Evaluation of GeneXpert MTB/RIF assay as a potential diagnostic tool of Mycobacterium tuberculosis (MTB) in Chittagong metropolitan city

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ABSTRACT

Aims: Precise diagnosis of Mycobacterium tuberculosis (MTB) is key to treating and managing tuberculosis (TB) patients effectively. This study was carried out to evaluate the performance of the GeneXpert MTB/RIF assay and compare its efficiency with other conventional TB detection methods.

Methodology and results: In total, 110 samples were collected from pulmonary presumptive TB patients in Chittagong metropolitan city and subjected to GeneXpert MTB/RIF assay, MTB culture and microscopic examination to compare their performance in the diagnosis of TB. The sensitivity, specificity, and positive and negative predictive values (PPV, NPV) of these methods were calculated using culture as the reference method. The TB-positive results found for GeneXpert, ZN staining, Auramine O staining and culture were 30 (27.27%), 21 (19.09%), 26 (23.64%) and 18 (16.36%), respectively. GeneXpert showed higher sensitivity (100%) than that of ZN stain (44.44%) and Auramine O stain (72.22%). Moreover, the specificity for detecting MTB in sputum samples observed for GeneXpert was 86.96%, whereas ZN and Auramine stain showed similar specificity (85.86%).

Conclusion, significance and impact of study: In this study, GeneXpert was found as the rapid and sensitive method for early detection of MTB compared to other diagnostic methods. Identification of MTB by GeneXpert assay might improve the quality and reduce the time of diagnosis in developing countries like Bangladesh.

Keywords: GeneXpert MTB/RIF assay, Mycobacterium tuberculosis, sensitivity, specificity, tuberculosis

INTRODUCTION

Tuberculosis (TB) is an airborne disease caused by the nine species of the genus Mycobacterium known collectively as M. tuberculosis complex (MTBC). Three species of the MTBC group are more prevalent as identified causative agents of TB in humans and they are M. tuberculosis (MTB), M. africanum and M. canetti (Malone and Gordon, 2017; Piriñata et al., 2018; Riojas et al., 2018). One of the critical complications in TB management arises from the drug-resistance development in the identified species in MTBC. Global statistical data indicates a rise in the development of drug-resistant TB (DR-TB) and multidrug-resistant TB (MDR-TB). For instance, the MDR-TB strain that is resistant to both isoniazid (INH) and rifampicin (RIF) was diagnosed in 600,000 cases in 2016. However, in 2014, approximately 480,000 patients were diagnosed with MDR-TB for INH and RIF, indicating a rapid rise in the spread of DR-TB (WHO, 2017). Bangladesh ranked 5th among the 30 high TB burden countries and 10th among the high MDR-TB countries in the world (Banu et al., 2017; Chowdhury et al., 2021). In the first six months of 2020, the reported TB cases in Bangladesh were 99,000. Due to the insufficiency of screening or asymptomatic condition of the disease, approximately 247 cases remain undetected per day (ICDDR,B, 2020).

Above 90% of RIF Resistant (RR) TB strains grew resistance against other drugs, which enabled researchers to use it as a primary indicator of potential MDR-TB (Rahman et al., 2016; Fadeyi et al., 2017). RIF is the most potent antibiotic that binds to the β-subunit of DNA-dependent RNA polymerase (rpoB) and thereby inhibits transcription initiation (Campbell et al., 2001; Koch et al., 2014). Primarily, the rpoB gene region between the codon 507 to 533 was mutated in an 81 bp-long hot spot region in 95% of the RR strains (Campbell et al., 2001; Wang et al., 2013). This highly mutated segment is termed Rifampicin Determining Region (RRDR) as it was crucially associated with the RR development in TB strains.

Approximately 500,000 patients in 2019 were diagnosed with rifampicin-resistant tuberculosis (RR-TB), of which 78% had multidrug-resistant TB (MDR-TB)
(WHO, 2020). Moreover, about 20% of previously treated and 3.3% of new TB patients become infected with MDR-TB, leading to higher mortality rates (Denkinger et al., 2014).

