RESEARCH ARTICLE

Evaluation of Rifampicin-resistant Tuberculosis in Pediatric Patients by GeneXpert MTB/RIF

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ABSTRACT

Objectives: India has the largest burden of MDR-TB worldwide, with an annual incidence of 1,30 000 patients. Multidrug-resistant tuberculosis (MDR-TB) carries a poor prognosis, a high mortality rate, and treatment success rates as low as 65%. The mortality in India is estimated to be about 480,000 per year. The aim of the study was to evaluate Rifampicin-resistant Tuberculosis and its distribution by GeneXpert in a tertiary care hospital.

Methods: A total of 2864 samples were processed from the patients attending outpatient departments and indoor wards as per the pediatrician's request. Acid-fast bacilli (AFB) smear microscopy was done on all samples by Acid-fast staining for early diagnosis followed by GeneXpert MTB/RIF (CBNAAT) testing. Specimens were transported and stored at 2–8 °C prior to processing for CBNAAT. Results were read and reported within 2 hours.

Results: A total of 2864 samples were tested for TB using CBNAAT (including 645 [22.5%] extra-pulmonary and 2219 [77.5%] pulmonary samples). The test results were positive in a total of 346 (12%) samples by CBNAAT. The positivity is highest in the age group >10 years and in sputum samples (37.28%) followed by pus samples (23.26%) in pulmonary and extrapulmonary distribution. Out ZN staining was positive in 244 (8.5%) specimens. There were 102 (29.5%) CBNAAT positive specimens which showed negative results for Acid-fast bacilli (70.52% Sensitivity). Among 346 TB-positive patients, 10.46% samples were pulmonary and 17.67% were extrapulmonary. It was observed that in positive CBNAAT patients, the prevalence of Rifampicin resistance was 12.72% i.e. 44 samples which include 31(13.36%) and 13(11.40%) pulmonary and extra-pulmonary samples respectively. Among the Rifampicin resistant samples, there were 29.55% samples that were extrapulmonary.

Conclusion: GeneXpert MTB/RIF is a very rapid diagnostic assay that provides information regarding the mutation pattern of RIF resistance in MTB isolates. *J Microbiol Infect Dis 2021; 11(2):81-87.*

Keywords: *CBNAAT, Tuberculosis, GeneXpert MTB/RIF*

INTRODUCTION

Tuberculosis is considered one of the major causes of mortality worldwide [1]. India accounts for the highest-burden of TB (27%) and MDR-TB (24%) as per the Global Tuberculosis Report 2018 [2]. Drug-resistant tuberculosis is caused by genetic mutation of bacilli, an inadequate or poorly administered treatment regimen, and weak services program that leads to delay in detection and effective treatment of drug resistance and are unequipped to support patients to keep adherence to treatment [3].

Multidrug-resistant tuberculosis (MDR-TB), defined as tuberculosis caused by strains of Mycobacterium tuberculosis (MTB) resistant to the two most important antituberculous drugs – isoniazid and rifampicin – carries a poor prognosis, a high mortality rate, and treatment success rates as low as 65% [4]. The mortality in India is estimated to be about 480,000 per year [5]. The exact data of pediatric MDR cases is not available due to the is not There is variation in the viral content of tuberculosis. As it can be a low viral load that limits the detection rate of Multidrug-resistant Tuberculosis. The difficulty in

the diagnosis of the disease can be observed due to the low bacterial content [6]. According to some studies in 2010, there has been an estimation of 850,000-1,000,000 cases of tuberculosis, out of which cases of MDR-TB had been observed to be around 25,000-32,000 [7-8].

Extrapulmonary TB accounts for about 25% of all cases of TB and an even higher percentage of cases in children and in people who are immunocompromised. Diagnosing extrapulmonary TB is often challenging, requiring the clinician to obtain specimens for microscopy, culture, and histopathology from the suspected sites of involvement [9]. Diagnosis of pulmonary TB in children is also challenging, more so in resource-limited, tuberculosis-endemic countries, and is largely based on clinical and radiological findings and medical history [10]. Isolation of mycobacteria by culture, while considered as the gold standard for diagnosing TB, takes 4–8 weeks and often requires expensive and sophisticated laboratory facilities which cannot be afforded in most resource-limited settings [11]. Due to poor sensitivity and specificity, it is not recommended to use antigen and antibody TB ELISA tests for the diagnosis of tuberculosis [12]. The latent TB infection is being detected by Interferon-gamma release assays (IGRAs) which had replaced skin tests in low prevalence countries. However, these tests are expensive and lack in differentiation the TB infection from disease. In case of a high burden, the use of these tests is still not clear [13].

