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Baseline Evaluation of the Shorter Regimen for Multidrug Resistant Tuberculosis in South-South, Nigeria.Mac-Fiberesima, G. ^{*1.}, Ayodele, M.B.O.² and Aaron, U.U.²*South-South Tuberculosis Zonal Reference Laboratory Department of Microbiology and Parasitology University of Port Harcourt Teaching Hospital, Nigeria¹, Department of Medical Microbiology and Parasitology, University of Port Harcourt, Nigeria²**Author for Correspondence*: macfibs04@yahoo.com/+234-803-707-9310***Abstract**

Multidrug resistant Tuberculosis (MDR-TB) is a serious public health challenge and a security risk for childhood and adult life all over the world. The shorter regimen for multidrug resistant tuberculosis is a developmental stride to manage patients quickly and rapidly. The emergence of MDR-TB together with pre-extensively drug resistant tuberculosis had made treatment complicated for the patient and care givers. Selection of criteria from various studies was carried out by World Health Organization in 2016 which resulted to the recommendation on the use of the shorter MDR-TB regimen under specific conditions. However, the program was faced with challenges beginning with shortage in culture-based facilities and the Line Probe Assay which is not widely available. A total number of 168 sputum samples were collected and processed in a biosafety level 2 laboratory with level 3 practices in Port Harcourt. Sputum samples were decontaminated using sodium hydroxide citrate method. A random sampling technique was used to select samples for analysis at every stage. The aim of this study was to evaluate the baseline MDR-TB among patients seeking diagnostic services to be included in shorter regimen. There was a statistically significant proportion for males (46.67%) among age group 31-45 and age group 16-30 for females, (31.75%) (Fisher's exact test $p=0.02$). There was also a statistically significant higher prevalence for Rifampicin resistance using Line Probe Assay compared to Culture (59.76% vs. 40.24%; $p=0.01$). Resistance to fluoroquinolones was 3.5% in ages 16-30 and 31-45. Second line treatment failure co-infected with Non- *Mycobacterium tuberculosis*

Complex (NMTBC) requires an urgent resolution. Pragmatic efforts are needed to stop the deadly MDR-TB and baseline investigation for second line anti TB drug resistance should be strengthened to sustain the shorter regimen program.

Keywords: *Baseline, Tuberculosis, Multidrug-Resistance, Pre extensively Drug Resistance and Shorter Regimen.*

Introduction

Multidrug resistant Tuberculosis (MDR-TB) is a serious public health challenge and a security risk for childhood and adult life all over the world. The emergence of Multidrug Resistant Tuberculosis (MDR-TB), Pre- Extensively Drug Resistant TB (Pre- XDR-TB) and Extensively Drug Resistant TB (XDR-TB) makes it more complicated for the patient and care givers. The shorter regimen for multidrug resistant tuberculosis is a developmental stride to manage patients quickly and rapidly. As a result, MDR-TB eventually became a global alarm that attracted attention for stakeholders to come up with criteria to categorize patients that qualify for the shorter regimen. Selection of criteria from various studies was carried out by WHO in 2016 which resulted to the recommendation on the use of the shorter MDR-TB regimen under specific conditions. However, the program was faced with challenges beginning with shortage in culture- based facilities and Line Probe Assay which was not widely available. Moreover, there was fear that the program may include patients whose profile is different from those that were included in the cohort study. Globally, 4.1% of MDR-TB new cases in 2016 and 19% of

previously treated cases was reported which could develop to pre- XDR-TB and XDR-TB Worldwide. It was also reported from drug resistant surveillance that 240,000 died from MDR-TB which may involve Pre-extensively drug resistant and Extensively drug resistant TB (Natasha and Manormoney, 2017; WHO, 2016) emanating from unconditional use of the shorter regimen.

