GENETIC DETERMINANTS OF DRUG-RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS* AMONG FOLLOW-UP TUBERCULOSIS PATIENTS ATTENDING CHEST CLINIC IN AMINU KANO TEACHING HOSPITAL, KANO, NIGERIA.

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DECLARATION

I, Mohammad Aminu Bashir, hereby declare that this research is my work and has not been presented anywhere for any academic award in any other university.

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Sign..... 24/04/2576 Date..... 24/04/2576.....

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APPROVAL

This dissertation "Genetic determinants of drug-resistance in *Mycobacterium tuberculosis* among follow-up tuberculosis patients attending chest clinic at Aminu Kano Teaching Hospital, Kano Nigeria" has been submitted with our approval as supervisors.

Sign..... Date 23-04-2016

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Dedication

This research is dedicated to my family for their prayers, encouragement, tolerance, patience and understanding during the study.

Acknowledgement

I wish to express my gratitude Almighty God (SWT) for making this study possible. I have to thank all members of microbiology department KIU WC, my supervisors, and management of Aminu Kano Teaching Hospital, Kano Nigeria. I also thank members of staff microbiology department Aminu Kano Teaching Hospital, Kano Nigeria (more especially Molecular Biology North-West TB Reference Laboratory for all the assistance they rendered during this study) and members of staff DOTS clinic AKTH, Kano, Nigeria. I finally, thank members of my family for their patience and endurance during this study.

List of Acronyms

AKTH : Aminu Kano Teaching Hospital

ASM : American Society for Microbiology

CDC : Centres for Disease Control and Prevention

DOTS : Directly Observed Treatment Short course

DR-TB : Drug-Resistant tuberculosis

FMOHN : Federal Ministry of Health Nigeria

HIV : Human Immunodeficiency Virus

IUATLD : International Union Against Tuberculosis and Lung Disease.

MDR-TB : Multi-Drugs Resistant tuberculosis

MTB : *Mycobacterium tuberculosis*

PTB : Pulmonary tuberculosis

TB : Tuberculosis

TDR-TB : Total Drugs Resistant tuberculosis

XDR-TB : Extensively Drugs Resistant tuberculosis

WHO : World Health Organization

Definition of key terms

Amplification: Multiply small amount of DNA into million copies of the same DNA using PCR machine (thermocyler).

Category-one anti-TB drugs: These are first line anti-TB drugs (rifampicin, isoniazid, ethambutol and pyrazinamide).

Category-one follow-up tuberculosis patients: These are the TB patients that treated with first line (rifampicin, isoniazid, ethambutol & pyrazinamide) category-one anti-TB drugs for the period of two months (intensive phase).

Continuation phase: Is a period of four months after the intensive phase, in which TB patient continue with his/her medication (rifampicin and isoniazid).

Decontamination: Removal of other bacteria (contaminants) from the sputum sample.

Deoxyribonucleic acid (DNA): Is a double stranded molecule that contains genetic information.

Incidence: is the number of new cases of a disease or event occurring in a define population during a specified period of time.

Mycobacterium tuberculosis: This is the bacterium that causes a disease called tuberculosis.

Polymerase Chain Reaction (PCR): Is a method used for amplification and detection of specific DNA in an organism.

Susceptibility: Means both inhibition and sensitivity micro-organism.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

1.1.1 Historical background of drug-resistant tuberculosis

Tuberculosis is a disease transmitted by a bacterium called *M. Tuberculosis* (*MTB*),a disease which is global threat (WHO, 2014). New drugs and vaccines are urgently needed to fight this poverty-related disease (CDC, 2010). Multidrug-resistance tuberculosis may have developed from the inadequate treatment; it causes growing threat in many countries including Nigeria (FMOHN, 2014). Therefore, the exploration of the evolutionary patterns of TB bacteria may help predicting future patterns of the disease (FMOHN, 2011).Selman, (1940) isolated actinomycin; later found to be toxic in humans and animals (Idiegbe *et al.*, 2010). In 1943 there was clinical trial using laboratory animals, streptomycin, purified from *Streptomyces griseus,* showed maximal inhibition of *M. tuberculosis.* In November 20th1944, the antibiotic was administered for the first time to a critically ill TB patient (Akaninyene *et al.*, 2013).

