Original Research Article

Utility of GeneXpert for Direct Detection of *Mycobacterium tuberculosis* in Stool Specimens in Children with Presumptive Pulmonary Tuberculosis

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Abstract

Background: Tuberculosis, caused by *Mycobacterium tuberculosis*, continues to be one of the world's deadliest communicable diseases. As per WHO Global Report 2018, India has the highest TB burden in the world accounting for 27% of the global cases with an estimated incidence of 2.74 million of which 2,24,000 were in the age group 0-14 years. Hence childhood TB is a great concern in India. In December 2010, a novel, rapid, automated, cartridge-based nucleic acid amplification test (CBNAAT), known as the Xpert MTB/RIF assay was endorsed by WHO for diagnosis of TB and simultaneous detection of rifampicin resistance which would be completed within 2 hours. **Aims and Objectives:** Comparison of Xpert MTB/RIF on stool specimen from children with presumptive pulmonary tuberculosis with Xpert MTB/RIF on gastric lavage specimen for detection of pulmonary TB in children. Comparison of Xpert MTB/RIF on stool specimen from children with presumptive pulmonary tuberculosis with conventional methods for detection of pulmonary TB in children. **Methodology:** The present study was conducted in a tertiary care hospital to test the utility of GeneXpert for detection of *Mycobacterium tuberculosis* in stool specimens in children with presumptive pulmonary tuberculosis. 120 clinically suspected cases of pulmonary TB in children in the age group 0 to 10 years were analysed for clinical profile and risk factors. Single gastric lavage and stool sample were collected from each patient. Each gastric lavage (GL) sample was subjected to conventional methods of TB detection like Acid Fast Bacilli (AFB) staining by Ziehl-Neelsen (ZN) technique and culture on Lowenstein Jensen (LJ) medium followed by GeneXpert analysis. Each stool sample was subjected to only GeneXpert analysis. The results of Stool GeneXpert were compared with results of GL GeneXpert and conventional methods of diagnosis of pulmonary TB in children. **Results:** Out of 120 GL and stool samples tested, highest positivity was seen on GL LJ Culture (10%) followed by GL Xpert (9.2%), Stool Xpert (7.5%) and GL ZN stain (5%). Considering GL LJ culture as Gold Standard, Stool GeneXpert showed sensitivity of 75%, specificity of 100%, PPV of 100% and NPV of 97.30%. When compared with GL ZN stain, Stool GeneXpert showed sensitivity of 100%, specificity of 97.37%, PPV of 66.67% and NPV of 100%.When compared with GL- Xpert, Stool-Xpert showed sensitivity of 81.82%, specificity of 100%, PPV of 100% and NPV of 98.20%. **Conclusion:** Stool specimen for GeneXpert can be used to detect pulmonary TB in children as an alternative to Gastric Lavage specimen for GeneXpert for its ease of collection.

Key words: GeneXpert, children, pulmonary tuberculosis, stool.

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INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis*, still continues to be one of the world's deadliest communicable diseases. It typically affects the lungs (pulmonary TB), but other sites can also be affected (extra-pulmonary TB) such as pleura, lymph nodes, meninges, abdomen, genitourinary system, spine, bones and joints, etc. As per WHO Global Report 2018, an estimated 10.0 million people developed TB and 1.6 million died from the disease in the year 2017. Among those who developed TB disease in 2017, 5.8 million were men, 3.2 million were women and 1.0 million were children. India has the highest TB burden in the world accounting for 27% of the global cases with an estimated incidence of 2.74 million of which 2,24,000 were in the age group 0-14 years.[1] Conventional pulmonary TB diagnosis is based mainly upon sputum microscopy, isolation of the causative organism on culture (solid or liquid medium), radiological or histopathological findings and clinical suspicion.[2] Other diagnostic tests that are available include Nucleic Acid Amplification Tests (NAAT) such as the Xpert MTB/RIF, Enhanced Amplified *Mycobacterium Tuberculosis* Direct Test (E-MTD), loop-mediated isothermal amplification assay (TB-LAMP) and lateral flow urine lipoarabinomannan assay (LF-LAM). Children rarely expectorate spontaneously so it is highly difficult to obtain a representative sample from the lower respiratory tract. Another important reason for difficult diagnosis of childhood pulmonary TB is its paucibacillary nature. Hence, as compared to adult cases, microbiological confirmation is less frequently achieved in children.[3,4] Since children tend to swallow sputum, gastric lavage is performed in order to obtain sample from a child's stomach to diagnose pulmonary TB.

