Original Article
DOI: https://doi.org/10.70749/ijbr.v3i7.2004

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Prevalence of Rifampicin Resistance in Pediatric Patients with Mycobacterium Tuberculosis Using Gene Expert in Gulab Devi Hospital

Ayesha Tariq[1], Muhammad Naeem[2]

[1]Department of Paediatrics, Gulab Devi Hospital, Lahore, Punjab, Pakistan.
[2]Professor of Paediatrics, Gulab Devi Hospital, Lahore, Punjab, Pakistan.

ARTICLE INFO

Keywords: Pediatric Tuberculosis, Rifampicin Resistance, GeneXpert, Xpert MTB/RIF Assay, Malnourishment, Cross-sectional Study.

Correspondence to: Ayesha Tariq, Department of Paediatrics, Gulab Devi Hospital, Lahore, Punjab, Pakistan.
Email: tariqayesha369@gmail.com

Declaration
Authors' Contribution
Both authors equally contributed to the study and approved the final manuscript

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 03-06-2025 Revised: 21-06-2025
Accepted: 06-07-2025 Published: 15-07-2025

ABSTRACT

Background: The prevalence of rifampicin resistance in pediatric patients with Mycobacterium tuberculosis is a growing concern, particularly in regions with high TB burden. Early and accurate detection of drug resistance is crucial for effective treatment and management, especially in children who are more vulnerable to complications. **Objective:** This study aims to assess prevalence of rifampicin resistance in pediatric patients with mycobacterium tuberculosis using gene expert in gulab devi hospital. **Methods:** The study was conducted at the Department of Paediatrics at Gulab Devi Hospital Lahore, using a cross-sectional design over a period of six months. A total of 150 pediatric patients, selected through non-probability consecutive sampling, were included based on the inclusion criteria, including newly diagnosed MTB cases. Sputum or gastric lavage samples were collected and processed using the Xpert MTB/RIF assay for the diagnosis of tuberculosis and detection of rifampicin resistance. **Results:** The mean age of the participants was 10.51 years, with a standard deviation of 2.692 years. The gender distribution was relatively balanced, with 47.3% male and 52.7% female participants. Regarding rifampicin resistance, 8.0% of the participants exhibited rifampicin resistance. **Conclusion:** The study demonstrated a relatively low prevalence of rifampicin resistance (8.0%) in pediatric patients with Mycobacterium tuberculosis as detected by GeneXpert.

INTRODUCTION

Tuberculosis is a prevalent infectious illness globally, particularly in impoverished nations, caused by acid-fast bacilli that transmit by droplet infection from an infected individual to a non-infected individual in close proximity. [1,2] Prompt detection and treatment of a disease may result in its elimination and avert consequences. Pakistan is rated fifth overall among nations with a tuberculosis burden and fourth in terms of medication resistance. Pakistan is prevalent for tuberculosis, with around 510,000 cases reported year, with an estimated 150,000 individuals acquiring drug-resistant tuberculosis each year. [3]

Like other kinds of tuberculosis, drug-resistant tuberculosis impacts individuals across all age demographics, including children under 14 years of age. It is estimated that annually, around 25,000 to 32,000 children acquire multidrug-resistant tuberculosis (MDR-TB), constituting 3% of all pediatric tuberculosis cases. [4]

The primary first-line anti-tuberculous medications include isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin. These medications effectively treat the majority of cases; nevertheless, some instances do not respond to first-line antituberculosis agents. A patient is classified as having MDR-TB if resistant to at least Isoniazid or Rifampicin. MDR-TB is a significant problem for healthcare professionals. [5]

Prompt identification of MTB is essential for effective illness treatment. Suboptimal dose, inadequate duration, insufficient treatment adherence, misdiagnosed conditions, and diminished therapeutic effectiveness contribute to resistance. [6,7] A drug susceptibility test is essential to prevent resistance, since obtaining cultures and testing for susceptibility is a time-consuming procedure. The GeneXpert is a fast tool for diagnosing and detecting resistance to Isoniazid and Rifampicin. [8]

