MULTIDRUG RESISTANT MYCOBACTERIUM TUBERCULOSIS IN ADAMAWA STATE, NIGERIA

Nusirat Omotayo Omisore,[1] Mukaila Oyeleke Oyewole[1] and Ezekiel Olugbenga Akinkunmi[2*]

[1]Department of Pharmacology and [2]Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

*Corresponding Author's E-mail: eoakinmi@oauife.edu.ng or akinkeroo@yahoo.com

Abstract

Background: There is a need to have regular updates from regions where high burden of tuberculosis (TB) have been reported in order to assist the local and global bodies in their objective to curtail the spread of drug resistant TB (DRTB). This study presents a situation report of DRTB in Adamawa State which has been identified as one of the States with high burden of TB in Nigeria.
Materials and Methods: Sputum culture in Lowenstein-Jensen Media, drug sensitivity tests and the GeneXpert MTB/Rif analysis were used in the identification and drug susceptibility studies of _M. tuberculosis_ isolates obtained from forty TB patients who were enrolled from three selected hospitals with DOTS facilities in the State.
Results: The age of TB patients range from 17 to 70 years (median = 30 years). Twenty (50 %) _M. tuberculosis_ isolates were detected by the GeneXpertMTB/Rif analysis while the media culture detected 31 (77.5%). The two methods however detected rifampicin resistance in 4 (10%) of the total isolates. All rifampicin resistant isolates were multidrug resistant TB (MDRTB) and three of them were from male patients aged 30, 38 and 45. There was only one case of resistance to streptomycin, 3 to ethambutol and 6 to isoniazid. Monoresistance were only observed for ethambutol and isoniazid and it was found in two isolates for each.
Conclusion: There is a need to provide interventions to control MDRTB in the state and to make such interventions available and closer to the patients.

Keywords: MDRTB, Rifampicin resistance, Adamawa State, GeneXpert MTB/Rif

Introduction

Drug resistance complicates the management of tuberculosis (TB) and represents one of the most important emerging challenges in the control of TB worldwide (Orenstein _et al.,_ 2009; WHO, 2016). It has been shown that resistant strains of _Mycobacterium tuberculosis_, the causative organism of TB, develop as a result of inadequate treatment and can propagate to other individuals in the same manner as drug-susceptible ones (Elzinga _et al.,_ 2004).

The drugs used in the treatment of TB are classified into two broad types, which are First Line anti-TB Drugs and the Second Line anti-TB Drugs (Chan _et al.,_ 2004; Orenstein _et al.,_ 2009). The established First Line anti-TB drugs such as streptomycin, isoniazid, rifampicin and ethambutol as well as the Second line drugs which include pyrazinamide, kanamycin, levofloxacin, capreomycin and etionamide and are used in the two phases of a TB drug treatment regimen (Tessema _et al.,_ 2009; Kabir _et al.,_ 2010; WHO, 2016).

These two phases of a TB treatment regimen consists of the initial intensive phase of two to three months of fully supervised daily administration of drugs and the continuation phase of four to six months applied in the Directly Observed Treatment Short Course (DOTS) recommended by the National Tuberculosis and Leprosy Control Programme (NTBLCP) (Chan _et al.,_ 2004; Orenstein _et al.,_ 2009; Kabir _et al._, 2010). The first line drugs are presented as fixed-dose combinations to slow down the possibility of the development of resistance (Kabir _et al._, 2010).

Further to these two classes of drugs, various medicinal agents and compounds, such as moxifloxacin, gatifloxacin, bedaquiline, imipenem, clofazimine, prochlorperazine and metronidazole as well as β-lactam combinations with clavulanate and linezolid have emerged as new classes of anti-_M. tuberculosis_ drugs, offering improvements over previously existing treatments (Ginsberg, 2008; Dauby _et al.,_ 2011; Wayne _et al.,_ 2011; Merle _et al.,_ 2014; Ruan _et al.,_ 2016; Shrivastava _et al.,_ 2017).

