DETECTION OF MULTIDRUG RESISTANT TUBERCULOSIS (MDR-TB) AMONG RIFAMPICIN-RESISTANT TB PATIENTS USING LINE PROBE ASSAY (LPA) IN KANO, NIGERIA

*1Aminu, A.I. and 2Tukur, A.D.
[1] Department of Microbiology, Bayero University, Kano, Nigeria
[2]National Tuberculosis (TB) Reference Laboratory North-West Zone, Aminu Kano Teaching Hospital, Kano, Nigeria.
*Correspondence author: aishatuaminuibrahim@gmail.com, 080545033260

ABSTRACT
The Emergence of drug-resistant Mycobacterium tuberculosis strains especially multidrug resistant-TB (MDR-TB) and indeed extensively drug resistant TB (XDR-TB) is considered a real threat to achieving TB control. Thus, the WHO identified the need for accelerated access to rapid testing for rifampicin resistance to improve case detection in all patients with suspected MDR and XDR-TB. This study aimed at detecting MDR status of rifampicin-resistant TB patients in north western part of Nigeria, as such information was found to be rare in this part of Nigeria. It was a descriptive cross-sectional study conducted at Aminu Kano Teaching Hospital, North-West TB reference Laboratory and purposive sampling technique was used in selecting patients for the study. Data generated was presented using descriptive statistics. Sputum samples were collected and processed according to standard mycobacteriological procedures. The presence of M. tuberculosis complex (MTBC) was confirmed using SD BIOLINE TB Ag MPT64 Rapid test. Genotypic assays were carried out on these samples by the GenoType® MTBDRplus molecular line prove assay (LPA). The results of the study revealed that of the 88 sputum samples collected from rifampicin resistant (confirmed by Genxpert from referring centres) TB patients, 74 (84.09%) were males and 14 (15.9%) were females. Most of them were aged 21-30 years and 31-40 years. Also, 86 of the sputum samples were confirmed as MTBC. Genotypic assays revealed that 48/88 (54.5%) of the samples were identified as MDR-TB, 29 (32.9%) were rifampicin mono-resistant cases while only 1(1.36%) was resistant to isoniazid and 8 samples were found to be susceptible to both isoniazid and rifampicin. The pattern of genetic mutations revealed that rifampicin resistance due to the missing rhoB WT gene and the presence of rhoB MUT gene was seen in 77/78 (98.7%) and 51/78 (61.4%) of the rifampicin-resistance samples respectively and isoniazid resistance due to katG missing WT gene and katG MUT gene region was seen in 35/78 (44.9%) of the samples. Among the MDR-TB cases rifampicin resistance was associated with rpoB WT gene and rpoB MUT gene in 100% and 62% of the isolates, and INH resistance was associated with katG WT gene and katG MUT gene in 70.8% and 72.9% of the isolates respectively. INH resistance in the MDR-TB cases was further associated with InhA WT and InhA MUT in 16.67% and 4.17% of the isolates respectively. The study concludes by identifying that the reported MDR-TB rate of 54.5% among rifampicin-resistant TB patients in the study was rather alarming and also identifies the need for expertize in the conduct and diagnosis of rifampicin-resistance status of TB patients using the GeneXpert techniques lack of which may be detrimental to the health of the patient.Thus, the study solicits for a much more committed TB control programmesincluding provision and training of manpower by the relevant agencies as a well as putting more efforts towards rapid and accurate diagnosis of TB patients, and proper treatment and management of the infected cases to minimize the spread and evolution of MDR-TB and indeed XDR-TB whose treatment is presented with more challenges.
Keywords: Rifampicin resistance, MDR-TB, LPA, North-Western zone, Nigeria.

INTRODUCTION

Tuberculosis (TB) a curable disease currently remains one of the most important infectious diseases not only in Nigeria but worldwide, with drug resistance and multidrug resistance (MDR) identified as the major factors affecting the TB control strategies. The World Health Organization (2013a) revealed that 3.7% of new tuberculosis (TB) patients in the world have multidrug-resistant strains (MDR-TB) and levels are much higher in those previously treated– about 20%.The annual global MDR-TB burden is estimated at around 400 000 cases (WHO, 2010), and Nigeria as at 2011 has an estimated MDR-TB burden of >1000 cases with only 1% of the cases enrolled on treatment (WHO, 2013a).

The WHO (2006) stated that although causes of MDR-TB are microbial, clinical and programmatic, drug-resistant TB is essentially a man-made phenomenon, mainly attributed to patience non-compliance, inadequate supply and inappropriate administration of drug regimens, and inadequacies to monitor treatment and most importantly the presence of poorly organized or TB control program. The appearance of extra multidrug resistance (XDR) as documented by many works further compounded

the MDR nature of TB mainly due to the fact that treatment of patients with XDR-TB is challenging because of the lack of potent anti-TB drugs (Barnard *et al.*, 2012).

