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DEMOGRAPHIC AND PHENOTYPIC CHARACTERIZATION OF DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* ISOLATED FROM PATIENTS RESIDENT IN RIVERS STATE, NIGERIA

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ABSTRACT
Tuberculosis (TB) remains a leading public health problem worldwide. In Nigeria the burden of TB is high however only a few cases have been reported especially in Rivers State. The aim of this study is to phenotypically characterize *Mycobacterium tuberculosis* isolates recovered from clinical specimens of patients infected with drug resistant tuberculosis. Three hundred and ninety (390) sputum samples were collected from the study participants from six different locations in Rivers State. The samples were received and analyzed by decontaminating with NaOH-Citrate N-acetyl-L-Cystein method before conventionally inoculating them unto Lowenstein Jensen culture slants and incubated at 37^{0}C for 8weeks. Total number of participants were higher in females (61.53%) than in males (38.46%) $p<0.05$ and there was no significant statistical difference in prevalence of TB with respect to study sites. Participants whose age were between 21-40 years (50.0%) were more vulnerable to the disease $p<0.05$. The age bracket that was closer amongst others was 41-60 years (32.69%). Also occupation as a risk factor for TB was statistically significant with (P=0.001). TB-HIV co-morbidity among the study participants showed a statistical significance ($p<0.05$). Highest TB-HIV co-morbidity was seen among age group 21-40 (3.07%). Prevalence of MTB-NTM co-infection was 14.10% and TB morbidity with HIV and NTM highest prevalence among age groups was 16(4.10%) between age 21-40 occurring highest in males 10(2.6%) and age 41-60 as least 2(0.59%) among females 7(1.79%). Of the 390 samples tested, 45(11.53%) were positive for MTB out of which 10(2.56%) were resistant for rifampicin. HIV co-morbidity with TB and NTM is endemic in Rivers State, Nigeria. Non Tuberculous Mycobacterium infection is an emerging neglected tropical disease. All stake holders need to take drastic efforts to control these deadly infectious diseases.

KEYWORDS: Demographic, Phenotypic, *Mycobacterium tuberculosis, drug resistance, Rivers State, Nigeria.*

INTRODUCTION
Tuberculosis (TB) is considered to be one of the life threatening diseases and a target for global control for which Nigeria is noted as one of the countries that has the highest burden (WHO, 2010). TB is contagious and highly infectious so can easily spread from person to person through the air contaminated with the bacilli of the TB bacterium known as *Mycobacteriumtuberculosis.*

In 2011, the World Health Organization (WHO) reported an estimate of 280,000 incident cases of TB equating to a 68% prevalence which was notified in the WHO global report of 2012. Also records have it that Nigeria was ranked 4th position in 2007 and gradually attained 10th position in 2012 among the 22 high TB burden countries in the world and from 1st to 4th highest TB burden in Africa. TB has been reported to be a major cause of death and Drug Resistant TB (DRTB) is very difficult to treat because of the long term treatment period required (18-24 months). "It is more toxic, more expensive and involves a whole lot of challenges such as treatment failure and disease retrogression (Orenstein *et al.*, 2009, and Grandhi *et al.*, 2012 and WHO, 2014). DRTB is often triggered by the rapid genetic mutations and irregular adherence to anti TB drug regimen by patients which subsequently develop to Multidrug Resistant TB (MDR- TB). Although TB is a risk for all ages, the population that is mostly affected are adults who are in their productive age (WHO, 2015).

In Nigeria areas where TB is highly prevalent, culture positive TB are mostly of the MTB complex. Globally,

Non-Tuberculous Mycobacteria (NTM) isolates have been increasing gradually (Koh *et al.*, 2003). NTM promotes disease progression and true infections can be important clinically (Wagner and Young, 2003). Data on NTM disease in Nigeria are limited owing majorly to the fact that culture facilities are few as well as difficulty in identification for Mycobacterial species are paramount. As a result, many Laboratories do not differentiate between MTB and NTM (Chang, *et al.*, 2001) and treatment in most African countries especially Nigeria, relies majorly on sputum smear microscopy which is implicated for the inappropriate management of TB with first line anti TB drugs. This worsens the condition of the patient giving rise to the risk of drug resistant TB (DRTB) (Yim and Han,2005).

