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Investigation of Conventional and Molecular Methods of *Mycobacterium tuberculosis* Diagnosis: Smear Microscopy Versus GeneXpert MTB/RIF Assay

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Abstract

The present study was conducted to determine the diagnostic performance of the molecular technique GeneXpert MTB/RIF assay in comparison with conventional method of Acid-fast staining for the detection of *Mycobacterium tuberculosis* from pulmonary specimens. Moreover, it aimed to evaluate the Rifampicin resistance at the molecular level. A total of 610 patients suspected of TB were enrolled for diagnosis using GeneXpert MTB\RIF assay and Ziehl-Neelsen (ZN) smear microscopy. The data revealed that out of 610 patients, 45.41% (*n*=277) were females and 54.59% (*n*=333) were male patients. *M. tuberculosis* was detected in 60.82% (*n*=371) samples using GeneXpert MTB/RIF assay, while 63.44% (*n*=387) were detected by ZN staining. Moreover, Rifampicin resistance was detected in 11.15% (*n*=68) of patients. In summary, the GeneXpert MTB/RIF assay demonstrated high sensitivity for rapid diagnosis of tuberculosis, particularly in smear-negative cases, and exhibited better diagnostic performance compared with conventional Ziehl-Neelsen staining.

Keywords: *Mycobacterium tuberculosis, MTB, GeneXpert, Acid-fast bacilli, Rifampicin Resistance.*

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I. INTRODUCTION

Mycobacterium tuberculosis causes tuberculosis (TB), a serious and highly contagious disease that affects around one-third of the world's population (Jagielski, Augustynowicz-Kopec, & Zwolska, 2010). Although tuberculosis is predominantly a pulmonary illness caused by *M. tuberculosis*, which is present as aerosol droplets that impact lung alveolar surfaces, it affects numerous organs of the body, including the bones and central nervous system (Smith, 2003). Latent tuberculosis is a phenomenon in which *M. tuberculosis* may live within a human host for years without developing illness. Since one-third of people on the planet have latent TB, they are susceptible to developing active TB. Future TB control heavily depends on understanding the processes by which Mycobacterium tuberculosis creates a latent metabolic state, evades immune monitoring, and reacts to stimuli that trigger reactivation (Parrish, Dick, & Bishai, 1998).

Multidrug-resistant tuberculosis (MDR-TB) is caused by *M. tuberculosis* that is resistant to at least two anti-tubercular drugs, isoniazid and rifampicin (Marahatta, 2010). Both MDR-TB and extensively drug resistant tuberculosis (XDR-TB) pose significant threats to global tuberculosis control efforts (Prasad, 2010). *M. tuberculosis* and Rifampin resistance can be directly detected from clinical specimens using the GeneXpert MTB/RIF assay, a real-time PCR technique (Helb et al., 2010). The assay simultaneously identifies *M. tuberculosis* and rifampicin resistance by amplifying the 81-bp region of the *rpo*B gene and detecting mutations associated with rifampicin resistance (Blakemore et al., 2010; Helb et al., 2010).

Culture remains the gold standard for definitive diagnosis; however, it is time consuming and may require 2 to 8 weeks to yield results. Although smear microscopy for acid-fast bacilli is inexpensive and rapid, it has limited sensitivity and a low positive predictive value. Therefore, rapid identification requires nucleic acid amplification techniques, which are essential for better patient outcomes, an earlier start to therapy, and more effective public health initiatives (Acharya *et al*., 2020). The purpose of the current study was to assess the prevalence of tuberculosis in Sindh, to compare the effectiveness of molecular and conventional methods for diagnosing the disease, and to examine the pattern of rifampicin resistance among *M. tuberculosis* circulating in different areas of Sindh.

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II. Materials And Methods

A. Study design

The present study was conducted to analyze GeneXpert assay and smear staining data for the diagnosis of TB and detection of Rifampicin resistance among the samples referred to the Civil Hospital, Hyderabad. Patients were instructed to rinse their mouths with water prior to sample collection and then expectorate a deep cough sputum specimen into a sterile, screw-capped container. To ensure optimal sample quality, specimens were preferably collected in the morning. Fixed cell preparations were stored at room temperature. Unfixed specimens intended for examination within 48 hours of collection were stored at 4ºC.