The currently recommended treatment for new cases of drug-susceptible TB is a regimen of four-line drugs: Isoniazid (INH), Rifampicin (RMP), Ethambutol(ETM) and Pyrazimide(Barnard *et al.*, 2012). Treatment for the multidrug-resistant TB, defined as resistant to Isoniazid (INH) and Rifampicin (RMP) – the two most powerful anti-TB drugs- is longer, and requires more expensive and more toxic drugs. According to Barnard *et al.* (2012) XDR-TB is defined as resistance to at least INH, RMP, a fluoroquinolone, and one of three injectables (amikacin, kanamycin, or capreomycin).

Resistance-associated point mutations, deletions, or insertions have been described for all first-line drugs and for several second-line and newer drugs (ethionamide, fluoroquinolones, macrolides and nitroimidazopyrans)(Barnard *et al.*, 2012).Barnard *et al.* (2012) revealed that INH resistance involves mutations in several genes such as *katG*, which encodes the activating enzyme, catalase, and other regulatory genes such as *inhA*, *ahpC-oxyR*, *ndh*and *furA*.RIF on the other hand specifically inhibited the elongation of full-length transcripts,subsequently, the *rpo*B locus from MTB was characterized and mutations conferring the resistant trait were identified(Rattan *et al.*, 1998). It is interesting to note that mono-resistance to INH is relatively common while mono-resistance to RMP is quite rare (Barnard *et al.*, 2012). In fact, nearly 90% of RMP-resistant strains are also INH-resistant. Therefore, resistance to RMP may be used as a surrogate marker for MDR-TB.

Nathason*et al.* (2010) identified that, preventing initial infectionwith MDR tuberculosis and managing the treatmentof existing cases appropriately are the keysto containing the spread of this disease. Thus, rapid laboratory detection of MDR- and XDR-TB is critical, as late diagnosis could cause delays in treatment and serves as a major contributor to increased transmission of TB in the community and may compromise the efficacy of therapy (Barnard *et al.*, 2012). In 2013 the World Health Organization in its policy statement indicated that effort should be made to reach the 3million new TB case that are missed every year by the health system 70% of which live India, South Africa, Bangladesh, Pakistan, china, DR Congo, Mozambique, Nigeria, Ethiopia, phliphines and Myanmar (WHO, 2013b) and especially bearing the fact that only 7% of the estimated MDR-TB cases are detected (WHO, 2010). Barnard *et al.* (2012) emphasizes the need for accelerated access to rapid testing for rifampicin resistance to improve case detection in all patients with suspected MDR and XDR-TB and to identify the pattern of genetic mutations associated with the resistant strains. Thus, case detection is one of most important factor that determines the successful implementation of TB control strategies or otherwise.

In view of the above, coupled with the fact that information on MDR status of TB patients is rare in Nigeria, this study was aimed at revealing the MDR profiles of rifampicin-resistant TB patients that reported to the National TB Reference Laboratory of Aminu Kano Teaching Hospital, Kano from the northwestern part of Nigeria using Line Prove Assay (LPA).

MATERIALS AND METHODS
The study was conducted at Aminu Kano Teaching Hospital, North West TB reference Laboratory, Kano, Nigeria after receiving ethical clearance from the Ethical Review Committee of the hospital (Appendix I). The study was a descriptive cross-sectional study and purposive sampling technique was used in selecting patients for the study, which included all TB patients that reported to the laboratory and were rifampicin resistant cases (confirmed by Genxpert) from their respective health centers across North-Western Zone of Nigeria. Furthermore, except for three, all the patients were originally diagnosed with TB and were treated with World Health Organization category I therapy (*i.e.* treatment with INH, RIF, PZA and EMB for 2 months, followed by treatment with INH and RIF or INH, RIF, and PZA for an additional 4 months). New-smear positive TB patients were excluded from the study. A total of 88 sputum samples were collected from the TB patients that were referred to the laboratory from September 2013 to October 2015 upon their consent. Data generated from the study was presented using descriptive statistics.