Gene Xpert identifies Mycobacterium tuberculosis using PCR amplification of the rpo gene and assesses rifampicin resistance by searching for mutations in this area associated with resistance. The sensitivity is 90.4% and the specificity is 98.4%. Culture serves as the definitive standard for diagnosis; nevertheless, it requires 8 to 12 weeks, so postponing the initiation of anti-tuberculous medication. [9] Gene Xpert reduces the detection time from days to just hours; it can identify Rifampicin and Isoniazid resistance in under 2 hours. [10]

A research indicated that the percentage of individuals who tested positive for Gene-Xpert MTB and also exhibited rifampicin resistance was 10.9% (95% CI: 8.2–13.6%).[11] A local investigation including all age groups (0-45+) found a rifampicin resistance prevalence of 75 cases (5.43%) (95% CI 4.35-6.74) among 1,382 identified lung isolates of Mycobacterium tuberculosis (MTB).[7]

This study aims to determine the prevalence of Rifampicin resistance in pediatric patients (aged ≤ 14 years) using GeneXpert within our local population, as existing local studies encompass all age groups, while studies conducted on other pediatric populations have reported a prevalence of 10.9%.[11] Consequently, this research aims to determine the prevalence of rifampicin resistance by assessing higher frequencies across all patients, since rifampicin is a primary therapeutic choice. The superior therapy choice may be used to address patient care, which will undoubtedly facilitate recuperation and enhance health outcomes.

MATERIAL AND METHODS

Study setting: Department of Paediatrics Gulab Devi Hospital Lahore

Study design: This is a cross-sectional study.

Duration of study: 6 months after approval of synopsis from 1st December 2004 to 1st June 2025.

Sample size: A total of 150 cases, are estimated, using proportion rifampicin resistance having MTB on Gene-Xpert as 10.9%.[11] The sample size is calculated using 5% margin of error and 95% confidence level.

Sampling Technique*:* We used non –probability consecutive sampling

Sample Selection

Inclusion Criteria

1. Pediatric age group < 14 year.
2. Either gender.
3. Newly diagnosed cases of MTB as per operational definition.

Exclusion Criteria

1. Patients with extra pulmonary tuberculosis (was confirmed on medical record)
2. Patients having HIV diagnosis along with MTB
3. Any prior history of drug resistance
4. Hematological disorders such as aplastic anemia, leukemia, and lymphoma,

Data Collection Procedure

After receiving approval from the ethical committee and obtaining informed consent from the parents or attendants of 150 patients who met the inclusion criteria, participants were recruited at the hospital using a consecutive sampling technique. Demographic data such as age, gender, and contact information, as well as clinical data, were collected for all patients. A single sputum sample was obtained from children who were able to expectorate sputum, while gastric lavage samples were collected from younger children unable to provide sputum. These samples were collected for the diagnosis of all presumptive TB patients using the Xpert MTB/RIF assay.

Briefly, after collecting the sputum or gastric lavage sample, it was mixed with a sample reagent buffer in a 1:2 (sample: sample reagent buffer) volume ratio. The mixture was then tightly closed, vortexed for 15 seconds, and allowed to stand at room temperature for 10 minutes. Afterward, it was vortexed again and allowed to stand for an additional 5 minutes, totaling 15 minutes. The processed sample was then placed into the Xpert MTB/RIF cartridge using the standard kit. The cartridge with the specimen was loaded into the GeneXpert machine, and results were collected after two hours.

Mycobacterium tuberculosis was considered positive on PCR if amplification of specific DNA sequences corresponding to M. tuberculosis genomic regions targeted by the PCR assay was observed. This amplification indicated the presence of M. tuberculosis DNA in the clinical specimen. Newly diagnosed cases were defined as patients diagnosed for the first time, without any prior medication history for MTB. Rifampicin resistance with Mycobacterium tuberculosis using GeneXpert was identified if specific mutations in the rpoB gene of M. tuberculosis, associated with resistance to rifampicin (a key first-line drug in TB treatment), were detected. The results were collected from the laboratory and labeled according to the operational definition. All data were collected on the attached proforma. Data was analyzed using SPSS version 20. Qualitative variables such as gender, malnourishment, and rifampicin resistance were presented as frequencies and percentages. Quantitative variables like age, duration of symptoms, and BMI were calculated using mean ± SD. Effect modifiers such as age, gender, malnourishment, and BMI (underweight, normal weight, overweight, and obesity) were assessed using the Chi-square test, with a p-value ≤ 0.05 considered significant.