Different levels of drug resistance in tuberculosis are known. A patient is said to have Drug-Resistant TB (DRTB) if there is a laboratory confirmation of resistance to one or more of the first line anti-TB drugs. Further to this,

a patient who has resistant bacilli to one of the first line anti-TB drugs is said to have Mono-Resistance TB (MR-TB). Poly-Resistance TB (PR-TB) occurs when there is resistance to more than one of the first line drugs other than rifampicin and isoniazid. Patients who have resistant bacilli to both rifampicin and isoniazid are said to be Multi-Drug Resistant (MDR-TB) (Kabir *et al.,* 2010; Lamikanra, 2010).

The final category of DRTB patients involve those with MDR-TB plus resistance to any of the fluoroquinolones (ofloxacin, moxifloxacin, levofloxacin) and one of the second line injectables (amikacin, kanamycin, and capreomycin). These ones are said to be extended spectrum drug resistant (XDR-TB) (Kabir *et al.,* 2010). Extensively drug-resistant TB XDR-TB cases with even broader resistance patterns have been reported in more recent years from different countries (Glaziou *et al,* 2013).

It has been shown that the effective management of drug resistance is based on preventing the emergence of resistant strains through adequate basic treatment of patients, the early detection and proper treatment of existing patients with MDR-TB to stop transmission, and measures to limit transmission through infection control (Federal Ministry of Health, 2015; WHO, 2016).

Nigeria ranks fourth in the world and first in Africa in terms of the numbers of people with TB disease, with a projected 590,000 incident cases of TB in 2013 and over 600,000 new cases in 2014 (Federal Ministry of Health, 2015; Uplekar and Raviglione, 2015, WHO, 2016). The prevalence, incidence and death rate of the disease has always been ranked very high with values above 200/100,000, 140/100,000 and 20/100,000 population respectively consistently over the past decade (Federal Ministry of Health, 2015).

The implementation of the DOTS strategy began in Nigeria in 2004 and its uptake has increased since then leading to increased case notifications from 31,164 in 2002 to 100,401 in 2013 and greater success in TB management (Federal Ministry of Health, 2015). It has been reported that the fact that there are DOTS centres available may influence a client's willingness to seek for TB diagnostic services, because s/he knows that there is a facility for treatment should s/he be diagnosed of TB (Federal Ministry of Health, 2015).

Adamawa State is one of the states in Nigeria with a high burden of TB (John et al., 2015). As at 2013, it has the highest burden of all the states in the North Eastern geographical zone of the country with a value of about 60/100,000 population (Federal Ministry of Health, 2015). However, drug susceptibility testing is not a routine practice in the state (Toma, 2014). As of 2013, out of the 87 centres in Nigeria with the GeneXpert facility, Adamawa state has only one which was then at the Specialist Hospital, Yola. Sputum smear microscopy still remains the mainstay of TB diagnosis in the State.

As far as we know, there is presently no report of the prevalence of MDR-TB in Adamawa State in the literature. Reports indicate that patients are assumed to have drug-sensitive TB and are generally placed on first line anti-TB regimen with the exception of few cases with clear evidence of MDR-TB who are referred to the National TB and Leprosy Research Centre, Zaria (Toma, 2014).

Furthermore, for about a decade, Adamawa State has been involved in dense occurrence of civil unrest due mainly to the Boko Haram insurgents, resulting in great socio-economic problems which have been recognized as important risk factors in the transmission and rapid spread of TB in high burden communities (Elzinga et al., 2004; Glaziou et al., 2013).

There is a need to have regular updates from regions where high burden of TB have been reported in order to assist the local and global bodies in their objective to curtail the spread of DRTB. This study was conducted in order to present a situation report of the DRTB in Adamawa State. It is expected that the results obtained from the study would guide policy formulation, planning interventions and resource utilization in the enhancement of effective TB treatment that should have local and global benefits.

