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Drug Resistant *Mycobacterium tuberculosis* in Tertiary Hospital South East, Nigeria

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Abstract

Objective: Drug resistant Mycobacterium tuberculosis is a public health threat globally. We described the drug resistance pattern of *M. tuberculosis* to first line anti-TB drugs and the prevalence of Multidrug resistant TB among TB patients at tertiary hospital Nnewi, Nigeria.

Methods: Sputum specimens from 550 suspected TB patients were analyzed for AFB. The smear positive samples were subjected to culture and drug susceptibility testing to first line anti-TB drugs on Lowenstein-Jensen medium.

Result: Out of 180 (32.7%) culture positive samples subjected to DST, 97 (53.8%) were susceptible to all first line anti-TB drugs while 83 (46.1%) were resistant to one or two anti- TB drugs. The level of resistance was significant at $p<0.05$ identifying three patterns, Mono-drug resistance in 34(18.8%) patients, Multi-drug resistance in 14 (7.7%) and Poly drug resistance in 35 (19.4%) patients. The proportion of TB cases with resistance to single drugs ranged from 5 (2.7%) for rifampicin to 12 (6.6%) for isoniazid and previously treated TB 8 (4.4%) patients was a significant factor ($P<0.000$) to development of MDR-TB compared to new TB patients 6(3.3%) within the age range of 21-40 years. Other factors such as age, gender and HIV positive status were not significantly associated with the development of any resistance.

Conclusion: The investigation highlights the presence of drug resistant TB with high prevalence of MDR-TB in the studied community. Larger studies are urgently recommended to improve TB clinical management and control efforts.

Keywords: Tuberculosis; *Mycobacterium tuberculosis;* Multi-drug resistant TB; New and previously treated TB cases; Nigeria

Introduction

The emergence of *M. tuberculosis* (MTB) resistance to anti- TB drugs has been a major public health obstacle to achieve the goal of effective global TB control [1]. Drug resistance in M. tuberculosis isolates arises from spontaneous genetic mutations and can be enhanced by poor adherence of patients to anti-TB drugs [2]. Multi-drug resistant TB (MDR-TB) portrays a very grave danger and particularly challenging to treatment interventions while Extensively drug resistance TB (XDR-TB) is virtually untreatable and should by all means be avoided. World Health Organization reported that, the global resistance to any of the anti-TB drug accounted for 20% of all reported TB cases and estimated 500,000 multi-drug resistant TB (resistant to at least isoniazid and rifampicin) cases to emerge each year worldwide with 150,000 deaths from MDR-TB [3]. About 5.3% of all reported TB cases had MDR-TB, between 5-7% develop XDR-TB and globally only 10% with MDR-TB receive treatment leading more spread of MDR-TB which is difficult to treat [4]. The pandemic of HIV/AIDS have been attributed to upsurge of TB/MDR-TB globally and attack rate is heavy on the wage earning community in the age group of 15-49 years [3]. Global surveillance programme showed variation in the magnitude and trends of drug resistance in different countries. Migration of population has also been reported to strengthen the transmission dynamics of TB as well as antimicrobial drug resistant organism.

Nigeria moved from 4th position in 2007 to 10th in 2012 among the 22 high TB burden countries in the world and from 1st to 4th highest TB burden in Africa [5]. Nigeria is the third highest burden of HIV in the world with 4.4% prevalence rate and an estimated rate of 3 million individuals are infected with 21% TB/HIV co-infection [6]. The implementation of Directly Observed Therapy (DOTs) strategy in Nigeria since 1993 has achieved a case detection rate of 30% and treatment success rate of 79% which is still below the global target of 70% detection and 85% cure rate respectively [7]. This implies that majority of active cases are still not detected within the communities and this will continue to transmit TB infection.

Although there are limited information on the rate of drug resistant TB in Nigeria due to lack of laboratory facilities and poor DOTs programme but key studies from private tertiary care facilities in Nigeria reported increasing trends of MDR-TB in new and re-treatment cases. The current estimates of MDR-TB prevalence in Nigeria is 2.2% and 9.4% among new and retreatment cases respectively [3]. Recent national survey on prevalence of drug resistance TB reported 4.8% MDR-TB in Nigeria, 2.9% MDR-TB among new cases and 14.3% MDR-TB in re-treatment TB cases [5]. Some private care facilities reported 4% new cases and 18% re-treatment cases of MDR-TB in Jos, Nigeria [8] and 53.6% MDR-TB at Ibadan [9].

