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Impact of GeneXpert MTB/RIF for Rapid Tuberculosis Diagnosis and Rifampicin Resistance Detection in South-Western NigeriaSaheed Opeyemi Usman¹, Tolulope Adaran³, Daniel Chinenyeike Offie¹, Femi Emmanuel Owolagba¹, Jay Osi-Samuels² & Eke Ofuche²

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Abstract

Mycobacterium tuberculosis (MTB) still remains a major public health issue, with the disease estimated to cause approximately 9 million cases annually and 1.5 million deaths. Xpert MTB/RIF is described as a game changer in tuberculosis control of tuberculosis. This study assessed the impact of using Xpert for tuberculosis diagnosis and the efficiency in terms of tuberculosis treatment initiation in selected tuberculosis clinics in South-Western Nigeria. A total of 300 consenting patients between 15 to 60 years of age attending tuberculosis clinic were recruited into this prospective cohort study from January 2015 to June 2017 and evaluated for pulmonary or extrapulmonary tuberculosis. Written consent was obtained from every participant and ethical clearance was obtained from the Ethical Review Committee of the Federal Teaching Hospital Ido Ekiti, Nigeria. Data was analysed using SPSS software, with significance fixed at $p < 0.05$. The mean age \pm SD is 37.11 ± 15.27 years. One hundred and thirty-five (45.0%) of them are males while one hundred and sixty-five (55.0%) are females. Two hundred and eighty-four patients (94.7%) were diagnosed of pulmonary tuberculosis (PTB), with two hundred and eighty-two (94.0%) being new tuberculosis patient. MTB detected and rifampicin (RIF) resistance not detected was detected in two hundred and seventy-six patients (92.0%) while MTB detected; RIF resistance detected was reported in two (0.7%) of the patients who tested smear microscopy positive at varying levels before treatment commencement. There was a significant association between site of infection and tuberculosis treatment outcome, as 233 (77.7%) of the patients were declared cured. The Xpert MTB/RIF technique has great performance for rapid diagnosis of Mycobacterium tuberculosis with 93% sensitivity especially as it helps fast track tuberculosis treatment initiation.

Keywords: Tuberculosis, Xpert, Diagnosis, Nigeria

Introduction

Despite renewed effort to end the disease, tuberculosis has remained a major cause of morbidity and mortality worldwide including among Persons Living with HIV and AIDS (PLWHA). It is estimated that there are about 9 million cases of tuberculosis annually, with approximately 1.5 million deaths. Most of these deaths are in low and middle-income countries (Cox *et al.*, 2010). Accurate and prompt diagnosis of tuberculosis and timely initiation of appropriate treatment decreases tuberculosis transmission and mortality. Tuberculosis (TB) is a chronic, infectious disease caused by bacteria generally referred to as 'Mycobacterium tuberculosis complex'. Almost every organ in the body can be affected, but involvement of the lungs accounts for more than 80% of TB cases. TB affecting the lungs is called Pulmonary Tuberculosis (PTB), while that affecting other organs is called Extra-Pulmonary TB (EPTB). The most important source of infection is an untreated Pulmonary TB (PTB) patient. When such a person coughs, spits or sneezes, tiny droplet nuclei containing the tubercle germ are released. Transmission is through inhaling these droplet nuclei. A person with Extra-Pulmonary TB (EPTB) will show symptoms depending on the affected organ and some general symptoms such as weight loss, persistent fever and night sweats. Organs that can be affected include vertebral spine, bone, joints, kidney, urinary tract, upper respiratory tract (larynx), pleural membrane of lungs and etcetera (Lienhardt *et al.*, 2001).

Until recently, the mainstay of tuberculosis was sputum smear microscopy, which is a cheap and easy to implement technique, it however has low sensitivity thus detecting only tuberculosis cases with substantial bacterial load and advanced disease.

