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Original Research Article

USE OF GENE XPERT MTB/RIF ASSAY FOR DETECTING *MYCOBACTERIUM TUBERCULOSIS* AND RIFAMPICIN RESISTANCE

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Abstract: *The current study was designed to assess the specificity and sensitivity of MTB/RIF assay for early detection of Mycobacterium tuberculosis and RIF resistance in smear-positive and smear-negative pulmonary and extra-pulmonary samples. This retrospective study was conducted in Bakhtawar Amin Trust Hospital Multan from July 2021 to July 2022. Total 240 pulmonary samples (gastric fluid specimens, post-bronchoscopic sputum, bronchoscopic aspirate, bronchoalveolar lavage and sputum) and 170 extra-pulmonary samples (urine specimens, skin biopsy, pericardial fluid, cerebrospinal fluid, ascitic fluid, disc material, lymph node biopsy and pleural fluid) were collected. After culture growth and identification of species, drug susceptibility testing (DST) was done. All samples underwent Xpert MTB/RIF assay. A total of 240 pulmonary and 170 extra-pulmonary samples from 410 patients were collected. The mean age of the patients was 46.5 ± 10 years. Of 410 subjects, 100 had TB, and 310 had no evidence of TB. Of 100 TB patients, the specificity of the MTB/RIF assay was 100 % (100), sensitivity was 69% (69), PPV was 100% (100), and NPV was 91% (91). According to the MTB/RIF assay, 79 samples were RIF-susceptible, and 1 was RIF-resistant; DST confirmed the results. MTB/RIF assay is a valuable tool for detecting Mycobacterium tuberculosis and rifampicin resistance. It is not technique sensitive and provides results in less than three hours*.

Keywords: *Mycobacterium tuberculosis*, rifampicin resistance, culture, Gene Xpert

Introduction

Tuberculosis (TB) is a significant health concern worldwide. According to WHO, TB affects over ten million people and results in 1.2 million deaths; 90% of TB patients lie in the reproductive age group (Chakaya et al., 2022). Recently, there has been an increase in multidrug resistance TB (MDR-TB) which pose a challenge for health workers. Rifampicin-resistant TB (RR-TB) constitutes a large portion of MDR-TB(Chakaya et al., 2022). Moreover, drug-resistant strains are increasingly transmitted because of empirical treatment (Zignol et al., 2016). Developing countries have a high burden of MDR-Tb, TB and TB/HIV. Early diagnosis and drug sensitivity test for TB is central management of TB. However, the low sensitivity of diagnostic tools, specifically in immune-compromised and smear-negative individuals hinder early detection. Xpert MTB/RIF assay was endorsed by WHO in 2010. It is a rapid molecular system for detecting *Mycobacterium tuberculosis* and rifampicin resistance. It is a cartridge-based simple diagnostic tool that can be used for pulmonary and extra pulmonary samples to detect TB and rifampicin resistance (Diandé et al., 2019; Perdigão et al., 2020; Schwoebel, 2020). Xpert MTB/RIF assay have shown better results as compared to culture and other conventional methods. After WHO's endorsement, early case detection using Xpert MTB/RIF assay improved immensely. *M. tuberculosis* (MTB) and rifampicin resistance (RIF) is detected by MTB/RIF assay through PCR amplification of *M. tuberculosis rpoB* gene followed by regional probing to detect mutations leading to RIF resistance. It takes less than two hours to generate results (Georghiou et al., 2021; Phetsuksiri et al., 2020). The study aimed to assess the specificity and sensitivity of MTB/RIF assay for early detection of tuberculosis and RIF resistance in smear-positive and negative pulmonary and extra-pulmonary samples. The assay results were compared with those of phenotypic susceptibility testing and culture.

Methodology

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After culture growth and specie identification, drug susceptibility testing (DST) was done. Geno Type MTBDR plus assay was used to confirm *M. tuberculosis* in growth cultures. RIF resistance was tested by the proportional method using the 7H10 agar medium. All samples underwent Xpert MTB/RIF assay. A sample reagent was added to dilute the sample. After 15 minutes, 2ml of the mixture was put in an Xpert cartridge and placed in the Xpert machine. All MTB/RIF test-negative and culture-positive samples and MTB/RIF-test positive and culture-negative samples were tested twice. Final reports were analyzed. Subjects were categorized into these groups: I) subjects with culture and smear-positive TB, II) subjects with culture-positive and smear-negative TB, III) those with culture and smear-negative TB but were treated for TB based on radiological, pathological and clinical findings, and IV) those with no evidence of TB. A clinician diagnosed culture-negative samples. SPSS version 20 was used for data analysis. Numerical variables were represented as mean and standard deviation. Student *t*-test was used for assessing differences among groups. The Chi-square test was used for assessing categorical variables.

A *P-value* < 0.05 was considered statistically significant.