There is quite a variation seen in the specificities and sensitivities of the polymerase chain reaction (PCR) based diagnostic tests [13]. Further, these tests involve multiple manual steps and long turnaround times, making them unsuitable for decentralized deployment. A series of meta-analyses have shown cartridge-based nucleic acid amplification test (CBNAAT)/ Xpert MTB/ RIF to have a high specificity with variable sensitivity in different types of specimens for TB diagnosis [14]. In 2013, the WHO endorsed the use of CBNAAT for TB diagnosis in pediatric presumptive pulmonary and extra-pulmonary tuberculosis (EPTB) cases [15].

The recent introduction of the CBNAAT assay has significantly revolutionized the diagnostics of tuberculosis in adults, but its application for the diagnosis of pediatric TB is under evaluation. To date, there are only a few studies on the application of CBNAAT for the diagnosis of pediatric tuberculosis in India, more so in eastern India. The Gene Xpert MTB/Rif assay is a cartridge-based fully automated NAAT (nucleic acid amplification test) for TB case detection and rifampicin resistance testing, suitable for use in disease endemic countries. This nucleic acid amplification test (CBNAAT) is a recently introduced polymerase chain reaction-based method for the detection of TB. It is an MTB-specific automated, cartridge-based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real-time PCR, providing results within 100 minutes [16]. It has a highly specific primer and five unique molecular probes to target the rpo B gene of M. tuberculosis, which is the critical gene associated with rifampicin resistance [17]. No cross-reactions have been observed with many other bacterial species tested, including a comprehensive band of mycobacteria, thereby excluding non-tubercular bacteria. This study was conducted for the knowledge of the pattern of mutations present in RIF-resistant isolates which can provide information regarding the epidemiology of RIF-resistant MTB isolates of this particular area.

The aim of the study was to evaluate Rifampicin-resistant Tuberculosis and its distribution by GeneXpert in a tertiary care hospital.

METHODS

This retrospective study was conducted in the Department of Microbiology, Lady Hardinge Medical College, New Delhi from January 2019 to December 2019. The Lady Hardinge Medical College has accepted samples for CBNAAT testing from connected 377 bedded Kalawati Saran Children Hospital which is limited to pediatric patients. The retrospective data of 1 year was collected from the patients' records maintained in the laboratory. In the study period, a total of 2864 samples were collected and processed from the pediatric patients attending outpatient departments and indoor wards as per pediatrician request in the age group. Acid-fast bacilli (AFB) smear microscopy was done on all samples by Acid-fast staining for early diagnosis followed by CBNAAT testing.

Specimen Collection and Processing

Pulmonary samples and Extrapulmonary samples (CSF, Ascitic Fluid, Pus, Pleural Fluid, Urine, Synovial Aspirate, Lymph node aspirates, Pericardial fluid) were collected in leak-proof sterile containers and under aseptic precautions, wherever necessary. The samples were processed as soon as possible. In case of delay, they were refrigerated at 4°C for not more than 24 hours and then processed accordingly. All specimen processing was done in Biosafety cabinet class II A2.

AFB Staining

Ziehl-Neelsen (ZN) staining was performed according to the standard protocol [18]. From each sample, a smear was made on a new, clean, unscratched glass slide. Smear was allowed to air-dried and fixed by heat then the slide was stained by Z-N stain and examined under immersion oil (x100). Acid-fast bacilli appear bright red with a beaded appearance.

GeneXpert

Processing of specimens for CBNAAT was done as per standard protocol given by WHO [19]. According to the standard operating procedure of GeneXpert MTB/RIF, the sampling reagent (containing NaOH and isopropanol) was added at 2:1 ratio to the sample and then incubated for 10 minutes at room temperature with intermittent vigorous shaking After that 2 ml of sample reagent mixture was transferred into GeneXpert cartridge and loaded into GeneXpert instrument. Results were obtained after 2 hours, the presence or absence of MTB was reported by the systems with the bacterial load as very low, low, medium, or high and simultaneously giving results for susceptibility to rifampicin drug.