Unfortunately, there has not been a report of community based data on the rate of Multi-drug resistance TB in Nnewi, Anambra State due to the fact that ,drug susceptibility test (DST) is not routinely carried out as a part of DOTs programme policy , public health laboratory infrastructure is limited and poorly equipped to cope with large scale testing. Only two reference laboratory NIMR in Lagos and Zanklin TB laboratory with culture based and drug susceptibility testing are functioning in Nigeria.

This study assessed a community based pattern of drug resistance M. tuberculosis to determine the prevalence and risk factors associated with MDR-TB in new and previously treated TB patients attending NAUTH Nnewi and distant peripheral DOTs centers in Anambra state, South East Nigeria.

Material and Methods

The laboratory based study was conducted among patient with pulmonary tuberculosis within the age of 7 to 82 years attending Nnamdi Azikiwe University Teaching Hospital (NAUTH) DOTS Clinic and distant peripheral hospitals at Nnewi, Anambra state in South-Eastern geopolitical zone Nigeria from January 2009 to March 2011.

A total of 550 sputa of suspected new and previously treated patients were examined for Acid Fast Bacilli (AFB) and screened for HIV status. Patients with sputum smear positive samples were selected and subjected for culture and drug susceptibility testing at TB research laboratory Zankli medical center Abuja. A standard questionnaire was completed for each recruited patient to collect demographic, laboratory data and clinical/treatment history. The study was approved by the Ethical committee of Nnamdi Azikiwe University Teaching Hospital Nnewi and informed consent was obtained from each patient.

Laboratory investigation

Each patient 5mls of venous blood was screened for HIV using serial algorithm method. Determine and stat-paks test kits were used according to manufacturers direction [10].

Three sputum samples were collected from each patient into well-labeled containers, processed and analyzed microscopically for AFB using Ziehl- Neelsen (Z.N) method as described previously. Results were recorded according to the grading system by IUATLD [11]. The best quality sputum sample of each smear positive patient was cultured using modified petroff's method onto Lowenstein Jensen (LJ) medium slope, incubated at 37°C for 4-6 weeks. In-house known strain and H37RV Mycobacterium tuberculosis strain was used as a positive control while sterile L.J medium was used as a negative control under biosafty level II hood. The growth and morphology of the colonies were noted and biochemical test was performed on the colonies and confirm with ZN method to identify Mycobacterium tuberculosis [12].

Drug Susceptibility Testing (DST)

All culture positive *M. tuberculosis* identified colonies were subjected to drug sensitivity test using proportional method as described by WHO [13]. The concentration of streptomycin (S) 4 µg/ml, isoniazid (I) 0.2 µg/ml, rifampicin (R) 40 µg/ml and ethambutol (E) 2 µg/ml was incorporated into LJ medium slop and 0.1ml of the cell suspension of each *M. tuberculosis* strain was inoculated and incubated for 2-4weeks at 37°C. The critical proportion was taken at 1% for all drugs. If the bacteria growth on the medium with the specific drug was >1% compared to the control, the strain was identified as resistant to the specific drug but sensitive to the drug if the growth rate was <1% compared to the control. In-house strain and H37RV standard Mycobacterium tuberculosis strain was used as positive control while inoculated slope without drug was used as negative control.

Internal quality control was routinely performed using known MTB strains and negative samples for every batch stained, cultured and for DST. Appropriate SOPs was developed and quarterly External Quality Control (EQC) for smear positive microscopy was conducted at NAUTH by NTBLCP.

Data was analyzed using Statistical Package for Social Sciences (SPSS version 2.0), frequencies and proportions (%) were described and compared using chi-squared test. P-value of less than 0.05 ($P<0.05$) was considered statistical significant, variables were described by means and Standard Deviation (SD).