Materials and Methods
Study Area and Centres

The study area is Adamawa State. Adamawa State is located in North Eastern part of Nigeria and lies between latitude 80N and 110N, and longitude 11.50E and 13.50E. The state is bounded by Borno State to the North, Gombe and Taraba States to the North/West and South/West respectively, and the Cameroons extending throughout its Eastern border. Adamawa state has three Senatorial zones (central, north and south) and 21 local government areas (LGAs). Yola is the state capital and sit of the government. Adamawa state has a population density of over 95 person/km2 arising from a population of over 3.7 million (projected from 2006 National Population Census) with a land mass of 39,742.12 km^2. Adamawa State has over 1,300 health facilities which included; 1 Federal Medical Centre and 6 General and 7 Cottage Hospitals. The remaining were Clinics, Dispensaries, and Health Posts. Over 1,160 facilities were public while about 140 were private (~12%); (https://www.informationng.com/tag/adamawa-state).

Three Centre were chosen based on the availability of DOTS facilities and from three different geographical zones of the State: Specialist Hospital, Yola in the centre, General Hospital, Hong in the North and Cottage Hospital, Fufore in the East. As a result of civil unrest due to the activities of the Boko-haram insurgents towards the northern part of the state, access to the General Hospital, Hong was reduced. Therefore, the Federal Medical Centre (FMC), Yola was used in replacement. The three Centre eventually used for the study were: FMC, Yola; Specialist Hospital, Yola and Cottage Hospital, Fufore. These hospitals receive patients from within the state as well as communities in the

adjoining states: Borno, Gombe and Taraba. All patients with suspected TB infection, whether or not they elected to participate in the study, were diagnosed and managed at these Centres.

Ethical Clearance

Ethical clearances were obtained from the Ministry of Health and Federal Medical Centre (FMC), Yola, Adamawa State, Nigeria (Ref. No: S/MOH/s.524/VOL.1/94 and FMCY/SUB/S.9). The study protocol was submitted to each hospital Ethics Committee and approved before data collection. Study was conducted with strict adherence to guidelines.

Inclusion criteria

Subjects consist of patients in these hospitals who have been presumptively diagnosed as TB patients and who gave written informed consents for participation in the study. The diagnoses of TB were based on the WHO criteria for the diagnosis of TB in resource-poor setting (WHO, 2016) and were done by the physician in charge. The diagnoses were made using Ziehl-Neelsen (ZN) staining of sputum to identify acid-fast bacilli (AFB). Sputum samples were taken from patients before commencement of treatment.

Exclusion criteria

Pulmonary TB patients who were currently receiving anti-TB chemotherapy, patients with extra pulmonary TB and those who could not expectorate were excluded.

Sample Size Determination

Sample size determination was done using the formula for sample size calculation for prevalence studies given by Araoye (2003).

$n = a^2bc/d^2$, where n = sample size, a = Z statistic for a level of confidence, b = prevalence and d = precision or confidence interval.

The level of confidence of 95% is conventional at which the value for 'a' is 1.96 and d is 0.05. Based on WHO estimation of DRTB for Africa in 2010 which put DRTB at approximately 5.4% (WHO, 2010), the value of 0.054 was used for 'b'. The equation gave a numerical value for 'n' of 79.

During the collection of samples for the study, the civil unrests in the state also resulted in low attendance for care in the three hospitals, hence a smaller number of patients were attended to at the hospitals at the time and these adversely affected enrolments for the study. Sample collection started in June 2014 and ended in November 2014 when 40 subjects had been assessed and difficulty in getting more volunteers aggravated.

Collection and Analysis of Sputum Specimen

Sputum was collected into three separate properly labeled, dry, clean, transparent, leak proof and open-mouthed sputum sample bottles with cover. One of the sputum samples was collected early in the morning by the patient himself after being well instructed on the procedure of collection as previously described (WHO, 2016; Audu *et al.,* 2017). This specimen was transported through cold-chain for the probe assay using the GeneXpert MTB/Rif machine.

The remaining two samples were collected following the same procedure used for the first sample but these were collected on the spot at the clinic. One of the samples was preserved using a 5-ml cetyl pyridinium chloride solution (1%) and stored at room temperature while the other, which serves to authenticate the results obtained from the first one, was kept without a preservative in a refrigerator. These two specimens were used for the culture, isolation, identification and drug susceptibility testing. Sputum samples without preservatives were sent through cold-chain medium while those containing preservatives were sent in cold-box at room temperature.

