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Clinical Evaluation of the Xpert MTB/XDR Assay for Rapid Susceptibility Testing of Mycobacterium tuberculosis in a Region of High TB Burden

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ABSTRACT

Background: Drug-resistant tuberculosis (DR-TB) poses a significant threat to TB control programs in Pakistan. Conventional drug susceptibility testing (DST) methods are slow and technically demanding, leading to delays in appropriate treatment. Rapid molecular tests like the Xpert MTB/XDR are crucial for timely diagnosis and improving patient outcomes in high-burden settings like Peshawar.

Objective: To evaluate the diagnostic accuracy of the Xpert MTB/XDR test for the detection of resistance to first- and second-line anti-tuberculosis drugs in Peshawar, Pakistan.

Methodology: This study was a cross-sectional, blinded, lab-based inquiry that used culture isolates or sputum samples. The Xpert® MTB/XDR test's diagnosis accuracy was compared to that of the MGIT960, the reference drug susceptibility testing (DST) techniques, and the Hain Genotype® MTBDR plus and MDRsl assays (LPA).

Results: The patients were divided into three groups based on technique used i.e., Pre-LPA or DST, Post-LPA and Ten color Xpert MTB/XDR and followed up for their culture conversion. In this study,180 positive MDR-TB patients were included. Gene-Xpert MTB/RIF showed 93% cases were positive and on DST all cases showed positive results. For Drug-resistance in DST, LPA and Gene-Xpert MTB/XDR, 61.1% patients were resistant to three first-line drugs and 11.1% resistant to second line.

Conclusion: This study concludes that based on timely initiation of treatment and culture conversion reports, DST and LPA are less efficient than Ten-color Gene Xpert MTB/XDR assay.

Keywords: MDR-TB; LPA; DST; Xpert MTB/XDR

Introduction

In spite of its high morbidity and mortality rate, tuberculosis (TB), which is caused by M. tuberculosis (MTB), continues to pose serious threats to world health. In addition, the emergence of drug-resistant tuberculosis (DR-TB) in several nations poses a concern. According to reports, the number of new cases of DR-TB worldwide increased between 2020 and 2021, with an expected 450,000 (95% UI: 399,000–501,000) instances of RR-TB[1]. It is still crucial to prioritize DR-TB diagnosis as soon as possible and to start suitable therapy as soon as possible.[1,2]

Pakistan is ranked as the 5th highest TB burden country and annually contributes to nearly 5.8% of new cases of TB worldwide (WHO Global TB report, 2021). Out of the 573,000 new cases estimated for 2020, a total of 276736 (48%) were notified. With 2,689 laboratory confirmed cases of MDR-TB and another 839 patients identified with XDR-TB in 2020, Pakistan is also included in the list of 30 High Burden countries for Drug Resistant TB.[3,4]

Enhancing the identification and initiation of therapy for drug-resistant tuberculosis (DR-TB) is still highly demanded. The liquid-based Mycobacterial Growth Indicator Tube (MGIT960®) system made by Becton Dickinson in Sparks, Maryland, and the solid agar proportions method are the main techniques used in Pakistan for drug susceptibility testing (DST) for Mycobacterium tuberculosis (MTB). The MGIT system is available as a ready-to-use kit, however the agar proportions approach usually requires media preparation in a laboratory environment. Culture-based DST needs to be carried out in specialist labs with highly trained staff members and state-of-the-art biosafety precautions.

Therefore, rather than depending entirely on culture-based DST, it may be advantageous to use a genotypic susceptibility testing strategy that makes use of a kit format and automated DNA-based technology to identify resistance-associated mutations. Limited accessibility regardless, the Line Probe Assay (LPA) provides a quick molecular diagnosis for MDR-TB and pre XDR-TB. Examples of such assays include Genotype® MTBDR plus and MDRsl from Hain Life Sciences in Nehren, Germany. Compared to culture-based methods, LPA yields DST results for rifampicin, isoniazid (INH), fluoroquinolones (FQs), and second-line injectable agents (IAs) faster, but it also requires greater technical knowledge and certain infrastructure requirements, such as the need for three separate rooms (pre-amplification, amplification, and post-application).

