Volume-6, Issue-1, January - 2017 • ISSN No 2277 - 8160 IF : 3.62 | IC Value 80.26

Original Research Paper Microbiology

"Evaluation of Gene Xpert Mycobacterium tuberculosis Assay for the Detection of Rifampicin Resistant Mycobacterium tuberculosis among Pulmonary Patients: A Hospital Based Study"

Dr Basavaraj M S Associate Professor, Department of Microbiology, Heritage Institute of Medical Science, Varanasi

ABSTRACT *Conventional diagnostic methods for Mycobacterium tuberculosis are slow and/or lack sensitivity. A number of new diagnostic approaches have brought incremental improvements to detection and drug susceptibility testing; however, the technical complexity of these assays and their dependence on dedicated laboratory infrastructure have limited their adoption, especially in low-resource, high-burden settings. It ranks as the second leading cause of death from an infectious disease worldwide. My aim was to evaluate the performance of GeneXpert system for the detection of Mycobacterium tuberculosis with its Rifampicin resistance. The concentration of M. tuberculosis bacteria in clinical sputum samples can vary from over 107 to less than 20 CFU/ml. Sputum samples were collected from 135 clinically suspected multidrug resistant pulmonary tuberculosis (MDR-TB) patients.*

KEYWORDS : Culture & MTB/RIF GeneXpert Pulmonary Tuberculosiss

Introduction:

Conventional diagnostic methods for *Mycobacterium tuberculosis* are slow and/or lack sensitivity. A number of new diagnostic approaches have brought incremental improvements to detection and drug susceptibility testing; however, the technical complexity of these assays and their dependence on dedicated laboratory infrastructure have limited their adoption, especially in low-resource, high-burden settings.[1,2,3,4] Tuberculosis remains a major global health problem. It ranks as the second leading cause of death from an infectious disease worldwide. The latest estimates included that there were 8.6 million new Tuberculosis cases in 2013 and 1.5 million Tuberculosis deaths.[5] Globally in 2012, 3.6% of new Tuberculosis cases and 20.2% of previously treated cases were estimated to have MDR-TB; furthermore, there were approximately 170,000 deaths from MDR-TB; however, extensively drug-resistant Tuberculosis (XDR-TB) has been reported by 105 countries in 2014.[6] There is growing concern that the increase in the global prevalence of multidrug- and extensively drug-resistant tuberculosis (M/XDR-TB), as well as the emergence of what is being called "totally drug-resistant TB",[7–9] may compromise the recent successes seen by global TB control efforts.[10] Conventional diagnostic method for Mycobacterium tuberculosis are slow and/or lack sensitivity. A number of new diagnostic approaches have brought incremental improvements to detection and drug susceptibility testing; however, the technical complexity of these assays and their dependence on dedicated laboratory infrastructure have limited their adoption, especially in low-resource, high-burden settings.[11,12,13,14] The recently introduced Xpert MTB/RIF (manufactured and marketed by Cepheid, Sunnyvale, CA) assay simultaneously detects the presence of M. tuberculosis and its susceptibility to the important first-line drug rifampin (RIF).[15] A sample processing system and an automated heminested real-time PCR assay are integrated into a single disposable cartridge. The assay can be performed directly from a clinical sputum sample or from a decontaminated sputum pellet and can generally be completed in less than 2 hrs.[15] The Xpert MTB/RIF assay detects M. tuberculosis and RIF resistance by PCR amplification of the rifampin resistancedetermining region (RRDR) of the M. tuberculosis rpoB gene and subsequent probing of this region for mutations that are associated with RIF resistance. Approximately 95% of RIF resistant tuberculosis cases contain mutations in this 81-bp region.[16] Our previous work has established that the Xpert MTB/RIF assay has a limit of detection (LOD), defined as the minimum number of bacilli that can be detected with 95% confidence) of 131 CFU per ml of clinical sputum.[11] The assay was also able to identify RIF resistance in samples containing 23 common clinically occurring rpoB mutations. None of the 20 nontuberculosis mycobacteria (NTM) species tested, including the NTM species commonly described as causing human disease were falsely identified as M. Tuberculosis,15 suggesting high specificity. Several small studies using clinical samples demonstrated 98% to 100% sensitivity overall, 72% sensitivity in smear-negative patients, and a specificity of 100%.[15] My aim was to evaluate the performance of GeneXpert system for the detection of Mycobacterium tuberculosis with its Rifampicin resistance.

