

HUMAN MYCOBACTERIUM TUBERCULOSIS AND MULTI-DRUG RESISTANCE IN BENUE, NIGERIA

ABSTRACT

Tuberculosis is an important opportunistic infection in HIV/AIDS. Benue Nigeria is the highest HIV endemic state in the country, needs to be investigated for tuberculosis and its resistance patterns. A retrospective study with a review of reports of pulmonary *Mycobacterium tuberculosis* and its rifampicin resistance detected by the Cepheid GeneXpert MTB/RIF system from July, 2012 to September, 2013 in Federal Medical Centre, Makurdi and 45 Nigeria Airforce Hospital, Makurdi was carried out. Pulmonary *Mycobacterium tuberculosis* was detected in 21.5% (n=303/1407) of the total sample. The average prevalence of rifampicin resistance in the study was 13.9% (n=42/303). Re-treatment tuberculosis patients constituted 11.3%[n=34/303] and new tuberculosis patients 2.6%[n=8/303]($\chi^2 = 6.51$; $p < 0.05$). The mean age was 30 years and there was no significant statistical difference in gender. Rifampicin resistance which is an excellent surrogate marker for multi-drug resistant tuberculosis is prevalent in Benue state Nigeria especially, in the young adults. Therefore, laboratory facilities for rapid diagnosis of *Mycobacterium tuberculosis* and its multi-drug resistance should be scaled

up across the country. This remains an important step to achieve maximal impact in managing TB drug resistance in Nigeria.

KEY-WORDS: Mycobacterium tuberculosis, multi-drug resistance, rifampicin resistance, GeneXpert system, Makurdi, Nigeria

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.

One in three people in the world is infected with TB bacteria ². Only when the bacteria become active such as in HIV, advancing age, or some medical conditions that can reduce the person's immunity do people become ill with TB ^{2,3}. It has been estimated that about 3 million deaths are due to TB world wide every year [2]. Nigeria ranks 10th among the high burden countries for TB in the world , while it is the 15th among the 27 heavy burden countries world wide for MDR-TB ^{3,4}. Multi-drug-resistant TB (MDR-TB), defined by resistance to INH and RIF, is a growing global health problem ^{4,5} . In 2011, Nigeria detected a total of 93,050 cases of TB, which translated into a 43% case detection rate. Cumulatively, only 142 confirmed 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 ⁴. Similarly, the emergence of multi-drug

resistant TB(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 ⁶. 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 ^{1,6}. Unfortunately, new difficult – to- treat MDR –TB cases have been described, named XDR- or TDR –TB (extensively or totally drug –resistant tuberculosis, respectively)¹.

Like other forms of TB, multi drug resistance- TB is spread through the air following coughing, sneezing, talking or spitting by the infectious TB patient. A person needs only to inhale a small number of these to be infected and will not necessarily become sick because the immune system walls off the TB bacilli which is protected by a thick waxy coat, can lie dormant for years. Drug resistance 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 to anti-TB ⁶. Mechanisms for chromosomal resistance vary; Rifampicin-resistance is associated with the alterations in the β -subunit of RNA polymerase (*rpoB*) gene. Isoniazid – resistance is associated with deletion/mutation of catalase-peroxidase gene(*kat G*) & alterations in the *inh A* gene, which encodes enzyme for mycolic acid synthesis ⁶. Multi-drug resistance TB (MDR-TB) is resistance to at least the two major first line anti-tuberculosis drugs, isoniazid and rifampicin ⁴. *Mycobacterium tuberculosis* (MTB) is the most common bacterial agent responsible for TB, however, *M. bovis*, *M. microti*, *M. canettii* and *M. africanum* can also result in TB. MTB is 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 ⁶.

The GeneXpert MTB/ RIF is a novel automated molecular assay recently endorsed by the World Health Organization for the early diagnosis of both *M.tuberculosis* infection and rifampicin resistance ⁷.

National Tuberculosis & 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% ⁸ with 3.3 million people living with HIV, represents the second largest burden of disease on the continent ⁹. Being the highest HIV/AIDS(12.7%) endemic state in the country, Benue state therefore has a high risk for TB burden in Nigeria ¹⁰. A strong relationship exists between drug resistance TB and HIV/AIDS has been discovered by some studies ⁶. Therefore, the study of resistance pattern of TB has become necessary in Benue, Nigeria.

