ORIGINAL ARTICLE

Isolation and Immunological Detection of *Mycobacterium Tuberculosis* from HIV and Non-HIV Patients in Benue State, Nigeria

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ABSTRACT

BACKGROUND: *Immunological techniques are important tools for tuberculosis epidemiology; although its use is underutilized in Nigeria. In this study, we report the epidemiological outlook of Mycobacterium tuberculosis among HIV patients in Benue State, Nigeria.*

METHODS: *Sputum samples were collected from 425 suspected TB patients from July 2016 to February 2018 and subjected to acid-fast microscopy, GeneXpert MTB/RIF, processed using NALC-NaOH and cultured on Lowenstein-Jensen media. The isolates obtained were identified by SD-Bioline® assay.*

RESULTS: *The prevalence of TB by acid-fast microscopy was 35(15.9%). The prevalence of TB by acid-fast bacilli was significantly (χ^2 = 8.458; P = 0.003) highest among the 15-34 years age group (22.0%) compared with other age groups. TB prevalence was significantly (χ^2 = 4.751; P = 0.029) higher among patients from rural areas than those from urban center (23.8% vs 14.1%). GeneXpert assay detected 64(15.1%) TB cases of which patients from rural areas had significantly (χ^2 = 8.104; P = 0.017) higher prevalence of TB than patients from urban areas (23.8% vs 12.9%). The overall rifampicin resistance TB was 3.1%. Also, patients from rural areas had significantly (χ^2 = 10.625; P = 0.005) higher rifampicin resistance compared with patient from urban areas (8.3% vs 1.3%). Of the 126(29.7%) mycobacterial isolates, 42(33.33%) were identified as MTBC and 84 (66.67%) as NTM by SD-Bioline® assay.*

CONCLUSIONS: *The study revealed that Mycobacterium tuberculosis infection is still a major public health problem, with relatively high prevalence rate of rifampicin resistance among HIV positive patients. Further studies are needed for early detection and treatment intervention necessary for infection control.*

KEYWORDS: *Benue State, Immunology, Infection, Prevalence, Rifampicin Resistance, Tuberculosis*

INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is both a preventable and treatable disease but it is the leading cause of death globally (1). The organism is responsible for over 9 million TB cases and 1.7 million deaths annually (2). TB and Human immunodeficiency Virus (HIV) coinfection and the emergency of drug resistance TB are the drivers for the reemergence of TB in Nigeria (3).

Globally, Nigeria ranks among the 22 high TB burdened countries and the second in Africa (4). Benue State in Nigeria has high TB prevalence which is worsened due to high rate of HIV/AIDS and poverty (5). There exists strong relationship between TB and HIV/AIDS. For example, an HIV infected individual has high risk of rapid disease progression. Similarly, there is high rate of TB treatment failure among HIV infected patient than HIV negative TB patients. Also, TB patients with HIV infection demonstrate greater mortality as high as 20-30% in resource limited settings like Nigeria (6–8).

There have been serval reports of the prevalence of TB in different parts of Nigeria. A prevalence of 10.5% was reported among HIV positive patients in Kano State (9). Ojiezeh et al., (10) reported a prevalence of 14.0% among HIV individuals in Owo, Southwest Nigeria. Other studies include: 6.1% in Jos (11); 12.7% in Ife (12); 14.7% in another study in Kano (13); 19.0% in Maiduguri (14); 21.5% in Benue State (15) and 28.1% in Ibadan (16). These studies were mostly based on retrospective data which has inherent problems such as poor record keeping and several human limitations. Also, the lack of isolation and identification of *Mycobacterium tuberculosis* in earlier studies in Benue State may account for the under reporting of the impact of the disease in Benue State (17,18). The aim of this study was to determine the prevalence of tuberculosis and rifampicin resistance among HIV and non-HIV patients in Benue State.

