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Antimicrobial Susceptibility Profile of *Mycobacterium tuberculosis* among Patients with Pulmonary Tuberculosis in Anambra State, Southeast Nigeria

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Abstract

Background: *Mycobacterium tuberculosis* drug resistance i*s* a threat to global control of tuberculosis especially in resource limited settings. This study evaluated the anti-tuberculosis drug susceptibility pattern of *Mycobacterium tuberculosis*, strains isolated from patients with pulmonary tuberculosis in Anambra state, southeast Nigeria.

Methods: This was a cross-sectional study that involved the analysis of sputum samples of tuberculosis patients seen from 2010-2012 in randomly selected public hospitals in Anambra state, southeast Nigeria. All the sputum samples were processed according to standard operating procedure and cultured using Lowenstein Jensen medium (LJ). One hundred and eighty colonies of *Mycobacterium tuberculosis* strains grown were identified on the basis of morphology, pigment production and biochemical characteristics. Drug susceptibility testing of each isolate to the first line anti-TB drugs (Streptomycin, Isoniazid, Rifampicin and Ethambutol) was performed by proportional method on LJ medium.

Results: Of the 180 *M. tuberculosis* isolates, 143 (79.4%) were from new TB cases and 37 (20.6%) from retreatment TB cases. A total of 95 (52.8%) {95% CL: 45.34 - 59.76} strains were susceptible to either one or more Tb drugs, of which 86 (47.8%) were from new TB cases while 9 (5%) were from retreatment TB cases. A total of 85 (47.2%) {95% CI: 22.68 - 69.32. P = 0.0001} were drug resistant strains. Of these resistant strains, 57 (31.7%) {95% CI: 23.59 - 39.40} were from new TB cases, while 28 (15.6%) {95% CI: 7.58 - 22.42} were retreatment TB cases. The rates of the sensitivity to a single first line anti-TB drug were: Streptomycin 78%, Isoniazid 68%, Rifampicin 85% and Ethambutol 75%. The rates of resistance to a single anti-TB drug were: Streptomycin 22%, Isoniazid 32%, Rifampicin 15% and Ethambutol 25%). Resistance to drugs were higher with Isoniazid compared to other drugs while Rifampicin was more susceptible than other anti-TB drugs. Among the total of 180 culture positive TB cases, 34(18.9%) were found to be mono-resistance {95% CI: 10.45-26.58}. The prevalence rate 23(12.8%) among new TB cases was significantly higher than the retreatment 11(6.1%) diagnosed TB cases. About 16(8.9%) were MDR-TB [95%CI:2.34-15.55} with 7 (3.9%) new TB cases and 9(5%) retreatment TB cases . There was an association between retreatment TB cases and MDR-TB (P=0.0001). Of the total culture positive TB cases 35(19.4%) were Poly-resistant [95% CI: 11.66-27.34} of which the prevalence rate 27(15%) among new TB cases was significantly higher than 8(4.4%) retreatment TB cases and the difference was statistically significant. Drug resistance increased in both the new and retreatment TB cases. Also, of the 85 (47.2%) drug resistant TB, 14 (7.8%) (95% CL: 3.037: P = .386} were HIV positive while 71 (39.4%) were HIV negative. Those older than 21 years were most likely to have drug resistant TB (95% CI: 27.449: P = .156).

Conclusion: Retreatment cases of Pulmonary TB is significantly associated with drug resistance and patients who were treated at NAUTH Nnewi site had significantly higher rate of Multi-drug resistant TB compared to other hospitals investigated.

***Keywords**: Drug Resistance; Mycobacterium tuberculosis; Pulmonary Tuberculosis; Anambra State*

Abbreviations

Mono Drug Resistant: Resistance to One Drug Only; Multi Drug Resistant: Resistance to Isoniazid and Rifampicin; Poly Drug Resistant: Resistance to I or R, plus any one or two other drugs; S: Streptomycin; I: Isoniazid; R: Rifampicin; E: Ethambutol; DR-TB: Drug Resistant Tuberculosis; DSM: Direct Smear Microscopy; DST: Drug Susceptibility Testing; CAT 1: Category 1: New TB Cases; CAT11: Category 11: Retreatment TB Cases; TB: Tuberculosis; Pos: Positive; Neg: Negative; LJ: Lowenstein Jensen; NTM: Non- Tuberculosis Mycobacteria; ZN: Ziehl-Nielsen; AFB: Acid Fast Bacilli; HIV: Human Immunodeficiency Virus; NAUTH: Nnamdi Azikiwe University Teaching Hospital, Nnewi; GHO: General Hospital Onitsha; COOTH: Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka; OHI: Our Lady of Lourds Hospital Ihiala; *MTB: Mycobacterium tuberculosis*; PH: Positive HIV; NH: Negative HIV; NT: New TB; RT: Retreatment TB; PTB: Pulmonary Tuberculosis; SPSS: Statistical Package for Social Sciences, F:Female; M: Male.

