RIFAMPICIN RESISTANCE *MYCOBACTERIA TUBERCULOSIS* AND ASSOCIATED RISK FACTORS AMONG PATIENTS ATTENDING FEDERAL MEDICAL CENTRE BIRNIN KUD JIGAWA STATE.

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ABSTRACT

Background of study: The emergence and spreading of multidrug (MDR) and extensively (XDR) drug-resistant *M. tuberculosis* complex (MTBC) strains poses significant challenges to TB control and drug resistance *M. tuberculosis* is found in all countries of the world.

Aim: This study is set out to determine the pattern of Rifampicin- resistance mycobacteria tuberculosis among patients attending Federal Medical Centre Birnin Kudu, Jigawa state, Nigeria.

Methods: A cross-sectional study was conducted between April to December, 2019 at the Federal Medical Centre Birnin – Kudu (FMC, BKD), Jigawa state. Subjects presenting with any of the following symptoms were recruited: the presence of symptoms suggestive of TB like chronic cough for a period of ≥ 2 weeks, night sweats, fatigue, unexpected loss of weight, and fever. Each eligible patient (272) who signed written consent provided clinical specimens. From each patients presumptive of pulmonary TB, 4 ml of sputum sample was collected. In the case of presumptive extra-pulmonary TB, four ml of either pus, CSF samples was collected. Samples were immediately processed for Gene Xpert MTB/RIF assay. Testing for HIV were done according to the current national algorithm recommended by the Federal Ministry of Health of Nigeria. Two rapid HIV tests, HIV Determine rapid test strip and Stat-Pak were run simultaneously.

Results: A total of 272 presumptive TB or DR-TB patients participated in the study. Most 157 (57.7%) were males. The age range of participants was 9 to 80 years with mean age of 32.5 years. Majority 194 (71.3%) of participants were rural dwellers. Prevalence of HIV was 50 (18.4%) among study participants. Of the 52 *M. tuberculosis* cases, 3 (5.8%) were resistant to rifampicin, of, which all were previously treated, rural dwellers, pulmonary and presumptive DR-TB patients. Two rifampicin-resistant *M. tuberculosis* was noticed from all patients with MTB/HIV co-infection (16.7%).

Conclusion: This study showed low prevalence of rifampicin resistance tuberculosis in this environment. Previous treatment with anti-TB drugs was significantly associated with rifampicin resistance.

Key words: *M .tuberculosis*, Rifampicin resistance, Sputum, Gene Xpert.

INTRODUCTION

Tuberculosis (TB) stands as a major global health problem, ranking as the second highest cause of death from an infectious disease globally, after the human immunodeficiency virus (HIV). The World Health Organization (WHO) estimates that 10.0 million people developed TB in 2019, of whom, 13% were HIV positive individuals. Among the incident cases, 44% were from the South-East Asian and Western Pacific

Regions 18% and one quarter were from Africa. The African continent accounts for the highest rates of cases and deaths relative to population (WHO, 2019).

In 2019, WHO estimates that 1.2 million deaths occurred due to TB (251 000 of whom were HIV positive).Among these deaths 210 000 were from multidrug resistance (MDR) patients, representing 43.75% of the total incident cases of MDR-TB.TB is a major public health problem in Nigeria with about 407,000 people infected, new TB cases of 120,000 with 154,000 death from TB in 2019 (WHO, 2020. TB factsheet).

TB is a disease of poverty (Spence, *et al.* 1993). A lack of basic health services, malnutrition, social disruption, tobacco consumption and inadequate living conditions all contribute to the dissemination of TB and its impact in the community. HIV infection and Acquired Immune Deficiency Syndrome (AIDS) amongst others are the strongest risk factor for TB (WHO, 2019). The observed increase in TB incidence in sub-Saharan Africa may have resulted from several of these factors. The ability of a bacterial cell to survive the presence of a drug at a concentration that normally kills or inhibits growth is called resistance. Drug resistant TB is a particular problem because of the prolonged therapy of at least six months that makes patient compliance very difficult, frequently creating drug resistant *Mycobacterium tuberculosis* complex strains. Other factors that contribute to the development of resistance are the inadequate use of antimicrobials, low compliance and completion of treatments, together with poor TB control programs and lack of access to drugs (Sharma and Mohan, 2006).