Results

A total of 550 sputum specimens analyzed microscopically and cultured yielded, 370 (67%) negative sputum samples and 180(33%) culture positive strains. The proportion of males 109 (60.5%) were statistically higher compared to females 71(39.4%) TB patients. The age group most affected with M. tuberculosis was 21- 40 years, 117(65%) patients followed by 41-60 years 35 (19.4%) age group with the average mean (SD) age of 35 years as shown in Table 1. There was no statistically significant difference in the mean age for male and female. Demographic characteristics of 180 strains analyzed as shown in Table 2, identified 151(83.8%) new TB cases and 29 (16.1%) previously treated TB cases.

Age (Years)	Males	Female	Total	%
1 – 20	7(3.8%)	10(5.5%)	17	9.40%
21 – 40	65(36.1%)	52(28.8%)	117	65.00%
41 – 60	27(15%)	8(4.4%)	35	19.4%s
61 – 80	9(5%)	1(0.5%)	10	5.50%
81 – 100	1(0.5%)	-	1	0.50%
Total examined	109(60.5%)	71(39.4%)	180	100%

Table 1: Age and Sex distribution of the study population

Data	New cases	Previously treated	Total	%
HIV status				
Positive	19(10.5%)	15(8.3%)	34	18.80%
Negative	132 (73.3%)	14(7.7%)	146	
Total examined	151(83.8%)	29(16.1%)	180	100%
Occupation				
Business	64(35.5%)	13(7.2%)	77	42.80%
Student	43(23.8%)	3(1.6%)	46	25.50%
Workers	32(17.7%)	7(3.8%)	39	21.50%
Civil servants	11(6.1%)	3(1.6%)	14	7.70%
None	1(0.5%)	3(1.6%)	4	2.10%
Total examined	151(83.8%)	29(16.1%)	180	100%
Education				
Tertiary	45(25%)	5(2.7%)	50	27.70%
Secondary	90(50%)	17(9.7%)	107	59.40%
Primary	16(8.8%)	7(3.8%)	23	12.70%
Total examined	151(83.8%)	29(16.1%)	180	100%
History of contact				
Unknown	62(34.4%)	16(8.8%)	78	43.20%
Yes	43(23.8%)	10(5.5%)	53	29.40%
No	46(25.5%)	3(1.6%)	49	27.10%
Symptoms				
Cough	87(48.3%)	22(12.2%)	109	60.50%
Fever	44(24.4%)	1(0.5%)	45	25%
Chest pain	20(11.1%)	6(3.3%)	26	14.40%

Table 2: Demographic characteristics of tuberculosis patients

The risk of drug resistance tuberculosis

Drug sensitivity testing done on 180 MTB positive strains yielded, 97 (53.8%) MTB strains susceptible to the four first line anti-TB drugs tested and 83 (46.1%) strains resistance to at least one or two drugs. The prevalence rate of drug resistance 46.1% was statistically significant at $p<0.05$ and varied to the four anti-TB drugs tested identifying three patterns of resistance as shown in Table 3. Mono-drug resistant pattern was observed in 34 (18.8%) patients, MDR-TB in 14 (7.7%) patients and poly- drug resistant pattern seen in 35 (19.4%) patients. The proportion of TB cases with resistance to single drug ranged from 5(2.7%) for rifampicin, 8(4.4%) for streptomycin, 9(5%) for ethambutol and 12(6.6%) for isoniazid. Higher proportion of drug resistance were seen on new TB cases than to MDR-TB higher in previously treated TB cases. Tables 4 and 5 describe the factors associated with the development of any resistance to first line anti TB drugs. The most significant factor associated with the development of MDR-TB was the history of previously anti-TB treatment .Other factors such as HIV, age and gender were not significantly associated with development of MDR-TB. However, when compared MDR-TB and Non MDR-TB, there was no significant difference in age and gender of the patients.

