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Diagnostic Evaluation of GeneXpert MTB/RIF Assay for the Detection of Rifampicin Resistant *Mycobacterium tuberculosis* among Pulmonary Tuberculosis Patients in Bangladesh

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Abstract

Background: The emergence of multidrug resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) has highlighted the need for early accurate detection and drug susceptibility. Objective: The purpose of the present study was to evaluate the accuracy of GeneXpert MTB/RIF assay for the detection of *Mycobacterium tuberculosis* and rifampicin resistance. Methodology: This cross sectional study was done in the Department of Microbiology at Sir Salimullah Medical College, Dhaka and National Institute of Chest Disease & Hospital (NIDCH), Dhaka during the period of January 2014 to December 2014 for a period of 1 (one) year. Sputum samples from suspected MDR-TB patients were collected by purposive sampling technique from OPD of Sir Salimullah Medical College (SSMC) and NIDCH. Microscopy, liquid culture in liquid MGIT 960 media and GeneXpert MTB/RIF were done for MTB diagnosis and detection of rifampicin resistance. MGIT 960 media were also used for determination of drug resistance. Result: Liquid culture yielded higher growth (68%) from 100 samples while GeneXpert MTB assay showed similar result

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(67% positive and 33% negative). Drug susceptibility test in MGIT 960 media showed that out of 68 positive cases Rifampicin resistant cases were 15 (22.05%) whereas GeneXpert MTB assay detected 14 (20.89%) were Rifampicin resistant out of 67 MTB positive samples. When compared to liquid culture the calculated sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy of GeneXpert MTB were 98.52%, 100%, 96.96%, 100% and 99%. Conclusion: GeneXpert MTB/RIF assay is high detection rate of pulmonary tuberculosis and multidrug resistant tuberculosis.

Keywords

MDR-TB, GeneXpert MTB/RIF, Liquid Culture, Pulmonary Tuberculosis

1. Introduction

Tuberculosis (TB) 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 TB cases in 2013 and 1.5 million TB deaths [1]. Globally in 2012, 3.6% of new TB 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 TB (XDR-TB) has been reported by 105 countries in 2014 [2]. On average, an estimated 9.7% of people with MDR-TB have XDR-TB in Bangladesh ranks sixth among 22 highest burden tuberculosis countries in the world; however, total 173,619 cases were notified in 2012, and total retreatment cases were 8001 and 1.5% of the new and 29% of the retreatment TB cases were MDR-TB [3]. Therefore, rapid detection is now an urgent need. In this regard, rapid tools for TB detection developed over the last decade in the industrialized world are largely Nucleic Acid Amplification Tests (NAAT) based on amplification of nucleic acids (DNA or RNA). Recently, line-probe assays (LPAs) and Xpert MTB/RIF have been formally endorsed by the WHO and are now in routine use in many TB laboratories in high and middle-income countries [4]. The Xpert MTB/RIF assay has been described as a potential "game changer" for TB controls [5]. INNO-LiPA was the first line probe assay which can detect only RIF resistant and has high sensitivity and specificity when culture isolates are used. This assay was less sensitive for the detection of *M. tuberculosis* complex and less accurate when the test is applied to clinical specimens. The Xpert MTB/RIF assay is simple and robust enough to be performed by personnel with minimal training [6]-[10]. Total hands-on time is less than 5 minutes and results are available within 1 hour 55 minutes. Instrumentation costs for the GeneXpert system are similar to those of automated liquid culture system for tuberculosis, and per-assay running costs are also in the same range as culture, despite vastly superior performance in terms of speed, bio-safety, and ease of use [11] [12]. The purpose of this study was to evaluate the performance of GeneXpert system for the detection of *Mycobacterium tuberculosis* with its Rifampicin resistance within shortest possible time.

2. Methodology

This cross sectional study was done in the Department of Microbiology of Sir Salimullah Medical College, Dhaka and National Tuberculosis Reference Laboratory (NTRL), Dhaka during the period of January 2014 to December 2014. Suspected cases of MDR-TB patients who were attended in the OPD and IPD of NIDCH and SSMC 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 [8]. 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 [8]. Liquid culture and Drug Susceptibility Test (DST) were performed in BACTEC MGIT 960 Media [13]. 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 960 System for loading on the same day [14]. 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, each patient was interviewed and informed written consent was taken from patients or legal guardian of patients and relevant information were recorded systematically in a pre-designed data sheet. The protocol was approved by the ethical review committee of Sir Salimullah Medical College, Dhaka. Confidentiality of the data was preserved.

3. Results

Sputum samples were collected from 100 clinically suspected multidrug resistant pulmonary tuberculosis (MDR-TB) patients. Liquid culture had yielded the highest growth of *Mycobacterium tuberculosis* which was 68 (68%) cases. GeneXpert MTB assay showed 67% positive and 33% negative (**Table 1**). 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 960 media for detection of *M. tuberculosis*. **Table 3** compares the rifampicin resistance detection by GeneXpert

Table 1. Comparison of results of liquid culture and GeneXpert MTB assay (n = 100).

GeneXpert	Culture		Total
	Positive	Negative	
Positive	67	0	67
Negative	1	32	33
Total	68	32	100

Table 2. Performance of GeneXpert MTB/RIF Assay for detection of *M. tuberculosis* (n = 100).

Variables	Values
Sensitivity	98.52%
Specificity	100%
NPV	96.96%
PPV	100%
Accuracy	99%

Note: NPV: Negative Predictive Value; PPV: Positive Predictive Value.

Table 3. Detection of rifampicin resistant *M. tuberculosis* by GeneXpert MTB/RIF assay and Drug Susceptibility Test (DST) on liquid media (MGIT 960) culture.

