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ORIGINAL ARTICLE

Evaluation of GeneXpert MTB/RIF for the Detection of *Mycobacterium Tuberculosis* in a Tertiary Care Hospital, Pakistan

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ABSTRACT

Objectives: To evaluate the diagnostic accuracy of GeneXpert MTB/RIF assay for the analysis of pulmonary tuberculosis and detection of Rifampicin resistance using sputum samples.

Methodology: The retrospective descriptive cross-sectional study was conducted at the department of microbiology, Federal Government Polyclinic (FGPC), Islamabad, Pakistan, from August 2023 to July 2024. A total of 771 participants, referred by their physicians as TB suspects, were consecutively enrolled in the study from various wards of FGPC and other healthcare facilities in Islamabad, Pakistan. Sputum samples were collected from these participants and examined using light microscopy following Ziehl-Neelsen (ZN) staining. All samples were processed using the GeneXpert MTB/RIF Assay.

Results: Out of the 771 sputum samples, *Mycobacterium tuberculosis* was detected in 89 (11.54%) cases using the GeneXpert MTB/RIF assay. Rifampicin-resistant *M. tuberculosis* was found in 8 out of these 89 cases (8.98 %). Male and female ratio of the patients was almost equal. Males were found to be 406 (57.4%) while 365 (42.5%) were females. The data were expressed as mean, median, and mode, and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0. Simple descriptive statistics (frequencies and percentages) were calculated for each categorical variable. The diagnostic accuracy, i.e., sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the GeneXpert MTB/RIF assay compared to traditional methods was calculated.

Conclusion: The GeneXpert MTB/RIF has higher sensitivity and specificity to conventional ZN smear microscopy. It is a simple, rapid, and accurate test for detecting Mycobacterium tuberculosis in sputum specimens.

Keywords: GeneXpert MTB/RIF; Pakistan; Pulmonary tuberculosis; Rifampicin resistance.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [5,6]Data analysis; Manuscript Editing.

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Introduction

Tuberculosis (TB) is one of the oldest diseases known to affect humans.[1] It remains one of the world's largest threats. In 2014, approximately 9.6 million people suffered due to TB and 1.5 million people expired because of the universal spread of TB.[2] According to WHO unprecedented progress is reported in 2023 a record 7.5 million diagnoses out of an estimated 10.3 million cases in 2022 - the highest number of people with TB ever diagnosed and treated in a year. Although culture has been

declared as the gold standard procedure for detecting TB, it is slow and may take 2 to 8 weeks. Correspondingly sputum smear microscopy for acid-fast bacilli (AFB) is declared as quick and low-priced diagnostic technique and it has poor sensitivity as well.[3] Therefore, swift detection of MTB, which is indispensable for prompt diagnosis and management, refining patients' outcomes, and taking impressive actions for public health and safety, trusts on nucleic acid amplification practices.[4] Numerous molecular techniques have been established in current years for speedy detection of MTB and drug resistance in clinical isolates, including line probe assays and real-time polymerase chain reaction (PCR).

GeneXpert mycobacterium tuberculosis (MTB)/rifampicin (RIF) is a cartridge-based, automated and quick molecular investigative equipment that accomplishes sample handling and hemi-nested real-time PCR scrutiny in a single, hands-free step for detecting MTB and rapid recognition of rifampicin (RIF) resistance in sputum samples.[5] The GeneXpert MTB/RIF assay has proven to play a fundamental tool in the quick diagnosis of Mycobacterium tuberculosis (MTB) and its resistance to rifampicin, ominously influence tuberculosis (TB) control efforts worldwide. This molecular diagnostic technique gives a substantial benefit over traditional methods like acid-fast bacilli (AFB) smear microscopy and culture, specifically in categorizing cases that are smear negative. It has been reported that the GeneXpert MTB/RIF assay shows sensitivity rates as high as 90.91%[6], as it is helpful in detecting *Mycobacterium tuberculosis* in smear-negative cases where traditional methods may fail. In addition, the assay provides rapid results within a few hours, assisting with timely initiation of treatment, which is critical in TB management, particularly in high-burden clinical settings.[7] The performance of GeneXpert MTB/RIF has been validated across diverse populations and healthcare settings, confirming its effectiveness in diagnosing both pulmonary and extrapulmonary TB. For instance, studies indicate that this technique is advantageous in accurately detecting extrapulmonary TB, including cases involving lymph nodes and other tissues, thereby extending its diagnostic utilizations beyond respiratory specimens.[8]