To reduce TB transmission as well as to achieve the elimination of TB, early, rapid and accurate diagnosis and treatment of TB patients is inevitable. The gold standard technique for the diagnosis of TB is bacteriological culture, which can also be used for testing drug resistance. Similarly, such techniques also need complex laboratory infrastructure and take a long time to obtain results (Platek et al., 2013). Despite being a rapid and inexpensive technique, the sensitivity of Acid-fast bacilli (AFB) microscopy with ZN staining is variable (20-80%) (WHO, 2011). Currently, WHO recommended GeneXpert MTB/RIF assay as the rapid molecular test for the diagnosis of tuberculosis by dint of which it is possible to detect not only the presence of the MTBC genome but also the mutations liable for rpoB gene mutation in patient specimens within 2 hours (Zhang et al., 2018). An automated and cartridge-based real-time PCR system called Xpert assay, employs five molecular beacon probes that overlap with one another and target the RRDR region of the rpoB gene. These probes are used to detect MTBC coupled with the mutations that give RR. Afterward, the probes will hybridize to the corresponding sequence of the RRDR of the rpoB gene, which exists between the codons 507-511 (A), codons 512-518 (B), codons 518-523 (C), codons 523-529 (D) and codons 529-533 (E). MTBC is identified when a minimum of two out of five probes exhibits a cycle threshold ( Ct) ≤38. Any strain in which one or perhaps more probes do not hybridize or when the discrepancy between the first and final Ct is greater than 3.5 is designated as an RR case (Lawn and Nicol, 2011). Previous studies demonstrated that the GeneXpert assay had a specificity of 99% and sensitivity of 98% in AFB smear positive (AFB+) and 67% in AFB- smear specimens in mostly high tuberculosis-prevalence settings (Steingart et al., 2014).

This study aimed to monitor the efficiency of GeneXpert in diagnosing TB in Bangladesh, a tuberculosis-endemic nation. Furthermore, it aimed to form a comparative assessment in the context of performance by GeneXpert MTB/RIF to that of GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) relative to other common TB detection techniques.

MATERIALS AND METHODS

Study area, population and sample collection

The pulmonary samples (Sputum) of TB presumptive patients (n=110) were collected from the Bangladesh Institute of Tropical and Infectious Diseases (BITID) Hospital of Chattogram. Out of this presumptive, 67 were males and 43 were females; they ranged in age from 0 to 80 years. For each patient, a detailed history was taken from patients, or their guardians and they were informed of the purpose of the study. The inclusion criteria for the presumptive TB cases were productive cough for two or more weeks accompanied by one or more of the following: persistent fever, loss of appetite and weight, chest pain, shortness of breath and fatigue. Criteria for exclusion were patients with chest disease other than TB or non-consenting people. Samples were collected in a wide, clean, dry sputum container and stored at 4 °C until further use. Each sputum received in the lab as per the collection and transportation policy of the laboratory was divided into three parts; one part was immediately tested using GeneXpert, the second part was used for Auramine O and ZN smear microscopy and the third part was decontaminated and inoculated on Lowenstein-Jensen (LJ) media. The study was approved by the Ethical Review Committee (ERC) of BITID hospital.

GeneXpert MTB/RIF assay

Samples were processed following the manufacturer's instructions. Briefly, samples were diluted with GeneXpert sample reagent in (1:2 ratios). The tube was vortexed twice during a 10 min incubation period at room temperature. Then, 2.5 mL of processed solution was taken to a plastic cartridge which was then inserted into the GeneXpert device. The results were obtained not more than two hours.

Acid-fast bacilli (AFB) microscopy

Preparation of smears

For a direct sputum smear, a small portion of purulent/mucopurulent material was selected and transferred to the slide using a sterile application stick. The material was spread over an area equal to about 1-2 cm, air-dried at room temperature and fixed by passing through a flame three times in quick succession.

Auramine O (AO) stain

The auramine solution was poured over the smears and left for 20 min. Then the slides were meticulously cleaned using water that had been previously distilled. Afterward, the acid alcohol solution (decolorizing solution) was poured over the slides allowing 3 min for action. Next, the smears were carefully cleaned with distilled water and then submerged in methylene blue for 1 min. Then, they were washed with purified water and left to dry in the air. Smears were examined by fluorescent microscopes using 25× or 40× objective together with a 10× objective lens.

Ziehl-Neelsen (ZN) stain

Freshly filtered Carbol Fuchsin solution was poured over the slides and the heat was given from below and left for 10-15 additional min. The slides were thoroughly rinsed with distilled water and acid alcohol was poured over the slides to decolorize the primary stain allowing it to stay for 3 min. Then the slides were rinsed with distilled water and counterstained with 1% methylene blue solution for 1 min.
and then washed off in running tap water and air-dried. The slides were inspected with an optimized light microscope using a 1000× immersion oil objective lens.

**Culture on Lowenstein-Jensen media (L-J media)**

Sputum N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) (Petoff’s method) were used to disinfect samples (Denkinger et al., 2014). McCartney bottles containing egg-based Lowenstein-Jensen media (L-J media) were labeled with the patient’s ID number and date. Two slopes of each decontaminated sputum sample were inoculated in L-J media and incubated at 37 °C for 8 weeks before being reported as negative. For PCR, residual processed samples were stored in the refrigerator for one week. Each week, the development of colonies was examined and compromised cultures were eliminated. Results were reported immediately after detection and identification. The growth of M. tuberculosis was confirmed by AO stain and ZN stain, as previously mentioned.

