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Detection Of Mycobacterium Tuberculosis and Rifampicin Resistance Using GeneXpert MTB/RIF Assay

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ABSTRACT

Background: Tuberculosis (TB) remains a major global health challenge, especially in developing countries, due to its high morbidity and mortality rates. The emergence of rifampicin-resistant Mycobacterium tuberculosis (MTB) strains further complicates disease management and control strategies.

Objective: To evaluate the diagnostic performance of the GeneXpert MTB/RIF assay in detecting MTB and rifampicin resistance among suspected TB patients in Lahore, Pakistan.

Methods: This cross-sectional study analyzed 865 sputum samples from patients presenting with TB symptoms at selected hospitals in Lahore. The GeneXpert MTB/RIF assay was employed for simultaneous detection of MTB and rifampicin resistance. Demographic data, including age and gender, were also recorded and analyzed.

Results: Out of 865 patients, 192 (22.19%) tested positive for MTB. The majority of positive cases were males and individuals aged 46 to 65 years. Rifampicin resistance was identified in 41.66% of the MTB-positive cases, indicating a high prevalence of drug-resistant TB. The GeneXpert system demonstrated rapid and reliable detection capabilities, significantly reducing diagnostic delays.

Conclusion: The GeneXpert MTB/RIF assay proves to be a valuable diagnostic tool in TB-endemic regions, offering timely detection of both MTB and rifampicin resistance. The findings highlight the urgent need for improved biosafety measures, regular drug susceptibility testing, and strengthened public health strategies to contain the spread of drug-resistant TB.

Keywords: Tuberculosis; Mycobacterium tuberculosis; GeneXpert; Rifampicin resistance

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INTRODUCTION

Tuberculosis (TB) constitutes one of the world's worst infectious diseases because it causes more than one million annual deaths according to the last ten-year time period [1]. Pulmonary Tuberculosis (PTB) stands as the most common contagious manifestation of TB since the infection first appears in the lungs although it has the ability to impact different body organs. A tuberculosis-infected person spreading the sickness occurs through tiny airborne droplet nuclei that develop from coughing, sneezing or speaking activities thus permitting prolonged airborne existence. The bacilli enter the lungs of susceptible persons after they inhale the contaminated droplet particles from infected persons.

TB spreads rapidly among crowded living areas together with communal housing facilities and immune-compromised individuals especially those affected by HIV/AIDS. Antibody-deficient people develop active tuberculosis in much higher numbers than those with healthy immune systems. Early detection combined with

accurate etiological diagnosis of TB remains essential for stopping disease transmission while it allows proper treatment initiation. The low number of bacteria in clinical samples makes diagnosis difficult because it is a paucibacillary disease[2] .

The worldwide TB epidemic faces increased difficulty because drug-resistant bacterial strains continue to appear and spread around the globe. The public health community faces a critical challenge because Multidrug-resistant TB (MDR-TB) exists when bacteria show resistance to isoniazid and rifampicin. These two drugs represent the strongest first-line treatment drugs for tuberculosis. Extensively drug-resistant TB (XDR-TB), a more severe form, exhibits resistance to isoniazid and rifampicin, in addition to any fluoroquinolone and at least one of the second-line injectable drugs such as amikacin, capreomycin, or kanamycin. The global rifampicin-resistant TB (RR-TB) count reached 450,000 cases in 2021 which led to almost 191,000 deaths during the reporting period [3].

Drug-resistant TB diagnosis must occur rapidly because this enables the use of proper medications that help stop disease transmission. The BACTEC MGIT 960 stands as the standard reference test for drug sensitivity assessment but requires substantial time to deliver results that typically span between 4 and 6 weeks. The use of molecular methods changed TB detection by bringing more sensitive testing which provides rapid results to patients. WHO approves Xpert® MTB/RIF (Cepheid, USA) and line probe assays (LPA) from Hain Lifescience, Germany as methods for prompt MDR and XDR strain identification[4] . These molecular assays perform DNA amplification by PCR to detect mutations in the rifampicin resistance-determining region (RRDR) of the rpoB gene according to research [5].