The emergence of drug resistance is a serious threat to global efforts to control TB.

Rifampicin resistance, defined as resistance to rifampicin based on phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampicin, whether monoresistance, MDR, polydrug resistance or XDR. The emergence of HIV had a unique impact on the epidemiology of infectious diseases in general and particularly on TB. Individuals with latent *M. tuberculosis* infection who contract HIV are at risk of developing active TB at a rate of 7 to 10% per year, compared to approximately 8% per lifetime for HIV negative individuals (Selwyn, et *al.* 1992). Thus, the dissemination of the HIV infection has contributed to the expansion of TB, which is the main cause of mortality among HIV patients. In Africa, the proportion of TB cases co-infected with HIV is the highest (WHO, 2019). WHO estimated that in 2018, 64% of TB cases were co-infected with HIV in the continent, accounting for 78% of TB cases among people living with HIV worldwide. Although there are limited information on the rate of drug resistant TB in Nigeria due to lack of laboratory facilities and poor DOTs programme but key studies from private tertiary care facilities in Nigeria reported increasing trends of MDR-TB in new and re-treatment TB cases. The current estimates of MDR-TB prevalence in Nigeria is 4.8% and 9.4% among new and re-treatment TB cases respectively (WHO, 2018).There is limited documented information relating to magnitude of MDR-TB (Rif\R) and associated risks among presumptive pulmonary TB patients in Jigawa state. Therefore, this study is set out to determine the pattern of Rifampicin-resistance mycobacteria tuberculosis and associated risk factors among patients attending Federal Medical Centre birnin kudu, Jigawa state, Nigeria.

MATERIALS AND METHODS

Study design, area and period

A cross-sectional study was conducted from April, 2019 to December, 2019 at the Federal Medical Centre Birnin – Kudu (FMC, BKD), Jigawa state. Subjects resenting with any of the following symptoms were recruited: the presence of symptoms suggestive of TB like chronic cough for a period of ≥ 2 weeks, night sweats, fatigue, unexpected loss of weight, and fever.

FMC, BKD has more than 340 beds offering different specialized services. It receives patients from the catchment area and referred from different areas of Jigawa, Kano, Bauchi and Yobe states. The hospital has TB/HIV clinic as well as DOTS-TB clinics used for diagnosis and treatment of TB patients. The Gene Xpert MTB/RIF assay was conducted at FMC, BKD tuberculosis laboratory.The main variables included in the study were age, sex, residence, reason for diagnosis, treatment history, and category of presumptive DR TB and site of tuberculosis.

Ethical approval

Ethical approval was obtained from the ethical committee of Federal Medical Centre, Birnin Kudu Hospital management and informed consent from the patients before sample collection.

Sample size

There are reports of 23.0% prevalence rates of pulmonary tuberculosis infections in Northern Nigeria (Aliyu, *et al.* 2013). Considering 95% confidence level and marginal error of 5%, the sample size was determined using the formula described by Naing, e*t al.*(2006). Therefore, the total samples required is 272.

Inclusion criteria

All subjects aged 9 years to 80years attending clinic during the study period and informed consent from the patients were the inclusion criteria into the study.

Exclusion criteria

Temporary residents like visitors, unwillingness to consent and patients who had been on TB treatment for more than one week were excluded from the study.

