

## Evaluation of Gene Xpert Test of Stool Specimen with that of Induced Sputum as Gold Standard

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### Abstract

### Original Research Article

**Background:** Globally Tuberculosis (TB) is a major infectious disease. In 2011, there were an estimated 8.7 million new cases with 6% childhood tuberculosis. The majority of the cases occurring in high TB burden countries worldwide. Confirmed diagnosis of childhood TB remains challenging for physicians. MT test and Chest X-ray usually has very little diagnostic value for the diagnosis of pulmonary tuberculosis in children who has symptom criteria suggestive of PTB. **Objectives:** This study was done to evaluate of Gene Xpert Test of Stool Specimen with that of Induced Sputum as Gold Standard. **Methods:** The cross-sectional study was conducted in the Department of Pediatrics, Sir Salimullah Medical College & Mitford Hospital from January 2020 to June 2021. A total of 48 subjects of both sexes with the age range from 1 month to 59 months were included in the study. Data were collected over a period of eleven months and analyzed by appropriate computer based programmed software Statistical Package for the Social Sciences (SPSS), version 22. **Results:** In this study, most of the children 14 (29.2%) lies between 13 months to 24 months. About most of the children 28 (58.33%) were male and 20 (41.67%) children were female. According to the clinical presentation majority of the children present with fever 39(75.0%), followed by cough (72.9%) and weight loss (50%), history of contact was present in 15 (31.3%) children. Chest X-ray of 54% children was suggestive of PTB (as Consolidation, Millitary mottling, patchy opacity etc) 46% showed normal X-ray findings. Gene X-pert of Induced sputum could detect M.Tuberculosis in case of 12.5% children and in 87.5% cases Gene X-pert could not detect M.Tuberculosis in induced sputum. M.Tuberculosis was detected by Stool X-pert in 18% of children who were clinically diagnosed as PTB. In 82% of patient stool X-pert could not detect M.Tuberculosis. **Conclusion:** Gene Xpert test of stool specimen is a relatively easy test for the diagnosis of pulmonary tuberculosis in younger children (below 5 years).

**Keywords:** Gene Xpert, *Mycobacterium tuberculosis*, Pulmonary tuberculosis, Children.

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## INTRODUCTION

Even after being declared a worldwide public health emergency for more than 20 years, tuberculosis (TB) is still a significant public health concern [1]. The World Health Organization (WHO) estimates that tuberculosis remains the second leading cause of death among the infectious diseases after Human immune Deficiency Virus (HIV) and that almost one third of the world's population (2.5 billion people) is infected with mycobacterium Tuberculosis. It is estimated that more than 1.3 million people die each year from TB [2].

According to the 2016 WHO Global Tuberculosis Report, estimated new cases of tuberculosis

(TB) were 10.4 million, of which about 1.0 million were children (<15 years of age). Children under 15 years of age account for about 33.8% of total population of Bangladesh. In 22 high-burden nations worldwide, children under the age of 15 are expected to contribute between 4 and 22 percent of the illness burden with proper diagnosis and reporting systems in place. 7984 (4.01%) of the 199,001 cases of tuberculosis overall in 2015 were in children; this percentage was 3.35% in 2014, 2.78% in 2013, and 3% in 2012 [3].

Undiagnosed or delayed diagnosis of adult TB contributes significantly to the childhood TB burden by creating a reservoir for child transmission [4]. Moreover, because of their developing immune systems, youngsters

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can develop tuberculosis (TB) extremely quickly (Newton *et al.*, 2008). Therefore, quicker treatment and better results might be possible with early identification of tuberculosis in children [5]. However, the diagnosis of tuberculosis (TB) in children is not as simple as it is in adult patients. Therefore, a comprehensive evaluation of all the information obtained from a meticulous history, clinical examination, and pertinent investigation is necessary. Examples of these investigations include smear microscopy, chest X-rays, Mantoux tests (MT), and other tests [3]. According to National recommendations for the management of tuberculosis in children (2016), a positive MT test does not always imply active illness; it can also be negative in children who are malnourished or in other vulnerable conditions. Moreover, the reading of the test of tuberculin test requires experience and care. Inexperience can lead to error. So, there may be a large chance of misinterpretation of MT test [2].

