Detection of Second Line Drug Resistant *Mycobacterium tuberculosis* among Patients Attending National Tuberculosis and Leprosy Training Center Zaria, Nigeria

Suleman, M[1,2]., Ado, S.A[1]. and Aliyu, M.S[1].
[1] Department of Microbiology, Ahmadu Bello University, Zaria.
[2] National Tuberculosis and Leprosy Training Center, Zaria.
*Correspondence author: mikhasulemada@yahoo.com

Abstract: Tuberculosis (TB) is an infectious disease cause by *Mycobacterium tuberculosis* and it remain one of the major public health problem. This study was to detect resistance to second line anti-tuberculosis among patient attending national tuberculosis and leprosy training centre Zaria, Nigeria using Lowenstein Jensen proportion (phenotypic) methods. A total 6125 patients were recruited, out of which 775 (12.6%) were MTB positive and 100 out of 775 were resistant to rifampicin by Xpert MTB/RIF with a prevalence of 13%. Out of 100, (90%) were culture positive while 7 (7%) were culture negative and 3 (3%) were contaminated. All of the ninety (90) samples that were culture positive were confirmed as *Mycobacterium tuberculosis* complex using immunochromatoghapic test. Seventy (77.7%) isolates were found to be pan susceptible while twelve (13.3%) and eight (9%) were resistant to Fluoroquinolones and Aminoglycoside respectively. Resistance of *Mycobacterium tuberculosis* to second line anti-TB drugs in this study was observed to be high among age groups 31-45 and 16-30 years who are male living in urban setting. It was also observed to be high among non-reactive HIV that have not taken alcohol before and among those that were not previously treated with TB drugs, even though there's no statically association between the drug resistance and social-demographic or risk factors in this study. This study has shown high prevalence of drug resistant tuberculosis among patient attending national tuberculosis and leprosy training centre Zaria, Nigeria. The proportions of resistance detected in this study serve as possible indicator of the future emergence of XDR-TB in Nigeria. There is a need for close monitoring of TB patients for proper treatment and compliance to prevent drug resistant tuberculosis. However for the correct management patients with resistance to any of the SLD, results must be confirmed by phenotypic drug susceptibility testing.

INTRODUCTION

Tuberculosis (TB) is one of the world's deadliest infectious disease, it is estimated that one-third of the global population is infected with *Mycobacterium tuberculosis* (MTB), it claims three lives every minute and it remained one of the top 10 causes of death worldwide in 2015 (WHO, 2016). Nigeria is ranked 4th among 20 high TB burden countries with tuberculosis; the country has an estimated burden of pulmonary TB of 219 per 100,000 populations between 15 years and above (WHO, 2019).

Multi-drug resistant tuberculosis (MDR-TB) is refers to strain of *Mycobacterium tuberculosis* that is resistant to at least two most important anti-TB drugs rifampicin (RIF) and Isoniazid (INH). Following WHO guidelines issued in May 2016, all cases of rifampicin–resistant TB (RR-TB), including those with multidrug-resistant tuberculosis (MDR-TB), should be treated with a second line MDR-TB regimen (WHO, 2016). There were an estimated 3.4% and 18% cases among new and previously treated cases that had MDR/RR-TB. There were about 214, 000 deaths from MDR/RR-TB in 2018 and in Nigeria, there were estimated 4.3% of news cases and 15% among previously treated cases with MDR/RR-TB (WHO, 2019). Extensively drug resistant tuberculosis is defined as multi drug-resistant tuberculosis (MDR-TB) plus resistance to a fluoroquinolones and at least one of the three injectable second line drugs (amikacin, kanamycin, and capreomycin) (WHO, 2016).

The emergence of multi drug resistant TB (MDR-TB) and extensively drug resistant TB (XD-TB) threatens the effort to reduce the global burden of tuberculosis (Jassal *et al*., 2009). The most effective control measure for checking the spread of TB is to detect it early and treat it optimally at the earliest. Culture on Lowenstein-Jensen solid medium require about 100 bacilli/ml of specimen for specimen for recovery of mycobacterium, and is the gold standard for

microbiological diagnosis of tuberculosis in developing countries (Morcillo *et al*., 2008). Patient with MDR-TB and in particular, XDR-TB have a significantly poorer prognosis than patients with TB caused by drug susceptibility *Mycobacterium tuberculosis*. They have a higher probability of death, treatment failure, longer hospitalization and treatment duration (Johnston *et al*., 2009). Conventional method that is proportional method (PM) is the gold standard for drug susceptibility testing (DST) of *Mycobacterium tuberculosis*, which is heavily rely on (Ajbani *et al*., 2012). Early detection of drug resistance is crucial to prevent the transmission of drug-resistant TB and averting mortality rate (Barnard *et al*., 2008).