Introduction

The scope of human immunodeficiency virus (HIV) pandemic and negligence in tuberculosis (TB) control in some developing countries have remarkably caused an increase in TB incidence worldwide. According to World Health organization, about 10 million people had tuberculosis with mortality rate of 1.7 million in 2019; 0.5 million were drug resistant and 56% were treated successfully [1]. Anti-TB drug resistance is one of the biggest public health challenges of our time. Fighting this threat is a public health priority that re- quires a collaborative global approach across all sectors of healthcare. Drug resistance in *M. tuberculosis* isolates arises from spontaneous genetic mutations and poor adherence to anti-TB drugs [2]. However, transmission of drug resistant TB is associated with the virulence of the drug resistant strain and the susceptibility of the population [3].

Nigeria is among the 14 high burden countries with TB/HIV and drug resistant TB [4]. One of the major issues with TB care in Nigeria is the low TB case finding for both adults and children. Out of 429,000 estimated TB cases in Nigeria only 24% receives appropriate treatment [1]. About 150,000 people die each year from TB with an estimated 39,000 of these deaths reported among people with HIV [5]. In Anambra state a TB prevalence ranging from 12.3-15.4% has been reported in different parts of the state with a HIV prevalence of 2.4% and TB/HIV co-infection of about 11.9% [6,7]. A total of 1,783 drug resistant TB cases were notified out of an estimated 5,200 in Nigeria [5]. According to the health system report, prevalence of drug-resistance varies depending on methods of drug susceptibility testing used and geographic region of Nigeria [4].

However continuous surveillance and frequent monitoring of drug resistance based on routine drug susceptibility testing of TB patients is important to assess the prevalence and trends of anti-TB drug resistant especially in a country like Nigeria with high burden of tuberculosis..[8] Anambra state has limited public health laboratory infrastructure and capacity to perform cultureand drug susceptibility testing of *M. tuberculosis,* even in patients known to have drug resistant strains. The consequence is that majority of active TB and MDR-TB cases remain undiagnosed and this impacts negatively on the global strategy of eliminating TB [5]. The geneXpert MTB/RIF® test was recommended as the primary TB diagnostic tool by the National Tuberculosis and Leprosy Control Programme but compared with the reference standard of culture, geneXpert MTB/RIF® has suboptimal sensitivity and specificity especially in individuals with smear-negative TB and drug resistant TB [9]. Furthermore, Nigeria has a low coverage of the geneXpert MTB/ RIF® technology for TB diagnosis and only 6.3% of primary health care facilities in the country had access to the test in 2017, leaving them to depend on identification of acid-fast bacilli (AFB) on direct sputum smear microscopy with Ziehl-Nielson staining technique [10].

There is paucity of information on the susceptibility of pattern of *M. tuberculosis* isolates against anti-tuberculosis drugs in Anambra state. Drug susceptibility pattern is an important aspect of TB control and surveillance because it helps in detection and monitoring of the extent of drug resistance and multi drug resistant and the quality of TB control in the country. We evaluated the anti-TB drug susceptibility of *M. tuberculosis* isolated from patients with pulmonary TB attending four public hospitals in Anambra state, Nigeria.

Materials and Methods

Study area

The study was carried out in 4 randomly selected public hospitals in Anambra state, southeast Nigeria : Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, General Hospital Onitsha (GHO) and Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka (COOTH) and Our Lourds Hospital Ihiala, (OHI).

Study population: The study population was, all suspected TB patients and those currently receiving treatment for pulmonary TB in the selected hospitals**.**

Inclusion criteria: All new pulmonary TB suspects of all ages and known pulmonary TB patients who had failed first line anti-TB treatment who gave a written informed consent for the study and HIV counseling and testing**.**

Exclusion criteria: Suspected and old patients on TB treatment that refused consent to undergo HIV testing and to participate in the study**.**

Ethical consideration

Ethical approval was obtained from the ethics committee of NAUTH Nnewi. A signed informed consent was obtained from each participant after detailed explanation of the study objectives to them. The study was conducted in accordance with the declaration of Helsinki.

Laboratory diagnosis Specimen collection

This cross sectional study was carried out within the period 2010 - 2012. Three sputum samples were collected from each of the 180 known TB and previously treated patients (spot, early morning sputum and spot in sterile screw capped containers) and maintained at temperature of 4^0C - 8^0C**.**

Laboratory procedure

Sputum samples of each patient were processed within 24hours of collection and quantified using standard operating procedures [11]. Sputum samples were sent to Zanklin Medical Centre Abuja in cold ice pack where cultures on Lowenstein -Jensen solid medium were done using modified Petroffs method [12]. Sputum samples were decontaminated by centrifugation at 3000g for 15 minutes. Sediments were re-suspended in 2 ml phosphate buffer at pH of 6.4 and 2 drops of the sediments were inoculated on slopes of already prepared LJ medium.

The inoculated LJ slopes were incubated at 37^0C and examined on the 4th day for contamination and weekly for 6 - 8 weeks for growth of

M. tuberculosis [13]. The growth and morphology of the colonies were noted and biochemical tests such as niacin, nitrate reduction and catalase test were performed on the colonies and confirmed with ZN stain which identified serpentine cords typical of *Mycobacterium tuberculosis* (MTB) [14].