According to National guidelines for the management of tuberculosis in children, 2016, Chest X-ray detection of pediatric pulmonary TB is also quite challenging. Because X-rays in children are frequently interpreted differently and are not always specific. Variable nonspecific signs may be discovered; however, they do not necessarily point to a current illness. Instead, they may suggest PTB. According to WHO, a chest X-ray has a relatively low specificity of 52% (55–58) for confirming the diagnosis of pulmonary tuberculosis. Therefore, a chest X-ray and MT alone cannot be used to diagnose pulmonary tuberculosis in children. To diagnose pulmonary tuberculosis, respiratory specimens such as sputum, bronchoalveolar lavage, or gastric lavage must be obtained. Microscopy testing has a high specificity (98%) and sensitivity (63%) for detecting AFB [2]. Culture test is gold standard to detect M.Tuberculosis.

However, as children are less prone to develop cavity lesions in their lungs that would confine the bacilli, their sputum is frequently paucibacillary [6]. For young children, sputum microscopy may not always be feasible since they are unable to cough up enough material for microscopic inspection. Therefore, there is currently a big effort to collect sputum samples from older children who are able to produce sputum. Sputum induction has been shown to be a successful technique

for collecting specimens from younger children these days [3]. Testing early morning stomach aspirates is another method for diagnosing tuberculosis in children [7]. However, the intrusive specimen collecting process results in a diagnostic yield of just 20–40% [4].

As a result, children are frequently treated empirically for tuberculosis (TB) based on clinical characteristics, results from chest X-rays, tuberculin skin tests, and contact with an index patient. This method could result in both excessive and insufficient therapy [8]. When children cough, they frequently ingest the sputum, which allows M. tuberculosis to persist in the digestive system [9]. Thus, in order to diagnose pulmonary tuberculosis in younger children, a stool specimen can identify M. tuberculosis. Thus, it is simple to gather samples in clinics in the field [10]. Furthermore, the disease can advance quickly in young children and take several weeks for a culture to confirm the diagnosis. Therefore, quick diagnosis techniques as PCR (Gene Xpert) MTB/RIF represent a significant advancement [11].

## METHODOLOGY

The cross-sectional study was conducted in the Department of Pediatrics, Sir Salimullah Medical College & Mitford Hospital from January 2020 to June 2021. A total of 48 subjects of both sexes with the age range from 1 month to 59 months were included in the study according to the physical sign-symptoms suggestive of pulmonary Tuberculosis along with history of contact within last 12 months. Tuberculin test & Chest X-ray were advised to all of the subjects. Then children were enrolled who fulfill the criteria 3 or more. After that induced sputum & stool sample was collected from each subject & was sent for AFB microscopy, PCR (Gene Xpert) & culture to the icddr'b laboratory. Data were collected over a period of eleven months and analyzed by appropriate computer based programmed software Statistical Package for the Social Sciences (SPSS), version 22.

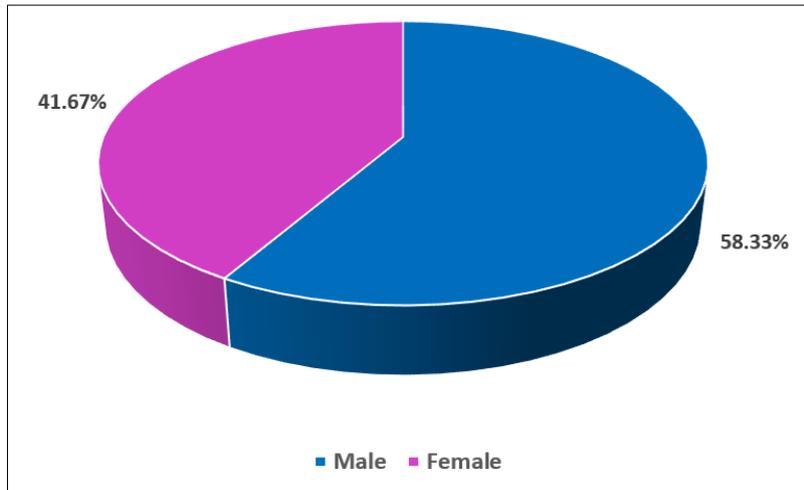
## RESULT

Table I shows age distribution of the children, where most of the children 14 (29.2%) lies between 13 months to 24 months.