Gastric lavage (GL) is a procedure by which gastric contents are aspirated using a nasogastric tube inserted through the child's nose. Gastric lavage sample thus obtained is subjected to conventional tests like Acid Fast Bacilli (AFB) staining by Ziehl-Neelsen (ZN) technique and culture (liquid or solid medium) followed by GeneXpert analysis for diagnosis of pulmonary TB in children. But, this procedure is invasive, requires expertise and can be traumatic for the child. Hence non-invasive TB diagnostic test which gives a fast and reliable result needs to be developed for young children. As children tend to swallow sputum and if the sputum contains TB bacilli, TB DNA can survive intestinal transit; therefore, testing stool using molecular methods like GeneXpert for detection of TB DNA can be utilized for detection of childhood pulmonary TB.[5] Stool GeneXpert could be a powerful tool for diagnosis of childhood pulmonary TB when ease of sample collection was taken into account.[5] There are a few studies from India using GeneXpert on stool samples for detection of pulmonary TB in children. Hence, this study was conducted to compare Xpert MTB/RIF on stool sample from children with presumptive pulmonary tuberculosis with Xpert MTB/RIF on gastric lavage and conventional methods used for detection of childhood pulmonary TB.

MATERIALS AND METHODS

Present study was a prospective study carried out in Department of Microbiology at a tertiary care hospital. Total 120 paediatric cases of Presumptive Pulmonary Tuberculosis were studied during 1st January, 2017 to 31st December, 2017.

Inclusion Criteria:

1. Children in the age group 0 to 10 years
2. Patients with clinical symptoms suggestive of pulmonary TB along with atleast any one factor like History of TB contact, Raised Erythrocyte Sedimentation Rate (ESR), Positive Mantoux Test [Positive Result- ≥10 mm], abnormal radiological findings suggestive of Pulmonary TB
3. Human Immunodeficiency Virus (HIV) negative patients.

Exclusion Criteria:

1. Age > 10 years
2. Patients on Anti-tuberculosis treatment (ATT)
3. Human Immunodeficiency Virus (HIV) positive patients.
4. Parent/ guardian not giving consent for the study.

Study was approved by ethical committee of the institute. A valid written consent was taken from parent/ guardian of the patient after explaining the study to them. Data was collected with pretested questionnaire. It included the demographic profile, presenting complaints, past history, investigations done for each patient and it was duly recorded in the case record form.

Single gastric lavage and stool sample were collected from each patient. Each gastric lavage sample was subjected to conventional methods of TB detection in

children like Acid Fast Bacilli (AFB) staining by Ziehl-Neelsen (ZN) technique and culture on Lowenstein Jensen (LJ) medium followed by GeneXpert analysis. Each stool sample was subjected to only GeneXpert analysis. In in-patient cases, gastric aspiration was carried first thing in the morning when the child woke up, at the child's bedside. In case of out-patients, gastric aspiration was carried in a procedure room in the ward. 5–10 ml of gastric aspirate was withdrawn. Gastric fluid from the syringe was transferred into a sterile container.[6] The gastric lavage samples were transported immediately to the laboratory and processed to prevent the killing action of the acid content in the gastric lavage on the tubercle bacilli. In the event of delay of more than 4 hours, the sample was neutralised with an equal volume of sterile 10% Sodium bicarbonate solution.[6,7] Parent/ guardian was instructed to collect one stool sample in a duly labelled dry, clean, leak-proof, screw capped wide-mouthed container. The parent/ guardian of the patients included in the study were instructed to collect the sample avoiding contamination with urine, water or dirt. All the stool samples were transported to the laboratory without any delay and processed immediately. Processing of samples was carried out in Bio Safety Cabinet (BSC) 2 and level 2 biosafety practices were followed.