MATERIALS AND METHODS

The National Tuberculosis and Leprosy Training Centre (NTBLTC) Saye, is located in Zaria, Kaduna State, Nigeria. The Centre has one of the two National Tuberculosis Reference Laboratories (NTRL) in Nigeria, which serve as the largest TB referral laboratory in the northern Nigeria. The NTRL is equipped with both biosafety level 2 and 3 (BSL 2&3). Ethical approval was obtained from research ethical committee of the NTBLTC, Zaria. The sample size of this study was determined using an estimated prevalence of the infection (MDR/RR-TB) is 4.3% in Nigeria (WHO, 2016). Six three (63) sample size was obtained but in order to increase precision and minimize error, the total number of sample size were rounded up to 100. Patients attending NTBLTC Saye, Zaria, serve as the study population. All Xpert MTB/RIF resistant patients who consented were enrolled and all those who did not consent and all consenting Xpert MTB/RIF negative patients were not enrolled.

Sample Collection

Two sputum samples were collected from the patients; the patient was asked to inhale deeply 2-3 times and cough deep from the lungs and spit the sputum carefully into the container to avoid contaminating the outside container. The sputum samples were collected in 50 ml capacity universal container, 3-5 ml and should be mucoid/purulents in appearance (NTBLCP, 2014).

Analyses of Samples

Xpert MTB/RIF assay

The Xpert MTB/RIF assay (ceipheid) is a real time polymerase chain reaction (RT-PCR) technology, which is self-enclosed, rapid molecular test for the simultaneous detection of *Mycobacterium tuberculosis* (MTB) and its resistance to rifampicin (RIF). The assay uses sputum sample which can give a result in less than 2 hours, 2:1 of the sample reagent buffer to the sputum would be mix together. The mixtures were mixed by shaking vigorously 20 times and allowed to stand for 10 minutes; it was then shaking 20 times and allows it to stand for 5 minutes. Two ml of the mixtures was then be inoculated into the cartridge and test is started. After the test, the result were printed and interpreted as MTB not detected as negative, MTB detected RIF not detected as positive but no resistance, while MTB detected RIF detected as positive and resistance to rifampicin (Boehme, 2010).

Media Preparation

For the growth of *Mycobacterium tuberculosis*, Lowenstein Jensen media base was prepared and used according to the manufacturer instruction (Difco laboratories, BD Company). The media was prepared by weighing 37.2g of the powder and was dissolved in 600ml of distilled water containing 12 ml glycerol, mixed thoroughly. The solution was then heated with frequent agitation to completely dissolve the powder. The solution was then autoclave at 121ºC for 15 minutes; it was allowed to cool down between 45ºC-60ºC. One thousand ml of fresh, homogenized egg was prepared and added aseptically into solution and mixed thoroughly. The media were then dispensed into sterile 15ml falcon tubes, slanted and inspissated at 85ºC for 45minutes, the prepared media was then incubated at 37ºC for 48 hours for sterility check control and reference strains of *Mycobacterium tuberculosis* and non

tuberculous Mycobacteria were used for performance check (Concepcion *et al*., 2001).

Digestion and Decontamination of Sputum Specimen.

All samples that are Xpert MTB/Rif positive were processed using NALC-NaOH-sodium citrate solution. The NALC-NaOH-sodium citrate solution was prepared as described by Kent and Kubica, 1985. To the 50ml falcon tube containing 3-5ml of sputum sample, an equal volume of NALC-NaOH- citrate reagent was added and the tubes were vortex for 30 second and allowed to stay for 15 min with vortexing in every 5 minutes interval. Following 15mins of incubation at room temperature, phosphate buffer saline was added to 45ml mark and centrifuge at 3000×g for 15min, the supernant was discarded and the pellet material were re-suspended with 1-2 ml phosphate buffer (p^H 6.8) and mixed by inversion. The pellet served as inoculums (Sharma *et al*., 2012).

Isolation of the Mycobacteria

Lowenstein Jensen media was inoculated with 0.1 ml of the inoculums from the sediment. All culture tubes were incubated at 37ºC in slanting position with the loosened caps and observe for the first three days. Caps were then tightened after three days of incubation and tubes were observed macroscopically for growth weekly thereafter for a period of 8weeks. Tubes showing evidence of growth within the period of incubation were removed and smear was made from the growth and stain using ziehl neelsen procedure (Concepcion *et al.*, 2001). Absence of growth at the end of eight (8) weeks were considered as negative culture. All contaminated samples were recorded separately (Sharma *et al*., 2012).

Identification of the Isolates

Mycobacterium tuberculosis complex were identified using rapid identification test, a rapid chromatographic immunoassay for the qualitative detection MPT64 protein fraction that is secreted from MTBc cell during culture. Colonies were harvested from AFB smear-positive culture tube and were suspended, emulsified in 2.0 ml cryovial tubes containing 200 µl of buffer, 100 µl of the emulsify colonies be used as the inoculums. If the MPT64 antigen is present in the sample, a pink to red color reaction is produced on both test and control lines and if the MPT64 antigen is absent in the sample, a color reaction produce pink to red line on the control line only while absence of control line was regarded as invalid (Becton Dickson, 2015).

Preparation of Lowenstein Jensen Medium Containing Drugs.

The second line anti-tuberculosis drugs, which include Amikacin and Ofloxacin were obtained from Molekula while Kanamycin and Capreomycin, were obtained from Sigma Aldrich (St.Lious, Missouri USA). Amikacin, Kanamycin, and Capreomycin were dissolved in sterile distilled water; while Ofloxacin was dissolved in 0.4% NaOH. The final concentration of Amikacin, Kanamycin, Capreomycin, and Ofloxacin were (30µg/ml),(30µg/ml),(40µg/ml) and (2.0µg/ml) respectively, and they were incorporated into the Lowenstein Jensen medium and inspissapated at 85ºC for 45 minutes, then the medium was incubated at 37ºC for 48 hours sterility check (WHO, 2012).

