Original Article

GeneXpert MTB/RIF Assay for Rapid Identification of Mycobacterium Tuberculosis and Rifampicin Resistance Directly from Sputum Sample

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Abstract

Background*: The World Health Organization has endorsed the use of molecular methods for the detection of tuberculosis (TB) and drug resistant TB as a rapid method. In Bangladesh, the Xpert MTB/RIF assay has been implemented into reference laboratories for diagnosis of TB and also MDR TB.* ***Objective****: Drug resistant tuberculosis has long been a common problem prevailing in our country. The present study focused on the rapid identification of Mycobacterium tuberculosis as well as drug resistance.* ***Materials and Methods****: Sputum samples from a total of 107 cases, assumed as multi-drug resistance tuberculosis, were studied through GeneXpert assay.* ***Results****: Out of 107 cases, 91 (85.05%) were detected having M. tuberculosis ─ 64 (59.81%) were rifampicin sensitive and 27 (25.23%) were rifampicin resistant. The sensitivity and specificity of the GeneXpert are 87.64% and 75% respectively.* ***Conclusion****: GeneXpert assay can be considered for the rapid diagnosis of drug resistant tuberculosis.*

Key words*: GeneXpert assay; MDR TB; Sensitivity; Specificity*

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Introduction

'If the importance of a disease for mankind is measured by the number of fatalities it causes, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera and the like. One in seven of all human beings died from tuberculosis. If one only considers the productive middle-age groups, tuberculosis carries away one-third, and often more.' − Robert Koch, 24 March 1882.[1]

Tuberculosis is an infectious disease caused by acid-fast bacillus which belongs to the Mycobacterium tuberculosis complex. It typically affects the lungs (pulmonary TB), but can affect other sites as well (extrapulmonary TB). The disease is spread in the air when people who are sick with pulmonary TB expel bacteria by coughing, sneezing or talking. Aerosols are formed in the lung and expelled. These aerosols contain bacilli, and can be inhaled by others. Overall, a relatively small proportion of people infected with M. tuberculosis will develop TB disease.[2]

Tuberculosis (TB), especially the drug resistant one, appears as a major health problem worldwide. The infection affects up to one-third of the world population, claiming over 1.4 million deaths and more than 9.4 million incident cases every year, throughout the world.[3-5] The latest estimates included that there were 1.5 million TB deaths and 1.1 million (13%) of the 9 million people who developed TB in 2013 were HIV-positive.[6] Mortality rate globally range from 1.6 to 2.2 million lives per year. The situation is further exacerbated with the increasing incidence of drug resistant TB.[7]

Globally in 2013, 3.5% of new TB cases and 20.5% of previously treated cases were estimated to have Multi-drug resistant tuberculosis (MDR-TB). Also, there were approximately 210,000 deaths from MDR-TB.[6]

Bangladesh ranked 6th among the 22 highest TB-burdened countries in the world and 9th among the

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25 high priority multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB-flourished countries.[5,7] As per National Tuberculosis Control Program (NTP) Report, 2009, it is estimated that MDR-TB rate in Bangladesh is 1.4% among new cases and 29% among previously treated cases.[8] Multi-drug resistant (MDR) TB is caused by Mycobacteria that are resistant to at least isoniazide (INH) and rifampicin (RIF) which are most effective anti-TB drugs.[7]

National TB Control Program (NTP), in Bangladesh, began implementing DOTS since 1993 and DOTS has already been proved with a high cure rate; with proper treatment almost all TB cases are curable. However, the success of tuberculosis control programs depends not only on successful completion of treatment but also on early diagnosis, steady monitoring, and response to treatment, because incomplete treatment carries a risk of development of resistance, increases disease transmission and increases morbidity and mortality.[9,10]

In order to overcome conventional methods, low sensitivity and diagnostic delays, Xpert MTB/RIF, a fully automated molecular test, has been introduced. It can detect the presence of Mycobacterium tuberculosis (MTB) complex DNA and mutations associated with rifampicin (RIF) resistance directly from sputum in less than 2 hours, and it minimizes staff manipulation and biosafety risk.[3]

Materials and Methods

This cross-sectional observational study was carried out at Department of Microbiology, Mymensingh Medical College, Mymensingh and National Tuberculosis Reference Laboratory (NTRL) of National Institute of Disease of the Chest and Hospital (NIDCH), Bangladesh from July 2014 to December 2015. The NTRL handles a substantial number of patients with complications referred from other hospitals and upazilla health complexes within the country. The laboratory is supervised and has been certified by Supranational Reference Laboratory (SRL), Antwerp, Belgium.

Study population

One hundred and seven suspected MDR TB patients of all ages and both sexes, who attended at NTRL during the study period, were enrolled in this study.

GeneXpert assay procedure

Sputum sediments procedure

Sputum was collected in a dry, wide-necked, leak-proof sterile container. After collecting sputum from a patient, sputum sediments were prepared according to the method of Kent and Kubica and re-suspended in 67 mL phosphate/H_2O and tested using Xpert MTB/RIF Assay. Minimum volume of sputum/sediment for one test was 0.5 mL and for fresh sputum 1.0 mL.

Each Xpert MTB/RIF cartridge was labeled with the sample ID, then 0.5 mL of the total re-suspended pellet was transferred to a conical, screw-capped tube for the Xpert MTB/RIF using a transfer pipette. About 1.5 mL of Xpert MTB/RIF Sample Reagent (SR) was transferred to 0.5 mL of re-suspended sediment using a transfer pipette and shaken vigorously 10 to 20 times or vortexed for at least 10 seconds. After that this specimen was incubated for 10 minutes at room temperature, and then again shaken vigorously 10 to 20 times or vortexed for at least 10 seconds. Specimen was again incubated at room temperature for an additional 5 minutes.

