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Prevalance of *Mycobacterium tuberculosis* Complex in HIV Sero-positive Patients Attending Dalhatu Araf Specialist Hospital, Lafia Nasarawa State Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Paper

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ABSTRACT

Tuberculosis is a public health problem especially in developing countries, including Nigeria. The aim of the study was to determine the prevalence of *Mycobacterium tuberculosis* complex in HIV patients attending Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria. A total of 399 sputum samples were collected from HIV patient and examined using culture technique, Gene-Xpert, and SD-Bioline techniques. SD-Bioline test was used to differentiate between MTBC and non-Tuberculosis mycobacteria (NTM). Risk factors for MTBC were investigated. One hundred and forty-eight (148) MTBC isolates were detected using the culture techniques, 88 MTBC by the Gene-Xpert technique and 37 by the Ziehl-Neelson staining method, and 88 by the rapid test (SD-Bioline test kit) differentiating MTBC from NTM. This result indicates that most of the samples contained NTM (77.9%; 311/399) signifying latent tuberculosis, and 22.1% (88/399) were MTBC.

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Rate of rifampicin resistance was 13.5% (n = 54) in the study population. RIF resistance was higher in samples from male patients (16.5%; 22/134) than in those from female patients (12.1%; 32/265).

Keywords: Tuberculosis; prevalence; HIV; sputum.

1. INTRODUCTION

Tuberculosis is caused by one of several Mycobacterial species that belongs to the *Mycobacterium tuberculosis* complex. The *Mycobacterium tuberculosis* complex is a genetically related group of Mycobacterium species that can cause tuberculosis in human or other living things, and they include *Mycobacterium tuberculosis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae Mycobacterium canettii, Mycobacterium pinnipedii, Mycobacterium caprae and Mycobacterium microti* [1,2].

Although tuberculosis (TB) is an age-old disease, its burden continues to present a public health problem especially in developing countries. The World Health Organization (WHO) estimated more than one million TB deaths in 2015, and an additional two-fifths of a million TB deaths among HIV-positive people. Most of the cases were male patients [3].

Previous reports have shown that the disease is preventable and curable, even though it has remained a significant cause of morbidity and mortality in resource poor nations [4] .The disease seems to have re-emerged in developed nations due to its synergy with Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS), demographic changes, and subsequent immigrations [5,6]. On the basis of Tuberculin reactivity, a sign of prior infection, one third of the world's population appears to have been infected with *Mycobacterium tuberculosis* complex, the etiological agent of the disease. Infected individuals are thus at risk of presenting with disease later in life as immunity wanes due to aging or as a result of HIV co-infection [1,7].

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Gene Xpert Laboratory of Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria. The hospital is situated along Shendam road Lafia, Nasarawa State Nigeria. The Gene Xpert laboratory was established in 2013 by the National TB program in conjunction with KNCV/TBCARE 1 to assist in the rapid diagnosis of TB in HIV patients and the diagnosis of drug resistant TB.

2.2 Inclusion and Exclusion Criteria

The inclusion criteria for the study were to give an inform consent and willingness to know the HIV status of the patients. Exclusion criteria were unwillingness to give an inform consent or refusal to disclose or take HIV test.

2.3 Study Population

The targeted population was all suspected TB cases attending Dalhatu Araf Specialist Hospital.

Since tuberculosis affects all ages, the study population comprised all ages, that is, less than one year and above.

2.4 Sample Size

The sample size was determined using the following equation as described by (Naing et al., 2006) and a prevalence of 32% from a previous study on TB at Dalhatu Araf Specialist Hospital was used.

$$n = \frac{Z2P \text{ X } (1-P)}{d2}$$

Where

n = Number of samples to be collected.
Z= Standard normal deviation stipulated at 1.96 at 95% confidence limit.
P= Prevalence of previous report (32%).
d= Allowable error taken as 5%.
By substitution the values into the formula above, we have
n= (1.962) x 0.32 x (1-0.32) 0.05 x 0.05 = 334

The calculated sample size was 334, but 400 sputum samples were collected for the study.

