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Original Research Article ***Open Access***

CLINICAL VALIDATION OF GENEXPERT MTB/RIF ASSAY IN THE RAPID DIAGNOSIS OF EXTRA PULMONARY TUBERCULOSIS

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Abstract *Tuberculosis (TB) remains a significant global health concern, with nearly 2 billion people latently infected worldwide, particularly concentrated in the 30 countries with the highest TB burden, including Pakistan, ranking fifth in this category. Pakistan also grapples with Multidrug-resistant TB strains, complicating treatment options. Early diagnosis is crucial for timely TB treatment initiation and prevention of further transmission. Among various diagnostic tests, the Gene Xpert MTB/RIF assay stands out as a promising tool endorsed by the World Health Organization for diagnosing TB in the lungs and other organs. The current study aims to evaluate the diagnostic performance of this assay in the current setting of Peshawar, Khyber Pakhtunkhwa for the diagnosis of extrapulmonary TB. The study included 529 extrapulmonary specimens from suspected TB patients, meeting the specified criteria, anonymously and consecutively. These specimens underwent decontamination using the NALC-NaOH method and were then inoculated onto Lowenstein Jensen media slants. The remaining pellet from each specimen was subjected to testing using the Xpert assay to detect TB bacilli. The study comprised 529 specimens, including 181 pleural fluid, 117 CSF, 89 tissue biopsies, 84 pus, and 58 ascitic fluid samples. The Xpert assay exhibited an overall sensitivity of 78.3% and a specificity of 92% compared to culture techniques. Its positive predictive value stood at 42.6%, with a negative predictive value of 98.2%. Notably, the sensitivity of the Xpert assay was particularly high for pus and tissue biopsy specimens, while it was moderate for body fluids. Specificity remained consistent across various non-respiratory specimens. The study recommends the use of the Xpert assay for diagnosing extrapulmonary TB in Peshawar, Pakistan, due to its simplicity and very short turnaround time. Despite its moderate sensitivity in body fluids, this can be enhanced by considering additional clinical findings.*

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Keywords: *Tuberculosis; Extra pulmonary Tuberculosis; Gene Xpert MTB/RIF assay; Closed system PCR; non-respiratory TB*

Introduction

Tuberculosis (TB) remains a significant global public health concern, claiming 1.3 million lives and generating 5.3 million new cases in 2020. It is the ninth leading cause of mortality worldwide. Despite a decrease in TB incidence since 2000, the emergence of drug-resistant strains hampers control efforts (Wei et al., 2021). The highest burden of TB is found in 30 countries, accounting for 87% of the global TB load. Pakistan ranks fifth among these high TB-burden countries, with an incidence rate of 268 per 100,000 population and a notable burden of drug-resistant TB, including multidrug-resistant TB (MDR-TB), with an incidence rate of 14 per 100,000. Annually, Pakistan records 510,000 new TB cases, including 15,000 pediatric cases, resulting in 70,000 deaths (Ullah et al., 2021). TB is caused by the Mycobacterium tuberculosis complex (MTBc), consisting of five genetically similar species. MTBc species share DNA sequences, including parts of the 16S rRNA gene, but differ in host preferences and phenotypes (Alexander et al., 2010) (Brosch et al., 2001; De Vos et al., 2013). Transmission of TB primarily occurs indoors in settings like homes and healthcare facilities where infectious aerosols are less diluted. TB is airborne, often infecting alveolar macrophages. Aerosols range from 1 to 5µm and are produced by patients with active TB or during certain laboratory and medical procedures (Bacaër et al., 2008; Hutton et al., 1990; Orme, 2014; Robertson et al., 2011). TB can be pulmonary (PTB) or extra pulmonary (EPTB). PTB