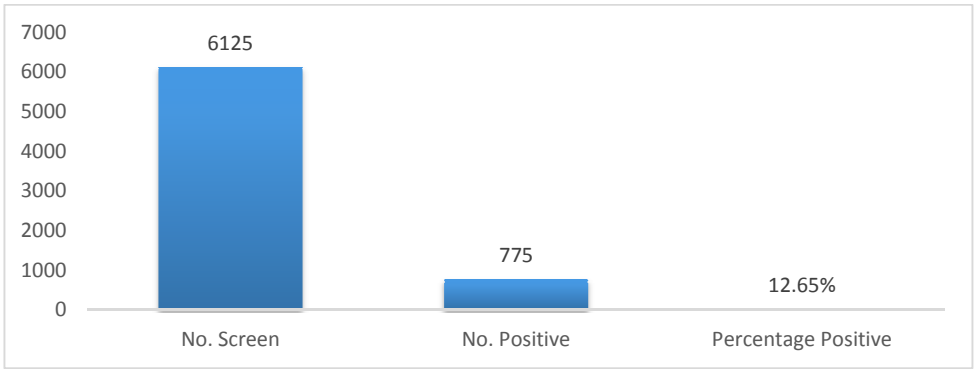
Drug Susceptibility Testing by Proportion Method

The drug susceptibility test was carried-out using indirect proportion method on Lowenstein Jensen medium. The *Mycobacterium tuberculosis* colonies from culture were harvested using a sterile loop and suspended in 15 mls falcon tubes containing sterile distilled water, and 5 sterile glass beads, vortexed for 30 seconds and allow to stand for 15 minutes for the larger bacteria aggregates to settle down. The homogenous upper part of the supernatant was transferred aseptically into another tube, bacterial suspension was adjusted equal to McFarland standard 1 for visual comparison. The bacteria suspension equivalents to Mcfarland No.1 was diluted into tenfold serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}.

The tubes were arranged in order of C_1, C_2, C_3, Kn, Amk, Cp, and Ofl, representing control 1, control 2, control 3, kanamycin, amikacin, capreomycin, and ofloxacin respectively. 100 µl of the bacteria suspension was taken from 10^{-4} dilution and inoculated into C_3, likewise 100µl from 10^{-3} dilution was inoculated into C_2 while 100µl from 10^{-2} dilution was inoculated into C_1 and other tubes containing both first and second line drugs. Incubation was in a slanting position with loosen caps for 24 hours and caps would be tighten and return to upright position at 37ºC for 4-6 weeks. Result were read at 4 weeks and final result was reported after 6weeks, growth on culture would be recorded and reported as follows: No growth as Negative, 1-50 colonies as actual count, 50-100 colonies as +, 100-200 colonies as ++ (innumerable colonies) and ≥200 colonies as +++ (confluent growth). The resistance or susceptibility to a given drug was determined using the proportion (Bwanga, 2009). *Mycobacterium tuberculosis* reference strain for second line drugs with the following pattern (SRRR) which is susceptible ofloxacin but resistance to kanamycin, amikacin and capreomycin and RSSS resistance to Ofloxacin but susceptible to kanamycin, amikacin and capreomycin serve as controls. All strains were subculture in Lowenstein- Jensen medium for a maximum of 8 weeks (Sagonda *et al*., 2014).

RESULTS

A total of six thousand one hundred and twenty five (6125) samples were screened, out of which 775 were positive by Xpert MTB/RIF assay with a prevalence of 12.65% and one hundred (100) out of 775 positive samples were resistance to rifampicin with a prevalence of 13% (Figure 1 and 2).



Fiqure 1. Prevalence of *Mycobacterium Tuberculosis* Using Xpert MTB/RIF Assay

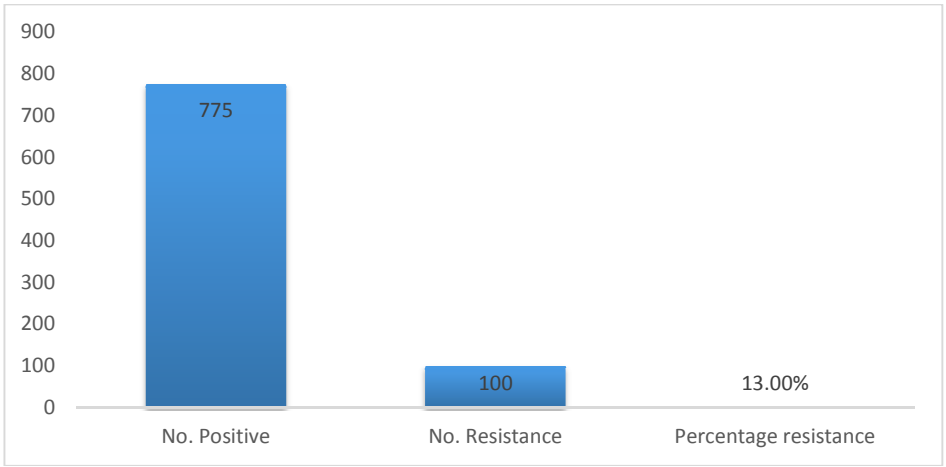


Figure 2: Prevalence of Rifampicin Resistant *Mycobacterium Tuberculosis* Using Xpert

