Original Article

Molecular detection of sputum *Mycobacterium tuberculosis*/rifampicin resistance among presumptive pulmonary tuberculosis cases in Borno state, North-Eastern Nigeria

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Abstract

Background: Drug resistance to anti-tuberculosis (anti-TB) medication and human immunodeficiency virus (HIV) infection undermines global control of TB.

Aim: The aim of this study is to determine sputum mycobacteria/rifampicin resistance obtained from Xpert MTB/RIF molecular assay in five health facilities in Borno state.

Methods: Records of 5518 presumptive TB cases that presented for care from September 2014 to December 2017 were retrieved from TB registers in this multicentre descriptive study.

Results: A total of 5518 pulmonary TB presumptive cases, out of which 5484 were drug-sensitive TB (DSTB) and 34 drug-resistant TB (DRTB). The MTB detection rate was 19.1% and it was higher among DRTB with rate of 41.2% than DSTB of 19.0%. The prevalence of rifampicin resistance was 6.1%, with higher preponderance rate of 78.6% among DRTB cases than 5.1% among DSTB cases. Only 2566 (46.5%) had HIV counselling and testing. The MTB detection rate of 22.1% in HIV-negative patients was significantly higher than 16.5% in HIV patients, $P = 0.001$, 95% confidence interval (CI) = 2.27–8.93. Conversely, RIF resistance of 7.0% obtained in HIV patients was significantly higher than 4.8% in HIV-negative patients, $P = 0.03$, 95% CI = 0.06–4.34. Previous TB treatment was significantly associated with RIF resistance, $P = 0.000$, odd ratio = 1318.1, 95% CI = 302.1–6318.3.

Conclusion: GeneXpert is a valuable tool for the detection of both MTB and RIF resistance. It is therefore useful for both management and TB infection prevention and control. Given the observed strong association between previous exposure to anti-TB medication and RIF resistance in this report, we advocate mandatory resistance test for TB patients with previous exposure to TB medication in addition to good adherence to TB medication.

Keywords: GeneXpert MTB/RIF, medical records, *Mycobacterium tuberculosis*, North-Eastern Nigeria, resistance, rifampicin

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INTRODUCTION

Despite global tuberculosis (TB) control strategies, resistance to TB medication and human immunodeficiency virus (HIV) infection undermines and has a potential of reversing effective TB control programmes. TB is a leading cause of death due to the single infectious agent and is listed among the 10 causes of death worldwide.[1] It is the main cause of deaths related to antimicrobial resistance and the leading cause of morbidity and mortality among persons living with HIV infection.[1] Globally, in 2016, there were an estimated 10.4 million new (incident) TB cases, of which 6.2 million were men, 3.2 million were women and 1 million were children. People living with HIV accounted for 10% of the total global estimate. However, of the estimated global 10.4 million new cases, about 6.3 million new TB cases were notified to national authorities and reported to the World Health Organization (WHO).[1] This reflects a 4.1 million gap between incident and notified cases, with India, Indonesia and Nigeria accounting for almost half of this gap. [1,2] Nigeria is among the seven countries (others are India, Indonesia, China, Philippines, Pakistan and South Africa) that accounted for 64% of 2016 global new TB cases.[1-3]

In Nigeria, TB remains a major public health problem with an annual estimated 460,000 cases. Case detection of TB and treatment success rates are among the lowest of the high TB burden countries despite the National TB and Leprosy Control Programme and the adoption of the World Health Organization's strategy of Directly Observed Treatment with Short Course (DOTS) strategy.[4] Anti-TB drug resistance among TB patients in some high TB burden countries including Nigeria has not only increased in the last decade but also has progressed from multiple drug resistance (MDR) to extensive drug resistance and to extreme and total drug resistance.[5-7]

Early detection of drug resistance TB is crucial for patient management and infection control in TB-positive cases. Acid-fast staining remains the main diagnostic method in resource-limited settings despite its low sensitivity in detection of *Mycobacterium tuberculosis*.[8,9] Mycobacterial culture is the gold standard and the most sensitive method for TB diagnosis; however, its use in clinical practice is limited due to a slow turnaround time, biosafety requirements and high cost.[8,9] In 2011, the WHO introduced the wide use of Xpert MTB/RIF assay. It is a fully automated diagnostic molecular test using real-time polymerase chain reaction (PCR) technology to simultaneously detect *M. tuberculosis* and rifampicin resistance mutations in the gene encoding the beta-subunit of RNA polymerase (rpoB).[10] The Xpert assay is highly rapid, sensitive and specific in the diagnosis of both pulmonary and extrapulmonary TB.[8-11]