MATERIALS AND METHODS

Study areas: Benue State lies between latitudes 6°25'N and 8°8'N and longitudes 7°47'E and 10°E' (Figure 1). It is surrounded by five States, namely Nassarawa to the north, Taraba to the northeast, Cross River to the south, Anambra to the southwest and Kogi to the west. There is also a short international boundary between the State and the Republic of Cameroun along Nigeria's southern border. The State has a population of over 4 million people (19).

The climatic condition of Benue State is influenced by two air masses: the warm, moist south westerly air mass and the dry northeasterly air mass. The rainy season begins in the month of May and ends in October (19). Benue State is known as the food basket of the nation because of its high agricultural activity and level of food production.

Makurdi, Gwer East and Otukpo Local government areas represent the major local governments in two of the three senatorial zones in Benue State where tuberculosis referral centres are located. Also, Federal Medical Centre (FMC), Makurdi; General Hospital, North Bank; St. Vincent Hospital, Aliade and General Hospital, Otukpo are the main hospitals for tuberculosis diagnosis and treatment in Benue State which are designated as tuberculosis referral centers.

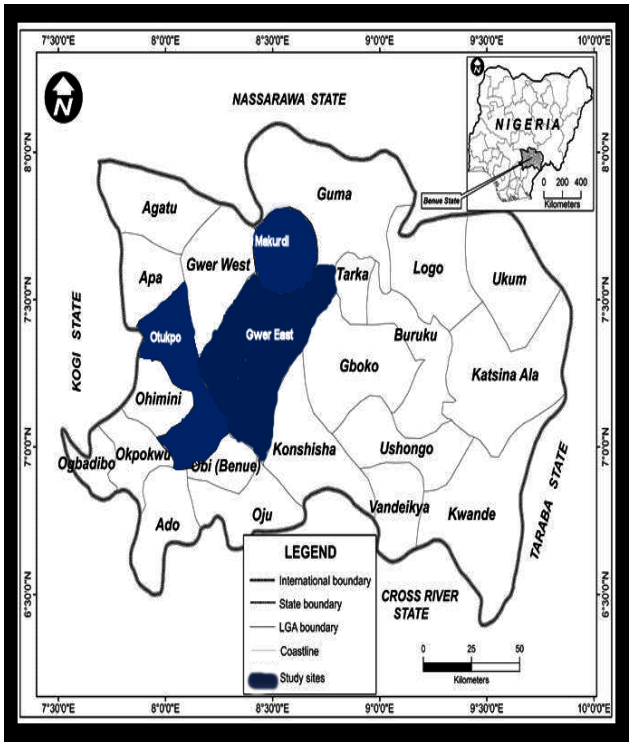

***Figure 1**: Map of Benue state showing the study area*

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Study design: A case-control study based on tuberculosis cases was carried out in Directly Observed Treatment (DOT) centers and hospitals in Makurdi, Otukpo and Gwer East to evaluate the prevalence of tuberculosis due to *Mycobacterium tuberculosis* complex among HIV and non-HIV patients in Benue State, Nigeria.

Ethical approval: The permission (BSUTH/MKD/HREC2013B/2017/0011) to conduct this study was obtained from the Health Research Ethics Committee, Benue State University Teaching Hospital, Makurdi, and the Research and Ethics Committee of the Federal Medical Centre of Makurdi (FMC/MED108/VOL1/X), Nigeria. There is no way to trace patients' identities from the results generated in this study.

Inclusion Criteria

Location: The locations of this study were Makurdi general hospital, Federal Medical Center, Apir, St. Vincent Hospital, Aliade, Gwer East and General hospital Otukpo.

Conditions: The wiliness to participate in the study, suspected tuberculosis patients that are attending tuberculosis clinic and was tested for HIV infection.

Collection of sputum samples from suspected human TB Patients: Regular visits were made to tuberculosis and chest hospitals and clinics in Benue State from July 2016 to February 2017. The hospitals include: General Hospital, Otukpo; St. Vincent Hospital, Aliade; Federal Medical Centre, Makurdi and General Hospital North Bank, Makurdi. Sputum from suspected TB/HIV co-infected individuals and TB patients who were willing to participate in the study were collected on daily basis until the end of February 2017.

