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Drug Resistant *Mycobacterium tuberculosis* in Benue, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author SIN designed the study. Authors SIN and IO Managed the literature searches. Authors GCE and SIN wrote the protocol author EOPN performed statistical analysis. Authors NCE, AA and FA managed the analysis of the study. Authors IO and GCE wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Tuberculosis is an important opportunistic infection in HIV/AIDS. Benue state is the highest HIV endemic state in Nigeria and investigation of tuberculosis cases and Mycobacterial resistance patterns are needed. A retrospective study with a review of reports of Mycobacterium tuberculosis and the rifampicin resistance was detected by the Cepheid GeneXpert MTB/RIF system was carried out from July, 2012 to September, 2013 in Federal Medical Centre, Makurdi and Nigeria Airforce Hospital, Makurdi. Pulmonary tuberculosis was detected in 21.5% (n=303/1407) of the total sample. Rifampicin resistance of 13.5% (31/230) and 15.1% (11/73) of the pulmonary tuberculosis positive cases was detected in Federal Medical Centre and Nigeria Airforce Base Hospital respectively, with an average prevalence of 13.9% (42/303). Re-treatment cases comprised 81% (34/42) whereas new cases constituted 19% (8/42) of all Rifampicin resistant Mycobacterium tuberculosis positive cases ($X^2 = 6.51$; $p<0.05$). Mean age was

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30 years and there was no significant statistical difference in gender. Rifampicin resistant Mycobacterium tuberculosis is prevalent in Benue state, Nigeria especially, in the young adults. Therefore, laboratory facilities for rapid diagnosis of the drug resistant M. tuberculosis should be scaled up across the country. This remains an important step to achieve maximal impact in managing drug resistance in Nigeria.

Keywords: Mycobacterium tuberculosis; rifampicin resistance; GeneXpert system; Makurdi; Nigeria.

1. INTRODUCTION

Tuberculosis is a re-emerging disease in both industrialized and developing world, essentially due to worldwide epidemics of HIV/AIDS, where it presents as an opportunistic infection. Unfortunately, the re-emergence of this disease has been fueled by the disruption of TB control programmes with accompanying TB drug resistance [1].

About one- third of the world's population has latent TB, which means people have been infected by TB bacteria, but are not ill yet with the disease and can not transmit the disease [2]. Only when the bacteria become active such as in HIV positive cases, advancing age or some medical conditions that can reduce the person's immunity do people become ill with *TB* [2,3]. In 2012, 8.6 million people fell ill with *TB* and 1.3 million died from *TB* world wide [4]. Tuberculosis is endemic in Nigeria, and is ranked by the World Health Organization (WHO) as 10th among the high burden countries for *TB* in the world, while it is the 15th among the 27 heavy burden countries for Multi-drug-resistant TB(MDR-TB) [3,5]. Multi-drug-resistant TB defined by resistance to at least the two major first line anti-tuberculosis drugs, isoniazid (INH) and rifampicin (RIF) is a growing global health problem [5,6]. According to WHO statistics 2011, Nigeria reported over 93,050 new *TB* cases, which translated into a 43% case detection rate [7]. According to the National Drug Resistant Survey of 2010, commissioned by the Federal Ministry of Health, the MDR-TB prevalence in Nigeria was 2.9%. MDR-TB, which often results from misuse or mismanagement of traditional treatment drugs, presents an increasing threat to TB control in the country. Nigeria was considered among the countries in the global progress in implementing national surveys of the prevalence of TB disease [7].

Cumulatively, only 142 confirmed cases of MDR-TB cases out of the estimated 9,000-13,000 cases have been notified to the National TB and Leprosy Control Programme (NTBLCP) between 2006 and 2011 [5]. Similarly, the emergence of MDR-TB with an estimated 2.2% among new smear positive cases and 9.4% among re-treatment cases [3], has set back some of the progress made in TB control. MDR-TB results in significantly higher mortality rates in HIV-infected patients than drug susceptible TB [8]. Family Health International (FHI 360) recently opened Nigeria's only laboratory equipped for first – and second - line drug susceptibility testing, in Lagos, filling a significant gap in the diagnosis of multidrug – resistant tuberculosis. Currently, the World Health Organization estimates at least 600,000 MDR-TB cases worldwide, particularly in China, India, South Africa, and in former Soviet Union countries [1,8]. Unfortunately, a new form of extensively drug – resistant TB (XDR-TB) is emerging, which is resistant to at least four of the core ant - TB drugs. XDR-TB involves resistance to the two most powerful anti-TB drugs, isoniazid and rifampicin, also known as multi-drug-resistance, in addition to resistance to any of the fluoroquinolones (such as moxifloxacin or ofloxacin) and to at least one of three injectable second – line drugs (amikacin, capreomycin or kanamycin). Both MDR –TB and XDR-TB take substantially

