

Multi-drug resistant *Mycobacterium tuberculosis* among presumptive individuals attending clinic in North Central, Nigeria

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ABSTRACT

Increasing resistance to antimicrobial medicines is posing a serious threat to public health and patient management globally. In recent years the problem of tuberculosis (TB) has been compounded by the emergence of multidrug-resistant (MDR) strains. Due to rapid growth in the population of the states within the capital region of the country, the study was carried out to give an insight to major areas attention is needed for interventions in the control of drug resistant tuberculosis (DR TB) and patient management. Six hundred and ninety-six sputum samples were decontaminated (using N-acetyl-L-cysteine sodium hydroxide - NALC NaOH), concentrated (via centrifugation) and resuspended in 1.5 ml. of sterile phosphate buffer using 'Petroff' method. Each specimen was inoculated into two Lowenstein Jensen slants prepared according to the standard operating procedure (SOP). Ninety-seven culture positive isolates were subjected to drug susceptibility testing (DST) with First-Line anti-TB drugs using 'proportion' method by following the SOP. The mean age of the subjects was 33.61 ± 10.92 , while the age group with the highest frequency (282, 40.5%) was 29 to 39 years. The study population had a higher representation of males (473, 68%), than females (223, 32%). Six hundred and ninety-six sputum samples were cultured out of which 97 (13.94 %) were culture positive, 542 (78.9%) were culture negative and 57 (8.2 %) were culture contaminated. Samples from Niger State had the highest culture-positive (21, 21.65 %) result. Forty-nine isolates (50.5%) were resistant to at least one of the first-line anti-TB drugs. Thirty six percent (36%) of the isolates exhibited the highest level of resistance to Streptomycin while 4.1% was noted against ethambutol. In total, 32 isolates (33%) were mono-resistant and 9 isolates (9.3%) were poly-resistant. MDR TB was detected in 8 cases (8.2%). A total of 9 different susceptibility profiles were identified in the study. In conclusion, that the study revealed 50.5% of the isolates being resistant to at least one of the first-line anti-TB drugs cannot be ignored. Nasarawa, Benue, Niger, FCT and Kogi from the study seem like 'hot spots' for which interventions such as active case finding, contact tracing and the use of radio jingles and Information, Education and Communication (IEC) materials to create more awareness of increasing cases of DR TB in the growing population.

Keywords: Multi-drug resistance, drug susceptibility testing, prevalence, North Central Nigeria.

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INTRODUCTION

Increasing resistance to antimicrobial medicines is posing a serious threat to public health and patient management globally (Otokunefor et al., 2018). In recent years the problem of tuberculosis has been compounded by the emergence of multidrug-resistant (MDR) strains (Otokunefor et al., 2018). An estimate of 110,000

individuals die annually due to MDR-TB (Asgedom et al., 2018).

Many countries have put in efforts in testing, detection and treatment of multidrug resistance/Rifampicin resistance (MDR/RR) TB, especially between 2017 and 2018 (WHO, 2019). MDR/RR-TB cases notified, has

been on the rise. In 2016, the total notified globally was 153,119 (TBFACTS.ORG; Onyedum et al., 2017) then in 2018 a total of 186,772 cases of MDR/RR-TB were detected and notified and 156,071 cases were enrolled on treatment. Nigeria is among the 10 countries that contributed to 75% of the global gap between treatment enrolment and estimated number of new cases of MDR/RR-TB (WHO, 2019).

WHO in their 2016 global TB report noted that MDR TB is caused by certain strains of *Mycobacterium tuberculosis* that is resistant to both isoniazid and rifampicin (WHO, 2016). Apart from MDR-TB other forms of drug-resistant TB exist (WHO, 2008). Two pathways give rise to drug resistant TB: acquired (secondary) and primary drug resistance (WHO, 2019). Drug Susceptibility Testing (DST) is used to classify drug resistant cases in clinical isolates that are culture positive. This classification includes Mono-resistance, Poly-resistance, Multidrug resistance (MDR), Extensive drug resistance (XDR) and Rifampicin resistance (RR). WHO consolidated guideline on drug resistant tuberculosis treatment, 2019, further described RR-TB strains as strains that are not susceptible to rifampicin based on DST (WHO, 2019).