TB has co-morbidity with Human Immunodeficiency Virus (HIV). According to Lawson *et al.*, 2011, Nigeria has a high HIV burden which has positioned her to be third highest in the world with a prevalence rate of 4.4% leaving Nigeria with an estimated rate of 3 million individuals (21%) with TB-HIV co-infection. There is no doubt that the Directly Observed Treatment Short-course (DOTS) program achieved successes. The Federal Ministry of Health in (2011) reported that the DOTS strategy recorded a case detection success rate of 30% and 79% treatment rate leaving several undiagnosed TB cases as a result of the DOTS limitations. This is obviously below the global target rate of 70% to 85% rates respectively.

The findings of this study will be a reference source for knowledge nationally and internationally. It will provide information on tuberculosis for people who have not heard or are unaware of the national algorithm of the disease, mode of transmission, risk factors, diagnosis and management. Also to re-address the minds of those who have been misinformed that TB is a spiritual disease, genetic and is incurable. This will reduce the stigma on the social status of the population especially young people who are within the age of the work force. Its menace on the economy, labour and health of the population will be a thing of the past. To the health providers, it will update them on the need to counsel their clients to engage in screening hence equip themselves with continuous training to be alert by providing timely diagnostic and therapeutic measures. It will also help them to focus on the age group that is most affected so that measures on how to control spread within the age bracket will be provided. The findings of this study will further help Government Agencies and Non-Governmental Organizations (NGOs) in the planning and implementation of intervention programs that are related to the control and prevention of TB. Finally, it will be of immense help to researchers who are interested in carrying out further studies on TB by serving as a guide and template for direction. Policy makers will also find it as an important tool to formulate new policies and ideas to improve the health of the masses. The aim of this study is to phenotypically characterize *Mycobacterium tuberculosis* isolates recovered from clinical specimens of patients infected with DRTB.

MATERIALS AND METHODS

Study Area

The study area that was selected for this study was Rivers State that has her capital city as Port Harcourt. It is a tourist dream center with magnificent architecture that attracts both national and international attention. It is endowed with natural treasure such as crude oil. It is the largest city in Rivers State and the fifth largest in Nigeria with a reported estimated population of 1,865,000. It lies along the Bonny River located in Niger Delta. Rivers State was selected as the study area because of the vast classes of people with their different lifestyles based on tradition, culture, beliefs and orientation. The study was carried out in South-South Tuberculosis Zonal Reference Laboratory, University of Port Harcourt Teaching Hospital Choba, Rivers State, Nigeria.

Study Design

A cross sectional study design was adopted for this study. The study design examines the relationship between diseases and other related health characteristics and variables of interest such as age, sex, marital status, area of residence. It is adequate for quantifying the prevalence of a disease or risk factors and for estimating the accuracy of a diagnostic test. Clinical specimens were randomly collected from six different locations which represent a subgroup of Rivers State in Nigeria. Individuals and sites were randomly selected based on symptoms and prevalence.

Sample Collection

Two hundred and thirty two males (252) and One hundred and fifty eight females (158) who met the criteria for the study made up the study population. Each participant was given two sample collection containers to collect sputum. On return, the sample was given a unique identification number. Two samples were collected to enable the researcher have enough sample to conduct the different analysis.

Sample Collection Procedure

Two 50ml falcon tubes with lid were given to each participant to expectorate sputum. Participants were counseled to wash their mouth with clean water in the morning before brushing their mouth or chewing stick. Thereafter, they were asked to breathe in and out up to four times to expel air into their lungs which will help them to regurgitate to cough. Sputum was then produced into the falcon tubes and then tightly covered with the lid. Identification numbers were given to them and then transported in cold chain with triple packaging to the South-South Tuberculosis Zonal Reference Laboratory Port Harcourt.

Laboratory Procedure Macroscopic Examination

Two sputum samples were collected from each

participant. They were examined macroscopically to ensure that they do not contain blood products and food particles. They were also examined to ensure that there was no leakage or spillage and the accompanying participant form contains correct information of the participant. Demographic characteristics such as age, sex, address, state of origin, HIV status, date of sample production were indicated on the forms.

Phenotypic Cultivation of Mycobacteria

Following the manufacturer's instructions, phenotypic culture media such as the Lowenstein-Jensen's (LJ) media was prepared in slants and used as isolation media for the cultivation of *Mycobacterium tuberculosis*. Decontaminated sputum collected in sterile Pasteur pipette was inoculated onto them and incubated at 37°C for eight weeks. The slants were read weekly to observe the growth of *Mycobacterium tuberculosis* (Guz*et al.*, 2018).