The burden of MDR-TB in Nigeria is on the increase (WHO, 2016) and pre- XDR-TB and XDR-TB are gradually increasing. Efforts made to control the spread is staggered as a result of poverty, overcrowding, treatment default, lack of awareness, inadequate provision of trained man power, insufficient diagnostic tools, untimely health assessment, specimen transport challenges, sample contamination, and inadequate number of facilities to handle the life-threatening disease (WHO, 2016). In a bid to reduce the risks associated with the long treatment of MDR-TB, pre- XDR-TB and XDR-TB, the World Health Organization launched the shorter regimen program in 2014 which shortens the conventional TB treatment from 20-24 months to 9-12 months (WHO, 2016). A survey carried out by WHO Global Task Force on TB Impact Measurement in 2012 reported that the TB burden in the country was much higher than previously estimated (Zhao *et al.*, 2012). It was obvious that the Directly Observed Treatment Short Course (DOTS) strategy had failed in militating against further spread. This prompted WHO to endorse the Xpert MTB/RIF diagnostic test with promising outcome. Nevertheless, it's challenges and increasing impact of MDR-TB, pre-XDR-TB and XDR-TB raised concerns. Therefore, The Line Probe Assay (LPA) which is a molecular test that signals gene mutations for the two most potent anti TB drugs and also for the amino glycosides and fluoroquinolones became necessary for use by WHO for screening patients at risk of Drug resistant TB.

Recent studies have published that resistance to second line anti TB drugs have been discovered leading to the emergence of the militants in the community known as pre- XDR and XDR-TB which is occurring in 105 countries globally (WHO, 2016). XDR-TB is MDR-TB together with resistance to fluoroquinolone and at least

one of the second line aminoglycosides which are the injectables. On the other hand, pre-XDR-TB is MDR-TB plus resistance to the fluoroquinolones. (Gandhi *et al.*, 2010). The two forms of TB are characterized by alarming mortality. To manage these health challenging factors successfully, an effective and timely patient management and diagnostic structure has to be in place which is now taught to be the baseline investigation otherwise the shorter regimen. It will be of help firstly to appreciate the resultant effects of delay in diagnosis and misuse of second line anti-TB drugs.

To this end, baseline studies for TB shorter regimen became necessary to curtail the challenges associated with the prolonged duration of second line anti TB drugs. Baseline screening of Multidrug resistant tuberculosis targets at eliminating the doubts and delays connected to conventional methods but compel patient to molecular methods by enrolling into GeneXpert screening at zero month and simultaneously with the Line Probe Assay. Patient management with the baseline testing is a better and timely strategy for the control of MDR, pre- XDR-TB, and XDR-TB and the associated numerous adverse effects. The outcome automatically enrolls patients into the shorter regimen program. The aim of this study was to evaluate the baseline MDR-TB in relation to pre- XDR-TB among patients seeking diagnostic care for enrollment into the shorter TB regimen.

Materials and Method

Eligibility Criteria:

Patients receiving TB diagnostic care from the South-South Zonal TB Reference Laboratory, University of Port Harcourt Teaching Hospital that have not been tested previously for TB diagnosis and who have not been enrolled into the TB program were recruited eligible for this study.

Sample Collection:

Samples for baseline investigation of MDR-TB were routinely transported in cold chain to the Zonal reference Laboratory in University of Port Harcourt Teaching Hospital by dispatch riders for help and courier delivery. At the sample reception unit of the laboratory was a trained

receptionist who received the samples and labels them with identification numbers. The samples were registered as baseline and follow up. A total of 168 baseline samples were processed for this study. They were dispatched to the culture laboratory for decontamination through the sample reception hatch with the sample manifest.

GeneXpert Assay:

All baseline sputum samples were screened for GeneXpert according to Wachukwu *et al.* (2018).

Decontamination of Samples:

The Sodium Hydroxide Citrate NALC method was used for decontamination of samples according to standard preparations. All biosafety and quality measures were applied to ensure best practices. Pellets from decontaminated sputum samples were cultured onto Plain Lowenstein Jensen (LJ) and Pyruvate slants and incubated at 37°C. They were read weekly and results recorded in the sample reading worksheet. Suspected growth on the plain LJ slants were

smear on grease free slides labeled with sample identification number, stained with Ziehl-Neelsen (ZN) stain and viewed with a light microscope (Angra, 2007). Positive ZN smears were noted and recorded. Acid Fast bacilli (AFB) positive samples were further tested with the Standard Diagnostic (SD) Bioline rapid kits. Only the Standard Diagnostic (SD) Bioline positive samples were considered as *Mycobacterium tuberculosis*. First line Drug Susceptible Testing (DST) was done on them with the First line anti TB drugs. DST Slants were read weekly and results recorded (Mac-Fiberesima *et al.*, 2018).