A total of 425 sputum samples were collected from suspected TB patients in Benue State. Three hundred and fifty-two samples were collected from suspected patient in Federal Medical Center Makurdi and General hospital, North Bank, 59 sputum samples from Otukpo General Hospital and 17 sputum samples from suspected TB patients from St. Vincent Hospital, Aliade. Suspected sputa were collected in a sterile sample container and stored at -20°C in the medical laboratory of the Federal Medical Centre Makurdi and the General Hospital Otukpo for a period of one to two weeks before transportation to the tuberculosis laboratory, University of Ibadan for bacteriological culture and identification.

Mycobacteria culture of specimens from sputum samples: Processing and culture of sputum samples were done as described by GLI (2014). This includes digestion and decontamination of samples with NALC-(N-acetyl-L-cysteine)-NaOH. It was then followed by inoculation on paired Lowenstein Jensen media with glycerol and pyruvate and incubated at 37°C for between 8 and 12 weeks. Routine precautions were taken to avoid laboratory cross-contamination. Three sub-cultures of each positive culture were made and stored at -20°C for further use.

Immunological Identification of Mycobacterium tuberculosis complex: Immunological identification of isolates was done by rapid detection of MPT64 protein. The 28kDa (MPT64) early secretory protein had been identified as one of the candidates for immunological diagnosis of TB. The protein is encoded by RD2, a genomic region of difference that is deleted in all M. bovis BCG but conserved among other members of the MTBC (20,21). An immunochromatographic assay, SD BIOLINE TB Ag MPT64 RAPID® (SD Bioline assay) which is based on the MPT64 protein was used to differentiate M. tuberculosis from non-tuberculous mycobacteria.

The SD bioline assay was performed on acid-fast bacilli in 7H9 broth, as described by the manufacturer. Liquid culture (0.1 ml) was put onto the sample well. The test cassettes strips were incubated for 15-30 minutes at room temperature. The pink band in the 'C' region confirmed the test validity. An additional pink band in the 'T' region was interpreted as positive for the MPT64 Ag. Only the pink band in the 'C' region and no band in the 'T' region were considered negative for the MPT 64 antigen. No band in 'C' region was interpreted as an invalid test (22–25).

Statistical analysis: Data were cross tabulated, Chi-square was used to compare differences in TB detection rate among demographic variable using SPSS version 17. Results obtained were considered significant at a level of P value < 0.05.

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RESULTS

Demographic characteristics of study participants: During July 2016 to February 2017 total of 425 suspected TB patients participated in the study. Of the 425 participants, 220(51.8%) were males. One hundred and fifty-nine (37.4%) and 163(38.4%) were in the age group of 15 – 34 and 35-54 years. One hundred and fifty-nine (37.4%) and 243(57.2%) were HIV positive and HIV negative. Two hundred and ninty-seven (69.9%) do no consume local milk products. Three hundred and forty-one (80.2%) lived in the urban areas. Two hundred and sixty seven (62.8%) were Tiv and 119 (28.0%) were Idoma. Three hundred and thirty-four (78.6%) attended FMC, Makurdi and 59(13.9%) attended General hospital, Otukpo (Table 1).

Table 1: Demographic Characteristics of Human Study Participants

Characteristics	Frequency (%), N = 425
Sex	
Male	220(51.8)
Female	205(48.2)
Age group	
0 – 14	24(5.7)
15 – 34	159(37.4)
35 – 54	163(38.4)
≥ 55	79(18.6)
HIV Status	
Positive	159(37.4)
Negative	243(57.2)
Unknown	21(4.9)
Consumption of Nono	
Yes	128(30.1)
No	297(69.9)
Location	
Urban	341(80.2)
Rural	84(19.8)
Ethnicity	
Tiv	267(62.8)
Idoma	119(28.0)
others	39(9.2)
Hospital	
FMC	334(78.6)
GHOTKP	59(13.9)
GHNB	15(3.5)
St. VH	17(4.0)
Total	**425(100.0)**