MTB/RIF Assay

Out of one hundred (100) samples that were rifampicin resistant, 90 (90%) were culture positive while 7 (7%) were culture negative and 3 (3%) were contaminated. All of the ninety (90) samples that were culture positive were confirmed using rapid immunochromatoghapic test as *Mycobacterium tuberculosis* complex (Table 1). Twelve (13.3%) out of the ninety *Mycobacterium tuberculosis* isolates were resistance to ofloxacin and seventy eight (86.7%) were found to be pan susceptible to the second line anti-TB drugs. Eight (9%) were resistance to kanamycin and amikacin while seven (8%) were only resistance to capreomycin (Table 2).

Table 1. Culture Results Using Lowenstein Jensen Medium (N=100)

Culture	Frequency	Percentage (%)
Positive	90	90
Negative	7	7
Contaminated	3	3
Total	100	100

Table 2. Drug Susceptibility of *Mycobacterium Tuberculosis* Complex By Lowenstein Jensen Proportion Method (LJPM)

Drugs	Ofloxacin	Kanamycin	Amikacin	Capreomycin
Resistance (%)	12 (13.3)	8 (9)	8 (9)	7 (8)
Susceptible (%)	78 (86.7)	82 (91)	82 (91)	83 (92)

No XDR-TB was detected while pre extensively drug resistant tuberculosis (pre XDR-TB) due to fluoroquinolone (ofloxacin) resistance was observed to be 13.3% and pre XDR-TB due to aminoglycosides (kanamycin or/and amikacin or/and capreomycin) resistance was observed to 9% and 77.7% were pan susceptible to the second line anti-TB drugs (Table 3).

Table 3: Drug Resistance Pattern By Lowenstein Jensen Proportion Method

Resistance pattern	LJ Proportion method	Percentage (%)
XDR-TB	0	0
Pre XDR-TB (Ofloxacin)	12	13.3
Pre XDR-TB (Kanamycin/Amikacin/Capreomycin)	8	9
Pan susceptible	70	77.7
Total	90	100

Key: XDR-TB: Extensively drug resistant tuberculosis, Pre XDR-TB**:** pre extensively drug resistant tuberculosis

The age distribution among the respondents showed that no XDR-TB was detected between the age groups. However, resistance due to ofloxacin or aminoglucosides (kanamycin or/and amikacin or/and capreomycin) was detected to be high between the age groups 31-45 and 16-30 while no resistance due to SLDs was detected among the age groups 0-15 and >60 and there were more male who lives in urban settings with resistance to SLDs in this study. The highest prevalence of SLDs resistance obtained in this study was among non-reactive HIV, those without history of taken alcohol and those that were not previously exposed to TB treatment. However, all of the social-demographic and risk factors observed in this study were not statistically significant $p \leq 0.05$ (Table 4).

Table 4. Socio-Demographic and Risk Factors in Relation to Tuberculosis By LJ Proportion Method

Demographic and risk factors	No. positive (Pre XDR-TB)	Percentage (%)	No. positive (XDR-TB)	Odd ratio	P value
Age (Years)					
0-15	0	0	0		0.5026
16-30	7	35	0		
31-45	12	60	0		
46-60	1	5	0		
>60	0	0	0		
Gender					
Male	13	65	0	0.69 (0.24-2.0)	0.4839
Female	7	35	0		
Residence					
Rural	8	40	0	1.06 (0.38-2.93)	0.8875
Urban	12	60	0		
HIV status					
Reactive	1	5	0		0.5945
Non-reactive	12	60	0		
Unknown	7	35	0		
Alcohol					
Yes	2	10	0	2.48 (0.39-15.99)	0.3349
No	18	90	0		
Previous treated TB					
Yes	3	15	0	0.71 (0.18-2.75)	0.6468
No	17	85	0		

Key:
Pre XDR-TB= pre extensively drug resistant *Mycobacterium tuberculosis*
XDR-TB= extensively drug resistant *Mycobacterium tuberculosis*

DISCUSSION

The prevalence of *Mycobacterium tuberculosis* was found to be 12.65% by Xpert MTB/RIF assay this could be due to the study site which is referral hospital and decentralization of this Genexpert machine to other hospital. Our findings in this study were lower than 22.9% reported by Ikuebe *et al*., (2018) in Bayelsa and that of Panwal *et al*., (2018) who reported 27.8% in Kaduna. Resistance due to rifampicin by Xpert MTB/RIF assay was found to be 13%, this could be attributed to the study site which is referral hospital and decentralization of this Genexpert machine to other hospital. Resistance to rifampicin obtained in this study agrees with the work of Ikuebe *et al*., (2018) who reported 14.7% in Bayelsa but lower than 16.2% reported by Panwal *et al*., (2018) in Kaduna and higher than the 8.9% as reported by Akanbi *et al.,* (2017) in Plateau State.