2.5 Sample Collection

A total of 400 samples of sputum were collected from patients attending TB Laboratory Dalhatu Araf Specialist Hospital from March 2017 to October 2017. Sputum samples were collected from HIV patients suspected to be TB patients

especially those with persistent cough that had lasted for more than three weeks, having blood-stained sputum, weight loss and prolonged tiredness, and those without symptoms.

Patients were instructed on appropriate sample collections as follows: patients were asked to rinse mouth with clean water, take 3-4 deep breaths holding for 3-5 seconds after each inhalation; cough after the last inhalation, empty the sputum produced into a clean sterile, transparent, wide mouthed container that is provided, taking care not to contaminate the outside cup. The sputum container was then tightly closed with appropriate cover and stored at 2-8 0C until ready for processing.

2.6 Sample Analysis

Samples collected were examined using three different methods: GeneXpert method, microscopy method using Ziehl Neelsen Stain, and Solid Culture Method (MTBC and NTM)

2.7 Detection of Mycobacteria by GeneXpert

The Xpert MTB/RIF assay is a nucleic acid amplification test that uses a disposable cartridge with the GeneXpert Instrument system. Sputum sample collected from the patient with suspected TB was mixed with the reagent that is provided with the assay kit, and the cartridge containing this mixture was placed in the GeneXpert machine. All processes from this point were fully automated. At least 1ml of sputum was required. The sample was inspected for quality, and sample reagent containing sodium hydroxide (5-10%) and isopropyl alcohol (10-20%) was added to the sample in a ratio of 2:1. The sample reagent was added to sputum in order to break down the mucous component of sputum. With the lid closed, the sample was shaken 10-20 times and was incubated for approximately 10 minutes. The sample was then shaken and incubated for another 5 minutes. Two-ml of liquefied sample was transferred into the Gene Xpert cartridge in the Gene Xpert MTB/RIF assay system. The computerized system was used for analysis according to standard operating procedures.

The GeneXpert Dx instrument was turned on, in the Dx system window, the option “Create Test” was clicked, a scan cartridge barcode dialog box appeared, the barcode on the cartridge was scanned, and the software automatically fills the boxes for the following field, Cartridge serial number, Reagent Lot ID, Expiration Date. In the sample ID dialogue box, all the patient’s information were correctly entered and the “Start Test” button was selected, the instrument module door was opened with blinking green light and the cartridge was loaded, the module door was closed, after which the test started and the green light stop blinking, when the test finished, the light turns off, at this point the module door was opened and all cartridges were removed.

On the GeneXpert DX system, the option “Viewed Result” was clicked, the window on the mean bar and the result was displayed. This result indicates whether or not MTBC was detected in the sample. If the result reads “Invalid”, the test was repeated. Result also states whether resistance to “Rifampicin” was detected or not.

2.8 Detection of Mycobacteria in Sputum Using Solid Culture Method

All supplies and reagent needed were arranged in a Bio-safety Cabinet. At least 2 ml of the sputum was required. Decontaminating solution (NALC-NAOH) (2 ml) was added to each sample in the conical tubes, and the tubes were vortexed for 20 seconds at a moderate speed. The tubes were properly mixed and were allowed to stand for 15 minutes after which sterile 0.067M phosphate buffered saline (pH 6.8) was added to the digested and decontaminated specimen to reduce the continued action of NaOH and lower the viscosity of the mixture. The tubes were recapped tightly, the content mixed, and the tubes were centrifuged (4oC, 15 minutes, 3000 x gravity (g). After centrifugation the supernatant from each tube was carefully poured into a discard container (with funnel to avoid splash or aerosol generation) containing Lysol (a disinfectant). The sediment was re-suspended in 2 ml phosphate buffer saline.

2.9 Preparation of Lowenstein Jensen Media for the Isolation of MTB

Lowenstein-Jensen, a selective medium for cultivation and isolation of Mycobacterium species was used to isolate Mycobacterium species. Preparation of this medium was as specified by the manufacturers.