The diagnosis of routine TB relied on smear microscopy as its main method but this technique demonstrated limited sensitivity when detecting poorly visible bacterial numbers in patients without strong TB presentation or those who have HIV. Hospitals use readily available and cost-effective smear tests but these methods show poor results in early stages of TB infection or when bacterial counts are low resulting in delayed diagnosis and extra transmission[6].

The World Health Organization approved the GeneXpert MTB/RIF assay as an automated real-time polymerase chain reaction system that strengthened TB control operations mostly in resource-constrained areas. Through this assay both Mycobacterium tuberculosis (MTB) detection and rifampicin resistance (RR) identification take place simultaneously in less than two hours[7] .

This diagnostic tool successfully detects tuberculosis because it delivers highly precise results through quick testing procedures which require minimal hands-on skills. Although molecular diagnostics, such as GeneXpert, have been globally endorsed, their local performance achievements and diagnostic effect in a given point of high-burden, including Lahore, Pakistan, are not well investigated. Lahore, the site selected in this study, has a high prevalence of TB presenting a good opportunity to assess the need to shorten delays in TB diagnosis and manage TB drug resistance [8]. This article set out to establish the usefulness of the GeneXpert MTB/RIF test to ascertain Mycobacterium tuberculosis and rifampicin resistant in pulmonary specimens taken on Lahore patients. The study aims at preventing the discrepancy in the clinical context of the timely diagnosis that is critical to the timely initiation of a proper therapy and avoiding additional spread by determining the correctness and applicability of this approach in a real-world clinical scenario.

Lahore was chosen because it has a well distributed population and a comparatively large disease burden of TB and has very few rapid diagnostic equipment in the government health care facility.

The broad application of quick molecular detection tools like GeneXpert becomes essential since multidrug-resistant and extensively drug-resistant Tuberculosis strains continue to increase in high-burden and low-income regions throughout the world. Fast and exact detection through these tools enables doctors to start proper treatment and control resistant-strain spread for enhanced patient survival and TB control achievements[9] .

The primary objective of this study was to assess the diagnostic properties of the GeneXpert MTB/RIF assay in detecting Mycobacterium tuberculosis (MTB) and rifampicin resistance in pulmonary specimens collected from patients in Lahore, Pakistan. The study also aimed to achieve several secondary objectives. One of these was to compare the turnaround time of the GeneXpert assay with that of traditional diagnostic methods, including smear microscopy and culture. Another key objective was to evaluate the relevance and feasibility of implementing the GeneXpert test in resource-constrained, high TB-burden settings such as Pakistan [10].

In terms of study design, specific variables were defined. The independent variables included the presence or absence of pulmonary TB infection, the status of rifampicin resistance, and the type of diagnostic method used either GeneXpert or conventional approaches. The dependent variables measured were the sensitivity of MTB detection, the rate of rifampicin resistance, the accuracy of the diagnostic results, and the overall turnaround time for reporting [11].

The central hypothesis of the study proposed that the GeneXpert MTB/RIF assay would demonstrate higher sensitivity and specificity, while also being less time-consuming for diagnosing pulmonary TB and rifampicin resistance when compared to traditional diagnostic methods. It was further hypothesized that this assay would be especially suitable for deployment in resource-limited

settings such as Lahore, where the burden of TB is particularly high and rapid diagnosis is crucial for effective disease control [12].

MATERIAL AND METHOD

Study design: Research data was collected at Jinnah Hospital along with Bahria International Hospital in Lahore between December 15th 2021 to June 1st 2022 through a cross-sectional descriptive study. The research included 192 patients who showed tuberculosis indicators by experiencing long-term fever together with persistent coughing longer than two weeks with night sweats. These patients received study group designation while the control group consisted of 673 healthy participants. Both male and female participants across the age range of 05 to 85 years joined the research assessment.