Sample collection and laboratory procedures

Each eligible patient who signed written consent provided clinical specimens. From each patient presumptive of pulmonary TB, 4 ml of sputum sample was collected. In the case of presumptive extra-pulmonary TB, four ml of either pus, CSF samples were collected. Samples were immediately processed for Gene Xpert MTB/RIF assay. Clinical samples were diluted and decontaminated and Xpert MTB/RIF assay (Cepheid) was performed according to manufacturer's instruction. The Xpert® MTB/RIF purifies and concentrates *M. tuberculosis* bacilli from clinical samples. Genomic material isolated from the captured bacteria by sonication and subsequently amplifies the genomic DNA by polymerase chain reaction (PCR). Furthermore, the process identifies all the clinically relevant rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the *M. tuberculosis* genome in a real time format using fluorescent probescalled molecular beacons.

HIV testing

Testing for HIV was done according to the current national algorithm recommended by the Federal Ministry of Health of Nigeria. Two rapid HIV tests, HIV Determine rapid test strip and Stat-Pak were run simultaneously. Samples was tested first with Determine. Positive samples was confirmed with Stat-Pak. Discordant results were resolved using a third confirmatory testing kit, HIV-1/2 Unigold Recombinant assay. Pre and post-test HIV counseling was provided for all consenting individuals. Using a structured questionnaire data was collected by both face to face patient interviews and patients' clinical record review. The main variables included in the study were age, sex, residence, reason for diagnosis, treatment history, and category of presumptive DR TB and site of tuberculosis.

Quality assurance

Both SPC and PCC internal controls used during Gene Xpert MTB/RIF assay. The specimen were excluded from the analysis if it was an invalid sample for Xpert assay or sample error according to Cepheid package insert. All procedures were done using standard operating methods.

Data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS® 20, USA). Descriptive statistics was used to describe the study participants in relation to relevant variables. Chi-square and logistic regression analysis was computed to identify the associated factors of *M. tuberculosis* and rifampicin-resistance.

RESULTS

Patient characteristics

A total of 272 presumptive TB or DR-TB patients participated in the study. Most 157 (57.7%) were males. The age range of participants was 9 to 80 years with mean age of 32.5 year. Majority 194 (71.3%) of participants were rural dwellers. Of the total, 271 (99.6%) were presumptive for pulmonary TB while 1 (0.4%) was presumptive for extra-pulmonary TB. Prevalence of HIV was 50 (18.4%) among study participants (Table 1).

Rifampicin- resistant *M. tuberculosis*

Of the 52 *M. tuberculosis* cases, 3 (5.8%) were resistant to rifampicin, of which all were previously treated, rural dwellers, pulmonary and presumptive DR-TB patients. Two rifampicin-resistant *M. tuberculosis* was noticed from all patients with MTB/HIV co-infection (16.7%). (Table 2).

Associated risk factors

Out of the total number of 52 TB positive only 3 (5.8%) were rifampicin resistant. The distribution of Rifampicin resistant *Mycobateria* across the socio demography and the TB associated risk factor was displayed in Table 2. Only "reason for diagnosis" showed a significant ($p<0.05$) relationship with rifampicin resistance. Presumptive DR-TB has significant higher prevalence of rifampicin resistance than presumptive TB. Previous anti-TB drug treatment has a higher prevalence of rifampicin resistance, but the difference in the prevalence was statistically not significant ($p>0.05$). (Table 2)

Table 1: Socio-demographic and biological characteristics distribution across the study population

Variables	Groups	Frequency	Percentage
Age group	≤9	21	7.7
	10-19	18	6.6
	20-29	91	33.5
	30-39	72	26.5
	40-49	35	12.9
	50-59	22	8.1
	60-80	13	4.8
	Total	272	100
Sex	Female	115	42.3
	Male	157	57.7
Residence	Rural	194	71.3
	Urban	78	28.7
	Total	272	100
HIV status	Negative	222	81.6
	Positive	50	18.4
Treatment history	Previously Untreated	51	18.8
	Previously Treated	221	81.3
Tuberculosis status	Negative	220	80.9
	Positive	52	19.1
Diagnosis	Presumptive DR-TB	6	2.2
	Presumptive TB	266	97.8
RIF	Sensitive	269	98.9
	Resistant	3	1.1
Site	Extra-pulmonary	1	0.4
	Pulmonary	271	99.6