accounts for 85-90% of TB cases and is marked by symptoms like prolonged cough, fever, night sweats, and weight loss. EPTB, affecting other organs, accounts for 10-15% of cases and is more common in immunocompromised patients. In Pakistan, EPTB constitutes 20% of TB cases (Isticato and Ricca, 2016; Ramirez-Lapausa et al., 2015; Sharma et al., 2021; Tahseen et al., 2020). Diagnosis of TB has evolved from traditional microscopy to advanced molecular techniques. Conventional microscopy, despite its simplicity, has low sensitivity and cannot differentiate drug-resistant strains (Singhal and Myneedu, 2015). Fluorescence microscopy, recommended by WHO, offers higher sensitivity but is costly (Zijenah, 2018). Culture methods considered the gold standard, are more sensitive but time-consuming (Demers et al., 2012). Line probe assays (LPA) and the GeneXpert MTB/RIF assay provide rapid detection of drug resistance, with GeneXpert being especially useful for detecting rifampicin resistance (Sanchez-Padilla et al., 2015; Tomasicchio et al., 2016). Whole genome sequencing (WGS) offers comprehensive insights into TB genetics and drug resistance profiles, surpassing the capabilities of other molecular methods (Vogel et al., 2021).

Materials and Methods

This clinical validation study was conducted in the Provincial Reference Laboratory for TB, Hayatabad Medical Complex, and Peshawar, Pakistan from January 1, 2023, to June 30, 2023.

Study Design and Sample Collection

The study consecutively and anonymously collected non-respiratory specimens, including pleural fluid, cerebrospinal fluid (CSF), tissue biopsy, pus, and ascitic fluid, from patients referred by their physicians on suspicion of extrapulmonary tuberculosis (EPTB). All patients suspected of having EPTB were included, except those providing specimens for follow-up purposes.

Sample Processing

All the specimens recruited in the study were divided into two equal aliquots. One aliquot was processed by the standard N-acetyl L-cystine (NALC) sodium hydroxide. (NaOH) method for TB culture by solid techniques whilst the other aliquot was processed by Xpert assay according to the instructions provided by the manufacturer.

NALC-NaOH Decontamination Method for EPTB Specimens

For the diagnosis of EPTB, specimen decontamination was conducted using the N-acetyl L-cysteine (NALC)-sodium hydroxide (NaOH) method, as described by (Kumari et al., 2016). In this process, a 4% NaOH solution and a 2.9% sodium citrate solution were prepared separately, then mixed in equal volumes and sterilized to form the NALC-NaOH decontamination solution. Following decontamination, specimens were cultured on Lowenstein-Jensen (LJ) media. A phosphate buffer solution was also prepared to dilute the NALC-NaOH solution post-decontamination following WHO guidelines (Ahmed, 2017).

Phosphate Buffer Preparation (pH 6.8)

To prepare a phosphate buffer with a pH of 6.8, two stock solutions were created. Solution A was prepared by dissolving 9.74 grams of Na_2HPO_4 in 1,000 ml of distilled water, and Solution B was made by dissolving 9.07 grams of KH_2PO_4 in 1,000 ml of distilled water. Equal parts of Solution A and Solution B were then combined and sterilized via autoclaving for 15 minutes, as per (Wallace et al., 2021).

Preparation of Lowenstein-Jensen (LJ) Egg-Based Medium

The Lowenstein-Jensen (LJ) medium, an egg-based medium widely used for culturing, was sourced from Oxoid Company (Satta, 2018). For its preparation, 37.3 grams of the medium powder was added to 600 ml of distilled water along with 12.01 ml of glycerol. This mixture was stirred on a hot plate until the solution became clear (Preece, 2016). After autoclaving the medium for 15 minutes, it was cooled to 55°C. Then, the cooled medium was combined with a freshly prepared homogenized egg suspension. Approximately 7 ml of the final mixture was transferred into screw-cap bottles, which were subsequently placed in an inspissator at 85°C for 45 minutes to complete inspissation (Lewis and Fleming, 1995).

