Comparative Study between Genexpert and Smear Microscopy for the Diagnosis of Tuberculosis in Samples of Patients Suspected of Pulmonary Tuberculosis – the Case Study of General Hospital, Awo-Omamma, Imo State, Nigeria

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Abstract

Tuberculosis (TB), caused by Mycobacterium tuberculosis, has remained a major scourge of humanity all over the world, with the greatest mortality occurrences noted, in developing countries. The cannot-be-over- emphasized burden of TB in Nigeria is among the highest in Africa. The study on hand was therefore aimed at comparing Cepheid GeneXpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis Complex (MTBC) and Rifampicin (RIF) resistance with the traditional smear microscopy method-the ZN technique. Sensitivity and specificity of diagnostic yields were high points of comparison. A carefullydesigned cross-sectional study was drawn and executed at the General Hospital, Awo-Omamma, covering patients' inflow from August, 2016 to May 2017. Amongst the numerous patients presenting, a total of 120 samples were collected from patients with highest pulmonary concerns, having been assessed prognostically.

Sixty-two patients (51.67%) were males, fifty-eight (48.33%) were females and all having mean ages of 42.2+16 years. Thirty patients (25%) had chronic lung diseases. Out of the 120 samples examined, 36 samples (30.00%) were MTBC positive by Smear microscopy while 42 (35.00%) were positive by GeneXpert. Placing both methods (GeneXpert and Smear microscopy) side-by-side, GeneXpert gave 85% sensitivity and 98.5% specificity GeneXpert indeed detected 6 (7.2%) additional positive cases as compared to Smear microscopy. Only 5 clinical isolates of the entire patients were resistant to Rifampicin. The study therefore conduced that GeneXpert was a better and more reliable diagnostic tool compared to Smear microscopy and can significantly reduce false-negatives and very interestingly, rules out the unnecessary delays often experienced hitherto with Smear microscopy in treatment initiation.

Keywords: Smear, Microscopy, GeneXpert, Tuberculosis.

Introduction

The global burden of TB remains enormous. More than 9million newMycobacterium tuberculosis cases occur annually worldwide. TB is responsible for 1.7million deaths per year; the vast preponderance in resource limited settings [1].

Culture and sensitivity technique offers a "gold-standard" for final determination, and also permits drug susceptibility testing. It remains largely inaccessible in resource limited settings as a result of infrastructure and cost limitations .Even where accessible, culture results are typically unavailable for 2-6weeks [2].

Traditionally, sputum Smear microscopy is easier to do and is very cheap and combined with chest X-ray, has been used for a long time by TB control agencies worldwide. However, the sputum Smear microscopy (sputum AFB) test has some problems HIV-positive patients, children andin cases of multi-drug resistance as mostly indicated by injudicious use of drugs [3,4]. Thus for rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, newer methods of detection are required [5].

WHO has recently recommended a real-time PCR test called CBNAAT (Cartridge Based Nucleic Acid Amplification Test) / GeneXpert as a revolutionary and primary diagnostic modality for detection of TB due to its better accuracy [6].

GeneXpert, as it is more commonly now referred to, is a semi-quantitative nested real-time PCR in-vitro diagnostic test with two uses:

(a) The detection of *M. tuberculosis* Complex DNA in sputum that are either acidfast bacilli Smear positive or negative, and

(b) The detection of rifampicin resistance associated mutations of the RopB gene in samples from patients of rifampicin resistance [7,8].

In view of the above disparities, this on-hand study was to evaluate Cepheid GeneXpert MTB/RIF assay for direct detection of *M. tuberculosis* and RIF resistance and compare it with traditional sputum Smear microscopy. Specificity, Sensitivity and Resistance were key determinative factors underlying the study.

Materials

This is a comparative study conducted in the Department of Medical Laboratory Services (TB Specialty Laboratory) of General Hospital, Awo-Omamma, Imo State, Nigeria between August, 2016 to May, 2017.

Being a referral centre, enormous cases abound, amongst which a total of 120 samples (sputum) were collected from patients with highest pulmonary concerns, all having been assessed prognostically.
• 	Inclusion criteria: Age group > 16years.
Males and females alike.
• 	Exclusion criteria: All patients < 16 years.

Methods

Principle and procedure: Cepheid GeneXpert.
GeneXpert RIF system, an automated instrument works on the principle, thus: sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The assay utilizes single use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing DNA extraction and heminested rt-PCR [9, 10].