longer time to treat than the drug –susceptible TB, and require the use of second –line anti-TB drugs, which are more expensive and have more side –effects than the first –line drugs used for drug-susceptible TB [9]. Like other forms of TB, MDR-TB is spread through the air following coughing, sneezing, talking or spitting by the infectious TB patient. An immune competent person will not necessarily become sick, when infected because the immune system walls off the TB bacilli, which are protected by a thick waxy coat and can stay dormant for years. Drug resistant TB occurs when drug resistant bacilli outgrow drug susceptible bacilli [3]. Drug resistance is produced by random mutations in the drug target genes in the bacterial chromosome even before the strains are exposed the anti-TB [8]. Mechanisms for chromosomal resistance vary; rifampicin resistance is associated with the alterations in the ß-subunit of RNA polymerase (rpo ß) gene. Isoniazid resistance is associated with deletion /mutation of catalase-peroxidase gene *kat* G [8], and alterations in the *inh* A gene, which encodes an enoyl acyl reductase involved in mycolic acid biosynthesis, is associated with reduced susceptibility to INH in mycobacteria [10].

M. tuberculosis (MTB) is the most common bacterial agent responsible for TB, however, infections with *M. bovis, M. microti,* and *M. africanum* can also result in TB. *Mycobacterium spp* are all obligate aerobic and acid –fast bacterium. The cell wall has three major components: mycolic acids, cord factors and Wax-D. High lipid shield is highly conducive to the antibacterial resistance formation, protects against cationic proteins, lysozyme and oxygen radicals of phagocytosis [8].

The GeneXpert MTB/RIF is a novel automated molecular assay recently endorsed by the World Health Organization for the early diagnosis of both MTB infection and RIF resistance [11]. National TB and Leprosy Control Programme has recorded an impact of HIV/AIDS which has a prevalence rate of 27% among patients with TB in the country [3].

Nigeria having an estimated national adult prevalence of HIV of 3.6% [12] with 3.3 million people living with HIV, and represents the second largest burden of disease on the continent [13]. Benue state has a high risk for TB burden in Nigeria being the highest HIV/AIDS (12.7%) endemic state in the country [14]. However, a strong relationship existing between drug resistance TB and HIV/AIDS has been discovered by some studies [8]. Therefore, the study of resistance pattern of TB has become necessary in Benue state, Nigeria.

2. MATERIALS AND METHODS

A total of 1407 sputum samples were collected from two geographic distinct sites in Makurdi, namely Nigeria Airforce Hospital (NAF) (1159 samples) and Federal Medical Centre (FMC) (248 samples). Data from NAF Makurdi Hospital, hosting the facilities for CDC laboratory were collected from July, 2012 to September, 2013, while the data from FMC, hosting the facilities for APIN laboratory, Makurdi were collected from February to September, 2013. Presently, those are the only two centres with GeneXpert testing for MTB drug resistance in Benue state. The study included all patients suspected of pulmonary TB, treatment-naïve (new) and re-treated cases irrespective of HIV status. All ages were included in the study,with a mean age of 30 years. Out of the 1407 cases, 700 were males, and 707 were females. The study was approved by the ethics committees of the hospital.

The GenXpert MTB/RIF detects DNA sequences specific for MTB and RIF resistance by polymerase chain reaction. The Cepheid GeneXpert system is a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). It purifies and concentrates TB bacilli from one sputum samples collected from patients with chronic cough suspected of

pulmonary TB. Sample reagent was added in a 3:1 ratio to 0.5 ml of decontaminated specimen. The closed tube was manually agitated twice during a 15 – minute incubation period at room temperature before 2ml of the reagent- sample mixture was transferred to the Xpert test cartridge. The loaded cartridges were inserted into the Xpert device. The system isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR and identifies all the clinically relevant RIF resistance inducing mutations in the RNA polymerase beta (rpoß) gene in the TB genome in a real time format using fluorescent probes called molecular beacons [15]. Results were obtained from the sputum samples in 90 minutes, according to standard procedures [3,15] and very little technical training is required to operate.

