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Research Article

Genexpert MTB/RIF Diagnostic Yield of *Mycobacterium tuberculosis* and Rifampicin Resistance in Uyo, Nigeria

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Abstract

Tuberculosis (TB) is a public health problem worldwide, with the highest mortality and methods used for the laboratory diagnosis of tuberculosis are continually evolving in order to achieve more rapid, less expensive, and accurate results. Though acid-fast staining and culture for mycobacteria remain at the core of any diagnostic algorithm, a 2-hour identification system for *Mycobacterium tuberculosis* is describe here. This system has significantly increased sensitivity compared to conventional smear microscopy and provides results within a matter of hours compared to weeks for solid culture, which is the current gold standard. This study assessed the diagnostic accuracy of GeneXpert MTB/RIF assay in the diagnosis of TB and MDR-TB in Uyo. Acid fast bacilli test and genexpert assay were used to test one hundred and forty sputum specimens at the TB laboratory, St, Luke's Hospital, Uyo, Akwa Ibom State. The results showed that GeneXpert had a positivity rate of 22.9% (32/140), which was significantly higher than that AFB smear (19.3%, 27/140). Sputum quantity and type were both significantly associated ($p<0.05$) with MTB positivity in Xpert MTB/RIF results. Genexpert MTB/RIF detected 1(3.1%) case of rifampicin resistance (also termed MDR-TB) among 32 *M. tuberculosis* positive samples. In conclusion, the study data provides important hints to formulate an appropriate diagnostic algorithm for pulmonary tuberculosis based on the appearance of sputum samples and also demonstrates that GeneXpert can be used as an initial diagnostic test for tuberculosis detection and rifampicin resistance in patients suspected of having tuberculosis.

Keywords: Tuberculosis; Modified Petroff's method; Direct Smear; Concentrated smear microscopy; Sputum smear microscopy

Introduction

Tuberculosis (TB) is still a substantial public health problem worldwide because of the high mortality associated with this disease. A major challenge to the reduction of the global burden of tuberculosis (TB) [1] has been multi drug resistance, has a significant impact on the effectiveness of antimicrobial agents, hence, resulting in prolonged time of infection in patient [2] and the poor sensitivity and timeliness of conventional diagnostic tests, such as smear microscopy and mycobacterial culture [3]. The control of TB involves early diagnosis and early treatment and in recent time, molecular diagnostic techniques have been developed to address this issue [4]. These include line probe assay (LPAs), PCR based RD-typing, spoligotyping, real time PCR and Genexpert MTB/RIF (Xpert MTB/RIF) [5]. Conventional culture-based drug susceptibility testing is time consuming, which brings about prolonged diagnosis and this negatively impacts on the transmission of TB or the drug-resistant strain in the community [6]. The Genexpert which was endorsed by the WHO in December 2010 [7], is an automated technology that rapidly and simultaneously detects *Mycobacterium tuberculosis* and Rifampin (RIF) resistance mutation in the *rpoB* gene within 2 h [8,9]. The Xpert assay procedure for the detection of *M. tuberculosis* from sputum has been well described [8,10]. In brief, the sputum sample is mixed with a "Sample Reagent" (SR) that is used to liquefy the sample, reduce biohazard, and inactivate PCR inhibitors. Two millilitres of the mixture are placed into the Xpert cartridge, and the cartridge is inserted into the Genexpert instrument, where fully automated PCR is completed to detect both *M. tuberculosis* and RIF resistance [8,10]. The Xpert MTB/RIF can be performed on either raw, unprocessed sputa or concentrated sputum pellets obtained after the sputum is liquefied and decontaminated with N-acetyl cysteine-NaOH, followed by centrifugation [3]. In this study, we evaluated the diagnostic performance of Genexpert and Smear AFB in the diagnosis of MTB and RIF resistance.

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Materials and Methods

Study area and design

The study area was Uyo, Akwa Ibom State. Uyo is located in the Southern part of Nigeria. The study was a cross-sectional study conducted among suspects and patients of MTB infection attending TB Clinic in St, Luke's Hospital, Anua, Uyo, from May 2019 to August

2019. Study participants were recruited based on the following criteria.

Study participants

Individuals of all ages who were clinically assessed by a physician as suspected TB patients were included in the study. Patient's information was collated from hospital records following approval from appropriate authority.

Sample collection

Sputum samples were collected at an interval of 24 hours from 140 participants. Samples were labeled, and all microbiological testing for the study were carried out in the TB laboratory, St, Luke's Hospital, Uyo, Akwa Ibom State.

Microbiological analysis

Acid-fast microscopy: A drop of raw sputum was placed on a grease free glass slide and evenly spread, dried, and heat fixed. Thereafter, Ziehl-Neelson staining was done according to previously described methods [11].

