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PREVALENCE OF *MYCOBACTERIUM TUBERCULOSIS* AND RIFAMPICIN RESISTANCE USING GENEXPERT MTB/RIF ASSAY IN JHANG, PAKISTAN

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Abstract

Tuberculosis remains one of the leading public health challenges in developing countries such as Pakistan. With the increasing resistance to antibacterial drugs, *Mycobacterium tuberculosis* is also showing resistance to the first-line drug rifampicin. Resistance to rifampicin is a key indicator of multidrug-resistant tuberculosis (MDR-TB). Accurate and timely diagnosis is crucial for effective tuberculosis treatment, as early detection of resistant strains is essential for appropriate patient management. This study was designed to find out the prevalence of Mycobacterium Tuberculosis and growing resistance to these bacteria, tested at DHQ Hospital Jhang with the help of Genexpert

MTB/RIF method. The study design was a retrospective cross-sectional study, and the duration was six months, from June to November 2024. Patient reports were inspected, and a total of 5074 suspected patients were included in our study. Male patients made up a slightly higher percentage (54.2%), and 0.9% of cases had rifampicin resistance. The study reveals a low frequency of rifampicin resistance and a significant burden of MTB in the community under investigation. These results emphasize how crucial early diagnosis and ongoing drug-resistance monitoring are to successful tuberculosis control.

INTRODUCTION

Mycobacterium tuberculosis, an obligatory aerobic, acid-fast bacillus with a highly complex lipid-rich cell wall that adds to its pathogenicity and resistance to many common antimicrobial drugs, is the causative agent of tuberculosis (TB), a potentially fatal infectious illness [1]. Even with the advent of efficient chemotherapy treatments, tuberculosis (TB) continues to rank among the world's top causes of illness and mortality, especially in low- and middle-income nations. Malnutrition, a lack of resources for healthcare, and a strong correlation with HIV co-infection all contribute to the global burden of tuberculosis [2].

Rifampicin is regarded as the cornerstone of short-course chemotherapy and is one of the first-line anti-tubercular medications. This strong bactericidal drug works by stopping RNA synthesis, which stops bacterial replication, by inhibiting the DNA-dependent RNA polymerase enzyme. Rifampicin's introduction enhanced treatment results and drastically shortened the length of TB therapy [2,3]. However, a significant barrier to successful TB control initiatives around the world is the rise of multidrug-resistant tuberculosis (MDR-TB) and Rifampicin-resistant M. tuberculosis strains. Mutations in the rpoB gene, which codes for the β-subunit of RNA polymerase, frequently result in resistance, which reduces the effectiveness of rifampicin. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (M. tuberculosis), remains one of the leading infectious diseases globally despite decades of intensive research and therapeutic advancements. According to the World Health Organization (WHO), nearly 10.6 million people developed TB in 2022, with 1.3 million deaths, making it the

second leading cause of infectious mortality after COVID-19 [4]. The disease burden is disproportionately higher in low- and middle-income countries, particularly in South Asia and Sub-Saharan Africa, where malnutrition, overcrowding, and co-infection with HIV exacerbate transmission and poor outcomes.

The unique lipid-rich cell wall of *M. tuberculosis*, enriched with mycolic acids, glycolipids, and lipoproteins, contributes to its pathogenicity and intrinsic drug resistance. This complex structure hinders drug penetration, enhances persistence within macrophages, and induces a strong inflammatory response. The bacillus is an obligate aerobe, slow-growing, and highly adaptive, allowing it to survive latent within host tissues for decades [5]. Its ability to evade immune responses makes eradication challenging, even with effective antimycobacterial therapy. The introduction of rifampicin in the 1970s revolutionized TB treatment by significantly shortening therapy duration from 18–24 months to 6–9 months [6]. Rifampicin, a rifamycin derivative, exerts potent bactericidal activity by inhibiting the DNA-dependent RNA polymerase enzyme, thereby blocking RNA synthesis. As one of the four cornerstone first-line drugs (with isoniazid, pyrazinamide, and ethambutol), rifampicin is indispensable in short-course chemotherapy regimens. Its high sterilizing activity against both replicating and semi-dormant bacilli plays a key role in preventing relapses and ensuring cure.