RESULTS

The descriptive statistics for age, duration of symptoms, and BMI (n=150) reveal important details about the study participants. The mean age of the participants was 10.51 years, with a standard deviation of 2.692 years, suggesting that the age distribution was relatively consistent. The range of ages spanned from 1 year to 13 years, indicating a broad inclusion of both younger and older children. **Table -1**

The gender distribution was relatively balanced, with 47.3% male and 52.7% female participants. **Fig-1** The duration of symptoms had a mean of 4.517 months with a standard deviation of 2.607 months, showing variability in how long symptoms persisted before the subjects were tested. The BMI had a mean of 18.965, with a standard deviation of 1.9789, and ranged from 12.0 to 23.2, suggesting that most of the participants had a BMI within the normal to underweight range, with minimal extremes. **Table -1** The overwhelming majority of participants were malnourished (90.0%), while only 10.0% were not, highlighting a significant nutritional concern in the study population. **Fig-1**

Regarding rifampicin resistance, 8.0% of the participants exhibited rifampicin resistance, while 92.0% did not, which indicates a relatively low prevalence of resistance among the subjects. **Fig-1**

In Table-2, the comparison of rifampicin resistance across various demographic and clinical factors is

presented. For gender, the p-value was 0.847, which suggests no significant difference in the prevalence of rifampicin resistance between males and females. Similarly, the comparison across age groups showed no significant difference (p-value=0.291), with the younger age group (<8 years) showing a slightly lower percentage of rifampicin resistance (25.0%) compared to the older group (75.0%). For malnourishment, the p-value was 0.229, indicating no significant relationship between malnutrition and rifampicin resistance, with both malnourished and non-malnourished children showing similar resistance rates. However, the BMI comparison showed a statistically significant result (p-value < 0.0001), where a higher proportion of underweight children (58.3%) exhibited rifampicin resistance compared to those with normal weight (4.3%). This suggests that underweight children may be at a higher risk for rifampicin resistance, which could be due to various factors such as weaker immune responses or previous treatments.

Table 1

Descriptive statistics for age, duration of symptoms and BMI [n=150]

	Age (years)	Duration of symptoms (months)	BMI
Mean	10.51	4.517	18.965
Std. Deviation	2.692	2.607	1.9789
Range	12	11.0	11.2
Minimum	1	1.0	12.0
Maximum	13	12.0	23.2

Figure 1

Gender, nourishment and Rifampicin resistance status

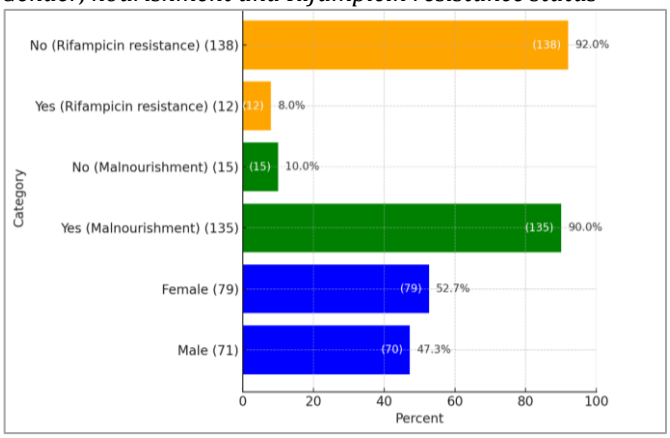

Table 2

Comparison of Rifampicin Resistance versus age, gender, malnourishment and BMI [n=150]