Since the endorsement of Xpert MTB/RIF assay by the WHO, Nigeria has incorporated it into the diagnostic algorithm for TB. This 3-year retrospective study evaluated the sputum mycobacteria yield/rifampicin resistance using Xpert MTB/RIF molecular assay in presumptive TB cases in five hospitals that performed the molecular assay within the study period in Borno State.

METHODS

Study area

The study was conducted in the GeneXpert laboratories established by the Federal Government of Nigeria through the National TB control programme in Borno State. Five GeneXpert laboratories exist in Borno state such as University of Maiduguri Teaching Hospital (UMTH, tertiary health facility), three specialist hospitals, i.e., State Specialist Hospital Maiduguri (SSHM), Mohammad Shuwa Specialist Hospital (MSSH) and Umaru Shehu Ultra-Modern Hospital (USUMH) and General Hospital (GH) Biu. The laboratories began operation in September 2014. They process sample from their host hospital and other secondary and primary health facilities including private hospitals in the State.

Study participants

Records of 5518 presumptive TB cases, with complete information in the register from September 2014 to December 2017, were abstracted for this multicentre descriptive study. Data were retrieved from the TB registers of the five hospitals analysing sputum sample using the GeneXpert molecular assay machine. Sputum samples were collected according to the standard procedure and protocol.

Detection of *Mycobacterium tuberculosis*/rifampicin resistance

Sputum samples were analysed for the presences of DNA sequences specific for MTB and RIF resistance using the GeneXpert MTB/RIF according to the manufacturer's instruction. Records of patient data generated for each test were used for this study. The GeneXpert MTB/RIF is an automated molecular test that detects DNA sequences specific for MTB and RIF resistance by PCR with fully integrated sample processing in patients suspected of drug-sensitive or multidrug-resistant pulmonary TB (PTB).

One millilitre of sputum sample was mixed with 2 ml of buffer (Cepheid AB Rontgenvagen 5 SE-171 54, Solna), to liquefy the sputum and incubate at room temperature for 10 min. Thereafter, 2 ml of the diluted sample was transferred to the cartridge (Cepheid AB Rontgenvagen

5 SE-171 54, Solna) for ultrasonic lysis of mycobacteria to release target DNA.[12] The cartridge was loaded into the GeneXpert machine (Cepheid) to proceed with the rest protocol. After 2 h, the comprehensive test result was read on computer screen. Results were automatically generated indicating if MTB was detected or not. Where MTB was detected, the GeneXpert automatically generated result indicating if the MTB was RIF resistant or not resistant.[12]

Ethical consideration

Permission to conduct this study was obtained from the Ethics and Research Committee of the five institutions and the Borno state Ministry of Health.

Data management

Data retrieved from the TB registers of the five health institution were entered into an Excel workbook and cleaned. Missing data were filled in where available and duplications removed. Records with incomplete information were excluded from the study. The data were then exported into SPSS version 16 (SPSS Inc., Chicago, IL, USA) and variables coded for analysis.

Statistical analysis

Statistical comparisons were made using the Chi-squared test of hypothesis or Fisher's exact test, where appropriate. *P* values were two-tailed and $P < 0.05$ was considered statistically significant. All analyses were done using SPSS version 16.