Sample size: The study included all patients considered possible cases of pulmonary or extrapulmonary TB whose full information appeared in the registration book from the study period. The study excluded patients with missing information from their records including age along with sex details and GeneXpert MTB/RIF results.

The sample size (n=192) was calculated using the following formula:

$n = Z^2 \times p(1-p) / d^2$,

where Z = 1.96 (95% confidence level), p = estimated prevalence of MTB (0.14), and d = margin of error (0.05).

The total of 192 TB-suspected and 673 control subjects ensured sufficient statistical power.

A standardized checklist enabled the retrospective retrieval of hospital registration data. The examination process employed EpiData to enter the gathered data while SPSS version 21 performed the analysis of this information. The research established a p-value less than 0.05 which indicated statistical significance.

The data was summarized by using descriptive statistics (mean, frequency, percentage). Chi-square test was used to determine the levels of association related to categorical variables whereas the t-test was used to test continuous variables. The confidence interval was set at 95 percent and the p-values of less than 0.05 were reported as statistically significant.

Variables:

- **Independent variables:** TB status (suspected/healthy), age, sex, diagnostic method (GeneXpert)
- **Dependent variables:** MTB detection rate, rifampicin resistance, time to diagnosis, test accuracy

Data collection tool and procedure:

Specimen collection: The entire group of suspected tuberculosis participants provided early-morning sputum samples at their initial screening spot. Participants were given deep coughing instructions for producing sputum generated in lower parts of the lungs. All specimen collection took place within a room with proper ventilation. All participants received Falcon tubes alongside written instructions to supply at least 3 ml of sputum into them. The laboratory staff accepted only samples containing 3 ml in volume or above and the samples must be in appropriate containers and undamaged tubes and must have accompanying clinical forms to pass inspection. The laboratory kept accepted samples secured in tightly sealed containers or refrigerators before initiating their laboratory tests.

Laboratory method by gene expert: The GeneXpert MTB/RIF system (Cepheid USA) processed all sputum specimens. Shipping of 0.5 ml sputum specimens required hand mixing with 1.5 ml sample reagent (NaOH and isopropanol mixture) before vigorous hand shaking. Random vortexing lasted 30 seconds before placing the samples in an incubator for 15 minutes at temperatures between 20–30°C while manually shaking intermittently.

The processed sample received a sterile Pasteur pipette transfer into the Xpert cartridge before machine automated testing at the GeneXpert machine. The results window of the GeneXpert machine showed the detection status of MTB DNA. When detecting MTB, the test would confirm whether rifampicin resistance was present or absent and when indeterminate results occurred. When a test gave an invalid result, the procedure required using a fresh sample for another test**.**

RESULTS

GeneXpert MTB / RIF analysis reviewed 865 samples of patients to identify the MTB detection. Among them, 192 (22.20%; 95% CI: 18.99 25.73) have been infected by Mycobacterium tuberculosis (MTB). Of the 525 male patients, 124 (23.61%) were positive with MTB, whereas 68 out of 340 females (20.00%) had positive results. The rate of MTB positivity was greater in male patients when compared with female patients as shown in fig 1.

Patients belonging to the 46–65 years' age bracket composed the largest MTB-positive group with 33.33% of all cases. Results showed that individuals between the ages of 5 to 25 composed 29.16% of MTB-positive cases followed by those between 26 to 45 years of age (25%) and people within the 66 to 85 years range (12.5%). The figure depicts residents of overcrowded urban areas comprised 54.16% (104 patients) of MTB-positive cases and the rural participants made up 45.83% (88 patients) of the total as shown in fig 2.

MTB-positive cases distribution regarding residency, emphasizing that there was a visible disparity in TB prevalence between the urban and rural groups. Out of the 192 definite MTB positive patients, 104 (54.17%) have been urban, 88 (45.83%) have been rural. The study shows that the burden of tuberculosis is relatively higher in the urban population and it can be blamed on overcrowding, poor ventilation, high population density and increased contact between people which are the predisposing factors of TB. Furthermore, cities are associated with an excessive amount of air pollution and a shortage of health care

services in impoverished neighborhoods that lead to late diagnosis of the disease as well as the spreading of it.

