ORIGINAL ARTICLE

GeneXpert: A new tool for the rapid detection of rifampicin resistance in mycobacterium tuberculosis

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Abstract

Objective: To evaluate the diagnostic accuracy of GeneXpert assay for the detection of rifampicin resistance in mycobacterium tuberculosis using conventional drug susceptibility testing as gold standard.

Methods: This cross-sectional study was conducted at Jinnah Hospital, Lahore, / Allama Iqbal Medical College, Lahore, Pakistan, from January 2012 to December 2014, and comprised clinically and radiologically diagnosed tuberculosis suspected cases. Pulmonary and extra-pulmonary specimens were collected from strong tuberculosis suspects. All specimens were processed for Ziehl Neelsen staining, Lowenstein-Jensen culture and GeneXpert assay. All mycobacterium tuberculosis positive cases on Lowenstein-Jensen culture were further processed for drug susceptibility testing.

Results: Of the 2,200 cases, 840(49.46%) were positive for mycobacterium tuberculosis on GeneXpert assay. Of them, 134(15.6%) cases showed rifampicin resistance on GeneXpert assay. The sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert assay for rifampicin resistance were 127(98.3%), 704(99.1%), 127(94.7%) and 704(99.4%), respectively, by comparing the results with drug susceptibility testing.

Conclusion: GeneXpert assay was an extremely helpful diagnostic tool for the detection of rifampicin resistance in tuberculosis suspects with fairly high sensitivity and specificity along with short turnout time.

Keywords: GeneXpert MTB/RIF assay, Mycobacterium tuberculosis, Rifampicin resistance, Drug susceptibility testing. (JPMA 67: 270; 2017)

Introduction

The emergence and increase of multidrug-resistant tuberculosis (MDR-TB) strains poses significant challenges to control tuberculosis (TB) around the globe. It is alarming that Pakistan is 5th among countries with highest burden of TB and ranked 4th in the world with highest MDR-TB cases.[1] In 2013, an estimated 480,000 new MDR-TB active cases were reported from the world.[2] The five first-line anti-TB drugs are pyrazinamide, ethambutol, isoniazid or isonicotinylhydrazide (INH), streptomycin and rifampicin (RIF). A patient is diagnosed as MDR-TB when he develops resistance against INH and RIF. This resistance may be present with or without resistance to any other primary drug.[3] The reasons behind the development of MRD-TB might be the use of low-quality drugs, incomplete treatment of diagnosed patients and poor management of TB cases in health sectors.

There are chances of transmission of drug resistance strain of TB from an individual infected with susceptible TB strains to other healthy contacts which will lead to development of new MDR-TB cases. Every undiagnosed, untreated active TB case can infect 10-15 persons per year.[4] Early disease management and detection of RIF resistance is very important to prevent transmission and limit death rate. Since resistance arises from genetic mutations, many deoxyribonucleic (DNA) sequencing studies have confirmed that more than 95% of RIF-resistant mycobacterium tuberculosis (MTB) strains have hot spot region of 81 bp of ribonucleic acid (RNA) polymerase beta-subunit (rpoB) gene with mutation.[5]

In recent years several rapid molecular assays for the diagnosis of MTB and MDR-TB have been introduced to reduce diagnostic turnaround time. Currently, the World Health Organisation (WHO) has endorsed molecular beacon technology for the direct detection of MTB by using ultra-sensitive polymerase chain reaction (PCR) technology. In December 2010, the WHO recommended the GeneXpert (MTB/RIF) assay (Cepheid, Sunnyvale, California, United States) in TB endemic countries for diagnosis of TB and affirmed it a major landmark for TB management.[6] Later in October 2013, the WHO released a policy update on GeneXpert (MTB/ RIF) which expands the recommended use of GeneXpert (MTB/RIF) as the initial diagnostic test in all individuals (smear negative, paediatric, MDR-TB and human immunodeficiency [HIV]) suspected of having pulmonary and extrapulmonary tuberculosis (EPTB).[7]