RESULTS

A total of 2864 samples were tested for TB using CBNAAT (including 645 [22.5%] extra-pulmonary and 2219 [77.5%] pulmonary samples). The test results were positive in a total of 346 (12%) samples by CBNAAT. The positivity is highest in age group >10 years (44.8%) followed by 6-10 years (37.6%), <=2 years (11.6%), and 3-5 years (6%) with majority occurrence of Tuberculosis in the females 183(53%). The specimen distribution in CBNAAT positive is contributed maximum by Sputum samples (37.9%) followed by Pus

samples (23.7%), Gastric aspirate (18.2%), Lymph node aspirates (17.6%), CSF (2%), Pleural fluid (0.9%), Bronchoalveolar lavage (0.9%) and Ascitic fluid (0.6%) (Figure 2). ZN staining was positive in 244 (8.5%) specimens. Among extrapulmonary samples, maximum positivity is seen in Lymph node aspirates (44.79%).

Table 1. Age distribution of GeneXpert MTB/RIF positive samples (N=346).

Age Groups	GeneXpert MTB/RIF Positive
≤2 Years	40 (11.6%)
3-5 Years	21 (6%)
6-10 Years	130 (37.6%)
>10 Years	155 (44.8%)

There were 102 (29.5%) CBNAAT positive specimens which showed negative results for Acid-fast bacilli (Table 2). With CBNAAT as the gold standard, the sensitivity and specificity of the ZN staining were observed to be 70.52% and 100% respectively while the positive predictive value and negative predictive value of ZN staining were found to be 100% and 96.1% respectively. Among the TB-positive patients, 10.46% samples were pulmonary and 17.67% were extrapulmonary (Figure 1). The Rifampicin resistance was seen in 44(12.72%) patients which included 29.55% extrapulmonary samples (Figure 3). Among the CBNAAT positive samples, the prevalence of Rifampicin resistance was 31(13.36%) and 13(11.40%) in pulmonary and extra-pulmonary respectively (Table 3) with maximum resistance seen in the Sputum samples (50%) and followed by Pus (34%), lymph node aspirates (7%) and Gastric Aspirates (5%).

Table 2. Distribution of positivity of ZN staining in comparison to GeneXpert MTB/RIF samples (n=2864).

Variables	CBNAAT Positive	CBNAAT Negative	Total
ZN Staining Positive	244	0	244
ZN Staining Negative	102	2518	2620
Total	346	2518	2864

DISCUSSION

CBNAAT (Xpert MTB/RIF assay) is a very rapid and accurate diagnostic tool for the early detection of TB. In our study prevalence of Mycobacterium Tuberculosis by CBNAAT among 2864 samples was observed to be 346 (12%). This is similar to studies done in Ethiopia (13.6%) [20] and South Africa (13%) [21] and Uganda (14%) [22]. There has been increasing positivity of tuberculosis in the age group of >10 years [23] which is similar to the finding in our study.
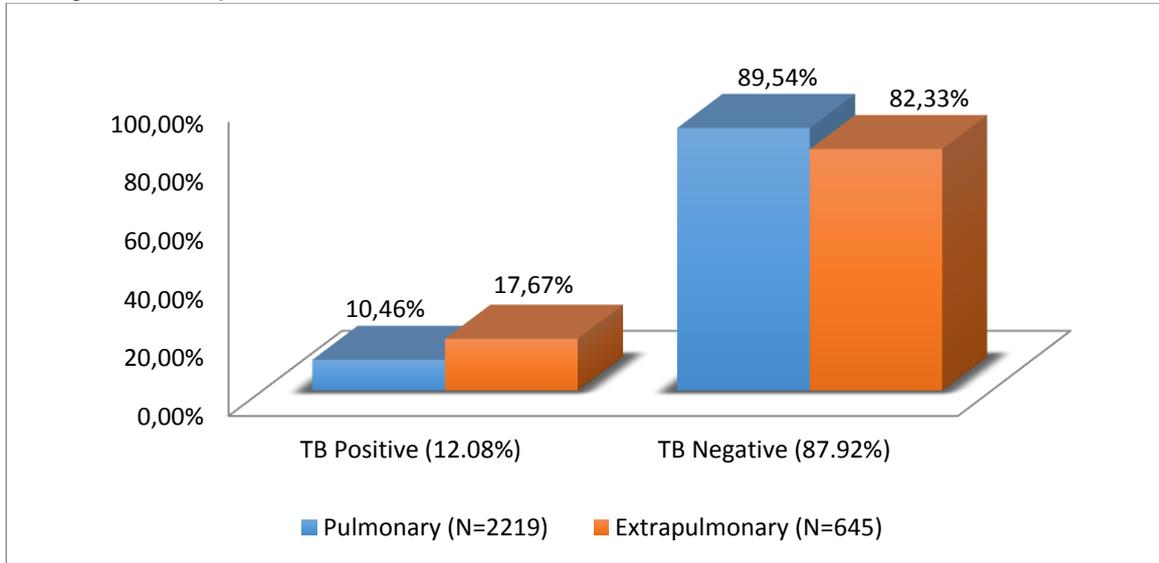