Gastric lavage : Gastric lavage was decontaminated using Modified Petroff's Method.[7] Smear was prepared using sterile loop and stained by Ziehl-Neelsen (ZN) staining technique as per RNTCP protocol.[7] From the sediment, two slopes of LJ medium were inoculated using a sterile 5 mm Nichrome wire loop (22 SWG). After inoculation, all cultures were incubated aerobically at 37°C. All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter cultures were examined weekly, on a specified day of the week, up to growth was detected or 8 weeks whichever was later. If no growth was detected after 8 weeks of incubation, then the cultures were discarded as negative. Any growth observed on LJ medium was identified with smear stained by ZN technique and standard biochemical tests.[8]

GL GeneXpert Analysis: The sample reagent buffer containing NaOH and isopropanol was added to decontaminated gastric lavage sample in the ratio of 3:1, and incubated for 15 min at room temperature. 2ml of the treated sample was then transferred into the Xpert MTB/RIF cartridge containing the wash buffer, lyophilized reagents for DNA extraction and PCR amplification. The cartridge was loaded in the Xpert MTB/RIF instrument after proper mixing. The instrument automatically performs specimen mixing, sonication of the *Mycobacterial* bacilli and internal control (spores), DNA release and mixing with the PCR reagents followed by a hemi-nested real time-PCR amplification, target detection by five-color fluorescence molecular beacon probes and internal control *in-situ*.[9] Results were generated after 2 hr and reported as *M. tuberculosis* - negative or positive. If the result was positive then a semi-quantified bacillary load as high, medium, low or very low and whether the pathogen was RIF sensitive or resistant was also generated.[9]

Stool sample: Single stool sample was collected from each participant and analyzed immediately. About 2 ml of stool specimen was suspended in 10 ml of distilled water and homogenized by vortex. It was then left at room temperature until solid particles settled. Supernatant was taken and centrifuged at 4000 rpm for 20 minutes. The resulting sediment was decontaminated by 10 ml of 3% N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) for 15 min at room temperature and then mixed with 40 ml of phosphate buffer (PB) at pH 6.8 and centrifuged for 20 min.[5]

Stool GeneXpert Analysis: The resulting sediment was suspended in 1 ml of PB and mixed with 2 ml of sample reagent. This was then left at room temperature for 15 min and after that transferred to GeneXpert MTB/RIF cartridge and analyzed using GeneXpert system.[5] The instrument automatically performs specimen mixing, sonication of the *Mycobacterial* bacilli and internal control (spores), DNA release and mixing with the PCR reagents followed by a hemi-nested real time-PCR amplification, target detection by five-color fluorescence molecular beacon probes and internal control *in-situ*.[9] Results were generated after 2 hr and reported as *M. tuberculosis* - negative or positive. If the result was positive then a semi-quantified bacillary load as high, medium, low or very low and whether the pathogen was RIF sensitive or resistant was also generated.[9]

Interpretation of the Results[10]:

MTB NOT DETECTED was reported when there was only one or no positive probe.

MTB DETECTED was reported when at least two of the five *rpoB*-specific molecular beacons gave a positive signal with cycle threshold (C_T) values that were ≤38 and that differed by no more than two cycles.

RIF Resistance NOT DETECTED was reported if the ΔCT Max [The difference in C_T between the first (early C_T) and last (later C_T) *M. tuberculosis*-specific molecular beacon] was ≤3.5 cycles.

RIF Resistance DETECTED was reported if the ΔC_T Max was >3.5 cycles.

RIF Resistance INDETERMINATE was reported when the last probe returned a C_T of >38 and the first probe had a C_T value of >34.5 cycles.

Data was analysed with appropriate statistical tests.

Statistical Analysis:

Statistical analysis was performed with the software package: SPSS statistic 20 for Windows. The significance of difference of proportion of categorical variables among groups was examined by the chi-square test for large samples and Fischer's exact test for small samples. A value of P of ≤ 0.05 was considered significant for all statistical analyses and was marked with an asterisk. Gastric Lavage LJ culture was taken as the Gold Standard.