Proficiency and Sterility Testing of LJ Media

All prepared slants of LJ media underwent both proficiency and sterility testing. Sterility testing involved overnight incubation at 35°C; slants showing no growth were refrigerated at 4°C for storage. For proficiency testing, an ATCC control strain of Mycobacterium H37RV was inoculated on the slants. Growth observed after incubation indicated proficiency, and these slants were also stored at 4°C (Tiwari, 2009).

NALC-NaOH Method and LJ Medium Culture Procedure

In the NALC-NaOH method, non-sterile specimens of extrapulmonary tuberculosis (EPTB) were processed by adding equal volumes of specimen and NALC-NaOH solution in a Falcon tube. After vortexing to liquefy the specimen, the mixture was left for 15 minutes for decontamination. Sterile specimens like CSF were excluded from NALC-NaOH treatment. The mixture was diluted with buffer to neutralize NaOH and adjust pH, then centrifuged at 3500 RCF for 15 minutes in a refrigerated centrifuge. After allowing 5 minutes for the aerosol to settle, the supernatant was carefully discarded into a splash-proof container with anti-mycobacterial solution. The sediment was then inoculated onto LJ medium for solid culture. LJ media were incubated for bacterial growth, with results declared negative if no growth appeared within 56 days. Positive results underwent a nitrate reduction test for identification (Wang et al., 2022).

Xpert assay

The Xpert assay, also known as closed system PCR, is an automated nucleic acid amplification-based test utilizing single-use GeneXpert cartridges. Each cartridge contains segregated compartments with PCR reagents, ensuring minimal risk of contamination (Organization, 2014). During analysis, specimens were mixed with decontamination reagent in a 2:1 ratio and incubated for 15 to 20 minutes after vortexing. A 2ml sample of the mixture was then added to a sterilized cartridge provided in the test kits and placed in the instrument for processing according to standard instructions.

Statistical Analysis

The study variables included sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Xpert assay, which were calculated using standard formulas. Sensitivity was determined as [a / (a + c)] x 100, where a represents true positives and c represents false negatives. Specificity was calculated as [d / (b + d)] x 100, with d representing true negatives and b representing false positives. PPV was computed using [a / (a + b)] x 100, and NPV as [d / (c + d)] x 100. True positive and true negative values were determined using TB culture as the gold standard.

Results

The study included 529 extrapulmonary samples: 181 pleural fluid, 117 CSF, 89 tissue biopsy, 84 pus, and 58 ascitic fluid**,** collected from 307 males and 222 females aged 16 to 75 years as shown in Figure 1.

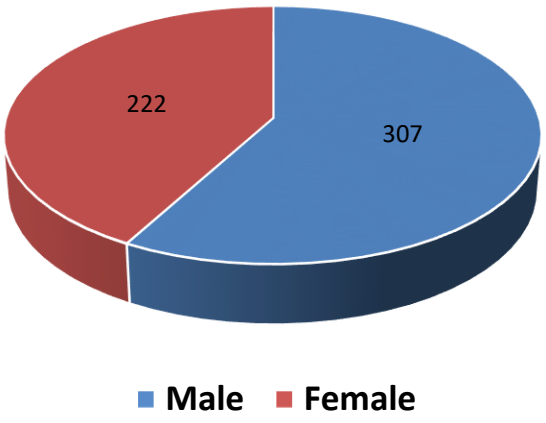

Figure 1. Gender wise distribution of EPTB

Out of these, 37 samples were MTBc positive on culture, and 492 were negative. The Xpert assay detected MTBc in 68 samples and 461 were negative shown in figure 2.