**Table I: Distribution of the patients according to age (n = 48)**

Age (in months)	Frequency	Percent
1 - 12	12	25.0
13 - 24	14	29.2
25 - 36	6	12.5
37 - 48	11	22.9
49 - 59	5	10.4
<b>Total</b>	<b>48</b>	<b>100.0</b>

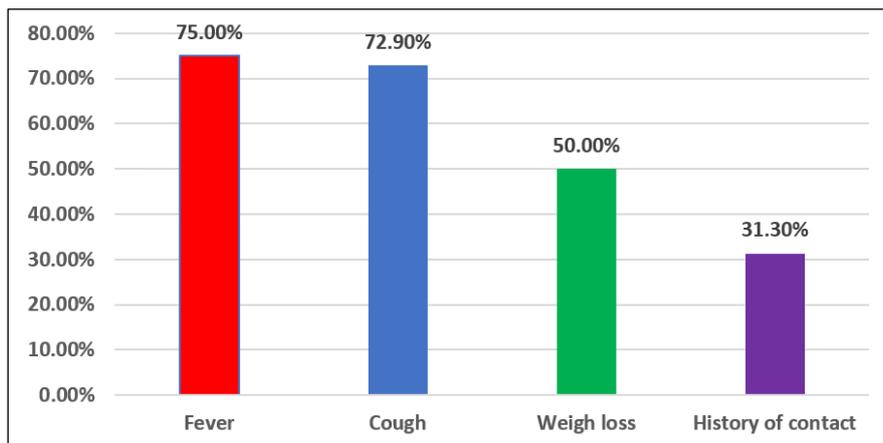
This figure shows that most of the children 28 (58.33%) were male and 20 (41.67%) children were female.



**Figure I: Distribution of the patients according to sex (n=48)**

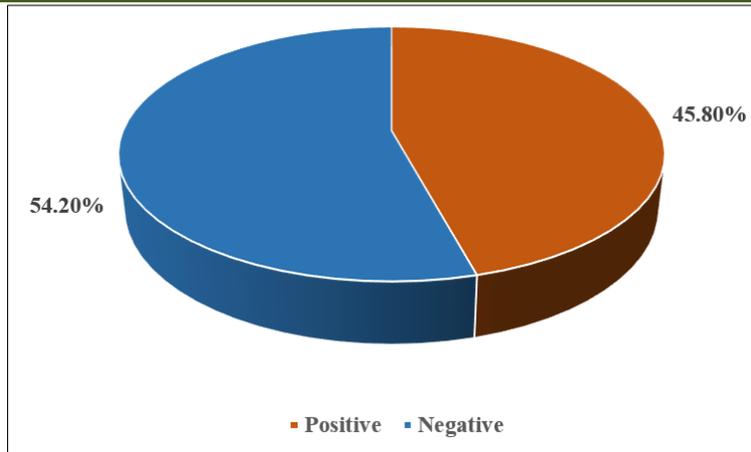
Figure II this Bar diagram shows the distribution of patients according to symptoms, where most of the children 36 (75.0%) presented with Fever,

then cough 35 (72.9%), weight loss 24 (50%) and History of contact was present in 15 (31.3%) children.