Epidemiologic data and history of previous treatment were collected from the patients. The laboratory investigations were done free of charge courtesy of AIDS Prevention Initiative in Nigeria (APIN)/ PEPFAR/CDC- 45 NAF.

2.1 Statistical Analysis

The results were analysed using SPSS 11.0 statistical softwares. Chi-square (X^2) was used to compare the association between proportion and P-values <0.05 was considered significant at 95.0% confidence level.

3. RESULTS

A total of 1407 one spot sputum sample was analysed and MTB was detected in 303/1407 (21.5%) sample. Out of the 303 MTB positive samples RIF resistance was detected in 42/303(13.9%): 13.5% in NAF (31/230) and 15.1% in FMC (11/73) Table 1. The age groups 21 -30 and 31– 40 years had the highest number of RIF resistance which added to 65% at risk of having RIF resistant MTB infection Table 1. There was no statistically significant difference in gender. The RIF resistant MTB positive cases were much higher in the re-treated cases 81% (34/42) where as, new TB cases constituted 19% (8/42) ($X^2 = 6.51$; df=1; p<0.05) Table 2.

Table 1. Age distribution of patients with MTB in both FMC and NAF Hospital, Makurdi, Nigeria

	Federal Medical Center	Nigerian Air Force Hospital	
Age (years)	**MTB (RR)**	**MTB (RR)**	**Total MTB (RR)**
<1-10	-	-	-
11-20	6(1)	20(3)	26(4)
21-30	25(4)	100(12)	125(16)
31-40	30(4)	70(10)	100(14)
41-50	9(1)	30(5)	39(6)
51-60	-	10(1)	10(1)
61-70	3(1)	-	3(1)
≥71	-	-	-
Total	73(11)	230(31)	303(42)

X^2=5.72; df=1, p>0.05; MTB=Mycobacterium Tuberculosis; RR=Rifampicin Resistant Mycobacterium Tuberculosis

Eighty-two per cent (n=1159/1407) sample was from NAF hospital, Makurdi. Twenty per cent (n=230/1159) sample was MTB positive and 13.5% (n=31/230) of the MTB was RIF resistant. Forty-five per cent (n=14/31) of the RIF resistant MTB patients was male while fifty-five per cent (n=17/31) was female Table 3.

The collected samples from FMC, were 248/1407(18%) of the total samples. Of these 73/248(29%) were MTB positive and 11/73(15.1%) were RIF resistant. Five of the 11 RIF resistant MTB patients (45%) were males, while 6 (55%) were females Table 4.

Table 2. Treatment distribution of the patients with RIF resistant MTB

Age(Years)	Re-treated (%)	Treatment- naïve (%)	Total (%)
<1-10	-	-	-
11-20	-	4	4
21-30	12	3	15
31-40	12	1	13
41-50	8	-	8
51-60	1	-	1
61-70	1	-	1
≥71	-	-	-
Total	34(80.9)	8(19.1)	42(100)

X^2 = 6.51; df = 1; p<0.05

Table 3. Age and sex distribution of the RIF resistant MTB patients in NAF hospital, Makurdi

Age(Years)	Males (%)	Females (%)	Total (%)
<1-10	-	-	-
11-20	2	1	3
21-30	2	10	12
31-40	7	3	10
41-50	3	2	5
51-60	-	1	1
61-70	-	-	-
≥71	-	-	-
Total	14(45.2)	17(54.8)	31(100.0)

df = 1, p> 0.05

Table 4. Age and sex distribution of the RIF resistant TB patients in FMC, Makurdi

Age (Years)	Males (%)	Females (%)	Total (%)
<1-10	-	-	-
11-20	-	1	1
21-30	2	2	4
31-40	1	3	4
41-50	1	-	1
51-60	-	-	-
61-70	1	-	1
≥71	-	-	-
Total	5(45.4.)	6(54.6)	11(100.0)

df = 1, p>0.05

4. DISCUSSION

The study was set to determine the prevalence of MTB and to assess its RIF resistance in Benue, which is an HIV/AIDS endemic region in Nigeria in order to achieve effective medical management of the condition.