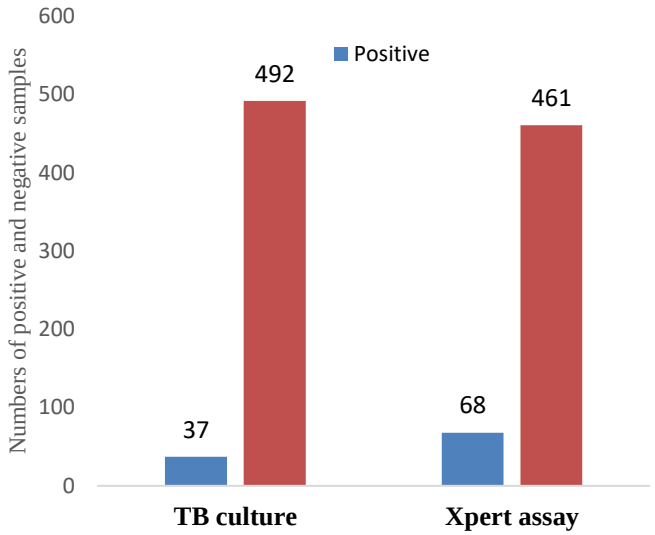

Figure 2. Overall results of TB culture and Xpert assay

True positive, false positive, true negative, and false negative results were defined based on Xpert assay and culture outcomes as; True positive: MTBC detected positive on both culture and Xpert assay

Tru negative: MTBC detected negative on both culture and Xpert assay False positive: MTBC detected positive on Xpert assay and negative on culture False negative: MTBC detected positive on culture and negative on Xpert assay

The overall sensitivity and specificity of the Xpert assay were 78.3% and 92%, respectively, with PPV at 42.6% and NPV at 98.2% as dipicted through figure 3.

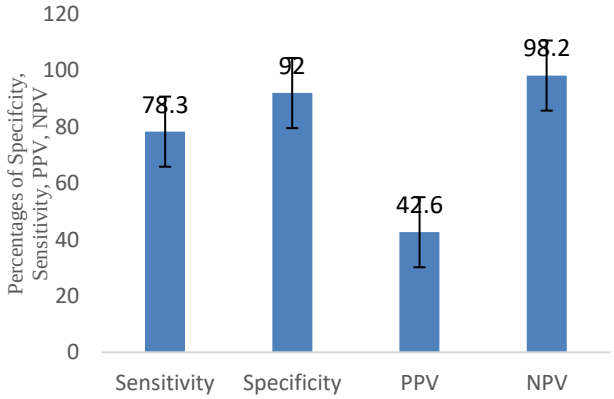

Figure 3. Overall Sensitivity, Specificity, PPV and NPV of Xpert assay

The assay showed the highest sensitivity for tissue biopsy, highest specificity for ascitic fluid, highest PPV for ascitic fluid, and highest NPV for tissue biopsy, as detailed in Tables 1 to 6.

Table. 1 Performance of Xpert assay for Pleural fluid

Total specimen	End results after comparison of Xpert and Culture result			Sensitivity	Specificity	PPV	NPV
181	Positive	True	8	80%	96%	53%	99%
		False	7				

	Negative	True	164				
		False	2				

Table 2 Performance of Xpert assay for CSF

Total specimen	End results after comparison of Xpert and Culture result			Sensitivity	Specificity	PPV	NPV
117	Positive	True	7	70%	92%	44%	97%
		False	9				
	Negative	True	98				
		False	3				

Table 3 Performance of Xpert assay for Tissue biopsy

Total specimen	End results after comparison of Xpert and Culture result			Sensitivity	Specificity	PPV	NPV
89	Positive	True	3	100%	88%	23%	100%
		False	10				
	Negative	True	76				
		False	0				

Table 4 Performance of Xpert assay for Pus

Total specimen	End results after comparison of Xpert and Culture result			Sensitivity	Specificity	PPV	NPV
84	Positive	True	9	82%	84%	43%	97%
		False	12				
	Negative	True	61				
		False	2				

Table 5 Performance of Xpert assay for Ascitic fluid

Total specimen	End results after comparison of Xpert and Culture result			Sensitivity	Specificity	PPV	NPV
58	Positive	True	2	67%	98%	67%	98%
		False	1				
	Negative	True	54				
		False	1				

Table 6 Comparative performance of Xpert assay for different specimens

Specimen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Tissue Biopsy	100	88	23	100
Pus	82	84	43	97
Pleural fluid	80	96	53	91
CSF	70	92	44	97
Ascitic fluid	67	98	67	98

The comparative presentation of sensitivity, specificity, PPV and NPV of Xpert assay for the EPTB specimens included in the current study has been shown in figure 4, 5, 6, and 7.