**Figure II: Distribution of patients according to the symptoms criteria**

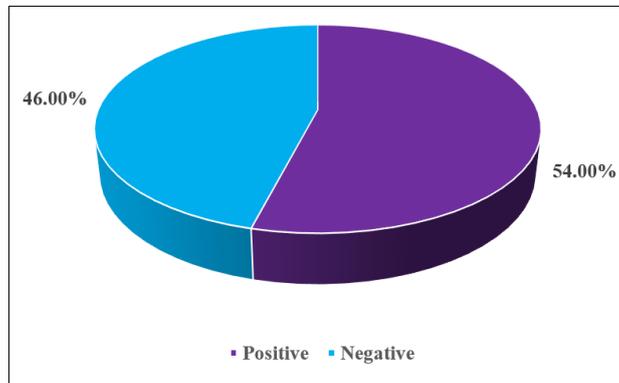
Figure III shows that 45.8% of children had positive MT test.



**Figure III: Distribution of patients according to MT test (n=48)**

Figure IV shows Chest X-ray of 54% children was suggestive of PTB (as Consolidation, Millitary

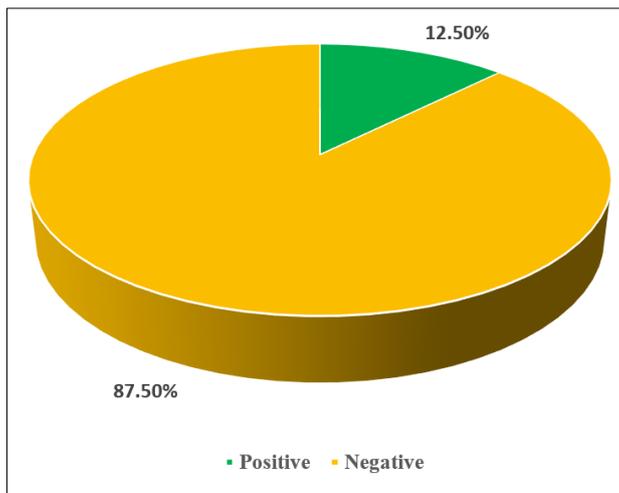
mottling, patchy opacity etc) 46% showed normal X-ray findings.



**Figure IV: Distribution of the patients according to chest X-ray (n = 48)**

Figure V shows Gene X-pert of Induced sputum could detect M. Tuberculosis in case of 12.5% children.

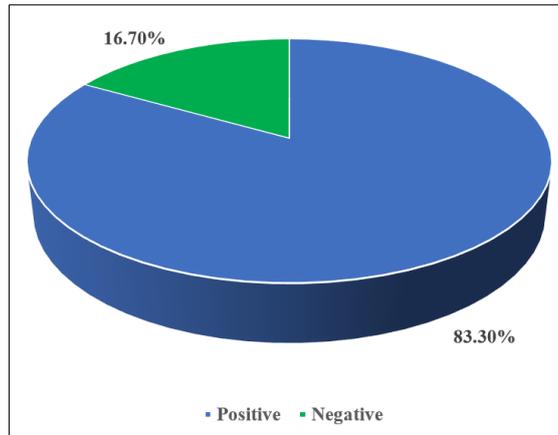
In 87.5% cases Gene X-pert could not detect M. Tuberculosis in induced sputum.



**Figure V: Distribution of the patients according to Gene X-pert test of induced sputum (n = 48)**

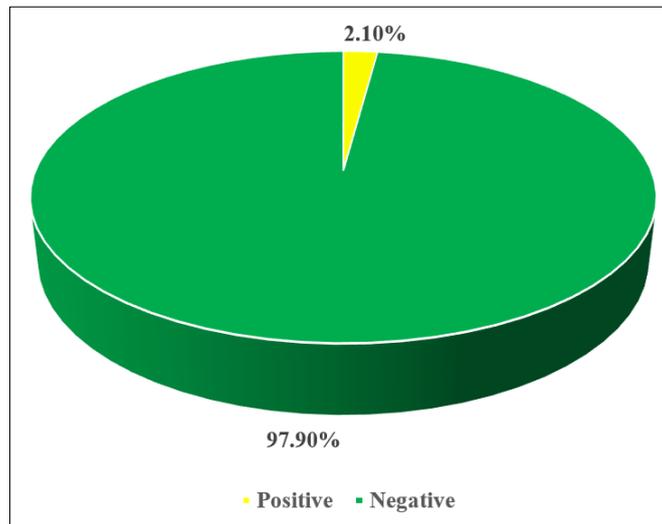
Figure VI shows that M. Tuberculosis was detected by Stool X-pert in 16.7% of children who were

clinically diagnosed as PTB. In 83.3% of patient stool X-pert could not detect M. Tuberculosis.