Ninety (90%) of the isolate were culture positive and confirmed using rapid immunochromatographic test as *Mycobacterium tuberculosis* complex (MTBC), this may be due to primary screening tool (Xpert MTB/RIF) used in this study which can only detect MTB/RIF resistance and all the samples were MTB/RIF resistance, the use of moderate decontaminant (4% NaOH) which allow more recovery of Mycobacteria in sputum sample, this agrees with the finding of Chihota *et al*., (2010) who reported 88.9% in South Africa and that of Mamuda *et al.*

(2017) who reported 86% in Kaduna State but higher than 79.5% obtained by Molina-moya *et al*., (2018) in Abuja . Seven (7%) were culture negative, this could be due to presence of dead bacilli, longer time of exposure of the viable organism to the action of sodium hydroxide (NaOH) used during decontamination or low number of viable tuberculosis (TB) bacilli that cannot be detected by LJ media, this was in agreement with the findings of Mamuda *et al*., (2017) who reported 8% in Kaduna State and that of Molina-moya *et al*., (2018) who reported 8.6% in Abuja but higher than 4.9% reported by Chihota *et al*., (2010) in South Africa. While contamination rate was found to be 3% which is within the acceptable limit of World Health Organization (WHO) of 3-5%; this could be due to high quality standard of the laboratory used for the study and this could also be due to the number of samples used in this study, this contamination rate is lower than 9.3% reported by Chihota *et al*., (2010) in South Africa, 6% reported by Mamuda *et al*., (2017) in Kaduna and 6% as reported Aliyu M.S. (2015) in Kaduna state as well as 11.9% reported by Molina-moya *et al*., (2018) in Abuja.

The ofloxacin resistance was found to be 13.3%, this might be due inappropriate usage of these drugs especially FLQs including ofloxacin due to its broad spectrum of activity against gram-negative and gram-positive organisms most commonly prescribed and used in the treatment of respiratory tract infections, other infections other than tuberculosis and are readily available in pharmacies without prescription with easy access and inappropriate use of these drugs increase the risk for drug resistant TB emergence. Our findings agrees with the findings of Tasnim *et al*., (2018) in Bangladesh who reported 13.24%, Hu *et al*., (2013) who reported 12.6% in China and that of Kim *et al*., (2010) who reported 11.3% in South Korea but higher than 6% reported by Adam *et al*., (2017) in Sudan and 7% reported by Tuelo *et al*., (2019) in Botswana but lower than 17.9%, 22.2%, 26.3% and 55.94% reported by Hoa *et al*., (2016) in VietNam, Oudghiri *et al*., (2018) in Morocco, Jain *et al*., (2012) in India and Adwani *et al*., (2016) in India respectively. Resistance due to kanamycin (KM), amikacin (AM) and capreomycin (CM) were found to be 9%, 9% and 8% respectively in this study all TB cases who were resistant to KM were also resistant to AM and CM with the exception of one capreomycin which is contaminated, using a combination of aminoglycoside (KAN or AMK) and cyclic peptide (CAP) is equivalent to using a single drug with respect to the development of drug resistance.

Our findings agrees with the study conducted by Jain *et al*., (2012) who reported 13.5% in India, Hu *et al*., (2013) in China reported 15% for kanamycin, 11.6% for amikacin and 10.8% for Capreomycin, and that of Kim *et al*., (2010) who reported 8.3% in South Korea but higher than the 2.22% for kanamycin, 3.33% for amikacin and 1.11% for capreomycin reported by Oudghiri *et al*., (2018) and 2.94% reported by Tasnim *et al*., (2018) in Bangladesh and that of Hoa *et a*l., (2016) who reported 6.0% in VietNam .

Our findings revealed that no XDR-TB was detected in this study, a high proportion of pre XDR-TB due to ofloxacin resistance was observed to be 13.3% while pre XDR-TB due to kanamycin, amikacin and capreomycin resistance was observed to be 9%. The exposure of rifampicin or multidrug resistant TB patients to fluoroquinolones such as ofloxacin, ciprofloxacin and levofloxacin is common because these drugs are readily available in the open market for the treatment of other infections. The indiscriminate use of these antibiotics may have contributed to the evolution of resistant pre-XDR-TB cases found in this study and it could be due to inadequate treatment by health providers, drugs may be of poor quality and low compliance to full therapy by TB patients and some mutation that were express by this organism does not confer any resistance and in some cases the mutations identified are silent and are not always related to the acquisition of resistant. Our findings in this study was higher than that of

Daniel *et al*., (2013) in Nigeria who reported 16.7%, 17.9% reported by Hoa *et al*., in VietNam (2016) and 19.2% reported by of Gallo *et al*., (2018) in Brazil, but lower than 27%, 39.5% and 42.3% as reported by Sagonda *et al*., (2014) in Zimbabwe, Rao *et al*., (2015) in Karachi and Jain *et al*., (2012) in India respectively. There's an increase in the rate of pre XDR-TB reported globally. A large proportion of RR-TB isolates 77.7% were susceptible to second line anti-TB drugs, this is in agreement with 73% reported by Sagonda *et al.*, (2014) in Zimbabwe.