The blood samples of all patients (5 mls) were collected in a dry sterile screw capped containers for HIV screening, using stat-pak and kits as directed by the manufacturer.

Drug susceptibility testing (DST)

All culture positive *M. tuberculosis* identified colonies were subjected to drug sensitivity testing on LJ medium slope using proportional method. The four first line anti-TB drugs were incorporated into the LJ slop with known concentration; Streptomycin (1.00 µg/

ml), Isoniazid (0.10 µg/ml), Rifampicin (1.00 µg/ml) and Ethambutol (5.00 µg/ml) [12]. The medium was incubated at 35 - 37^{0}C for 6 - 8 weeks.

Quality assurance

External and internal quality control using appropriate standard operating procedure was developed and followed. In-house known positive strain and H37RV *Mycobacterium tuberculosis* positive strain was used as positive control strain and sterile LJ medium was used as negative control culture. Results were recorded according to a grading system [11].

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 21. Relevant frequencies and proportions were calculated. Statistical significance was set at 95% confidence level. The sensitivity patterns of new and previously treated TB cases were described for patients with and without HIV.

Results

Out of 180 culture positive *M. tuberculosis strains i*solated from TB patients, 109 (61%) were from males and 71 (39%) females within the age range of 1-80 years. The age group most affected with tuberculosis was 21- 40 years, 117 (65.0%), with the average mean (SD) age of 35 years, followed by 41- 60 years, 35 (19.0%). Out of 180 TB patients 34 (19.0%) were HIV positive while 146 (81.0%) were HIV negative TB cases. Categorically,143 (79%) were new TB cases and 37 (21%) retreatment TB cases. The majority of the study participants, 77 (42.8%) were occupation- ally in business (Table 1).

Category	NAUTH	%	GHO	%	COOTH	%	OHI	%	Total	%
CAT 1	81	45	60	33.3	1	0.5	1	0.5	143	79
CAT 11	28	15.6	9	5	-		-	-	37	21
Total	109	61	69	38	1	0.5	1	0.5	180	100
Age										
1 - 20	10	5.6	4	2.2	-	-	-	-	14	8
21 - 40	70	38.9	45	25	1	0.5	1	0.5	117	65
41 - 60	23	12.8	12	6.7	-	-	-	-	35	19
61 >	6	3.3	8	4.4	-	-	-	-	14	8
Total	109	61	69	38	1	0.5	1	0.5	180	100
Gender										
Male	63	35	45	25	1	0.5	-	-	109	61
Female	46	25.6	24	13.3			1	0.5	71	39
Total	109	61	69	38	1	0.5	1	0.5	180	100
HIV										
Positive	24	13.3	10	5.6	-	-	-	-	34	19
Negative	85	47.2	59	32.8	1	0.5	1	0.5	146	81
Total	109	61	69	38	1	0.5	1	0.5	180	100
Occupation										
Business	42	23.3	34	18.9	1	0.5	-	-	77	43
Students	31	17.2	15	8.3	-	-	-	-	46	26
Workers	26	14.4	12	6.7		0.5	1	0.5	38	21
Civil servants	8	4.4	5	2.8	-	-	-	-	13	7
None	2	1.1	3	1.7	-	-	-	-	5	3
Total	109	61	69	38	1	0.5	1	0.5	180	100

Table 1: *Demographic characteristics of TB patients by hospitals.*

Drug susceptibility test results showed that, of 180 *M. tuberculosis strains* isolated from TB patients, a total of 95 (52.8%) {95% CL: 45.34 - 59.76} strains were susceptible to either one or more Tb drugs, of which 86 (47.8%) were from new TB cases while 9 (5%) were from retreatment TB cases. Also a total 85 (47.2%) {95% CI: 22.68 - 69.32. P = 0.0001} were drug resistantTB of which, 57 (31.7%) {95%CI: 23.59 - 39.4} were new TB cases and 28 (15.6%) {95%CI: 7.58 - 22.42} were retreatment TB cases [Table 2].

Susceptibility rate of the drugs	F	%	M	%	NT	%	F	%	M	%	RT	%	TNT/RT	%
Sensitive	32	17.8	54	30	86	47.8	4	2.2	5	2.8	9	5	95	52.8
Resistance	27	15	30	16.7	57	31.7	8	4.4	20	11.1	28	15,6	85	47.2
Total	59	32.8	84	46.7	143	79.4	12	6.6	25	13.9	37	20.6	180	100
P = 0.0001														

Table 2: *Total susceptibility rate of M. tuberculosis strains isolated from 180 TB patients to all drugs. F=Female. M=male.TN/R=Total new TB /Retreatment TB.*

The highest rate of sensitivity to a single first line anti-TB drug indicated, Rifampicin (R)85% followed by Streptomycin(S) 78%, Ethambutol(E) 75% and Isoniazid(I) 68%. Also Isoniazid (I) 32% showed the highest rate of resistance followed by Ethambutol (E)25%, then Streptomycin (S)22% with lowest rate of resistance to Rifampicin(R) 15% [Table 3].