Storage Media

Trypton soy broth incorporated with 6mls of glycerol for every 500mls was prepared and used as a storage media for *Mycobacteria* following the manufacturer's instructions which were stored in -86°C freezer.

Digestion and Decontamination of Sputum Samples

Sputum samples were digested and decontaminated before culturing unto the *Mycobacteria* cultivation media. NaOH and N-Acetyl-L-Cysteine (NALC) was prepared to digest and decontaminate sputum samples. Fifty milliliters of NaOH was used to dissolve 0.25g of NALC. Five milliters of this solution was used to decontaminate 5mls of sputum sample in a 50ml falcon tube in a batch of 12 samples which were vortexed for 15minutes (Mac-Fiberesima*et al.*, 2018).

Neutralizing Solution

Phosphate Buffer Saline (PBS) was prepared and used to neutralize the action of NaOH by lowering the specific gravity. Forty milliliters of PBS was used to add to the specimen to the 50mls mark. It was voetexed and centrifuged in a refrigerated centrifuge for 15minutes at 3000RM. The supernatant was decanted and then 2mls of PBS was added again to re-suspend the deposit which was used to inoculate unto the LJ slants (Mac-Fiberesima*et al.*,2018).

Sterility Testing

Nutrient agar was also prepared and used to check the sterility of NaOH, Phosphate Buffer Saline (PBS), N-Acetyl-L-Cystein (NALC) and Sterile Distilled water (Cheesebrough, 2006).

Confirmatory Test

Mycobacterium tuberculosis isolates were confirmed with the Standard (SD) Bioline rapid immuno-chromatographic assay specific for MPT64, a protein predominantly excreted by *Mycobacterium tuberculosis* Complex strains. A colony of positive Acid Fast Bacilli (AFB) was emulsified in 100ul SD Bioline extraction buffer. It was vortexed and 100ul of the bacterial suspension was collected with a sterile Pasteur pipette which was dispensed in the placing area of the test plate and allowed to wait for 15minutes. A positive test showed a red band on the test and control line while a negative test showed a red band on the control area alone (Mac-Fiberesima *et al*., 2018).

Microscopic Examination

Sputum samples were microscopically examined by making a smear on grease free slides labeled with unique identification numbers. The slides were left to air dry and later stained with the stains.

Staining Process

"The slides were arranged including positive and negative controls to be stained on a staining rack". "The slides were flooded with carbolfuchsin working solution and thereafter heated to steaming intermittently 3 times using a lit alcohol soaked cotton swab. They were allowed to stain for 10minutes". The slides were washed with running tap water to remove excess carbolfuchsin until no visible color. Excess rinsing water was drained off and later flooded with 3% acid alcohol for 3minutes to decolorize". This process was repeated until the red colour disappears". The slides were washed with running tap water and excess water was drained off. "Slides were later flood with methylene blue to counter stain for 30-60seconds". At the expiration of this time, they were rinsed with tap water, drained and dried (Angra, 2007).

Microscopic Examination

Reading of Smears Stained with ZiehlNeelsen Stain

"The microscope was switched onn and dried slides were placed on the stage of the microscope. Using the x10 objective, examination was done at low power to identify fields to be examined". By placing a drop of immersion oil, the objective was changed to the x100 and smears were examined for the presence of acid fast bacilli". "A minimum of 100 fields were examined in a systematic fashion before a smear was reported as negative"(Angra, 2007).

"Microscopic Grading"

"Grading was done according to the quantity of bacilli seen per 100 fields". "When no AFB were seen after examining 100 fields, it was considered as No AFB Seen. AFB ranging from 1-9 was reported exactly as the actual number seen". "Every 10- 99 AFB seen in 100 fields was reported as 1+". "Every 1-10 AFB seen per field in at least 50 fields was reported as 2+". "AFB seen above 10 per field in at least 20 fields was reported as 3+"(Angra, 2007).

Drug Susceptibility Testing (DST)

First Line Phenotypic DST

All culture positive of *Mycobacterium tuberculosis* were subjected to DST using the two most potent anti-TB drugs Isoniazide (INH) 0.2mg/L and Rifampicin (RIF)

2.0mg/L, according to international recommendations. Culture positive samples were processed for DST in an egg based Lowenstein Jenson media incorporated with drugs at the South- South Tuberculosis Zonal Reference Laboratory as described by Ardito *et al* (2001) and Lawson *et al* (2010). Results were read after 6weeks of incubation.

