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Prevalence of *M. tuberculosis* and Associated Risk Factors Among Suspected Patients in Federal Medical Centre Birnin Kudu, Jigawa State.

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Abstract

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 aim of this study was to identify mycobacteria tuberculosis at the molecular level in symptomatic presumptive TB subjects in Federal Medical Centre, Birnin Kudu Jigawa State, Nigeria. 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 $\geq$ 2 weeks, night sweats, fatigue, unexpected loss of weight, and fever. Each eligible subject (272) who signed written consent and provided clinical specimens were recruited into the study. Four milliliters (4ml) of sputum sample were collected. In the case of presumptive extra-pulmonary TB, four milliliters of either pus, CSF samples was collected. Samples were immediately processed for Gene Xpert MTB/RIF assay. Testing for HIV was done according to the current national algorithm recommended by the Federal Ministry of Health of Nigeria. **T**otal number of 52 subjects were *M. tuberculosis* positive with a total prevalence of 19.1% and total prevalence of 1.1% rifampicin resistance. Most 157 (57.7%) were males. Age groups 20-29, 30-39, and 40-49 have TB positivity rate of 33.5%, 26.5%, and 12.9% respectively. Majority 194 (71.3%) of participants were rural dwellers. Prevalence of HIV was 50 (18.4%) among study participants. The measure of association showed that there was significant association ($p<0.05$) between TB positivity with type of residence, history of previous TB treatment and reason for diagnosis. Urban residents were 3.23 times likely of being TB positive compared to rural dwellers. The prevalence of Rifampicin-resistant *M. tuberculosis* is low in pulmonary tuberculosis cases in the study area.

Keywords: *M. tuberculosis,* Rifampicin resistant tuberculosis, Sputum, tuberculosis.

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 while 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).

This study was undertaken to identify and characterize mycobacteria tuberculosis at the molecular level among symptomatic presumptive TB subjects in 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. Individuals presenting with any of the following symptoms was recruited: the presence of symptoms suggestive of TB like chronic cough for a period of $\geq$ 2 weeks, night sweats, fatigue, unexpected loss of weight, and fever.

FMC, BKD has more than 300 beds offering different specialized services. It receives patients from the catchment area 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.

Ethical approval

Ethical approval was obtained from the ethical committee of Federal Medical Centre, Birnin Kudu Hospital management and written 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 *et. al.* (2006). The calculated sample size was 272.

Inclusion criteria

All subjects aged 9 years to 80 years attending clinic during the study period and those that offered written informed consent to participate in the study were recruited 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 were collected. In the case of presumptive extra-pulmonary TB, four milliliters of either pus, CSF samples was 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 are subsequently amplified by genomic DNA testing 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 probes called 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 were tested first with Determine. Positive samples were 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 were 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, gender, residence, reason for diagnosis, treatment history, and category of presumptive DR TB and site of tuberculosis.

Quality assurance

Both SPC and PCC internal controls were used during Gene Xpert MTB/RIF assay. The specimens 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 were 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. Total number of 52 subjects were *M. tuberculosis* positive with a total prevalence of 19.1% and total prevalence of 1.1% rifampicin resistance. Most 157 (57.7%) were males and 115(42.3%) were females. The age range of participants was 9 to 80 years with mean age of 32.5 years. Age groups 20-29, 30-39, and 40-49 years have TB positivity rate of 33.5%, 26.5%, and 12.9% respectively. Majority 194 (71.3%) of participants were rural dwellers. Of the total, 271 (99.6%) were presumptive for pulmonary TB while 1 (0.4%) were presumptive for extra-pulmonary TB. Prevalence of HIV was 50 (18.4%) among study participants (Table 1).

Prevalence of *M. tuberculosis* and Rifampicin resistance TB

Total number of 52 subjects were *M. tuberculosis* positive with a total prevalence of 19.1% and total prevalence of 1.1% rifampicin resistance. The measure of association showed that there was significant association ($p<0.05$) between TB positivity with type of residence, history of previous TB treatment and reason for diagnosis. Subjects resident in urban areas were 3.23 times more likely to be TB positive compared to rural dweller. Preciously untreated subject has a higher risk of TB positivity. TB\HIV infection positivity rate was 24 %. 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 and 3).

Associated risk factors

Out of the total number of 52 TB positive only 3(5.8%) was rifampicin resistant. In the multivariate analysis, type of residence (Urban settlement) (AOR=4.58; 95%CI, 2.06 – 10.2) was independently associated with TB positivity. Only "reason for diagnosis" showed a significant ($p<0.05$) relationship with rifampicin resistance. Subjects with a previous anti-TB drug treatment had a higher prevalence of rifampicin resistance. However, the difference in the prevalence was statistically not significant ($p>0.05$) (Table 4).

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
Gender	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 *M. tuberculosis* among presumptive TB patients referred to FMC, Brinin Kudu using Gene Xpert MTB/RIF assay, 2019.