Results

A total of 240 pulmonary and 170 extra-pulmonary samples from 410 patients were collected. The mean age of the patients was 46.5 ± 10 years. Of 410 subjects, 100 had TB, and 310 had no evidence of TB. Of 100 TB patients, 80 were culture positive, and 20 were culture-negative; however, their history and investigative evidence indicated TB. Of 240 pulmonary Samples, 60 were diagnosed with pulmonary TB after combining clinical data and culture results. Of 170 extra-pulmonary samples, 40 were diagnosed with extra-pulmonary TB after combining clinical data and culture results. Of 100 TB patients, the specificity of the MTB/RIF assay was 100 % (100), sensitivity was 69% (69), positive predictive value (PPV) was 100% (100), and negative predictive value (NPV) was 91% (91). For pulmonary samples, the specificity and sensitivity of the MTB/RIF assay were 100% and 83%, respectively. For extra-pulmonary samples, specificity and sensitivity were 100% and 53%, respectively. According to the results of the culture, the specificity of the MTB/RIF assay was 99%, sensitivity was 79.6%, PPV was 92.1%, and NPV was 95%. The sensitivity of the MTB/RIF assay for smear-positive pulmonary samples was 100%, and for smear-negative pulmonary samples, it was 75.4%. The assay sensitivity for smear-positive extra-pulmonary samples was 100%, and for smear-negative extra-pulmonary samples, it was 64%. Tables I and II summarize the diagnostic value of MTB/RIF test. All culture-positive samples underwent susceptibility testing. Seventy-three patients had MTB strains susceptible to pharmacotherapy, 4 patients had single-drug resistant (3 to isoniazid, 1 to ethambutol), and 3 patients had multi-drug resistant strains (2 to EMB and INH, 1 to INH and RIF). According to the MTB/RIF assay, 79 samples were RIF-susceptible, and 1 was RIF-resistant; DST confirmed the results.

Table I Diagnostic Value of Various Modalities

Modality and sample type	Pulmonary Sample				Extra-pulmonary sample			
	Sensitivity	Specificity	Negative Predictive value	Positive Predictive value	Sensitivity	Specificity	Negative predictive value	Positive predictive value
Smear	42.6	97.6	85.5	95	9.2	100	75	100
Smear-positive	100	99	99	99	100	99	99	99
Smear-negative	69	100	95	100	48	100	84.3	100
Culture	92.5	100	98	100	65	100	88.2	100

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MTB/RIF assay	83	100	95	100	53	100	84	100

*All values are in percentage(%)

Table II Diagnostic Value of Various Modalities Based on Culture Results

Modality and sample type	**Pulmonary Sample**				**Extra-pulmonary sample**			
	Sensitivity	Specificity	Negative Predictive value	Positive Predictive value	Sensitivity	Specificity	Negative predictive value	Positive predictive value
Smear	45.6	98.5	85.2	95.3	13	100	85	100
Smear-positive	100	99	99	99	100	99	99	99
Smear-negative	75.4	98.5	97	96	64	98	92.2	77.1
MTB/RIF assay	86.3	95.4	97	99	68	95.6	92	81

*All values are in percentage(%)

Discussion

The current study evaluated the significance of MTB/RIF assay in pulmonary and extra pulmonary samples. Previous studies show that MTB/RIF assay had a sensitivity of 56%-77% for culture-positive, smear-negative pulmonary TB and 99%-100% for culture-positive, smear-positive pulmonary TB. The specificity of the assay was 99%-100% (Anand et al., 2018; Bahraminia et al., 2021; Katoch et al., 2021). In the current study, the sensitivity of the assay for culture and smear-positive pulmonary samples was 100%, in line with previous studies. In this study, sensitivity for smear-negative pulmonary samples was 75.4%, higher than reported in a previous study (Kolia-Diafouka et al., 2019). A study reported the assay's sensitivity for smear-positive and negative extra-pulmonary samples to be 100% and 36%, respectively (Piersimoni et al., 2019). In the current study, sensitivity for the smear-positive extra pulmonary sample was also 100%, but for the smear negative extra pulmonary sample, it was 64, unlike the previous study's findings. In all TB samples, assay sensitivity for respiratory samples was significantly more significant compared to extra-pulmonary samples (P = 0.001). It may be due to the extra-pulmonary specimen's higher smear negative rate. Although the sensitivity of the MTB/RIF assay for the smear-negative, culture-positive pulmonary sample was higher than the sensitivity for smear negative, culture-positive extra-pulmonary samples, the difference was not statistically significant. The mean turnaround time in culture-positive samples was 18 ± 9 days. For smear-positive samples, the mean turnaround time was significantly shorter for pulmonary samples as compared to extra-pulmonary. Moreover, turnaround time was shorter in MTB/RIF test-positive and smear-positive samples (P = 0.001). This may be because the extra-pulmonary sample had a lower number of organisms. Another study reported that the limit of detection of MTB/RIF assay was 129 CFU/ml sputum, and it detected only 9 CFU/ml sputum in 34% of specimens (Bonney et al., 2019). In the current study, the MTB/RIF test-negative samples had a long turnout time because of the lower number of organisms under the detection limit. MTB/RIF test gives results much faster as compared to culture. Other studies reported the assay's sensitivity for detecting rifampicin resistance to be 95% - 100%. Specificity was 99%-100%(Fakhreddine et al., 2020; Penn-Nicholson et al., 2021). In the current study, MTB/RIF assay showed 79 samples were RIF-susceptible, and 1 was RIF-resistant. The culture gave positive in a single pulmonary sample, while the MTB/RIF test yielded negative results. The limitation of our was a small sample size; a larger study is required for further analysis.

Conclusion

MTB/RIF assay is a valuable tool for detecting *Mycobacterium tuberculosis* and rifampicin resistance. It is not technique sensitive and provides results in less than three hours.

Conflict of interest

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The authors declare no conflict of interest.

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