HIV Screening

HIV Screening was conducted with the serial testing algorithm using the Determine (Abbott Laboratories, Abbott Park, IL), STAT-PAK (Chembio Diagnostics), and Uni-Gold™ Recombigen· Patients that tested HIV negative on Determine were reported as negative. Patients that tested positive on Determine, negative on STAT-PAK and negative on Uni-Gold were also reported as negative. According to the national algorithm, samples that tested positive on Determine, negative on STAT-PAK and then positive on a Uni-Gold (tie-breaker test) was considered and reported as positive (Steven *et al*., 2012).

RESULTS

Table 1: Demographic Characteristics of the TB Risk Factors Stratified by Site.

Sample Collection Sites	UPTH Freq (%)	BMSH freq (%)	Bori Freq (%)	Chest Clinic freq (%)	Ahoada freq (%)	Degema freq (%)	Total Freq (%)
No of samples collected	65	65	65	65	65	65	390
Total Infected	9	10	12	8	7	6	52
Male	3(4.61)	4 (6.15)	3(4.61)	5(7.69)	2(3.07)	3(4.61)	20(38.46)
Female	6(9.23)	6(9.23)	9(13.84)	3(4.61)	5(7.69)	3(4.61)	32(61.53)
X2 (*p-value*)							5.54***(0.02)****
AGE							
<20	1(1.53)	2(3.07)	0(0.00)	1(1.53)	0(0.00)	0(0.00)	4(7.69)
21-40	3(4.61)	5(7.69)	8(15.38)	5(7.69)	2(3.07)	3(4.61)	26(50.00)
41-60	4(6.15)	3(4.61)	3(4.61)	2(3.07)	4(6.15)	1(1.53)	17(32.69)
>60	1(1.53)	0(0.00)	1(1.53)	0(0.00)	1(1.53)	2(3.07)	5(9.61)
X2 (*p-value*)							33.85***(0.001)****
Residence							
Urban	6(9.23)	3(4.61)	8(15.38)	2(3.07)	2(3.07)	3(4.61)	23(44.23)
Rural	3(4.61)	7(10.76)	4(6.15)	6(9.23)	5(7.69)	3(4.61)	29(55.76)
X2 (*p-value*)							1.38***(0.239)***
Occupation							
Trader	2(3.07)	4(6.15)	6(11.53)	3(4.61)	4(6.15)	1(1.53)	17(32.69)
House wife	3(4.61)	2(3.07)	1(1.53)	1(1.53)	1(1.53)	2(3.07)	10(28.84)
Student	2(3.07)	2(3.07)	4(6.15)	1(1.53)	1(1.53)	3(4.61)	13(23.07)
Health Provider	0(0.00)	0(0.00)	1(1.53)	0(0.00)	0(0.00)	0(0.00)	1(1.92)
Others	2(3.07)	2(3.07)	0(0.00)	3(4.61)	1(1.53)	0(0.00)	8(15.38)
X2 (*p value*)							19.10***(0.001)****

Key:

UPTH University of Port Harcourt Teaching Hospital
BMSH Braitewaite Memorial Specialist Hospital
Freq Frequency

Table 2: Prevalence of HIV Risk Factor Stratified by Site.

	UPTH Freq (%)	BMSH Freq (%)	Bori Freq (%)	ChestClinic freq(%)	AhoadaFreq (%)	Degema Freq (%)	Total Freq (%)	X2 (*p-value*)
Total	9	10	12	8	7	6	52	
MTB Alone	9(13.38)	10(15.38)	12(18.46)	8(12.30)	7(10.76)	6(9.23)	52(100.0)	2.64 (*0.755*)
HIV Status								
HIV Positive	3(4.61)	4(6.16)	4 (6.16)	2 (3.07)	1 (1.53)	3 (4.61)	17(32.69)	2.356
HIV Negative	6(9.23)	6(9.23)	8(12.30)	6 (9.23)	6 (9.23)	3 (4.61)	35(67.30)	(*0.798*)

No statistical significance (p>0.05).

Table 3: Prevalence of TB Co-morbidity Among the Study Participants.