To address these issues and fill the diagnostic gap for MDR-TB, the World Health Organization (WHO) recommended in 2011 that the GeneXpert MTB/RIF test (Xpert; Cepheid, Sunnyvale, CA) be used as the main diagnostic tool for TB and people at high risk of RR-TB.[5] In 2017, GeneXpert Ultra (Ultra) was upgraded to replace the original test. Following this, the WHO's End TB policy promoted the use of more strong medications in shorter regimens, targeted TB therapy based on drug resistance profiles, and universal screening for DR-TB.[6] However, in many low- and middle-income countries (LMICs), putting these suggestions into practice still presents a substantial challenge[7,8]. Patients diagnosed with RR-TB by Xpert testing are started on MDR-TB medication, and in nations such as Pakistan, MDR-TB treatment is initiated solely based on Xpert results alone. All individuals who test positive for RR-TB using Xpert are started on MDR-TB medication; very few receive second-line DST before starting treatment.

Launched in 2021, Cepheid announced a new cartridge called the Xpert® MTB/XDR test. The purpose of this cartridge is to assess a patient's susceptibility to second-line injectable agents (IAs), fluoroquinolones (FQs), and isoniazid (INH). For tuberculosis (TB) patients with high-risk INH resistance and those with a high probability of resistance to second-line anti-TB medications, it is now advised as a reflex test. The World Health Organization (WHO) recommended further field assessments before to providing final approval for the Xpert® MTB/XDR test in 2021. Using biobanked specimens, the current study seeks to evaluate the efficacy of the Xpert® MTB/XDR test for MTB susceptibility testing in patients suspected of having extensively drug-resistant tuberculosis (XDR-TB). Metrics related to test performance were compared to those from the Xpert Ultra test.

Objective

To evaluate the diagnostic accuracy of the Xpert MTB/XDR test for the detection of resistance to first- and second-line anti-tuberculosis drugs in Peshawar, Pakistan.

Methodology

This single-blinded, cross-sectional research was conducted in a laboratory setting of Programmatic Management of Drug-Resistant TB (PMDT) Unit of Lady Reading Hospital (LRH) in Peshawar, between March 2021 and March 2022 with the goal of evaluating the efficacy of the Xpert® MTB/XDR test utilizing sputum samples. To identify resistance to INH and/or second-line fluoroquinolones (FQs) and injectable agents (IAs), a total of 180 samples were included. These samples were obtained from patients who were demonstrating rifampicin resistance and/or isoniazid (INH) resistance, as well as those who were at high risk of INH-monoresistance. As a reference comparison, the results were compared to those from the WHO-endorsed MGIT 960 DST or Hain MDR plus and MTBDRsl.

This study's sputum samples were obtained and kept at -80°C. The Provincial TB Reference Laboratory (PTRL) at

the Biosafety Level 3 (BSL-3) Hayatabad Medical Complex Peshawar reported the results for these samples. The Xpert® MTB/XDR test utilized in this investigation can identify a patient's susceptibility to injectable drugs (amikacin, kanamycin, and capreomycin), ethionamide, fluoroquinolones (FQs), and isoniazid (INH)—drugs that are essential for treating multidrug-resistant tuberculosis (MDR-TB). It uses the geneXpert platform to conduct semi-quantitative nested PCR and high-resolution technologies.