A total of three first-morning sputum specimens were collected from the patients and were subjected to pretreatment involving digestion, homogenization, decontamination and concentration in Biosafety Cabinet Level II (BSL II)using NALC-NaOH solution, 0.067M phosphate buffer (pH 6.8) and buffered Saline (PBS) according to NTBLCP SOP Manual (2011) and NCCLS (2000). Sputum samples were also Ziehl-Neelsen stained and acid fast bacilli were detected. All sputum samples collected, reagents and equipment used were handled according to standard mycobacteriological procedures as described by NTBLCP SOP Manual (2011) and NCCLS (2000)

The SD BIOLINE TB Ag MPT64 Rapid test was used to confirm the identity of *M. tuberculosis* complex (MTBC) contained in the samples according to manufacturer's specifications(SD Bioline Kit, Standard Diagnostics, Inc., Korea, 2015). It is a rapid immunochromatographic identification test for the *M. tuberculosis* complex (MTBC) that uses mouse monoclonal anti-MPT64 which has sensitivity and specificity of 98.6% and 100% respectively. The cassette was removed from the foil pouch and placed on a flat dry surface thus exposing the sample well. For each sample, one hundred microlitres (100 µl) of the treated sputum sample was then added into the sample well. After 15minnutes of sample application, the appearance of two colour (purple) bands ("T" test band and "C" control band) within the result window was considered a positive result. Samples that were confirmed as being MTBC were then used for further analysis.

Genotypic assays were carried out on each of the confirmed MTBC samples in three separate rooms using the GenoType® MTBDR*plus* molecular line probe assay (LPA) according to the manufacturer's specification (HainLifescienceGmbH, Nehren, Germany, 2015) and WHO (2008) recommendations in three stages indicated below.

Additionally DNA extraction was performed in the BSL III laboratory, master mix preparation in a second room, and PCR and hybridization were performed in a third laboratory.

1- DNA EXTRACTION PROCEDURE WITH GENOLYSE

DNA extraction procedure with genolyse was done according to manufacturer's specifications (HainLifescienceGmbH, Nehren, Germany,2015) and WHO (2008).Using a sterile graduated pipette 0.5 ml (500 µl) of the decontaminated sputum sample was transferred into microcentrifuge tube. This was done for all the samples, after which the tubes were closed and centrifuged for 15 minutes at 10000xg. The supernatant was discarded and 100µl lysis buffer (A-LYS) was added and re-suspended by vortexing gently for 30 sec. The tubes were arranged in a floater inside the BSC II and incubated for 5minutes in a water bath at 95^0C. Then, 100µl Neutralisation buffer (A-NB) was added and vortexed for 30 seconds and the tubes were centrifuged at maximum speed (10,000xg). The heavier debris formed the pellet and the lighter DNA (free from impurities) was suspended in the supernatant which was transferred into clean labeled micro-centrifuge tubes for further use.

2- PCR amplification of the extracted DNA

The master mix preparation was done according to manufacturer's specification (HainLifescienceGmbH, Nehren, Germany, 2015) and WHO (2008). The master mix was made up of 10µl of the AM-A and 35µl of AM-B Reagent which was placed in a PCR tube labeled with sample number and mixed very well.This was prepared inside dead air box in a clean DNA free room. Then 5µl of each sample (containing the extracted DNA from above) was added to the corresponding tube containing the master mix and then mixed gently by pipetting up and down a few times. The PCR tubes were then placed in a 30 cycle (10 + 20) thermal cycler program for amplification. After amplification the DNA contained in the amplicons were denatured in the TwinCubator® which was pre-warmed to 45 °C and 20µl of denaturation solution (NaOH) was added to each labeled well of the TwinCubator® tray followed by the addition of 20µl of the amplicons respectively. The mixture was mixed gently by pipetting up and down five times and then incubated at room temperature for 5mins.

3- Hybridization and detection

Hybridization and detection procedures were carried out according to according manufacturer's specification (HainLifescienceGmbH, Nehren, Germany, 2015) and WHO (2008). After denaturation of the amplicons, 1ml of the pre-warmed hybridization buffer (HYB) was carefully added to the wells using a pipette and thoroughly mixed. The tray was placed on the TwinCubator® and labeled strips were added to each well ensuring that the strips were completely covered by the liquid and incubated at 45°C for 20mins. After incubation, the HYB buffer was aspirated completely from each well and 1ml of the pre-warmed red stringent wash buffer (STR) was then dispensed into the tray. After 10 minutes incubation at 45 °C in the TwinCubator®, STR buffer was

aspirated and was washed off with 1 ml of Rinse solution (RIN) for 1 minute. Then 1ml of the Conjugate (CON) solution was dispensed into each well and incubated for 20 minute on the TwinCubator®. The strips were washed twice with 1 ml of Rinse solution (RIN) for 1 minute in the TwinCubator®. Then sterile distilled water was added and a 1 minute wash performed on the TwinCubator® to wash off the RIN solution after which the distilled water was completely decanted. One (1) ml of the Substrate solution was then dispensed into each well and incubated for 10 minutes on the TwinCubator® after which the Substrate solution was aspirated and the strips washed twice with sterile distilled water. A pair of clean tweezers was used to remove the strips from the TwinCubator® tray and placed onto absorbent paper. The developed strips were partially dried and transferred to the GenoType® MTBDR*plus* score sheet for interpretation.