**Statistical analysis**

Data was calculated in percentages using Microsoft Excel. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of these methods were calculated, and all the values were expressed as percentages.

**RESULTS AND DISCUSSION**

A total of 110 pulmonary presumptive TB samples were collected in the present study among which 67 (60.91%) were males and 43 (39.09%) were females (Table 1 and Figure 1). The TB presumptive were divided into 4 age groups; 32 (29.09%) suspects were between the ages of 10 and 30 years old, 49 (44.55%) were between the ages of 31 and 50 years old, 23 (20.91%) were between the ages of 51 and 70 years old and 6 (5.45%) suspects were between the ages of 71 and 90 years old (Table 1 and Figure 2). The demographic breakdown revealed that the majority of presumptive ages of patients ranged from 31 to 50 years (44.55%). A male predominance was observed, which is due to them being actively populated in the community. Moreover, males are more likely to come into contact with people infected with TB. Enhanced vulnerability of men to respiratory disease may be attributable to their habits of alcoholism and smoking (Okonkwo et al., 2017).

In the present study, GeneXpert MTB/RIF assay revealed that 30 out of 110 samples (27.27%) were MTB-positive. According to the AO staining, 26 out of 110 sputa (23.64%) samples were found positive whereas, in the ZN stain test, 21 (19.09%) samples showed positive results. Overall, 16.36% of the (18/110) samples tested positive for mycobacteria on the LJ medium. On LJ medium at 37 °C, the morphological characters of all 18 slow-growing isolates of MTBC were almost similar. All

![Figure 1](Image)

**Figure 1:** Pie chart of percentages of male and female suspects of pulmonary tuberculosis presumptive (N=110). N is the sample size.

**Table 1:** Demographic characteristics of pulmonary tuberculosis presumptive.

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<td>Female</td>
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The colonies were found to be rough, dry and buff-colored. The isolates' growth rate ranged from 3 days to 6 weeks. After 3 weeks, the majority of isolates exhibited noticeable development. The MTBC-like colonies were later confirmed by ZN stain and AO stain.

Among 110 sputum samples, 18 were both culture and GeneXpert positive, 12 were GeneXpert positive but culture-negative and 80 showed negative results in both tests. Out of 110 samples, 13 samples were culture and AO stain test positive, 13 samples were only AO stain positive and 5 samples only culture positive. Both ZN stain and culture-positive samples were 8 and ZN-positive but culture-negative samples were 13 (Table 2).

The comparison of overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for GeneXpert, Auramine O stain and ZN stain is shown in Table 3. The sensitivity of the Auramine O stain was 72.22% and specificity was 85.86%, with a PPV of 50% and NPV of 94.05%. ZN stain was found to be 44.44% sensitive and 85.86% specific in TB detection, with PPV of 38.09% and NPV of 88.76%. GeneXpert showed 100% sensitivity and 86.96% specificity, with a PPV of 60% and NPV of 100% (Table 3).

In the present study, the Auramine O stain (72.22%) test was found more sensitive than the ZN stain (44.44%). However, both tests had equal specificity (85.86%) (Table 3). This fluorochrome staining of sputum smears is a better microscopy method in comparison with ZN stain. Osman et al. (2014) reported the sensitivity and specificity of microscopic techniques based on results of culture as...
gold standard techniques as follows: 59.38%, 43.75%, sensitive for Auramine and ZN, respectively, and 98.18%, 89.79% specific, respectively. Our study found 100% sensitivity and 86.96% specificity for GeneXpert (Table 3). Bilgin et al. (2016) reported 100% sensitivity and 98% specificity of GeneXpert. Ejeh et al. (2018) reported in their study that the sensitivity and specificity of GeneXpert were 93.75% and 86.03%, respectively.

GeneXpert was found as a more sensitive method than both of the AFB staining methods. The most commonly used technique for diagnosing pulmonary TB is sputum smear microscopy because it is simple, rapid and inexpensive, despite its low sensitivity (60-70%) (WHO, 2002). Culture is the criterion of the gold standard, yet it requires several weeks to give results. In the domain of tuberculosis (TB) diagnosis, molecular approaches have undergone a radical transformation and have been shown to produce speedy and sensitive findings.

The GeneXpert technique is an excellent and user-friendly instrument for obtaining speedy, accurate and sensitive results.

CONCLUSION

In conclusion, superior performance was observed for GeneXpert over Auramine O stain and ZN stain as it had higher sensitivity and specificity. Hence, GeneXpert can be used as a viable alternative to the other conventional diagnostic methods in the early diagnosis of tuberculosis.

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REFERENCES