Genexpert: Genexpert test was performed according to manufacturer's instruction. Briefly, with the aid of a sterile pipette, 2 ml of Genexpert reagent was added to 1 ml of sputum sample and incubated at room temperature for 15 min. The mixture was agitated twice at 5 min interval. The liquefied mixture was transferred into the Genexpert cartridge using a sterile pipette and loaded into Genexpert instrument. Results were available within 2 h.

Statistical analysis

Data obtained were cross tabulated and Chi square test was used to find the significant differences between two methods at 95% confidence interval. A p-value less than 0.05 was considered statistically significant.

Ethics statement

Ethical approval for this study was obtained from the Akwa Ibom State Ministry of Health, Akwa Ibom State, Nigeria with Ref: MH/PRS/99/Vol.V/631. All clinical isolates were collected for routine diagnosis by the hospital. No patient information was used in this study; informed consent from participants was not required.

Results

Classification of the study population with their characteristics

A total of 140 individuals' submitted sputum samples comprising of 71(50.7%) male and 69(49.3%) female which were classified into different groups according to demographic and clinical characteristics (Table 1). The highest number of participants 37(26.4%) was seen in age group 20-29 and 30-39 years each, followed by age group 40-49 years (21.4%), 50-59 and >60 years (11.4%) each, 10-19 years (2.1%), while the lowest percentage was seen in age group 1-9 years (0.7%). All participants had their HIV status known and 44(31.4%) of the total population were HIV positives while 96(68.6%) were HIV negatives (Table 1).

Of the total 140 sputum samples submitted, majority of samples were mucoid 126(90%), with salivary, blood-stained and purulent being less common sample types with 8(5.7%), 4(2.9%) and 2(1.4%) respectively.

Detection of MTB by Smear AFB and GeneXpert Techniques according to their Characteristics

The microbiological characteristics of all 140 sputum samples subjected to smear AFB microscopy and GeneXpert MTB/RIF assay are shown in Table 2. GeneXpert assay had 32 (22.9%) MTB positive sputum samples while smear AFB had 27 (19.3%) positive samples. Seven (5%) sputum samples subjected to GeneXpert MTB/RIF assay were invalid which were interpreted by the machine as "error".

Among 71 males that submitted sputum samples, Genexpert had highest number of MTB detection with 20(28.2%) while AFB was 15(21.1). Out of the 69 females, MTB detection by Smear AFB and Genexpert was 12(17.4%) each (Table 1).

In the study, it was observed that age range 30-39 had the highest MTB detection rate with 10(27%) to Genexpert assay and 8(21.6) by Smear AFB method. Age range 10-19 had the lowest MTB detection rate with 1(33.3%) each to Genexpert assay and Smear AFB method (Table 1).

Among 44 HIV positive individuals, 6(13.6%) samples each were MTB positive for the two methods (Smear AFB and Genexpert). In 96 HIV negative individuals, 26(27.1%) samples were MTB positives by Genexpert, and 21(21.9) samples were positive by Smear AFB (Table 1).

Detection of MTB by genexpert according to sputum quantity

As a strategy to improve the sensitivity of nucleic acid-based testing (GeneXpert Assay), the volumes of sputum 1, 3 and 5mL were used for the analysis. Of the 140 sputa analyzed, 1 (3.1%) MTB positivity, 7 (6.9%) MTB negative, 7 (100%) error (invalid/failed test) was recorded for 1ml sputa. Twenty eight (87.5%) MTB positivity, 89 (88.1%) MTB negative with no failed test (error) was recorded for 3ml sputa. Three (9.4%) MTB positivity, 5 (1%) MTB negative with no failed test (error) was recorded for 5 ml sputa. Sputum volume was significantly related ($p<0.05$) to MTB detection by Xpert MTB/RIF assay (Table 3).

Detection of MTB using smear AFB and GeneXpert Techniques according to Sputum Macroscopy

Mucoid sputum samples had a substantially higher proportion of MTB positives by Xpert and Smear AFB, 26(81.2%) and 25(80.6%) respectively, followed by salivary with 4(12.5%) by Xpert and 0 by AFB and blood stained with 2(7.4%) by AFB, 2(6.2%) by Xpert. There was no significant relationship between sputum type and MTB yield by AFB compared to Xpert that showed a significant relationship between sputum type and MTB yield with $p<0.005$ (Table 4).

Detection of MDR-TB by GeneXpert MTB/RIF Assay

Genexpert MTB/RIF detected 1(0.7%) case of *Mycobacterium tuberculosis* which was rifampicin resistant (also termed MDR-TB) among 31 *M. tuberculosis* positive samples (Table 2).