RESULTS

The results of the study revealed that of the 88 sputum samples collected from rifampicin- resistant (confirmed by Genxpert from referring centres) TB patients that were originally on the World Health Organization category I therapythat reported to the Aminu Kano Teaching Hospital, Northwest TB Reference Laboratory, 74 (84.09%) were males and 14 (15.9%) were females (Table 1). Most of the patients were in the age groups 21-30 years and 31-40 years. Age group 11-20 years had the least number of patients (15). Only 1 patient was aged 70 years (Table 1).

Table 2 revealed that 86 (97.72%)of the sputum samples were confirmed as MTBC using theSD BIOLINE TB Ag MPT64 Rapid test while two were invalid.

Results of the GenoType® MTBDR*plus* molecular line probe assay (LPA) were shown in Table 3, which reveals that of the 88 rifampicin-resistant (by Genexpert) sputum samples, 48 (54.5%) were identified as MDR-TB, 29 (32.9%) were rifampicin mono-resistant TB cases while only 1(1.36%) was resistant to isoniazid. Furthermore, 8 sputum samples were found to be susceptible to both isoniazid and rifampicin.

Table 4 describes the pattern of genetic mutations of the rifampicin-resistant samples. According to the study, among the 78 rifampicin-resistant TB samples (i.e., MDR=48, RIF=29 and INH=1), detection of rifampicin resistance due to the *rhoB*missing WT geneand the presence of *rhoB*MUT gene was seen in 77 (98.7%) and 51 (61.4%) of them respectively.

Similarly, Table 4 further describes that among the 78 rifampicin-resistant samples, detection of isoniazid resistance due to *katG* missing WT gene and presence of *katG* MUT gene was seen equally in 35 (44.9%) of them.

Among the 78 rifampicin-resistant isolates, resistance due to *inhA* missing WT gene was observed in only 9 (11.5%) of them, whereas resistance due the presence of *inhA* MUT gene was only seen in 2 (4.17 %) of the samples (Table 4)

3

Among the 48 MDR-TB samples, detection of rifampicinresistance due to *rhoB*missing WT gene was seen in all (100%) of them while 34 (70.8%) of them exhibited resistance due to the presence of *rhoB*MUT gene (Table 4). Similarly detection of isoniazid resistance due to *katG* missing WT gene and the presence of *katG* MUT gene was seen in 34 (70.8%) and 35 (72.9%) of the MDR-TB samples respectively.INH resistance in the MDR-TB cases was further associated with InhA WT and InhA MUT in 16.67% and 4.17% of the isolates respectively

Among the 29 rifampicin mono-resistant sample, resistance attributed to*rhoB*missing WT gene was observed in all the 29 (100%) samples, whereas 21 (72%) of the isolates exhibited resistance due the presence of *rhoB*MUT gene (Table 4). And, finally, the only isoniazid mono-resistant isolate among the 29 rifampicin mono-resistant samples revealed a 100% resistance pattern to both *katG* missing WT gene and *inhA* missing WT gene respectively.

Table 1: Distribution of age and sex among rifampicin resistantTB patients attending Aminu Kano Teaching Hospital, Northwest TB Reference Laboratory,Kano Nigeria.

AGE (years)	SEX MaleFemale No (%)	No (%)	Total
11-20	11 4		15
21-30	254		29
31-40	223		25
41-50	151		16
51-60	11		2
61-70	01		1
TOTAL	**74 (84.09)**	**14(15.91)**	**88**

Table 2: Status of AFB Smear Positive Rifampicin-Resistance TB sputum samples from patients attendingAminu Kano Teaching hospital, Northwest TB Reference Laboratory, Kano, Nigeria using SD BIOLINE TB Ag MPT64 Rapid test.

Organism identifiedAFB Smear Positive Rifampicin-Resistance Sputum Samples(SD BIOLINE TB Ag MPT64)Number (%)	
Mycobacterium tuberculosis complex	86 (97.72)
Invalid	2 (2.28)
Total	**88**

Table 3: Drug resistance and susceptibility profile of 88 rifampicin-resistant TB patients attending Aminu Kano Teaching Hospital, Northwest TB Reference Laboratory, Kano, Nigeria.