FMC = Federal Medical Centre; GHOTKP = General Hospital Otukpo; GHNB = General Hospital, North Bank; St. VH = Saint Vincent Hospital

Prevalence of tuberculosis in Benue State based on AFB test: A total of 425 suspected TB patients were screened for tuberculosis using AFB test. The overall prevalence of tuberculosis by AFB was 16.0%. Prevalence of TB between sex by AFB test was not significantly ($\chi2$ = 0.003, P = 0.958) different-(35 (15.9%) males vs 33 (16.1%) females). Prevalence of TB among the age group of 15-34 years (22.0%) was significantly ($\chi2$ = 8.458, P = 0.037) higher than other age groups. HIV positive patients had higher prevalence of TB than those who were HIV negative (19.5% vs 15.4%). However, the difference was not statistically significant ($\chi2$ = 5.594, P = 0.061). Also, the prevalence of TB among those who consume local milk products and those who do not consume local milk product was not significantly ($\chi2$ = 0.526, p = 0.467) different. Prevalence of TB was significantly ($\chi2$ = 4.751, P = 0.029) higher among rural dwellers, 20(23.8%), than those from urban areas, 48(14.1%). Prevalence of TB diagnosed at General Hospital, Otukpo, was higher ($\chi2$ = 20.122, P = 0.000) than other hospitals in this study areas (Table 2).

Prevalence of TB in Benue State using GeneXpert assay: Results from GeneXpert assay for the 425 participants in this study showed an overall prevalence of 15.1%, of which 37(16.8%) males and 27 (13.2%) females were positive to TB. Prevalence of TB among all age groups was not significantly ($\chi2$ = 2.003, p = 0.919) different. Although the prevalence (11.4%) was lowest among people who are greater or equal to 55 years of age and highest among 15-34 age group. Prevalence of TB by GeneXpert assay was highest among HIV patient than non-HIV patients (18.2% vs 14.3%). There was no positive TB case among patients with unknown HIV status. Patients who consume local milk products had higher prevalence than those who do not (18.0% vs 13.8%). However, the difference was not significant ($\chi2$ = 1.831, P = 0.400) statistically. The prevalence of TB using GeneXpert by location varies significantly ($\chi2$ = 8.104, P = 0.017); patients from rural areas had 20 (23.8%) and patients from urban areas had 40 (12.9%). Also, the prevalence of TB among the different ethnic groups was significantly ($\chi2$ = 29.758, p = 0.000) different. The Idoma ethnic group had 25(27.7%) compared with Tiv 33 (9.4%)

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Table 2: Prevalence of tuberculosis in Benue State based on Acid-Fast Bacilli Test.

Variables	No. Sampled	AFB Test (%)	$\chi 2$	P value
Sex				
Male	220	35(15.9)	0.003	0.958
Female	205	33(16.1)		
Age group				
0 – 14	24	1(4.2)	**8.458**	**0.037**
15 – 34	159	35(22.0)		
35 – 54	163	23(14.1)		
≥ 55	79	9(11.4)		
HIV Status				
HIV Positive	159	31(19.5)	5.594	**0.061**
HIV Negative	243	37(15.4)		
Unknown	21	0(0.0)		
Consumption of nono				
Yes	128	23(18.0)		
No	297	45(15.2)	0.528	0.467
Location				
Urban	341	48(14.1)	**4.751**	**0.029**
Rural	84	20(23.8)		
Ethnicity				
Tiv	267	37(13.9)	3.144	0.208
Idoma	119	25(21.0)		
others	39	6(15.4)		
Hospital				
FMC	334	42(12.6)	**20.122**	**0.000**
GHOTKP	59	19(32.2)		
GHNB	15	1(6.7)		
St. VH	17	6(35.3)		
Total	**425**	**68(16.0)**		

and other 6(15.4) ethnic groups. Patients diagnosed at General Hospital, Otukpo, and St. Vincent Hospital, Aliade, had higher prevalence of TB (49.2% and 41.2%) compared with patients from Federal Medical Centre, Makurdi (8.4%). GeneXpert assay was not available at the General Hospital, Northbank, at the time of this study (Table 3).