RESULTS

A total of 5518 PTB presumptive cases, comprising 5484 presumptive drug-sensitive TB (DSTB) (Category I) and 34 drug-resistant TB (DRTB) (Category II), were considered in this descriptive study. The overall MTB detection rate was 19.1% and it was higher among DRTB with rate of 41.2% than DSTB of 19.0%. The prevalence of rifampicin mono-resistance in MTB-detected cases was 6.1%, with higher preponderance rate of 78.6% among DRTB cases than 5.1% among DSTB cases. The MTB and rifampicin resistance detection rates are presented in Table 1. The MTB detection of 19.7% obtained from cases in tertiary facility, UMTH, was similar to 22.2%, 20.1% and 18.2% obtained from the three specialist's hospitals, i.e., SSHM, USUMH and MSSH, respectively. However, the MTB detection rate of 12% obtained from GH, i.e., GH Biu was lower than rates obtained from tertiary and specialist hospitals. The highest RIF resistance detection rate of 8.9% was obtained from UMTH and it was followed by 6.8%, 5.1%, 4.1% and 3.7% from SSHM, MSSHM, GH Biu and UMUSHM, respectively. The MTB and rifampicin resistance detection rates based on health facility are depicted in Table 2. Of the studied participants, 2566 (46.5%) had HIV counselling and testing (HCT). The MTB detection rate of 22.1% in HIV-negative patients was significantly higher than 16.5% in HIV patients, $P = 0.001$, 95% confidence interval (CI) = 2.27 8.93. Conversely, RIF resistance of 7.0% obtained in HIV patients was significantly higher than 4.8% in HIV-negative patients, $P = 0.03$, 95% CI = 0.06–4.34. The MTB and rifampicin resistance based on HIV sero-status is presented in Table 3. Previous TB treatment (TB treatment Category II) was significantly associated with rifampicin resistance, $P = 0.000$, odds ratio = 1318.1, 95% CI = 302.1–6318.3. Table 4 shows the multivariate analysis of factors associated with RIF resistance. As shown in Figure 1, TB cases are higher in age groups between the second and fourth decades and also in males than females. The proportion of RIF resistance within defined age group is presented in Figure 2.

Table 1: Distribution of cases based on the *Mycobacterium tuberculosis* and rifampicin resistance detection

	Presumptive PTB cases		Total
	DSTB	DRTB	
MTB negative	4442	20	4462
MTB positive, RIF negative	989	3	992
MTB positive, RIF positive	43	9	52
MTB positive, RIF resistance intermediate	10	2	12
Total	5484	34	5518
Percentage MTB detected	19.0	41.2	19.1
Percentage MTB/RIF resistance	5.1	78.6	6.1

DSTB: Cases with no history of TB treatment (Category I), DRTB: Cases with history of TB treatment (Category II). MTB: *Mycobacterium tuberculosis*, RIF: Rifampicin, TB: Tuberculosis, PTB: Pulmonary TB

DISCUSSION

The WHO advocates the use of rapid molecular methods for the diagnosis of TB. Rapid tests can provide results within few hours and therefore enable identification, monitoring, prompt and appropriate therapy, decrease morbidity and mortality and interrupt transmission.[11,13] The GeneXpert MTB/RIF is a rapid assay endorsed by the WHO as a point-of-care molecular assay that can simultaneously

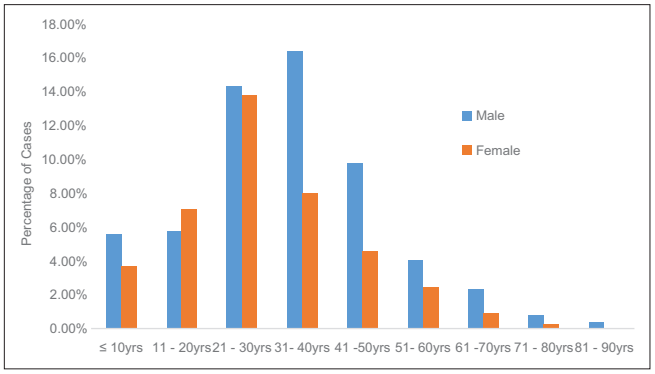

Figure 1: MTB-detected cases in defined age group stratified by gender

Table 2: Distribution of *Mycobacterium tuberculosis* and rifampicin resistance detection based on health facility

	Health facility				
	UMTH*	SSHM†	USUMH†	MSSH†	GH Biu‡
MTB negative	966	1074	1174	529	719
MTB positive, RIF -ve	216	286	284	112	94
MTB positive, RIF positive	18	19	9	4	2
MTB positive, RIF resistance intermediate	3	2	3	2	2
Total	1203	1381	1470	647	817
Percentage MTB detected	19.7	22.2	20.1	18.2	12.0
Percentage MTB/RIF resistance	8.9	6.8	3.7	5.1	4.1