Furthermore, the assay's ability to simultaneously detect rifampicin resistance has established it as a mandatory tool in combating multidrug-resistant TB (MDR-TB). Recent studies highlight its significance in detecting resistance-associated mutations in the *rpoB* gene, a common hallmark of these resistant strains.[9] Resource-limited settings get benefit from GeneXpert MTB/RIF assay, as these regions often face a high burden of TB compounded by widespread drug-resistant strains.[10] Although the GeneXpert MTB/RIF assay represents an innovative diagnostic strategy, it is not without its limitations. In endemic regions with a high TB burden, false-positive and false-negative results have been reported, making conclusive clinical decisions difficult.[11]

Furthermore, while this assay significantly improves the diagnostic precision over AFB smear microscopy, its sensitivity may vary based on the type of specimen and the bacterial load present.[12]

Rapid diagnosis of pulmonary tuberculosis and early detection of rifampicin resistance are essential for effective management and disease control. The GeneXpert MTB/RIF assay provides simultaneous detection of *Mycobacterium tuberculosis* and rifampicin resistance using sputum samples, facilitating timely and appropriate clinical management. The study aimed to assess the diagnostic performance of GeneXpert for detecting *Mycobacterium tuberculosis* using a composite reference standard (CRS) in a real-world clinical setting where culture and histopathology data were not consistently available

Methodology

This was a retrospective, cross- sectional study conducted at the Department of Microbiology

Federal Government Polyclinic (FGPC), Islamabad, Pakistan from August 2023 to July 2024. Data was obtained from existing laboratory records, patient files of individuals whose GeneXpert MTB/RIF testing was done during the study period, and electronic hospital databases. All samples tested for *Mycobacterium tuberculosis* using the GeneXpert MTB/RIF assay within this timeframe were included. Duplicate entries and records with incomplete data were excluded.

A consecutive sampling method was employed for statistical inquiry.

A demographic Performa specially designed for this purpose was filled. This study involved participation of 771 patients aged one year and older. All samples were subjected to ZN smear microscopy (traditional method) and GeneXpert, automated molecular test detecting MTB and Rifampicin resistance.

The study included primary cases of Tuberculosis and early morning specimens. Spot specimens were excluded. Non-probability consecutive sampling was done and data collection included demographic information of patients, sputum samples and cases from different wards. Non-sterile clinical sample (sputum) was pre-treated according to the conventional N-acetyl-L-cystine-NaOH decontamination procedure. Each sample was subjected to ZN smear microscopy. The effectiveness of ZN smear microscopy, as a diagnostic test, was assessed using Gene Xpert MTB/RIF as the standard investigation for further analysis

All the samples were sorted out in the laboratory as per standard measures for the Gene Xpert procedure (Cepheid Inc., Sunnyvale, CA, USA).[13] The collected isolates were mixed with sample reagent (SR) at a ratio of 2:1 and shack manually 10 to 15 times. These treated samples were then incubated for 15 minutes at room temperature. After this incubation period, 2 ml of the treated sample was transported into the sample chamber of the Xpert cartridge, looked over the barcode, and encumbered into the Gene Xpert appliance following the constructor's directions. Following two hours of sample incubation, the effects were inferred by the Gene Xpert diagnosis system from the measured fluorescent signals and revealed automatically as identified, not perceived, or invalid/error for MTB.

All ethical considerations were duly addressed and approved by the Institutional Review Committee (IRC - No. FGPC. 1/12/24/E) before commencement of this study.

Results

An overall of 771 suspected TB specimens were scrutinized. Table I show distribution of the patients according to age and gender. The lowest age of patients was 8 months while extreme was 90 years. More than 130 (17.3%) of the patients were found to be in an age interval of 61 - 70 years and 123 (15.9%) of the patients were in the age group 51 - 60 years. The mean age of participants was instituted to be 46 $\pm$19.37. Male and female ratio of the patients was almost equal. Males were found to be 406 (57.4%) while 365 (42.5%) were females (Table I).

The majority of the cases were reported from Islamabad and Rawalpindi 542 (70.29%) followed by cases reported from rural areas in Punjab 229 (29.70%). The Smear examination (ZN staining) was positive in 73 (9.46%) patients. When GeneXpert assay was performed on all samples, 89 (11.54 %) samples showed positive result for TB. RIF (Rifampicin) resistance was detected in 8 (8.4%) samples as shown in Table II.