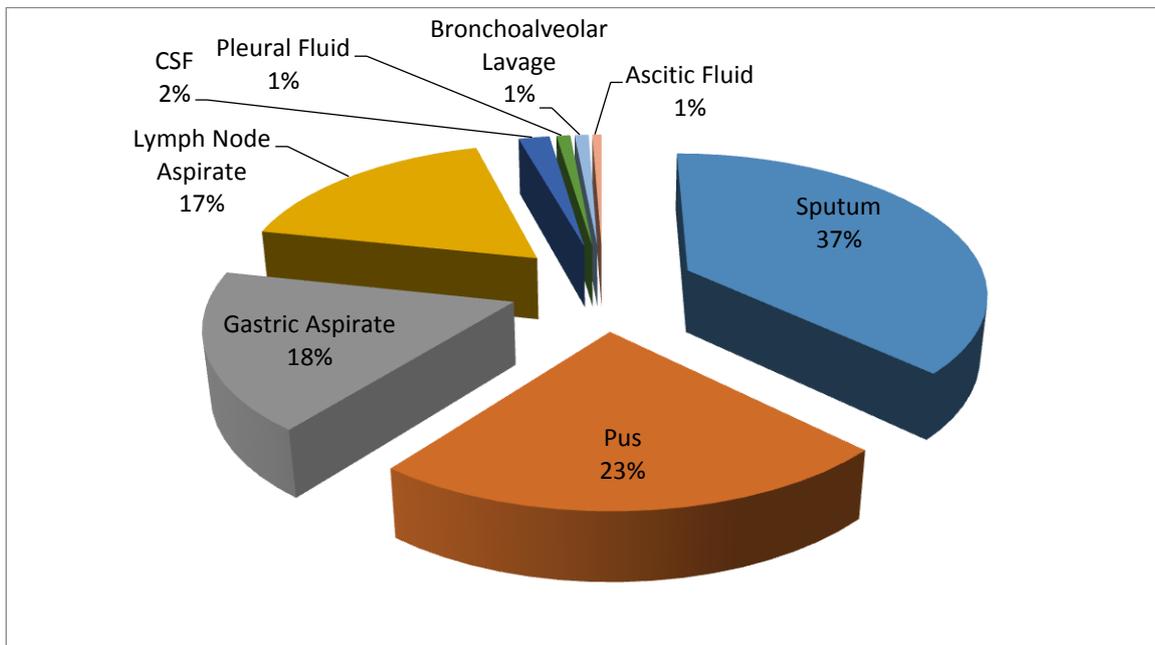Figure 1 : Broad distribution of CBNAAT positivity (n=2864).

Figure 2. Distribution of CBNAAT positivity contribution according to samples (n=346).

Table 3. Distribution of susceptibility of Rifampicin according to Positive CBNAAT samples (n=346).