Extraction of DNA:

DNA was extracted from decontaminated samples using Genolyse based on the manufacturer's direction. The DNA extracted was collected in micro tubes to the Molecular unit. The Genotype MTBDRplus and MTBDRsl is produced by Hain Life Science was used for Master mix, amplification and detection. The results were read and recorded (Mac-Fiberesima *et al.*, 2018).

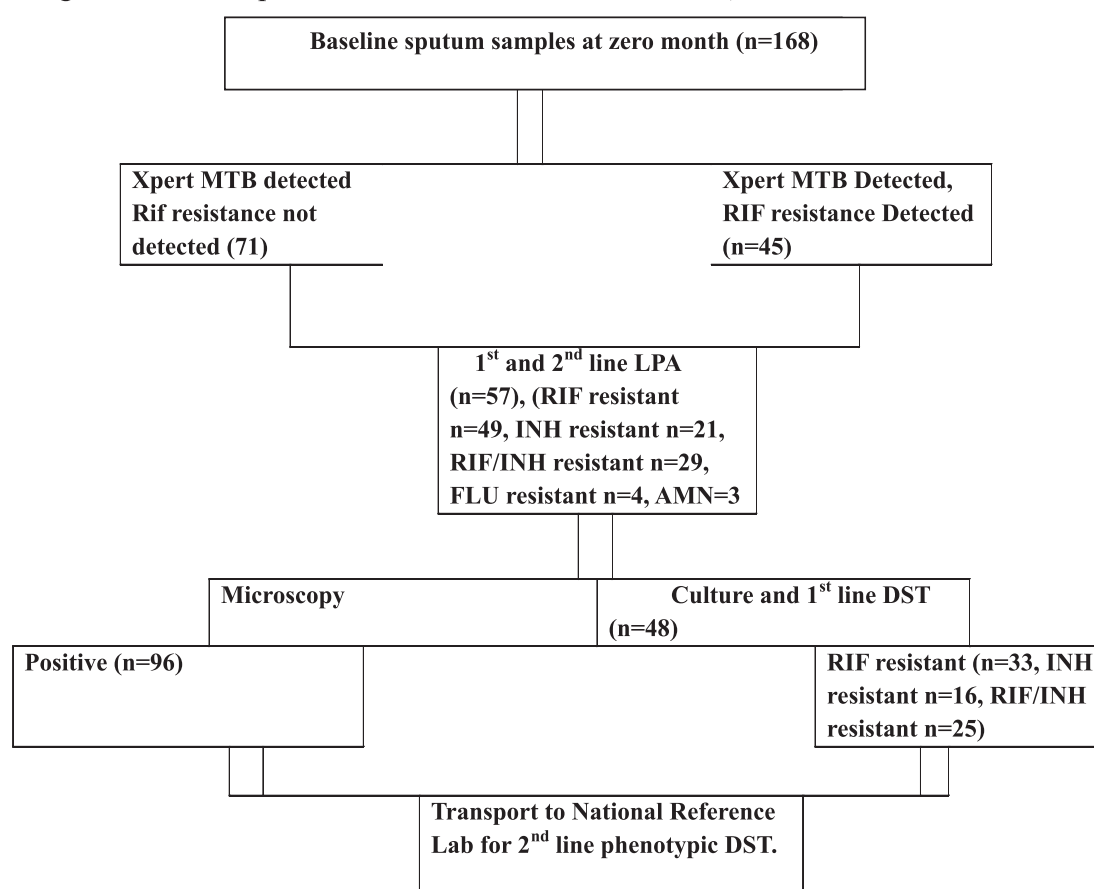


Figure 1: Flow Chart of Sample Collection for Baseline TB Diagnosis

Inclusion and Exclusion Criteria:

All baseline sputum samples were included in the study. Age ranging from 0-75years. Both male and female gender was considered in the study. Only Rifampicin (RIF) and Isoniazide (INH) drug resistance for First line anti TB drugs were included in the study. Phenotypic drug resistance for second line was not conducted but Second line LPA was done for all baseline samples simultaneously with the First line LPA.

Statistical Evaluation

Chi-square significance at $p < 0.05$ was used to evaluate all the data generated for this study.

Ethical Approval:

Ethical approval was obtained from the Ethical

Committee of University of Port Harcourt Teaching Hospital.

Conflict of Interest:

No conflict of interest was established by any of the authors.

Data Availability

All data generated during this study are included in this article.

Funding Support

There was no provisional funding support for this study.