Despite its effectiveness, the widespread emergence of rifampicin resistance poses a serious global health concern. Rifampicin-resistant TB (RR-TB) and multidrug-resistant TB (MDR-TB), defined as resistance to at least rifampicin and isoniazid, are increasingly reported worldwide. The WHO estimated nearly 410,000 cases of MDR/RR-TB in 2022, with less than 60% treatment success [7]. Resistance is primarily associated with mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase. More than 95% of rifampicin resistance–associated mutations occur within an 81-bp region of *rpoB* known as the rifampicin resistance-determining region (RRDR). Common mutations include S531L, H526Y, and D516V, which alter rifampicin binding affinity and reduce drug efficacy. The development of rifampicin resistance complicates TB control because treatment regimens for MDR/RR-TB are longer (18–24 months), more toxic, and less effective compared to

standard therapy [8]. Second-line drugs such as fluoroquinolones, aminoglycosides, and newer agents like bedaquiline and linezolid are associated with significant side effects, high costs, and limited availability in resource-constrained settings. Consequently, patient adherence is poor, treatment outcomes are suboptimal, and the risk of transmission of resistant strains increases.

Advances in molecular diagnostics have improved the rapid detection of rifampicin resistance. Techniques such as GeneXpert MTB/RIF assay and line probe assays (LPAs) allow identification of *rpoB* mutations directly from patient samples, enabling earlier initiation of appropriate therapy [9]. Whole-genome sequencing (WGS) has further enhanced understanding of the molecular epidemiology of resistant strains, revealing regional variations in mutation patterns and transmission dynamics. These tools are vital for surveillance, outbreak tracking, and tailoring treatment strategies. Efforts to address rifampicin resistance include both pharmacological and programmatic interventions. On the pharmacological side, novel drugs (bedaquiline, pretomanid, delamanid) and repurposed agents (linezolid, clofazimine) are being incorporated into shorter, all-oral regimens that show promising results in MDR-TB management. From a public health perspective, strengthening adherence support, ensuring uninterrupted drug supply, and integrating TB and HIV programs are crucial. Preventive therapy for high-risk populations and improved nutritional support further aid in reducing disease burden.

This study aimed to determine the prevalence of *Mycobacterium tuberculosis* and assess rifampicin resistance among suspected tuberculosis patients at DHQ Hospital Jhang using the GeneXpert MTB/RIF assay, to support early diagnosis and effective management of drug-resistant tuberculosis. The emergence of rifampicin resistance in *M. tuberculosis* represents a critical challenge to global tuberculosis elimination efforts, as rifampicin remains a cornerstone of first-line therapy. Mutations in the *rpoB* gene have contributed to the increasing burden of multidrug-resistant and rifampicin-resistant tuberculosis (MDR/RR-TB), threatening decades of progress in disease control. Although molecular diagnostic tools such as GeneXpert have significantly improved the rapid detection and surveillance of drug resistance, there remains an urgent need for the development of novel therapeutic agents, shorter treatment regimens, and integrated strategies that address the social

determinants of tuberculosis. Therefore, understanding the prevalence and resistance patterns in local settings is essential for guiding public health interventions, improving treatment outcomes, and contributing to global efforts such as the World Health Organization End TB Strategy.

1.1. Problem Statement

i. Global TB control is at risk due to the rising prevalence of rifampicin resistance. Researching resistance mechanisms is crucial.

ii. To research the prevalence of resistant strains and develop better therapeutic approaches.

1.2. Rationale of the Study

Addressing the growing problem of Rifampicin resistance in Mycobacterium TB requires this research. This study intends to improve TB diagnosis, prevention, and treatment methods by investigating the molecular mechanisms driving drug resistance, tracking resistance trends, and assessing innovative therapeutic options. In order to support international programs like the World Health Organization's "End TB Strategy," which aims to eradicate TB as a hazard to public health, such activities are essential.