GeneXpert MTB/Rif Analysis

The GeneXpert MTB/Rif machine was used for the identification and typing of pathogen as well as for the probing of resistance to Rifampicin. Sputum samples were processed according to the operating procedures for Gene Xpert MTB/RIF assay as previously described (Audu *et al.,* 2017). Results were automatically generated indicating if *M. tuberculosis* was detected or not. Where it was detected, the GeneXpert MTB/Rif automatically generated result indicating if the organism was rifampicin resistant or not.

Culture of Samples

Cultures of the two specimens were done in Lowenstein-Jensen (LJ) Media (both solid and liquid). The recommendation of the International Union Against Tuberculosis (IUAT) were followed in the procedures (Ananthanarayan and Panicker, 2009) and the culturing took between 4 and 6 weeks for each batch of specimen.

Drugs Sensitivity Test (DST)

After the isolation and identification of the organism in LJ media, drug sensitivity was performed by the proportion method. The LJ media were prepared for each of the four first line Anti-TB drugs with final concentrations of 0.2 μg isoniazid, 2 μg ethambutol, 40 μg rifampicin, and 4 μg streptomycin. Bacterial suspensions were inoculated into drug-free and drug-containing slopes of LJ media. Susceptibility or resistance was recorded when the proportion of bacteria in drug-containing medium to that of drug-free medium is <1 or ≥1, respectively (Otu *et al.,* 2013).

Results

Of the 40 subjects studied, 24 were males. The age ranges from 17 to 70 years with a median of 30 years.

From the GeneXpert MTB/Rif analysis, 20 (50 %) of the patients had *M. tuberculosis,* 32.5 % did not show *M. tuberculosis* infection and the remaining 7.5 % were rejected by the GeneXpert MTB/Rif machine. Four of the *M. tuberculosis* showed rifampicin resistance.

Culture results in LJ media detected *M. tuberculosis* in 31 (77.5%) of the patients. A total of 23 (57.5%) of the patients had isolates which were susceptible to all the first-line drugs while eight (20%) had isolates which showed resistance, four (10%) to only one of the drugs (DR-TB) and the remaining four to more than one (MDR – TB). The remaining nine did not grow in culture.

There was only one case (2.5%) of the total samples resistant to streptomycin, 6 (15%) to isoniazid, 4 (10%) to rifampicin and 3 (7.5%) to ethambutol. The one case resistant to streptomycin was simultaneously resistant to isoniazid and rifampicin. Also, of the three cases resistant to ethambutol one was resistant to the duo of isoniazid and rifampicin. The remaining two MDRTB were resistant to both isoniazid and rifampicin only. All the four isolates that were resistant to rifampicin were simultaneously resistant to isoniazid. The remaining two isoniazid resistant isolates were mono drug resistant. The four mono drug resistant TB are two cases each for isoniazid and ethambutol (Table 1).

Three of the four mono drug resistant strains were from male patients of age 24, 29 and 36 while the only female was 35 years of age. The 24 and 29 years old patients were resistant to isoniazid while the 36 years old male patient and 35 year old female were resistant to ethambutol.

Of the four MDRTB patients three were males of age 30, 38 and 45 years while the only female was a 70 year old woman. The 30 years old male patient was the one showing MDR to three of the first line drugs: isoniazid, rifampicin and ethambutol. While the 45 year old man and the 70 year old woman were the ones with resistance to both isoniazid and rifampicin, the 38 year old male patient showed MDR to the three of isoniazid, rifampicin and streptomycin (Table 1).

Discussion

The emergence of MDR-TB poses a threat which, if not effectively addressed, may wipe out the achievements of previous efforts in controlling TB. The World Health Organization (WHO) recommends that where possible, patients with MDR-TB are treated using ambulatory care rather than models of care based principally on hospitalization (WHO, 2016). It has been strongly recommended that any patient identified as suspects for DR-TB require urgent intervention to prevent further damage to their lungs and the risk of infection to other patients, particularly those debilitated by other medical conditions such as HIV and diabetes (Glaziou *et al.*, 2013). According to a report on TB surveillance in Nigeria in 2012, TB has 10 – 15 index rate of being infectious, if one comes in contact with somebody who is an active TB patient (Kabir et al., 2010).