Drug resistance and Susceptibility status	Number of isolates (n =88) No	%
INH resistance	1	1.36
RIF resistance	29	32.9
MDR TB	48	54.5
Susceptible	8	9.0
Invalid	2	2.3

Table 4: Pattern of genetic mutations of MTBC confirmed sputum samples from rifampicin-resistant TB patients at Aminu Kano Teaching hospital, Northwest TB Reference Laboratory, Kano,Nigeria.

Gene	Band type/ mutation region	Resistance status			Total (n=78)
		MDR (n=48)	RIF-R (n=29)	INH-R (n=1)	
rho B	WT missing	48 (100%)	29 (100%)	0	77 (98.7%)
	MUT present	30 (62.5%)	21 (72%)	0	51 (61.4%)
kat G	WT missing	34 (70.8%)	0	1 (100%)	35 (44.9%)
	MUT present	35 (72.9%)	0	0	35 (44.9%)
Inh A	WT missing	8 (16.67%)	0	1 (100%)	9 (11.5%)
	MUT present	2 (4.17)	0	0	2 (2.6%)

DISCUSSION

The backbone of a successful TB case management depends largely on, accurate and rapid detection of new cases and, prompts and proper monitoring of infected cases to avert the evolution and spread of drug resistant TB and indeed MDR-TB which could lead to a more complicated XDR-TB, thus jeopardizing efforts to control TB. In this regard several rapid diagnostic tests have been evolved including GenoType® MTBDR *plus*assay which was used in this study to describe the multidrug resistant profile of TB patients initially identified with rifampicin-resistance (by GeneXpert) referred from various centers across the Northwestern zone of Nigeria.

This study revealed that of the 88 initially confirmed rifampicin-resistance patients who were on WHO category I anti-tuberculosis drugs that reported to the Aminu Kano Teaching Hospital, Northwest TB Reference Laboratory, Kano 48(54.5%) were identified as MDR-TB (by line prove assay) and this was higher than the reports by Qazi*etal.*(2014) and Tripathi*et al.* (2012) who reported MDR-TB rate of 38% among resistant cases in Pakistan and 43.37% in India from patients that were refractory to chemotherapy for >12 month respectively. However, the results were similar to 54% of MDR-TB in Georgia, USA reported by Tukvadze*et al.* (2012). Thus, our study identifies that in a setting typified with inadequate provisions of well-organized TB reference laboratories the reported MDR-TB rate of 54% is rather alarming especially bearing the fact that the WHO (2013a) revealed that the prevalence of MDR-TB among previously treated cases was about 20%.

The study revealed that 29 (32.9%) of the TB patients were identified with rifampicin mono-resistance.

The 8 susceptible cases and 1 INH mono-resistant case reported in this study were earlier identified as RIF resistance by GeneXpert at their initial healthcare center, however our report detected them as susceptibleland as INH mono resistant respectively by line prove assay. More so, 3 out of the 8 cases were patients that were primarily not on any anti-TB drug but identified as RIF resistance meaning that it could be an acquired drug resistance. So many factors might account for the observed discrepancy. Firstly, the initial report of RIF resistance status of the patients by their respective health facility could be an inappropriate diagnosis, which indicated the need to conduct and report the TB diagnosis with expertise and caution by the appropriate authority lack of which could lead to inappropriate administration of anti-TB drugs which is detrimental to the health of the patient and encourages development and spread of drug resistance, MDR and XDR-TB. Secondly, Barnard *et al.* (2012)described that LPA results are based on banding patterns detected on a strip following hybridization with PCR products amplified from target DNA in a patient specimen, thus the molecular test may not include probes for all possible mutations. Tripathi*et al.* (2012) also explained that INH and RIF's resistance in MTB complex (MTC) isolates are mainly based on mutations in a limited number of genes.Thus, in this study there is every possibility that the gene regions responsible for rifampicin resistance of the 8 susceptible cases in our study were not identified by the LPA method used.

Barnard *et al.* (2012) further elaborated that often, confusion arises if a wild-type band (found in drug-susceptible strains) is missing, but a corresponding mutation band (found in drug-resistant strains) is not present. They identified that this banding pattern is the result of a drug resistance-associated mutation different from the common ones identified by the specific probes on the strip. They further revealed that, there is a slight possibility that the pattern represents a silent mutation, one that does not result in an amino acid change and concluded that this is one of the reasons that mutations observed with molecular methods should be confirmed with a phenotypic assay silent mutation. Qazi*et al.* (2014) also expounded that five (20%) MDR-TB isolates in their study lack any mutation in the rifampicin resistance determining regions (RRDR) region of the *rpoB*gene, though these isolates were phenotypically resistant to RIF. They indicated that thisdifference might persist due to genotype variations prevailing worldwide. Another important observations made by Barmankulova (2015) was that although Xpert MTB/RIF showed higher accuracy in detecting rifampicin resistance TB cases with a sensitivity and specificity of 96.8% and 91.8%respectively in their study, yet they identified that furtherstudies ofXpert MTB/RIF in a general population with better adherence to laboratory protocol and solid recording systems may yield more accurate information.Albert *et al.* (2010) earlier stressed that careful attention must be paid to training, supervision andadherence to stringent laboratory protocols to ensure high quality results during routine implementation.