Variables	*M. tuberculosis* Detected N (%)	*M. tuberculosis* Not detected N (%)	Total N (%)	P-Value	OR (95%CI)
Age (Years)					
≤	0(0.0)	21 (100)	21 (7.7)	0.11	0
10 – 19	2 (11.1)	16 (88.9)	18 (6.6)		1
20 – 29	21 (23.1)	70 (76.9)	91 (33.5)		2.40 (0.5-11.3)
30 – 39	18 (25.0)	54 (75.0)	72 (26.5)		2.67 (0.6-12.7)
40 – 49	5 (14.3)	30 (85.7)	35 (12.9)		1.33 (0.23-7.7)
50 – 59	3 (13.6)	19 (86.4)	22 (8.1)		1.26 (0.19-8.5)
60 – 80	3 (23.1)	10 (76.9)	13 (4.8)		2.40 (0.3-17.0)
Gender					
Female	18 (15.7)	97 (84.3)	115 (42.3)	0.21	1
Male	34 (12.5)	123 (73.3)	157 (57.7)		1.49 (0.8-2.8)
Residence					
Urban	26 (33.3)	52 (66.7)	79 (28.7)	<0.001	3.23 (1.7-6.0)
Rural	26 (13.4)	168 (86.6)	194 (71.3)		1
HIV Infection					
Positive	12 (24.0)	38 (76.0)	50 (18.4)	0.33	1.43 (0.7-3.0)
Negative	40 (18.0)	182 (82.0)	222(81.6)		1
Treatment History with Anti- TB Drugs					
Previously treated	37 (16.7)	184 (83.3)	221(81.2)	0.04	0.48 (0.2-0.97)
Previously untreated	15 (29.4)	36 (70.6)	51 (18.8)		1
Reason for Diagnosis					
Presumptive TB	46 (16.9)	220 (82.7)	266(97.9)	<0.001	
Presumptive DR-TB	06 (100)	0 (0)	6 (2.2)		
Site of Presumptive TB					
Pulmonary	51 (18.8)	220 (81.2)	271(99.6)	0.19	
Extra – pulmonary	01(100)	0 (0)	1(0.4)		
Total	**52(19)**	**220(81)**	**272(100)**		

Table 3: Prevalence of Rifampicin-resistant *M. tuberculosis* in each variable 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)					
≤	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)		
Gender					
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)		

Table 4: Multivariate analysis showing the associated predictors of *M. tuberculosis* in FMC, Brinin Kudu, 2019.

Characters	*M. tuberculosis* Detected N (%)	*M. tuberculosis* Not detected N (%)	Total N (%)	P. Value	AOR (95%CI)
Age (Years)					
≤	0(0.0)	21 (100)	21 (7.7)	0.25	0
10 – 19	2 (11.1)	16 (88.9)	18 (6.6)		1
20 – 29	21 (23.1)	70 (76.9)	91 (33.5)		2.79 (0.5-15.2)
30 – 39	18 (25.0)	54 (75.0)	72 (26.5)		4.04 (0.7-22.8)
40 – 49	5 (14.3)	30 (85.7)	35 (12.9)		1.15 (0.2-7.6)
50 – 59	3 (13.6)	19 (86.4)	22 (8.1)		0.99 (0.11-8.9)
60 – 80	3 (23.1)	10 (76.9)	13 (4.8)		2.26 (0.28-18.4)
Gender					
Female	18 (15.7)	97 (84.3)	115 (42.3)	0.15	1
Male	34 (12.5)	123 (73.3)	157 (57.7)		1.75 (0.82-3.7)
Residence					
Urban	26 (33.3)	52 (66.7)	79 (28.7)	<0.001	4.58 (2.06-10.2)
Rural	26 (13.4)	168 (86.6)	194 (71.3)		1
HIV Infection					
Positive	12 (24.0)	38 (76.0)	50 (18.4)	0.81	0.89 (0.36-2.2)
Negative	40 (18.0)	182 (82.0)	222(81.6)		1
Treatment History with Anti- TB Drugs					
Previously treated	37 (16.7)	184 (83.3)	221(81.2)	0.09	0.50 (0.2-1.13)
Previously untreated	15 (29.4)	36 (70.6)	51 (18.8)		1
Reason for Diagnosis					
Presumptive TB	46 (16.9)	220 (82.7)	266(97.9)	<0.001	
Presumptive DR-TB	06 (100)	0 (0)	6 (2.2)		
Site of Presumptive TB					
Pulmonary	51 (18.8)	220 (81.2)	271(99.6)	0.19	
Extra – pulmonary	01(100)	0 (0)	1(0.4)		
Total	52(19)	220(81)	272(100)		

Discussion

In this study, we observed a 19.1% prevalence of *M. tuberculosis* infection. Our finding is similar with previous reports (Cox, *et., al.*, 2014) from South Africa (26%), (Aliyu *et al.,* 2013) from Northern Nigeria (23%) and (Alvarex-uria *et al.,* 2012) from India (27.6%). However, it is lower compared to reports on multi-drug-resistant tuberculosis in Northern Pakistan by (Adeniyi, *et al.,* 2004) of (37%). The lower proportion rate of confirmed *M. tuberculosis* in the present study compared to other studies could be due to the fact that we included presumptive cases to identify *M. tuberculosis* while other studies included identified cases of *M. tuberculosis* to check Gene Xpert technique. In contrast, it is higher than studies conducted by (Deribew *et al.,* 2011) in Ethiopia (10.4%) and (Sharma *et al.,* 2014) in India (12.0%). The discrepancy might be due to difference in methods of detection of *M. tuberculosis,* community, study design and geographical area.