In this study, both the GeneXpert MTB/Rif analysis recommended by the WHO and the culture techniques were used for the determination of drug resistant TB strains. Both methods were also used for the species typing of the TB pathogen in sputum samples. The two methods showed good correlation in the results obtained for rifampicin resistance as both detected four of the isolates that demonstrated resistance to this agent.

By definition, TB cases that showed resistance to rifampicin are taken as MDR – TB (Abanda *et al.,* 2017). This definition is supported by the results of this study in which case all the rifampicin resistant cases also showed resistance to at least one other first line drug.

Results indicate that monoresistance were observed for ethambutol (5%) and isoniazid (5%), but not for streptomycin and, as already indicated, rifampicin. The high degree of monoresistance shown to the former agents corroborates similar reports from studies conducted in other sections of the country such as seen for Otu et al. (2013) in Calabar and Audu et al. (2017) in Nasarawa. This observation has been associated to the fact that these agents are less

effective being mainly bacteriostatic in action. The comparatively higher susceptibility of the *M. tuberculosis* strains to rifampicin and streptomycin has also been linked to their high bactericidal activity (Lamikanra et al., 2010; Otu et al., 2013).

The results of this study have also shown that most of the rifampicin resistant cases are found in the adult males. This corresponds to results obtained in similar studies from different regions of the country such as South-western States (Daniel and Osman, 2011), health institutions in Calabar, South-East (Otu *et al.,* 2013), and Nasarawa State in North-central Nigera (Audu *et al.,* 2017) as well as other areas outside of Nigeria (Coovadia *et al.,* 2013). The importance of this could be viewed from two perspectives: health and economic.

From the perspective of health, young adults have high risk index of aggravating TB incidence as well as prevalence. This is due to the fact that people in this age range are normally socially vibrant both in term of human movements as well as sexual activity. From the economic perspective, adult males are supposed to be the most productive in any labour market. They are naturally seen as bread-winners. This is particularly so in a State like Adamawa where women have their sustenance culturally attached to males. Hence, economic empowerment of the citizens will likely be compromised because of the high rate of prevalence of TB in this group.

Results indicate that strains with codes 2471, 2633, 2875, 2528 are MDR- TB Group while those with code 2439, 2486, 2653 and 2488 are mono- resistant group. Since it has been shown that patients with mono-resistance TB usually benefit from drug substitution, it is suggested that another drug should be substituted for these cases based on the clinical experience of the physician and the pharmacotherapist as well as the results obtained from laboratory drug sensitivity tests. For example, patients with strains code 2473 and 2653 that were resistant to isoniazid could be substituted with pyrazinamide, one of the highly active second line drugs. In this case, ethionamide should not be chosen as substitute because this agent is structurally related to isoniazid and their mechanisms of action and activity are also similar. In addition to this, it has been shown that cross-resistance exists between these two agents (Petri, 2006).

Although resistance to one drug within a class generally means resistance to all drugs within that class, a notable exception is shown in rifabutin. Although rifabutin is structurally similar to rifampicin, rifampicin-resistance does not always mean rifabutin-resistance. Thus the laboratory should be asked to test for it to be used as possible substitute in cases of rifampicin resistance (Petri, 2006).

Furthermore, isolates with code 2486 and 2488 that demonstrated mono resistance to ethambutol will also enjoy substitution with pyrazinamide. However, in each of these cases, certain clinical considerations such as adverse drug reaction should be given priority when substitutions are considered (Lamikanra, 2010). This emphasized the need for appropriate pharmaceutical care in the management of these cases.