Results

Table 1: Demographic association of Gender and age range in the study

Age range	Gender		Total (%)	df	Fisher's P
	Male Freq (%)	Female Freq (%)			
0-15	1(0.95)	3(4.76)	4(2.38)		
16-30	20(19.05)	20(31.75)	40(23.80)		
31-45	49(46.67)	15(23.81)	64(30.10)	4	0.02*
46-60	28(26.67)	19(30.16)	47(27.98)		
61-75	7(6.67)	6(9.52)	13(7.74)		
Total	105	63	168		

*Statistically significant ($p < 0.05$)

Table 2: Participant's status of Tuberculosis to type of test

Type of test p-value	Tuberculosis		Total	Chi Square (X ²)	
	Positive Freq (%)	Negative Freq (%)			
Microscopy	61(36.30)	107(63.69)	168 (100.0)	4.66	0.198
GeneXpert	45(26.78)	123(73.21)	168 (100.0)		
LPA	57(33.92)	111(66.07)	168 (100.0)		
Culture	48(28.57)	120(71.42)	168 (100.0)		

Table 3: Prevalence of MDR-TB among the study participants.

	NMTBC	Monoresistance	Multi-resistance	
		RIF	INH	RIF/INH
Culture	15(8.92)	33(19.64)	16(9.52)	25(14.88)
LPA	24(14.28)	49(29.16)	21(12.50)	29(17.26)
Total	39	82	37	54
X²	4.15	6.24	1.35	0.59
(p-value)	(0.04) *	(0.01) *	(0.254)	(0.441)

*Statistically significant ($p < 0.05$)

Key

NMTBC: Non- Mycobacterium tuberculosis Complex

LPA: Line Probe Assay

RIF: Rifampicin

INH: Isoniazid

MDR: Multidrug Resistant

Table 4: Baseline studies of participant's response to second line drugs

Age group (years)	No Tested	Second line drugs (Resistance)	
		AMN	FLU (%)
0-15	7(12.28)	0 (0.00)	0 (0.0)
16-30	16(28.07)	0 (0.00)	2 (3.5)
31-45	21(36.84)	2 (3.50)	2 (3.5)
46-60	9 (15.78)	1(1.72)	0 (0.0)
61-75	4(7.01)	0(0.00)	0(0.00)
Total	57 (100.0)	0 (5.22)	4 (7.00)

Discussion

The growing trend of MDR-TB and subsequent emergence of XDR-TB calls for serious concerns. The growing trend of MDR-TB burden locally and internationally is synonymous to a bug that gently eats deep into the marrows. In our study, we considered the baseline investigation of sputum samples (Figure 1). A total of 168 samples were collected for the baseline investigation at zero month out of which 45(26.78%) were Rifampicin (RIF) resistant for GeneXpert. 57(30.35%) were assayed for first- and second-line LPA out of which 49(29.16%) were RIF resistant and 21(12.50%) were Isoniazid (INH) resistant. 29(17.26%) were multi resistant for RIF and INH and 4(2.38%) were resistant for Fluoroquinolones which is suggestive of pre-XDR-TB. 48(28.57%) were culture positive and 61(36.30%) were smear positive. Phenotypic resistance for RIF was 33(19.68%), INH resistant was 16(9.52%) and multidrug resistance for RIF and INH was 25(14.88%). Association of age and gender in TB diagnosis was also studied. The age group 31-45 showed a statistically significant higher proportion for males, 49 (46.67%) and the age group 16-30 for females, 20 (31.75%) (Fisher's $p=0.02$) (Table 1). Our report is far close to the prevalence of TB 13.8% in males and 12.8% in females residing in urban areas reported by (Kumar *et al.*, 2016) but closely related to the report given for rural areas which was 37.5% and 35.9% for males and females respectively. From the results of this study, a barrier to seeking health in relation to gender was observed. Only one third of the studied female population accessed health which are those of the child bearing class. The challenges that accompany early marital life such as pregnancy, postnatal activities, housekeeping and child upbringing could be possible causes of the default. Other factors may be ignorance, lack of exposure, illiteracy, cultural differences, stigma, deprivation of right to personal opinion, and absence of leverage to control personal funds. The idea that a woman should completely become a house wife does not promote health seeking habits. Only a few that are independent can readily access and maintain health seeking habits. The participant's TB status to type of test conducted in this study did not

show any statistical significance $X^2=4.66$; $p=0.198$) (Table 2). This is owing to the study site that was used and secondly the type of statistical tool employed in the study. We were unable to apply a wider range of studies due to time constraints.