Results

A combined total of 180 MDR-TB samples were used. As diagnostic procedures play main role in the initiation of treatment. Here in this selected unit different diagnostic techniques are used smear AFB, Gene-Xpert MTB/RIF, Gene-Xpert MTB/XDR, Culture and Line Probe Assay. All the individuals enrolled in the study fulfilled the inclusion criteria and were diagnosed as MDR-TB patients. For study purpose all study cases were categorized into three groups. The first group contains 60 patients who only used Drug susceptibility testing/DST for the detection of MDR-TB known as the pre-LPA group. The second group also contains 60 isolates but manual 'Line Probe Assay' was used for the detection of drug-resistance in the MDR-TB patients. In contrast to these two groups, Ten-color Gene-Xpert MTB/RIF Ultra was used by the third group for the speedy detection of drug-resistance in the remaining 60 samples, respectively.

WHO-endorsed Gene Xpert was used in this study, allowing for the speedy diagnosis of MDR-/RR-TB cases. Positive cases were 167(93%) and remaining 13(07%) were false negative cases which were later confirmed by DST and culture reports to be MDR-TB occurrences. Drug susceptibility testing is considered as the gold standard therefor it was used for the confirmation of the presence as well as drug resistance in all the patients. Line Probe assay, Drug susceptibility testing and Ten-color Gene Xpert MTB/RIF Ultra Assay was used for initial drug-resistance in each patient for the initiation of individual treatment for the MDR-TB patients. Drug-resistance has a significant p-value < 0.05. In the pre-LPA or DST group 53 (88.3%) patients showed resistance in three first line drugs (FLDs) called rifampicin-RIF, isoniazid- INH and pyrazinamide-PZA. Similarly, 16 (26.6%) patients from post-LPA and 41 (68.3%) patients from Ten-color Gene Xpert MTB/RIF Ultra Assay showed drug resistance in first line (FLDs) Rifampicin-RIF, Isoniazid- INH and Pyrazinamide-PZA drugs. Secondly, drug resistance of RIF+INH was reported in 26 (14.4%) patients in our study. From Second Line Drugs, majority patients 20 (11.1%) had drug resistance related to Isoniazid-INH, Rifampicin-RIF and Fluoroquinolones-FQ. Isoniazid-INH, Rifampicin-RIF and Ethambutol-EMB resistance was expressed in 8 (4.4%) patients and also 8(4.4%) patients showed Rifampicin drugs resistance only.

The median time duration of patients suspecting MDR-TB to submission of samples in Lab was 2 (1-3) days for majority of the patients for all three groups. The total median time taken by Laboratory for the confirmation of presence of this disease till treatment initiation in post-LPA, pre-LPA and DST was 1 day. Laboratory time taken for MDR-TB confirmation had the main role in the overall time consumption for the treatment initiation and was reduced to 1 day by Ten color Gene-Xpert MTB/XDR, compared to 18 days (post-LPA) and 55 days (pre-LPA/DST) in Table 1.3 Median time taken for the results to reach the DR-TB center for treatment initiation in pre-LPA/DST group is 35 days, post-LPA is 2 days and Ten-color GeneXpert is maximum 2 days. Overall median time for pre-LPA/DST group is 95 days (IQR- 72.5), post-LPA is 25 days (IQR-20) and Ten-color GeneXpert is 5 days (IQR-2.5), respectively.

Discussion

Results of the study show that the Xpert® MTB/XDR is a successful method for the identification of drug resistance regarding isoniazid (INH) and fluoroquinolone (FQs) compared to phenotypic and line probe assay (LPA) Drug Susceptibility Testing (DST). This present study was designed to understand the impact that Line Probe Assay had on the timely initiation of treatment given to the MDR-TB patients, and to identify the treatment outcomes. 180 positive MDR-TB cases were reported in this study. These study patients were split into three groups on the basis of Diagnostic techniques that were used for the detection of drug resistance in patients for the better understanding of time taken by Line Probe Assay and other techniques. These three groups were compared for the time taken in their treatment initiation as well as culture conversion time of patients in each group.