**Figure VI: Distribution of the patients according to Gene X-pert test of stool (n = 48)**

Figure VII shows only one patient had positive C/S of MTB in stool specimen.



**Figure VII: Distribution of the patients according to culture test of stool for MTB (n = 48)**

## DISCUSSION

Among the 22 countries with the highest rate of tuberculosis cases worldwide, Bangladesh is one of them. The majority of tuberculosis patients are under the age of 15, which primarily leads to pulmonary tuberculosis. The majority of children remain underdiagnosed because pulmonary tuberculosis in children lacks identifiable signs and symptoms, and since chest X-rays and MT tests have limited diagnostic utility. Therefore, in these situations, a quick and simple method for diagnosing childhood PTB is required. The use of PCR (Gene Xpert) as a preliminary test for the diagnosis of PTB in children was advised by the National Guidelines for Childhood TB. Still the diagnosis of PTB in children has typically been low due to the challenge of getting appropriate specimens for testing.

This Cross-sectional study was done in the Department of Pediatrics in Sir Salimullah Medical College and Mitford Hospital, Dhaka during the period from January 2020 to June 2021 to evaluate of Gene Xpert Test of Stool Specimen with that of Induced Sputum as Gold Standard.

In this study, according to the clinical presentation majority of the children present with fever 39(75.0%), followed by cough (72.9%) and weight loss (50%), history of contact was present in 15 (31.3%) children. Which is similar with the study of Welday *et al.*, 2014, where fever was 88%, cough (84%) and weight loss (45%) [10].

Hasan *et al.*, 2017 showed that in under 5 children contact history was present in 52%, and in this

study contact history was present in 16 children that is 32% [12].

In the present study MT test was positive in 22 children (45.8%) and Chest x-ray suggestive of PTB was in 26 children (54.2%). But among the 48 children 11 children were confirmed diagnosis of pulmonary tuberculosis by induced sputum and stool PCR (Gene Xpert) and culture that is 22%.

In the present study PCR (Gene Xpert) test of induced sputum detected M.Tuberculosis in 6 children out of 48 that is 12.5% and stool PCR (Gene Xpert) detected MTB in 8 children out of 48, that means 16.7%. Among the 8 children of stool Gene Xpert positive, the result of Gene Xpert test of IS was also positive in 2 children. That means stool PCR detected M.Tuberculosis solely in 7 children. But it is very unlikely to get more M.Tuberculosis in stool than in induced sputum. It may be due to the collection technique as the children did not allow to collect the induced sputum, it have to collect forcefully, so, there may be inadequate amount of induced sputum to detect M.Tuberculosis, as MTB is paucibacillary in children. But in case of collection of stool there is no such problem as it is a natural process.

In another cross-sectional study in Durban showed that stool GeneXpert was positive in 68% and IS Gene Xpert was positive in 79%, where p value was 0.24, not significant [13].

The present study detected MTB on stool culture only in 1 patient out of 48 (2.1%) and no MTB was detected on IS culture. A Cohort study was done in Cape town where showed that Stool culture was positive in 6/37 (16.2%) children with confirmed TB, where sensitivity was 33.3% (95% CI 11.8 to 61.6%). They conclude stool culture for TB diagnosis cannot currently be recommended for the diagnosis of PTB in children [14].

## CONCLUSION

Gene Xpert test of stool specimen is a relatively easy test for the diagnosis of pulmonary tuberculosis in younger children (below 5 years). Gene Xpert provided timely results with quite acceptability.

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