Sample reagent was added to the specimen in a ratio of 2:1, manually agitated and kept for 10mins at room temperature, then shaken again and kept for 5mins; 2ml of the inactivated material was transferred to the test cartridge and inserted into the test platform. Electronic results were available in > 2hours.

Principle and Procedure: Sputum Smear Microscopy (by Ziehl-Neelsen (ZN) technique). The primary stain (strong Carbol fuchsin) binds to the mycolic acid in the mycobacterial cell wall. After staining, an acid decolourizing solution is applied. This removes the red dye from the background cells, tissue fibres, and any organisms in the Smear except mycobacteria which retains (hold fast to) the dye and are therefore referred to as acid fast bacilli. Following decolourization, the Smear is counterstained with malachite green or methylene blue which stains the background material, providing a contrast colour against which the red AFB can be seen[11].

Results

Sputum smear microscopy by ZN technique was done for 120 samples of the patients who were having history suspected of pulmonary tuberculosis. Out of these, 36 (30.00%) sputum samples were AFB/MTBC positive, while 84 (70.00%) were AFB/MTBC negative.

Furthermore, the samples were subjected to a GeneXpert MTB/RIF assay. Out of the 120 sputum samples, 42 (35.00%) were positive, 77 (64.16%) were negative while 1 (0.84%) showed false-negative. The results of GeneXpert and AFB/ZN staining are compared in our study.

It is evident from the table below that GeneXpert MTB/RIF is more useful than ZN staining. As compared to ZN staining, it can detect MTB even in 1ml of sputum. Thus GeneXpert indeed detected 6 (5.00%) additional positive cases as compared to Smear microscopy.

Worthily noted was the other advantage of GeneXpert over ZN staining is that it also detected rifampicin resistance, and thus now helps us to diagnose multi-drug resistant tuberculosis (MDR TB). In this study, 5 (4.16%) clinical isolates out of the entire patients were resistant to rifampicin; which was confirmed with drug susceptibility.

Table 1

	Sputum for AFB +ve	Sputum for AFB –ve	Total samples
GeneXpert MTB +ve	36	6	42
GeneXpert MTB -ve	1	77	78
Total samples	37	83	120

Table 2

	MTB +ve	MTB – ve	Total samples
RIF Resistance Not DETECTED	32	83	115
RIF Resistance DETECTED	5	0	5
Total samples	37	83	-

All results were analysed statistically by applying Chi-square test

$$X^2 = \sum (0i- E i)^2$$

Pvalue was < 0.001, all results were highly significant.

Discussion

Performance studies as to sensitivity, specificity and associated disparities in MTB/RIF assay with pulmonary specimens obtained during the clinical routines have been investigated. In the on-hand study, the MRB/RIF test detected the agent in 42 out of 120 pulmonary specimens (35% detection rate) whereas sputum for AFB was able to detect only 36 out of the 120 pulmonary specimens (30% detection rate).

A review study found that the MTB/RIF assay had a calculated limit of detection of 131 CFU/ml of sputum in 35% of samples compared with approximately 10,000 CFU/ml with conventional smear microscopy [12]. User's skills reliance is a less considerable factor in MTB/RIF assay procedure, thus routine staff with minimal training and guidance can use the test. Technicians can be trained in 1-2days; just 2 steps (addition of buffer and sputum sample) are manual; and results are available within 90minutes.

Each tabletop-sized module can process 4 samples daily (larger modules can run 200 tests in an 8-hour 1 day), and because it is a closed system, biosafety and contamination concerns are minimized. It has a short turn-around time and simultaneously detects *M. tuberculosis* and RIF resistance in less than 2hours.

Although the MTB/RIF test could be a useful tool for rapid identification of RIF-resistant *M. tuberculosis*, especially in smear-positive clinical samples, the test results must always be confirmed by culture [13].

Conclusion

The study concluded that as compared to the traditional technique of sputum smear AFB microscopy, GeneXpert is more sensitive and specific not only for acid-fast bacilli (AFB) detection but also for rifampicin (RIF) resistance. GeneXpert is therefore a better and more reliable diagnostic tool compared to smear microscopy and can significantly reduce false-negatives arising from staining inconsistences due to primary and counterstain failures etc and very interestingly, rules out the unnecessary delays often experienced hitherto with smear microscopy in treatment initiation, follow-up and general TB-patient management.

Acknowledgement

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