Isolation and identification of Mycobacterium tuberculosis complex from TB patient in Benue State: The overall isolation rate of MTBC and NTM was 42(33.3%) and 84(66.7%) respectively. Mycobacterium isolation on LJ media was significantly ($\chi 2 = 3.879$, $P = 0.049$) higher among males, 68(30.9%) than among females 46(22.1%) patients. Identification by Immunological identification of MP64 protein (SD-Bioline assay) showed that MTBC isolation was higher among male than female TB patients. The difference was not statistically significant ($\chi 2 = 5.609$, $P = 0.061$). HIV negative patients and those whose HIV status was unknown had higher isolation rates of Mycobacterium (26.4% and 27.8%). Identification of MTBC was highest among HIV positive patients;

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patients whose HIV status was unknown were negative for MTBC. Other ethnic group had the highest mycobacteria isolation rate; it also had the lowest rate of MTBC. Tiv and Idoma ethnic groups had the highest rate of MTBC respectively. The isolation rate of MTBC among ethnic groups was significantly ($\chi2 = 53.192$, $P = 0.000$) different. Of the 6(40%) mycobacteria isolated from patients who attended General Hospital, Northbank, 0(0.0%), was identified as MTBC. Samples from the Federal Medical Centre, Makurdi, had the highest rate (6.4%) of MTBC identification. Identification of MTBC varies significantly ($\chi2 = 156.000$, $P = 0.000$) among the hospitals in this study (Table 3).

Table 3: Prevalence of TB in Benue State using GeneXpert assay

Variables	No. Sampled	GeneXpert Assay (%)	$\chi2$	P value
Sex				
Male	220	37(16.8)	1.613	0.446
Female	205	27(13.2)		
Age group				
0 – 14	24	4(16.7)	2.003	0.919
15 – 34	159	27(17.0)		
35 – 54	163	24(14.7)		
≥ 55	79	9(11.4)		
HIV Status				
HIV Positive	159	29(18.2)	5.171	0.270
HIV Negative	243	35(14.3)		
Unknown	21	0(0.0)		
Consumption of Nono				
Yes	128	23(18.0)	1.831	0.400
No	297	41 (13.8)		
Location				
Urban	341	44(12.9)	8.104	0.017
Rural	84	20(23.8)		
Ethnicity				
Tiv	267	25(9.4)	29.758	0.000
Idoma	119	33(27.7)		
others	39	6(15.4)		
Hospital				
FMC	334	28(8.4)	120.3	0.000
GHOTKP	59	29(49.2)		
GHNB	15	0(0.0)		
St. VH	17	7(41.2)		
Total	425	64(15.1)		

Prevalence of rifampicin resistance TB in Benue State: The overall prevalence of rifampicin resistance TB in this study was 3.1%. Patients from rural areas had significantly ($\chi2 = 10.625$, $P = 0.005$) higher rate of rifampicin resistance than those from urban areas (8.3% vs 1.8%). Rifampicin resistance was highest among the Idoma ethnic group, 5(4.2%), Tiv while the other ethnic groups had 2.6% respectively. The difference in the prevalence of rifampicin resistance among the different ethnic

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groups was statistically significant ($\chi 2 = 17.923$, P = 0.001). Prevalence of rifampicin resistance TB was most detected at St. Vincent Hospital, Aliade, i.e. 2(11.8%), followed by General Hospital, Otukpo, 3(5.1%). The difference in the detection of rifampicin resistance TB was significantly ($\chi 2 = 79.273$, P = 0.000) different among the hospitals (Table 4).