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GeneXpert (MTB/RIF) assay is the only independent cartridge-based, novel integrated diagnostic instrument used as RNA testing platform to identify MTB along with detection of RIF resistance in a single tube by using specific primers and unique molecular probes to make sure a high-level specificity.[8] Mono-resistance to RIF is less common and almost 90% RIF resistance cases also exhibit resistant to INH.[9] Therefore, detection of RIF resistance may serve as a surrogate marker for MDR-TB.[10]

This molecular technology first purifies and concentrates target bacilli in specimen. After purification, the captured bacterium is subjected to process of isolation by sonication method and at the end genomic DNA is consequently amplified by PCR. The GeneXpert (MTB/RIF) technology recognises all the resistance associated with RIF in rpoB gene in real-time format. With little technical skills results are obtained within 105 minutes, with least biohazards.[11]

Treatment of TB patients without analysis for drug susceptibility resistance can lead to failure of treatment and emergence of MDR-TB cases. To fight against this huge burden of disease in Pakistan, GeneXpert (MTB/RIF) was implemented to ensure rapid diagnosis of RIF resistance in MTB.

The present study was planned to detect MTB along with RIF resistance by using real-time PCR-based GeneXpert (MTB/RIF) and evaluate its diagnostic sensitivity and specificity by comparing with conventional techniques, keeping drug susceptibility testing (DST) as gold standard.

Materials and Methods

This cross-sectional study was conducted at Jinnah Hospital, Lahore (JHL), Lahore, / Allama Iqbal Medical College, Lahore, Pakistan, from January 2012 to December 2014, and comprised clinically and radiologically diagnosed TB suspected cases. The cases were selected from the indoor and outpatients (OPD) of the hospital's Department of Pulmonology. The study was approved by the institutional ethical review board. Pulmonary specimens (sputum, bronco alveolar lavage, bronchial washings) and extrapulmonary (EP) specimens (pleural fluid, ascitic fluid, cerebrospinal fluid and pus) were included. All samples collected were sent to TB laboratory of the Allama Iqbal Medical College (AIMC)/JHL for further analysis.

All the pulmonary specimens were subjected to (i) Ziehl Neelsen (ZN) staining for examination of acid-fast bacilli (AFB) on smear as per WHO protocol,[12] (ii) GeneXpert (MTB/RIF) was performed directly on samples, as per manufacturer's instructions,[11] (iii) Lowenstein-Jensen (LJ) culture: all sputum samples were inoculated on LJ media after decontamination as per standard WHO protocol.

All the samples in which MTB was detected on GeneXpert (MTB/RIF) and/or LJ culture were subjected to DST for RIF.

EP specimens were concentrated by cyto-centrifugation and deposit was processed for ZN staining, GeneXpert (MTB/RIF), LJ culture and DST as per WHO recommendation.[13]

Results

Of the 2,200 specimens, 1,702(77.4%) were pulmonary and 498(22.6%) were EP.

Of all, the highest detection rate for MTB was found on LJ culture 859(39%) followed by GeneXpert 840(38%) and ZN smear 490(22.3%) (Figure-1). The sensitivity and specificity of GeneXpert (MTB/RIF) were also calculated by comparing the results of GeneXpert (MTB/RIF) with gold standard (LJ culture). The sensitivity of GeneXpert (MTB/RIF) was 840(97.5%) and specificity was 1,326(98.8%). The sensitivity was 490(100%) and 147(42%)

Table-1: Sensitivity and specificity of GeneXpert (MTB/RIF) for the detection of MTB by comparing with LJ Gold Standard.