Variables	Source of M. tuberculosis	Rifampicin-Resistant	Rifampicin-Sensitive	Total
Positives	Pulmonary	13.36%	86.64%	100.0%

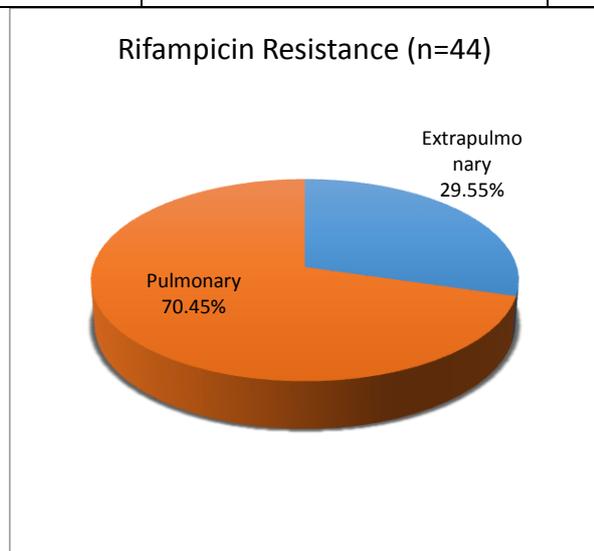|	Extrapulmonary	11.40%	88.60%	100.0%
Total	Pulmonary + Extrapulmonary	12.72%	87.28%	100.0%

Figure 3. Distribution of TB positivity among Rifampicin resistance samples.

There is as such no proper reason for the increasing cases in this age group. A possible hypothetical reason may be the trigger induced by various variables in cognition or behavior may have led to tuberculosis. Among the extrapulmonary samples, Lymph node aspirate has shown the highest positivity which is almost similar (42.8%) according to a study done in Bangalore, India [24].

By using Xpert MTB/RIF assay, including Rifampicin resistance, a surrogate marker for MDR strains, we observed RIF resistance which is a surrogate marker of MDR-TB in 12.7% of suspected cases. This finding is similar to the studies reported in Jaipur (11.09%) [25] and Punjab (10%) [26].

Xpert will divert treatment away from false smear-negative cases" to true" smear-negative TB cases, thereby increasing the accuracy of treatment and cost-effectiveness, while reducing the burdens of toxicity and cost of treatment in patients who do not in fact have TB. In our study, there were 144 (29.5%) CBNAAT positive specimens that had shown negative results for Acid-fast bacilli (70% Sensitivity and 96.1% Negative predictive value). The findings were similar to the study done in Maharashtra which had shown that (8/25) 32% samples had been missed for TB diagnosis by AFB smear which was positive for CBNAAT [27].

Extrapulmonary Tuberculosis is also a significant contributor to Tuberculosis and should be diagnosed the earliest along with pulmonary samples. In an earlier review done in an Indian study, EPTB (Extrapulmonary Tuberculosis) was estimated to constitute about 15 to 20 percent of all cases of tuberculosis [28]. This is quite similar to our study which showed 17.67% positivity for extrapulmonary samples.

Multidrug-resistant cases have also been observed to be caused by extrapulmonary Tuberculosis which poses difficulty in the treatment. Our study has shown Extrapulmonary MDR tuberculosis (MDR-EPTB) prevalence of 11.40% which is almost similar to the reported prevalence in studies in Brazil (14%)[29] and Nepal(12.5%)[30]. It was also observed that rifampicin-resistant pulmonary cases (13.36%) were more as compared to extrapulmonary cases (11.40%) (Table 3). A study done by Chakraborty et al in India found similar results with 13.7% from pulmonary and 8.6% from extra-pulmonary cases being rifampicin resistant [31].

Conclusions

The problem of MDR in extrapulmonary tuberculosis (EPTB) cannot be overlooked as being a major health problem. The key factor being the development of MDR- TB which might be delayed and incorrect diagnosis which can impose an extra load of drug administration and its side effect which ultimately might lead to extensively drug-resistant tuberculosis (XDR-TB). Rapid diagnosis of drug resistance using Gene Xpert is crucial to the early treatment of drug-resistant tuberculosis and prevention of its transmission. This is a very useful assay for implementation in the TB control program as it is helpful for information regarding the mutation pattern of RIF resistance in MTB isolates which further provides an understanding of the epidemiology of the disease and identification of hot spots.

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