B. Acid fast staining

AFB testing was used as a rapid test to detect *Mycobacterium tuberculosis,* causing tuberculosis from sputum samples. Mycobacteria were stained with Ziehl-Neelsen Staining as per standard protocol. Briefly, the sputum was spread evenly onto a clean slide. The slide was then air-dried for about 30 minutes. The dried smear was heat-fixed and covered with carbol fuchsin stain. Following heating for five minutes, the slide was gently rinsed with clean water and then decolorized with 3% (v/v) acid alcohol for 2-5 minutes, until the smear became pale pink in appearance. The slide was then washed with clean water. Malachite green stain was poured over the smear for 1-2 minutes, followed by washing with clean water. After air-drying, the slide was examined microscopically using a 100× oil immersion objective lens. AFB were identified as red, straight, or slightly curved rod-shaped organisms, appearing as single cells or in small clusters**.**

C. GeneXpert MTB/RIFAssay

The GeneXpert system uses reverse transcriptase PCR and real-time PCR to automate nucleic acid amplification, sample processing, and target sequence detection in simple samples. The reagents for identifying MTB and rifampicin resistance are included in the Xpert MTB/RIF assay. The assay utilizes specific primers to amplify a segment of the *rpoB* gene that encompasses the 81-base pair core region associated with rifampicin resistance. The probes are able to distinguish mutations in the core region associated with rifampicin resistance and the conserved wild-type sequence (Lawn & Nicol, 2011).

III. Results

A. Prevalence of Mycobacterium tuberculosis in Hyderabad

In order to assess the prevalence of TB and the diagnostic accuracy of conventional and molecular techniques, 610 samples from TB patients were collected and examined smear microscopy and GeneXpert assay. The sputum specimens were investigated during the months of January to August 2015. Analysis of all samples revealed that highest number of samples was collected during the month of august, whereas lowest number of samples was collected in February.

B. District-wise distribution of all samples

The samples were collected from nine different areas of Sindh province (Figure 1). The data revealed that highest number of samples was collected from Hyderabad, followed by T. M. Khan, Matiyari and Sanghar. Whilst the lowest number of samples was from districts Tando Allah Yar, Karachi, Umer Kot and Jamshoro (Fig. 1).

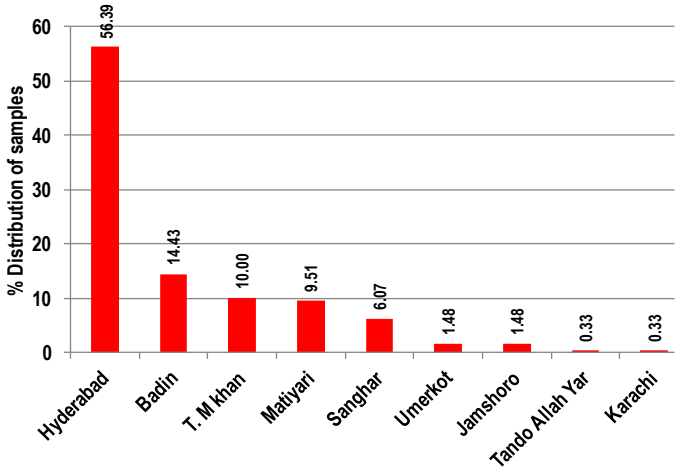

Figure 1. Graph showing district wise distribution of samples

C. Genderwise distribution of total samples

Among the 610 samples collected and investigated for detection of rifampicin resistance and MTB pathogen, females were 45.41% (n=277) whereas the 54.59% (n=333) were male patients.

D. Comparative analysis of GeneXpert and Smear positive cases

M. tuberculosis isolates were detected by the GeneXpert MTB/RIF assay in 371/610 (60.82%). whereas ZN staining demonstrated smear positivity in 387/610 (63.44%). Rifampicin resistance was detected in 68/610 patients (11.15%) (Figure 2). GeneXpert provides greater sensitivity and rapid diagnosis of pulmonary tuberculosis compared with microscopy, and its scalability can improve access to TB diagnosis. However, its sensitivity remains lower than culture-based method, therefore, a culture negative test does not exclude tuberculosis. Clinical judgment remains essential when initiating anti-tuberculosis therapy.