Antibiotic pattern	No of Isolates	No Sensitive %		No Resistance %	
S	180	141	78%	39	22%
I	180	123	68%	57	32%
R	180	153	85%	27	15%
E	180	135	75%	45	25%

Table 3: *Susceptibility rate of Mycobacterium tuberculosis to a single drug. S = Streptomycin, I = Isoniazid, R = Rifampicin, E = Ethambutol.*

Among the 180 culture positive TB cases identified by hospitals, 34(18.9%) M.TB strains were found to be mono- drug resistant {95% CI: 10.45-26.58}. The prevalence rate 23(12.8%) among new TB cases was significantly higher than the retreatment 11(6.1%) diagnosed TB cases. About 16(8.9%) M.TB strains were MDR-TB [95%CI:2.34-15.55} with 7 (3.9%) new TB cases and 9(5%) retreatment TB cases . There was an association between retreatment TB cases and MDR-TB (P=0.0001). Of the total culture positive TB cases, 35(19.4%) M.TB strains were found to be Poly- drug resistant [95% CI: 11.66-27.34} of which the prevalence rate 27(15%) among new TB cases was significantly higher than 8(4.4%) retreatment TB cases and the difference was statistically significant [Table 4].

Also about 50 (27.8%) males and females 35(19.4%)(95% CI: 0.766 : P=.858) were drug resistance-TB patients while 59 (32.8%) males and 36(20%) females were susceptible to all anti-tuberculosis drugs. Of 85 (47.2%) drug resistant TB, 14 (7.8%) (95% CL: 3.037: P=.386} were HIV positive while 71(39.4%) were HIV negative drug resistant TB. HIV positivity have no association with drug resistant TB. The age groups older than 21years were most likely to have drug resistant TB (95% CI: 27.449: P=.156) [Table5].

Resistant pattern	NAUTH				GHO				COOTH				OHI	Total				Total%	
Mono-drug resistance	NT	%	RT	%	NT	%	RT	%	NT	%	RT	%	-	NT	%	RT	%	N/R	%
S	4	2.2	1	0.5	2	1.1	1	0.5	-	-	-	-	-	6	3.3	2	1.1	8	4.4
I	2	1.1	2	1.1	4	2.2	3	1.7	1	0.5	-	-	-	7	3.9	5	2.8	12	6.6
R	1	1.7	1	0.5	2	1.1	1	0.5	-	-	-	-	-	3	1.7	2	1.1	5	2.8
E	5	2.8	2	1.1	2	1.1	-	-	-	-	-	-	-	7	3.9	2	1.1	9	5
Total	12	6.6	6	3.3	10	5.5	5	2.8	1	0.5	-	-	-	23	12.8	11	6.1	34	18.9

Resistant pattern	NAUTH				GHO				COOTH				OHI	Total NT		Total RT		Total N/RT	
Multi-drug resistance	NT	%	RT	%	NT	%	RT	%	NT	%	RT	%	-	NT	%	RT	%	N/R	%
I/R	-	-	1	0.5	-	-	-	-	-	-	-	-	-	-	-	1	0.5	1	0.5
S/I/R	1	0.5	3	1.6	1	0.5	-	-	-	-	-	-	-	2	1.1	3	1.6	5	2.8
I/R/E	1	0.5	1	0.5	1	-	1	0.5	-	-	-	-	-	1	0.5	2	1.1	3	1.6
S/I/R/E	4	2.2	3	1.6	-	-	-	-	-	-	-	-	-	4	2.2	3	1.6	7	3.9
Total	6	3.3	8	4.4	1	0.5	1	0.5	-	-	-	-	-	7	3.9	9	5.0	16	8.9

Resistant pattern	NAUTH				GHO				COOTH				OHI	Total NT		Total RT		Total N/RT	
Poly-drug resistance	NT	%	RT	%	NT	%	RT	%	NT	%	RT	%	NT	%	RT	%	N/R	%	%
I/E	5	2.8	2	1.1	6	3.3	1	0.5	-	-	-	-	-	11	6.1	3	1.7	14	7.8
S/E	2	1.1	-	-	-	-	-	-	-	-	-	-	-	2	1.1	-	-	2	1.1
S/I	1	0.5	1	0.5	2	1.1	-	-	-	-	-	-	-	3	1.7	1	0.5	4	2.2
S/I/E	6	3.3	2	1.1	2	1.1	1	0.5	-	-	-	-	-	8	4.4	3	1.7	11	6.1
S/R/E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S/R	1	0.5	1	0.5	2	1.1	-	-	-	-	-	-	-	3	1.7	1	0.5	4	2.2
Total	15	8.3	6	3.3	12	6.6	2	1.1	-	-	-	-	-	27	15	8	4.4	35	19.4

Table 4: *Drug resistance pattern of M. tuberculosis isolated from new and retreatment TB patients at different hospitals.*

P = 0.0001.

