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Original Article

Prevalence and Diagnosis of Rifampicin-Resistant *Mycobacterium Tuberculosis* using the GeneXpert MTB/RIF Assay at a Tertiary Care Children's Hospital

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ABSTRACT

Rifampicin is a primary anti-tuberculosis medication. Diagnosing multidrug-resistant tuberculosis (MDR-TB) remains a persistent challenge when examining both pulmonary and extra-pulmonary samples. Rapid detection of rifampicin resistance is essential for the timely treatment and prevention of the spread of tuberculosis. Recently, GeneXpert MTB/RIF assay has emerged as an advanced technique for the rapid diagnosis of *Mycobacterium tuberculosis*. **Objectives:** To assess the prevalence of rifampicin resistance in samples from pediatric patients in Pakistan, encompassing both pulmonary and extra-pulmonary cases using GeneXpert MTB/RIF assay. **Methods:** A cross-sectional study was conducted in the Children's Hospital, Lahore for a duration of six months. Pulmonary and extra pulmonary samples of patients under age 16 were examined by GeneXpert MTB/RIF assay. **Results:** A total of 1320 samples were examined, and among them, 110 tested positive for *M. tuberculosis* (MTB) infection. Out of these 110 positive samples, 5 exhibited resistances to rifampicin, 18 showed indeterminate resistance, while rifampicin resistance was not detected in the remaining 87 samples. Additionally, 3 of the rifampicin-resistant samples displayed a very low load of MTB, and 2 samples exhibited a low MTB load. **Conclusions:** This study revealed 4.5% prevalence of MDR-TB in pediatric population. The study also showed that GeneXpert is a highly advanced technique for the diagnosis of rifampicin resistance in pulmonary and extra-pulmonary samples.

INTRODUCTION

In spite of many advances in the discipline of medicine, tuberculosis (TB) is still considered as one of the most lethal diseases. Moreover, the increasing trend in multidrug-resistant tuberculosis (MDR-TB) cases also bring significant challenges to control TB [1, 2]. It is concerning that Pakistan ranks fifth among nations with highest TB burden and fourth globally for MDR-TB cases (National TB Control Program - Pakistan) (https://ntp.gov.pk/) [3]. Pakistan has significant tuberculosis (TB) burden, with approximately 510,000 new cases emerging annually and around 15,000 developing drug-resistant TB each year. Pakistan accounts for 61% of the TB burden in the WHO's Eastern Mediterranean Region. The main factors contributing to the rise of drug-resistant TB in Pakistan include delayed diagnosis, inadequate or unsupervised treatment, poor follow-up, and a lack of social support programs for high-risk populations (WHO 2024). Rifampicin is considered to be one of the most effective first line anti-TB drugs. Other drugs used for treatment of TB include pyrazinamide, ethambutol, isoniazid and streptomycin [4]. Rifampicin has its mode of action by halting the transcription and elongation of RNA by binding to the RNA polymerase [5, 6]. Rifampicin resistance is therefore a surrogate marker in diagnosis of MDR-TB. Drug resistance

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against MTB is classified as monoresistance, MDR-TB and extensive drug resistant TB (XDR-TB). TB which is resistant to one of the drugs from first-line therapy (rifampicin, isoniazid, pyrazinamide and ethambutol) is called Mono-resistant TB while resistance against both rifampicin and isoniazid is classified as MDR-TB. XDR-TB is combination of MDR-TB plus resistance to one of the injectable drugs from the second line therapy. Resistance to rifampicin is due to mutation in RNA polymerase subunit B (rpoB gene) [6]. Drug resistant MTB is rapidly transmitted from an infected individual to other healthy individuals which leads to an increase in the frequency of MDR-TB. According to a survey, every undiagnosed, untreated active TB case can infect 10-15 healthy persons per year. So, in order to reduce transmission of infection, early diagnosis and detection of rifampicin resistance is very important [7]. In high incidence environments, where reinforcement is common, an undetermined percentage of previously treated TB patients have acquired MDR-TB unelated to their initial infection. Other risk factors for transmission of MDR-TB include close contact with the infected person and younger age. Minimum 18 to 24 months are required for the current standard treatment of MDR-TB using at least five drugs but none of them is as effective as rifampicin and all more toxic and less well tolerated [7]. Culture method and DST method are the gold standard methods to diagnose TB and drug resistance, but they have limitations as requirement of long time and high costs. The newer and faster technologies include microscopic observation, drug susceptibility assay, MTB growth indicator tube and calorimetric assays but the places where these techniques are most required frequently lack the specific knowledge and biosafety laboratories needed for these techniques [8]. Molecular assays such as GeneXpert have altered the panorama of diagnosis and proved to be economical, time saving solution to this issue worldwide. GeneXpert uses a real time PCR to detect the specific sequence for MTB as well as that of rifampicin resistance [9] and is considered a better approach than the conventional methods as Ziehl-Neelsen technique [10]. GeneXpert is a WHO endorsed, cartridge based novel diagnostic instrument that performs sample processing and real-time PCR analysis in a single cartridge for simultaneous diagnosis of TB infection and RIF resistance by PCR amplification of the resistance responsible fragment of the rpoB gene. It also identifies other possible mutations that are associated with RIF resistance [11]. The specificity and sensitivity of GeneXpert is found to be 98.3% and 93% [12].

