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Original Research Article

Evaluation of cepheid GeneXpert MTB/RIF assay for *Mycobacterium tuberculosis* detection and rifampicin resistance in clinical specimen

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ABSTRACT

Background: Timely diagnosis and treatment of tuberculosis is important to treat the disease and to reduce transmission. The WHO recommends using GeneXpert MTB in developing, high-burden countries. A study was conducted to evaluate the performance of Xpert assay for the detection of *M. tuberculosis* and rifampicin resistance in clinical specimen.
Methods: About 615 consecutive samples were simultaneously subjected to culture and phenotypic drug susceptibility test for *M. tuberculosis* and analysis by GeneXpert assay. Confirmed *Mycobacterium tuberculosis* in a positive culture was used as a reference standard for TB diagnosis.
Results: The assay achieved a sensitivity of 96.75% (268/277) and 76.47% (26/34) for smear positive and smear negative pulmonary specimen respectively. In extrapulmonary specimen, the sensitivity was 50% (1/2) and 42.8% (3/7) for smear positive and smear negative specimen respectively. An additional 48 *M. tuberculosis* were detected by Xpert assay which were smear and culture negative. The Xpert assay identified 100% of the phenotypic rifampicin susceptible isolates and 74.19% of the phenotypic rifampicin resistant isolates. Discordant results were seen in 8 (2.76%) isolates. 6 of these isolates were confirmed to be rifampicin resistant by the reference lab.
Conclusions: Present study indicates that Xpert MTB/RIF assay is an effective and rapid tool for the rapid diagnosis of Mycobacterium tuberculosis. The sensitivity is comparable to culture in smear positive specimen but less sensitive than culture for smear negative specimen. In cases with high index of suspicion or discordance for rifampicin results, confirmation should be done by other methods due to false negative results on Xpert assay.

Keywords: Discordance, Extra pulmonary, Gene Xpert, Pulmonary, Rifampicin resistance, Sensitivity, Specificity, Tuberculosis

INTRODUCTION

Tuberculosis is one of the leading causes of morbidity and mortality across the world. The problem is compounded with the emergence of multidrug resistant tuberculosis (MDR-TB), which is defined as resistance to rifampicin and isoniazid. MDR-TB treatment is complex, lengthy and extensive. Timely diagnosis is crucial for initiation of appropriate treatment and to interrupt the transmission of disease.[1] Conventional diagnostic methods used are time consuming and/or insensitive. Although smear microscopy for acid fast bacilli is rapid and inexpensive, it has poor sensitivity and poor positive

predictive value. Traditionally, a diagnosis of MDR-TB is made by mycobacterial culture and phenotypic drug susceptibility testing (DST). This approach requires relatively advanced laboratory capacity, is labor intensive, and takes about 3 months before results are available.[2] Diagnosis by molecular methods has led to incremental improvements in the detection and drug susceptibility testing of *M. tuberculosis*; however their use in low resource, high burden countries is limited by the need for technical expertise, laboratory infrastructure and complexity of the test.[3,4]

The world health organization in 2010, endorsed the use of GeneXpert MTB/RIF (Xpert; cepheid, Sunnyvale, CA), an automated nucleic acid amplification test for simultaneous detection of mycobacterium tuberculosis complex (MTBC) and its resistance to rifampin directly from clinical samples. Rifampicin resistance serves as a surrogate marker for multidrug resistance tuberculosis (MDR-TB. The assay does not require sample processing but requires the addition of sample diluents to chemically inactivate the specimen and results are available within 2 hours. Therefore the process is simple, less time consuming and does not require special technical expertise and biosafety requirements.[5,6] The objective of this study was to evaluate the performance of GeneXpert assay for the direct detection of *M. tuberculosis* in smear positive and smear negative pulmonary and extrapulmonary clinical specimens and to evaluate the ability of the assay to detect rifampicin resistance.