2. Materials and Methods

2.1. Study Design, Area, and Period

This was a retrospective cross-sectional study conducted at the THQ Hospital Jhang, Department of Health Sciences Technology, located in Islamabad. The study covered 6 months (June-November 2024).

2.2. Study Population and Eligibility Criteria

The study population included patients with a confirmed diagnosis of pulmonary tuberculosis whose laboratory records contained results of GeneXpert MTB/RIF testing.

2.3. Inclusion criteria

- All patients with complete GeneXpert MTB/RIF reports.
- Both male and female patients of all age groups.

2.4. Exclusion criteria

- Records with incomplete or missing GeneXpert data.
- Patients are diagnosed with extrapulmonary TB.

2.5. Sampling Technique and Data Collection

A non-probability convenience sampling technique was used. Data were collected retrospectively from hospital laboratory records and patient files following previous standard protocol [11]. The following variables were extracted and entered into a pre-designed data collection sheet:

- Demographics (age, gender)
- GeneXpert MTB detection status (positive/negative)
- Rifampicin resistance status (sensitive/resistant)

2.6. GeneXpert Assay Procedure

The GeneXpert MTB/RIF assay (Cepheid, USA) had been routinely performed in the hospital diagnostic laboratory as part of patient care following previous documented protocol [12]. The test simultaneously detects the presence of *M. tuberculosis* complex DNA and mutations in the *rpoB* gene responsible for rifampicin resistance. Laboratory technicians followed standard operating procedures at the time of testing. For this retrospective study, existing test results were retrieved from hospital records without repeating laboratory procedures.

3. Results

Data was entered and analyzed by using SPSS version 24. Descriptive statistics such as frequencies and percentages were used to summarize categorical data (gender, age group, resistance pattern). Chi-

square test was applied to assess the association between rifampicin resistance and demographic factors. A p-value ≤ 0.05 was considered statistically significant.

Table 4.1: Identification of Mycobacterium tuberculosis (MTB)

Result (MTB)	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Detected	2	.0	.0	.0
Detected (HIGH)	786	15.5	15.5	15.5
Detected (MEDIUM)	320	6.3	6.3	21.8
Detected (LOW)	423	8.3	8.3	30.2
Detected (VERY LOW)	155	3.1	3.1	33.2
Detected (TRACE)	217	4.3	4.3	37.5
Not Detected	3170	62.5	62.5	100.0
Not Detected	1	0	0	100.0
Total	5074	100.0	100.0	

A total of 5074 samples were tested for Mycobacterium tuberculosis (MTB). The total percentage of MTB positive was 37.5%, and the remaining 62.5% of MTB samples were found to be negative. Among the samples analyzed, 786 (15.5%) showed high bacterial loads. This was followed by medium (6.3%), low (8.3%), very low (3.1%), and trace bacterial loads (4.3%). These findings showed that a significant population of patients had MTB-positive cases, and most of these positive cases had high or medium bacterial loads, which are suggestive of active medical problems.

Table 4.2. Resistance Pattern to Rifampicin

(Rifampicin)	Frequency	Percent	Valid Percent	Cumulative Percent
Valid Detected	46	0.9	0.9	0.9
Not Detected	4822	95.0	95.0	95.0
IND	206	4.1	4.1	100.0
Total	5074	100.0	100.0	

The rifampicin resistance analysis revealed that 46 (0.9%) of the 5074 samples analyzed were rifampicin resistant, while 4822 (95.0%) were not. Additionally, 206 (4.1%) samples were categorized as uncertain (IND) since the test was unable to determine resistance or susceptibility. These results demonstrate that the majority of MTB-positive people were responsive to rifampicin, which is crucial to take into account in cases with potential drug-resistant tuberculosis (DR-TB), even though a small fraction demonstrated resistance.