Method	RIF sensitive	RIF resistant
GeneXpert MTB/RIF	53	14 (20.9%)
Drug Susceptibility Test	53	15 (22.1%)

MTB/RIF and liquid culture on MGIT 960 media. GeneXpert MTB/RIF detected 14 (20.9%) cases of *Mycobacterium tuberculosis* which were rifampicin resistant among 67 *M. tuberculosis* positive samples. On the other hand liquid (MGIT 960) culture detected 15 (22.06%) out of 68 *M. tuberculosis* positive samples.

4. Discussion

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, out of 100 samples, *M. tuberculosis* was detected in 68 (68%) samples when cultured in MGIT 960 medium. In Vietnam Helb *et al*. [4] isolated 81 (76%) *M. tuberculosis* out of 107 clinical sputum samples which was similar to the current study. On the other hand, Hasan *et al*. [15] examined 421 specimens from TB suspects of Bangladesh and was recovered 45 (10.6%) *Mycobacterium tuberculosis* isolates in MGIT 960 cultures. It is much lower than present finding and may be related to difference in case selection.

Out of 100 cases, GeneXpert MTB/RIF assay detected *M. tuberculosis* in 67 (67%) samples in this study. Helb *et al.* [4] showed that GeneXpert was detected 62 (58%) *Mycobacterium tuberculosis* out of 107 clinical sputum samples which was similar to the present study. However, in Turkey, Zeka *et al.* [16] detected 51 (20.16%) *Mycobacterium tuberculosis* out of 253 sputum samples which was much lower than the current study. Haider *et al.* [17] from Malaysia showed that out of 125 clinical sputum samples GeneXpert could detect tuberculosis from only 8 (6.4%) samples. These variations might be due to geographical difference and difference in case selection.

In this study, MGIT 960 culture yielded highest (68%) isolation of *M. tuberculosis* from sputum samples. GeneXpert MTB assay showed equal 67% positivity. Chien *et al.* [18] reported that recovery rates were 94% with BACTEC MGIT 960. These were less than current study findings as well as previous results. Helb *et al.* [4] in their study showed that positivity for, MGIT 960 liquid culture and GeneXpert assay were 76% and 58% respectively. Scott *et al*. [19] in their study showed positive MGIT culture in 38% participants and GeneXpert positive result in 36.6% of participants. However, Zeka *et al.* [16] had 93% MGIT culture positive and 44% GeneXpert positive results. These variations might be due to difference in population and mycobacterial characteristics, sampling techniques and microbiological methods applied.

Liquid culture systems have many advantages. Several studies have shown that they have a shorter time to detection and have a higher recovery rate of *Mycobacteria* when compared to solid culture. This difference may be due to the added enrichment of the liquid culture media or the ability of bacteria within a liquid medium to spread through the media and access to all the nutrients [8]. In this study drug susceptibility test (DST) was performed on MGIT 960 media. Out of 68 MTB positive cases, 05 (7.35%) mono-INH resistant, one (1.47%) mono-RIF resistant and 14 (20.59%) multidrug resistant organisms (MDR) resistant to INH and RIF were detected. Lawson *et al.* conducted DST on BACTEC-MGIT-960 and showed 5.1% mono-INH resistance, 4.1% mono-RIF resistance and 7.5% MDR. These discrepancies might be related to sample size, sampling technique, geographical and bacteriological variations.

The present study showed that 14 (20.9%) out of 67 GeneXpert positive samples were Rifampicin resistant. It included both mono-RIF resistant and multi-drug resistant samples. According to GXMTB/RIF-10 manual, 60 (17.44%) out of 344 GeneXpert positive samples showed Rifampicin resistance, similar to the current findings. The primers in the GeneXpert MTB/RIF Assay amplify a portion of the rpoB gene containing the 81 base pair "core" region specific to members of the *Mycobacterium tuberculosis* complex and probe for mutations within the rifampicin resistance determining region (RRDR) of rpoB gene [5]. *Mycobacterium tuberculosis* is detected by the five overlapping molecular probes (probes A-E) or beacons that collectively are complementary to the entire bp rpoB core region. *Mycobacterium tuberculosis* is identified when at least two of five probes give positive signals [10].

In the present study, liquid culture on MGIT 960 yielded much early (TTD-17.5 ± 3.8 days) positive result. Most (88%) of the positive results in liquid culture were found within 7 to 21 days. Somoskovi *et al.* [20] showed in their study the mean time of detection of *M. tuberculosis* in smear-positive specimens was 12.6 days for BACTEC MGIT 960 medium and in smear-negative specimens it was 15.8 days for BACTEC MGIT 960

medium.

In this study, the performance of GeneXpert MTB assay was compared with MGIT 960 culture for detection of MTB in sputum samples. The calculated sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy of GeneXpert MTB/RIF assay was 98.52%, 1005, 96.96%, 100% and 99% respectively. Zeka *et al*. [16] showed for smear positive pulmonary samples the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of GeneXpert assay all were 100% but for smear negative samples these were 74.2%, 99.4%, 96% and 95.8% respectively. In the study conducted by Scott *et al.* 19 the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of GeneXpert were 86%, 97%, 95% and 92% respectively. Helb *et al.* [4] showed 100% sensitivity for smear positive samples and 71.7% sensitivity for smear negative samples but the specificity was100% for both. All these results are encouraging for the use of GeneXpert. Rodrigues *et al.* [21] showed that overall sensitivity of MGIT 960 was 97% and specificity was 100%. All these studies showed results comparable to this study.

In present study, 15 (22.06%) out of 68 MGIT positive samples and 14 (20.9%) out of 67 GeneXpert MTB positive samples were shown to be Rifampicin (RIF) resistant. So there was one phenotypic (DST on MGIT 960) 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*. [22] 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. This means that results were comparable.

5. Conclusion

From this study, it can be concluded that 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.

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