*Tertiary facility, †Specialist facility, ‡GH. SSHM: State Specialist Hospital Maiduguri, GH: General hospital, UMTH: University of Maiduguri Teaching Hospital, MTB: *Mycobacterium tuberculosis*, RIF: Rifampicin, USUMH: Umaru Shehu Ultra-Modern hospital, MSSH: Mohammad Shuwa Specialist hospital

Table 3: *Mycobacterium tuberculosis* and rifampicin resistance detection based on human immunodeficiency virus sero-status

	HIV status		
	HIV positive	HIV negative	Unknown
MTB negative	652	1389	2421
MTB positive, RIF negative	120	377	495
MTB positive, RIF positive	5	16	31
MTB positive, RIF resistance intermediate	4	3	5
Total	781	1785	2952
Percentage MTB detected	16.5	22.1	17.9
Percentage MTB/RIF resistance	7.0	4.8	6.8
Percentage with known HIV status	31.9	68.1	-
Percentage with unknown HIV status	-	-	63.0

HIV: Human immunodeficiency virus, MTB: *Mycobacterium tuberculosis*, RIF: Rifampicin

Table 4: Multivariate analysis of factors associated with rifampicin resistance

Risk factor	OR	95% CI	*P*
Gender			
Male	0.316	0.052–1.926	0.212
Female	1		
Age group (years)			
<40	1.682	0.419–6.747	0.463
≥40	1		
HIV status			
HIV positive	2.817	0.550–14.420	0.241
HIV negative	1		
Occupation			
Unemployed	1.907	0.164–22.121	0.606
Employed	1		
TB Rx category			
II	1318.1	302.1–6318.3	0.000*
I	1		

*Statistically significant. CI: Confidence interval, OR: Odd ratio, HIV: Human immunodeficiency virus, TB: Tuberculosis, Rx: Treatment

establish the diagnosis of TB and detect rifampicin resistance within 2 h.[14] The Xpert assay can detect mutations in five regions of the beta-subunit of the RNA polymerase enzyme (rpoB) gene using five overlapping probes (A, B, C, D and E). Studies indicate that >90% of rifampicin (RIF) resistance *M. tuberculosis* is also resistance to isoniazid, suggesting RIF resistance as a good surrogate marker for MDR-TB.[8,9,14] Studies have reported the increasing trend

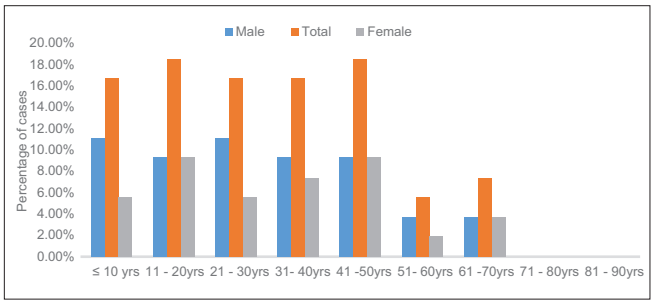

Figure 2: Proportion of rifampicin resistance in defined age group

in the incidence of MDR-TB in several high TB burden countries.[15] This unfortunate trend has the potential of reversing the gains achieved in TB prevention and control. Since the endorsement of GeneXpert by the WHO in 2010, several countries in low- and middle-income countries including Nigeria have incorporated it into their diagnostic algorithm for TB management and control programme.[14,15] This is because new molecular biology technique such as GeneXpert drug (genotypic) testing has an advantage over conventional culture and drug (phenotypic) susceptibility testing as it has a short turnaround time and can be operated by trained workers in health facilities without technical expertise.[8,14,15] Conventional culture and drug sensitivity testing, on the other hand, is time-consuming, costly and characterised by technical difficulties to control the inoculums size and stability of the compound in culture media.[14,15] There is also associated safety concerns and therefore need for safety cabinet as the slow process and cumbersome drug testing may be associated with biohazard due to the risk of disseminating *M. tuberculosis*.[8,9,14,15]