RESULTS

120 Gastric Lavage (GL) specimens in the study were subjected to conventional microscopy, culture on Lowenstein Jensen (LJ) medium and GeneXpert analysis. 120 stool samples were subjected to only GeneXpert analysis. Gastric Lavage LJ culture was taken as the Gold Standard.

Table 1: Percentage positivity of tests done on Gastric Lavage (GL) and Stool (n=120)

Test	Positive	Percentage (%)
GL-ZN Stain	6	5
GL LJ Culture	12	10
GL Xpert MTB	11	9.2
Stool Xpert MTB	9	7.5

Chi Square Value- 21.1 P Value - 0.001*

120 paediatric cases were tested for GL-ZN Stain, GL LJ Culture, GL-Xpert MTB and Stool-Xpert MTB. Out of 120 Gastric Lavage samples tested, 5% were positive by ZN stain, 10% were positive by LJ culture and 9.2% were positive by GeneXpert. Out of 120 Stool samples tested, 7.5% were positive by GeneXpert.

Highest positivity was seen on GL LJ Culture (10%) followed by GL Xpert (9.2%), Stool Xpert (7.5%) and GL ZN stain (5%). This difference in percent positivity in various tests performed was found to be statistically significant.

Table 2: Comparison of GL-ZN Smear with GL LJ Culture

GL-ZN Smear	GL LJ Culture		Total
	MTB Grown	No Growth	
AFB seen	6 (5%)	0 (0%)	6 (5%)
AFB not seen	6 (5%)	108 (90%)	114 (95%)
Total	12 (10%)	108 (90%)	120 (100%)

Chi Square Value-56.84 P Value- <0.001*

5% of cases were positive by both GL- ZN smear and GL LJ Culture. 5% of cases were negative by GL- ZN smear but positive by GL LJ Culture. 90% cases were negative by both GL- ZN smear and GL LJ Culture. Considering GL LJ culture as Gold Standard, GL ZN smear showed Sensitivity: 50.00%, Specificity: 100% Positive Predictive Value (PPV): 100% Negative Predictive Value (NPV): 94.74%. This was found to be statistically significant.

Table 3: Comparison of GL GeneXpert with GL LJ Culture

GL-Xpert	GL LJ CULTURE		Total
	MTB Grown	No Growth	
Detected	11 (9.2%)	0 (0%)	11 (9.2%)
Not detected	1 (0.8%)	108 (90%)	109 (90.8%)
Total	12 (10%)	108 (90%)	120 (100%)

Chi Square value-108.99 P value- <0.001*

9.2% of cases were positive by both GL-Xpert and GL LJ Culture. 0.8% of cases were negative by GL- Xpert but positive by GL LJ Culture. 90% cases were negative by both GL- Xpert and GL LJ Culture. Considering GL LJ culture as Gold Standard, GL GeneXpert showed Sensitivity: 91.67%, Specificity: 100%, Positive Predictive Value (PPV): 100% Negative Predictive Value (NPV): 99.08%. This was found to be statistically significant.

Table 4: Comparison of Stool GeneXpert with GL ZN stain

Stool-Xpert	GL-ZN stain		Total
	AFB seen	AFB not seen	
Detected	6 (5%)	3 (2.5%)	9 (7.5%)
Not detected	0 (0%)	111 (92.5%)	111 (92.5%)
Total	6 (5%)	114 (95%)	120 (100%)

Chi Square Value- 77.89 P Value-<0.001*

5% of cases were positive by both Stool-Xpert and GL ZN stain. 2.5% of cases were positive by Stool- Xpert but negative by GL ZN stain. 92.5% cases were negative by both Stool- Xpert and GL ZN stain. When compared with GL ZN stain, Stool GeneXpert showed Sensitivity: 100%, Specificity: 97.37%, Positive Predictive Value (PPV): 66.67 %, Negative Predictive Value (NPV): 100 %. This was found to be statistically significant.