Type of infection	Age group (years)	No infected (%)	Gender (%)	
			Male Freq (%)	Female Freq (%)
MTB alone	< 20	4(1.02)	3(5.7)	1(0.25)
	21-40	26(6.6)	17(4.35)	9(2.30)
	41-60	17(4.35)	9(2.30)	8(2.05)
	>60	5(1.28)	1(0.25)	4(1.02)
X2 (*p-value*)		33.85 ***(0.001)****	4.189 (*0.242*)	
MTB + HIV	< 20	1(0.25)	1(0.25)	0(0.00)
	20-40	12(3.07)	9(2.30)	3(0.76)
	41-60	6(1.53)	4(1.02)	2(0.51)
	>60	8(2.05)	4(1.02)	4(1.02)
X2 (*p-value*)		12.40 ***(0.01)****	1.875 (*0.599*)	
MTB + NTM	< 20	1(0.25)	1(0.25)	0(0.00)
	20-40	6(1.53)	4(1.02)	2(0.51)
	41-60	4(1.02)	3(0.76)	1(0.25)
	>60	3(0.76)	1(0.25)	2(0.51)
X2 (*p-value*)		4.95 (*0.175*)	2.022 (*0.568*)	
MTB + NTM + HIV	< 20	1(0.25)	1(0.25)	0(0.00)
	20-40	16(4.10)	10(2.56)	6(1.53)
	41-60	10(2.56)	8(2.05)	2(0.51)
	>60	12(3.07)	5(1.28)	7(1.79)
X2 (*p-value*)		16.51 ***(0.001)****	4.073 (*0.254*)	

Table 4: Prevalence of TB-NTM Co-morbidity.

T Type of infection	Positive culture (%)	Positive SD Bioline (%)	Positive smear (%)	Negative culture (%)	Negative SD Bioline (%)	Negative smear (%)
NTM & MTB	55 (14.10)	39 (10.00)	55 (14.10)	335(85.89)	16 (4.10)	335(85.89)
NTM alone	16 (4.10)	00 (0.00)	16(4.10)	374(95.89)	16 (4.10)	374(95.89)
MTB alone	39 (10.00)	39 (10.00)	39 (10.00)	351 (90.00)	00 (0.00)	351(90.00)

Key:
MTB *Mycobacterium tuberculosis*
NTM Non Tuberculous Mycobacterium
SD Standard Diagnostics

Table 5: Phenotypic DST for Rifampicin and Isoniazide.

Phenotypic Assay	MTB isolated (%)	RIF and INH Resistance (%)	RIF monoresistance (%)	INH monoresistance (%)
Culture	351(90.00)	13(3.33)	9(2.3)	4(1.02)

DISCUSSION

Sex differences in TB burden over the years have shown that males have a higher prevalence of TB compared to females. In this study, total number of participants recruited for the study was 390. Total number recruited for this study was higher in females (61.53%) than in males (38.46%) ($p<0.05$), Table 1. Sixty-five participants were recruited for each study location. They were University of Port Harcourt Teaching Hospital (UPTH), Braitewaite Memorial Specialist Hospital (BMSH), Bori, Chest Clinic Port Harcourt, Ahoada and Degema. There was no significant statistical difference in relation to site. A meta-analysis conducted by Katherine *et al* (2016) comprising of 56 TB surveys of 2.2 million in 28 countries gave a male to female ratio of 3.8 versus 3.2 which is similar to the report given in South East Asia and West Pacific (2.0-1.9). TB is a disease that is connected to poverty affecting all groups that are exposed and the majority of TB mortality emanates from gender inequality from the developing countries such as Nigeria. Inadequate provision of food and malnourishment can increase the risk of contracting the disease among women especially those that are pregnant. Global report has indicated that more men than women are infected with TB annually, nevertheless, Afganistan, Pakinstan and Iran have recorded more morbidity and mortality in women than men (WHO, 2013). This report is in line with the result of our study. Stigma, discrimination and maltreatment on women in our setting have denied most women of their civil rights. They are

excommunicated from their families and the society. Cultural beliefs, unemployment, lack of exposure and financial barriers are major challenges for women to develop health seeking habits. It is obvious that women become more vulnerable to the disease when they are in their reproductive stage and economically active. In some areas in Nigeria, some families deliberately deny their female children from having the Western education. The reason being that women are expected to be in the kitchen hence it's abortive to give the girl child a formal education. Conversely, informed women tend to seek health more than their counterparts that are not informed especially those of them that are in the reproductive age. During antenatal and post-natal stage of life, informed women find it most worthy to seek health care. This could be a reason why the prevalence of TB could be more in women than in men.