Pattern of Antibiotics SIRE	F	M	PH	NH	NT	%	F	M	HV	NH	RT	%	T	%
Mono-resistance	9	14	3	20	23	12.8	4	7	1	10	11	6.1	34	18.9
MDR to IR	3	4	-	7	7	3.9	3	6	3	6	9	5.0	16	8.9
Poly-resistance	15	12	6	21	27	15	1	7	1	7	8	4.4	35	19.4
Total resistance	27	30	9	48	57	31.7	8	20	5	23	28	15.6	85	47.2
Mono susceptible	3	6	5	4	9	5	2	3	6	1	7	3.9	17	9.4
Other susceptible	29	48	8	69	77	42.7	2	2	1	1	2	1.1	78	43.3
Total susceptible	32	54	13	73	86	47.8	4	5	7	2	9	5	95	52.8
Total	59	84	22	121	143	79.4	12	25	12	25	37	20.6	180	100

Table 5: *Drug susceptibility pattern of M. tuberculosis by gender and HIV status of TB patients.*

Abbreviation

NT = New TB patients; RT = Retreatment TB patients; % = Percentage; NAUTH = Nnamdi Azikiwe University Teaching Hospital Nnewi; GHO = General Hospital Onitsha; COOTH = Chukwuemeka Odimegwu Ojukwu Teaching Hospital Awka; OHI = Our Lady of Lourds Hospital Ihiala; F = Female; M = Male; PH = Positive HIV; NH = Negative HIV.

Discussion

This study reported a high prevalence rate of 47.2% drug resistant TB against all first line anti-TB drugs in Anambra state. This is consistent with reports of 46.7% in port Harcourt [15], 42% in Calabar [16], 40% in Benin [17], 41.6% in Iraq [18] reflecting insufficient health services in the country. However, this was higher than previous reports of 20.23% in Ethiopia [19], 22.3% in Addis Ababa [17] and 20% for any form of anti-TB drug resistance globally [20], and 34.0%, 23.3%, 32.3% reported inother parts of Nigeria [4,21,22]. Other studies have reported a higher prevalence of resistance cases such as, 60.8% in Addis Ababa [23] 58.7% in Ethiopia [24], 57.8% in India [25] and 56% in Lagos Nigeria [26], showing variations of results in different countries according to methods for drug susceptibility testing. Of the 47.2% drug resistant TB, 31.7% were new TB cases while 15.6% retreatment TB cases, indicating a high level of emerging *M. tuberculosis* resistance to new TB cases. This observed increase in drug resistance along with differences between new TB cases and retreatment TB patients may suggest poor TB control programs and improper usage of anti-TB drugs which have led to accumulation and multiplication of resistance strains. Similar studies have documented high level of drug resistance TB in new cases; this may complicate patients' management globally [27].

Out of 180 culture positive samples analyzed for drug susceptibility test , 95 (52.8%) were sensitive to all four anti-TB first line drugs and 85 (47.2%) were drug resistant to one or more drugs. Of the 52.8% sensitive drugs, the rate at which each strains of *M. tu-berculosis* was susceptible to a single drug was; Streptomycin 78%, Isoniazid 68%, Rifampicin 85% and Ethambutol 75% while of 47.2% drugs resistance, the rate for each drug resistant was; Streptomycin 22%, Isoniazid 32%, Rifampicin 15% and Ethambutol 25% . Isoniazid 32% showed high level of resistance followed by Ethambutol 25% among others while Rifampicin 85% was more susceptible followed by Streptomycin 78% than other drugs. This result identified that, Isoniazid 32% mono-drug resistance was higher in proportion than others, according to a report Isoniazid reduces the effectiveness of conventional first line TB regimens and this often leads to poor response to treatment [28]. Resistance to streptomycin rarely impacts on outcomes because other agents can counter its effect while high resistance to isoniazid has a detrimental effect on clinical outcome because there is high risk of developing rifampicin resistance [28].

The pattern of drug resistant TB observed among the patients in different hospitals studied was, mono drug resistant 34 (18.9%), multi drug resistant 16 (8.9%) and poly drug resistant 35 (19.4%). Assessment of the drug resistance trend in both new and retreatment cases between the hospitals showed a significant increase in resistance to any anti-TB drug with variation in proportion to isoniazid 12 (6.6%), ethambutol 9 (5%) and streptomycin 8 (4.4%) and Rifampicin 5 (2.8%) with total mono-drug resistant of 34 (18.9%). Of the 18.9% mono-drug resistant TB cases, new Tb patients 23 (12.8%) were higher in number than retreatment TB patients 11 (6.1%). Individual hospitals showed; NAUTH had 12 (6.6%) new TB mono-drug resistant patients of which Ethambutol 5 (2.8%) was observed to be more resistant among patients followed by Streptomycin 4 (2.2%) than others while in retreatment TB patients was 6 (3.3%) and Ethambutol 2 (1.1%) was found to be at equal rate with Isoniazid 2 (1.1%) resistance than other drugs among the patients thus given a total mono- drug resistant rate of 18 (10%) at NAUTH Nnewi. GHO had 10 (5.5%) new TB mono-drug resistant patients of which Isoniazid 4 (2.2%) was more resistance than other drugs while in retreatment TB patients 5 (2.8%) and Isoniazid 3 (1.7%) was also found to be more resistance than others thus given a total mono-drug resistant of 15 (8.3%) at GHO Onitsha. COOTH Awka had 1 (0.5%) new mono- drug resistance TB case while OHI Ihiala did not have any drug resistance TB case. The result of the study indicated a higher mono-drug resistance TB cases 10% at NAUTH Nnewi followed by 8.3% at GHO Onitsha . Similar studies in Calabar Nigeria reported resistance to Ethambutol [8%], Streptomycin [7%] [16] and in Port Harcourt Nigeria resistance to Isoniazid (13.3%), streptomycin (26.7%), Ethambutol (20%) and rifampicin (10%) [29] while in Ethiopia resistance was Isoniazid (6.7%), rifampicin (3.89%), streptomycin (7.87%), Ethambutol (6.74%) [19]. The 2.8% resistance observed in this study for rifampicin was the lowest and it is in agreement with 2% resistance to rifampicin in a study from Lagos, Nigeria [26]. This observation has an important implication for national and global MDR-TB diagnosis because rifampicin resistance has been used as a maker for molecular detection of MDR-TB. When a significant proportion of rifampicin resistant isolated in a population are non MDR-TB, the predictive value of rifampicin resistance for MDR-TB is significantly reduced, although dif-