This study aimed to detect rifampicin-resistant MTB and assess its prevalence in the pediatric population diagnosed with tuberculosis, using the GeneXpert assay.

METHODS

This cross-sectional study was conducted in the Children's Hospital during the period of six months from August 2022 to February 2023. A total of 1320 samples which were suspected of tuberculosis were examined and out of these 110 samples which were positive for Mycobacterium infection were examined to analyze rifampicin resistance. The patient population consisted of children under age 16 of both genders, admitted to hospital due to any infection. Subjects enrolled were considered irrespective of any other disease or history of disease. Patients with tuberculosis were confirmed with the help of GeneXpert MTB/RIF assay. Sample reagent and sample were added in 2:1 and allowed to stand for 5 min. Sample container was shaken for 10 to 20 min using forth and back movements and incubated for 10 min at room temperature. Xpert MTB/RIF cartridge was labelled with laboratory number on the side of cartridge. With the help of a transfer pipette, 2ml sample was transferred into the sample column of Xpert/MTB cartridge. Then cartridge was installed in the GeneXpert MTB/RIF assay machine. After 1 h and 50 min, results were displayed on the GeneXpert® Dx System. The used cartridge was discarded immediately. PPE kits, safety goggles, nitrile gloves and a procedure mask were used for this purpose. The data were entered and analyzed using IBM-SPSS version 23.0. Categorical variables like gender are described in the form of frequencies. Ethical approval was taken from the Institutional review board of the University of Child Health Sciences, The Children's Hospital, Lahore, under the (Ref no 1221/SAHS) before the conduction of this study. Confidentiality of each patient enrolled in this study was maintained. Patients consent was taken before enrolling in our study.

RESULTS

Total 110 eligible samples were analyzed using GeneXpert MTB/RIF assay following complete safety protocol. Out of 110 MTB detected patients, 5 patients were detected with rifampicin resistant MTB while 18 had indeterminate resistance and 87 patients were not detected with rifampicin resistance (Table 1).

Table 1: Frequency Distribution of Rifampicin Resistance

Rifampicin resistance	Frequency (%)
Not detected	87 (79,1)
Detected	05 (4.5)
Indeterminate	18 (16.4)
Total	110 (100)

In terms of the gender distribution among the patients, almost equal representation of tuberculosis cases in males 54 (49.1%) and 56 females (50.9%) were observed. Out of all the samples that tested positive for tuberculosis, five were found to exhibit rifampicin resistance. Among these cases, four were male patients, while one was female. This result indicates a higher prevalence of rifampicin resistance

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among males (80%) compared to females (20%). It was concluded that both genders exhibit a similar susceptibility to tuberculosis, indicating no discernible gender-based dominance in tuberculosis prevalence. However, when the distribution of rifampicin resistance was assessed among both genders, a higher incidence of resistance was observed among male patients in comparison to their female counterparts (Table 2).

Table 2: Frequency Distribution of Association between Gender and Rifampicin Resistance

Gender	Not Detected	Detected (%)	Indeterminate	Total (%)
Male	39	4 (80)	11	54 (49)
Female	48	1 (20)	7	56 (51)
Total	87	5 (100)	18	110 (100)

GeneXpert analysis also provides information on the MTB load in the detected samples. Among the five samples where rifampicin resistance was detected, two samples exhibited a low load of MTB, while three had a very low load of MTB. None of the samples with rifampicin resistant samples showed a high or medium load (Table 3).

