Research Article

Evidence-based comparison of molecular-genexpert and microscopic method in the diagnosis of *mycobacterium tuberculosis* among subjects in the limited resource setting of Niger delta

Abstract

Increasingly, pragmatic disease diagnosis is important for treatment, as well as disease prevention and control strategy, thus timely and accurate diagnostic outcome, may help to prevent or reduce the negative consequences of the disease in the body and the population at large, even as well as reduce mortality index cases in the affected community or region. This study was carried out to determine an effective method for the detection of *Mycobacterium tuberculosis* (MTB) among suspected tuberculosis patients visiting the University of Port Harcourt Teaching Hospital (UPTH) and the Braithwaite Memorial Specialist Hospital (BMSH), all in Port Harcourt, Nigeria. A total of 286 and 269 sputum samples were collected from the UPTH and BMSH respectively, making a total of 555 samples. The two methods explored were ZN-Microscopy and the GeneXpert assay method. Using GeneXpert (Modules Thermal and Optical System Cepheid, Serial Number, 805757, USA) 26.2% and 30.9% of the samples were positive for MTB in UPTH and BMSH, respectively. While microcopy detected 15.4% and 29.4% MTB samples from UPTH and BMSH respectively. Four (4) positive cases were detected by microscopy which was not detected by GeneXpert. Nonetheless, in overall, the sensitivity of GeneXpert assay was higher than that of microscopy. It is therefore strongly believed that the combination of the conventional method and the molecular assay or improvement of the molecular method may enhance detection of MTB in clinical samples especially in endemic regions where majority of the populace may be at risk due to seemingly poor living condition, poverty and limited access to prompt health education and robust health facility.

Keywords: *Mycobacterium tuberculosis*, genexpert, microscopy, comparison, Niger delta, diagnosis, disease

Wokem Gloria Ngozika,[1] Azuonwu Obioma,[1] Josiah Nkereawaji Appollus[2]

[1]Department of Medical Laboratory Science, Rivers State University of Science and Technology, Nigeria

[2]Department of Medical Microbiology, University of Port Harcourt Teaching Hospital Choba East-West Road, Nigeria

Correspondence: Azuonwu Obioma, Department of Medical Laboratory Science, Rivers State University of Science and Technology Nkpolu Orowhorukwo, Port Harcourt, Nigeria, Tel 234 803551968, Email bimajacobs@yahoo.co.uk

Introduction

At of 2014, tuberculosis (TB) was termed the world's deadliest communicable disease and is caused by *Mycobacterium tuberculosis* (MTB).[1] The disease is transmitted from one infected person to a healthy individual(s) through air droplets or inhalation of aerosols from an infected subject.[2] Worldwide, the tuberculosis burden is high with an estimation of 8.7 million new cases in 2012.[3] Nevertheless, 80% of this was from 22 countries, the majority of which were diagnosed as smear-negative pulmonary tuberculosis (SN-PTB). These countries rely on the conventional method of detection of MTB using smear microscopy whose reliability ranges from 20% to 60%.[4,5] Nonetheless, PTB is a popular opportunistic infection in Sub-Saharan Africa (Nigeria inclusive) where there is a high prevalence of HIV; usually persons with TB/HIV co-infection present with SN-PTB. This is as a result of the poorly formed lung granulomas/cavities in TB patients which leads to a reduced quantity of MTB in the lesions and subsequently cause difficulties during diagnosis of the infection.[6,7]

Overtime, early diagnosis has proved to be important in the early management and possible cure of the disease and its clinical complications. Tuberculosis is one of such diseases that require careful and accurate diagnosis outcome. It is caused by the bacterium-*Mycobacterium tuberculosis* (MTB). Three methods are most often used in the diagnosis of TB and these are microscopy, culture and GeneXpert. Of these, microscopy is not as sensitive and specific as GeneXpert which is described as a molecular technique for MTB detection.[8] However, in most nations, the conventional microscopy, culture and drug susceptibility testing (DST) method is used in TB diagnosis.[9] There is however, a possibility of a misdiagnosis leading to a false positive or false negative result in cases where culture is done. Furthermore, culture is time consuming, as it requires about 2-6 weeks to get a result and also requires technical expertise (1,6,7). In 2014, 68.1% of the PTB cases recorded in Italy were smear-negative.[10] The WHO has thus approved the GeneXpert assay for the diagnosis of TB, as it not only detects MTB, but also detects rifampicin resistance strains, and finally it is able to produce results within 2 hours.[11] This is, therefore, commendable, as it tends to promote health outcome of massive benefit to the supposedly patients.