The age of the study participants as a risk factor for TB burden was also considered. This present study reported that participants whose age were between 21-40 years (50.0%) were more vulnerable to the disease ($p<0.05$). This age bracket was about twice more statistically significant compared to the other age brackets. The age bracket that was closer amongst others was 41-60 years (32.69%) Table 1. Also occupation as a risk factor for TB was statistically significant with (P=0.001). Among occupations engaged by the study participants, traders were reported to be statistically significant (32.69%) compared to house wives (28.84%) students (23.87%) and health providers (1.92%) Table 1. Risk factors with respect to HIV was statistically not significant. In a study conducted by Hassan *et al* (2017) showed that there was a higher proportion of females (53%) than males and over 60% were between the age range of 21- 40years and petty trading (28%) was the highest consistent source of livelihood among the respondents followed by farming(17%). According to WHO, (1999) young people ranging between the age bracket of 10-24 are more prone to HIV hence are likely to contract TB faster than ever taught. Denhe *et al* (2005) described youths and adolescents as people that are more vulnerable to HIV/AIDS so can contract TB faster than necessary since HIV/AIDS is a risk factor for TB.

Although, the report of this current study did not show obvious significance for HIV, the study participants may be incubating the virus until such a time when they become deficient in immune competence to wade off the agent. Youths at this age are eager to attempt new things such as sex, smoking, hard, injectable drugs and alcoholic drinks which are capable of exposing them to contract the TB disease easily. Most of the youths engaging in these unholy behavior reside in remote areas that are overcrowded and deficient of good ventilation. They may also not be economically capable of providing quality food not to talk about accessing health care. All these are risk factors that can promote disease. According to NACA, 2016, a nationally acceptable HIV strategy for youths and adolescent had been developed to narrow HIV infection in young and adolescent age. The most predominant negative behaviors promoting HIV were identified and addressed. They include nonchalant attitudes of health care providers towards adolescent and young people, sexual competence, confidentiality and bias, poor health seeking habits, trained and sensitized health care workers and socio-cultural norms about sex. The goal is to reduce new HIV infections by 2020 among youths. Data on TB concerning youths and young people are scarce. Age-specific burden of disease estimation for this age bracket is complicated by incomplete age disaggregation of tuberculosis data, highlighting the importance of continued surveillance system strengthening to enhance accurate collation.

Majority of the TB cases reported in London were between 15-44years which is the reproductive age Group (Corbett *et al*., 2003). It is a great economic loss to have this age bracket being tormented by the killer TB disease. Pregnant women who come down with the disease pose additional psychological and financial trauma for their spouses, relations and health providers. TB in women can cause much more health disturbances than earlier imagined. According to Fallahian and Tikhani, (2006), case control studies have reported amenorrhea and hypomenorrhea in extragenital pulmonary TB. In the developed countries once TB has been reported or diagnosed, and it is treated, it rarely aggravates the condition of pregnancy but this cannot be said of developing countries like Nigeria where majority of the pregnant women do not report to the gynecologist for prompt management. Moreover, when it is complicated with MDR-TB or XDR-TB, it becomes more difficult to manage. Most of them consider it a spiritual disease so confine themselves to churches and other spiritual aids. Incidentally mother and child may be lost to the deadly disease. What a great economic loss. Kothari *et al*, (2006) reported in United Kingdom that 53-77% of pregnant women are infected with extra-pulmonary TB which is similar to the report given in Mexico (Figueroa*et al*.,2001).

Early diagnosis of TB may be difficult when associated with pregnancy as it may mimic some of the physiological changes that occur during pregnancy which includes but not limited to increased respiratory rate, fatigue, cough, weight loss and fever. Malaise and fatigue were found to be a common presenting manifestation of TB with pregnancy. It can be easily seen that except weight loss (which to some extent can be compensated by weight gain during pregnancy), none of the other symptoms can warn the caregiver that they may be dealing with HIV associated TB.

It is not surprising to note in this study that traders have a statistically significant risk for TB (Table 1). As people move about in search of greener pasture possibly to TB endemic areas they become more susceptible to contracting the disease. Regional migration due to occupation tops up the TB prevalence. TB is a social

disease so migrating from one place to the other to make fortune increases the TB burden. Depending on the type of trade that one is engaged influences the social status of the person. It is however advised for traders to regularly check their status so that they can be treated early enough.