Prevalence of pre XDR-TB was high among age groups 31-45 and 16-30 years, the probable cause of the higher numbers of drug resistant TB in this groups might be because they are economically and reproductive active groups and may also be attributed due to their frequent movement/interaction, greater exposure to the environment, coming in contact with more people outdoors and higher case notification due to greater health awareness and concern among young adults and usually good quality sputum are obtained within these active groups, this agrees with the study by Murase *et al*., (2010) who reported between 21-40 years in Japan, 20-39 years reported by Tuelo *et al*., (2019) in Botswana but slightly differ from the study conducted in India by Adwani *et al*., (2016) and Daniel *et al*., (2013) in Nigeria who found higher numbers of pre XDR-TB cases among the young adult group, with ages ranging from 18-25 years and 15-29 years respectively and that of Tasnim *et al*., (2018) in Bangladesh who reported higher number of pre XDR-TB cases in the age group of 21 - 30 years . Pre XDR-TB was found to be higher in male (65%) compared to the female (35%), this agree with the findings of Mirza *et al*., (2013) in Pakistan who reported 66.6% for male and 33.3% for female but higher than the study reported by Adwani *et al*., (2016) in India who reported 55.1% and 44.9% among male and female respectively and lower than 78% for male and 22% for female reported of Hoa *et al*., (2016) in Viet Nam, this was thought to be related to alcohol and smoking dependency, imprisonment status where more male than female are involve and it could be due to less number of female participants in this study.

The high prevalence of pre XDR-TB was observed to be slightly higher (60%) among urban resident than those living in rural setting (40%), this could be as a results of less awareness of the disease and its mode of transmission, congestion and overcrowding commonly found in urban market places, mosque, church, and football watching centers, it could also be attributed due to the lesser number of patient from rural setting than urban setting in this study, there's no association between pre XDR-TB and type of residency. The high prevalence of pre XDR-TB in this study was observed to be among non-reactive HIV patients (60%), this could be related to the fact that HIV does not cause drug resistance but rather predispose individuals to TB/drug resistant TB infection or disease progression, our findings agrees with the study reported by Hoa *et al*., (2016) in Viet Nam who reported high prevalence of drug resistance (pre/XDR-TB) were among HIV sero-negative only but disagree with the study by Tuelo *et al*., (2019) who reported high prevalence among HIV sero-positive. Statistical analysis showed there's no statistically association between HIV and drug resistance (pre XDR-TB/XDR-TB) with $p \leq 0.05$.

The prevalence of pre XDR-TB in relation to alcohol drinking was high among those that have not taken alcohol before (90%) compared to those that have taken alcohol before (10%). Statistically analysis revealed no association between alcohol consumption and drug resistance (pre XDR-TB/XDR-TB) with $p \leq 0.05$; this could be due to the number of respondent for alcohol consumption and location of this study. The high prevalence of pre XDR-TB was among those that were new cases or not exposed to TB drugs were 85%, this could be due to the transmission of this infectious organism directly from patient who have drug resistant strains to another person and could be due to the sampling, by which majority of the

sample in this study were from patient who were not treated with anti-TB drugs. Statistical analysis showed that no association exist between previous TB and drug resistance, our findings agrees with the study by Hoa *et a*l., (2016) in VietNam who reported high prevalence of pre XDR-TB among new cases (19.5%) than in previously treated cases (16.3%).

CONCLUSION

This study detects high resistance of *M. tuberculosis* to either ofloxacin or second line injectables (amikacin, kanamycin or capreomycin). This study identifies the need for more awareness to the general public on the mode of transmission of tuberculosis and there's need to monitor over the counter sales of some of the drugs used in the treatment of TB patients specifically quinolones in our pharmacies and medical stores for sales without prescription. Likewise there's need for close monitoring of the TB patients for proper treatment and compliance to prevent the emergence of drug resistant tuberculosis and its subsequent transmission/spread in to the community. Clinicians need to be sensitized on the rational in use of fluoroquinolones in patients suspected of having respiratory tract infections or having other disease than that cause by TB. Furthermore, the study identified the need for rapid identification and confirmation of drug resistance among TB patients which will assist clinicians to monitor these patients in order to prevent the progression to XDR-TB which is more difficult to treat and with poor treatment success.

REFERENCE

Adam, M. A. M., Ali, H. M. H., and Khalil, E. A. G (2017). Initial second-line drug resistance of *Mycobacterium tuberculosis* isolates from Sudanese retreatment-patients. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases,* 9: 21-23

Adwani, S., Desani, D.U. and Joshi, M.J. (2016). Prevalence of Pre-Extensively Drug-Resistant Tuberculosis (Pre XDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB) among Pulmonary Multidrug Resistant Tuberculosis (MDR-TB) at a Tertiary Care Center in Mumbai. *Journal of Krishna Institute of Medical Sciences University*, **5**:13-19.