The choice of GeneXpert as a method of MTB detection was preceded by developments in the area of molecular biology and diagnostics approach, which is currently playing a major role in the swift dynamism of the diagnosis and response to TB health issues

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across the globe.[9] These advances including those in vaccines and drugs have enhanced the worldwide hope for TB control and prevention.[12] Molecular methods for the detection of TB are INNO LIPA Rif.TB (Innogenetics, Ghent, Belgium) and GeneType MTBDRplus (Hain Lifescience GmbH, Nehren, Germany) which are line probe assays and GeneXpert MTB/RIF (Cepheid, Sunnyvale, USA) which is a real-time polymerase chain reaction (PCR).[13]

GeneXpert assay involves lysis of the bacteria, extraction of DNA, amplification and amplicon detection.[14] Its advantages are that it is less complex, requires less time, has high specificity and sensitivity and requires less safety concerns for whoever that will be handling the samples.[15–19] Nonetheless, this study was carried out to determine which method of assay is best and more sensitive in the detection of MTB in sputum samples from two major health facilities in Port Harcourt, using GeneXpert assay and microscopy. It is strongly believed that data generated would help to improve prompt and precise diagnostic outcome of the disease burden, and thus in the end, patients care and overall case management strategy would be strongly underpinned, especially in our remote communities in Niger Delta where access to functional health care Centre remains a massive challenge.

Methodology

Sampling area

This study was carried out in Braithwaite Memorial Specialist Hospital and the University of Port Harcourt Teaching Hospital, which are two hospitals in the state with a GeneXpert Machine. BMSH is located in the Government Residential Area (GRA), Port Harcourt; latitude 12.9416°N and 77.5669°E. It is a state government owned hospital operated by the Rivers State Hospitals Management Board and named after Eldred Curwen Braithwaite, a British surgeon and pioneer surgeon in Rivers State. When it was established in 1925 as Braithwaite Memorial Hospital (BMH), it served as a medical facility for senior civil servants. Later, it became a general hospital but has now become a Specialist Health Institution. It has 731 medical staff and 375 licensed beds.

The UPTH is a federal government owned hospital located in East West Road, Port Harcourt; latitude 4.8996°N and 6.9286°E. It is a major tertiary-care teaching and research health facility in the Niger Delta region of Nigeria established in 1980 but officially commissioned in 1985. It presently has a 500-bed capacity. The city where both hospitals are located is the largest in Rivers State. The State has 23 Local Government Areas of which Port Harcourt Local Government is one.

Study population

The city is home to persons from different tribes of the country as well as foreigners who indulge in oil exploration. The dwellers are predominantly civil servants while some indulge in trading, farming and fishing. Suspected PTB patients were used for the study after ethical approval from the State Ministry of health as well as the UPTH had been obtained and the consent of the patients had been sought and obtained after informing them on the reason and relevance of the study. The patients who provided their sputum in sterile sample containers were the test population while non-TB subjects who had tested negative to MTB infection were the control population.

Ethical clearance and subject consent

Ethical clearance was obtained from the UPTH and the Rivers State Ministry of Health following due process. Informed consent of the patients was also obtained and the confidentiality of the results assured before their sputa was collected. Confidentiality of results was ensured by assigned numbers to samples collected as codes. The study was carried out between January 2016 and September 2016. All willing suspected PTB patients regardless of their sex and age were included in the study through convenience sampling design. Health workers with TB symptoms were also included. These included those with prior PTB history, and those with signs and symptoms of PTB and/or chest x-ray suggestive of PTB from the Directly Observed Therapy Short Course (DOTS) clinic.