Material and methods:

The present study was conducted in the departments of Department of Microbiology, Heritage Institute of medical Science and hospital, Varanasi during the period from November, 2015 to July 2016. Suspected cases of MDR-TB patients who were attended in the OPD and IPD of HIMS were selected as study population. Patients were excluded who were undergoing treatment or having extra-pulmonary tuberculosis or were new pulmonary tuberculosis cases. Fresh sputum were collected from suspected multidrug resistant pulmonary tuberculosis (MDR-TB) patients with all aseptic precaution and sputum samples were digested and decontaminated by N-acetyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) method.[17] The sediment of processed sputum was used for microscopic examination by Ziehl-Neelsen (Z-N) staining and by auramine staining, culture and drug susceptibility test (DST) on MGIT 960 media[17] Liquid culture and Drug Susceptibility Test (DST) were performed in BACTEC MGIT 965 Media.[18] MGIT growth supplement/PANTA was aseptically added to the appropriately labeled MGIT tube and then well mixed concentrated specimen was added to each MGIT tube. Inoculated tubes were placed on a rack and were carried to BACTEC MIGT 965 System for loading on the same day.[19] The instrument was monitored for the entered susceptibility test set. The susceptibility Set Carrier was scanned and the report was printed. The instrument printout indicated susceptibility results for each drug. GeneXpert MTB/RIF Assay (GXMTB/RIF-10) was performed. The primers in the Xpert MTB/RIF Assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with Rifampicin resistance. Each Xpert MTB/RIF cartridge was labeled with the sample ID. The sample was then transferred into the sample chamber of the labeled Xpert MTB/RIF cartridge and lid was closed firmly. The barcode on the Xpert MTB/RIF cartridge was scanned. The instrument module door opened with the blinking green light and the cartridge was loaded. The results were interpreted by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and displayed in the "View Results" window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template. Before collecting specimens, informed written consent was taken from patients. The study protocol was approved by the ethical committee of Heritage Institute of medical Science, Varanasi.

Results and Discussion:

The concentration of M. tuberculosis bacteria in clinical sputum

samples can vary from over 107 to less than 20 CFU/ml.[20] Sputum samples were collected from 135 clinically suspected multidrug resistant pulmonary tuberculosis (MDR-TB) patients. Liquid culture had yielded the highest growth of Mycobacterium tuberculosis which was 89 (65.9%) cases. GeneXpert MTB assay showed 64.4% positive and 34.07% negative (Table-1).

Table-1: Comparison of results of liquid culture and GeneXpert MTB assay:

GeneXpert	Culture (n = 135)		Total
	Positive	Negative	
Positive	87	0	87
Negative	2	46	48
Total	89	46	135

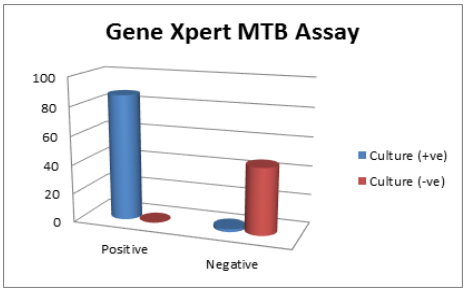

Fig-1: Shows the comparison of results of liquid culture and GeneXpert MTB assay:

Table-2: Performance of GeneXpert MTB/RIF Assay for detection of M. Tuberculosis:

Method	RIF sensitive	RIF resistant
GeneXpert MTB/RIF	69	18
Drug Susceptibility Test	69	20