Table 5: Comparison of Stool GeneXpert with GL LJ Culture

Stool-Xpert	GL LJ Culture		Total
	MTB Grown	No Growth	
Detected	9 (7.5%)	0 (0%)	9 (7.5%)
Not detected	3 (2.5%)	108 (90%)	111(92.5%)
Total	12 (10%)	108 (90%)	120 (100%)

Chi Square value-57.59 P Value- <0.001*

7.5% of cases were positive by both Stool-Xpert and GL LJ Culture. 2.5% of cases were negative by Stool- Xpert but positive by GL LJ Culture. 90% cases were negative by both Stool- Xpert and GL LJ Culture. Considering GL LJ culture as Gold Standard, Stool GeneXpert showed Sensitivity: 75%, Specificity: 100%, Positive Predictive Value (PPV): 100% Negative Predictive Value (NPV): 97.30%. This was found to be statistically significant.

Table 6: Comparison of Stool Gene Xpert with GL Gene Xpert for MTB

Stool-Xpert	GL-Xpert		Total
	Detected	Not detected	
Detected	9 (7.5%)	0 (0%)	9 (7.5%)
Not detected	2 (1.7%)	109 (90.8%)	111 (92.5%)
Total	11(9.2%)	109 (90.8%)	120 (100%)

Chi Square Value-96.41 P Value- <0.001*

7.5% of cases were positive by both Stool-Xpert and GL-Xpert. 1.7% of cases were negative by Stool- Xpert but positive by GL- Xpert. 90.8% cases were negative by both Stool- Xpert and GL- Xpert. When compared with GL- Xpert, Stool- Xpert showed Sensitivity: 81.82% Specificity: 100% Positive Predictive Value (PPV): 100% Negative Predictive Value (NPV): 98.20%. This was found to be statistically significant.

DISCUSSION

In the present study, out of 120 gastric lavage samples, 12 (10%) samples were positive by LJ culture, 6 (5%) samples were positive by ZN stain and 11 (9.2%) samples were positive by GeneXpert analysis. Out of 120 stool samples, 9 (7.5%) samples were positive by GeneXpert analysis. Thus, highest positivity was seen on GL LJ Culture (10%) followed by GL Xpert (9.2%), Stool Xpert (7.5%) and GL ZN stain (5%). In the present study, out of 120 gastric lavage samples, 12 (10%) samples were positive by LJ culture. In a study by Hasan Z *et al.*[11] in 2017, out of 49 gastric lavage samples, 9 (18.3%) samples were positive by culture. In a study by Singh M *et al.*[12] in 2000, out of 58 gastric lavage samples, 10 (17.2%) samples were positive by LJ culture. These findings well correlated with present study. In a study by Çakır E *et al.*[13] in 2018, out of 40 gastric lavage samples, 9 (22.5%) samples were positive by LJ culture. These findings were discordant with present study. In the present study, when GL- ZN smear was compared with GL LJ culture, out of 120 gastric lavage samples, 5% of cases were positive by both GL- ZN smear and GL LJ Culture. Additional 5% cases were positive by GL LJ Culture alone. None of the cases were GL -ZN smear positive but GL LJ culture negative. Considering GL LJ culture as Gold Standard, GL ZN smear showed sensitivity of 50% and specificity of 100%. A study by Kalu EI *et al.*[14] in 2013, showed that considering culture as Gold Standard, the sensitivity and specificity of GL ZN smear was 29.9% and 97.6% respectively showing lower sensitivity than the present study but comparable specificity. In a study by Bahammam A *et al.*[15] in 1999, the sensitivity and specificity of GL ZN smear was 19% and 100% respectively when compared to culture showing lower sensitivity than the present study but similar specificity. In a study by Gómez Pastrana Durán D *et al.*[16] in 2000, the sensitivity and specificity of GL ZN smear was 13% and 96.8% respectively when compared with culture showing lower sensitivity than the present study but comparable specificity. The result of the present study was in concordance with the study conducted by Arora A *et al.*[17] in 2018 which showed that considering culture as Gold Standard, the sensitivity and specificity of GL ZN smear was 47.6% and 98.7% respectively. These differences are quite likely due to paucibacillary nature of the sample. In the present study, the detection rate of GL- ZN smear was found to be statistically significant when compared to GL LJ culture [P value- <0.001*]. In the present study, when GL- Xpert was compared with GL LJ culture, out of 120 gastric lavage samples, 9.2% of cases were positive by both GL-Xpert and GL LJ Culture. 0.8% of cases were negative by GL- Xpert but positive by GL LJ Culture. Considering GL LJ culture as Gold Standard, GL GeneXpert showed sensitivity of 91.67% and specificity of 100%. A study by Hasan Z *et al.*[11] in 2017 showed that considering culture as Gold Standard, GL GeneXpert had sensitivity of 100% and specificity of 95%. This was in concordance with the present study. A study by Memon SS *et al.*[18] in 2018 showed that the sensitivity and specificity of GL Xpert with reference to culture was 76.92% and 77.03% respectively. This finding was discordant with the present study. In the present study, the detection rate of GL GeneXpert was found to be statistically significant when compared to GL LJ culture [P value- <0.001*].