Sample collection

Minimum sputum volume of 2 ml was collected from each patient by deep cough method in the morning of patient's enrollment. An early morning sputum was collected from each patient and tested using the microscopy technique and GeneXpert assay. Each sample was assigned a unique identification number, with the date, age and sex of the patient recorded accordingly. Well-structured questionnaires were given to each subject where the subjects provided demographic information as well as stated their reason for testing for TB. The questionnaires were completed by the patients and returned for analysis.

Experimental analysis

GeneXpert sample assay

This assay is made up of a one-time use multi-chambered plastic cartridge, filled with liquid buffer and lyophilized reagent beads needed for processing of the sample, DNA extraction and heminested real-time Polymerase Chain Reaction (PCR). The cartridge contained lyophilized *Bacillus globigii* spores that served as internal sample processing and PCR control. The collected sputa were treated with NaOH and a sample reagent (SR) containing isopropanol. In a ratio of 2:1, the SR was added to the sputum and incubated at room temperature for 15 minutes. The mixture was then transferred to the cartridge, the cartridge put into the GeneXpert instrument and an automated process completed the remaining steps. During the sample processing step, the *B. globigii* spores are resuspended automatically and processed during the sample processing step and the resulting DNA amplified during the PCR step. Amplification increases the amount of DNA molecules so as to enable easy detection of the MTB genomeThe standard user interface indicated the absence or presence of MTB and a semi quantitative estimate of the concentration of MTB (very low, low, medium and high). Negative assays were reported as invalid.

Microscopy analysis

Detection of MTB using microscopy is done by first staining the sputum sample using the Ziehl-Neelsen (Z-N) stain before viewing under the microscope. This staining method is based on the fact that the bacterium is an acid-fast one. A smear of the sputum is made on a clean dry slide and fixed. It is then covered with carbol fuchsin for 3 minutes; heated, rinsed with running water and decolourized with acid-alcohol for 5 seconds. It is then flooded with methylene blue

Evidence-based comparison of molecular-genexpert and microscopic method in the diagnosis of mycobacterium tuberculosis among subjects in the limited resource setting of Niger delta

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(counterstain) for 30 seconds, rinsed with running water and allowed to air dry, it was viewed under the microscope using the x10 to focus and X100 oil immersion lens for identification. The bacterium usually appears as a red beaded rod; 0.2-0.5 μm wide and 2-4 μm long.

Results

The 555 sputum samples collected from the two hospitals were assayed for the presence of MTB using both microscopy and GeneXpert assay method. Nevertheless, GeneXpert assay was able to detect 26.2% and 30.9% MTB positive samples in the UPTH and BMSH samples respectively, while microscopy detected 15.4% and 29.4% MTB positive samples respectively. Among the seven age groups investigated, ages 30-39 had a higher sample size of 171 and next to it were ages 40-49 while persons less than 10 years had lowest sample size of 21. GeneXpert identified 33.3% MTB samples among 30-39 age groups while it identified 9.5% MTB positive samples among ≤10 age group. Microscopy identified 25.1% and 4.8% of age group 30-39 and ≤10 respectively. Among males 34.2% and 28.1 was detected using GeneXpert and microscopy, respectively. Among females, 22.1% and 15.6% MTB positive samples was detected using GeneXpert and microscopy, respectively (Figure 1 & 2) (Table 1).
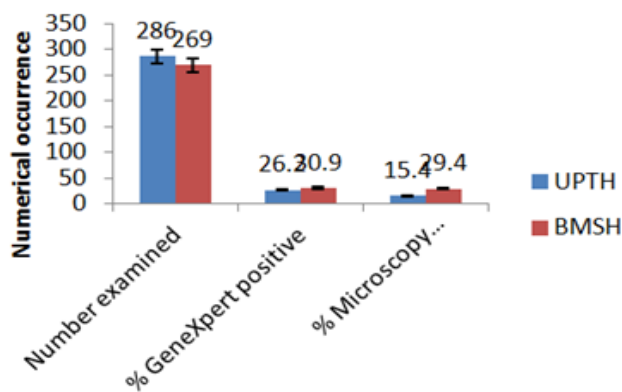