Rifampicin resistance was found out to be associated with transcriptional process that target the mycobacterial RNA polymerase leading to mutations in the *rpoB* locus, thus conferring conformational changes leading to defective binding of the drug and consequently resistance (Jin and Gross, 1988). Yang *et al.* (2011) further revealed that *rpoB* mutations are more likely segregated in the 81-bp regions called the rifampicin resistance determining regions (RRDR). And because up to 90% ofrifampicin resistance strains carry RRDR mutations within codons 516 (WT4), 526 (MUT2) and 531(WT8) they are termed as hot spots and are being rapidly used to identify RIF resistant isolates . Changes in the codon Ser531 (MUT8) and His526 (MUT2) have been documented in more than 70% of RIF resistant isolates (Rattan *et al.* 1988).

This study also illustrated the pattern of genetic mutations of the studied cases, in which resistance due to the missing *rpoB* WT gene was seen in 77 (98.7%) of the 78 rifampicin resistant TB cases and resistance due to the mutations in *rpoB* MUT gene was observed in 51/78 (61.4%) of them. This report is comparable to observations by Qazi*et al.* (2014) who indicated that more than 90% of the mutations were reported in the *rhoB* WT gene region. Similarly, Tukvadze*et al.* (2012) revealed that the most common genetic abnormality in their study was associated with lack of binding to the WT8 in 80 % of the isolates, followed by the S531L (MUT3) in 68%. However Barnard *et al.*, (2008) in a study in Cape Town, South Africa reported that detection of rifampicin resistance of 70.5% was due to mutations of S531L (MUT3).

This study further recorded a100% rifampicin resistance due tomissing *rpoB* WT gene among the MDR (48) and rifampicin mono-resistant (29) cases in our study, while Barnard *et al.* (2008) reported that 76.4% and 37.5% of MDR and RIF mono-resistant isolates exhibited resistance due to mutations in *rpoB* MUT gene region. Our study supports the earlier documented observations that detection of rifampicin resistance could be used as a surrogate marker for development of MDR-TB.

The observations of our study correlate with documented works that reported frequency inresistant strains in respect to rifampicin is attributed to *rpoB* in >95% and that to *kat G* was 60-70% (Rattan *et al.*, 1998) and most *katG*mutations are found between codons 138 and 328 with the most commonly observed gene alteration at codon 315 (WT) (60 - 80% of cases) (Barnard *et al.*, 2012). Moreover, most frequent mutation at codon 315 is a Ser→Thr substitution that is estimated to occur in 30 – 60% of all INH-resistant isolates. This study revealed that detection of isoniazid resistance due to *katG* missing WT gene and *katG* MUT gene was seen in 35/78 (44.9%) of the cases and this was lower than the reports of Barnard *et al.* (2008) who revealed that of all INH resistant strains 64.1% had mutations in the *katG* gene region. Furthermore, among the 48 MDR cases in this study, INH resistance due to*katG* missing WT gene was recorded in 34 (70.8%) and this was similar to the reports of Barnard *et al.* (2008) who

also recorded 70.8% INH resistance in MDR strains to be due to mutations in the *katG* gene.

Efforts to determine factors involved in resistance to INH led to the discovery of *inhA*locus, which was proposed as the primary target of coresistance to INH and ethionamide (Rattan *et al.*, 1998). According to Barnard *et al.* (2012),*inhA*encodes a mycobacterial enoyl-ACP reductase enzyme that is probably involved in the biosynthesis of mycobacterial cell wall fatty acids. The most common mutations of *inhA*occur in the promoter region of the gene and are most frequently seen at positions -24 (g→t), -16 (a→g), or -8 (t→g/a) and -15 (ct→).