Conversely, unhealthy access to healthcare, as well as underdiagnosis, might similarly affect rural populations, but the slightly smaller percentage in the group may be indicative of the lower detection rates and not the actual lower burden. These results support the relevance of region-specific TB control measures, prioritizing a high-risk urban environment, in which dynamics of transmission differ in comparison to the rural environment as shown in fig 3.

Of the 192 validated pulmonary tuberculosis (TB) cases, 80 people (41.66 percent) had rifampicin-resistant TB (RR-TB). Gender based analysis showed that the resistance towards rifampicin was high on male patients as compared to the female patients indicating a potential blame between gender and incidences of drug-resistant TB in the target population as shown in fig 4.

The patterns of socio-demographic features that were observed among TB-positive patients that got screened via GeneXpert in the Jinnah and Bahria International Hospitals include gender, place of residential location, and age. The statistics exhibited that TB is more prevalent in male patients than in females and the age group that is most affected by this condition is between 46 and 65 years. Also, urban areas were the source of TB-positive cases to a larger extent compared to the rural environment, which implies the role of collective and environmental conditions on the distribution of the disease. Such results point to the necessity of population-specific TB control measures, being mounted on the basis of population-specific risk factors.

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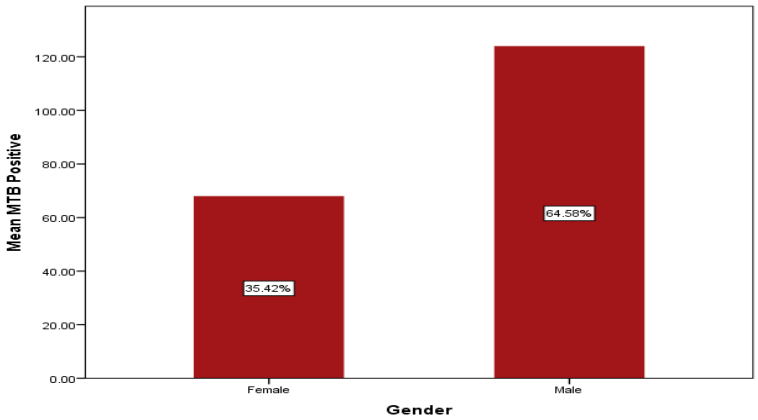

Figure 1: Distribution of MTB-positive cases by gender: The bar chart illustrates the proportion of Mycobacterium tuberculosis (MTB)-positive patients among male and female participants. Males accounted for 64.58% of positive cases, while females represented 35.42%, indicating a higher prevalence among males.

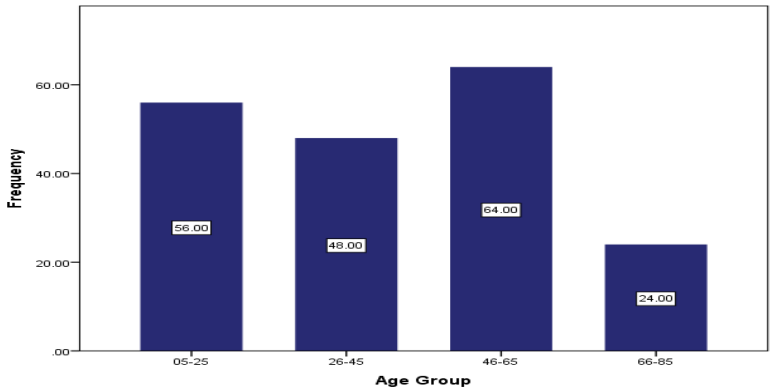

Figure 2: Age-wise distribution of MTB-positive cases, with the highest frequency in the 46–65 age group.