The treatment of MDR TB takes a long time and requires close supervision as a result of complications that may arise due to the toxicity of the regimen involved. Several factors may be involved that might lead to the acquisition of *Mycobacterium tuberculosis* resistance. Such factors may include; use of substandard drugs (van der Werf et al., 2012), previous exposure to certain antibiotics like quinolones (Deutschendorf et al., 2012), past treatment of drug susceptible TB whereby patient refuses to adhere to treatment (Zhao et al., 2012; Bloëndal, 2007), or lack the knowledge of why it is important to complete treatment and sometimes high human immunodeficiency virus co-infection (Jindani and Enarson, 2004; Suchindran et al., 2009).

Nigeria is among the 14 high burden countries for TB, TB/HIV and Multi Drug Resistant TB. The country is ranked seventh among the 30 high TB burden countries and second in Africa (WHO, 2015; WHO, 2018). There is need for renewed effort in detecting the missing cases of TB and building capacity for diagnosing and treating drug resistant TB in specific geographic areas in the country where the prevalence is high. The estimated incidence of TB in Nigeria is 322 per 100,000 population (WHO, 2016). The estimation for MDR/RR-TB according to the same report was 4.3% among new cases and 25% among previously treated (WHO, 2016).

Nigeria adopted the molecular Xpert MTB/RIF assay as the first point of TB diagnosis in 2016 (Gidado et al., 2019). DR-TB management is based on laboratory confirmation of TB supported by drug susceptibility testing to ensure accurate diagnosis and placement on the appropriate treatment regimen (Deutschendorf et al., 2012). A good number of research works have reported

on the rates of DR-TB in different settings in the country (Otokunefor et al., 2018; Adejumo et al., 2018). Some have done a comprehensive analysis of DR-TB burden as well (Onyedum et al., 2017; Fadeyi et al., 2017).

The North Central Zone (NCZ) is made up of 7 States and due to rapid growth in the population, there is a corresponding expansion of business activities in the zone. The States that make up the NCZ include; Benue, Nasarawa, Niger, Kogi, Kwara, Plateau and FCT. Data are available on the prevalence of DR-TB in Nigeria and some other parts of the country, but there is a dearth of information on the same topic in the north-central zone. The study, therefore, gives an insight into the extent of DR TB in the zone where the capital of the country is situated. This will guide the choice of interventions that can be put in place to control DR TB and patient management in the zone.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Federal Ministry of Health, Abuja. The Ethics approval number is as stated: NHREC Approval Number NHREC/01/01/2007-16/03/2018. Informed consent was obtained from all participants in the study.

Study area

The study was conducted in Zankli TB Reference laboratory in Bingham University Karu, Nasarawa State. This laboratory is centrally located and is of National TB Reference laboratory standard. Samples were collected from presumptive TB individuals attending clinics in some high-volume facilities in north central states (Figure 1).

Study population

Participants between the ages of 18 and 75-years were enrolled in the study. In each state most of the samples came from high volume facilities offering TB services irrespective of whether it is public or private facility. It was difficult to get the estimated number of clinic attendees that needed TB services since samples were from different facilities. Within the period of sampling, March, 2017 to May, 2018, TB presumptive individuals (with signs and symptoms of TB {cough for 2 weeks or more}) that consented to participate were enrolled and samples were collected according to standard operating procedures.

Sample size

A total of 696 samples from 696 presumptive individuals were used for the study. The number of samples collected from each state is as in Table 1. The sample size did not put into consideration the burden of TB as well as the population of various states.