The effect was impressive because his advanced disease was visibly arrested, the bacteria disappeared from his sputum, and he made a rapid recovery. However, the new drug had side effects, especially on the inner ear, (Akaninyene *et al.*, 2013). Multi-drug-resistant tuberculosis (M*DR-TB*) is an emerging problem of great importance to public health worldwide (WHO, 2014). The modern era of tuberculosis has recently been characterized by a rise in the number of cases of M*DR-TB*, which causes higher mortality rates than drug-sensitive tuberculosis(WHO, 2011).Drug-resistant tuberculosis (*DR-TB*) is a term used to describe all those strains of *M. tuberculosis* that show resistance to one or more of the common first-line anti TB drugs [Isoniazid (INH), rifampicin (RMP), ethambutol (ETB) and pyrazinamide (PYZ)] (WHO, 2014). Monodrug-resistant tuberculosis (mono *DR-TB*), describes TB that is resistant to any one first-line drug (WHO, 2014). Multidrug-resistant tuberculosis (M*DR-TB*) is defined as TB that is resistant to isoniazid and rifampicin, the two most powerful first-line TB drugs. Extensively drug-resistance TB (X*DR-TB*) describe the form of TB caused by *M. tuberculosis*(*MTB*) strain,

which is resistance to Isoniazid (INH), rifampicin (RMP), M*DR-TB* as well as fluoroquinolones (FQ), aminoglycosides (second line anti-TB injectable) and macrolides(IUATLD, 2011).

1.1.2 Theoretical Background

M. tuberculosis belongs to the family Mycobacteriaceae (Ismael *et al.,*2004). Tuberculosis is an infectious disease that causes mortality and morbidity worldwide especially in Africa (WHO, 2013).

The mechanisms of action for anti-TB-drugs are based on:

- Inhibiting of Mycobacterial cell wall formation e.g. Ethambutol.
- Protein synthesis inhibition e.g. Isoniazid, Ethionamide, Aminoglycoside, Macrolides.
- Anti-metabolites e.g. Pyrazinamide.
- DNA or RNA inhibition e.g. Rifampicin, Quinolones (First and second generation fluoroquinolones) (WHO, 2008).

The usage of anti-TB drugs are based on outline principle listed below.

Mechanism of anti-TB drugs: The anti-TB drugs that are used to treat tuberculosis are classified into first-line and second-line agents (McCann *et al.*, 2009). First-line essential anti-tuberculosis agents are the most effective, and are necessary components of any short-course therapeutic regimens (WHO, 2010).The drugs in this category are isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin (WHO, 2012). Second-line anti-tuberculosis drugs are clinically much less effective than first-line agents and elicit severe reactions (McCann *et al.*, 2009). These drugs include para-aminosalicylic acid (PAS), ethionamide, cycloserine, amikacin and capreomycin (FMOHN, 2014). New drugs, which are yet to be assigned to the above categories, include rifapentine, levofloxacin, gatifloxacin and moxifloxacin. Recently there has been development in the molecular pharmacology of anti-tuberculosis drugs (WHO, 2007).