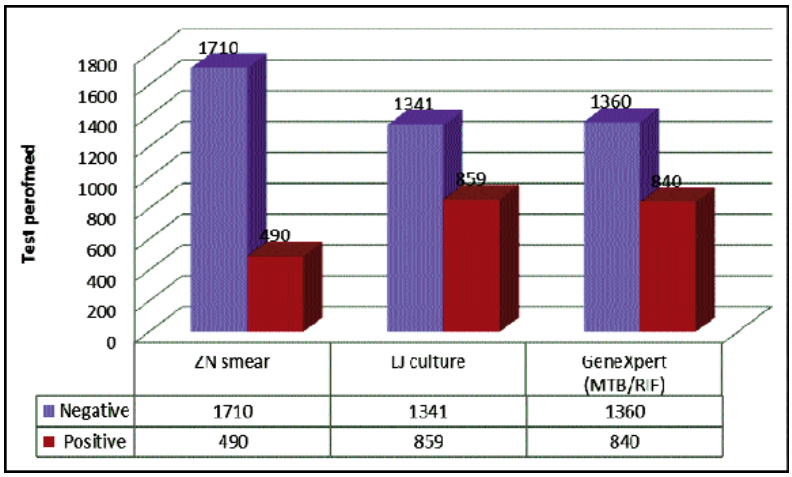
Tools	Total sample (n=2200) Reference test = LJ culture	
	Sensitivity %	Specificity %
ZN smear	57%	100%
GeneXpert	97.5%	99.1%
GeneXpert in Smear +ve cases	100%	100%
GeneXpert in smear -ve cases	42%	100%

MTB: Mycobacterium tuberculosis
RIF: Rifampicin
LJ: Lowenstein-Jensen
ZN: Ziehl Neelsen

Table-2: DST versus GeneXpert (MTB/RIF) assay for RIF resistance.

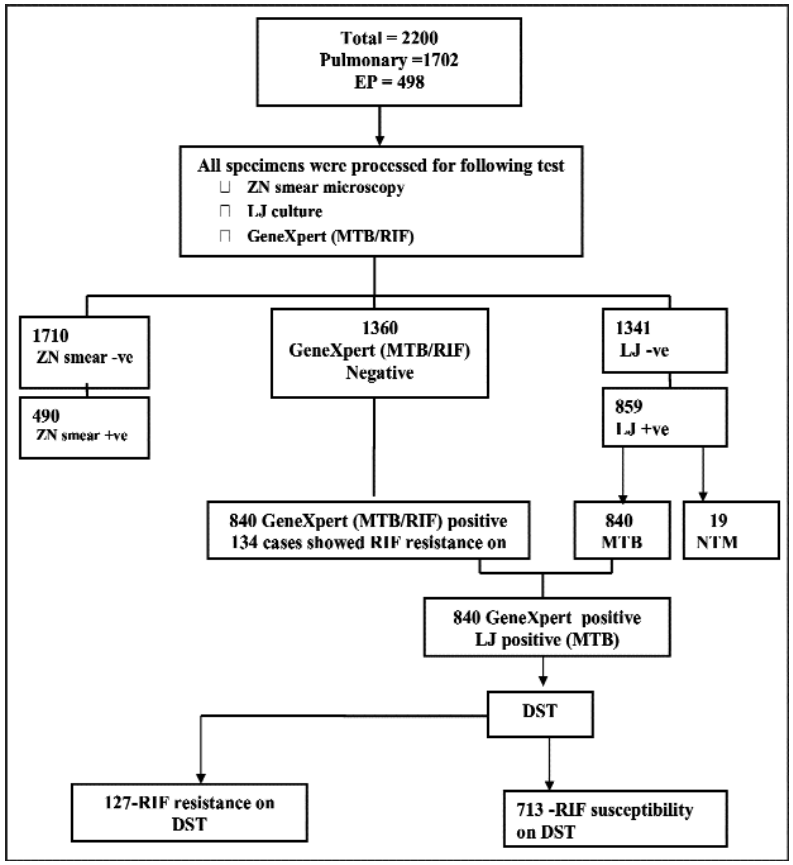
		DST (Gold standard)		Total
		+ve	-ve	
GeneXpert	+ve	127	7	134
	-ve	2	704	706
	Total	129	711	840
Sensitivity			(127/129) 98.3%	
Specificity			(704/711) 99.1%	
PPV			(127/134) 94.7 %	
NPV			(704/706) 99.4%	

MTB: Mycobacterium tuberculosis
RIF: Rifampicin
DST: Drug susceptibility testing
PPV: Positive predictive value
NPV: Negative predictive value.