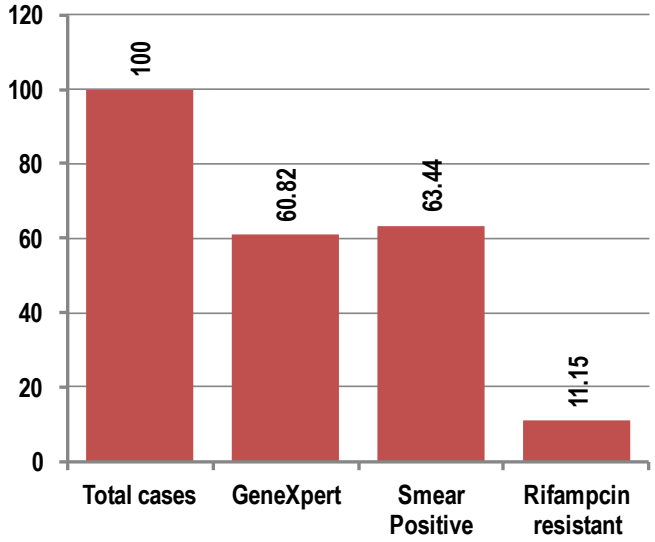

Figure 2. Graph showing the distribution of Gene Xpert, Smear positive, and Rifampicin resistance

IV. Discussion

In developing countries, TB remains a significant public health concern. Effective disease control strategies depends on early and precise diagnosis of the disease (Zumla et al., 2015). The present study focused on diagnosis of TB using conventional and molecular techniques. Samples of sputum were collected from TB patients from various parts of Sindh province and investigated using smear microscopy and the GeneXpert MTB/RIF assay. Demographic analysis revealed that most samples belonged to Hyderabad region, followed by Tando Muhammad Khan, Matiyari, and Sanghar, while lowest samples were from Tando Allah Yar, Karachi, Umer Kot, and Jamshoro. These area wise variation in TB sample distribution may possibly be the result of availability of diagnostic facilities, awareness among the population (Dye, Glaziou, Floyd, & Raviglione, 2013).

Gender-wise distribution of samples revealed that male patients (54.6%) were higher as compared to the female patients (45.4%). The male dominance in sampling is in agreement with global TB epidemiology, which indicates that men have higher TB rates, which may be associated to socioeconomic determinants, smoking, occupational exposure, and obstacles to women access to healthcare in certain situations (Horton, MacPherson, Houben, White, & Corbett, 2016). The data of smear microscopy revealed 63.44% of samples as positive whereas GeneXpert MTB/RIF assay detected 60.82% of cases. Although smear microscopy demonstrated a slightly higher positivity rate in this study, GeneXpert offers significant advantages, including rapid diagnosis and simultaneous detection of rifampicin resistance and TB (Boehme et al., 2010; Byanyima et al., 2022). The identification of rifampicin resistance in 11.15% of patients highlights the growing burden of drug-resistant tuberculosis and highlights the need for routine molecular resistance testing (Bagcchi, 2023).

Several studies have demonstrated that GeneXpert has higher sensitivity than smear microscopy, particularly in smear-negative and HIV-associated TB cases (Jameel et al., 2024; Lawn & Nicol, 2011; Steingart et al., 2013). While smear microscopy remains widely used in resource-limited settings due to its low cost, it lacks sensitivity and does not provide information on drug resistance. In contrast, GeneXpert enables rapid diagnosis within two hours, allowing early initiation of appropriate therapy (Abayneh et al., 2025; Boehme et al., 2010). Despite these advantages, the sensitivity of GeneXpert remains lower than that of mycobacterial culture, known as the gold standard method of diagnosis of TB (Pai et al., 2016). Therefore, a negative GeneXpert result does not exclude TB, particularly in patients with low bacterial load. Clinical judgment, radiological findings, and follow-up investigations remain essential before interrupting the anti-tuberculosis treatment (Zumla et al., 2015).

V. Conclusions

Overall, the findings support the use of the GeneXpert MTB/RIF assay as a valuable tool for rapid TB diagnosis and rifampicin resistance detection. Integrating GeneXpert with conventional microscopy can strengthen TB control strategies and improve patient outcomes. Further research is needed to enhance diagnostic accuracy and expand access to advanced TB diagnostics facilities in high-burden areas.

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