ferent populations may have different levels of accessing anti-TB drug over time. Multi-drug resistant TB (MDR-TB) 16 (8.9%) was found in this study with 7 (3.9%) new cases and 9 (5%) retreatment cases. The rate of acquired MDR-TB was higher 5% than the rate of primary MDR-TB 3.9%. There was an association between retreatment TB cases and MDR-TB (P = 0.0001). The finding was similar with the report in India 3.4% new cases of MDR-TB and 25% of retreatment MDR-TB cases, Nepal, 20.5% and 1.3% of acquired MDR-TB and primary MDR-TB (38) respectively and similar to other studies reported elsewhere. This is higher than the rate of MDR-TB 6.0% reported in other parts of Nigeria but agreed with an initial report of variation in different communities in the state [4]. According to individual hospitals evaluated, NAUTH had 6 (3.3%) new MDR-TB cases with 8 (4.4%) MDR retreatment TB cases thus indicated a total of 14 (7.8%) MDR-TB at NAUTH Nnewi. GHO Onitsha had 2 (1.1%) MDR.TB while other hospitals did not have MDR-TB cases. The high prevalence of MDR-TB reported in NAUTH Nnewi 7.8% is an indication of a larger epidemic than previously suspected in other parts of the country and even globally. The high rate of MDR-TB 8.9% cases in this study may be due to late identification of suspected MDR-TB cases. Also identification of all MDR-TB required culture and drug susceptibility testing of tuberculosis suspects as reference standard but in Nigeria the culture and sensitivity facilities for *M. tuberculosis* are only in Nigeria Institute of Medical Research Yaba, Lagos and a private TB laboratory, (Zankli Medical Laboratory Abuja) which are very far from the study sites. The culture method are not commonly implemented in Nigeria. Due to lack of funding and infrastructural requirements.

The gene Xpert MTB/RIF® test is recommended as the primary TB diagnostic tool in Nigeria by the National Tuberculosis and Leprosy Control Programme [9]. However, compared with the reference standard of culture,geneXpert MTB/RIF® has suboptimal sensitivity (especially in individuals with smear-negative TB and people living with HIV) and specificity. Furthermore, Nigeria has a low coverage of the gene Xpert MTB/RIF® technology for TB diagnosis. Only 6.3% of primary health care facilities in the country had access to the test in 2017, leaving them to resort to diagnosing of TB by identification of acid-fast bacilli (AFB) on direct sputum smear microscopy with Ziehl-Neelson staining technique [10]. MDR-TB may also be attributed to the quality of sputum used as well as the proficiency of laboratory scientists in reading the culture thus influencing the result. A missed diagnosis of MDR-TB positive patient prolongs morbidity and can ultimately lead to death in an individual. It also places a heavy financial burden on the patients, their families and country as a whole. Another concern is the high level of poly drug resistance 35 (19.4%) and majority of these were seen among new cases 27 (15%) than retreatment TB patients 8 (4.4%). The 19.4% is similar to the report of state of Qatar with 15% anti-TB drug resistant to one or more drugs among 406 cases of pulmonary TB cases [39] and other similar report poly-drug resistant TB cases elsewhere. The high rate of poly-drug resistance among new TB cases indicated that drug resistant strains are circulating and are being transmitted from patient to patient in the studied community and Nigeria as a country. A history of previous treatment for TB was a risk factor found to be associated with drug resistant TB. Several studies documented the association between a previous history of TB and anti-TB drug resistance. Epidemiologically, delay in obtaining early results translates to the drug resistant-TB cases remaining in the community for two months before effective clinical intervention. The pattern of drug resistance in this study noted that, both primary and acquired resistance have contributed to the drug resistant TB in the study area. Transmission of already resistant strains is a serious problem and threat, as it is difficult to treat patients who are infected with drug resistance TB. Therefore, it is important for a TB controlprogramme to have reliable laboratory facilities for susceptibility testing of *M. tuberculosis* isolates. Also, majority of the study population were in business as occupation and this may have contributed to the observed increase in drug resistance due to crowded environment and lack of information on personal distancing at business area.