In an earlier study, it has been suggested that, when sensitivities are known and the isolate is confirmed as resistant to both isoniazid and rifampicin (MDR), five drugs should be chosen in the following order (based on known sensitivities). The suggestions given are: an aminoglycoside (e.g., amikacin, kanamycin) or polypeptide antibiotic (e.g., capreomycin), PZA, EMB, a fluoroquinolones: with preference for the C-8-methoxy-fluoroquinolones such as moxifloxacin or gatifloxacin rather than ciprofloxacin the use of which is more likely to result in the development of quinolone resistance (Petri, 2006). It has also been shown that it is not recommended to use more than one injectable (STM, capreomycin or amikacin), because the toxic effects of these drugs are additive. Other drugs that are suggested are rifabutin, cycloserine, a thioamide: prothionamide or ethionamide, PAS, a macrolide: e.g., clarithromycin, linezolid, high-dose isoniazid (if low-level resistance), interferon-γ, thioridazine and meropenem and clavulanic acid (Petri, 2006; Glaziou *et al.,* 2013).

Further to this, these groups of patients require special attention and facilities which if not available in the present hospitals, the patients should be immediately referred to those centres with such facilities. It should be noted that a holistic approach is imperative in handling resistant TB cases. Hence, no stone should be left untouched in identifying and treating prospective resistant TB cases as urgently as possible given the magnitude of the menace such pose to the eradication of TB.

43

Table 1: Result of Gene Expert Analysis, Culture and Drug Susceptibility Testing for Subjects

Serial number of Isolates	Isolates Codes	Age of Patient	Sex of Patient	Results of GeneXpert MTB/Rif Analysis	Results of Culture	Drug Sensitivity Testing Result to the First Line Anti-TB Drugs*				REMARK
						Streptomycin	Isoniazid	Rifampicin	Ethambutol	
1	2420	20	F	+	+	S	S	S	S	Non Resistant
2	689	45	M	NC	+	S	S	S	S	Non Resistant
3	2457	20	M	+	_	NT	NT	NT	NT	Not Applicable
4	693	67	F	NC	+	S	S	S	S	Non Resistant
5	695	18	M	NC	+	S	S	S	S	Non Resistant
6	2445	18	M	Error	+	S	S	S	S	Non Resistant
7	2437	29	M	+	+	S	R	S	S	DR-TB
8	580	17	F	NC	+	S	S	S	S	Non Resistant
9	2463	41	M	+	+	S	S	S	S	Non Resistant
10	2854	38	F	Error	_	NT	NT	NT	NT	Not Applicable
11	2469	35	M	+	+	S	S	S	S	Non Resistant
12	2471	30	M	Resistant	+	S	R	R	R	MDR-TB
13	2472	30	M	_	+	S	S	S	S	Non Resistant
14	2480	42	M	+	+	S	S	S	S	Non Resistant
15	471	17	M	Error	_	NT	NT	NT	NT	Not Applicable
16	2486	36	M	_	+	S	S	S	R	DR-TB
17	2483	41	M	+	+	S	S	S	S	Non Resistant
18	2488	35	F	+	+	S	S	S	R	DR-TB
19	2529	29	M	+	+	S	S	S	S	Non Resistant
20	2525	22	M	+	+	S	S	S	S	Non Resistant
21	2633	70	F	Resistant	+	S	R	R	S	MDR-TB
22	2653	24	M	+	+	S	R	S	S	DR-TB
23	2652	18	M	+	+	S	S	S	S	Non Resistant
24	2528	38	M	Resistant	+	R	R	R	S	MDR-TB
25	2675	21	F	+	+	S	S	S	S	Non Resistant
26	XXX	44	F	+	+	S	S	S	S	Non Resistant
27	2884	40	F	+	+	S	S	S	S	Non Resistant
28	2836	40	M	_	_	NT	NT	NT	NT	Not Applicable
29	2839	40	M	+	+	S	S	S	S	Non Resistant
30	2821	52	M	+	+	S	S	S	S	Non Resistant
31	658	29	M	NC	+	S	S	S	S	Non Resistant
32	2875	45	M	Resistant	+	S	R	R	S	MDR-TB
33	703	25	F	_	_	NT	NT	NT	NT	Not Applicable