Out of a total of 390 participants, 52(13.33%) were reported to have TB (P<0.05). Table 3 illustrates the distribution of the disease among different age groups were 4(1.02%) for age group <20, for males 3(5.7%) and females 1(0.25%), 26(6.6%) for age group 21-40, 17(4.35%) for males and 9(2.30%) for females, 17(4.35%)for age group 41-60,9(2.3%)for males and 8(2.50%) for females and age group >60 5(1.28%) where 1(0.25%) was for males and 4(1.02%) for females. In June, 2018 Professor Adewale, Minister of Health remarked that Nigeria was the 7th TB burden country in the world and 2nd in Africa. He made this known at a symposium on tuberculosis themed: "Raising Future Leaders to end TB in Nigeria". Irrespective of the Nigerian TB strategies aligning with global TB control trends, Nigeria is still listed among the highest 30 TB burden countries in the world. A systematic TB meta-analysis conducted in Nassarawa State by Onyedum *et al*, (2007) reported the prevalence rate of any drug resistance among new TB cases to be 32.0%. In another study conducted in Calabar by Kooffreh *et al*, (2016) reported the burden of TB as 44.6% in 2015. Also the study reported by Sani *et al* (2015) in Mina revealed that 25.5% (153/600) of the studied participants were positive for *Mycobacterium tuberculosis* and the most affected age group was between 11-40 years with the percentage prevalence of 81.6%. All these reports on TB prevalence are higher than what was reported in this current study. The disparity could be as a result of difference in study sites. Despite efforts made to reduce the TB burden in Nigeria by WHO and other funding partners, the disease thrives to extend its tentacles more to colonize fresh susceptible people and even become worse in people who have been previously diagnosed or treated. The following reasons may have made it possible. Firstly, care givers still depend on passive case findings by only subjecting those with clinical signs and symptoms to diagnosis and treatment forgetting that there are other latent TB cases that may become active with time. Secondly, a lot more facilities lack the expertise, manpower and the right technology to screen TB at the early stage. Thirdly, there is also a deficit among health workers to collaborate and discharge their sense of team play for better TB management. Fourthly, incessant industrial actions displayed by health workers to drive home their demands is a strong factor for a shortfall in the proper management of those that have been infected. This contributed in no small way to boost the relapse cases and further encourage MDR-TB which can develop further to XDR-TB among new and old cases. Fifthly, there is a knowledge disconnect with practice on the aspect of newer diagnostic tools and lastly, there appears to be a great shortfall in funding and human resource.

TB-HIV co-morbidity among the study participants showed a statistical significance (p<0.05) Table 2. Highest TB-HIV co-morbidity was seen among age group 20-40 (3.07%) which was closely related to what was reported in United States between 1993-2004 by Rachel *et al*, (2017) where the TB-HIV co-morbidity was highest among age bracket 25 to 44 years with a prevalence of 13.8%. It is also in agreement with the reports of Erhabor *et al*., (2010); Teklu *et al*., (2012); Christopher *et al*., (2012); Okonkwo *et al*., (2013); Olanrewaju *et al*., (2013); Eyasu, (2014); Smart and Aleru, (2015), but contrary to significant difference between female and male by Akinleye *et al*. (2015). A cross matched incident TB-HIV co-morbidity of 57,527 TB cases carried out by John *et al*, (2013) in California reported between 1993-2008 gave 3,904 (7%) to be positive for HIV.

Studies conducted in Nigeria within 10 years period in Kano by Maiyaki *et al*, (2015) had a TB incidence of 47(13.62%). In another research conducted in Nasarawa State by Umeh *et al*, (2007) had 12.8% TB-co-infection, Olusola *et al*, (2017) also reported TB/HIV co-infection as 21.6% in lagos. Grace *et al*, (2010) reported 42.2% TB- HIV co-morbidity in northern Nigeria and Onidipe *et al*, (1999) reported 12.0% in Ile-Ife, 10.0% in Kano by Iliyasu & Babashani (2009), Daniel *et al*, (2004) in Sagamu reported 14.9% and in Ibadan 28.12% by Odaibo *et al*., (2006) with a greater HIV prevalence found in females than males. While Lawson *et al*, (2008) reported that women were more likely to be infected than males Oladipo *et al* (2006), did not record any significant co-infection rate with respect to gender. In Bayelsa, Omo-Emmanuel *et al*, (2017) reported 21.8% with highest prevalence among females within the 31-40 age bracket. Alau *et al*, (2016) in Abuja reported that 8.3% out of 19,572 participants were TB-HIV co-infected. They further stated that in 2014 co- infection rate was 6.67%, in 2015 it was 7.6% and over all co-infection rate for three years was 7.5%. Highest co-infection was recorded in Edo (0.8%) which was low for HIV prevalence, followed by Rivers (15.6%) and the least co-infection rate was recorded in Ondo (1.7%) and Abia (1.8%).