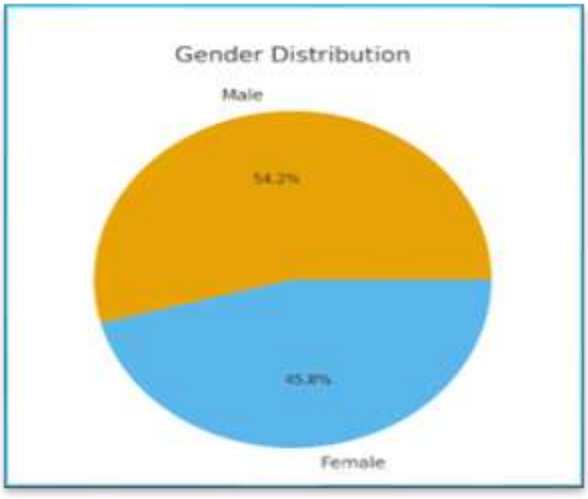

Figure 1. Gender distribution of study participants

Table 4.3. Distribution of Patients by Gender

Gender	Frequency	Percent (%)
Male	2748	54.2
Female	2326	45.8
Total	5074	100.0

This study included a total of 5074 participants, of whom 2326 (45.8%) were female, and 2748 (54.2%) were male. This implies that testing for tuberculosis is slightly more common in males than in females.

4. Discussion

Tuberculosis (TB) continues to pose a significant global health challenge, affecting an estimated 2.2 billion people. Despite advancements in diagnostic techniques and treatment strategies, the disease remains difficult to control, particularly in regions with high transmission rates [1, 13]. The World Health Organization (WHO) estimates that nearly 10 million people develop active tuberculosis annually, leading to more than 1.5 million deaths. As a result, TB remains one of the most lethal infectious diseases worldwide [2,14]. The majority of TB infections remain latent, with approximately 95% resolving without medical treatment. However, some cases progress to active disease later in life. The rise of multidrug-resistant tuberculosis (MDR-TB) further complicates disease control, as rifampicin (RIF) resistance is often associated with concurrent resistance to isoniazid (INH). These challenges underscore the critical need for early diagnosis and timely intervention to prevent the spread of TB [3, 15, 16].

The study presents a detailed analysis of Mycobacterium tuberculosis (MTB) detection rates and rifampicin resistance patterns in a cohort of 5,074 individuals. MTB was detected in 37.5% of the

participants, with bacterial load distribution as follows: high (15.5%), medium (6.3%), low (8.3%), very low (3.1%), and trace levels (4.3%). The remaining 62.5% of samples tested negative, indicating either the absence of active infection or bacterial loads below the detection limit. These findings emphasize the considerable TB burden in the studied population. These are relevant to previous studies [17,18,19].

Comparison with previous research indicates that the distribution of MTB detection levels in this study aligns with global trends, where TB cases are frequently observed in populations with high bacterial loads. For instance, a study conducted in South Africa reported that 18% of TB cases had a high bacterial load, closely matching the 15.5% identified in the present study [4, 20]. However, the proportion of "Detected (low)" and "Detected (very low)" cases in this study (8.3% and 3.1%, respectively) exceeds the levels reported in some low-burden TB regions, such as the United States, where cases with low bacterial loads typically constitute less than 5% of total detections [5, 21,22].

The detection of rifampicin resistance is critical for tuberculosis management, as it serves as a primary indicator of multidrug-resistant tuberculosis (MDR-TB). In this study, among the 5,074 analyzed samples, only 46 (0.9%) tested positive for rifampicin resistance, while 95% showed no resistance. Additionally, 4.1% of cases were classified as indeterminate (IND), where resistance status could not be definitively determined. Compared to high-burden TB regions such as India and China, where rifampicin resistance rates often exceed 5%, the prevalence in this study remains considerably lower. For example, a 2020 study in India reported that 6.2% of MTB isolates were rifampicin-resistant, a substantially higher proportion than the 0.9% observed here [6, 23, 24].