Table 2: Prevalence of Rifampicin-resistant *M. tuberculosis* in each variables among the total *M. tuberculosis* cases using gene xpert MTB/RIF assay, FMC Brinin Kudu, 2019

Characters	**No. of Sensitive (%)**	**Resistant No. (%)**	**Total N0. (%)**	**P-Value**	**OR (95%CI)**
Age (years)					
≤ 9	0 (0)	0 (0)	0 (0)	1	-
10 – 19	2 (100)	0 (0)	2 (3.8)		
20 – 29	19 (90.5	2 (9.5)	21 (40.4)		
30 – 39	17 (94.4)	1 (5.6)	18 (34.6)		
40 – 49	5 (100)	0 (0)	5 (9.6)		
50 – 59	3 (100)	0 (0)	3 (5.8)		
60 – 80	3 (100)	0 (0)	3 (5.8)		
Sex					
Male	32(94.1)	2(5.9)	34 (65.4)	0.96	1
Female	17(94.4)	1(5.6)	18 (34.6)		1.06 (0.09-12.6)
Residence					
Urban	26(100)	0(0)	26 (50.0)	0.24	
Rural	23 (88.5)	3(11.5)	26 (50.0)		
HIV infection					
Positive	10(83.3)	2(16.7)	12 (23.1)	0.11	7.80 (0.64-94.9)
Negative	39(97.5)	1(2.5)	40 (76.9)		1
Treatment history with anti – TB drugs					
Previously treated	34(91.9)	3(8.1)	37 (71.2)	0.55	
Previously untreated	15(100)	0(0)	15 (28.8)		
Reason for diagnosis					
Presumptive TB	46(100)	0(0)	46 (88.5)	<0.001	
Presumptive DR – TB	3(50.0)	3(50.0)	6 (11.5)		
Site of presumptive TB					
Pulmonary	48 (94.1)	3 (5.9)	51 (98.1)	1	
Extra – pulmonary	1 (100)	0 (0)	1 (1.9)		
Total	49(94.2)	3 (5.8)	52 (100)		