Comparison of Stool GeneXpert with Conventional Methods

Comparison of Stool GeneXpert with GL ZN smear

In the present study, when Stool GeneXpert was compared with GL ZN smear, out of 120 cases, 5% of cases were positive by both Stool-Xpert and GL ZN stain. 2.5% of cases were positive by Stool- Xpert but negative by GL ZN stain. When compared with GL ZN stain, Stool GeneXpert showed sensitivity of 100% and specificity of 97.37%. Results obtained by Kokuto *et al.*.[19] in 2015 in adults, revealed that the sensitivity of the Stool GeneXpert was 100% for detection of MTB in specimens from sputum AFB smear positive (1+ to 3+) patients, 81.0% in specimens from sputum AFB smear scanty positive patients, and 50.0% in specimens from sputum AFB smear negative patients with 100% specificity. This was comparable with the present study. These differences are quite likely due to paucibacillary nature of the sample. In the present study, the detection rate of Stool- GeneXpert was found to be statistically significant when compared to GL ZN stain [P value- <0.001*].

Comparison of Stool GeneXpert with GL LJ culture

In the present study, when Stool GeneXpert was compared with GL LJ culture, out of 120 cases, 7.5% of cases were positive by both Stool-Xpert and GL LJ Culture. 2.5% of cases were negative by Stool- Xpert but positive by GL LJ Culture. Considering GL LJ culture as Gold Standard, Stool GeneXpert showed sensitivity of 75% and specificity of 100%. In a study by Moussa HS *et al.*.[5] in 2016, Stool GeneXpert had a sensitivity of 83.33% and specificity of 98.73% when compared with culture

which were in concordance with present study. In a study by Hasan Z *et al.*[11] in 2017, when compared with culture, Stool GeneXpert had a sensitivity of 88.9% and specificity of 95% showing higher sensitivity than the present study but comparable specificity. In a study by Memon SS *et al.*[18] in 2018, the sensitivity and specificity of stool GeneXpert was 11.53% and 98.65% as compared to culture, showing lower sensitivity than the present study but comparable specificity. In a study by Walters E *et al.*.[20] in 2012, the sensitivity was 75% as compared to culture positive cases which was in concordance with the present study. In a study by Nicol MP *et al.*[21] in 2013, the sensitivity and specificity of stool GeneXpert was 47.1% and 99% as compared to culture, showing lower sensitivity than the present study but comparable specificity. In the present study, the detection rate of Stool- GeneXpert was found to be statistically significant when compared to GL LJ culture [P value- <0.001*].

Comparison of Stool GeneXpert with GL GeneXpert

In the present study, when Stool GeneXpert was compared with GL GeneXpert, out of 120 cases, 7.5% of cases were positive by both Stool-Xpert and GL-Xpert. 1.7% of cases were negative by Stool- Xpert but positive by GL- Xpert. When compared with GL- Xpert, Stool-Xpert showed sensitivity of 81.82% and specificity of 100%. In a study by Hasan Z *et al.*[11] in 2017, Stool GeneXpert versus GL GeneXpert had sensitivity 81.8% and specificity 94.7%. This finding was concordant with the present study. In the present study, the detection rate of Stool- GeneXpert was found to be statistically significant when compared to GL GeneXpert [P value- <0.001*]

CONCLUSION

Stool specimen for GeneXpert can be used to detect pulmonary TB in children as an alternative to Gastric Lavage specimen for GeneXpert for its ease of collection.

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