Ajbani, K., Nikam, C., Kazi, M., Gray, C., Boehme, C., Balan, K., Shetty, A., and Rodrigues, C. (2012) Evaluation of GenoType MTBDRsl Assay to Detect Drug Resistance Associated with Fluoroquinolones, Aminoglycosides and Ethambutol on Clinical Sediments. *Plos ONE* **7**(11): e4933.doi:10.1371

Akanbi, M.O., Achenbach, C., Taiwo, B., Idoko, J., Ani, A., Isa, Y., Agbaji, O., Ukoli, C., Akande, P., Maiga, M. and Murphy, R.L. (2017). Evaluation of gene xpert for routine diagnosis of HIV-associated tuberculosis in Nigeria: A prospective cohort study, *BMC Pulmonary Medicine.* **17**: 87. doi: 10.1186/s12890-017-0430-6

Aliyu M.S. (2015). Prevalence of multi drug resistance mycobacterium in kaduna state (Doctor dissertation, Ahmadu Bello UniversityZaria, Kaduna Nigeria).

Barnard, M., Albert, H., Coetzee, G., Obrien, R. and Bosman, M.E. (2008). Rapid Molecular screening for multidrug resistant tuberculosis in high volume public health laboratory in South Africa. *American Journal of Critical Care Medicine*, 177(7):787-792.

Becton, Dicson and Company, (2015). BD MGIT™ TBc ID idetification test package insert, BD document L 8085917 (02) 2015-04. BD Dignostic System Sparks MD 21152 USA.

Boehme, C.C., Nabeta, P., Hillemann, D., Nicol, M.P., Shenai, S., Krapp, F., Allen, J., Tahirli, R., Blakemore, R., Rustomjee, R., Milovic, A., Jones, M., O'Brien, S.M., Persing, D.H., Ruesch-Gerdes, S., Gotuzzo, E., Rodrigues, C.,Alland, D., and Perkins, M.D. (2010). Rapid molecular detection of tuberculosis and rifampicin resistance. *New England Journal of Medicine*, 363:1005–1015

Bwanga, F., Hoffner, S., Haile, M. and Joloba, M.L. (2009). Direct susceptibility testing for multidrug resistant tuberculosis: A meta-analysis. *Bio Med Central of Infectious Disease* **9**:67.

Chihota, V.N., Grant, A.D., Fielding, K., Ndibongo, B., van Zyl, A., Muirhead, D. and Churchyard, G.J. (2010). Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *International Journal of Tuberculosis and Lung Disease*, **14**(8):1024-31.

Concepcion, F.A., R.M.T., Myrna, T.M., M.D., Heidi, R.S., Regina C.O., Carmela, P.E., Wilma C.B. and Aileen, M.A. (2001). Isolation rate of mycobacterium tuberculosis from smear-negative and smear-positive sputum specimen using the ogawa culture technique and the standard Lowenstein Jensen culture technique. *Philippine Journal of Microbiology and Infectious Disease;* **30**(2):37-39.

Daniel, O., Osman, E., Oladimeji, O. and Dairo, O.G. (2013). Pre-Extensive Drug Resistant Tuberculosis (Pre-XDR-TB) among MDR-TB Patents in Nigeria. *Global Advanced Research Journal of Microbiology,* **2**:22-25.

Gallo, J.F.**,** Pinhata**,** J.M.W., Simonsen**,** V., Galesi, V.M.N., Ferrazoli**,** L. and Oliveira**,** R.S. (2018). Prevalence, associated factors, outcomes and transmission of extensively drug-resistant tuberculosis among multidrug-resistant tuberculosis patients in São Paulo, Brazil: a cross-sectional study. *Clinical Microbiology and Infection***, 24:** 889-895

Hoa, B.N., Nhung, V.N., Huong, T.G.T., Hai, V.N. and Quyen, T.T.B. (2016). Prevalence of resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients in Viet Nam. *Western Pacific Surveillence Response Journal*. **7**(2): 35–40.

Hu, Y. Hoffner, S. Wu, L. Zhao, Q. Jiang, W. and Xu, B. (2013). Prevalence and genetic characterization of second-line drug-resistant and extensively drug-resistant Mycobacterium tuberculosis in Rural China. Antimicrobial Agents Chemotheraphy. 57, 3857–3863.

Ikuabe, P.O and Ebuenyi, I.D (2018). Prevalence of rifampicin resistance by automated Genexpert rifampicin assay in patients with pulmonary tuberculosis in Yenagoa, Nigeria. *Pan African Medical Journal*. **29**: 204. doi: 10.11604/pamj.2018.29.204.14579

Jain, A., Dixit, P and Prasad, R. (20120. Pre-XDR & XDR in MDR and Ofloxacin and Kanamycin resistance in non-MDR Mycobacterium tuberculosis isolates. *Tuberculosis* (Edinb). **92**(5):404–6.

Jassal, M. and Bishai, W.R. (2009). Extensively drug resistant tuberculosis, *Lancet of infectious Disease* **9** (1):19-30.

Johnston, J.C., Shahidi, N.C., Sada, S.M. and Fizgoad, J.M. (2009). Treatment outcomes for multidrug resistant tuberculosis: a systematic review and meta-analysis.4, e6914.

Kim, D.H., Kim H.J., Park S.K., Kong S.J., Kim Y.S., Kim T.H., Kim E.K., Lee K.M., Lee S.S., Park J.S. and Koh W.J. (2010). Treatment outcomes and survival based on drug resistance patterns in multidrug-resistant tuberculosis South Korea. American Journal of Respiratory and Critical Care Medicine. **182**(1): 113–9.