2.10 Inoculation and Incubation

Sputum sediment (0.2 ml) was inoculated into a slant of Lowenstein-Jensen medium, and the

slants were incubated (35o C, 8 weeks). Smears from positive culture were made in Biosafety Cabinet by taking a portion of the visible growth with inoculating loop and placing it on a clean grease-free slide and spreading it on a part of the slide. The smear was air-dried, heat-fixed, and stained by Ziehl-Neelsen stained technique. Cultures that were positive for acid fast bacilli (AFB) were identified using the rapid identification (SD Bioline) kit.

2.11 Interpretation of Sputum Culture Result

The culture result was recorded as positive if the growth on Lowenstein-Jensen medium is confirmed Ziel-Neelson stain and SD-Bioline to be acid bacilli. However, the result was considered negative when no growth was observed in the L-J medium media, hence using the SD-Bioline assay detects None-*Tuberculosis Mycobacterium* (NTM).

2.12 Confirmation of Positive Culture Using Ziehl-Neelsen Stain

A heat-fixed smear of each of the positive culture on a grease-free slide was flooded with a solution of carbol fuchsin and heated until the steam arose. The solution was allowed to stand for 10 minutes after which the slide was rinsed with water, and was flooded with 3% acid alcohol. After further washing with water, a counter stain 0.2% aqueous solution of methylene blue was applied. The methylene blue stained the background material providing a contrasting colour against which the red AFB could be seen under the microscope. The result was interpreted based on the standard recommended by International Union against Tuberculosis and Lung Diseases (IUATLD).

2.13 Identification of Positive Samples using Rapid Kit

The culture positive samples were processed for identification of the MTB complex using the SD Bioline rapid test kit. This is a one-step immuno-chromatographic identifier which tests for MPT64 protein secreted by MTBC and is found in the culture fluid of only strains of MTBC. Using a sterile pipette, buffer (100 ml) was transferred into a tube, and colonies from the culture plate were suspended into vial tubes containing the 100 ml of buffer. The vial tube was vortexed for 5 minutes and the suspended solid cultures (100 ul) in buffer was transferred into a sample well. A purples color moving across the result window in the center of the test device was an indication that test reaction is working. After 15 minutes, the result was read. Purple red color on both the test and control areas was to indicate a positive result, while only one purple red color on the control area signified a negative result. All samples positive by Rapid Identification were regarded as true.

2.14 Data Analysis

SPSS version 20.0 (2016 Model) was the software used for the statistical analyses of data. Descriptive (e. g., mean, percentages) and inferential statistics (e. g. chi-square) were the statistical techniques used to analyze results. Chi-square test was used to determine associations between variables. Statistical level of significance was set at 0.05 level.

3. RESULTS

The study was carried out to determine the prevalence of *Mycobacterium tuberculosis* complex in HIV patients at Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. The culture method detected 37.1% (n = 148) from the 399 cultured specimens. Sputum smear microscopy using the Ziehl-Neelsen staining technique detected 9.3% (n = 37) acid fast bacilli (AFB).

The male patients had a higher prevalence by culture 39.5% (n=53) than the female patients 35.8 % (n = 95), thus having (χ^2=0.725, df =2, P=0.696) and (χ^2=1.199, df =2, P=0.549) showing no association with gender. A prevalence of 22.6% was obtained among the married patients by Gene-Xpert method (χ^2=1.365, df =2, P=0.505) and 37% by culture method (χ^2=0.725, df =2, P=0.640). The single patients had a prevalence of 22.1% and 38.9% for Gene-Xpert and culture method respectively. The lowest prevalence 8.3% was found among the widowed patients by GeneXpert. Hence, this analysis shows no statistical association with marital status.

67.7% of the study populations reported that they consumed raw milk, whereas only 32.9% reported they were not. Many of the patients admitted to taking alcohol (66.4%), and many were not smoking (54.1%).