Sample collection and processing

One sputum sample was collected from each participant and were transported in cold chain (triple packaged) to Zankli laboratory in

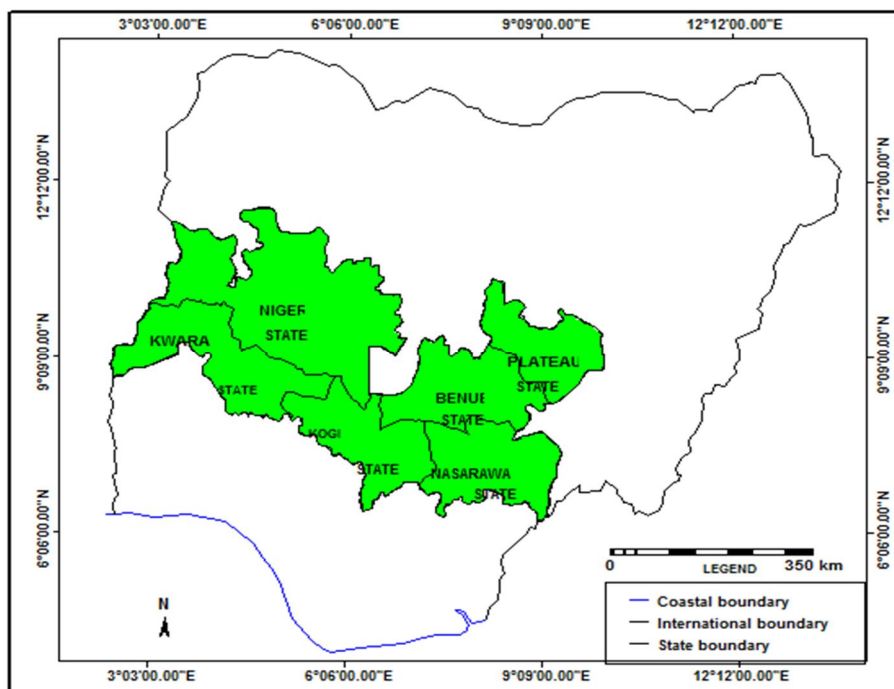


Figure 1. Map of North Central showing the 7 States that made it up. Adopted from: <https://www.google.com/search?q=map+of+north+central+states+of+nigeria&tbm>.

Table 1. Number of samples collected from each State.

S/N	State	Number of presumptives sampled
1	Nasarawa	100
2	Niger	100
3	Plateau	100
4	Benue	100
5	FCT	96
6	Kogi	100
7	Kwara	100
	Total	696

Bingham University, Karu, Nasarawa State where they were decontaminated (using 4% N-acetyl-L-cysteine sodium hydroxide {NALC NaOH}), concentrated (via centrifugation and resuspended in 1.5 ml of sterile phosphate buffer) using Petroff method. Furthermore, each specimen was inoculated into two Lowenstein Jensen slants (one containing pyruvate which supports the growth of *Mycobacterium bovis* and the other containing glycerol which supports the growth of other *Mycobacterium tuberculosis* complex) prepared according to the Standard Operating Procedure (SOP) (Canetti et al., 1969). The set up was incubated at 37°C and inspected for characteristic growth daily in order to track growth of contaminants. The set up was monitored for a period of 8 weeks to give enough room for the growth of the organism since *Mycobacterium tuberculosis* complex are slow growers.

In order to guide appropriate therapy for the treatment of tuberculosis, each first culture of *M. tuberculosis* complex (MTBC) isolated from a patient is tested for susceptibility to each of the four first line anti-TB drugs. Out of the 696 sputum samples from the study participants 97 were culture positive. Drug Susceptibility

Testing (DST) was performed on the culture positive samples for isoniazid (INH), rifampicin (RMP), ethambutol (EMB) and Streptomycin (STR) using proportion method which determines the percentage of growth (number of colonies) of a defined inoculum on a drug-free control medium versus growth on culture media containing the critical concentration of an anti-TB drug.²⁰ Each drug has varying strengths and so have different working concentrations that could achieve minimal growth of the organism. Calibrated bacterial suspension from positive culture were inoculated on media containing INH (0.2 µg/ml), RMP (40 µg/ml), EMB (2 µg/ml) and STR (4 µg/ml). The objective of the technique is to achieve a growth of greater than 5 colony forming units on the growth control (drug free) medium using the most dilute suspension for inoculation. The inoculated medium was incubated at temperature of 36 ± 1°C. The set up was examined daily for contamination and DST interpretation was carried out after 4 and 6 weeks of incubation.