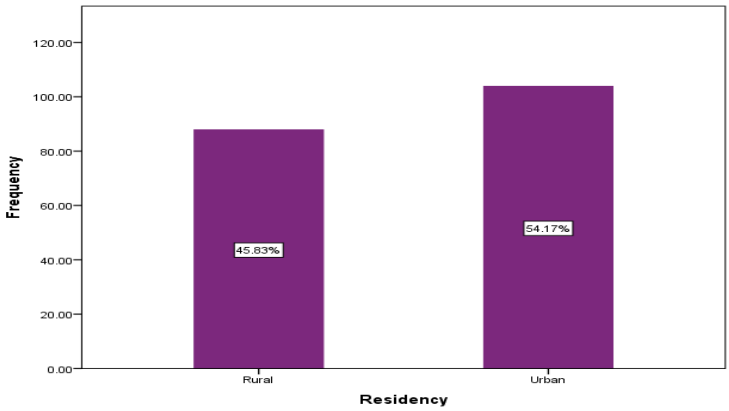

Figure 3: Residency-wise distribution of MTB-positive cases. A higher proportion of cases was reported in urban areas (54.17%) compared to rural areas (45.83%).

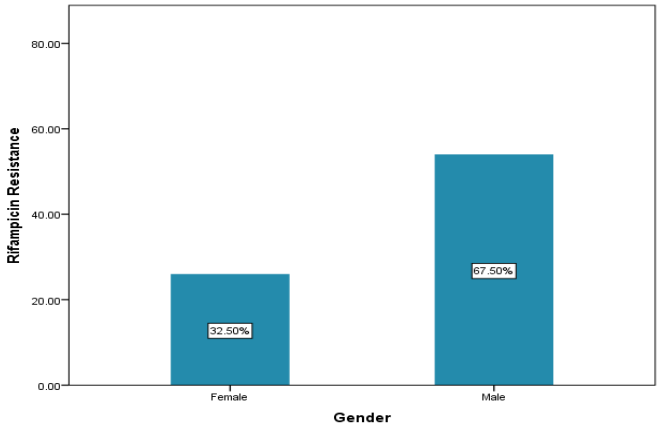

Figure 4: Gender-wise distribution of RR-TB cases showing higher resistance among males than females.

Table 1: Socio-demographic Characteristics of TB-Positive Patients (n = 192)

Variables	Categories	Frequency	Percentage (%)
Gender	Male	124	64.58%
	Female	68	35.41%
Residency	Urban	104	54.16%
	Rural	88	45.83%
Age (Years)	05 – 25	56	29.16%
	26 – 45	48	25.00%
	46 – 65	64	33.33%
	66 – 85	24	12.50%

Table 2: Distribution of Rifampicin-Resistant TB by Gender (n = 80)

Gender	RR Detected (n = 80)	Percentage (%)
Male	54	67.5%
Female	26	32.5%

DISCUSSION

The global outbreak of COVID-19 happened after TB remained as the main cause of disease deaths from one source among developing countries. Multiple study teams evaluated GeneXpert through testing of different specimen examples like paraffin-embedded materials and both blood and sterile fluids and bone samples [12,13]. The diagnostic device detects MTB together with rifampicin (RIF) resistance in less than two hours [14].

This study revealed TB prevalence to be 22.19% among all participants. The reported prevalence rate of 22.19% stands lower than studies performed at Enat Hospital (11.2%) in Central Ethiopia , Debre Berhan Referral Hospital (13%), Jimma University (14.2%), and Yirgalem Hospital (16.5%) in Southern Ethiopia [15]. The probable explanation for our study's diminished prevalence stems from its inclusion of presumptive TB cases in contrast to the confirmed cases in the studies previously mentioned [16,17].