All samples positive by GeneXpert were also positive by liquid culture and additionally one case was positive by liquid culture (Table-1). Table-2 shows the performance of GeneXpert MTB/RIF assay when compared to liquid culture on MGIT 965 media for detection of M. tuberculosis. Table-3 compares the rifampicin resistance detection by GeneXpert MTB/RIF and liquid culture on MGIT 965 media. GeneXpert MTB/RIF detected 18 (20.7%) cases of Mycobacterium tuberculosis which were rifampicin resistant among 87 M. tuberculosis positive samples. On the other hand liquid (MGIT 965) culture detected 20 (22.5%) out of 89 M. tuberculosis positive samples. An alarming increase in the global incidence of drug-resistance Mycobacterium tuberculosis infection has created a critical need for methods that can rapidly detect Mycobacterium tuberculosis and identify drug-resistant cases. Failure to quickly and effectively recognize and treat patients with drug-resistant tuberculosis, particularly multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis, leads to increased mortality, nosocomial outbreaks, economic burden and resistance to antitubercular drugs. In this study, 20 (22.5%) out of 89 MGIT positive samples and 18 (20.7%) out of 87 GeneXpert MTB positive samples were shown to be Rifampicin (RIF) resistant. So there was one phenotypic (DST on MGIT 965) and genotypic (GeneXpert assay) discordance—liquid culture detected one more sample as MTB positive which was Rifampicin resistant on culture-based drug susceptibility testing (DST). Ocheretina et al.[21] reported the phenotypic and genotypic characterization of 153 consecutive clinical Mycobacterium tuberculosis strains diagnosed as RIF-resistant by molecular tests in Port-au-Prince, Haiti. 133 (86.9%) isolates were resistant to both RIF and Isoniazid and 4 (2.6%) isolates were RIF mono-resistant in MGIT SIRE liquid culture-based DST. However the remaining 16 isolates (10.5%) were RIF-sensitive by the assay. According to CGXMTB/RIF-10 manual, 2009, GeneXpert MTB detected Rifampicin resistance from 16.86% and liquid culture detected from 17.44% sputum samples. The Xpert itself contains amplicons within the sealed cartridge, reducing or eliminating the need for the precautions typically associated with other nucleic acid amplification tests.

Conclusion:

In conclusion, the GeneXpert MTB/RIF is a rapid and highly dependable technique for identification of M. tuberculosis and rifampicin resistance from clinical sputum sample. The results are obtained within 2 hours with GeneXpert MTB/RIF assay. GeneXpert MTB/RIF assay should be used routinely for detection of M. tuberculosis and Rifampicin resistant M. tuberculosis from sputum sample of clinically suspected MDR patients where facilities are available. My assay can simultaneously confirm the presence of M. Tuberculosis and detect the mutations associated with resistance to anti-TB drugs, thereby diagnosing M/XDR-TB with good sensitivity and specificity. The Xpert itself contains amplicons within the sealed cartridge, reducing or eliminating the need for the precautions typically associated with other nucleic acid amplification tests.

References:

1. Balasingham, S. V., T. Davidsen, I. Szpinda, S. A. Frye, and T. Tonjum. 2009. Molecular diagnostics in tuberculosis: basis and implications for therapy. Mol. Diagn. Ther. 13:137–151.
2. Migliori, G. B., A. Matteelli, D. Cirillo, and M. Pai. 2008. Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: current standards and challenges. Can. J. Infect. Dis. Med. Microbiol. 19: 169–172.
3. Nyendak, M. R., D. A. Lewinsohn, and D. M. Lewinsohn. 2009. New diagnostic methods for tuberculosis. Curr. Opin. Infect. Dis. 22:174–182.
4. Young, D. B., M. D. Perkins, K. Duncan, and C. E. Barry III. 2008. Confronting the scientific obstacles to global control of tuberculosis. J. Clin. Invest. 118:1255–1265.
5. World Health Organization (2013) Global Tuberculosis Control: 2012. Information Resource Centre HTM/STB, World Health Organization, Geneva. www.who.int/tb
6. World Health Organization (2009) National Guideline and Operational Manual for Tuberculosis Control. 4th Edition, Tuberculosis Control Program.
7. Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. 2012. Totally drugresistant tuberculosis in India. Clin Infect Dis 54:579-U156.
8. Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, Ziazarifi AH, Hoffner SE. 2009. Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest 136:420–425.
9. Klopper M, Warren RM, Hayes C, van Pitttius CG, Streicher EM, Müller B, Sirgel FA, Chabula-Nxiweni M, Hoosain E, Coetzee G, van Helden DP, Victor TC, Trollip AP. 2013. Emergence and spread of extensively and totally drug resistant tuberculosis, South Africa. Emerg Infect Dis 19:449–455.
10. World Health Organization. 2011. Global tuberculosis control. World Health Organization, Geneva, Switzerland.
11. Balasingham, S. V., T. Davidsen, I. Szpinda, S. A. Frye, and T. Tonjum. 2009. Molecular diagnostics in tuberculosis: basis and implications for therapy. Mol. Diagn. Ther. 13:137–151.
12. Migliori, G. B., A. Matteelli, D. Cirillo, and M. Pai. 2008. Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: current standards and challenges. Can. J. Infect. Dis. Med. Microbiol. 19: 169–172.
13. Nyendak, M. R., D. A. Lewinsohn, and D. M. Lewinsohn. 2009. New diagnostic methods for tuberculosis. Curr. Opin. Infect. Dis. 22:174–182.
14. Young, D. B., M. D. Perkins, K. Duncan, and C. E. Barry III. 2008. Confronting the scientific obstacles to global control of tuberculosis. J. Clin. Invest. 118:1255–1265.
15. Helb, D., M. Jones, E. Story, C. Boehme, E. Wallace, K. Ho, J. Kop, M. R. Owens, R. Rodgers, P. Banada, H. Safi, R. Blakemore, N. T. Lan, E. C. Jones-Lopez, M. Levi, M. Burday, I. Ayakaka, R. D. Mugerwa, B. McMillan, E. Winn-Deen, L. Christel, P. Dailey, M. D. Perkins, D. H. Persing, and D. Alland. 2010. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J. Clin. Microbiol. 48:229–237.
16. Van Der Zanden, A. G., E. M. Te Koppele-Vije, N. Vijaya Bhanu, D. Van Soolingen, and L. M. Schouls. 2003. Use of DNA extracts from Ziehl- Neelsen-stained slides for molecular detection of rifampin resistance and spoligotyping of Mycobacterium tuberculosis. J. Clin. Microbiol. 41:1101– 1108.
17. Hines, N., Payeur, J.B. and Hoffman, L.J. (2006) Comparison of the Recovery of Mycobacterium bovis Isolates Using the BACTEC MGIT 960 System, BACTEC 460 System, and Middle Brook 7H10 and 7H11 Solid Media. Journal of Veterinary Diagnostic Investigation, 18, 243-250.
18. Siddiqi, S.H. and Gerdes, S.R. (2006) Foundation for Innovative New Diagnostics (FIND) MGITTM Procedure Manual for BACTEC™ MGIT 960™ TB System. Switzerland.
19. GXMTB/RIF-10, 301-0191 Rev. C, May 2012, 1-22.
20. Joloba, M. L., J. L. Johnson, A. Namale, A. Morrissey, A. E. Assegghai, R. D. Mugerwa, J. J. Ellner, and K. D. Eisenach. 2000. Quantitative sputum bacillary load during rifampin-containing short course chemotherapy in human immunodeficiency virus-infected and non-infected adults with pulmonary tuberculosis. Int. J. Tuber. Lung Dis. 4:528–536.
21. Ocheretina, O., Escuyer, V.E., Mabou, M.M., Mardi, G.R., Collins, S., Vilbrun, S.C., et al. (2014) 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, 9, 1-7.