44

34	2901	20	F	+	_	**NT**	**NT**	**NT**	**NT**	Not Applicable
35	2695	22	F	+	+	**S**	**S**	**S**	**S**	Non Resistant
36	2924	20	F	_	+	**S**	**S**	**S**	**S**	Non Resistant
37	2943	43	M	_	_	**NT**	**NT**	**NT**	**NT**	Not Applicable
38	2922	30	F	_	_	**NT**	**NT**	**NT**	**NT**	Not Applicable
39	2818	30	F	+	_	**NT**	**NT**	**NT**	**NT**	Not Applicable
40	580	17	F	**NC**	+	**S**	**S**	**S**	**S**	Non Resistant

KEY: M – Male, F – Female, + = Positive, - = Negative, NC = Not certain, NA = Not Applicable, S = Sensitive, R = Resistant, Error = Rejected by the Gene-Expert machine, DRTB = Drug Resistant TB, MDRTB = Multidrug resistance TB. *NT = Not Tested because culture was negative.

In conclusion, these results provide evidence for the circulation of MDRTB strains in Adamawa State. It also provides a confirmation for earlier reports that MDRTB are still prevalent in Nigeria. Therefore, this underscores the need to provide interventions to control MDR-TB in the state and to make such interventions available and closer to the patients.

References

1. Abanda, N.N., Djieugoue, J.Y., Lim, E., Pefura-Yone, E.W., Mbacham, W.F., Vernet, G., Penlap, V.M., Eyangoh, S.I., Taylor, D.W. and Leke, R.G.F. (2017). Diagnostic accuracy and usefulness of the genotype MTBDR plus assay in diagnosing multidrug resistant tuberculosis in Cameroon: a cross-sectional study. BMC Infectious Diseases **17**: 397.
2. Adamawa State Government. (2017). Available from https://www.informationng.com/tag/adamawa-state. Accessed 10.11.2017.
3. Ananthanarayan, R. and Panicker C. (2009). Textbook of Microbiology, 8th edition.
4. Araoye, M.O. (2003). Research Methodology with Statistics for Health and Social Sciences. Nathadex Publishers, Ilorin.
5. Audu, E.S., Gambo, M.S. and Yakubu, A.A. (2017). Rifampicin resistant mycobacterium tuberculosis in Nasarawa State, Nigeria. Nigerian Journal of Basic Clinical Sciences **14**: 21-25.
6. Chan, E.D., Laurel, V. and Strand, M.J. (2004). Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. American Journal of Respiratory and Critical Care Medicine **169**(10): 1103-1109.
7. Coovadia, Y.M., Mahomed, S., Pillay, M., Werner, L. and Mlisana, K. (2013). Rifampicin Mono-Resistance in *Mycobacterium tuberculosis* in Kwazulu-Natal, South Africa: A significant phenomenon in high prevalence Tb/HIV Region. PLos One **8**: e77712.
8. Daniel, O. and Osman, E. (2011). Prevalence and risk factors associated with drug resistant Tuberculosis in South West Nigeria. Asian Pacific Journal of Tropical Medicine **4**: 148-151.
9. Dauby, N., Muylle, I., Mouchet, F., Sergysels, R. and Payen, M.C. (2011). Meropenem/Clavulanate and Linezolid Treatment for Extensively Drug-Resistant Tuberculosis. Pediatric Infectious Diseases Journal **30**(9): 812–813.
10. Elzinga, G., Ravigllone, M.C. and Maher, D. (2004). Scale up: Meeting targets in global tuberculosis control. Lancet **363**(9411):841-849.
11. Federal Ministry of Health. (2015). The National Strategic Plan for Tuberculosis Control: Towards Universal Access to Prevention, Diagnosis and Treatment; 2015-2020. National Tuberculosis and Leprosy Control Programme, Department of Public Health, Federal Ministry of Health, Nigeria, p1-201.
12. Ginsberg, A.M. (2008). Emerging drugs for active tuberculosis. Seminar in Respiratory and Critical Care Medicine **29**: 552–559. https://doi.org/10.1055/s-0028-1085706.
13. Glaziou, P., Falzon D., Floyd, K. and Raviglione, M. (2013). Global epidemiology of Tuberculosis. Journal of Respiratory and Critical Care Medicine **34**(1): 1-26.
14. John, S., Gidado, M., Dahiru, T., Fanning, A., Codlin, A.J. and Creswell, J. (2015). Tuberculosis among nomads in Adamawa, Nigeria: outcomes from two years of active case finding. International Journal of Tuberculosis and Lung Diseases **19**(4): 1-7.
15. Kabir, M., Obasanya, O. and Van der Grinten, E. (2010). National Tuberculosis and Leprosy Control Programme, Workers Manual (5th Edition) Federal Ministry of Health, p. 1-64.
16. Lamikanra, A. (2010). Essential Microbiology. Amkra Books, pp. 395-404.
17. Merle, C.S., Fielding, K., Sow, O.B., Gninafon, M., Lo, M.B., Mthiyane, T., Odhiambo, J., Amukoye, E., Bah, B., Kassa, F.N., Diaye, A., Rustomjee, R., de Jong, B.C., Horton, J., Perronne, C., Sismanidis, C., Lapujade, O., Olliaro, P.L., Lienhardt, C. and OFLOTUB/Gatifloxacin for Tuberculosis Project. (2014). A four-month gatifloxacin-containing regimen for treating tuberculosis. North England Journal of Medicine **371**: 1588–1598. https://doi.org/10.1056/NEJMoa1315817.
18. Orenstein, E.W., Basu, S. and Shah, N.S. (2009). Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infectious Diseases **9**(3): 153–161.