Variations in rifampicin resistance prevalence may stem from differences in antibiotic prescription practices, adherence to treatment protocols, and the overall effectiveness of tuberculosis control programs across regions. The relatively low prevalence of rifampicin resistance in this study is an encouraging finding, indicating that drug-resistant tuberculosis is not yet a widespread concern within this population. However, the presence of indeterminate cases necessitates further investigation, as uncertainty in resistance status could complicate treatment decisions. To resolve

this, follow-up testing using phenotypic drug susceptibility testing (DST) or whole-genome sequencing is crucial for accurately characterizing the resistance profile of these cases [25,26].

The gender distribution of tested individuals indicates that 54.2% of the samples were from male patients, while 45.8% were from female patients. This trend aligns with global tuberculosis epidemiology, where TB cases are more commonly reported among males. Several factors may contribute to this disparity, including occupational exposure, variations in healthcare-seeking behavior, and possible biological susceptibility. Additionally, the proportion of MTB-positive individuals with high bacterial loads (15.5%) in this study exceeds the 12% reported by Khan et al. (2021) in Pakistan [15]. This difference may reflect regional variations in disease severity, diagnostic sensitivity, or underlying risk factors influencing bacterial load distribution [7, 27].

Future research should focus on identifying the key risk factors associated with high MTB bacterial loads to inform more effective intervention strategies. Additionally, whole-genome sequencing could provide deeper insights into the genetic mechanisms underlying rifampicin resistance, aiding in the development of improved diagnostic and treatment approaches. Longitudinal studies tracking MTB-positive individuals over time would be particularly valuable in evaluating disease progression, treatment responses, and the potential emergence of drug resistance. This study contributes to the growing body of research on Mycobacterium tuberculosis (MTB) detection and rifampicin resistance patterns. The results underscore the importance of employing highly sensitive diagnostic techniques, maintaining ongoing surveillance, and implementing targeted interventions to effectively manage tuberculosis and curb the spread of drug resistance. Comparisons with prior research suggest that, while significant progress has been made in TB control, substantial challenges remain. Continued scientific investigation and the development of innovative strategies are essential to advancing global efforts toward TB eradication.

5. Conclusion

This study highlights the continued burden of tuberculosis in the local population of DHQ Hospital Jhang, with a considerable number of suspected cases testing positive for *Mycobacterium tuberculosis*.

The findings demonstrate that although rifampicin resistance was detected at a relatively low frequency (0.9%), its presence remains clinically significant, as it serves as an important indicator of multidrug-resistant tuberculosis. The slightly higher proportion of male patients suggests possible gender-related differences in exposure, healthcare access, or disease susceptibility. The use of the GeneXpert MTB/RIF assay proved to be an effective tool for the rapid detection of both tuberculosis and rifampicin resistance, enabling timely clinical decision-making. Early diagnosis of resistant cases is essential to prevent disease transmission, reduce treatment failure, and improve patient outcomes. Overall, the study underscores the necessity of strengthening routine screening programs, expanding access to rapid molecular diagnostics, and maintaining continuous surveillance of drug resistance patterns. Such measures are vital for effective tuberculosis control and for limiting the emergence and spread of multidrug-resistant strains in the community.

Ethical Clearance

Ethical approval was obtained from the HST Department Ethical Review Committee (Approval No: HST-ERC/25/02). Since this was a retrospective record-based study, patient consent was waived; however, confidentiality and anonymity of patient records were strictly maintained.

Consent for publication: All the authors agree to publish the current work.

Data Availability Statement: Data will be available upon request from the corresponding author.

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Author Contributions

Study design and concept: Tooba Zahra, Rana Muhammad Yousaf. Interpretation, analysis of data: Arif Nadeem Saqib, Mishal Sikandar, drafting of the manuscript; Tooba Zahra, Samreen Gull, Rana Muhammad Yousaf. Critical revision of the manuscript: Dr. Muhammad Mudassir Jamal, Rashid Iqbal, and Asif Kamal.

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