Of the 85 (47.2%) drug resistant TB, males 50 (27.8%) were found higher in number than females 35 (19.4%), suggesting a potential role of gender in the epidemiology of drug resistant TB in the study population. The finding was in agreement with similar studies in other countries. Atlanta USA reported 74% males and 26% female TB cases among 1536 cases and Nepal reported 73.89% of males and26.10% of females TB among 295 TB cases [38]. The social roles of the male gender and cultural habits that influence risk of exposure have also been implicated as possible reasons for higher prevalence of drug resistance among males.

Also, among 85 (47.2%) drug resistant TB cases, the age group most likely to have drug resistant TB were > 21years (95% CI: 27.449: P= .156). A similar study noted that drug resistance was higher among the 25 - 35 years age group as this is the peak of infections in adults [30]. There was no relationship between drug resistance with age and gender. The result was in agreement with a similar study in Qatar

[39]. Of the 47.2% drug resistantTB patients, 14 (7.8%) were HIV positive while 69 (38.0%) were HIV negative (95% CL: 3.037: P = .386). No association was observed between the drug resistance and the HIV status of the patients. Similar studies in Nigeria and other African countries found no association between HIV status and drug resistant TB [16, 22, 31]. A similar report in Mumbai reported also that drug resistance TB does not appear to be greatly influenced by HIV infection [27].

Conclusion

This study has revealed a significantly high rate of drug resistant TB in among patients who were previously treated with anti-TB drugs. It also observed that patients who were treated at NAUTH Nnewi site had significantly higher rate of Multi-drug resistant TB compared to other hospitals investigated. It is hoped that the report of this study will add to the current knowledge of drug resistant TB situation in Nigeria and other countries of the world. Our findings emphasize strongly that, a national estimate was unable to capture local specific variations of drug resistant TB in the country because they originated from sites where a programmed operational factor was high**.** The high level of drug resistant-TB in the study area suggested, that a wider set of surveillance sites are needed to obtain a more realistic rate in Anambra State, Nigeria.

A systematic treatment is a key to effective management of TB in Nigeria and therefore, routine culture of *M. tuberculosis* test should be implemented as a reference standard for drug susceptibility testing of suspected TB patients to avoid low case detection rates using only sputum smear microscopy.

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Bibliography

1. World Health Organization. Global tuberculosis report 2019. Geneva: World Health Organizatio (2019).
2. Burk T. "Tuberculosis resistance funding and drug". *Lancet Infectious Disease* 10 (2010): 297-298.
3. JA Caminero. "Multi- resistant tuberculosis: Epidemiology, risk factors and case finding". *International Journal of Tuberculosis and Lung Disease* 14 (2010): 382-390.
4. Onyedum CC., *et al*. "Prevalence of drug resistant tuberculosis in Nigeria. A systematic review and Meta- analysis". *PloS One* 12.7 (2017): e0180996.
5. World Health Organization. Global tuberculosis report 2018. Geneva: World Health Organization (2018).
6. Nwachukwu NO., *et al*. "Prevalence of pulmonary tuberculosis and its associated risks factors in Anambra state, Nigeria". *FTSTJ* 1.2 (2016): 486-488.
7. Okonkwo RC., *et al*. "Prevalence of HIV infection in pulmonary tuberculosis suspects assessing the Nnamdi Azikewe University Teaching Hospital Nnewi, Nigeria". *Advances in life science and Technology* 14 (2013): 87-91.
8. World Health Organization. Multi drug-resistance tuberculosis (MDR-TB). Update 2013.Geneva World Health Organization (2013).
9. World Health Organisation. Global tuberculosis report 2020 country profiles (2020).

10. Gidado M., *et al*. "Assessment of GeneXpert MTB/RIF performance by type and level of health-care facilities in Nigeria". *Nigerian Medical Journal* 60 (2019): 33-39.

11. International Union Against Tuberculosis and Lung Disease(IUATLD) . Technical guide for sputum examination for tuberculosis by direct microscopy in low income countries (2000): 20-25.

12. World Health Organization. Guideline for surveillance of drug resistance in tuberculosis (2009).

13. Cheesbrough M. "District laboratory practice in tropical countries, part 2. 2nd edition. Cambridge: Cambridge University Press (2006).

14. World Health Organization. Laboratory services in tuberculosis control part III, culture. Geneva, Switzerland (1998).

15. Kome Otokunefor., *et al*. "Multi-drug resistant Mycobacterium tuberculosis in Port Harcourt, Nigeria". *African Journal of Laboratory Medicine* 7.2 (2018): 1-12.

16. Akaninyene Otu., *et al*. "Drug resistant among pulmonary tuberculosis patients in Calabar, Nigeria". *Pulmonary Medicine* (2013): 235190.