The prevalence mutations in *inhA* and *katG* genes seems to vary widely in different geographic locations (Barnard *et al.*, 2008), strains bearing mutations in the coding region of *inhA*show low-level resistance and studies revealed that evidence of mutations in the*inhA* locus and INH resistance were shown to have about 10% correlation (Barnard *et al.*, 2012; Rattan *et al.*, 1998). And this corroborated with the findings of our study which revealed that INH resistance due to missing *inhA* WT gene was reported in 9 (11.5%) TB cases who also exhibited mutation in the*katG* gene. Barnard *et al.* (2008) reported a similar finding of 12 (13.48%) of resistant strains had mutations in*katG* and *inhA* genes regions.Thus, it is interesting to note that INH resistance in some of our studied cases was due not only to mutations in the*katG* gene but also to mutations in the *inhA* gene. The low level of expression of INH resistance due to mutations in the*inhA* gene in this study might be attributed to the fact that the patients were on category I TB treatment regimens that does include ethionamide and Tukvadze*et al.* (2012) revealed thatethionamide inhibits*inhA*, thus if *inhA* mutations are present ethionamide resistance is highly likely.

Specifically, the INH resistance recorded in this study in 8(16.67%) of the MDR cases were due to missing*inhA* WT gene in addition to having mutations in the *katG* gene. However, contrary to the findings of this study Barnard *et al.* (2008) reported that 27% of the MDR isolates did not have mutations in the *katG* gene, but were identified as INH resistant as a result of mutations in the *inhA* WT gene region only. According toTukvadze*et al.* (2012) if mutations are detected in only the *inhA* gene the isolate likely has low level resistance to INH, thus high dose may have clinical effect.

In conclusion, this study revealed a 54% MDR-TB rate among rifampicin-resistant TB patients in Northwestern Nigeria.Amongthe MDR-TB casesRIF resistance was associated with *rpoB* WT gene and *rpoB* MUT gene in 100% and 62% of the isolates, and INH resistance was associated with *katG* WT gene and *katG* MUT gene in 70.8% and 72.9% of the isolates respectively. INH resistance in the MDR-TB cases was further associated with *InhA* WT and *InhA* MUT in 16.67% and 4.17% of the isolates respectively. The study also identifies the importance of expertise in the conduct and diagnosis of rifampicin-resistance status of TB patients by the GeneXpert methods lack of which may be detrimental to the health of the patient.

Thus, the study solicits for a much more committed TB control programmes (including provision and training of manpower) by the relevant agencies and recommends that efforts should be made towards rapid and accurate diagnosis of TB patients, and proper treatment and management of the infected cases to minimize the spread and evolution of MDR-

TB and indeed XDR-TB whose treatment is presented with more challenges.

REFERENCES

Albert, H.,Bwanga, F.,Mukkada, S.,Nyesiga, B.,Ademun, J.P., Lukyamuzi, G., Haile, M.,Hoffner, S.,Joloba, M. and O'Brien, R. (2010):Rapid screening of MDR-TB using molecular Line Probe Assay is feasible in Uganda. *BMC Infectious Diseases,*10 (41): 1-7. http://www.biomedcentral.com/1471-2334/10/41

Barmankulova A., Higuchi, M., Sarker, M.A., Alim, A. andHamajima, N. (2015): Tuberculosis And Rifampicin Resistance Among Migrants In Kyrgyzstan: Detection By A New Diagnostic Test. *Nagoya Journal of Med. Sci.* 77: 41-49.

Barnard, M., Parsons, L.,Miotto, P.,Cirillo, D.,Feldmann, K., Gutierrez, C. andSomoskovi, A. (2012): Molecular Detection of Drug-Resistant Tuberculosis By Line Probe Assay: Laboratory Manual for Resource-Limited Settings.*Foundation for Innovative New Diagnostics.* www.finddiagnostics.org.

Barnard, M., Albert, H., Coetzee, G., O'Brien, R. andBosman, E.M. (2008): Rapid Molecular Screening for Multidrug-Resistant Tuberculosis in a High-Volume Public Health Laboratory in South Africa. *American Journal of Respiratory and Critical Care Medicine*, **177**: 787–792. www.atsjournals.org

Genotype MTBDRplus VER 2.0 (For LPA); Manufactured by HAINLifeSCIENCE, GermanyMan Date: 2015-08-28; EXP 2016-03

Jin, D. and Gross, C. (1988): Mapping And Sequencing Of Mutations In*Escheria colirpoB* Gene That Lead To Rifampicin Resistance. *Journal of Molecular Biology*,**202**:45-48

Nathanson, E., Nunn, P., Uplekar, M., Floyd, K., Jaramillo, E., Lönnroth, K., Weil, D. andRaviglione, M. (2010): MDR Tuberculosis — Critical Steps for Prevention and Control. *New England Journal of Medicine*, **363** (11):1050-8.

National Committee for Clinical Laboratory Standards (NCCLS) (2000).Susceptibility of Mycobacteria, Norcadia, and Actinomycetes 2nd ed. Tentative Standards M24-T2 Wayne, P.A.

National Tuberculosis and Leprosy Programme Control (NBTLC) (2011). Manual on Technical SOPs for Tuberculosis Laboratories in Nigeria.