		Rifampicin Resistance		p-value
		Yes	No	
Gender	*Male (n=71)*	6 (50.0%)	65 (47.1%)	0.847
	Female (n= 79)	6 (50.0%)	73 (52.9%)	
Age (years)	*< 8 (n=22)*	3 (25.0%)	19 (13.8%)	0.291
	8-13 (n= 128)	9 (75.0%)	119 (86.2%)	
Malnourishment	*Yes (n= 135)*	12 (8.0%)	123 (82.0%)	0.229
	No (n= 15)	0 (0.0%)	15 (10.0%)	
BMI	*Underweight (n=13)*	7 (58.3%)	6 (4.3%)	<0.0001
	Normal (n= 137)	5 (41.7%)	132 (95.7%)	

DISCUSSION

Childhood tuberculosis (TB) substantially contributes to morbidity and mortality in developing countries, becoming a critical public health issue. The World Health Organization (WHO) states that pediatric TB accounts for about 10% of the global tuberculosis burden, underscoring the critical need for accurate and timely diagnostic methods.[12] This has made diagnosis of TB in children harder as the disease has a paucibacillary character so the bacterial load in children cases is lower compared with those of adults and therefore bacterial detection become more problematic. [11]

In 2014, WHO recommended that Gene-Xpert be the main diagnostic tool regarding a pediatric patient with TB since it presents an enhanced sensitivity and specificity in identifying Mycobacterium tuberculosis and rifampicin resistance promptly than the traditional methods of TB diagnostics.[12] The test is far superior as it gives a quick result and also detects rifampicin resistance that is an important factor signifying the multidrug-resistant tuberculosis (MDR-TB) and early detection of this resistance is crucial in administering proper treatment and preventing the resistant strains spread.[12,13]

On a thorough examination of 37,695 samples, Ramachandra et al. (2023) revealed that 7,156 (18.98%) samples had a confirmed result of tuberculosis, whereas 509 (7.11%) were rifampicin resistant. Further the study has revealed the good performance of Gene-Xpert MTB/RIF with respect to detecting pulmonary and extrapulmonary tuberculosis. Rapid molecular diagnosis has made it possible to receive timely diagnosis with the Gene-Xpert technology and detect rifampicin resistance in the effort to address the global challenge of MDR-TB.[14]

In the present study of rifampicin resistance, 8.0 percent of patients were resistant, and 92.0 percent were not [14]. The molecular diagnosis of tuberculosis was conducted on 2,864 samples, the diagnosis by Gene-Xpert revealed a positive rate of 12%, which reflects the goodness of molecular diagnosis of pulmonary and extrapulmonary tuberculosis.[15]

The positivity levels were also different depending on the type of sample, where in the pulmonary and extrapulmonary samples, sputum samples gave the highest of 37.28% followed by pus samples with 23.26%. This observation is an indication of why sample type is important in diagnostic accuracy. Notably, 102 (29.5%) of the Gene-Xpert positive specimens were acid-fast bacilli (AFB) negative with ZN staining with a sensitivity of 70.52%.[16] William et al. (2021) reported a similar prevalence of rifampicin resistance (12.72%) in the Gene-Xpert positive TB samples which is similar with findings from other studies.[15]

Of the rifampicin resistance samples, 29.55 percent were extrapulmonary that demonstrates the complex phenomenon of TB and the necessity to recognize both pulmonary and extrapulmonary cases in the management and therapeutic planning that indicates that rifampicin resistance can cause complication such extrapulmonary

TB. The study conducted by Dejene et al. (2023) reported a prevalence of 7.3% in children with suspected tuberculosis (TB) who have a bacteriologically confirmed Mycobacterium tuberculosis (MTB) infection and a rifampicin resistance rate of 10.9 percent.[17]

