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Comparative Evaluation of Genexpert MTB/RIF with AFB Smear Microscopy Methods in the Diagnosis of Tuberculosis

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Abstract
In recent times, emphasis has shifted from older phenotypically and biochemical methods of diagnosis to molecular methods especially Polymerase Chain Reaction (PCR) technique. This study aims at assessing the performance of Ziehl-Neelson (ZN) acid fast bacilli (AFB) smear microscopy in comparison with GeneXpert *Mycobacterium tuberculosis*/rifampicin assay (MTB Rif assay), a real time PCR method in the diagnosis of *Mycbacterium tuberculosis* (MTB) and to observe the diagnostic value of AFB smear at a tertiary hospital (University of Nigeria Teaching Hospital, Enugu) in Nigeria, a resource limited setting.Bacteriologic diagnosis and identification of MTB was made using ZN AFB smear microscopy and Cepheid GeneXpert MTB/RIF system. This research was a descriptive study based on retrospective data from a total of six hundred and fourteen (614) samples collected from presumptive TB patients who attended UNTH- ART TB laboratory. Frequency and percentages were used to summarize categorical variables while means and standard deviations were obtained for continuous variables. Associations between categorical variables were done using chi square while accuracy of the test was done using receiver operating characteristics. P value less than 0.05 were regarded as significant A total of 614 adults and paediatric patients were enrolled into the study, 381 (62%) were females while 233 (38%) were males. The mean age was 42.90yrs with standard deviation of 16.09yrs. Sixty-five (65) patients had pulmonary TB (PTB). Among the patients diagnosed with PTB, 51 (78.5%) were correctly classified positive with AFB smear result while 65 (100%) had MTB detected by Cepheid GeneXpert method We evaluated the outcome of the performance of GeneXpert with AFB smear microscopy methods in the diagnosis of PTB and observed 78% concordance between GeneXpert and smear result. Diagnostic value of ZN AFB smear method when compared to GeneXpert is significantly high and it is therefore an indispensable tool for the diagnosis of TB in Nigeria, a resource limited setting.
Keywords: Mycobacterium tuberculosis (MTB), GeneXpert MTB/RIF assay, Ziehl- Neelson Acid Fast Bacilli (ZN-AFB), Polymerase Chain Reaction (PCR). Human Immunodeficiency Virus (HIV).

Introduction
Mycobacterium tuberculosis complex (MTBC) is the transmissible bacteriological agent that is responsible for the infection of Tuberculosis (TB) and it constitutes a major public health problem in developing countries like Nigeria. Documented works show that MTBC is well characterised and consists of about nine bacterial species that cause TB in human beings and other mammals [Brosch *et al*, 2002]. It has been widely documented that a

third of the world's population has a latent infection of *M. tuberculosis* [WHO,2010]. However, not all the infections will ultimately result in tuberculosis disease. The bacilli can be dormant in the body for many years after infection and cause active TB disease if the immune system is compromised. TB still poses one of the biggest challenges worldwide and is the leading cause of avoidable deaths alongside Human Immunodeficiency Virus (HIV) especially in developing countries [unaids.org, 2014].The control of the disease is further hindered by its associated social stigma, economic and public health implications.

The TB problem is further compounded by a strong synergy with Human Immunodeficiency Virus infection (HIV). The Word Health Organization (WHO) report of 2013 estimates that of the 1.1 million TB cases co-infected with HIV, 80 % occurred in Africa and this calls for concern [Adwoa Asante-Poku et al, 2016]. Issues associated with poorly formulated policies and funding in the control of HIV in Sub-Saharan Africa are still major challenges. The impact of zoonotic TB because of the human-animal interface and some cultural practices such as open grazing, poorly regulated dairy production and high demand for meat encourages spread [Egbe *et al,* 2017]. Cattle keeping is an important means of livelihood for the Fulani tribe which constitutes the major people group in West Africa and Nigeria inclusive. Therefore, livestock keepers, abattoir workers and consumers are unknowingly at risk of zoonotic transmission of MTB through contact and consumption of infected animals and their untreated products respectively. In addition, lack of appropriate diagnostic tool and supporting infrastructure contribute a great deal in hindering successful control [Egbe *et al,* 2017]. According to WHO global TB report 2015, Africa has the most severe burden when compared to population [WHO Global TB Report, 2015].