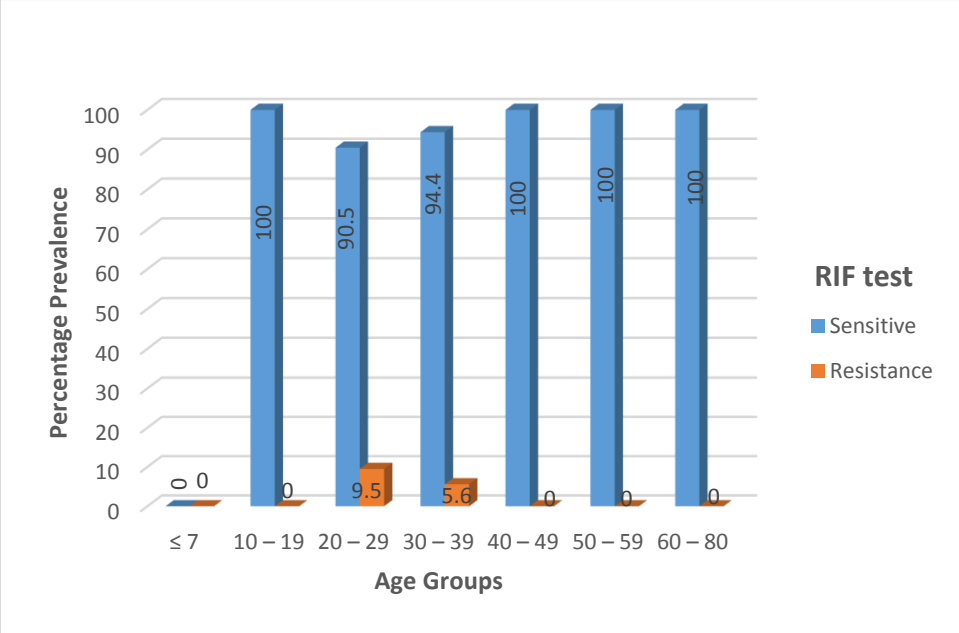

Figure 1: Pattern of RIF resistance among age groups

DISCUSSION

The emergence of drug resistance to *M. tuberculosis* has become a significant obstacle for TB control (WHO, 2019).The emergence and spreading of multidrug (MDR) and extensively (XDR) drug-resistant *M. tuberculosis* complex(MTBC) strains poses significant challenges to TB control (Ioannidis, *et al.* 2011). Drug resistant (DR)TB is widespread and is found in all countries of the world (WHO, 2019). MDR-TB caused by tubercle bacilli resistant to at least one of the first-line anti-TB drugs, Rifampicin (RMP) and or Isoniazid (INH) was declared a global burden (WHO, 2019). Both biological, as well as socioeconomic factors, have been responsible for the emergence of DR-TB, which is a purely manmade phenomenon; a result of sub-optimal chemotherapy (Pablos-Méndez, *et al*. 1998).

Although, Rifampicin-resistant *M. tuberculosis* is a serious health problem in the treatment and control of tuberculosis, the low proportion of rifampicin-resistant *M. tuberculosis of* (5.8%)in this study was in keeping with previous studies by (Nwadioha, *et al*. 2014), (Fadeyi *et al*. 2017) in Nigeria and Gupta *et al*., (2011) North India. In contrast, the proportion of rifampicin-resistant *M. tuberculosis* was lower than reports from Ethiopia by (Mekonnen, *etal*. 2014) and (Araya *et al*. 2011) from Chile. The variation could be due to difference in risk for HIV acquisition, exposure to anti-TB drugs, geographical location and national TB control program. In the present study, the proportion of rifampicin resistant *M. tuberculosis* was significantly higher among previously treated patients compared to treatment naïve patients which might be due to failure from previous treatment and contact with drug resistant TB patients (FMOH, 2019).

The level of rifampicin resistance among previously untreated cases (18.8%) in the analysis is close to the reported prevalence of rifampicin- resistant MTB (10.4%) by (Nwadioha *et al*. 2014) in Nigeria. This finding is of significant relevance in the current global and regional efforts to accurately and timely diagnose MDR-TB with the scale up of molecular technology like Gene Xpert MTB/RIF, providing quick results of rifampicin resistance as a proxy to MDR-TB.

In this study, high prevalence of rifampicin-resistant *M. tuberculosis* detected among HIV positive cases which are in accordance with a study done by (Abdella *et al*. 2015) in Ethiopia and (Walls *et al.* 2015) in Cambodia. However, in the present study, there was a lack of association between HIV infection and development of active tuberculosis as well as rifampicin resistance. This was consistent with the results of studies by (Mulu *et al.*, 2015) in Ethiopia and (Mboowa,*et al.* 2014) in Calabar, Nigeria.

In this present study, the proportion of pulmonary tuberculosis was significantly higher compared to extra-pulmonary tuberculosis in addition the proportion of rifampicin-resistant *M. tuberculosis* was higher in sputum specimens compared to non-respiratory samples. This was at variance with the study conducted by (Walls *et al.*2015) in Cambodia. This demonstrates that rifampicin-resistant pulmonary *M. tuberculosis* infection is a major health problem in resource-limited settings.

This study was able to detect *M. tuberculosis* and rifampicin resistance using the NTBLCP endorsed method Gene Xpert MTB/RIF assay from sputum and non-respiratory specimens. However, Gene Xpert could not determine the level of resistance to other anti-TB drugs and the finding of Gene Xpert was not compared to acid fast bacilli microscopy.

CONCLUSION

Although, this study showed low prevalence of rifampicin resistance tuberculosis in this environment. Previous treatment with anti-TB drugs was significantly associated with rifampicin resistance. This poses serious challenges to management and control of tuberculosis. Therefore, efforts should be intensify by the TB control program to ensure compliance and completion of treatments, use of GeneXpert is advocated for diagnosis of all TB suspects before commencement of treatments.

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