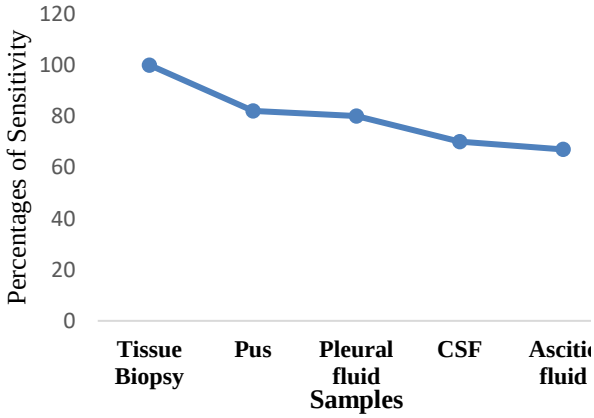

Figure 4. Comparative presentation of Sensitivity of Xpert assay for different specimens

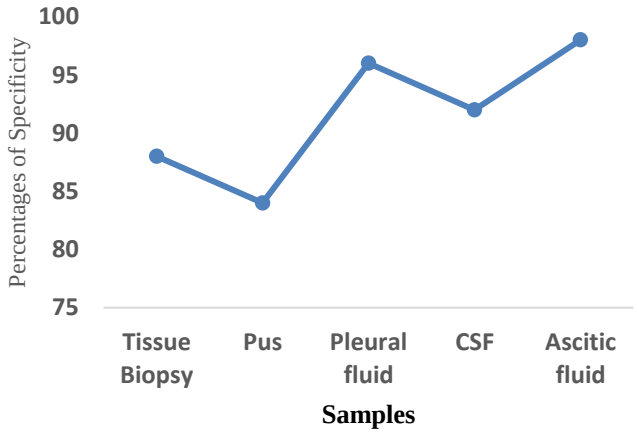

Figure 5. Comparative presentation of Specificity of Xpert assay for different specimen