Laboratories are essential in reaching an evident based diagnostic decision, even though they have remained the most neglected sector of the health care system especially in developing countries. The most widely used diagnostic techniques for the diagnosis of TB in Nigeria are: smear microscopy, growth of the TB bacilli in culture and more recently GeneXpert technology. Following endorsement by WHO for its use, GeneXpert was adopted by Nigeria's Federal Ministry of Health for the control of TB in 2010 especially for HIV/TB co-infection and retreatment cases. Nigeria is a country plagued by incessant power outages, inadequate infrastructures and generally poor financing and policies in the health sector. Hence, the need to advocate and encourage affordable and sustainable diagnostic system with minimal technology and infrastructure requirement.

Growth of TB Bacilli in Culture is a gold standard but is unable to provide early results and requires 4-6 weeks for ultimate diagnosis. Relatively faster radiometric: (BACTEC) and non-radiometric methods like fluorescent labelled mycobacterium growth indicator test (MGIT) methods are being used and provide results in 7-10 days. However, both methods are time consuming and their use in Nigeria is further limited by poor infrastructure, equipment and additional safety measures. Currently, there are only six zonal and functional TB culture laboratories in Nigeria.

AFB Smear Microscopy: There are two major stains used in the identification of M. tuberculosis –LED based fluorescent microscopy (more sensitive) and Ziehl Neelsen (ZN) stain AFB light microscopy (more specific). The ZN stain is a simple histological staining method used in the identification of MTB. ZN AFB smear microscopy is highly specific for MTB which appear as bright red, long, curved and beaded bacilli as against non-tuberculous mycobacteria which stain as red bacilli but with no specific morphology [Perkins *et al*, 2006]. The simplicity of this method makes it a popular method of choice for prompt diagnosis of MTB in most of the developing countries including Nigeria [Munir *et al,* 2014]. In resource-limited countries, microscopy will continue to be advocated as a means of microbiological diagnosis of TB for the foreseeable future because of its simplicity, low cost, rapidity, high specificity and poor infrastructure and technology prevalent in these settings.

GeneXpert MTB/RIF System: GeneXpert MTB/RIF system is an automated cartridge-based real time Polymerase Chain Reaction (PCR) technique used in the diagnosis of both MTB and mutations that lead to rifampicin resistance [Zeka *et al,* 2011]. Unprecedented rise in the MTB infection globally has necessitated the need for more rapid and sensitive technique in the diagnosis of the infection. Following its endorsement by WHO, GeneXpert MTB/RIF was adopted by Nigeria into the national diagnostic algorithm for the control of TB in 2010. GeneXpert was endorsed by WHO for use in TB prevalent resource limited countries as a first line diagnostic test in HIV-infected patients and for management of MDR-TB suspects [WHO Policy Statement, 2011]. Cepheid GeneXpert MTB/RIF was finally introduced in UNTH, Enugu in the year 2015 as a tool to improve diagnosis of TB and access to drug sensitivity test.

The use of GeneXpert is intended to improve diagnosis of PTB especially in HIV patients because of both its increased sensitivity, specificity and reduction in the turn-around time for diagnosis of DR-TB [Sharma *et al*, 2015].

Materials and Methods

Aim of Research: This study aims at assessing the performance of ZN AFB smear microscopy with GeneXpert MTB Rif assay (a real time PCR) method for the diagnosis of MTB and to observe the diagnostic value of AFB smear at a tertiary hospital in Nigeria, a resource.

Design and Setting of the Study

This is a descriptive study based on retrospective data from a total of six hundred and fourteen (614) samples collected from presumptive Tb patients who attended UNTH- ART TB laboratory in the years under review (2015-2016). Various samples of pulmonary and extra-pulmonary sources were used in this study, with pulmonary samples constituting the bulk. The research group comprise; females, males, people living with HIV (PLWH), HIV negative persons with symptoms of TB, retreatment cases and new cases. Both adults and paediatric suspects were involved. Bacteriologic diagnosis of MTB was made using AFB smear microscopy and Cepheid GeneXpert MTB/RIF system. Patients' data were collected in the laboratory during their visit and those whose HIV status were unknown were referred to the voluntary counselling and testing (VCT) team for retroviral screening. The study was approved by the University of Nigeria Teaching Hospital Health Ethics research committee.