Preparing the GeneXpert assay cartridge

At first cartridge lid was opened, and then the specimen container was opened. By using the provided transfer pipette, the liquefied sample was aspirated to the line on the pipette and transferred into the sample chamber of the Xpert MTB/RIF cartridge. The sample was dispended slowly to minimize the risk of aerosol formation. Lastly, the cartridge lid was closed firmly.

Starting the test

At first computer was turned on and then GeneXpert instrument was turned on. The GeneXpert software launched automatically. The GeneXpert diagnosis system software logged on using user name and password. In this system window, Create Test was clicked and then the Scan Sample ID dialog box appeared. The barcode on the Xpert MTB/RIF cartridge was scanned and after that the Create Test window appeared. By using the barcode information, the software automatically filled the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date. After that Start Test was clicked. Password was entered if requested. Then the instrument module door was opened with the blinking green light, the cartridge was loaded and the door was closed. The test started and the green light

stopped blinking. When the test was finished, the light turned off and the system released the door lock at the end of the run; then the module door was opened and the cartridge was removed. Finally results were recorded within 2 hours.[5]

Results

Out of 107 cases, M. tuberculosis were detected in 91 (85.05%) cases of which 64 (59.81%) were rifampicin sensitive and 27 (25.23%) were rifampicin resistant. M. tuberculosis were not detected in 16 (14.95%) cases. Among 64 (59.81%) rifampicin sensitive cases 34.58% were male and 25.23% were female. Among twenty seven (25.23%) rifampicin resistant cases 18.69%, were male and 6.54% cases were female. The sensitivity and specificity of the GeneXpert is 87.64% and 75% respectively using culture as gold standard.

Table I: Detection of M. tuberculosis and rifampicin (RIF) resistant M. tuberculosis by GeneXpert MTB assay

Resistance Pattern by GeneXpert Assay		Frequency (%)
M. tuberculosis detected		
	RIF resistance detected	27 (25.23)
	RIF sensitive detected	64 (59.81)
M. tuberculosis not detected		16 (14.95)
Total		107

Table II: Rifampicin sensitivity of MDR TB by GeneXpert according to sex

Sex	Rifampicin sensitive Number (%)	Rifampicin resistant Number (%)	Not detected Number (%)	Total Number (%)
Male	37 (34.58)	20 (18.69)	7 (6.54)	64 (59.81)
Female	27 (25.23)	7 (6.54)	9 (8.41)	43 (40.19)
Total	64 (59.81)	27 (25.23)	16 (14.95)	107 (100)

Table III: Age and sex distribution of suspected MDR TB patients

Age group	Male n (%)	Female n (%)	Total n (%)
<25	14 (13.08)	17 (15.89)	31 (28.97)
25−34	15 (14.01)	10 (9.34)	25 (23.36)
35−44	12 (11.21)	8 (7.48)	20 (18.69)
45−54	9 (8.41)	5 (4.67)	14 (13.08)
55−64	8 (7.48)	1(0.93)	9 (8.41)
>64	6 (5.61)	2 (1.87)	8 (7.48)
Total	64 (59.81)	43 (40.19)	107 (100)

Table IV: Sensitivity and specificity of GeneXpert considering culture as gold standard (n=105)

Liquid culture in MGIT960 System	GeneXpert		Total n (%)	Sensitivity (%)	Specificity (%)
	Positive n (%)	Negative n (%)			
Positive	78 (74.29)	4 (3.81)	82 (78.10)		
Negative	11 (10.48)	12 (11.43)	23 (21.90)	87.64	75
Total	89 (84.77)	16 (15.24)	105 (100)		

Discussion

Rapid diagnosis of mycobacterial disease is critical, and attempts to shorten the time to detection of such organisms deserve attention.[11] Smear examination is a rapid method for detection of mycobacteria in a clinical specimen, especially sputum but the limitations are low sensitivity and inability to diagnose rifampicin sensitivity pattern.

Increased prevalence of drug resistant tuberculosis in Bangladesh and other developing countries is a growing threat to tuberculosis control. So, early detection of MDR TB is crucial both for the patient management and infection control in TB positive cases.[5]

In our study, among 107 clinically suspected pulmonary tuberculosis cases 64 (59.81%) were male and 43 (40.19%) were female and male female ratio was 2:1.34. Torrea et al[12] reported that in their study out of 247 pulmonary tuberculosis patients, 160 (64.78%) were male and 87 (35.22%) were female with male female ratio 1.83:1. This is almost similar to our study. The reason of higher male tuberculosis cases than female cases might be explained by the fact that males are actively populated in the community and may come in contact with TB infected persons more frequently. But female members in Bangladesh still reside at home and therefore the chance of exposure is comparatively less.[7]

In this study, GeneXpert assay detected M. tuberculosis in 91 (85.05%) cases. Aurin et al[5] detected M. tuberculosis in 283 (94.33%) samples out of 300, which is very close to the findings of this study.

Out of 91 M. tuberculosis positive cases, GeneXpert assay detected 64 (59.81%) rifampicin sensitive and 27 (25.23%) rifampicin resistant cases. Sensitivity and specificity of GeneXpert were 87.64% and 75% respectively. Scott et al[13] showed that sensitivity and specificity of GeneXpert were 86% and 97% respectively which is similar to the findings in our study.

Analyzing the findings of the present study it can be concluded that the GeneXpert technique is a rapid, simple and highly dependable method for the diagnosis of pulmonary tuberculosis and it is equally effective for detection of MDR TB within 2 hours.

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