The socio-demographic profile of patients affiliated to our present study informs that majority of the patients are from age group 20 - 45 years with approximately 106 (59%) females patients in majority, Conflicting results were shown in a study conducted in India disease is more common in males with double increase in the risk[9]. We have found that 122 (68%) participants in our study were educated, probably due to the location of our PMDT center. People mostly came from the urban areas with formal education. Similar to this study, a study done in Bangladesh showed that TB is more common in urban areas. Contradicting to our study, another study suggested that more than half of the participants were rural residents with rural participants having double increase in prevalence rate of TB disease.[10]

It is repeatedly observed that low socioeconomic status can give rise to TB and household can also affect the outspread of TB disease. Our study suggested that majority (59%) of cases had large family size and joint

Table 1. Sociological and demographic characteristics of MDR-TB patients according to the methods used for the diagnosis of resistance in DR-TB

Variable	Pre-LPA or DST group n=60(%)	Post-LPA group n=60 (%)	Ten-Color Gene-Xpert MTB/XDR n=60 (%)	Total n=180	P-value
Gender					
Male	21(35%)	27(45%)	26(43%)	74(41%)	0.491
Female	39(65%)	33(55%)	34(57%)	106(59%)	
Age (years)					
≤ 20	15(25%)	24(40%)	19(32)	58(32%)	0.170
21 – 40	34(57%)	22(37%)	24(40)	80(44%)	
41 – 60	10(17%)	11(18%)	11(18)	32(18%)	
>60	1(2%)	3(5%)	6(10)	10(6%)	
Marital Status					
Unmarried	24(40%)	30(50%)	27(45%)	81(45%)	0.545
Married	36(60%)	30(50%)	33(55%)	99(55%)	
Education					
Educated	40(67%)	41(68)	41(68%)	122(68%)	0.975
Uneducated	20(33%)	19(32%)	19(32%)	58(32%)	
Ethnicity					
Pathan	60(100%)	53(88%)	55(92%)	168(93%)	0.001
Hindko	-	-	4(07%)	4(2%)	
Others	-	7(12%)	1(2%)	8(4%)	
Occupation					
Employed	19(32%)	21(35%)	17(28%)	57(32%)	0.735
Unemployed	41(68%)	39(65%)	43(72%)	123(68%)	
House Hold					
Joint	42(70%)	33(55%)	32(53%)	107(59%)	0.123
Nuclear	18(30%)	27(45%)	28(47%)	73(41%)	

Table 2. Result of Data including all Diagnostic Techniques used for the detection and Drug Resistance in Tuberculosis disease found in MDR-TB patients of this study

Variable	Pre-LPA or DST group n=60(%)	Post-LPA group n=60 (%)	Ten Color Gene-Xpert MTB/XDR n=60 (%)	Total n=180	P-value
Gene-Xpert Results					.028
Positive	53(88%)	54(90%)	60(100%)	167(93%)	
Negative	7(12%)	6(10%)	-	13(07%)	
DST results					
Positive	60(100%)	60(100%)	60(100%)	180(100%)	
Negative	-	-	-	-	
Drug resistance					.000
Resistance to First Line Drugs					
INH Resistant	-	3(5%)	-	3(5%)	
RIF Resistant	2(3.3%)	4(6.6%)	2(3.3%)	8(4.4%)	
INH+RIF Resistant	3(5%)	17(28.3%)	6(10%)	26(14.4%)	
INH+RIF+EMB	1(1.6%)	1(1.6%)	6(10%)	8(4.4%)	
INH+RIF+PZA	53(88.3%)	16(26.6%)	41(68.3%)	110(61.1%)	
INH+PZA+EM	1(1.6%)	-	2(3.3%)	3(1.6%)	
Resistance to second line drugs					
INH+RIF+FQ Resistant	-	17(28.3%)	3(5%)	20(11.1%)	
RIF+FQ Resistant	-	1(1.6%)	-	1(0.5%)	
FQ Resistant	-	1(1.6%)	-	1(0.5%)	

living system. Similar to this, another examination also discovered that congested living condition is related with lack of resources for each individual and high chances of susceptibility to this disease.[11]