METHODS

This study was conducted in a RNTCP certified intermediate reference laboratory (IRL) which caters to all the districts of Kashmir valley and Ladakh region. Samples from individuals known or presumed to have tuberculosis, all retreatment categories and patients considered at high risk of MDR-TB are referred to IRL. Culture and drug susceptibility testing are being done by both phenotypic (modified proportion method) and genotypic methods (GeneXpert MTB/RIF and genotype MTBDR plus assay)

A prospective study was carried out for a period of one year from January 2014 to December 2014. Clinical specimen both pulmonary (sputum, broncho alveolar lavage/aspirate and gastric lavage) and non-pulmonary (pleural fluid, tissue biopsy, pus, CSF, ascitic fluid, pericardial fluid, etc.) obtained for routine mycobacterial testing were included in the study. Two samples were collected from each patient whenever possible; one for GeneXpert and another for AFB smear and culture.

Specimens from non-sterile site were processed by conventional N-acetyl-L-cysteine-NaOH method. After decontamination, smears were prepared and stained with Zeihl Neelson staining method and culture on LJ media was done using standard protocol. Sterile specimen was concentrated by centrifugation and the sediment was inoculated on the LJ medium and incubated at 37º C for growth. The AFB smear was graded as per RNTCP guidelines: scanty (1-9/100 fields), 1+(10-99/100 fields), 2+ (1-10/100 fields) and 3+(10/field). A person was taken as smear positive if at least one of the smears was graded scanty or higher. Cultures were incubated for 8 weeks before being declared as negative. Contamination by rapidly growing bacteria and those with morphologies inconsistent with MTBC were checked regularly. After the appearance of growth on LJ medium, identification of M. tuberculosis was done by ZN staining, biochemical tests and susceptibility to para nitro benzoic acid. In cases where M. tuberculosis was identified, drug susceptibility testing was performed by modified proportion method. Tests were performed with the standard critical concentration of rifampicin (40µg/ml).[7 8]

Analysis of samples by Xpert MTB/RIF assay

The assay was performed using version 4 cartridges according to the manufacturers' recommendations. Briefly the sample reagent (containing NaOH and isopropyl alcohol) was added at a 2:1 ratio to clinical specimen to kill the mycobacteria and liquefy the samples. For biopsy specimen, a 2:1 volume of sample reagent (SR) buffer was added to biopsy specimens after they had been chopped into very small pieces with a sterile blade in a sterile petri dish. Fluids were processed directly by the addition of a 2:1 volume of SR buffer, except for CSF (usually <1ml), which was raised to 2ml by the addition of SR buffer. The sample-SR mixture was shaken vigorously and incubated for 10 minutes before being shaken again and kept at room temperature for another 10 minutes. Two ml of the digested material was transferred to the cartridge. The cartridge was subsequently loaded in the GeneXpert instrument where all subsequent steps occurred automatically. In case the results were reported as invalid, error or no result, the sample was reprocessed and rerun, if sufficient material was available.

Data collection

The data collected included the patients' demographics, semi quantitative bacillary load by AFB microscopy, history of TB treatment and treatment category.

Statistical analysis

The patients were characterized using simple descriptive statistics. Sensitivity, specificity, positive predictive value, and negative predictive value of the Xpert assay for detecting MTBC and rifampin resistance was done using phenotypic DST as the reference standard.

RESULTS

Of the 615 patients included in the study, 568 were pulmonary and 47 were extra pulmonary. Out of these, 341 (55.4%) were males and 274 (44.6%) were females.

Mean age was 39 years. 285 were AFB smear positive while 330 were AFB smear negative. The specimen analyzed and their culture results are given in (Table 1). Overall 322/615 (52.36%) isolates were culture positive of which 320 (52.03%) isolates belonged to *M. tuberculosis* complex while two isolates were identified as non-tubercular mycobacteria (NTM).

These NTM were isolated from BAL and ascitic fluid and were excluded from the study.

Table 1: Specimen analyzed and their culture results.

Source of specimen		Culture Results			
Pulmonary		**Contaminated**	**Negative**	**MTBC**	**NTM**
Sputum	479	29	159	291	0
Bronchoalveolar lavage /aspirate	81	5	55	20	1
Gastric lavage/ aspirate	8	0	8		0
Total	568	34	222	311	1
Extrapulmonary					
Pleural fluid	14	0	10	4	0
Pus/ aspirate	9	0	8	1	0
Endometrial Bx	8	0	8	0	0
Lymph node	5	0	3	2	0
Bone	5	0	4	1	0
Urine	2	0	1	1	0
Tissue	1	0	1	0	0
Skin Bx	1	0	1	0	0
Ascitic fluid	2	0	1	0	1
Total	47	0	37	9	1

Culture contamination was observed in 34/615 (5.5%) and these isolates were also excluded from the study for further analysis. The remaining 579 isolates were included in the study.