19. Otu, A., Umoh, V., Habib, A., Ameh, S., Lawson, L. and Ansa, V. (2013). Drug Resistance among Pulmonary Tuberculosis patients in Calabar, Nigeria. Pulmonary Medicine 235190, http://dx.doi.org/10.1155/2013/235190.

20. Petri, W.A. (2006). Chemotherapy of Tuberculosis, *Mycobacterium avium* Complex Disease, and Leprosy, in Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th Edition, Ed: Brunton, L. L., Lazo, J. S. and Parker K. L. McGraw-Hill, USA, p 1203-1224.

21. Ruan, Q., Liu, Q., Sun, F., Shao, L., Jin, J., Yu, S., Ai, J., Zhang, B. and Zhang, W. (2016). Moxifloxacin and gatifloxacin for initial therapy of tuberculosis: a metaanalysis of randomized clinical trials. Emerging Microbes and Infections **5**: e12. https://doi.org/10.1038/emi.2016.12.

22. Shrivastava, S., Magombedze, G., Koeuth, T., Sherman, C., Pasipanodya, J. G., Raj, P., Wakeland, E., Deshpande, D. and Gumbo, T. (2017). Linezolid dose that maximizes sterilizing effect while minimizing toxicity and resistance emergence for tuberculosis. Antimicrobial Agents and Chemotherapy Doi:10.1128/AAC.00751-17.

23. Tessema, B., Muche, A., Bekele, A, Reissig, D, Emmrich, F. and Sack, U. (2009). Treatment outcome of tuberculosis patients at Gondar University Teaching Hospital, Northwest Ethiopia. A five-year retrospective study. BMC Public Health **9**: 371–378.

24. Toma, M.M. (2014). Determinants of Retreatment with First Line Anti Tuberculosis Drugs in Smear Positive Patients in Adamawa State, October 2012 – June 2013. MPH Dissertation, Ahmadu Bello University Zaria, Nigeria.

25. Uplekar, M. and Raviglione, M. (2015). WHO's end TB strategy: From stopping to ending the global TB epidemic. Indian Journal of Tuberculosis **62**: 196-199.

26. Wayne, L.G. and Sramek, H.A. (2011). Metronidanizole is bactericidal to dormant cells of *Mycobacterium tuberculosis*. Antimicrobial Agents and Chemotherapy **38**(9): 2054–2058.

27. World Health Organization. (2016). Global Tuberculosis Report, Geneva: WHO Press, pp. 1-26.