Pattern of drug resistance	New TB	Previous treated	Total %
No of patients	151(83.8%)	29(16.1%)	180(32.7%)
Susceptible drug			
SIRE	95(52.7)	2(1.1%)	97(53.8%)
Mono drug resistance	23(12.7)	11(6.1%)	34(18.8%)
S	6(3.3%)	2(1.1%)	8(4.4%)
I	7(3.8%)	5(2.7%)	12(6.6%)
R	3(1.6%)	2(1.1%)	5(2.7%)
E	7(3.8%)	2(1.1%)	9(5%)
Multi drug resistance	6(3.3%)	8(4.4%)	14(7.7%)
IR	1(0.5%)	-	1(0.5%)
SIR	2(1.1%)	3(1.6%)	5(2.7%)
EIR	1(0.5%)	2(1.1%)	3(1.6%)
SIRE	2(1.1%)	3(1.6%)	5(2.7%)
Poly-drug resistance	27(15%)	8(4.4%)	35(19.4%)
SIE	8(4.4%)	3(1.6%)	11(6.1%)
IE	11(6.1%)	3(1.6%)	14(7.7%)
SE	2(1.1%)	-	2(1.1%)
SI	3(1.6%)	1(0.5%)	4(2.2%)
SR	3(1.6%)	1(0.5%)	4(2.2%)
SRE	-	-	-
Total drug resistance	56(31.1%)	27(15%)	83(46.1%)
S: Streptomycin, I: Isoniazid, R: Rifampicin, E: Ethambutol			

Table 3: Pattern of drug resistance among new and previously treated TB cases

Discussion

This study is to our knowledge, the first TB epidemiology study designed to provide a view of drug resistant TB in programmatically vulnerable areas at Nnewi, Anambra State, South-Eastern geopolitical zone, Nigeria. Nnamdi Azikiwe University Teaching Hospital (NAUTH) was used as study area because it is a treatment and management centre for TB/HIV patients supported by Institute of Human Virology Nigeria (IHVN).

Although, there were limited information available on the level of drug resistant M. tuberculosis in the country. This study highlight a high prevalence of drug resistant M. tuberculosis (DR-TB) 46.1% which is statistically significant at $P<0.05$ with significant proportion

of MDR-TB 7.7%. Internal comparison also indicated a high degree of reproducibility for susceptible strains 97 (53.8%).

Demographic factor		No of culture positive samples	No of drug susceptible TB	No of drug resistant TB	X2 (95%CL)	P.value (P≤0.05)
		n=180 (%)	n=97 (54%)	n=83 (46%)		
Age Groups	≤ 20	18 (10%)	12 (7%)	6 (3%)	-	-
	≥ 21	162 (90%)	85(47%)	77(43%)	-	-
Total					27.449	P=.156
Gender:	Male	108 (60%)	58 (32%)	50 (28%)	-	-
	Female	72 (40%)	39 (22%)	33 (18%)	-	-
Total					0.766	P=.858
HIV	Positive	34 (19%)	20 (11%)	14 (8%)	-	-
	Negative	146 (81%)	77 (43%)	69(38%)	-	-
Total					3.037	P=.386
Category:						
New TB cases		151 (84%)	95(53%)	56 (31%)	39.42	P≤.000
Previously treated TB		29 (16%)	2 (1%)	27 (15%)	39.42	P≤.000

Table 4: Analysis of association between demographic factor and development of drug resistant TB to one or two anti TB drugs

The observed high susceptibility rate supports earlier reports which indicated that, well supervised short course chemotherapy regimens quite often result in a lower proportion of treatment failure [9]. Of the 83 drug resistance cases 56 (31.1%) were new TB cases compared to 27(15%) previously treated TB cases, indicating a high level of emerging M. tuberculosis being resistance to new TB cases. Similar studies have documented high level of drug resistance TB in new cases [13] and the rate 46.1% drug resistance TB is statistically greater than the global percentage 20% for any form of anti-TB drug resistance [3]. Three patterns of drug resistance TB were observed in this study, mono- drug resistance 34(18.8%), multi- drug resistant 14 (7.7%) and poly- drug resistant 35(19.4%). The proportion of isolates resistant to each of the four tested anti-TB drug varied. Isoniazid 12(6.6%) has the highest proportion of drug resistant while rifampicin 5(2.7%) has the lowest percentage of resistant (Table 3). The finding that rifampicin 2.7% was the lowest resistance among the four anti-TB drugs used 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.