Among the pulmonary isolates, 277 (52%) isolates were smear positive, culture positive; 34 (6.4%) were smear negative, culture positive; 222 (41.6%) were smear negative, culture negative. Among the extra pulmonary isolates, 2 (4.3%) were smear positive, culture positive; 7 (15.2%) were smear negative, culture positive; 37 (80.4%) were smear negative, culture negative. Phenotypic DST for the culture positive isolates revealed 283 (88.44%) isolates to be rifampicin susceptible and 37 (11.56%) isolates as rifampicin resistant.

About 577 (99.6%) isolates gave an interpretable Xpert MTB/RIF assay result; 346 isolates were positive and 229 were negative. Indeterminate results (1 invalid and 1 error) were observed in 2 isolates. These isolates were subsequently found to be negative on culture. Comparison of culture results and Xpert MTB/RIF assay for 577 isolates are shown in (Table 2). Of the respiratory isolates, 268 of the 277-smear positive, culture positive, were detected positive by Xpert MTB/RIF assay resulting in 96.75% sensitivity.

Table 2: Comparison between Xpert MTB/RIF and culture results.

	GeneXpert		Total
	pos	**neg**	
Pulmonary specimen			
smear +, culture positive (S+, C-)	268	9	277
smear -, culture positive (S-,C+)	26	8	34
smear negative, culture negative (S-, C-)	42	178	220
Total	336	195	531
Extrapulmonary			
smear+, culture positive (S+, C-)	1	1	2
smear -, culture positive (S-, C+)	3	4	7
smear negative, culture negative (S-, C-)	6	31	37
Total	10	36	46

Out of 34 smear negative, culture positive isolates, 26 were positive by this assay. The sensitivity for this group was 76.47%. Among the extra pulmonary isolates, 1 out of 2 smear positive, culture positive (sensitivity 50%) and 3 out of 7 (sensitivity 42.8%) smear negative, culture

positive isolates were detected positive by this assay In a subgroup of 220 pulmonary and 37 extra pulmonary isolates that were smear and culture negative, GeneXpert was positive in 42 (19.1%) and 6 (16.2%%) cases respectively. A summary of the assay performance is depicted in (Table 3).

Table 3: Sensitivity, specificity, and predictive values of GeneXpert MTB/RIF assay with culture method as reference.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
All samples	93.12	81.3	86.12	90.5
AFB positive	96.4	-	100	-
AFB negative	70.7	81.3	37	94.5
All pulmonary samples	94.5	80.9	87.5	91.3
AFB positive pulmonary samples	96.75	100	100	100
AFB negative pulmonary samples	76.47	80.7	38	95.6
All extrapulmonary samples	44.4	83.8	40	86.1
AFB positive extrapulmonary samples	50	100	100	100
AFB negative extrapulmonary samples	42.8	83.8	33.33	88.6

Both the phenotypic DST and Xpert assay detected RMP susceptibility and resistance in 268 and 22 samples respectively (Table 4).

Table 4: Comparison between GeneXpert MTB/RIF assays and phenotypic drug susceptibility tests (DST).

Rifampicin susceptibility result by phenotypic DST	Rifampicin susceptibility result by Xpert MTB/RIF	
	Susceptible	Resistant
Susceptible	268	0
Resistant	8	22

The sensitivity of Xpert test compared to phenotypic DST was found to be 100% for detecting RMP susceptibility and 74.19% for detecting RMP resistance. However, there were 8 (2.76%) patients that detected rifampicin resistance by phenotypic DST but were found to be sensitive on Xpert assay. The samples were subsequently sent to national reference laboratory (NRL) for gene sequencing. 2 of these patients had died before registration and their repeat samples could not be taken. The remaining 6 isolates were confirmed to be rifampicin resistant by the NRL.