Qazi, O., Rahman, H., Tahir, Z., Qasim, M., Khan, S., Anjum, A.A.,Yaqub, T., Tayyab, M., Ali, N. andFiryal, S. (2014): Mutation pattern in rifampicin resistance determining region of rpoB gene in multidrug-resistant *Mycobacterium tuberculosis* isolates from

Pakistan. *International Journal of Mycobacteriology*, **3**: 173-177

Rattan, A., Kalia, A. and Ahmad, N. (1998) Multidrug–Resistant *Mycobacterium Tuberculosis*: Molecular Perspectives. *Emerging Infectious Diseases*, **4** (2): 195-209

SD BIOLINE TB Ag MPT64 Rapid testmanufactured bySD STANDARD DIAGNOSTICS, INC 65,Borahagal-ro, Giheung-gu, Yongin-si,Gyeonggi-do, Republic of Korea: Exp; 2016: 12:05.

Tripathi D.K., Srivastava K., Kant, S. andSrivastava, K.K. (2012): Molecular Profiling of Drug Resistant Isolates of *Mycobacterium tuberculosis* in North India. *Advances in Microbiology*, **2**: 317-326. http://www.SciRP.org/journal/aim

Tukvadze, N., Kempker, R.R., Kalandadze, I., Kurbatova, E., Leonard M.K., Apsindzelashvili, R., Bablishvili, N., Kipiani, M. and Blumberg, H.M. (2012) Use of a Molecular Diagnostic Test in AFB Smear Positive Tuberculosis Suspects Greatly Reduces Time to Detection of Multidrug Resistant Tuberculosis. PLoS ONE7 (2): e31563. doi:10.1371/journal.pone.0031563

WHO (2006):Guidelines For The Programmatic Management Of Drug Resistant Tuberculosis.WHO/HTM/TB/2006.361

WHO Policy Statement(2008): Molecular Line Prove Assay For Rapid Screening Of Patients At Risk Of Multidrug Resistant Tuberculosis (MDR-TB).World Health Organization,Geneva. Switzerland.

http://www.who.int/tb/features_archive/policy_statement.pdf

WHO (2010): Multidrug Resistant And Extensively-Drug Resistant TB (M/XDR-TB) 2010 Global Report On Surveillance And Response WHO/HTM/TB/20103. Geneva. World Health Organization, 2010.

WHO (2013a): Multidrug Resistant TB (MDR-TB) 2013 Update. www.who.int/tb/2013.

WHO (2013b): Count Down To 2015. Global Tuberculosis Report 2013 Supplement, World Health Organization. WHO/HTM/TB/2013.1. Geneva. World Health Organization, 2010.

Yang, S., Zhong, M., Zhang, Y. and Wang, Y. (2011): Rapid Detection of *rpoB*And*katG* Genes from the Sputum of Multidrug-Resistant *Mycobacterium tuberculosis* by Polymerase Chain Reaction (PCR)-Direct Sequencing Analysis. *African Journal of Microbiology Research*, **5** (26): 4519-4523. http://www.academicjournals.org/AJMR

AMINU KANO TEACHING HOSPITAL

P. M. B. 3452, ZARIA ROAD, KANO.

(☎: 07068297399,) www.akth.org.ng, E-mail: enquiries@akth.org.ng, email: (akthkano@yahoo.com)

CHAIRMAN BOARD OF MANAGEMENT	CHIEF MEDICAL DIRECTOR	CHAIRMAN M. A. C.	DIRECTOR OF ADMINISTRATION
DR. SAIDU SAMAILA SAMBAWA	PROF. AMINU ZAKARI MOHAMMED,	DR. HADIZA S. GALADANCI,	ALH. MUHD. SULAIMAN,
B.Sc, MBA, ACA, FCA, ACTI, AMINIM, FCTI, FIMC, MNIM.	MBBS, FMCPath	MBBS, FWACS, FICS, MRCOG.	B. Ed, CHPM, AHAN

NHREC/21/08/2008/AKTH/EC/1414

AKTH/MAC/SUB/12A/P-3/VI/1514   15th May, 2015

Abubakar Tukur D/Kudu
Department of Microbiology
AKTH, Kano.

Ufs:

The Head of Department
Microbiology
AKTH, Kano.

ETHICS APPROVAL

Further to your application in respect of your research proposal titled "Drug Susceptibility Profile of *Mycobacterium Tuberculosis* in Kano North Western Nigeria", the Committee reviewed your proposal and noted same as a Prospective Study.

In view of the above, Ethics approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Research Ethics Committee.

Regards

Abubakar S. Mahmud
Secretary, Research Ethics Committee
For: Chairman