Meanwhile, sputum culture is considered the gold standard for pulmonary tuberculosis diagnosis. It is often available only at the level of reference laboratory and often too slow to guide therapy (WHO, 2011). Thus, in order to prevent transmission and improve outcomes for individual patients, rapid diagnostic test that detects tuberculosis disease at lower bacterial burden with rapid result delivery to hasten and guide treatment is required. However, to aid this prompt tuberculosis diagnosis, the World Health Organization (WHO) in 2010 endorsed the Cepheid Xpert MTB/RIF as a first line tool for diagnosis of pulmonary tuberculosis. Xpert is not recommended for monitoring the effects of drug therapy because bacterial DNA might persist following antimicrobial therapy (Rie *et al.*, 2010). The Xpert is a real-time Polymerase Chain Reaction (PCR)-based molecular assay and simultaneously detects MTB and rifampicin resistance and has demonstrated high sensitivity as well as shorter diagnostic turn-around time (TAT) (2 hours) when compared to tuberculosis culture (WHO, 2015; Boehme *et al.*, 2010; Helb *et al.*, 2010). As at 2015, 60% of countries recommend Xpert as the initial tuberculosis test for persons with possible drug-resistant (DR) tuberculosis and 69% recommended it as the initial diagnostic test in cases of presumptive HIV-associated tuberculosis (Akanbi *et al.*, 2017).

A 2011 prospective cohort study on the evaluation of GeneXpert showed that pulmonary tuberculosis was diagnosed in 24.5% of the patients, with 93.4% of them bacteriologically confirmed (Scott *et al.*, 2011). Xpert MTB/RIF technique was reported to detect tuberculosis in 61% of smear negative and culture positive samples in a research on Xpert MTB/RIF comparison (Moussa *et al.*, 2016). In another African research, it was reported that GeneXpert was positive in 93% of confirmed tuberculosis cases, with the researchers concluding that although GeneXpert is a rapid and promising technique with good sensitivity and specificity, it cannot be used as a standalone pulmonary tuberculosis diagnostic tool (Lawn *et al.*, 2011). Some South African researchers reported that among 211 participants with a positive Xpert result, smear results were available for 95.7%, with only 28.7% of them being sputum-smear positive. Some 80.3% of the patients had their HIV status known, with 59.0% positivity while 38.9% of the patient had previous tuberculosis treatment (Cox *et al.*, 2014). The aim of the study is to assess the impact of using Xpert for tuberculosis diagnosis and the efficiency in terms of tuberculosis treatment initiation in selected tuberculosis clinics in South-Western Nigeria.

Research Hypothesis

- 1) Gender does not significantly have effect on tuberculosis treatment outcome.
- 2) Site of disease does not significantly have effect on tuberculosis treatment outcome.
- 3) HIV status does not significantly have effect on tuberculosis treatment outcome.

Methods

This study was a prospective cohort study carried out in the tuberculosis clinics at randomly selected hospitals in Ekiti State, Western Nigeria. A total of 300 patients tested for tuberculosis were included in the study from January 2015 to June 2017. The subjects were aged 15 to 60 years, attending tuberculosis clinic for evaluation for pulmonary or extra pulmonary tuberculosis and were consecutively enrolled into the study cohort following consent. At baseline, expectorated sputum of each patient was examined using smear microscopy and Xpert technique for Acid Fast Bacilli (AFB) and Mycobacterium tuberculosis (MTB) respectively. Follow up test was done via smear microscopy and patient outcome determined after six months of treatment course. Written informed consent was obtained from every participant and ethical clearance was obtained from the Ethical Review Committee of the Federal Teaching Hospital Ido Ekiti, Nigeria. Demographic and socio-economic information were obtained using a questionnaire.

Protocol

Early morning specimen was obtained, as sputum collected at this time has the highest yield. The Xpert MTB/RIF assay can be applied to sputum samples or concentrated sputum sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative (Marlowe, 2011; Boehme, 2010; Helb, 2010).

Sputum Samples

1. Label each Xpert MTB/RIF cartridge with the corresponding specimen ID.
2. Transfer 1.0 ml expectorated sputum to a conical, screw-capped tube using a sterile transfer pipette. Alternatively, the entire specimen may be processed in the original leak-proof sputum collection container.
3. Add 2.0 ml Xpert MTB/RIF Sample Reagent (2:1; v/v) to the expectorated sputum using a sterile transfer pipette.
4. Replace the lid and shake the tube vigorously 10-20 times. Alternatively, the mixture may be vortexed (30 seconds).
5. Allow the tube to stand upright for 5 min at room

- temperature.
6. Shake the tube vigorously 10-20 times. Alternatively, use a vortex (30 seconds).
7. Allow the tube to stand upright for another 10 min at room temperature.
8. Inspect the specimens: samples should be liquefied with no visible clumps of sputum.