DISCUSSION

Delay in diagnosis of MDR-TB is associated with worse clinical outcomes and increased transmission. The use of rapid molecular test Gene Xpert MTB/RIF to diagnose tuberculosis and rifampicin resistance dramatically shortens time to diagnosis from months to hours. The assay is a heminested real time PCR based assay that uses molecular beacon technology to detect amplified DNA sequences. The test is based on the detection of mutations localized within 81bp core region of the bacterial RNA polymerase β subunit (rpoB) gene, which encodes the active site of the enzyme. Moreover, the core region is flanked by *M. tuberculosis* complex- specific sequences. Thus, *M. tuberculosis* and rifampicin resistance can be tested simultaneously by targeting one amplicon generated with PCR technology.[9]

In this study the performance of the MTB/RIF assay with pulmonary and extra pulmonary samples obtained routinely was investigated. In present study, the assay identified the agent in 336 of 531 and 10 of 46 pulmonary and extrapulmonary specimen respectively. There was no significant difference between sample type and MTB/RIF assay performance among extrapulmonary specimen. In our test the sensitivity for smear and culture positive pulmonary specimen was 96.75% while for smear negative pulmonary specimen the sensitivity was 76.47%. Previous studies of the MTB/RIF assay have reported sensitivities of 98 to 100% in smear and culture positive tuberculosis and 57 to 76.9% for smear negative, culture positive tuberculosis.[10-12]

In the present study, the sensitivity of the test was 50% for smear and culture positive extra pulmonary specimen and 48.2% for smear negative, culture positive specimen. Present study shows a low sensitivity for smear and culture positive extrapulmonary specimen compared to other studies. This can be attributed to a very small sample size of smear and culture positive extrapulmonary samples which have significantly altered the sensitivity of the assay and is one of the limitations of this study. The sensitivity of smear negative, culture positive specimen was however consistent with other studies.[13,14]

An additional 42 pulmonary samples and 6 extrapulmonary samples positive by Gene Xpert MTB/RIF assay were found negative by conventional methods, viz a via, microscopy and culture. Of these,

88.1% (37/42) of smear and culture negative patients were on anti-tubercular treatment for various periods of time when enrolled in the study. Excretion of residual persistent DNA from non-viable organism could be possible reason for a positive Xpert result and negative culture. In other cases, use of harsh decontamination methods like NALC-NaOH during specimen processing have deleterious effect on the viability of bacilli due to effect of NaOH on live bacilli, which can kill about 33% of mycobacteria in a clinical sample while as presence or absence of viable bacilli is not an issue in Xpert assay. Finally, the paucibacillary nature of smear negative specimen with a tendency of *M. tuberculosis* to form clumps leads to uneven distribution of the bacilli and false negative results.[15,16] This finding highlights the fact that the sensitivity and specificity of newer diagnostic methods is compromised when compared to culture as reference method. Culture poses an imperfect reference method against which new technologies are compared.

The sensitivity of Xpert assay for detecting rifampicin resistance has been reported to be between 60% to almost 100%, depending on the characteristics of the population being tested and the bacterial loads in their samples.[17-19] Inconsistent results between the Xpert assay and phenotypic DST have been recognized.[20,21] In present study, although no case with positive Xpert assay and negative phenotypic DST results for rifampicin resistance were identified, 8 discrepant results with negative Xpert but positive DST for the same was obtained. Two of these patients had died before registration, five patients were already on antitubercular treatment while one patient was a new case of tuberculosis. The reason for false negative Xpert assay could be the following: First, while the Xpert assay has the ability to simultaneously test for a large number of rpoB mutations, it is not able to detect all mutations that cause rifampicin resistance. Second, these patients could be infected with concurrent sub-populations of rifampicin resistant and rifampicin susceptible strains. In such cases of hetero resistance, the Xpert assay only detects the resistant strain if this strain is predominant. Prior studies have shown that the Xpert assay is capable of detecting the presence of rifampicin resistance mutations down to a concentration of 40%. This drawback is a substantial problem for clinical decision making as failure to treat resistant sub populations is associated with poor clinical outcomes.

CONCLUSION

The Xpert MTB/RIF assay could be used as a useful tool for the detection of M. tuberculosis and rifampicin resistance. It has the advantage of rapid turn-around time of around 2 hours, hand- on time of less than 5 min per specimen and minimal biosafety requirement. However, the adoption of the assay does not eliminate the need for conventional TB culture and phenotypic DST. The sensitivity of the test is comparable to culture for the smear positive specimen, but the assay is less sensitive for smear negative cases. Also, a high index of suspicion should be kept for patients in whom the first line therapy has failed despite the Xpert assay results showing rifampicin susceptibility.