17. Affolabi D., *et al*. "First insight into a Nationwide genotypic diversity of Mycobacterium tuberculosis among previously treated pulmonary tuberculosis cases in Benin, West Africa". *Canadian Journal of Infectious Diseases and Medical Microbiology* (2017): 6.

18. Masoud Shamaei., *et al*. "Fist-line anti-tuberculosis drug resistace patterns and trends at the national TB referral center in Iran eight years of surveillance". *International Journal of Infeactious Disease* 13.5 (2009): 236-240.

19. Kelemework Adane., *et al*. "Prevalence and drug resistance profile of Mycobacterium tuberculosis isolated from pulmonary tuberculosis patients attending two public hospitals in East Gojiam zone, northwest Ethiopia". *BMC Public Health* 15 (2015): 572.

20. World Health Organization. Multi drug and Extensively drug resistant TB (M/XDR-TB), Global report on surveillance and response Geneva, Switzerland (2010).

21. Tilahun M., *et al*. "Molecular epidemiology and drug sensitivity pattern of Mycobacterium tuberculosis strains isolated from pulmonary tuberculosis patients in and around Ambo town, Central Ethiopia". *PloS One* 13.2 (2018): e0193083.

22. Lawson L., *et al*. "Resistance to first – line tuberculosis drugs in three cities of Nigeria". *Tropical Medicine and International Health* 16.8 (2011): 974-980.

23. M Agnoafir., *et al*. "Phenotypic and genotypic analysis of multi-drug resistant tuberculosis in Ethiopia". *International Journal of Tuberculosis and Lung Disease* 16.5 (2010): 1259-1265.

24. K Desta., *et al*. "Prevalence of smear negative pulmonary tuberculosis among patients visiting St. Peters tuberculosis specialized Hospital. Addis Ababa, Ethiopia". *Ethiopia Medical Journal* 47.1 (2009): 17-24.

25. Vishal Goyal., *et al*. "Prevalence of drug resistant pulmonary tuberculosis in India: systematic review and meta- analysis". *BMC Public Health* 17.817 (2017): 1-21.

26. O Idigbe., *et al*. "Initial drug resistance among HIV seropositive prison inmates in Lagos, Nigeria". *International conference on AIDS* 12 (1998): 137.

27. Desiree TB., *et al*. "High level of multi drug resistant tuberculosis in new and treatment failure. Patients from the revised national tuberculosis control programme in an urban metropolis (Mumbai) in Western India". *Biomedical Central Public Health* 9 (2009): 211-215.

28. Guy Thwaites. "Tuberculosis. Manson Tropical infectious disease". *Twenty-Thirdedition* (2014): 468-505.

29. Kemo Otokunefor., *et al*. "Multi-drug resistant Mycobacterium tuberculosis in Portharcourt, Nigeria". *African Journal of Laboratory Medicine* 7.2 (2018).

30. Ani AT., *et al*. "Drug resistance profile of Mycobacterium tuberculosis isolated from pulmonary tuberculosis patients in Jos, Nigeria". *Transaction of the Royal Society of Tropical Medicine and Hygiene* 103 (2009): 87-71.

31. D Anastasis., *et al*. "A restrospective study of human immunodeficiency virus infection and drug resistant tuberculosis in Durban, South African". *International Journal of Tuberculosis and Lung Disease* 1.3 (1997): 220-222.

32. Nwankwo NC., *et al*. "Epidemiology of pulmonary tuberculosis in some parts of Abia state, Federal Republic of Nigeria". *Asian Journal of Epidemiology* 2 (2009): 13-19.

33. Nwachukwu NO., *et al*. "Delay in diagnosis of pulmonary tuberculosis among presumptive tuberculosis cases in parts of Anambra state, Nigeria". *Emerging Infectious Diseases* 1.4 (2016): 120.

34. Imam TS and Oyeyi TI. "Retrospective study of pulmonary tuberculosis (PTB) prevalence amongst patients attending infectious diseases hospital (IDH) in Kano, Nigeria". *BAJOPAS* 1.1 (2008): 10-15.

35. Muvunyi CM., *et al*. "Prevalence and diagnostic aspects of sputum smear positive tuberculosis cases at a tertiary care institution in Rwanda". *African Journal of Microbiology Research* 4.2 (2010): 88-91.

36. Onubuogu CC., *et al*. "Sensitivity of direct smear microscopy for the diagnosis TB in high HIV prevalent population". *Scientific Research and Essay* 7.5 (2012): 593-597.

37. American Thoracic society. "Diagnostic standard and classification of tuberculosis in adult and children". *American Journal of Respiratory and Critical Care Medicine* 161 (2000): 1376-1395.

38. Subba S., *et al*. "Antibiotic susceptibility pattern of Mycobacterium tuberculosis". *Journal of Nepal Health Research Council* 7.14 (2009): 33-41.

39. Al-Marri MRHA. "Pattern of mycobacteria resistance to four anti-tuberculosis drugs in pulmonary tuberculosis patients in the state of Qarter after the implementation of DOTS and limited expatriate screening programme". *The International Journal of Tuberculosis and Lung Disease* 5.12 (2001): 1116-1121.

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