Loading the Xpert MTB/RIF Cartridge

Note: Start the test within 30 minutes of adding the sample to the cartridge.

1. Check the labels on the Xpert MTB/RIF cartridge and the specimen ID.
2. Open the cartridge lid.

3. Using the sterile transfer pipette provided, aspirate the liquefied specimen into the transfer pipette until the meniscus is above the minimum mark and transfer the sample into the open port of the Xpert MTB/RIF cartridge.

Note: Dispense slowly to minimize the risk of aerosol formation!

Close the cartridge lid.

Smear Microscopy

The number of bacilli (AFB) seen in a smear reflects the patient's infectivity. The laboratory records the number of bacilli seen on each smear as follows:

ZN Staining

Number of bacilli seen on smear	Results reported
No AFB per 100 oil immersion field	0
1-9 AFB per 100 oil immersion field	Scanty
10-99 AFB per 100 oil immersion field	+
1-10 AFB per 1 oil immersion field (min 50 fields)	++
>10 AFB per 1 oil immersion field (min 20 fields)	+++

Statistical Analysis

Data was statistically analysed using Statistical Package for the Social Sciences (SPSS) for windows version 23.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as Mean ± Standard Deviation (SD). Frequency counts were generated for variables and statistical test of significance was performed with Chi-Square test. Significance was fixed $p \leq 0.05$.

Results

Socio-Demographic Data

A total of 300 consenting patients participated in the study. The mean age ± SD was 37.11 ± 15.27 years. One hundred and thirty-five (45.0%) of them are

males while one hundred and sixty-five (55.0%) are females. Two hundred and eighty-four patients (94.7%) were diagnosed of pulmonary tuberculosis (PTB), with two hundred and eighty-two (94.0%) being new tuberculosis patient. The smear microscopy result before treatment showed that majority of the patients had Positive (+) smear microscopy result with Xpert result before treatment resulting MTB detected; rifampicin (RIF) resistance not detected in two hundred and seventy-six patients (92.0%). Table 1 shows the socio-demographic data of respondents. Table 2 shows the patient testing and treatment outcomes. Table 3 shows the Chi square result indicating the factors influencing tuberculosis treatment outcome.

Table 1: Socio-demographic data of respondents

Variables	Frequency (%)
Age Group (years)	
< 15 years	18 (6.0)
15 - 19 years	10 (3.3)
20 - 24 years	35 (11.7)
25 - 49 years	177 (59.0)
50 years and above	60 (20.0)35 (13.7)
Gender	
Male	135 (45.0)
Female	165 (55.0)

Table 2: Patient testing and treatment outcomes

Variables	Frequency (%)
Site of disease	
Pulmonary	284 (94.7)
Extrapulmonary	16 (5.3)
Type of patient	
New tuberculosis patient	282 (94.0)
Relapse tuberculosis patient	9 (3.0)
Treatment after failure tuberculosis patient	9 (3.0)
Smear microscopy result before treatment	
Positive (+)	158 (52.7)
Positive (++)	62 (20.7)
Positive (+++)	37 (12.3)
Positive (2/100)	10 (3.3)
Positive (3/100)	20 (6.7)
Positive (4/100)	9 (3.0)
Positive (5/100)	1 (0.3)
Positive (6/100)	1 (0.3)
Positive (7/100)	1 (0.3)
Positive (9/100)	1 (0.3)
Xpert result before treatment	
Mycobacterium tuberculosis (MTB) not detected	22 (7.3)
MTB detected; rifampicin (RIF) resistance not detected	276 (92.0)
MTB detected; RIF resistance detected	2 (0.7)
Smear microscopy result at 2nd or 3rd month	
Positive (+)	4 (1.3)
Positive (++)	3 (1.0)
Positive (6/100)	1 (0.3)
Positive (8/100)	1 (0.3)
Negative	291 (97.0)
Smear microscopy result at 5th month	
Negative	262 (87.3)
Positive (+)	3 (1.0)
Positive (3/100)	3 (1.0)
Result not available	32 (10.7)