From the forgoing it is obvious that co-infection with TB is a more serious health issue that needs strategic efforts to halt especially in the rural areas where the people are less informed and hardly access health care. In this time of economic recession in Nigeria, all hands need to be on deck to flush any agent that seems to rage war against an already depressed economy. Intense steps should be taken to address the situation by adopting a community evidence based health education model with a multisectoral approach.

Evaluating TB morbidity with HIV and NTM serves as a back ground study in Rivers State as it is the first of its kind (Table 4). Out of the total 390 samples, 55(14.10%) were culture and smear positive. When confirmed with

standard diagnostic bioline rapid test, 39(10%) was positive for TB while 16(4.10%) was negative which were considered as NTM (Table 4). Prevalence of MTB-NTM co-infection was 14.10% and TB morbidity with HIV and NTM highest prevalence among age groups was 16(4.10%) between age 21-40 occurring highest in males 10(2.6%) and age group 41-60 as least 2(0.59%) among females 7(1.79%). Aliyu *et al,* (2013) in Northern Nigeria reported overall NTM prevalence as 15% and co-morbidity with HIV was 5.7%. These reports are in line with the current study.

Simeon *et al*, 2016 in their report entitled NTM isolated from TB suspects in Ibadan, Nigeria reported TB- NTM co infection to be 13% and 39% single infection for NTM. Also Nyamogoba *et al*, (2012) in Nigeria had NTM- TB co-infection as 1.7%. These two reports from Nigeria are closely away from what was reported in this study. Tefere *et al*, 2017 in Finlay isolated 46-66% TB-NTM co-infection and 67.5% NTM potential pathogens. In Sub Sharan Africa, Catherine *et al*, (2017) had a prevalence of 7.5% pulmonary NTM colonization.

Low income countries with high TB burden also contributed to this increasing number of NTM infection. Such countries lack adequate man power to equate with the growing need. There are little or no appropriate equipment to isolate NTM and probably they lack basic understanding of NTM so are not cable of identifying and reporting them. Smear microscopy may not be helpful in diagnosing NTM infection. It is low in specificity and sensitivity, therefore high Tb burden areas where microscopy is in use will have a high unidentifiable MTN epidemic with lasting effect. Perhaps a significant proportion of morbidity and mortality resulting from TB may be from NTM infections. Despite the fact that NTM is found in the environment and water which possibly may indicate contamination if isolated in the laboratory, it is otherwise seen as an opportunistic pathogen implicated with rifampicin resistant TB which requires serious attention to address (Mac-Fiberesima *et al*, (2018).

The findings of our study and others explained the effect of drug non compliance and improper management (Table 5). A systematic review and meta-analysis conducted by Cajetan *et al*., 2017 in Nigeria was 6% for multidrug resistance. Uzoewhulu, *et al*., 2014 also in Nigeria reported in their study 7.7% multidrug resistance and poly drug resistance of 19.4%. These reports are within a small margin similar to the report given by our study (4.12%) for LPA and (3.3%) for culture. A genotypic mono and multi-resistant pattern reported by Mac-Fiberesima *et al*, 2018 declared 18.82% for rifampicin, 10.50% for isoniazid and 4.71% for rifampicin and isoniazide.

Azuonwu and his colleagues in Bayelsa (2017) isolated only 6(2.9%) rifampicin resistance and correlation coefficient for age and sex was not significant. Also Lana *et al*, 2018 in Nigeria reported results closely related to our study 2.8% for rifampicin monoresistance, 1.4% isoniazid monoresistance and 3.6% multi drug resistance (Table 5). Drug resistance in the southern area has only a few reports with low prevalence compared to the reports of other sites. This is attributable to the fact that there are just a minimal number of facilities equipped with modern technology to diagnose TB drug resistance. In India Depika *et al*., (2017) reported resistant drug patterns for mono, multi, and totally drug resistant and lack of adequate knowledge. Therefore differences in result profiles can further influence management and compliance. A single case of misdiagnosis and management can intensify an epidemic bearing in mind that the disease is promoted by a number of risk factors such as overcrowding, poverty, poor quality of air, improperly treated TB, malnutrition, immune-suppresion, co-infection and other underlining diseases etc. This study therefore is a clarion call for biomedical scientists, public health experts and policy makers to generate a standard algorithm to diagnose, report and manage this disease.

CONCLUSION

Prevalence of TB morbidity with TB and NTM is another area that needs serious attention. Age differences with colonization of the various forms of diseases are more among the active workforce of the nation. Awareness creation, prompt isolation and treatment is key in the management of any infectious disease.

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