The results of the study show an increased risk of being positive of tuberculosis in older children (1115 years), adolescents (1617 years old), recurrent cases and lost to follow-up cases. The observation that relapse cases have a higher odds ratio of being positive with regards to MTB is an indicator that there is something wrong with the treatment methods and monitoring approaches. On the contrary, Sajid and Riaz (2018) report a local picture, stating that out of 65 suspect cases, 40 were positive for TB, and the Gene Xpert had a detection rate of 45%.[16] The reported sensitivity and specificity of 55 percent and 81 percent, respectively, could be due to numerous factors, such as the quality ranges of the samples, the collection location, and the prevalence of TB in the analyzed population. This cohort lacks the presence of rifampicin resistance as well as the large specificity of the test demonstrates difficulty in identifying medicine resistance in locations with lesser incidence.[16]

Jameel et al. (2024) performed research with 1320 samples of which 110 were tested positive for MTB infection. In the study, the prevalence of rifampicin resistance was found to be 5 per cent with 18 cases showing unclear resistance and 87 indicating the no resistance.[13]

The decrease in the burden of MTB in certain rifampicin-resistant samples indicate that drug resistance might not necessarily be correlated to increasing bacterial burden. This observation underlines the necessity of thorough testing, which would involve load testing, to enhance the understanding of rifampicin resistance and the aftereffects of this resistance on the treatment. The results of the research point to a higher likelihood of being positive in terms of tuberculosis on the part of adolescents and older children (1617 years old) and recurrent and lost to follow-up. The cases involving relapse have a higher odds ratio to be positive to MTB demonstrate the requirement of efficacious management protocol.

CONCLUSION

The study has shown that the prevalence of rifampicin resistance in pediatric patients with Mycobacterium tuberculosis identified using a GeneXpert is relatively low (8.0 per cent). There were no remarkably noticed associations between gender, age, malnutrition or resistance. The strong correlation between low BMI (underweight) and increased rifampicin resistance was also observed and this poses a threat to malnourished children. This has underlined the importance of specific treatment and close follow up of these patients.