Demographic factor		**No of drug resistant TB**	**No of Non MDR-TB**	**No of MDR-TB**	**X2 (95%CL)**	**P.value (P≤0.05)**
		n=83 (%)	n=69 (83%)	n=14 (17%)		
Age Groups	≤ 20	6 (7%)	6 (7%)	-	-	-
	≥ 21	77 (93%)	63 (76%)	14(17%)	-	-
Total					214.63	P=.227
Gender:	Male	50(60%)	42 (50%)	8(10%)	-	-
	Female	33 (40%)	27 (33%)	6(7%)	-	-
Total					1.56	P=.816
HIV	Positive	14(17%)	11(13%)	3(4%)	-	-
	Negative	69 (83%)	58 (70%)	11(13%)	-	-
Total					2.395	P=.664
Category:						
New TB cases		56 (67%)	50(60%)	6(7%)		26.521 P ≤ 0.000
Previously treated TB		27 (33%)	19(23%)	8(10%)		26.521 P ≤ 0.000

Table 5: Association of demographic factor and development 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 different population may have had different levels of access to different anti-TB drug over time. A major concern is the high level of poly drug resistant 35 (19.4%) which were seen more on new TB cases in comparison to MDR-TB. The history of previous treatment was the most significant factor associated with MDR-TB and the finding is consistent with surveys conducted in several countries by the World Health Organization on the risk factors for MDR-TB [14]. The rate of MDR-TB 7.7% in this study is statistically higher than the estimated 5.3% rate of global MDR-TB (15s) and significantly higher (P<0.05) also to the current rate of MDR-TB 4.8% in Nigeria [5]. The rate of MDR-TB 3.3% in newly diagnosed TB cases has no statistical different between the current estimated rate of MDR-TB 2.2% in new case in Nigeria and 2.9% new cases in current national survey although slightly higher but MDR-TB 4.4% in previously treated cases is lower compared to estimated rate of MDR-TB 9.4% [15] and 14.3% from current national survey on MDR-TB in Nigeria [5]. In contrast to the WHO estimates and national reports, key studies from private, tertiary care facilities in Nigeria confirmed the increasing trend of MDR-TB in new and previously treated cases in Nigeria. The finding is within the trends for African countries. A similar report indicated that, MDR-TB in Africa is 3.9-5.0% in new TB cases and 16.7% in previously treated cases [15]. Both identical and varied drug resistance pattern were seen in the same defined cluster, suggesting that both primary and acquired

resistance have contributed to the drug resistant TB epidemic in the study area.

Demographic analysis showed that, drug resistance cases were found to be more likely in male 27.7% than female 18.3%, suggesting a potential role of gender in the epidemiology of drug resistant TB in the study population. Similar findings have been seen in other studies [16]. This may be due to differences in health seeking habits of people arising from the stigma associated with tuberculosis. The social role male and cultural habits that influence risk of exposure have also been implicated as possible reasons. Majority of patient with drug resistant TB were within the age range of 21–40 years, but the age distribution did not differ statistically significantly between the resistant and susceptible groups. The age group is the economically active group and should be provided with good health care system, which agreed with similar finding that, drug resistance peak at the age of 25–35 years in resemblance to peak of adult TB patients [6]. There was no significant association between HIV positive TB 18.8% patients and MDR-TB (Table 5). This is similar to report that, the high proportion of MDR-TB in Mumbai does not appear to be greatly influence by HIV infection [13]. The rate 18.8% of HIV/TB co-infection in this study is lower than the rate 27% among TB/HIV patients reported in Nigeria [17]. This could be due to the established observation of most TB/HIV co-infected patients being smear negative as most of the TB patients who were HIV sero- positive will have smear negative disease.

Conclusion

It is hope that the up-to date knowledge presented in this study will add to the understanding of the current drug resistant/MDR-TB situation in Nigeria and different countries of the world. Our findings showed that a national estimate was unable to capture local specific variations of MDR-TB in the country because they originated from sites where a programmed operational factor was high. The high prevalence of drug resistance TB reported in this study is an indicative of a larger epidemic than previously suspected and we strongly suggest that, a wider set of surveillance sites are needed to obtain a more realistic view of the MDR-TB in Anambra State, Nigeria. The implication is that, a good number of MDR-TB is not detected and treated thus a large number of undiagnosed cases remain in the community spreading widely even when there are facilities for second line drug in Nigeria. Patients with previous treatment history are at higher risk for MDR-TB.

Monitoring and control of drug resistant TB should be emphasized by revised DOTs programme, through prompt case detection, routine culture, quality assured drug susceptibility testing for patients and systematic treatment observation in Nigeria.

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