al[16], where considering GL culture as "gold standard", GL GeneXpert using Generation IV assays were found to be having sensitivity of 91.6 % and specificity of 100%. We also showed similar results. In yet another study, Hasan et al[17] 2017 where they considered culture as "gold standard ", GeneXpert MTB/RIF was found to be having sensitivity of 100 % and specificity of 95%, respectively.

In Table 5. considering stool culture carries high risk of contamination and showing low sensitivity for MTB detection as per a study done by Walters et al[18], we took GL liquid culture as standard reference and found sensitivity of 97.7% and specificity of 100% of GeneXpert Ultra using stool sample. Our study shows similar results with the study done by Belew et al[19] where they found out that sensitivity and specificity of GeneXpert Ultra using stool samples were found to be 95.7% and 99.7% respectively considering GeneXpert ultra-assay of respiratory samples as one of the references.

In table 6. We found among the 43 stool samples found positive on GeneXpert Ultra, rifampicin resistance was not detected in 25 samples, while rifampicin resistance was observed in 13 stool samples. There were 05 stool samples which showed Rifampicin resistance status Indeterminate. These were the samples which were identified as Trace calls by GeneXpert Ultra. It was further found in the study that 02 GL samples which did not detect rifampicin resistance, (they detected very low amount of MTB on GeneXpert MTB/RIF), the stool GeneXpert Ultra of these patients did not detect MTB. One study which showed similar Rifampicin "Indeterminate status" was done by Feng et al[20] where the GeneXpert method had the highest detection rate of 1,150 cases. Among these, rifampicin-resistant strains were found in 133 cases, along with 14 cases that resulted in "indeterminate" results. A study done by Chakravorty et al[21]found, that Ultra also enables improved detection of RR mutations associated with Rifampicin resistance.

In table 7. We found that 0 to 5 years age group were affected the most with pulmonary tuberculosis. One study conducted by Haque et al[22]also found higher prevalence of TB in children under 5 years of age. Our study shows similar finding. This could be possibly due to the immune system which is still in the developing stage so cannot combat the disease and gets affected by a smaller amount of bacterial load.

CONCLUSION

Tuberculosis (TB) is a major cause of death in this paediatric age group. Diagnosis is difficult as it mimics other chronic respiratory illnesses in children. They are also unable to produce an effective quality sputum. Thus, we depend upon invasive procedures to collect respiratory specimen including gastric aspirate. WHO has endorsed the use of stool specimens from children for GeneXpert MTB/RIF Ultra testing as it can be a non-invasive procedure.[23] It is found to be having higher sensitivity in identifying paucibacillary cases in paediatric population, when compared with the GeneXpert MTB/RIF (Cepheid, Sunnyvale, California Generation IV).As per the study done by Babo et al [15]stool can be an alternative to respiratory samples and a non-invasive test. A new BPaLM **regimen** consisting of Bedaquiline, Pretomanid, Linezolid and Moxifloxacin which is shorter, safe and effective, has been introduced for patients who are diagnosed with drug-resistant tuberculosis[24].Hence, the study strongly encourages the use of stool for detection of pulmonary tuberculosis in children as it is a non -invasive procedure and sample can be collected easily.

Informed Consent Statement

Blanket consent was taken from all the patients/guardians

Conflicts of Interest-

The authors declare no conflict of interest.

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