METHODS

Two geographic distinct sites were used for the study, namely 45 Nigeria Airforce Hospital, Makurdi and Federal Medical Centre, Makurdi. Data from 45 Nigeria Airforce Hospital (hosting the facilities for CDC laboratory), Makurdi were collected from July, 2012 to September, 2013 while the data from Federal Medical Centre (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 GenXpert MTB/RIF detects DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin 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 *Mycobacterium tuberculosis* bacilli from one spot 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 Rifampicin resistance inducing mutations in the RNA polymerase beta (*rpoB*) gene in the *Mycobacterium tuberculosis* genome in a real time format using fluorescent probes called molecular beacons¹¹. Results were obtained from unprocessed sputum samples in 90 minutes, according to standard procedures^{3,11} and very little technical training 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.

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

RESULTS

A total 1407 one spot sputum sample was analysed and MTB was detected in 21.5%(n=303/1407) sample. Rifampicin resistance was detected in 13.9% (n=42/303) total MTB sample. Age group 21 – 40 years was 65% at risk. There was no statistically significant difference in gender. There was a significant difference in Rifampicin resistance in the population between the two geographic sites(NAF:n= 31/230; FMC:n=11/73)($X^2 = 5.72$; df=1; $p < 0.05$)(Table 1).

Eleven per cent(n=34/303) of the rifampicin resistant TB patients had previous treatment(re-treatment) (FMC: n=11; NAF: n=23) while 2.6%(n=8/303) consists of new patients with no previous treatment(treatment-naïve) (FMC: n=0; NAF: n= 8.). There was a significant difference between treatment naïve and re-treatment individuals($X^2 = 6.51$; df=1; $p < 0.05$)(Table 2).

Eighty-two per cent (n= 1159/ 1407) sample was from 45 Airforce hospital, Makurdi. Twenty per cent (n=230/1159) sample was MTB and 13.5%(n=31/230) of the MTB was rifampicin resistant. Forty-five per cent(n=14/31) of the rifampicin resistant MTB patients was male while fifty-five per cent(n=17/31) was female (Table 3).

Eighteen per cent (n=248/1407) sample was from Federal Medical Centre, Makurdi. Twenty - nine per cent (n=73/248) was MTB and 15.1%(n=11/73)MTB was

rifampicin resistant. Forty- five per cent (n=5/11)of the rifampicin resistant MTB patients was male while 55%(n=6/11) was female (Table 4).

Table 1. Age distribution of patients with rifampicin -resistant MTB in both Federal Medical Centre and 45 Nigerian Airforce Hosp. Makurdi, Nigeria

	* FMC	NAF	
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)

$$X^2 = 5.72; df = 1 ; P < 0.05.$$

*FMC= Federal Medical Center, Makurdi;
 NAF= 45 Nigerian Airforce Base Hospital;
 MTB=*Mycobacterium Tuberculosis*;
 RR=Rifampicin Resistant *Mycobacterium Tuberculosis*

Table 2. Treatment distribution of the study population

Age(Years)	Re-treatment(%)	Treatment-naive(%)	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 Rifampicin resistant TB patients in 45 Nigerian Airforce 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 Rifampicin resistant TB patients in Federal Medical Centre, 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.

DISCUSSION

The study was set to assess Mycobacterium tuberculosis multi-drug resistance in an HIV/AIDS endemic region such as Benue, Nigeria in order to achieve effective medical management of the condition.

The prevalence of rifampicin resistance in the study was 13.9%(Retreatment= 11.3%; Treatment naïve=2.6%) in 21.5% of mycobacterium tuberculosis complex detected. The mean age of patients was 30 years. There was no significant statistical difference in gender. Two distinct geographic study sites were employed which recorded rifampicin resistance of 13.5% and 15.1% respectively. There was significant difference between re-treatment (experienced) patients and treatment –naïve (new) patients across the two geographic sites.