Table 4: Prevalence of rifampicin resistance TB in Benue State

Variables	No. Sampled	RIF	$\chi 2$	P value
Sex				
Male	220	8(3.6)	0.697	0.706
Female	205	5(2.4)		
Age group				
0 – 14	24	0(0.0)	3.741	0.712
15 – 34	159	7(4.4)		
35 – 54	163	3(1.8)		
≥ 55	79	3(3.8)		
HIV Status				
HIV Positive	159	5(3.1)	1.549	0.818
HIV Negative	243	8 (3.3)		
Unknown	21	0(0.0)		
Consumption of Nono				
Yes	297	3(2.3)	1.750	0.417
No	128	10(3.4)		
Location				
Urban	341	6(1.8)	10.623	0.005
Rural	84	7(8.3)		
Ethnicity				
Tiv	267	7(2.6)	17.923	0.001
Idoma	119	5(4.2)		
others	39	1(2.6)		
Hospital				
FMC	334	8(2.4)	79.273	0.000
GHOTKP	59	3(5.1)		
GHNB	15	0(0.0)		
St. VH	17	2(11.8)		
Total	425	13(3.1)		

DISCUSSION

Epidemiologic investigation was conducted among 425 suspected TB patients of which 51.8% were males, 37.4% had HIV infection, 69.9% did not consume "nono" and 80.2% lived in urban areas. The overall prevalence of TB in this study was 16.0% by acid-fast microscopy and 15.1% by GeneXpert MTB/RIF assay. It was also observed that the prevalence of TB by culture on LJ media was about twice (29.7%) the results obtained for acid-fast microscopy and GeneXpert assay. The high prevalence of TB by culture in this study may be because of the high isolation (66.67%) of NTM which cannot be detected by GeneXpert. Also, acid-fast microcopy has low specificity compared with culture (26–28).

The study observed that the prevalence of TB based on acid-fast microscopy, and GeneXpert did not vary significantly between males and females. Also, the prevalence of rifampicin resistance TB was not different among sexes. This observation was

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consistent with previous studies in Nigeria (9,29). The reason for the lack of difference in the prevalence of TB between males and females in this study may be because both sexes are equally exposed to the pathogen in the study areas. However, results from culture indicated that males had significantly ($\chi2 = 3.879$, $P = 0.0497$) higher prevalence of TB than females.

Although results from GeneXpert and culture showed that the prevalence of TB was not statistically different among age groups, however, acid fast microscopy indicated that the prevalence of TB varies significantly (($\chi2 = 8.468$; $p = 0.037$) among age groups. Those in the age group of 15-34 and 35-54 years old were the most affected. These groups represent active age group in the study areas. Since Benue State is an agrarian community that depends largely on manpower, agricultural output would be adversely affected and thus impacts negatively on the economy and living standard of the people.

Evidently, there was no statistically significant difference in the prevalence of TB between those who consumed nono and those that did not. This may be due to increased awareness campaign on the public health important of hygienic practice and pasteurization of milk among pastoralist and local milk vendors in Nigeria (2).

In this study, the prevalence of TB was statistically significant ($P < 0.05$) among patients from rural areas than those from urban center as identified by acid-fast microscopy and GeneXpert assay. Also, the prevalence of rifampicin resistant TB was higher among TB patients from rural areas. The reason for the differences may be due to variation in access to quality healthcare; there are more health facilities in urban areas than in rural areas. Therefore, people in rural areas do not have adequate healthcare facilities. Most of the times, people from rural areas had to travel a long distance on bad road network to DOTs center, which may contribute to failure to adhere to treatment regime. Furthermore, non-availability of diagnostic facilities to accurately and timely diagnose TB and rifampicin resistant TB among rural dwellers may also contribute to the maintenance of the disease in rural areas (28,30).

In this study, the isolation rate of NTM was higher than MTBC (84(66.67%) vs 42(33.33%)) from suspected TB patients. This finding was consistent with Bonnet et al,. (31), who demonstrated that two factors were associated with isolation of NTM, history of TB and HIV infection. Also, the growing incidence of tuberculosis-like illness caused by NTM may be one of the reasons for the high rate of NTM in this study. In conclusion, the prevalence of TB and rifampicin resistance was highest in rural areas and the active age group. If this trend is left unchecked, it will have significant negative effect on the economy and living standard of the people. Also, it may have secondary negative effect on food security since Benue State is the major supplier of food in Nigeria.

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