Discussion

Early diagnosis of tuberculosis is essential for initiating an effective treatment regimen and preventing its transmission in the community [12]. Recent advances in diagnostic technologies emphasize the role of molecular diagnostics for detecting bacteria from clinical specimens with acceptable turnaround time [13]. A highly sensitive and specific tuberculosis diagnostic test would contribute immensely to achieve the 90% reduction in tuberculosis incidence by 2035 as established by the End-TB strategy [14]. Commonly used techniques for detecting MTB have low sensitivity in clinical specimens [15,16]. Molecular techniques, such as Genexpert systems, have changed the field of diagnosis of TB. These tests contain high-sensitivity and

Table 1: Baseline characteristics of patients and their diagnostic results.

Characteristics		GeneXpert			Smear AFB	
		Error	MTB positive	MTB negative	Positive	Negative
	n=140(%)	n=7(%)	n=32(%)	n=101(%)	n=27(%)	n=113(%)
Demographic characteristics						
Gender						
Male	71(50.7)	5(7.0)	20(28.2)	46(64.8)	15(21.1)	56(78.9)
Female	69(49.3)	2(2.9)	12(17.4)	55(79.7)	12(17.4)	57(82.6)
Age						
1-9	1(0.7)	0(0)	0(0)	1(100)	0(0)	1(100)
10-19	3(2.1)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	2(66.7)
20-29	37(26.4)	1(2.7)	9(24.3)	27(73)	7(18.9)	30(81.1)
30-39	37(26.4)	1(2.7)	10(27.0)	26(70.3)	8(21.6)	29(78.4)
40-49	30(21.4)	1(3.3)	4(13.3)	25(83.3)	3(10)	27(90)
50-59	16(11.4)	1(6.3)	4(25)	11(68.8)	4(25)	12(75)
60+	16(11.4)	2(12.5)	4(25)	10(62.5)	4(25)	12(75)
Clinical characteristics						
HIV Status						
Positive	44(31.4)	2(4.5)	6(13.6)	36(81.8)	6(13.6)	38(86.4)
Negative	96(68.6)	5(5.2)	26(27.1)	65(67.7)	21(21.9)	75(78.1)

specificity results. In December 2010, the World Health Organization approved the Xpert MTB/RIF Diagnostic Test for rapid diagnosis of TB and MDR-TB [16]. It had been emphasized that Xpert can be used as an initial diagnostic test for tuberculosis detection and rifampicin resistance in patients suspected of having tuberculosis, MDR-TB or HIV associated tuberculosis [17]. To ensure reliable, high quality laboratory services, quality assurance of sputum microscopy is essential. Sputum specimens are among the most frequently rejected by microbiology laboratories because of a failure to meet minimum standards set by quality control [18]. Macroscopic quality has long been emphasized in guidelines on the use of smear microscopy in TB evaluation. Despite this emphasis, some works demonstrated substantially higher sensitivity with purulent or bloody sputum as compared with mucoid or salivary sputum [19].

In this study, it was observed that Xpert assay is actually dependant on the quality of sputum. This can be seen in the seven "invalid/error" results by Genexpert in (Table 4). However, our finding is different from that done by Meyer et al. [20] which has it that salivary sputum does not have lower but perhaps higher diagnostic yield when testing for TB with Xpert. Thus, the conventional assumptions that salivary sputum is of lower quality and bloody sputum of higher quality for smear microscopy do not apply to samples tested with Xpert [20]. Therefore, the low rate of detected MTB from salivary sputum may be important for explaining the "error" and no detection rate of MTB using Xpert. It is interesting to explore the potential reason for such extraordinary situation. Reasons being that the Z-N smear method requires 5×10^3 bacilli/ml to 1×10^4 bacilli/ml of specimen to generate a positive result. However, the Genexpert assay only requires 131 bacilli/ml of specimen [21].

Assessing the quantity of sputum submitted for Xpert MTB/RIF test suggests that this strategy could increase MTB positivity yield, which is important because a single Xpert test on lesser volume sputum may miss a substantial MTB positivity due to an error result by Xpert MTB/RIF assay. Boehme et al. [22] and Luetkemeyer et al. [23] is in acceptance with assessing sputum volumes for Xpert test because Xpert test on standard volume sputum could potentially increase the yield of Xpert testing in sputum-smear-negative TB disease. However, in the present study, the volume of sputum was significantly associated ($p<0.05$) with MTB positivity by Xpert MTB/RIF assay. The result suggests that despite a lesser minimum required volume of sputum, especially for Xpert MTB/RIF testing, suboptimal volume of sputum affects MTB positivity. This finding is consistent with previous studies by Maurici-da-Silva et al. and Zimba et al. [24, 25] which show that sputum volume of 2 ml to <3 ml was predictive for a positive result but on the other hand, sputum volume 1 ml to <2 ml was less predictive of a positive Xpert MTB/RIF result.