REFERENCES

1. Gough, A. (2011). Pulmonary tuberculosis: Clinical features and patient management. *Nursing Standard*, *25*(47), 48-58. https://doi.org/10.7748/ns.25.47.48.s51
2. Martinez, L., Cords, O., Horsburgh, C. R., Andrews, J. R., Acuna-Villaorduna, C., Ahuja, S. D., ... & Zhu, L. (2020). The risk of tuberculosis in children after close exposure: a systematic review and individual-participant meta-analysis. *The Lancet*, *395*(10228), 973-984.
3. Farah, M. G., Rygh, J. H., Steen, T. W., Selmer, R., Heldal, E., & Bjune, G. (2006). Patient and health care system delays in the start of tuberculosis treatment in Norway. *BMC Infectious Diseases*, *6*(1). https://doi.org/10.1186/1471-2334-6-33
4. WHO, G. (2020). Global tuberculosis report 2020. *Glob. Tuberc. Rep*, *2020*.
5. Chakaya, J., Khan, M., Ntoumi, F., Aklillu, E., Fatima, R., Mwaba, P., Kapata, N., Mfinanga, S., Hasnain, S. E., Katoto, P. D., Bulabula, A. N., Sam-Agudu, N. A., Nachega, J. B., Tiberi, S., McHugh, T. D., Abubakar, I., & Zumla, A. (2021). Global tuberculosis report 2020 – Reflections on the global TB burden, treatment and prevention efforts. *International Journal of Infectious Diseases*, *113*, S7-S12. https://doi.org/10.1016/j.ijid.2021.02.107
6. Zhang, C., Wang, Y., Shi, G., Han, W., Zhao, H., Zhang, H., & Xi, X. (2015). Determinants of multidrug-resistant tuberculosis in Henan province in China: A case control study. *BMC Public Health*, *16*(1). https://doi.org/10.1186/s12889-016-2711-z
7. Khan, A. A., Khan, H. U., Khan, Z., Khan, I., Rahim, A., Rahman, A. U., Ur-Rehman, Q. N., Roman, M., & Sajjad, M. (2022). Prevalence of rifampicin resistance and probes identification of 81bp rrdr rpo-B gene in pulmonary tuberculosis patients of district bannu, Pakistan. *Gomal Journal of Medical Sciences*, *20*(2), 75-81. https://doi.org/10.46903/gjms/20.02.1122
8. Pandey, P., Pant, N. D., Rijal, K. R., Shrestha, B., Kattel, S., Banjara, M. R., Maharjan, B., & KC, R. (2017). Diagnostic accuracy of GeneXpert MTB/RIF assay in comparison to conventional drug susceptibility testing method for the diagnosis of multidrug-resistant tuberculosis. *PLOS ONE*, *12*(1), e0169798. https://doi.org/10.1371/journal.pone.0169798
9. Atashi, S., Izadi, B., Jalilian, S., Madani, S., Farahani, A., & Mohajeri, P. (2017). Evaluation of GeneXpert MTB/RIF for determination of rifampicin resistance among new tuberculosis cases in west and northwest Iran. *New Microbes and New Infections*, *19*, 117-120. https://doi.org/10.1016/j.nmni.2017.07.002
10. How, S. H., Kuan, Y. C., Ng, T. H., Razali, M. R., & Fauzi, A. R. (2014). Monitoring treatment response in sputum smear positive pulmonary tuberculosis patients: comparison of weight gain, sputum conversion and chest radiograph. *PubMed*, *36*(2), 91–96.
11. Dejene, T., Hailu, G., Kahsay, A., & Wasihun, A. (2023). Pulmonary tuberculosis and Rifampicin resistant mycobacterium tuberculosis in children and adolescents using gene Xpert MTB/RIF assay in Tigray, northern Ethiopia. *Infection and Drug Resistance*, *16*, 6757-6765. https://doi.org/10.2147/idr.s433789
12. Kumar, R., Sood, A. K., Bajaj, M., & Kanga, A. (2019). Utility of GeneXpert for detection of mycobacterium tuberculosis and Rifampicin resistance in pediatric tuberculosis. *Pediatric Infectious Disease*, *1*(4), 137-141. https://doi.org/10.5005/jp-journals-10081-1229
13. Jameel, R., Mehdi, N., Majeed, N., Zafar, A., Tahir, A., & Aroob, I. (2024). Prevalence and diagnosis of rifampicin-resistant mycobacterium tuberculosis using the GeneXpert MTB/RIF assay at a tertiary care Children's Hospital. *Pakistan Journal of Health Sciences*, 110-114. https://doi.org/10.54393/pjhs.v5i07.1764

14. Ramachandra, V., Brammacharry, U., Muralidhar, A., Muthukumar, A., Mani, R., Muthaiah, M., Soundappan, G., & Frederick, A. (2023). Assess the Diagnostic Accuracy of GeneXpert to Detect Mycobacterium tuberculosis and Rifampicin-Resistant Tuberculosis among Presumptive Tuberculosis and Presumptive Drug Resistant Tuberculosis Patients. *Microbiology Research*, *15*(1), 91–108. https://doi.org/10.3390/microbiolres15010006
15. WİLLİAM, A., RAI, Y., & KAUR, R. (2021). Evaluation of Rifampicin-resistant Tuberculosis in Pediatric Patients by GeneXpert MTB/RIF. *Journal of Microbiology and Infectious Diseases*, *11*(02), 81–87. https://doi.org/10.5799/jmid.951506
16. Sajid, A., & Riaz, S. (2018). ROLE OF GENE EXPERT/MTB TEST IN CHILDHOOD TUBERCULOSIS. *DOAJ (DOAJ: Directory of Open Access Journals)*, *9*(3), 1–5.
17. Dejene, T., Hailu, G., Kahsay, A., & Wasihun, A. (2023). Pulmonary Tuberculosis and Rifampicin Resistant Mycobacterium Tuberculosis in Children and Adolescents using Gene Xpert MTB/RIF Assay in Tigray, Northern Ethiopia. *Infection and Drug Resistance*, *Volume 16*, 6757–6765. https://doi.org/10.2147/idr.s433789