Table 1. *Mycobacterium tuberculosis* Complex detection rates in HIV Patients using Gene Xpert, Culture, and Ziehl-Neelsen Stain Techniques

Method Used	Total No. of Samples Tested	MTB Detected (%)	MTB Not Detected (%)	χ^2	Df	P Value
Gene Xpert	**399**	88(22.1)	311(77.9)	191.473	1	0.002
Culture	**399**	148(37.1)	251(62.9)	69.568	2	0.003
Z-N Stain on Sputum	**399**	37(9.3)	362(90.5)			

KEY: MTBC: Mycobacterium tuberculosis Complex, RIF: Rifampicin, HIV: Human Immunodeficiency Virus

Table 2. Prevalence of MTBC by gender, age and marital status using GeneXpert and Culture Methods

Variables	Total No. of Samples Examined (%)	No. Positive by Gene-Xpert (%)	No. Positive by Z-N Stain on Culture (%)	χ^2	Df	P Value
Gender						
Male	134(33.6)	32(23.8)	53(39.5)	0.725	2	0.696
Female	265(66.4)	56(21.1)	95(35.8)	1.199	2	0.549
Total	**399(100)**	**88(22.1)**	**148(37.1)**			
Age group (Years)						
1-10	7(1.8)	1(1.1)	3(2.0)	5.711	8	0.680
11-20	23(5.8)	3(3.4)	6(4.1)	0.088	8	0.527
21-30	147(36.8)	35(39.8)	57(38.5)			
31-40	125(31.3)	30(34.1)	52(35.1)			
41-50	55(13.8)	11(12.5)	15(10.1)			
51-60	29(7.3)	5(5.7)	10(6.8)			
61-70	5(1.3)	2(2.3)	3(2.0)			
71-80	5(1.3)	0(0.0)	1(0.7)			
81-90	2(0.5)	1(1.1)	1(0.7)			
Total	**399**	**88(22.1)**	**148(37.1)**			
Marital Status						
Married	292	66(22.6)	108(37.0)	1.365	2	0.505
Single	95	21(22.1)	37(38.9)	0.893	2	0.640
Widow	12	1(8.3)	3(25.0)			
Total (%)	**399**	**88(22.1)**	**148(37.1)**			

Table 3. Description of Study Population by Socio-behavioral Lifestyle

Raw Milk Consumption	Frequency	Percentage
No	129	32.3
Yes	270	67.7
Total	**399**	
Alcohol Intake		
No	265	66.4
Yes	134	33.6
Total	**399**	
Smoking		
No	216	54.1
Yes	183	45.9
Total	**399**	

Table 4. Distribution of MTBC and Rifapin Resistance by Gender, Age and Marital Status

Variables	Number of Sputum Examined (%)	MTB Detected (%)	RIF Resistance (%)	χ^2	df	P Value
Gender						
Male	134(33.6)	32(23.9)	22(16.4)	0.986	3	0.805
Female	265(66.4)	56(21.1)	32(12.1)	1.791	3	0.617
Total	**399**	**88(22.1)**	**54(13.5)**			
Age group (Years)						
1-10	7(1.8)	1(1.1)	1(1.9)	6.214	8	0.623
11-20	23(5.8)	3(3.4)	1(1.9)	5.711	8	0.680
21-30	147(36.8)	35(39.8)	21(38.9)			
31-40	125(31.3)	30(34.1)	17(31.5)			
41-50	56(14.0)	11(12.5)	7(13.0)			
51-60	29(7.3)	5(5.7)	5(9.3)			
61-70	5(1.3)	2(2.3)	2(3.7)			
71-80	5(1.3)	0(0.0)	0(0.0)			
81-90	2(0.5)	1(1.1)	0(0.0)			
Total	**399**	**88(22.1)**	**54(13.5)**			
Marital Status						
Married	292(73.2)	66(22.6)	40(13.7)	1.365	2	0.505
Single	95(23.8)	21(22.1)	14(14.7)	2.003	2	0.367
Widow	12(3.0)	1(8.3)	0(0.0)			
Total	**399**	**88(22.1)**	**54(13.5)**			

4. DISCUSSION

Mycobacterium Tuberculosis Complex detections rates of 22.1% by Gene-Xpert technique and 36.8% by culture method were obtained in this study. Similar results using culture methods have been obtained in Pakistan [8] and in Nigeria [9]. A higher prevalence obtained by the culture method than by other methods (e.g., Gene-Xpert techniques) could be as a result of the fact that culture method is the gold standard for MTB detection. Not properly processing the sputum specimens may be responsible for the lower rate of MTB detection obtained by the Gene-Xpert method. However, the low rate of MTB detection by the Gene X-pert technique agrees with a similar study in Nigeria [10], in India, and in Ethiopia (Fanosie et al., 2016).