Inclusion and exclusion criteria

Only presumptive TB individuals that agreed to participate were recruited in the study. Co-morbid conditions were not considered. Individuals with ages below 18 years and above 75 years were excluded. Patients currently on TB treatment regimen were also excluded. Extra Pulmonary TB (EPTB) was not considered. Patients were grafted from various clinics irrespective of public or private.

Data collection

The data instruments include a structured questionnaire to enable the collection of some demographic data such as state, age, gender, occupation and HIV status.

Data analysis

Data was analyzed using IBM SPSS Statistics 20 to calculate the mean age of the participants, the frequency of age groups and gender.

RESULTS

Demographic data

An analysis of the socio demographic characteristics of the study population showed that the mean age of the subjects was 33.61 ± 10.92 , while the age group with the highest frequency (40.5%) was 29 to 39 years (study participants in this age group are more in number compared to other age groups [Table 2]). The study population had a higher representation of males (473, 68%), than females (223, 32%). Majority of participants (60.1%) were self-employed while those under Government/Private firm employment were the least in occurrence (5.3%).

Culture positive results disaggregated by State

Six hundred and ninety-six sputum samples were cultured out of which 97 (13.94%) were confirmed as *M. tuberculosis* complex (as a result of the characteristic growth on LJ slant) and 57 (8.2%) were culture contaminated. Samples from Niger State had the highest culture positive (21.65%) result (Figure 2).

Rates of resistance to first-line anti-TB drugs

It was observed from the study that 49 isolates (50.5%) were resistant to at least one of the first-line anti-TB drugs which include; streptomycin, rifampicin, ethambutol and isoniazid. Thirty six percent (36%) of the isolates exhibited the highest level of resistance to Streptomycin while 4.1% was noted against ethambutol (Figure 3).

Drug resistant patterns in North Central Zone

In total, 32 isolates (33%) were mono-resistant and 9 isolates (9.3%) were poly-resistant (Table 3). MDR tuberculosis was detected in 8 cases (8.2%). A total of 9 different susceptibility profiles were identified in the study (Table 3).

DISCUSSION

Tuberculosis is a serious public health problem globally. The emergence of Drug resistance worsened the situation, that every attempt to control the disease are met with 'road blocks. Samples from two states, Kogi

Table 2. Socio demographic characteristics.

Variables	Frequency N=696	Percent
Age (in years)		
18 – 28	250	35.9
29 – 39	282	40.5
40 – 50	117	16.8
51 – 61	32	4.6
Above 61	15	2.2
Mean 33.61 ± 10.92		
Gender		
Male	473	68.0
Female	223	32.0
Occupation		
Govt/Private firm employed	37	5.3
Unemployed	145	20.8
Student	96	13.8
Self Employed	418	60.1
Culture Results		
Positives	97	13.94
Negatives	542	77.9
Contaminated	57	8.2
DST Results		
R*/S* to Streptomycin	35/62	5.0/8.9
R/S to Isoniazid	19/78	2.7/11.2
R/S to Rifampicin	11/86	1.6/12.4
R/S to Ethambutol	4/93	0.6/13.4

*R = Resistant, S = Susceptible

and Plateau recorded low culture positivity rate 7.22 and 5.15% respectively, this could be attributed to high contamination rate 14.2 and 12.7% respectively observed with samples from the two States.