The prevalence of RIF resistance in the study was 13.9% (Retreated= 11.3%; Treatment naïve = 2.6%) in 21.5% of MTB detected cases. The mean age of patients was 30 years. There was no statistically significant difference in gender. The RIF resistance in Airforce Hospital and Federal Medical Centre were of 13.5% and 15.1% respectively. There was significant difference in infections between re-treated and treatment –naïve (new) patients in the two sites.

Other studies from different parts of Nigeria reported a RIF resistance of 11.8 to 22% [8,16]. RIF resistance reported from America, Western Pacific region and Europe were 2.1%, 4.9% and 12%, in newly diagnosed cases to 12%, 23%, and 37% in re-treated respectively [2]. Rifampicin resistance of 2.6% found among treatment-naïve (new)patients and 11.3% among re-treated cases in the present study are higher than the WHO estimated prevalence of 2.2% of cases among the notified newly diagnosed pulmonary TB cases and 9.4% among re-treatment cases [2,5]. Rifampicin resistance of 2.6% among treatment-naïve (new) patients in the present study is low compared to the 5.52% reported in another bi-centre study on genetic determinants of drug-resistant tuberculosis(using MTB DR. plus) among HIV-infected patients in Jos and Lagos, Nigeria [8]. A possible reason for the difference could be due to one or more of the following; our study was not restricted to HIV/AIDS patients, while the other study was only for HIV/AIDS patients; again the other study did not consider the re-treated cases, while our study did. The high prevalence in our study could be attributed to the fact that it was carried out at a tertiary care hospital where patients are being referred from different parts of the state.

On the other hand, a phenotypic study done in Calabar [16], south Nigeria by using drug susceptibility testing (DST) (Lowenstein-Jensen egg-based slopes) recorded no strains with mono-resistance to RIF. Resistance to two drugs (RIF and IZN) was found in 22 %, while 1% was resistant to three or four drugs [16]. The DST study in Calabar, showed that strains with mono-resistance to RIF were rarely isolated. In other words, a greater degree of cases of RIF resistance occurs along with IZN resistance. The same study [16], recorded 22% multi-drug resistance to the two drugs, RIF and IZN. The phenotypic DST methodology in Calabar study required AFB positivity as a point of entry for enrollment into the studies, which is different from the current study. Thus, the TB/HIV co-infected persons who were sputum AFB negative were excluded. Furthermore, AFB sputum smears, using the Ziehl-Neelsen stain, lack sensitivity in identifying TB cases and some cases of MTB infections could have been missed in the Calabar study. Since resistant bacteria are more likely to be less fit than sensitive bacteria [17,18,19,20], and thereby cause paucibacilliary disease, therefore the phenotypic results might have represented an underestimate of drug resistance. The genotypic analysis of rpo *ß* for RIF resistance, therefore remains an advantage.

However, a study has found that it is controversial to use the genotypic analysis of *rpo ß* for RIF resistance in evaluating the public health threat of MTB [21]. The misdiagnosing of patients as MDR-TB when they are only RIF mono-resistant would lead to inappropriate second line treatment, in a world of limited second line drugs armamentarium. Therefore, we recommend a rapid multi-resistance TB drug diagnostics at least for RIF and IZN resistance.

Meanwhile, GenXpert MTB/RIF-TB system analysis serves as a screening test with a need for further DST testing.

The age groups at risk for RIF resistance were 21 to 40 and there was no statistically significant difference in gender. A similar finding was recorded in other regions of the country [8,16,22]. There was a significant difference between re-treated (experienced) patients and treatment – naïve (new) patients in the two geographic sites. This difference might be because all the patients tested in FMC were referred by the physicians following relapses or treatment failures. On the other hand, in NAF hospital, most of the patients came without referral; the hospital embarked on recruitment campaign for patients from different facilities in the state in order to test run their new Gene XpertMTB/RIF diagnostic equipment.

A large scale multi-centre study with uniformity in methodology across the state is recommended to determine resistance in TB. Inability to carry out such a large scale multi-centre study constitutes a limitation to the present study.

5. CONCLUSION

Rifampicin resistant *Mycobacterium tuberculosis* is prevalent in Benue state, Nigeria especially, in the young adults. Therefore, laboratory facilities for rapid diagnosis of drug resistant *M. tuberculosis* should be scaled up across the country. This remains an important step to achieve maximal impact in managing drug resistance in Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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