Majority of the residents in KP province are pashthoons, so it is reasonable to understand that 168 (93%) patients in our study were from this ethnic group. The number of married (55%) and unmarried (45%) patients was close and less than half of the patients 57 (32%) were employed at the time while rest 123 (68%) were unemployed. Unlike in our study, the number of married patients was much greater in another study.[12]

It is a key factor to understand that TB can be diagnosed by many other successful clinical pathways and settings. In our study, Xpert MTB/RIF assay was used for the initial diagnosis as it can detect MTB as well as 98% of rifampicin resistance in sputum samples taken from patients, which is a representative marker of MDR-TB. In our study, we had 167 out of 180 samples to be positive cases. Drug Susceptibility Testing is considered as the most efficient and precise standard for the detection of Resistance pattern in different patients and hence Short term regimen and Long term regimen was prescribed accordingly. All 180 patients had positive results in DST

Table 3. Median Time taken for patients from suspecting MDR-TB till treatment initiation

	Median time in days (IQR)		
	Pre-LPA or DST group n=60 (%)	**Post-LPA n=60 (%)**	**Ten Color Xpert MTB/XDRn n=60 (%)**
Median time taken by patient suspecting MDR-TB to submission of samples in Lab.	2 (1-3) days	2 (1-3) days	2 (1-3) days
Median time taken for the confirmation of MTB.	1 day (microscopy)	1 day (Gene-Xpert MTB/RIF)	1 day (Gene-Xpert MTB/RIF)
Laboratory Turn-around time.	55(50-60) days	18(15-21)days	1 day (3 hours)
Median time from result reaching the DR-TB center to treatment initiation.	35(30-40)days	4 (3-5) days	2 (1-3) days
Overall mean time from 1st visit to treatment initiation.	93 days	25 days	5 days
Inter-Quartile Range	72.5	20	2.5

report with highest resistance in three First Line Drugs called Rifampicin, Isoniazid and pyrazinamide. Out of 180 individuals, 110 (61%) were resistant to these three medicines and 53 individuals were from DST group. However, study done by (Khan et al., 2015) shows highest resistance in these three drugs as well as in Ethambutol. Post-LPA group shows results of resistance found in Rifampicin and Isoniazid as well as Flourouinolones which is (28.3%) and Ten-color Gene Xpert MTB/XDR has individuals with drug resistance in Rifampicin, Isoniazid and pyrazinamide as well. As mentioned in a study, Ten-color Gene Xpert MTB/XDR has better coverage for INH resistance, FLQ and other SLIDs. Kanamycin and Amikacin resistance can also be detected by this assay.[13] After the introduction of LPA the time was significantly reduced to diagnose MDR-TB in the laboratory. This proposed that it is a successful method for the identification of drug resistance. However, in our study showed a significant reduction in time taken for starting the treatment of MDR-TB infected individuals. Time taken for the treatment initiation for the post-LPA patients was 25 days while treatment time taken for the patients in Ten-color Gene-Xpert MTB/XDR group was only 5 days. Lastly, patients who waited for their DST reports started their treatment after 93 days, which took nearly three months as compared to the Ten-color Gene-Xpert MTB/XDR group.

From a society health point of view, reduction in the time duration taken between diagnosis and treatment initiation by these three diagnostic assays has validated the robust technology of Ten-color Gene-Xpert MTB/XDR.

Conclusion

This study definitively demonstrates that the ten-color Xpert MTB/XDR assay is a superior diagnostic strategy for managing MDR-TB in our setting. By delivering a comprehensive resistance profile directly from the specimen, it eliminates the diagnostic delays inherent to the sequential use of LPA and DST. The consequent timely initiation of appropriate therapy, as evidenced by significantly improved culture conversion rates, establishes this integrated molecular solution as critical for enhancing patient outcomes and strengthening TB control efforts in high-burden regions like Peshawar.

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