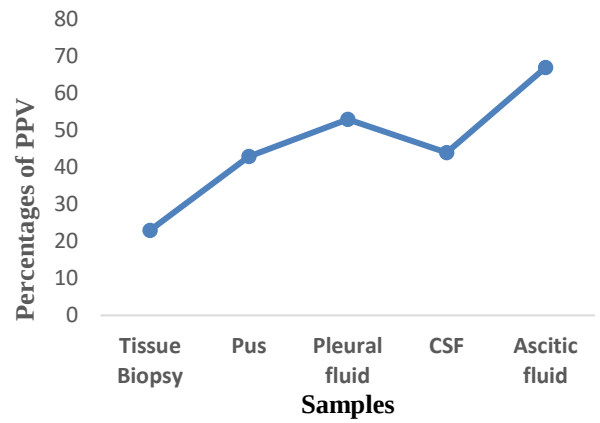

Figure 6. Comparative presentation of PPV of Xpert assay for different specimens

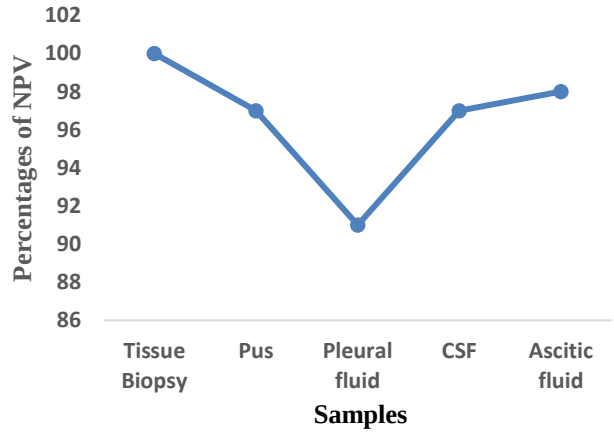

Figure 7. Comparative presentation of NPV of Xpert assay for different specimens

Discussion

Tuberculosis (TB) remains a major global public health challenge, persisting as a deadly disease since ancient times. Accurate and early diagnosis is critical but remains challenging, particularly in laboratory medicine. Traditionally, TB has been diagnosed using microscopy; however, this method lacks the sensitivity required for early detection, especially in samples with a low bacilli load (Bynum, 2012). The culture technique considered the gold standard for TB diagnosis, is hindered by the slow growth rate of TB bacilli, requiring 4-6 weeks for detection, and necessitates sophisticated infrastructure, trained personnel, and high costs (Campelo et al., 2021). The Xpert assay, introduced in 2010, revolutionized TB diagnosis by providing a rapid (98 minutes), simple, and efficient diagnostic tool that does not require sophisticated infrastructure or highly trained personnel (Zhang et al., 2020). Initially recommended for pulmonary TB (PTB), the assay was later endorsed by WHO for extrapulmonary TB (EPTB). However, the diagnostic performance of the Xpert assay varies across different EPTB samples and regions (Li T-x, 2023).

Our study aimed to evaluate the diagnostic performance of the Xpert assay for five different EPTB specimens: tissue biopsy, pus, CSF, pleural fluid, and ascitic fluid in Khyber Pakhtunkhwa. The overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 78.3%, 92%, 42.6%, and 98.2%, respectively. Recent studies, such as(Mechal et al., 2019) in Southwest China, reported a sensitivity of 80.7% and specificity of 70.9% for a diverse range of EPTB samples. Our results show higher specificity, likely due to the inclusion of fewer specimen types, which may enhance specificity. Similarly, Morocco reported sensitivity, specificity, PPV, and NPV values of 78.2%, 90.4%, 54%, and 97%, respectively, aligning closely with our findings. In contrast, Egypt and Ethiopia observed (Elbrolosy et al., 2021; Tadesse et al., 2019) different diagnostic accuracies, emphasizing the regional variability in the Xpert assay's performance. This variability highlights the necessity of evaluating the Xpert assay's performance in local settings.

In this study, we identified eight false-negative and 29 false-positive cases. The occurrence of false negatives could stem from differences in detection thresholds between the culture method and the Xpert assay. The culture method can identify bacterial loads as low as 10-100 CFU/ml, whereas the Xpert assay has a detection limit of 137 CFU/ml. The false positives, although initially classified as such, were later considered likely true positives based on follow-up data from other studies, suggesting that the Xpert assay may have higher diagnostic accuracy than initially estimated. The limitation of our study is the inability to conclusively confirm false-positive cases, as we relied solely on the culture method as the gold standard. Since the culture method might miss Mycobacterium tuberculosis complex (MTBc) in extrapulmonary tuberculosis (EPTB) cases due to low bacterial load (paucibacillary nature), this could lead to an overestimation of false positives and affect specificity. Future research should integrate additional confirmatory methods to better address these limitations and enhance diagnostic accuracy.

Conclusion

The study concluded that the Xpert assay is an effective tool for the early diagnosis of EPTB, showing strong alignment with clinical observations. It demonstrated high sensitivity and specificity, particularly in tissue biopsy and pus samples, though results were moderate for body fluids. Given its simplicity, open-bench setup, and rapid processing time for detecting MTBc, the Xpert assay is recommended for EPTB diagnosis in Pakistan. Its use may enhance early detection of EPTB, contributing to improved patient outcomes.

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Declarations

Declaration of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

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Author's contributions

MK and MN conducted the field trials and planned the experiment. AS, AU, S, NH analyzed the data. JU, AU and MAS assisted with data collection. All authors proofread the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for Publication

Not applicable