For comparative analysis between methods, a 2 x 2 table was made for ZN microscopy and GeneXpert MTB/RIF for four dissimilar categories: true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) (table III) The calculation of metrics involved sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Table I: Socio-demographic characteristics of study participants			
Variables	**Total n (%)**	**Results of Gene Xpert – n (%)**	
		Detected	**Not Detected**
Gender			
Male	406 (57.4)	49 (12.06)	357 (87.93%)
Female	365 (42.5)	40 (10.95)	325 (89.04)
Age in Years (groups)			
0-10 Years	8 (1.03)	1 (12.5)	7 (87.5)
11-20 Years	88 (11.4)	12 (13.6)	76 (86.3)
21-30 Years	105 (13.6)	7 (6.66)	98 (93.3)
31-40 Years	117(15.1)	17 (14.5)	100 (85.4)
41-50 Years	121 (15.6)	14 (11.5)	107 (88.4)
51-60 Years	123(15.9)	11 (8.9)	112 (91.05)
61-70 Years	134(17.3)	17 (12.6)	117(87.3)
71-80 Years	62(8.04)	9 (14.5)	53 (85.4)
81-90 Years	13(1. 6)	1 (7.6)	12 (92.13)
Residence			
Urban	542 (70.29)	57 (10.51)	485 (89.48)
Rural	229 (29.70)	32 (13.97)	197 (86.02)
HIV Status			
Yes	-	-	-
No	771 (100)	-	-

Table III and IV demonstrate a comparison and accuracy (Diagnostic Metrics) of test results of two methods under study. Positive cases detected by ZN staining were 73, whereas 698 were found to be negative. While GeneXpert detected 89 positive cases and 682 were found to be negative.

Table II: Frequency of ZN staining, GeneXpert and RIF Resistance		
ZN staining	MTB Detected	73 (9.46%)
	MTB Not Detected	698 (90.53%)
GeneXpert	MTB Detected	89 (11.54%)
	MTB Not Detected	682 (88.45%)
***M. Tb* Quantitative**	High	23 (2.98%)
	Medium	10 (1.29%)
	Low	45 (5.8%)
	Trace	11 (1.42%)
(Rifampicin) Resistance	Detected	8 (8.4%)
	Not Detected	81 (91.01%)

Table III: Comparative evaluation of the diagnostic performance of two methods.			
ZN Staining	**GeneXpert**		**Total**
	Positive	**Negative**	
Positive	73 (TP)	0 (FP)	73
Negative	16 (FN)	682(TN)	698
Total	89	682	771

TP: true positives; TN: true negatives; FP: false positives; FN: false negatives

Table IV: Evaluation and Accuracy of Diagnostic Tests		
Parameter	**Formula/ Findings**	**Results (%)**
Sensitivity	73/ (73+16) x 100	82.02
Specificity	682/ (682+0) x 100	100
PPV	73/ (73+0) x 100 = 100%	100
NPV	682/ (16+682) x 100	97.7

PPV: positive predictive value; **NPV**: negative predictive value

Sensitivity: TP/ (TP+FN) x 100

Specificity: TN/ (TN+FP) x 100

PPV: TP/ (TP+FP) x 100

NPV: TN/ (FN+TN) x 100

Data was expressed as mean, median, mode and evaluated by using Statistical Package for Social Sciences (SPSS) version 25.0. Simple descriptive indicators (frequencies, percentages) were reported for each categorical variable such as gender, ZN staining, sputum samples, Gene Xpert result and Rifampicin resistance. The Diagnostic Accuracy i.e., sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the GeneXpert MTB/RIF assay compared to traditional methods was calculated with 95% confidence intervals using the CRS as reference. Discrepant cases were independently reviewed by two clinicians who were blinded to molecular tests results.

Discussion

This study was designed to detect *M. tuberculosis* using the Gene Xpert assay among tuberculosis-suspected patients in the FGPC of Rawalpindi. Consequently, the general frequency of TB was 89 (11.54%) , which agrees with the research findings in Ethiopia, that expresses the usual prevalence of TB was 20.1%,11.2% and 12.6% respectively.[13,14]

The GeneXpert MTB/RIF assay is widely acknowledged for its substantial role in facilitating quick discovery of *Mycobacterium tuberculosis* (MTB) and its rigidity against actions of rifampicin. Regular systematic analyses and meta-analyses have established the extraordinary sensitivity and specificity of the Xpert assays in detecting both pulmonary and extrapulmonary tuberculosis. For instance, studies indicate that the Xpert MTB/RIF Ultra assay shows pooled sensitivity rates of approximately 85% and specificity rates of 98% in various populations.[15] Moreover, the rapid turnaround of the assay, to providing results within a few hours enables prompt treatment initiation, which is crucial in managing TB, especially in high-burden areas.[16]