MTB: Mycobacterium tuberculosis
RIF: Rifampicin
LJ: Lowenstein-Jensen
ZN: Ziehl Neelsen

Figure-1: Comparison of different techniques.



MTB: Mycobacterium tuberculosis. RIF: Rifampicin. LJ: Lowenstein-Jensen.
ZN: Ziehl Neelsen. DST: Drug susceptibility testing. EP: Extrapulmonary.

Figure-2: Flow Chart of Sample processing and summary of results.

in case of ZN smear +ve and ZN smear -ve samples, respectively (Table-1).

Of the 840 GeneXpert (MTB/RIF) positive samples for MTB, 134(16%) cases showed RIF resistance and diagnosed (labelled) as MDR-TB (Figure-2). Moreover, GeneXpert (MTB/RIF) was able to detect RIF resistance in 134(16%) cases while on DST 129(15.4%) cases were diagnosed as RIF resistant. There were 7(0.8%) cases that showed RIF resistance on GeneXpert (MTB/RIF) but they were RIF-sensitive on DST. Besides, 2(0.24%) cases were negative on GeneXpert (MTB/RIF) and positive on DST for RIF resistance. All RIF resistance cases were taken as MDR-TB. The sensitivity and specificity of GeneXpert (MTB/RIF) assay for RIF resistance were also calculated by keeping DST as a gold standard (Table-2).

Discussion

The alarming increase of MDR-TB in Pakistan and other developing countries is a serious threat to tuberculosis control. The major obstacles in treatment of TB are delayed diagnosis without drug sensitivity, which often leads to MDR-TB. Global prevalence of MDR-TB is markedly increasing which demands for the efficient and rapid method for the diagnosis. This will help pulmonologists in effective treatment, management and control of TB. Therefore, the present study was designed to evaluate the new PCR-based technology (GeneXpert) for the detection of MTB and RIF resistance and its comparison with conventional DST in terms of its sensitivity, specificity and detection time.

In the current study, the efficacy of the GeneXpert (MTB/RIF) for the diagnosis of MDR-TB from 2,200 clinical suspects of TB was investigated. Different studies about the performance of GeneXpert (MTB/ RIF) have showed the test sensitivities in the range of 57% to 76.9% in cases of culture +ve/smear -ve cases, while 98% to 100% sensitivities were observed in culture +ve/smear +ve cases. The overall specificity of GeneXpert remained at 99% to 100%.[8,11,14-16]

The results of the current study revealed that the sensitivity of the GeneXpert (MTB/RIF) with LJ positive specimens was 97.5% and the specificity was 98.1%, which are compatible

with results presented in previous studies. In case of ZN-ve samples, the sensitivity of the test was 42.5%, which is quite close to study conducted by Armand et al., 2011, who reported the sensitivity of GeneXpert (MTB/RIF) at 46% in smear -ve cases and congruent with the sensitivities of others.[14]

MDR-TB is an alarming challenge for clinician and researchers because conventional DST takes 60 days to confirm the MDR status of patients and only available in specific reference laboratories. When results were compared with conventional DST, GeneXpert (MTB/RIF) correctly identified 98.3% of RIF-resistant MTB cases with sensitivity and specificity of 98.4% and 99.1%, respectively, which is in agreement with other studies that reported the sensitivity of GeneXpert (MTB/RIF) assay at 90-98% and specificity at 95-100% for RIF resistance.[11,15,16] It was concluded that results of GeneXpert (MTB/RIF) for RIF resistance were quite compatible with reference test (i.e. DST) for MDR-TB in terms of sensitivity and specificity.