Table 3: Frequency Distribution of Load of Rifampicin Resistant MTB in Patients with Tuberculosis

Load	Frequency (%)
Very low	3 (60)
Low	2 (40)
Total	5 (100)

Out of 110 MTB samples, 81 samples were pulmonary and 29 were extra pulmonary (frequency distribution given in Table 4). Out of these, 4 pulmonary and 1 extra pulmonary sample was detected with rifampicin resistance. Among rifampicin resistant pulmonary samples, one was sputum and 3 were gastric aspirate. While in extra pulmonary samples, only CSF was detected with rifampicin resistance (Table 4).

Table 4: Frequency Distribution of Rifampicin Resistant MTB in Specimens Collected from Patients with Tuberculosis

-	Specimen	Frequency	Rifampicin Resistant (%)
Pulmonary samples	Sputum	23	1 (1.2)
	Gastric Aspartate	57	3 (3.7)
	NG Aspirate	1	-
	Total	81	4 (4.9)
Extrapulmonary samples	Cerebrospinal Fluid	24	1 (3.4)
	Pericardial Fluid	1	-
	Pleural Fluid	4	-
	Total	29	1 (3.4)
	Sum Total	110	5 (4.5)

DISCUSSION

Diagnosing MDR-TB is consistently challenging in healthcare settings. The primary reason for the spread of TB is our inability to detect drug-resistant TB using current diagnostic methods. Rapid diagnosis, on the other hand, results in timely treatment, ultimately contributing to better epidemiological control [13]. Moreover, there are many disputations regarding the diagnosis of childhood TB, and it is often ignored by TB control programs [14]. Consequently, TB remains a leading cause of pediatric mortality, posing numerous threats that drive TB progression [15]. Here in this study, we utilized the GeneXpert MTB/RIF assay to diagnose rifampicin-resistant TB in samples from both the pulmonary and extra-pulmonary sources of children under the age of 16. Out of the 1320 suspected samples obtained from patients, 8.33% were confirmed to have tuberculosis. Among these confirmed TB cases, 4.5% displayed rifampicin resistance. Previously, somehow similar prevalence of rifampicin resistance has been reported in similar studies from the African countries as, Zambia (5.9%) [15], Nigeria (6.9%) [16] and Ethiopia (5.7-9 %) [16-17]. In present study, we observed an approximately equal distribution of male and female patients with tuberculosis. However, rifampicin resistant *Mycobacterium* exhibited a higher prevalence, with 80% of cases occurring in male patients. This gender-based disparity is consistent with findings from a similar study conducted in Nigeria, where rifampicin resistance was predominantly identified in the male population (65.5%) [17]. Furthermore, when examining the load of MTB in the rifampicin-resistant MTB samples, it was observed that the majority had very low or low MTB load. None of the samples exhibited a high load of MTB. This observation suggests that effective control of drug resistance can be achieved through improved and early diagnosis, as well as the appropriate use of antibiotics. Nevertheless, the specific factors contributing to this predominance of rifampicin resistance among male remain unidentified [18-19]. The prevalence of rifampicin resistance in pulmonary samples was determined to be 4.9% in this study, which closely aligns with findings from a previous study conducted in a Nigerian population (4%) [20]. In extrapulmonary samples, we observed a prevalence of 3.4% rifampicin resistance. It is also worth noting that in this study, the prevalence of indeterminate rifampicin resistance was substantially higher (16.3%) compared to findings from a study in an Indian rural community where a prevalence of 1.5% was reported [21].

CONCLUSIONS

The prevalence of rifampicin-resistant MTB in pediatric population of Pakistan as diagnosed using the GeneXpert assay is 4.5%. Notably, all the patients included in this study who exhibited rifampicin-resistant MTB had low or very low MTB load. This finding underscores that the rifampicin resistance is posing a fitness cost on *Mycobacterium* and the resistant organisms are weakly stable in nature till date. The rifampicin-resistant MTB in our community can,

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therefore, be controlled through the implementation of precise antibiotic dosing protocols.

Authors Contribution

Conceptualization: AZ, NM[1]
Methodology: RJ, IA
Formal analysis: NM[2], AT
Writing, review and editing: IA, AT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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