Mamuda, K., Olonitola, O. S., Jatau, E. D. and Nicholas, E. (2017).Evaluation of Nitrate Reductase Assay for Detection of Multi-drug Resistant *Mycobacterium tuberculosis* among Patients at National Tuberculosis Reference Laboratory Zaria. Nigeria. *Journal of Advances in Medical and Pharmaceutical Sciences* **14**(1): 1-7

Mirza, I.A., Khan, F.A., Khan, K.A., Satti, L., Ghafoor, T. and Fayyaz, M. (2013). Extensively and Pre-Extensively Drug Resistant Tuberculosis in Clinical Isolates of Multi-Drug Resistant Tuberculosis Using Classical Second Line Drugs (Levofloxacin and Amikacin), *Journal of the College of*

Physicians and Surgeons Pakistan, **25** (5): 337-341

Molina moya B., Abdurrahaman S.T., Madukaji L.I., Gomgnimbou M.K., Spinasse L., Gomes-fernandes M., Gomes H.M., Kacimi S., Dacombe R.,Bimba J.S, Lawson L., Sola C., Cuevas L.E., Dominguez, J (2018). Genetic characterization of mycobacterium tuberculosis complex isolate circulating in Abuja, Nigeria. *Infectious and Drug resistance,* **11**:1617-1625

Morcillo, N., Imperaile, B. and Palomino, J.C. (2008). New simple decontamination method improves microscopic detection and culture of mycobacteria in clinical practice. *Infection and drug resistance,* **1:21**-26.

Murase Y.,Maeda S., Yamada H., Ohkado A.,Chikamatsu K., Mnuno K., Kato S.,Mitrai S (2010).Clonal expansion of multidrug-resistant and extensively drug resistant tuberculosis, Japan. Emerging Infectious Disease, **16**(6): 948-54

National Tuberculosis Leprosy Burulli Ulcer Management Control Programme Guidelines, 2014. 6th edition. Federal Ministry of Health.

Oudghiri, A., Karimi, H., Chetioui, F., Zakham, F., Eddine Bourkadi, J., Driss Elmessaoudi, M., Laglaoui, A., Chaoui, I. and Mohammed, E. M. (2018). Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrugresistant tuberculosis patients from high prevalence tuberculosis city in Morocco. *Bio Med Central of Infectious Diseases*, **18**:98

Panwal, M.T., Abubakar, A.G., Allanana, J.A., Nwofor, A.C., Ani, A., Ezati, N., Ahmadu, I., Peters, S., Ikeh, E.I. and Abimiku, A (2018). Evaluation of Genxpert Real Time PCR POC in the Diagnosis of Multidrug Resistant TB Among Patients Attending NTBLTC Saye-Zaria Nigeria. *Journal of Infectious Diseases and Pathogenesis* 1: 106

Rao, N., Baig, S., Hussain, N., Ahmed, N. and Rao, D. (2015). Prevalence of pre-XDR-TB, XDR-TB among MDR-TB Patients registered at Ojha Institute of Chest Diseases, Karachi. *European Respiratory Journal*, 46: PA2715; doi: 10.1183/13993003.congress-2015.PA2715

Sagonda T., Mupfumi, L., Manzou, R., Makamure, B., Tshabalala, M., Gwanzura, L., Mason, P., and Mutetwa, R. (2014). Prevalence of Extensively Drug Resistant Tuberculosis among Archived Multidrug Resisnt Tubculosis Isolates in Zimbabwe. Hindawi Publishing Corporation Tubrculosis Research and Treatment Vol. 2014, Article ID 349141, 8 pg.

Sharma, M., Misra, R.N., Gandham, N.R., Jadhav, S.V., Angadi, K., and Wilson, V. (2012). Comparison of modified petroff and N-acetyl-L-cystein- Sodium hydroxide method for sputum decontamination in tertiary care hospital in India. *Medical Journal of Dr. DY Patil University*; **5**:97-100.

Tasnim, T., Tarafder, S., Alam, F., Sattar, H. and Mostofa Kamal, S. (2018). Pre-Extensively Drug Resistant Tuberculosis (Pre-XDR-TB) among Pulmonary Multidrug Resistant Tuberculosis (MDR-TB) Patients in Bangladesh. *Journal of Tuberculosis Research*, **6**:199-206

Tuelo, M., Pinkie M., Brigitta, D., Serej, D. L., Elizabeth, M.S., Thato, I., Lucy, M., Margaret, M., Botshelo, K., Goabaone, R., Thusoyaone T.T., Ishmael, K., Sikhulile, M., Robin M. W., and Simani, G (2019). Detection of second line drug resistance among drug resistant *Mycobacterium tuberculosis* isolates in Botswana.*Pathogen* **8**:208

World Health Organisation (2012). Global TB programme, updated interim critical concentration for first line and second-line DST.

World Health Organization Report (2016). Global Tuberculosis Control: epidemiology, strategy, financing. WHO/HTM/TB/2016.13 www.who.int/tb/publications/global-report/2016.

World Health Organization Report (2019). Global Tuberculosis Control: epidemiology, strategy, financing. WHO/HTM/TB/2019.17 www.who.int/tb/publications/global-report/2019.