The TB prevalence in our study exceeded the rates observed in Debre Berhan and Dessie (2.6%) according to [18]. Extrapulmonary TB detection rates remain low because such cases usually contain small bacterial populations characteristic of paucibacillary conditions. The diagnostic results from GeneXpert MTB/RIF assay become better with pulmonary samples compared to extrapulmonary indications. Rifampicin-resistant MTB cases from extrapulmonary sites believe to be rising in importance as a public health challenge in TB-high burden areas according to recent research [19].

Among all confirmed TB cases we examined there were 41.66% which displayed resistance to rifampicin (RR-TB). These findings surpass the previous research in Northwest Ethiopia which reported 5.7% and 15.8% in Central Ethiopia [20].

The high rate of rifampicin resistance observed in this study may be attributed to several contributing factors. One major reason is the history of prior TB treatment or poor adherence to prescribed regimens, which often leads to the selection and propagation of drug-resistant Mycobacterium tuberculosis strains. In addition, inadequate access to quality healthcare services frequently results in interrupted or delayed treatment, providing a window for resistant strains to emerge and proliferate [21]. Another critical factor is the possibility of unrecorded co-infections, particularly HIV, which are known to complicate TB management and increase the risk of drug resistance due to immunosuppression. Furthermore, socioeconomic conditions such as overcrowding, malnutrition, and poor ventilation although not quantified in this study are well-established contributors to TB transmission and treatment challenges, particularly in densely populated urban areas. Lastly, referral bias may have played a role in the elevated resistance rate, as the study was conducted at major tertiary care centers which are more likely to receive complex or previously treated cases, including those with multidrug-resistant TB [22].

Unluckily, we had no access to the HIV status of patients, their previous TB treatment history, and indicators of socioeconomic determinants, which could have served as confounding variables in the expression of rifampicin resistance rates, because of the retrospective nature of our study. These data needs to be added to prospective studies conducted in the future, to have a better comprehension of the root causes [23].

All personnel working in TB laboratories must follow strict PPE guidelines to use lab coats with caps while wearing gloves and masks for preventing contaminations. Inappropriate work procedures with PPE actually create pathways through which DNA contamination may occur [24]. We dedicated time to validate our test samples as part of our troubleshooting efforts because contamination between samples is a common issue in automated test facilities. The extensive number of potential drug-resistant TB specimens increases the chances of specimen-handling errors which adds to inconsistent assessment results [25].

This research work was associated with a number of limitations. To begin with, it did not contain any information on the HIV status, history of treating TB and socioeconomic background, which could affect the level of TB infection and resistance. Second, pulmonary samples only were considered and it is limited in extra pulmonary TB. Third, the research was based on a cross-sectional study design, as it fails to determine cause and effect or treatment outcomes. Last, a population that is based in a hospital can over represent severe/drug-resistant cases, and have less extension to the community [22-25].

CONCLUSION

Research findings showed a substantial degree of rifampicin-resistant MTB (RR-MTB) along with PTB among the studied population. The main risk factors for drug-resistant TB development involved both HIV co-infection and retreatment cases which indicated particular groups in need of specialized intervention efforts. To address the critical situation, we need better detection methods for anti-TB drug resistance at the early stages. The rapid molecular tool GeneXpert as well as improved management of previous treatment patients need to be prioritized to halt the spread of drug-resistant TB while decreasing its health burden across the region.

Conflict of Interest: No conflict of interest

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Authors Contribution:

- FM (Article Writing)
- MTK (Statistician & Supervision)
- AYM (Plagiarism)
- AS (Plagiarism)
- FA (Formatting)
- ZA (Article writing)
- SB (Proof reading)
- SYF (Proof reading)
- IS (Formatting)
- WH (Proof reading)

Data Availability Statement: The datasets generated and analyzed during the current study are not publicly available due to institutional data protection policies but

are available from the corresponding author upon reasonable request.

Abbreviations:

- HIV: Human immunodeficiency virus
- MDR: Multidrug resistance
- MTB: Mycobacterium tuberculosis
- PTB: Pulmonary tuberculosis
- RIF: Rifampicin
- RR: Rifampicin resistance
- TB: Tuberculosis.

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