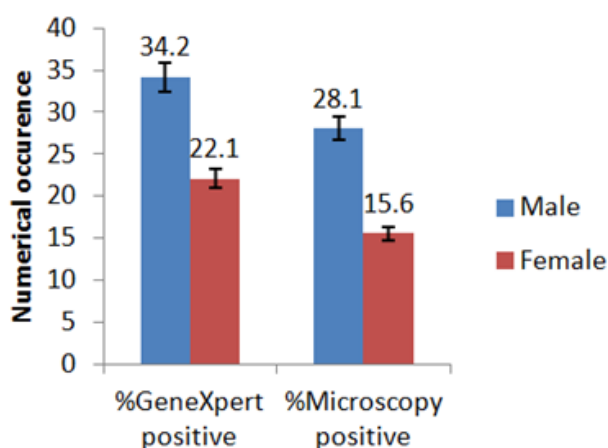**Figure 1** Percentage detection of MTB using GenXpert and Microscopy

Figure 2 Percentage detection of both methods based on gender

Table 1 Percentage detection of MTB by both methods based on age

Age group	Number of samples	%GeneXpert positive	% Microscopy positive
≤10	21	9.5	4.8
19-Nov	45	17.8	15.6
20-29	85	32.9	24.7
30-39	171	33.3	25.1
40-49	122	29.5	24.6
50-59	65	23.1	18.5
≥60	44	26.1	19.6

Discussion

Diagnosis of TB for several years had been through microscopy, however in recent times due to developments in molecular biology, GeneXpert assay has been used in the detection of MTB. Previous studies have, however, shown that the conventional methods for the detection of MTB in sputa have low sensitivity.[7] Although, research has proved that GeneXpert provides quick results in MTB detection, the extraction procedures as well as PCR inhibitors present in some of the samples serve as limitations to its use.[9] In South Africa a diagnostic sub-study of a tuberculosis prevalence survey was conducted in gold mining companies. Sputum samples were collected and tested using microscopy, liquid culture and GeneXpert. Out of 6893 participants 187 (2.7%) were positive for *Mycobacterium tuberculosis* in culture, 144 (2.1%) were positive by GeneXpert and 91(1.3%) were positive for acid fast bacilli by microscopy. Sensitivity, specificity, Positive predictive value (PV) and negative predictive value (NPV) for detection of *Mycobacterium tuberculosis* by GeneXpert were 62.6%, 99.6%, 81.3%, and 98.9; however, agreement between GeneXpert and culture were 98.5%. Sensitivity of microscopy was 17.6%. When individuals with a history of tuberculosis treatment were excluded from the analysis, GeneXpert specificity was 99.8% and positive predictive value (PPV) was 90.6% for detection of *Mycobacterium tuberculosis*.[20]

A multi-central study was conducted in Peru, Azerbaijan, South Africa, and India to assess the performance of GeneXpert assay in 730 patients with suspected pulmonary tuberculosis. Among culture-positive patients, a single, direct MTB/RIF (Mycobacterium tuberculosis/Rifampicin resistance) test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without TB (99.2%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 % and a third by 5.1 percentage points, to a total of 90.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampicin-resistant bacteria and 504 of 514 (98.1%) with rifampicin-sensitive bacteria. Sequencing resolved all but two cases in favor of the GeneXpert assay.[21] Nonetheless, an assessment by WHO Nigeria has shown that GeneXpert technology provides an opportunity for setting up routine drug resistance surveillance system in the country. Programme implementation data from 22 GeneXpert laboratories for period between September 2011 and December 2013

Evidence-based comparison of molecular-genexpert and microscopic method in the diagnosis of mycobacterium tuberculosis among subjects in the limited resource setting of Niger delta

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101

have already been analyzed by Mustapha et al. (2014) to evaluate outcomes and identify challenges and opportunities for strengthening tuberculosis detection in Nigeria.[22]