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REFERENCES

1. WHO. Global tuberculosis report 2012. Geneva, Switzerland: World Health Organization, 2012.Available at: https://www.who.int/tb/publications/global_report/gtbr12_main.pdf.
2. Farmer P, Bayona J, Becerra M, Furin J, Henry C, Hiatt H, et al. The dilemma of MDR-TB in the global era. Int J Tuberc Lung Dis. 1998;2:869-76.
3. Migliori GB, Matteelli A, Cirillo D, Pai M. Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: current standards and challenges. Can J Infect Dis Med Microbiol. 2008;19:169-72.
4. Wallis RS, Pai M, Menzies D, Doherty TM, Walzl G, Perkins MD, et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet. 2010;375(9729):1920-37.
5. Rapid implementation of the Xpert MTB/RIF diagnostic Test. World health organization.2011. Available at: https://www.who.int/tb/publications/tb-amplificationtechnology-implementation/en/.
6. Bwanga F, Hoffner S, Haile M, Joloba ML. Direct susceptibility testing for multi-drug resistant tuberculosis: a meta-analysis. BMC Infect Dis. 2009;9:67.
7. Pfyffer G. Mycobacterium: general characteristics, laboratory detection and staining procedures. In: Murray PJ, Baron EJ, Jorgensen JH, Landry ML and Pfaller MA, eds. Manual of clinical microbiology, Washington DC. 9th Ed. America society of Microbiology press; 2007:543-73.
8. Kent PT, Kubica GP. Public health mycobacteriology. A guide for a level III laboratory. Centers Dis Control, Atlanta, GA, 1985. Available at: https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB86216546.xhtml.
9. Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. Lancet Infect Dis. 2013;13(4):349-61.

10. lakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, et al. Evaluation of the Analytical Performance of the Xpert MTB/RIF Assay. J Clin Microbiol. 2010;48:2495-501.
11. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. New Eng J Med. 2010;363(11):1005-5.
12. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of Mycobacterium tuberculosis and rifampicin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48:229-37.
13. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol. 2011;49:4138.
14. Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaître N. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of Mycobacterium tuberculosis in respiratory and non respiratory specimens. J Clin Microbiol. 2011;49:1772-6.
15. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a New Pillar in Diagnosis of Extrapulmonary Tuberculosis?. J Clin Microbiol. 2011;49:2540.
16. Parsons LM, Somoskövi Á, Gutierrez C, Lee E, Paramasivan CN, Abimiku AL, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clinic Microb Reviews. 2011;24(2):314-50.
17. Boehme CC, Nicol MP, Nabeta P. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet. 2011;377:1495-505.
18. Theron G, Peter J, van Zyl-Smit R. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Respir Crit Care. 2011;184:132-40.
19. World Health Organization. Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational 'how-to' practical considerations. World health organization, Geneva, Switzerland, 2011. Available at: https://apps.who.int/iris/bitstream/handle/10665/44593/9789241501569_eng.pdf;jsessionid=B521B198B7F3D574E1F72987AFD28A3E?sequence=1.
20. Chakravorty S, Kothari H, Aladegbami B, Cho EJ, Lee JS, Roh SS, et al. Rapid, high-throughput detection of rifampin resistance and heteroresistance in mycobacterium tuberculosis by use of sloppy molecular beacon melting temperature coding. J Clin Microbiol. 2012;50:2194-202.
21. Ocheretina O, Escuyer VE, Mabou MM, Royal-Mardi G, Collins S, Vilbrun SC, et al. Correlation between genotypic and phenotypic testing for resistance to rifampin in mycobacterium tuberculosis clinical isolates in haiti: investigation of cases with discrepant susceptibility results. PLOS one. 2014;9:e90569.
22. Zetola NM, Shin SS, Tumedi KA, Moeti K, Ncube R, Nicol M, et al. Mixed Mycobacterium tuberculosis complex infections and false-negative results for rifampin resistance by GeneXpert MTB/RIF are associated with poor clinic Outcomes. J Clin Microbiol. 2014;52:2422-29.

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