1.1.3 Conceptual background

Infection can be cause by *MTB* complex or drug-resistant *MTB*, giving rise to primary tuberculosis or primary drug-resistance tuberculosis respectively (WHO, 2013).Screening of suspects with primary tuberculosis or drug-resistance tuberculosis with clinical symptoms (cough

with thick, cloudy purulent sputum, sometimes with blood, for more than two weeks; fever, chills and night sweats, fatigue, and muscle weakness; weight loss and in some cases shortness of breath and chest pain) (WHO, 2010), are done by sputum microscopy/PCR e.g. line probe assay (Hains, 2009).Sputum smear positive among the *DR-TB* suspects(follow-up patients) commence two months treatment (intensive phase), with first line drugs (Rifampicin, isoniazid, ethambutol and pyrazinamide) (FMOHN, 2014). If *DR-TB* is detected the patient will commence second line drugs (Aminoglycoside, First and second generation fluoroquinolones, and macrolides)(IUATLD, 2010).

Sputum conversion (smear positive to smear negative) after the intensive phase make patient to continuation phase for four months (continuation with rifampicin and isoniazid only), default or treatment failure result in development of secondary drug resistance tuberculosis by acquired or by mutation. On the other hand adequate compliance with the treatment, under functional Directly by Observe Treatment Short course (DOTS) programme with good nutrition and functional immunity could result in cure (IUATLD, 2013).

Drug resistance tuberculosis could be transmitted either to new susceptible host or developed due to treatment failure or re-infection (FMOHN, 2011).Tuberculosis is spread primarily by the air route and therefore, only patients with pulmonary tuberculosis are infectious (NTBLCP, 2014). Numerous studies and reviews have highlighted the various factors associated with infectivity (CDC, 2010). These factors include bacillary load, the severity of coughing and other forced expiratory manoeuvres such as sneezing, yelling and singing, the type and duration of anti-tuberculoses chemotherapy (Edward *et al.,* 2007). These factors lead to the production of droplet nuclei (aerosols), which are about (1-5μm in size) (CDC, 2012). After establishing residence in the immune cells of the lungs the organism often remains in a dormant state until the host immune system is compromised (Willey *et al.,* 2008).A patients with tuberculosis also generates larger particles which do not remain airborne, and if inhaled do not reach alveoli (Edward *et al.*, 2010). When organisms are deposited on intact mucosal or skins they do not invade tissues. When large particles are inhaled, they impact on the walls of the upper airway or tracheas, where they are trapped in the mucous blanket carried to the oropharynx and swallowed and expectorated (IUATLD, 2006).

1.1.4 Contextual background

Nigeria has incidence of about 399,000 cases of forms tuberculosis annually and 10% TB prevalence, ranking the fourth largest TB burden in the world and largest in Africa (FMOHN, 2005). Over 107,000 TB patients die from the disease each year (293 deaths occur daily), while 10%of multi-drug resistant tuberculosis (*MDR-TB*) death among new cases was also reported by World Health Organization,(2014). The World Health Organization stated that the incidence of MDR-TB was 0.31% implying 50% increase in the incidence rates in 2011 (WHO, 2012).

Taura *et al.*, (2008) in Kano, Nigeria, reported 6.5% TB prevalence which could be due to poor intervention plan, lack of effective prevention plan, poor infection control and inadequate continuing education on TB transmission.

1.2 Problem Statement

The emergence of drug-resistance TB in Nigeria is a public health concern (WHO, 2010). Medical strategies outline by various countries' ministry of health made a road block mounted by TB ability to challenge treatment regiments, even when the disease have been diagnosed properly (NTBLCP, 2014). There has been an increase in the follow-up positive tuberculosis patients in the hospitals of north-west Nigeria (FMOHN, 2012).

Despite the numerous interventions by Nigerian government and non-governmental organizations towards eradication of tuberculosis, the disease continues to rise in resource limited setting including Kano state (NTBLCP, 2014).

There is little or no data on prevalence of drug resistance tuberculosis, the patterns of DR-TB and the genes associated with drug resistance TB in the study area.

1.3Broad objective

To determine the presence and patterns of drug resistance in *M. tuberculosis* among category-one follow-up tuberculosis patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria.