A high level of resistance, 50.5%, was observed among the isolates with First-Line anti TB drugs. The highest resistance was with streptomycin, 36% of the isolates. This confirms the reason for removal of Streptomycin from First-Line regimen of anti-TB drugs. A higher RR-mono-resistance, 4.1% was recorded in the study compared to INH-mono-resistance, 3.1%. This was in line with what was observed in a study among PLHIV in Western India (Saldanhai et al., 2019), although with a high margin which recorded RR-mono-resistance of 12.5% as compared to INH-mono-resistance of 2.5%. Culture positive rate recorded in the study (13.94%) was lower than what was obtained in a similar study in Anambra State (33%) (Uzoewulu et al., 2016) and Lagos 37.7% (Olusola et al., 2018). The low culture positivity rate observed in the study may be due to excessive

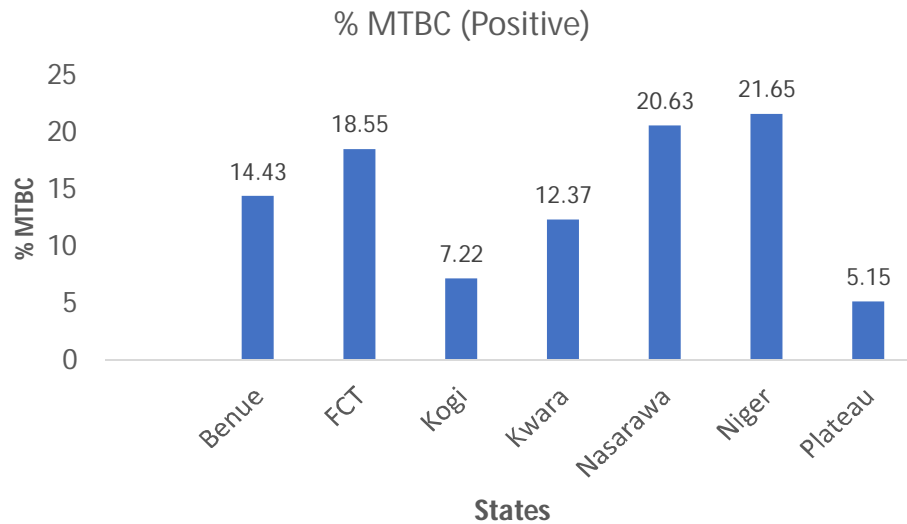


Figure 2. Solid culture positive results disaggregated by State. *MTBC: *Mycobacterium tuberculosis* complex.

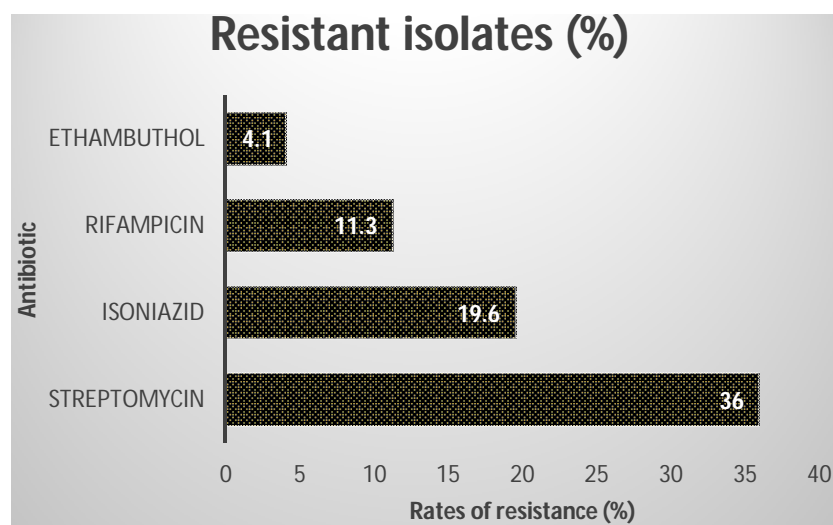


Figure 3. Rates of resistance to first-line anti-TB drugs.

decontamination of the sputum samples which is a critical process in culture procedure. The cumulative rate of monoresistance to first-line anti-TB drug in the north central zone was 33.0%. Isolates from Nasarawa (8) and Benue (8) States have the highest monoresistance to first-line anti-TB drugs followed by Niger (7) and FCT (6). On the other hand, the cumulative rate of polyresistance to first-line anti-TB drugs was 9.3% with isolates from Nasarawa (3) and Niger (3) leading.