Nevertheless, from their analysis, a total of 12,249 patients received a single GeneXpert test at 10 secondary, 10 tertiary and 2 private health facilities over 25 months. The tests were valid (ability to detect the presence or absence MTB) in 10,948 patients, and 3669/10948 (33.5%) were positive for Mycobacterium tuberculosis. In 815/3669 (22.2%) of the *Mycobacterium tuberculosis* cases, the bacteria were resistant to rifampicin. Rifampicin resistance was indeterminate in 509/12249 (4.2%) while the test failed in 792/12249 (6.5%). The conclusion was that the GeneXpert MTB/RIF programme implemented in Nigeria targets specific risk groups with a high number of cases detected.[22] Furthermore, in Abeokuta, Nigeria, Oluwaseun (2012) collected a total of five hundred and four (504) sputum samples by deep cough from TB suspects who registered for the first time at public health centres in Abeokuta. The researchers examined the sputum samples using the Zeihl-Neelsen staining method and GeneXpert assay and compared their results to composite reference standard Lowenstein Jensen culture (LJ) culture. The GeneXpert assay accurately detected 310 previously undiagnosed TB cases, resulting in sensitivity, specificity, positive predictive value and negative predictive value of 98.4% (95% CI 97.5-99.4%), 95.9% (95% CI 94.8-98.5%), 98.8% (95% CI 92.6-99.2%) and 94.0% (95% CI 88.5-97.2%) respectively. For rifampicin resistance detection, sensitivity of GeneXpert was 97.2% (95% CI 96.8-98.7%); Specificity was 99.6% (95% CI 92.6-99.8%); positive predictive value was 95.2% (95%CI 92.6-97.8%) and negative predictive value was 99.8% (95% CI 88.5-99.9%). Though the case of rifampicin resistance strain was not considered in this present work, the essence of mentioning it is to provide an evidence-based analysis of the sensitivity and specificity of GenXpert method when compared side by side with other methods such as Microscopy. Nevertheless, their conclusion was that GeneXpert performed well for TB diagnosis and rifampicin (RIF) resistance and outperformed smear microscopy.[23] This study however showed that GeneXpert was much more effective in the detection of MTB when compared with microscopy.

This present study recorded a 28.5% MTB detection by GeneXpert compared with the 22.2% detected by microscopy. This is in consonance with the study by Zeka *et al.* and Ndubuisi *et al* who recorded 20.2% and 25.9% MTB prevalence using GeneXpert assay.[24,25] Hosne et al. however recorded a 67% rate in Congo which is more than that recorded in this study.[26] In contrast to these, Haider et al. recorded a 6.4% MTB detection rate using GeneXpert which is much lower than that observed in this study.[27] However, the reasons for above variations is not very clear per se, although, this could be linked to difference in sample size, experimental design strategy and level of expertise and mastery of skills deployed during the sample collection and laboratory practical work outcome during experimental. Nevertheless, it will be evident to state very clearly that four (4) cases of positive cases identified by Microscopy in this study, was reported as negative by GeneXpert; however, the reason is still obscure. This could possibly be aligned to incorrect operation or improper calibration of the machine before use However; all the positives samples detected by GeneXpert were also detected by microscopy. However the importance of enhancing the diagnostic capabilities in our local communities cannot be over- emphasis given the visible absence of functional health care facilities and critical lack of trained and seasoned expertise in this direction to generate much

needed diagnostic information's that would obviously enhance the welfare and quick recovery of patients in good time.[28,29]

Conclusion

Several studies on the use of GeneXpert assay for MTB detection have been done by various researchers, to determine the efficacy of this method. However, this present evidence based study has further demonstrated that GeneXpert is more robust in terms of specificity and sensitivity in the prompt diagnosis of *Mycobacterium tuberculosis,* especially in endemic regions. But one critical setback that will promote non-accessibility of this method is its cost, especially in resource-limited settings like in the Niger Delta communities and Africa in general, where poverty remains a palpable challenge among citizens and health care providers. Nonetheless, it is strongly recommended that the use of both methods in synergy will certainly improve patients' outcome, even as we will continue to emphasize on the importance of the microscopy method especially in the remote Health Care Centre, in our villages, given its ability to detect MTB in some samples in which GeneXpert was unable to, as well as its relative cost advantage over GeneXpert methods.

Acknowledgments

Conflicts of interest

The authors declare no conflict of interest.

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Evidence-based comparison of molecular-genexpert and microscopic method in the diagnosis of mycobacterium tuberculosis among subjects in the limited resource setting of Niger delta

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