1.4 Specific objectives

i. To determine the prevalence of *DR-TB* among follow-up TB patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria.
ii. To determine the *M. Tuberculosis* drug-resistance pattern among follow-up TB patients in the study area.
iii. To determine the genes associated with *M. Tuberculosis* resistance among follow-up TB patients in the study area.

1.5 Research questions

i. What is the prevalence of the *DR-TB* among follow-up TB patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria?
ii. What are the *M. Tuberculosis* drug resistance patterns among follow-up TB patients in the study area?
iii. What are the genes associated with *M. Tuberculosis* resistance among follow-up TB patients in the study area?

1.6 Scope of the study

This study was restricted to the genetic determinant of drug resistance in *M. Tuberculosis* among the category-one follow-up TB patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria.

1.6.1 Content scope

This study determined drug resistance in *M. tuberculosis,* prevalence of *DR-TB*, the pattern of drug resistance in *M. Tuberculosis* and determine the genes associated with drug-resistance in *M. tuberculosis*.

1.6.2 Study site

The study was carried out in Aminu Kano Teaching Hospital, Kano, Nigeria. The hospital is located in Tarauni local government area, the state, Nigeria. Kano is the largest city in northern Nigeria and is located in the north-west Nigeria. The state has total population of 12,284, 335 (population census, 2006), fourty four (44) local government areas, the residents are mostly Hausa / Fulani people. It has one thousand three hundred and ninety six (1,396) health facilities (Public and Private). There are four referral hospitals (including the university teaching hospital and national orthopaedic hospital), three hundred and seventy nine (379) DOTS centres, eighty four microscopy sites (84), four GeneXpert distributed across the state, one MDR ward and a north-west zonal tuberculosis reference laboratory in the state. According to NTBLCP, (2014) 12,750 patients were given category one regimen in 2014.

1.6.3 Time scope

This study was carried out from April, 2015 to November, 2015.

1.7 Justification/Significance of the Study

This study determined the following:

i. DR-TB prevalence among the follow-up patients.

ii. Patterns of DR-TB among the participants.

iii. The genes associated with resistance in identified *M. tuberculosis*.

Achieving objectives above is of profound significance to the TB patients, community, hospital, policy makers, non-governmental organizations and science. Patients will benefit from improve treatment, community will benefit from reduced TB burden, hospital will adapt improved and updated treatment approach, non-governmental organizations will be more confident in investing for diagnosis, treatment and management of tuberculosis and drug resistance tuberculosis, science will benefit because of the new knowledge.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

2.1 Prevalence of drug resistance tuberculosis (DR-TB)

M. tuberculosis is the leading cause of death in the world (WHO, 2014). There were an estimated 9.6 million new cases of TB and 480,000 TB deaths among women (WHO, 2014).There were also an estimated 1.0 million cases of TB in children and 140,000 deaths (FMOHN, 2014). In 2012 more than 10 million children were orphaned as a result of their parent's death from TB (WHO, 2013).

Drug-resistant TB is defined as the non-responsiveness of one or more anti-TB drugs; these could be as a result of inadequate treatment of active pulmonary TB (Rijal *et al.*, 2010). This could also be as a result of inappropriate prescription with poor drug selection, insufficient treatment duration and poor compliance of patients to recommended treatment plan (Akaninyene *et al.*, 2013).Globally, 3.5% of new and 20.5% of the follow-up TB cases had *MDR-TB* in 2013 and 9.0% of patients with *MDR-TB* had extensively drug resistant TB (*XDR-TB*) (WHO, 2014). 6.1 million, new and follow-up TB patients tested (world-wide) for drug resistance in 2013 out of which 650,000 were MDR TB (WHO, 2014).The global study carried out by WHO (2013), showed that in some places about 19% *MDR-TB* were in fact *XDR-TB* (FMOHN, 2014). When second-line drugs to *MDR-TB* are mis-used, there is a possibility of *XDR-TB* arising (IUATLD, 2012). Seventy five percent (75%) of tuberculosis and drug resistance TB occur in developing countries, due to inadequate resources to enable proper treatment particularly where Human Immuno Deficiency Virus (HIV) infection may be common (CDC, 2012). In Ethiopia, Desta *et al.*, (2009), found even higher rates of TB drug resistance of 58.7%, this shows the high level of drug resistance TB in Africa. Another study in Ethiopia also reported high resistance rates of 21.4% to at least one anti TB drug (Asmamaw *et al.*, 2008).