(A). ZN AFB Microscopy:

Direct sputum samples were subjected to ZN AFB stain and microscopic view after the collection of two deep-cough sputum samples, on-the-spot and early morning specimens. Smears were prepared and stained using the standard method for ZN AFB smear microscopy technique.

Known positive and negative slides were included in each run of ZN staining.

A minimum of a hundred (100) microscopic fields using oil immersion technique (oil fields) were observed before reporting a smear as negative. Results were reported using the criteria of WHO/International Union of Tuberculosis and Lung Diseases (IUTALD).

(B) Cepheid GeneXpert MTB/RIF System:

Fresh specimen of deep cough sputum was collected.

Sputum samples were treated with sample reagent (SR) containing NaOH and isopropanol and analysed using the standard method for Cepheid Xpert MTB/RIF system, according to manufacturer's instruction. The Cepheid Xpert MTB/RIF assay includes internal quality controls that verify specimen processing, success of PCR and cartridge integrity. These controls are; (a) the probe check control (PCC) and (b) Sample processing control (SPC).

Statistical Analysis: Data collated was analysed with the aid of statistical package for social science (SPSS) version 22. Descriptive statistics which includes frequency and percentages were used to summarize categorical variables while means and standard deviations were obtained for continuous variables. Associations between categorical variables were done using chi square while accuracy of the test was done using receiver operating characteristics. P value less than 0.05 were regarded as significant.

Result

A total of 614 adults and paediatric patients were enrolled into the study, 381 (62%) were females while 233 (38%) were males. The mean age was 42.90yrs with standard deviation of 16.09yrs. Sixty-five (65) patients had pulmonary TB (PTB). Among the patients diagnosed with PTB, 51 (78.5%) were correctly classified positive with AFB smear result while 65 (100%) had MTB detected by Cepheid GeneXpert method. Table 1, which shows a sensitivity of 78% indicates that 51 patients out of a total of 65 with TB were correctly classified (true positives) while a specificity of 99% classified correctly 546 out of 549 patients without TB (True negatives). The positive predictive value shows that a patient who was diagnosed with TB using smear method, has 94% chance of actually having TB. The negative predictive value shows that a patient who was diagnosed without TB using smear method, has 98% chance of not actually having TB. The TB diagnostic outcome was poor among the paediatric age group and no positive result was obtained for both the smear and GeneXpert tests. More males were detected to have MTB than females, though more females presented themselves for diagnosis. Table 2 showed that he population group that was mostly affected by MTB were the economically and sexual active age (21-40 years).

Discussion:

We evaluated the outcome of the performance of GeneXpert with ZN AFB smear microscopy methods in the diagnosis of PTB and the turn-around time for the two methods. The Nigerian ministry of health has recently approved Xpert MTB/RIF as the first line in the diagnosis of TB and its use has improved the diagnosis of TB generally and HIV/TB co-infection in particular because of its increased sensitivity. However, we observed 78% concordance between Xpert and smear result. This partially agrees with the findings of Akanbi et al, 2017 in Jos University Teaching Hospital (JUTH), northern Nigeria who reported 65.8% concordance while Muhammad et al, 2015 in Pakistan TB research centre reported 67.5% positivity for smear result as against GeneXpert with positivity of 77.4%. There was no significant difference between the positive predictive values for the two techniques. Our observation about more men being infected than women agrees with findings in most part of the world but contradicts with the work of Bhembe et al, 2014 in South Africa who documented that more female (56.8%) were detected to have MTB than males (43.16%). Our findings which showed few positive MTB result

among the paediatric age group agrees with documented research findings which states that bacteriological diagnosis of Tb in children is often difficult and results are often negative even on culture, diagnosis are mostly done by clinical findings [Kanabus Annabel, 2017].