This study showed that MDR-TB is on the increase. It is an indication that the study area and the country as a whole is drawing closer to XDR-TB and the reason could possibly be that there is an acquired resistance to anti TB drugs (Jain *et al.*, 2012). Of the 168 sputum samples processed using LPA, Rifampicin monoresistance was 49(29.16%) and resistance to INH/RIF was 29(17.26%). There was also a statistically significant higher prevalence for RIF resistance in LPA compared to Culture (59.76% vs.40.24%; $p=0.01$) (Table 3). The impact of Rifampicin resistance by the TB bacterium cannot be over emphasized. The reasons for this high level of MDR-TB can be tied to untimely health seeking life style. Most of those that have been diagnosed MDR-TB may have picked the bacilli originally in the mere drug resistant form who failed to access health at the appropriate time but relied on self medication and other sources. Mutation is the basis of drug resistance. The TB bacilli are constantly changing their make up to compete and overcome newer drug structures for survival. The WHO report of MDR-TB in Nigeria (4.3%) is quite lower than what was reported in our study. This means that some records of MDR-TB in Nigeria were missing hence were not captured in the data base (Daniel *et al.*, 2013). It is therefore important to conduct a current nationwide meta-analysis to have an accurate data. However, a meta-analysis conducted in Nigeria reported 32.0% of previously treated MDR-TB which was slightly higher than that reported in our study (Ndubuisi *et al.*, 2016). The fact remains that the drivers of MDR-TB are mostly of those who default in treatment. They go back to the community to spread the disease unconsciously. At this point, the bacilli only relapse, modifies to fight back in a more fatal manner. Another reason could be the use of less potent drugs emanating from improper storage temperatures and wrong choice of drugs for patients by care givers.

Our study reported resistance to the second line TB drugs. Among the MDR-TB reported, two age groups 16-30 and 31-45 had a resistant prevalence for fluoroquinolones establishing a case of pre-XDR. Each age group having a prevalence of 3.5% (**Table 4**). This report is closely comparable to the report given previously in a study conducted in same site. It was found that age group 26-35 had the highest prevalence for Rifampicin resistance (Mac-Fiberesima *et al.*, 2018). Nevertheless, it is far lesser than the report (17%) given previously in Nigeria (Ashok and Padam 2010; Gandhi *et al.*, 2010). Also, in India 42% of the reported pre-XDR were recovered from MDR cases [Daniel *et al.*, 2013]. According to the reports obtained from these reports, the economy of Nigeria will be greatly affected since the tender school age class that will take over leadership are already ravaged by the deadly disease. The report of our study has further emphasized that Nigeria is already at risk of XDR-TB. Our report also recorded mixed infection with Non-*Mycobacterium tuberculosis* Complex (NMTBC) (Table 3). There was a statistically significant higher prevalence for NMTBC in LPA compared to Culture (61.54% vs.38.46%; $p=0.04<0.05$).

It can be deduced from this study that poor response to the second line TB drugs sometimes can be as a result of interference with NMTBC. Inaccuracy in diagnosing NMTBC co-infected with TB is another complication to treatment success and compliance. NMTBC prevalence for culture was 8.92% and LPA 14.28%. Despite global efforts made to control drug resistance, drug resistance to second line anti TB drugs is still increasing. Smear results of co-infection with NMTBC are consistently positive. The reason is that the bacterium is a bacillus that belongs to the *Mycobacterium* family. In India 6% of Non-Tuberculous *Mycobacterium* (NTM) was co-infected with pre- XDR-TB with a prevalence of 66.7% (Kumar *et al.*, 2016) and in Nigeria 15% of pulmonary TB reported was due to NTM (Aliyu *et al.*, 2013) which was a close margin compared to what was reported in this study.

Conclusion

There is no doubt that the TB bacilli are waging war against the human race. There is urgent need for stake holders, policy makers, patients, care givers and the entire public to be on toes in the control against MDR-TB, pre-XDR-TB and XDR-TB. Baseline investigation is a faster way of detecting multidrug resistant TB and simultaneously categorizing patients for proper management. Treatment failure resulting from other bacterial infections is a challenge therefore more pragmatic efforts are needed to combat this deadly menace.

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