The importance of assay in identifying rifampicin resistance has been highlighted, as identifies mutations in the rpoB gene, which play a vital role in the management of multidrug-resistant tuberculosis.[17] However, despite the significant advantages of the GeneXpert MTB/RIF assay several limitations and challenges remain. Few studies have documented issues of false positive and negative results, particularly in regions with a high TB burden, which can complicate accurate diagnosis and clinical decision-making.[18] For example, the assay's sensitivity may fluctuate based on specimen type and bacterial load, resulting in variations in diagnostic accuracy.[19] Moreover, alternative diagnostic tools, like the Abbott RealTime MTB assay, have demonstrated comparable or even higher sensitivity in certain specific settings, particularly for smear-negative samples.[20] This raises questions about the optimal integration of GeneXpert along with other diagnostic methods to improve overall diagnostic accuracy and enhance patient outcomes.[21] In summary, although the GeneXpert MTB/RIF assay represents a major significant advancement in diagnosing TB, its ongoing evaluation and integration with other tools are vital to address limitations and enhance its reliability across clinical settings. The emergence of alternative assays and the need for comprehensive diagnostic algorithms highlight the importance of continued research and innovation in the field of tuberculosis diagnostics.[22] Ultimately, a multifaceted approach that leverages the strengths of various diagnostic technologies may be the key to effectively combating the global TB epidemic.

In present study, nearly equal distribution of male and female patients with tuberculosis has been evaluated. However, rifampicin resistant was found to be predominant (80% of cases) in male patients suffering with Mycobacterium infections. This male to female discrepancy is supporting the findings from a similar study conducted in Pakistan, where rifampicin resistance was chiefly recognized in the male population (80%) [23] while this findings is in contrast with study conducted in Uganda which revealed female predominance.[24] Additionally, while

exploring the burden of MTB in the rifampicin-resistant MTB isolates, it was perceived that the mainstream of samples had very low or low MTB consignment while limited number of the samples displayed a high load of MTB. This statement proves the statement that effective regulator of drug resistance can be attained through upgraded and timely verdict, as well as the suitable selection of antibiotics. Nonetheless, the specific factors backing to this preponderance of rifampicin resistance among male remain unrevealed.

The demographic results of the present study revealed that the majority cases of tuberculosis fitted in the age bracket of 19-30 years, followed by the age group 41-60 years while the least number of cases belonged to the age of above 65 years. Furthermore, in this study, 57.4% Tuberculosis patients were males while 42.5% were females. Globally, the distribution of TB varies with age and gender. WHO documented variations in age and gender distribution across different continents where Africa showed a higher prevalence among younger age groups, Asia showed a more uniform distribution across all age brackets. A comprehensive study recorded a higher prevalence of tuberculosis among males aged 25-44 years.[25] Specificity of GeneXpert recorded in this study is 100% which is in alignment with a few studies which reported the same specificity of 100% regarding Gene Xpert.[26]

Similarly, a study conducted in Pakistan documented the specificity of 100% in the KPK population.[27] On the contrary, results of another research conducted in Bangladesh reported a significantly lower specificity of 88.3% showing that the variation in population can affect the range of specificity of Gene Xpert.[28]

Positive predictive value (PPV) and Negative predictive value (NPV) for Gene Xpert test in our study were documented as 100% and 97.7% respectively. Solanki and Karthek, both have reported a 100% PPV in their studies supporting our findings, whereas the NPV assessed by both had a contrasting difference of 93.88% and 56.5%.[29] NPV assessed by research conducted in Rawalpindi, Pakistan was calculated to be 99% underscoring the utility of Gene Xpert in correctly identifying the true positive cases of TB.[30]

Conclusion

The study concludes that GeneXpert MTB/RIF has higher sensitivity and specificity to conventional ZN smear microscopy. Regardless of the fact that overall frequency of *M. tuberculosis* being 12.3% in current study; supplementary intensified research findings may be obligatory with the anticipated approaches by the government of Pakistan to End TB by 2035, which beleaguered to condense TB prevalence to as low as 10 cases per 100,000 populations.

Premature recognition with enhanced analytical riggings and public health actions are imperative strategies to govern the transmission of TB infections and lessen the load of TB in the country.

Limitations and Outcome: This was a hospital-based study which may not be accurately illustrative of a total population. Although the results of Rifampicin resistance were not separately linked with other research readings in the discussion as it is insignificant, it can help in the selection of treatment regimen and prevention of spread of MDR-TB.

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