The study revealed MDR TB rate of 8.2% but did not distinguish the rate among new and retreatment cases. Similar study in China (Liang et al., 2012) revealed a high prevalence of MDR TB of 30.4% among retreatment cases and 7.2% among new patients. A systematic review and meta-analysis study in Nigeria revealed MDR

TB prevalence of 6.0% among new cases and 32.0% among retreatment cases (Onyedum et al., 2017). Similar review in India (Goyal et al., 2017) revealed a pooled estimate of MDR TB resistance higher in previously treated patient (29.8%) as compared to newly diagnosed cases (4.1%) (Deutschendorf et al., 2012). Isolates from TB patients in Kogi (3) had the highest number of MDR-TB followed by Benue (2) and Niger (2). The study recorded 9 different susceptibility profiles.

The study showed a relatively low prevalence of DR TB in north central zone when compared to what was obtained elsewhere around the world and within the country. Despite the limitations of the study which include the non-use of state population or annual DR TB notification of the states for sampling and high

Table 3. Drug resistant patterns of *Mycobacterium tuberculosis* isolates.

Resistance profile	Number of isolates							
	n = 97							
	Nasarawa	Niger	Plateau	Benue	FCT	Kogi	Kwara	Total
Mono-resistant								
INH ⁺	1				2			3 (3.1%)
EMB	3							3
RIF	1			1		2		4 (4.1%)
STR	3	7		7	4	1		22
Sub-total	8	7	0	8	6	3	0	32 (33%)
Poly-resistant								
STR-RIF	1	1						2
STR-INH	2	1		1	2			6
INH-EMB		1						1
Sub-total	3	3	0	1	2	0	0	9 (9.3%)
Multi-drug resistant								
INH-RIF		1				1	1	3
STR-INH-RIF		1		2		2		5
Sub-total		2	0	2	0	3	1	8 (8.2%)

+INH = Isoniazid, EMB = Ethambutol, RIF = Rifampicin, STR = Streptomycin.

contamination rate observed in the culture procedure, more work is still required in the area of drug resistant tuberculosis in the zone because a single case could have a multiplier effect when neglected.

Conclusion

The fact that 50.5% of the isolates were resistant to at least one of the first-line anti-TB drugs cannot be ignored. The study stands to guide choices of areas in the region that requires immediate interventions. Nasarawa, Benue, Niger, FCT and Kogi from the study seem like 'hot spots' for which interventions like active case finding, contact tracing and the use of radio jingles and Information, Education and Communication (IEC) materials to create more awareness of increasing cases of DR TB in the population.

Abbreviations: **TB**, Tuberculosis; **MDR TB**, Multidrug resistant Tuberculosis; **WHO**, World Health Organization; **DST**, Drug susceptibility testing; **MTBC**, *Mycobacterium tuberculosis* complex; **RIF**, Rifampicin; **HIV**, Human Immunodeficiency Virus; **AIDS**, Acquired Immunodeficiency Syndrome; **CT**, Contaminated; **Xpert**, GeneXpert; **NALC NaOH**, N-acetyl-L-cysteine sodium hydroxide; **NCZ**, North Central Zone; **DR TB**, Drug Resistant Tuberculosis; **SOP**, Standard Operating Procedure; **FCT**, Federal Capital Territory; **IEC**, Information Education and Communication; **RR TB**, Rifampicin Resistant Tuberculosis; **EPTB**, Extra

Pulmonary Tuberculosis; **XDR TB**, Extremely Drug Resistant Tuberculosis; **TB/HIV**, Tuberculosis/ Human Immunodeficiency Virus; **PLHIV**, People Living with Human Immunodeficiency Virus; **NHREC**, National Health Research Ethics Committee; **INH**, Isoniazid; **RMP**, Rifampicin; **EMB**, Ethambutol; **STR**, Streptomycin; **FL DST**, First Line Drug susceptibility testing; **LJ**, Loweisten Jensen; **IBM SPSS**, Statistical Package for Social Sciences version 20.

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