In this study, sputum samples were subjected to Xpert MTB/RIF and smear AFB method, the MTB detection rate was 22.9% by GeneXpert MTB/RIF, and this rate was higher than 19.3% by a smear AFB. This Xpert MTB detection rate is comparable to other studies by Ndubuisi et al. [17], Kakoma et al. [26], Tang et al. [27], Reechaipichitkul et al. [28] and Shi et al. [29] with 25.9%, 63.3%, 36.6%, 84% and 45.9% respectively which were higher than other techniques used. Xpert may also be valuable as an add-on test following microscopy for patients who have previously been found to be smear negative [30]. The performance of smear microscopy with Xpert MTB/RIF assay in MTB detection were compared and smear microscopy detected 27 (21.4%) MTB cases whereas Xpert MTB/RIF test correctly detected all 27 positive cases from smear microscopy with additional 5 positives among 108 smear negative results. Thus, making Xpert MTB/RIF outperform smear microscopy and establishing a significant proportion of presumptive pulmonary tuberculosis cases with smear negative tuberculosis. This is compatible with a study by Ndubuisi et al. [17] where ZN microscopy detected 15.8% positives while 25.9% positivity was with Xpert MTB/RIF. Same with another study by Darwish et al. [31], where ZN microscopy detected 15.8% positives while 25.9% positivity was with Xpert MTB/RIF. Same with another study by Darwish et al, (31) (2013), where positivity rate by ZN microscopy was 77% and 82.5% by Xpert MTB/RIF. Also, in agreement, studies by Geleta et al (32) (2015) and Chang et al, (33) (2012) reported smear microscopy to have detected only 9.3% (21/227) cases whereas Xpert MTB/RIF detected 16.7% (38/227) cases.

As shown in Table 1, the most affected age category with PTB was >20 to ≤ 39 years (26.4%), followed by >40 to ≤ 49 years (21.4%). This is comparable to a study by Shi et al. [29] with 34.66% for >25 to ≤ 44 as the most affected age range. The economically productive age groups are primarily affected and thus impacting the Akwa Ibom Society in terms of loss of economic productivity due to absenteeism, loss of potential tax revenue, loss of trained human resources and ever-rising health care costs. The age preponderance to PTB could also be due to the fact that these groups are sexually active, therefore encountering sexual partners in whom both TB and HIV are both prevalent [34].

Table 2: MTB positivity according to methods.

Methods	No.	%
Smear Results		
Positive	27	19.3
Negative	113	80.7
GeneXpert Results		
Error	7	5
MTB Detected		
Positive	32	22.9
Negative	101	72.1
RIF Detected		
Positive	1	0.7
Negative	139	99.3

Table 3: Relationship between specimen quantity and genexpert positivity.

Results		Sputum Quantity				p-value
		Total	1 ml (n=15)	3 ml (n=117)	5 ml (n=8)	
GeneXpert	Positive	32	1(3.1%)	28(87.5%)	3(9.4%)	0.000
	Negative	101	7(6.9%)	89(88.1%)	5(1%)	
	Error	7	7(100%)	0	0	

Of the 32(22.9%) Xpert MTB/RIF MTB positive cases, rifampicin resistance was detected in 1(3.1%) patient which served as a genotypic MDR resistance. This rate is lower than rates in studies by Gidado et al. [35] and Ndubuisi et al. [17], where rifampicin resistance was detected in 16(6.5%) out of 245 patients and 12(3.1%) out of 389 patients respectively. This is could be due to the low sample size. However, it is worrisome as MDR-TB spread in the community could be on-going. The rate of MDR-TB might be due to poor patient management, non-adherence to the prescribed regimen, irregular supply of drugs, poor quality of drugs and poor national TB control programme. The study from Ethiopia showed similar rate of MDR-TB ranged from 1.1% - 7.7% [36]. However, studies by Tilako et al. [37] conducted at the North Eastern part of Nigeria and by Ndubuisi et al. [17] conducted at Nnewi, Nigeria, showed the rate of MDR-TB was 7.33% in the survey carried out among three hundred (300) suspects and 3.1% respectively. Hence, it is urgent to strengthen capacity to perform Xpert MTB/RIF (genotypic) in order to prevent transmission of MDR-TB in the community.

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Table 4: Relationship between specimen macroscopy and mtb positivity.

Results		Total	Macroscopy				p-value
			Salivary (n=8)	Mucoid (n=126)	Bloodstained (n=4)	Purulent (n=2)	
AFBSmear	Positive	27	0	25(92.6%)	2(7.4%)	0	0.426
	Negative	113	8(7.1%)	101(89.4%)	2(1.8%)	2(1.8%)	
Xpert	Positive	32	3(9.4%)	26(81.2%)	2(6.2%)	0	0
	Negative	101	2(2%)	98(98%)	1(1%)	0	
	Error	7	3(42.9%)	1(14.3%)	1(14.3%)	2(28.6%)	

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