The prevalence of MDR-TB in the study was 13.9%. Other studies from different regions of the country report MDR-TB varying from 11.8 to 22% ^{6,12} . The data of MDR-TB from other regions of the world ranges from 2.1%, 4.9%, and 12%, in newly diagnosed cases to 12%, 23%, and 37% in re-treatment cases in the Region of the Americas, Western Pacific Region, and European Region respectively ² . Rifampicin resistance prevalence rate of 2.6% finding among treatment-naïve (new) patients and 11.3% among re-treatment cases in the present study are higher than the WHO estimated prevalence of 2.2 % of cases of MDR-TB among the notified newly diagnosed

pulmonary TB cases and 9.4% among re-treatment cases ^{2,4} . Rifampicin resistance prevalence rate of 2.6% finding among treatment-naïve (new) patients in the present study is low compared to the 5.52% recorded 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 ⁶ . A possible reason for the significant variation in the finding can be as follows; our study was not restricted to HIV/AIDS patients while the other study was only for HIV/AIDS population; again the other study did not take cognizance of the re-treatment 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 and its environ.

On the other hand, a phenotypic study done in Calabar ¹², south-south Nigeria by using Direct Sensitivity Testing (Lowenstein-Jensen (LJ) egg-based slopes) recorded no strains with mono-resistance to rifampicin. Resistance to two drugs(rifampicin and isoniazid) was found in 22 %, while 1% was resistant to both three and four drugs ¹² . The DST study in Calabar ¹² , showed that strains with mono-resistance to rifampicin very occasionally exists in isolation. In other words, a greater degree of cases of RIF resistance occurs along with isoniazid resistance. The study ¹², recorded 22% resistance rate for two drugs, rifampicin and isoniazid , which is synonymous to MDR-TB. This finding at Calabar ¹² , supported the findings of WHO global data ^{13,14} on GenXpert MTB/MDR-TB system that genotypic analysis of *rpoB* for RIF resistance is a surrogate marker for MDR-TB. According to the WHO global project data ¹⁴ , 82 % of new rifampicin resistant cases, and 87% of previously treated rifampicin resistant cases are also MDR-TB. Among high prevalence populations, the median proportion is closer to

92%¹⁴. The phenotypic DST methodology used in Calabar requires AFB positivity as a point of entry for enrollment into the studies, which is a limitation. 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 *M. tuberculosis* infections could have been missed in those other studies in Calabar. Since resistant bacteria are more likely to be less fit than sensitive bacteria^{15,16}, and thereby cause paucibacillary disease, therefore the phenotypic results might have represented an underestimate of drug resistance. The genotypic analysis of *rpoβ* for RIF resistance, therefore remains an advantage.

However, a study has found that the genotypic analysis of *rpoβ* for RIF resistance though sufficient for evaluating the public health threat of multi-drug resistant tuberculosis, remains controversial¹⁷. 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, by recommendation, a robust multi-resistance TB drug testing system that tests at least for rifampicin and isoniazid resistance and does not require AFB positivity as a point of entry for enrollment into the studies, stands an ideal development. In the interim, GenXpert MTB/RIF-TB system analysis serves as a screening test, there is a need for further DST testing.

The mean age was 30 years at risk for rifampicin resistance in our study while there was no significant statistical difference in gender. A similar finding was recorded in other regions of the country^{6,12,18}. There was a significant difference between re-treatment (experienced) patients and treatment-naïve (new) patients across the two

geographic sites. This difference might be due to the fact that all the patients tested in Federal Medical Centre, Makurdi were referred by the physicians after category two (CAT. 2) TB treatment(following relapses or treatment failures). On the other hand, in NAF hospital, Makurdi, 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 her new Gene XpertMTB/RIF diagnostic equipment. However, between these two geographically distinct study populations, a significantly higher number of rifampicin drug-resistant tuberculosis was found in NAF Hospital, Makurdi as compared to Federal Medical Centre, Makurdi. This phenomenon could be explained by the reason that a larger data from Nigeria Airforce Hospital , Makurdi were collected for a longer period (13 months) while the data from Federal Medical Centre , Makurdi were collected only for a shorter duration (7 months). The disparity in time of collecting data was owing to the difference in the period of installing the Gene Xpert/ MDR systems in the two centres.

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

CONCLUSION

In our study therefore, rifampicin drug resistant TB indicative of MDR-TB is highly prevalent in this locale. Treatment effort should be geared up with adequate provision of second line drugs. Patients who invariably are not of TB –drug resistant cases, should be given full and adequate measure of first line drug treatment, so that they will not convert into resistance. Finally, laboratory facilities for rapid MTB /MDR

diagnosis should be scaled up across the country for prompt and accurate diagnosis of *Mycobacterium tuberculosis* and its drug-resistant strains.

CONFLICT OF INTEREST

None

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