The results of MTB complex using Ziehl-Neelsen staining technique for sputum smear microscopy and culture microscopy shows “scanty positive” acid fast bacilli on sputum microscopy [11-13]. Sputum smear is a rapid test to detect the presence of acid-fast bacilli (AFB) but a single sputum test lacks sensitivity [14]. One of the reasons for low sensitivities of a single sputum test is that 10,000 microorganisms/ml of sputum are required foricroscopy [14] furthermore, sputum culture requires 10-100 colony forming units of AFB/ml of sputum to confirm the diagnosis of TB. As stated earlier, culture technique is the gold standard for TB detection due to its high sensitivity and specificity [15,16]. The low prevalence of MTB complex in this study as detected using microscopy falls within the range reported in other parts of Nigeria such as Abia State [17], Ebonyi State [9], Anambra State [18], Osun State [19], and Gombe State [20].

The prevalence of MTB/RIF resistance associated with gender indicates male patients have higher rifampicin resistance than the female patients. This finding is similar to reports from other parts of Nigeria [21,17] and in Benue State [10]. The high prevalence in the male gender could be due to factors such as high risk and poor health seeking behavior compared to their female counterparts [17].

The age group with the highest occurrence of rifampicin resistance in this study was between 21 and 30 years. Similar findings were observed in a study in South Africa where patients between ages 21 to 25 years had a higher prevalence of rifampicin resistance [4]. A large number of patients who are MTB positive and rifampicin resistant were aged between 22 and 40 years old. This is similar to reports from Kwara and Benue States in the North Central

Nigeria. Age was not significantly associated with MTBC. Similar findings were reported by Daniel and Eltayah [6].

The high number of positive cases as shown by Gene-Xpert and culture techniques in those aged 21-30 years could be because persons within these age groups are most exposed to TB risk factors such as smoking and alcohol intake. Patients in the age group 1-10 years had the least prevalence probably because of the immunity conferred on them by the BCG vaccine. This finding agrees with the results of the study carried out in Benin where patients within age 25-49 years had the highest proportional distribution [18].

Prevalence of MTB by Gene-Xpert and culture show no significant association with gender, age groups and marital status respectively. The male patients had a higher prevalence; this could be because of their lifestyle in relation to smoking and alcohol intake. This agrees with the study carried out in Malawi where males were more infected [5]. MTB disease was not significantly associated with marital status even though married patients had the highest prevalence. This could be because of their lifestyle, especially the married males who engage in several social activities that require them to have contact with a lot of people, who then infects their wives [22-24]. In contrast, a report from Ethiopia has it that marital status was significantly associated with TB disease: the unmarried (single) patients in that study had a higher prevalence than the married, the reason being that unmarried male patients can migrate from one town to another [14]. The results of this study revealed no statistical association between TB disease and geographical areas of residence. Patients coming from Lafia West had the highest prevalence. This could be because of the nature of their environment which tends to be dusty. This agrees with a similar study carried out in Kaduna State [25], Nigeria where patients in Northern Nigeria recorded a higher prevalence which could be attributed to dusty weather of the North, especially during the period of sample collection. There was no significant association between milk consumption and TB infection. Of the positive patients for TB, many had consumed raw milk, agreeing with a study in Kaduna State [25] where a lot of patients consumed raw milk and there was no association with TB and raw milk consumption. The data on smoking and alcohol consumption showed no association between that lifestyle and TB infection. A study in China reported that the correlation between TB and active smoking or alcohol intake remains controversial [26]. However, a study in Botswana attributed TB infection to smoking and alcohol consumption [17].

5. CONCLUSION

Prevalence of MTB complex was 22.1% by Gene-Xpert and 36.8% by culture methods. Patients aged 21-30 years and female participants were the most affected. Furthermore, MTB infection was not significantly associated with milk consumption. For instance, a large percentage (63.6% by Gene-Xpert and 68.2% by culture technique) of the 270 patients who consumed raw milk had/did not have the infection.

ETHICAL APPROVAL

Approval for the study was obtained from the research ethics committee of Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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