The MTB detection rate of 19.1% obtained in this study is similar to 19.0% and 18.8% reported from North-Central and South-Western Nigeria, respectively.[5,16] However, it was lower compared to earlier reports from South Africa (26%),[17] India (27.6%)[18] and Pakistan (37%).[19] Conversely, it was higher than earlier studies by other workers from high TB burden region.[20,21] The discrepancies in the proportion of confirmed *M. tuberculosis* could be due to several factors. For instance, our study considered

presumptive cases of TB; studies that returned higher MTB detection rate included identified cases of *M. tuberculosis* through microscopy to validate or make comparison with GeneXpert techniques. Other factors associated with variation besides methodology include the geographical area, setting such as hospital or community based of the study. In this study, the prevalence of RIF mono-resistance is 6.1% which is similar to 4.0%, 5.9% and 6.9% reported from studies conducted in Calabar South-South, Abeokuta South-Western and Nnewi South-Eastern Nigeria, respectively.[22-24] Our finding was, however, lower than 18.5%, 23% and 30% obtained in South-Western Nigeria.[25-27] This finding is in agreement with the wide variation in RIF resistance reported worldwide ranging from 1.7% in low TB region such as in Uruguay to 36.9% in high TB burden such as in Estonia.[28]

This is a multicentre study; we reviewed data obtained from five hospitals conducting GeneXpert test in Borno state, and the hospitals include a tertiary health facility, three specialist facilities and a GH. The high prevalence of RIF resistance from tertiary and secondary health facilities in this report may be due to referral of complicated TB or Category II TB cases at risk of drug resistance due to the past exposure to TB drug resistance. Studies conducted in high TB burden African countries also reported higher resistance rates. In Sub-Saharan Africa, pooled estimate of any DRTB prevalence among the new cases was 12.6%, and among previously treated patients, it was 27.2%.[29] This is in contrast with low prevalence of 2.1% reported from the United States, with low incidence of TB burden.[30]

Factors responsible for discrepancies in resistance studies in high TB burden countries include difference in research methodology, sample size, poor record keeping and capturing of data. In agreement with earlier studies, in this report, the past exposure to anti-TB medication was strongly associated with the risk of developing RIF resistance. Poor adherence to TB drugs in TB patients has been associated with risk of development of TB drug resistance. Under optimal treatment conditions, risk of developing resistance is decimal, at an estimate rate of 10–14 clearly indicating suboptimal treatment or poor drug resistance as a risk factor for resistance. In the present study, MTB detection rates were higher among males than females. This finding is in agreement with report by the WHO and several other studies.[31] Variation has been suggested to be due to the difference in social and health-seeking behaviours.[32] Males in our locality are often the breadwinners, working or trading in overcrowded environment, which may constitute risk of TB acquisition. Other factors responsible for vulnerability to TB in males may due to the higher incidences of smoking and alcoholism in males than females in our setting.[32]

The prevalence of MTB detection rate was higher among patients aged 20–40 years; this is in accord with several studies that reported a higher prevalence of TB among those within reproductive age group.[22] Person within reproductive age group that engages in risky sexual behaviours are at risk of both HIV infection and reactivated TB due to immunosuppression. In this study, the higher prevalence of RIF resistance *M. tuberculosis* was observed in HIV-positive cases, which is in agreement with earlier studies.[33-35] However, after adjusting for co-founders on multivariate analysis, HIV–TB co-infection was not associated with rifampicin resistance. Similar studies conducted in high TB burden countries such as Ethiopia,[36] Tanzania[37] and Brazil[38] also reported no association between drug resistance TB and HIV infections. The complex nature of both TB and HIV infections presents a challenge due to the overlapping toxicities and pill burden. Furthermore, mal-absorption due to diarrhoeal diseases associated with HIV may contribute to the development of drug resistance in the setting of HIV–TB co-infection. The Nigerian National TB Control Programme advocates universal HCT at service delivery points in health facilities nationwide. TB treatment centres, especially those under the Directly Observed Treatment with Short Course (DOTS), strategy offer free HIV testing to clients. However, only 37.0% of the patients with presumptive TB had documentation of their HIV status in this study.

CONCLUSION

The Xpert/RIF molecular assay is a valuable point-of-care facility for early detection of rifampicin resistance and therefore necessary for both management and TB infection prevention and control. The previous exposure to anti-TB medication is associated with rifampicin resistance; therefore, rational use of anti TB drugs is necessary as poor adherence can lead to anti TB drug resistance, especially in high TB burden setting. Universal access and rational use of TB medication are necessary to prevent the observed resistance to TB medication.

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Conflicts of interest
There are no conflicts of interest.

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