In our study, GeneXpert (MTB/RIF) detected 7 false positive cases which were RIF-sensitive on DST. The reason behind this might be the possibility of mixed population of RIF-sensitive and RIF-resistant bacterial strains in the sample.[17] Lawn and Nickol in 2011 also reported that for the detection of RIF resistance by the GeneXpert (MTB/RIF), the DNA must be present in the concentration of 65% and 100% form RIF-resistant isolates.[18] They recommended that in case of patients with mixed infections, there would be possibility that GeneXpert (MTB/RIF) only detects the strain which is present predominately. However, selection of resistant strains during the course of standard TB treatment might lead to an apparent switch from a susceptible to a resistant phenotype when comparing baseline testing with repeat testing during treatment. This may be the difference between the conventional phenotypic DST and the GeneXpert (MTB/RIF). The WHO recommended that if GeneXpert (MTB/RIF) detects RIF resistance in patients considered at risk of MDR-TB, an appropriate MDR-TB regimen should be started and additional sputum specimens are obtained for culture and DST.

In 2010, a multi-centred study was conducted to assess the reliability of GeneXpert (MTB/RIF) for determination of drug resistance in MDR-TB suspect patients. Their results showed sensitivity of 97.6% towards RIF resistance bacteria and specificity of 98.1% in RIF sensitive bacteria. These results are comparable with results of our study.[11] Similarly Boehme et al., performed a study in 2011 on GeneXpert (MTB/RIF) assay and reported the sensitivity and specificity of 94.4% and specificity of 98·3% in MDR cases, respectively.[15]

Time factor is matter of great importance in the diagnosis and management of TB. The conventional methods presently used for identification and antibiotic sensitivity of TB are either costly or time consuming. Definitive diagnosis of mycobacterium infection depends on rapid detection of the MTB in clinical samples. GeneXpert (MTB/RIF) detection time of MTB/RIF assay was only 2 hours as compared to LJ culture which takes 3-6 weeks. Similar results were reported by Boehme and its co-worker in terms of rapidity of GeneXpert (MTB/RIF) for the screening of TB and MDR-TB as well. Other studies reported mean detection time of 2 hours in comparison with 1 month on LJ culture and 24 hours for smear microscopy and 16 days for mycobacteria growth indicator tube (MGIT) by GeneXpert (MTB/RIF) for diagnosis of TB. In case of drug resistance detection the mean time is 20 days with line-probe assay and 106 days with DST.[15] GeneXpert (MTB/RIF) is not only helpful for rapid diagnosis of TB but also determines the patient's MDR status at the same time. It can be used as an effective tool for the screening and prompt accurate diagnosis of MDR-TB cases in resource-limited settings, thereby potentially decreasing morbidity associated with diagnostic delay, dropout and mistreatment.

An important difference between two diagnostic tools is cost. The cost of GeneXpert (MTB/RIF) assay is almost ten times more than the routine LJ culture and almost twenty times more than the ZN smear microscopy. When we talk about Third World countries like Pakistan, cost is a big issue; moreover, MTB is more prevalent among poor socio-economic group. So in such conditions cost becomes a big issue and major portion of the population being affected with MTB cannot afford GeneXpert (MTB/RIF) assay. Measures should be taken to reduce the cost of GeneXpert (MTB/RIF) assay or it should be subsidised by the government and other funding agencies so that this assay should be within the reach of most effected population.

Conclusion

GeneXpert (MTB/RIF) assay was found to be a simple rapid method well adapted to a routine laboratory. The assay was as sensitive as conventional DST for the diagnosis of MDR-TB and simultaneously detected MTB and RIF resistance in a short turnaround time of almost 2 hours. This extremely helpful diagnostic tool should be implemented for screening and management of MDR-TB in TB-endemic countries.

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Conflict of Interest: None.

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