In this study, the detection rate of *M. tuberculosis* was significantly higher in males than females. Reports from WHO (2019), Mekonnen *et al.* (2014) in Ethiopia and Yang *et al. (*2014) from Northeast China supports this finding. Also reports by Abdallah and Colleagues (2012) at Kasala State in Sudan showed high detection rates in males than females. The reason for this might be due to social and health seeking behavior difference and higher exposure of males to outer environment, smoking and alcoholism (WHO, 2019).

The highest proportion of Gene Xpert positive *M. tuberculosis* cases were seen in the age group of 20–29 years. This is consistent with previous reports by (Deribew *et al.*, 2011) on investigation outcomes of tuberculosis suspects in the health centers of Addis Ababa, Ethiopia. This might be due to more exposure to the outer environment, high workload and wide range of mobility of young people to acquire the TB bacilli. In the present study, the proportion of *M. tuberculosis* was significantly higher in presumptive TB compared to presumptive DR-TB patients ($p < 0.05$). Previous anti-TB drug treatment has a higher prevalence of rifampicin resistance, but the difference in the prevalence was statistically not significant ($p>0.05$). This might be due to treatment failure and acquiring of resistant bacilli from drug resistant TB contacts. Moreover, significantly higher proportion of *M. tuberculosis* was found among patients treated with anti-TB drugs compared to treatment naïve patients in the present study. This finding was comparable to a study conducted by Makamure *et al. (*2013) in Zimbabwe.

We observed an 18.4% HIV/TB co- infection in this study. Our finding is consistent with reports of many studies around the world. In a study conducted in Nigeria during 2013 intended to find the current factors affecting treatment outcomes of tuberculosis in a Tertiary Health Center in South Western Nigeria, the rate of pulmonary tuberculosis among HIV-infected patients was 20.0% (Babatunde *et al.*, 2013). Previous study conducted in India observed a 49.2% prevalence of TB among HIV-positive subjects in Gujarat –India (Ghiya *et al.*, 2009) which reflect a very high TB prevalence among PLWHA in Gujarat region in India. In rural Cambodia, Cain and Colleagues (2007) observed a prevalence rate of 38.0 % TB in HIV infected patients. Similarly, a previous report by Mihir and Colleagues (2011) among HIV Sero-positive patients attending a Counseling Center in Kolkata-India observed a prevalence of 33.0%. However, the prevalence rate observed in this study was to a significant extent higher compared to a study conducted in Accra city – Ghana that observed a 3.6% prevalence of TB among HIV Sero-positive patients (Essiam, 2013). The prevalence rate of TB in this study was higher than the results of a study carried out in Uganda to evaluate the prevalence, incidence and mortality associated with tuberculosis in HIV- infected patients initiating antiretroviral therapy in Rural Uganda which indicated a 7.2 % TB prevalence (Moore, *et., al.*, 2007). The variations in this study results and the above-mentioned studies could be multifactorial; different methodologies used in the study, diagnostic techniques used whether conventional or advanced beside the different study populations factors which have important roles.

Although, Rifampicin-resistant *M. tuberculosis* is a serious health problem in the treatment and control of tuberculosis, the low prevalence of

rifampicin-resistant *M. tuberculosis of* in this study was in keeping with previous studies in Nigeria (Nwadioha *et al*., 2014) and Gupta *et al*. (2011) North India. In contrast, the proportion of rifampicin-resistant *M. tuberculosis* was lower than reports in Ethiopia (Mekonnen *et al*., 2014) and Chile (Araya *et al*., 2011). The variation could be due to difference in risk for HIV acquisition, exposure to anti-TB drugs 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).

In this study, high prevalence of rifampicin-resistant *M. tuberculosis* was 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 finding is consistent with the results of studies by Mulu *et al*. (2014) in Ethiopia and Mboowa *et al*. (2014) in Calabar, Nigeria.

This study was able to detect *M. tuberculosis* and rifampicin resistance using Gene Xpert MTB/RIF assay from sputum and non-respiratory specimens. However, this study 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

Rifampicin-resistant *M. tuberculosis* prevalent is low in pulmonary tuberculosis cases in the study area. Previous treatment with anti-TB drugs was significantly associated with rifampicin resistance. The strong association of rifampicin resistance with previous treatment suggests that improved monitoring of treatment to limit the emergence of drug resistant *M. tuberculosis*. Hence, the use of Gene Xpert is advocated for diagnosis, management and expanded surveillance of drug-resistant *M. tuberculosis across the country.*

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