Smear microscopy result at 6th month

Negative	233 (77.7)
Positive (3/100)	1 (0.3)
Positive (4/100)	1 (0.3)
Result not available	65 (21.7)

Biopsy result suggestive of tuberculosis in patient

Yes	22 (7.3)
Not done/not available	286 (95.3)

X-ray report suggestive of tuberculosis in patient

Yes	4 (1.3)
Not done/not available	296 (98.7)

Patient HIV Status

Negative	262 (87.3)
Positive	38 (12.7)

Treatment Outcome

Cured	233 (77.7)
Treatment Default	32 (10.7)
Treatment Completed	22 (7.3)
Treatment Failed	2 (0.7)
Died	11 (3.7)

Table 3: Chi square result showing factors influencing tuberculosis treatment outcome

Variables (Hypotheses)	χ^2	Df	Critical value	Decision	p-value
Gender does not significantly have effect on tuberculosis treatment outcome	4.30	5	11.07	Accepted	0.506
Site of disease does not significantly have effect on tuberculosis treatment outcome	59.01	5	11.07	Rejected	0.001
HIV status does not significantly have effect on tuberculosis treatment outcome	9.17	5	11.07	Accepted	0.103

The null hypothesis is rejected when the test statistic is greater than the tabled value or critical value.

Discussion

This research outcome has shown that GeneXpert was positive in 92.7% of smear positive tuberculosis diagnosed patients. This is in agreement with a previous study which revealed that GeneXpert was positive in 93% of confirmed tuberculosis cases (Lawn *et al.*, 2011). Our finding is however at variance with a previous report (Scott *et al.*, 2011) which indicated that pulmonary tuberculosis was diagnosed in 24.5% of the patients, with 93.4% of them bacteriologically confirmed. Our finding is also at variance with a previous report (Moussa *et al.*, 2016) which detected tuberculosis in 61% of smear negative and culture positive samples on Xpert MTB/RIF comparison. Similarly, a South African research among 211 participants with a positive Xpert result for which smear results were available for 95.7%, indicated that only 28.7% of them being sputum-smear positive (Cox *et al.*, 2014).

The Chi Square analysis shows that gender and HIV status do not significantly affect tuberculosis treatment outcome. This is an indication that being male or even being positive for HIV does not necessarily impact the outcome of tuberculosis treatment. Meanwhile, the rejection of the hypothesis indicating that site of disease has effect on tuberculosis treatment outcome show that treatment failure tendencies were more associated with extra pulmonary tuberculosis as shown in this study.

This study demonstrates clear benefits from the provision of Xpert MTB/RIF testing for patients with presumptive tuberculosis (TB) over a diagnostic algorithm relying primarily on smear microscopy, as indicated in this study. The comparison of the two sputum detection techniques showed good sensitivity comparisons and very useful in the diagnosis of tuberculosis. Moreover, one of the principal benefits of Xpert for patients with rifampicin resistant TB as indicated in this study is more rapid treatment initiation, which is expected to reduce both early mortality and transmission. Controlling the TB epidemic in high-burden settings, particularly those with high HIV prevalence, will require the implementation of new tools and programmes that do more than just provide incremental benefit over current strategies, as implementation of Xpert for TB diagnosis in this setting has the potential to improve both programme and individual outcomes and therefore deliver the longer-term population outcomes. Thus, while Xpert is considerably costlier than smear microscopy, with implementation expected to result in substantially increased health system costs in the short to medium-term, it does

offer the chance to impact TB morbidity and mortality over the long-term especially as it is even not used in treatment follow up.

Conclusion

The Xpert MTB/RIF technique has great performance for rapid diagnosis of Mycobacterium tuberculosis with 93% sensitivity especially as it helps fast track tuberculosis treatment initiation.

Conflict of Interest

The authors declare that there is no conflict of interest.

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