Conclusion

Smear microscopy showed significant sensitivity (78.5%), specificity (99%), negative predictive value (98%) and positive predictive value (94%) result compared to GeneXpert method. Smear microscopy should therefore be scaled up in the national algorithm of resource limited settings by implementing quality assurance programs with method modifications in order to improve sensitivity. Negative smear and GeneXpert result should not be used to exclude the diagnosis of Tb in children, clinical findings is still the method of choice in developing countries for now.

Ethical Statements

This study was approved by the University of Nigeria Teaching Hospital Health Research Ethics Committee, reference number UNTH/CSA/329/OL.5.

The authors declare that the article is original, they have no conflict of interests or plagiarism issue and the article is not being considered elsewhere.

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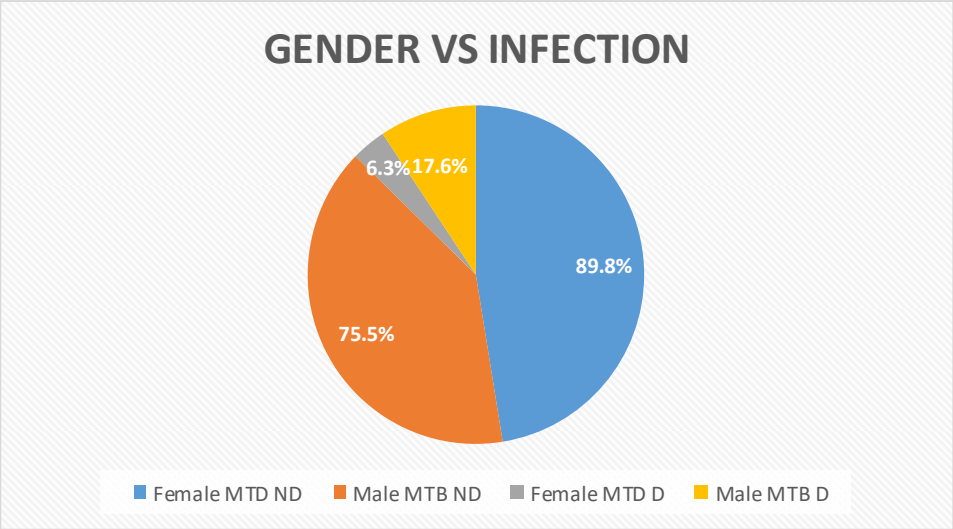
LEGEND

FIGURE 1: Result of TB Infection against Gender



TABLES

Table 1: comparison of sensitivity and specificity of AFB smear result against GeneXpert test

	GeneXpert		Sensitivity	Specificity	PPV	NPV
	Positive	**Negative**				
Smear						
Positive	51	3	78%	99%	94%	98%
Negative	14	546				

Table 2: The Prevalence of Tuberculosis Infection across Ages (Mean age ± SD = 42.90 ± 16.09)

AGE (Yrs)	MTB Detected (NO & %)	MTB Not detected (NO & %)	TOTAL (NO & %)
< 10	0 (0.0)	19	19 (3.10
11-20	4 (6.15)	27	31 (5.0)
21-30	14 (21.54)	60	74 (12.1)
31-40	20 (30.77)	147	167 (27.2)
41-50	11 (16.92)	137	148 (24.1)
51-60	11 (16.92)	73	84 (13.7)
61-70	4 (6.15)	60	64 (10.4)
>70	1 (1.54)	26	27 (4.4)
Total	65 (100)	549	614 (100)

TABLE 3: GENEXPERT MTB/RIF CARTRIGDE UTILIZATION VS AFB SMEAR RESULT

GeneXpert Result	AFB SMEAR POS (NO & % WITHIN SMEAR RESULT)	AFB SMEAR NEG (NO & % WITHIN SMEAR RESULT)	TOTAL
MTB DETECTED	51 (94.4%)	14 (2.5%)	65 (10.6%)
MTB NOT DETECTED	1 (1.9%)	517 (92.3%)	518 (84.4%)
INVALID	1 (1.9%)	18 (3.2%)	19 (3.1%)
ERROR	1 (1.9%)	11 (2.0%)	12 (2.0%)
TOTAL (NO & % WITHIN SMEAR RESULT)	54 (100.0%)	560 (100.0%)	614 (100.0%)