Nigeria has incidence of nearly 366,000 cases of all forms TB annually, ranking as the fourth TB burden in the world and largest in Africa (FMOHN, 2014). Taura *et al.*, (2008) reported 6.5% TB prevalence in Kano, Nigeria, due to poor intervention plan, lack of effective prevention plan, poor infection control and inadequate continuing education on TB transmission.

2.2 Patterns of drug resistance in *Mycobacterium tuberculosis*

Drug resistance patterns in *M. tuberculosis* are: Monodrug-resistant, multidrug-resistant tuberculosis (*MDR-TB*), poly drug resistant TB and extensively drug resistant TB (*XDR-TB*), they are global emergency (WHO, 2010). Monodrug-resistance occurs when *M. tuberculosis* develop or acquire resistance to a single anti TB drug. Multidrug-resistant tuberculosis (*MDR-TB*) is defined as resistance to isoniazid and rifampicin, with or without resistance to other first-line drugs (WHO, 2014). While poly drug resistance occur when *M. tuberculosis* develop or acquire resistance to two or more anti TB drugs (but it could be isoniazid or rifampicin and another drug) (IUATLD, 2006) and XDR-TB is defined as resistance to at least isoniazid and rifampicin, and to any fluoroquinolones, and to any of the three second-line injectable (amikacin, capreomycin, and kanamycin) (FMOHN, 2014).Resistance to isoniazid is due to mutations at one of two main sites, in either the *katG* or *inhA* genes (Zhang *et al.,* 2009; Piatek *et al.,* 2010).

2.3 Determination of genes associated with *Mycobacterium tuberculosis* resistance

Accurate and rapid detection of resistant strains is critical to provide appropriate treatment and to intercept the transmission of drug-resistant TB. However, lack of access to quality laboratory diagnostics continues to jeopardize efforts to control the worldwide transmission of TB (FIND, 2012). Poor diagnostic capacity and lack of infection control for both drug-susceptible and drug-resistant TB play a significant role in enhancing disease transmission (WHO, 2014).

The emergence of isoniazid (INH) resistance is multi-factorial and involves mutations in several genes such as *katG*, which encodes the activating enzyme, catalase, or regulatory genes such as *inhA*, *ahpC-oxyR*, *ndh*and *furA (*CDC, 2012*)*. More than 60% of INH-resistant strains of *M. tuberculosis* have missense mutations or small deletions/insertions in *katG (*Liu *et al.,* 2010*)*. Most *katG* mutations are found between codons 138 and 328 with the most commonly observed gene alteration at codon 315 (60 - 80% of cases) (FIND, 2012). The most frequent mutation at codon 315 is a Ser-Thr substitution that is estimated to occur in 30 – 60% of all INH-resistant strains (FIND, 2012). The *katG*463 (CGG-CTG) (Arg-Leu) amino acid substitutions is the most

common polymorphism found in *katG* and is not associated with isoniazid resistance (Hains, 2009). The high-level rifampicin (RMP) resistance is associated with mutations in codon 526 and 531, whereas alterations in codon 511, 514, 515, 516, 518, 521, 522 and 533 result in low-level rifampicin resistance (Hains 2009). Some mutations affecting codon 526 represent an exception and do not confer high-level resistance (Liu *et al.*, 2010).

To determine the genes associated with *M. tuberculosis* resistance, different molecular methods are used; these include: The sequences of the oligonucleotides primers are used for mutation analysis (Liu *et al.*, 2010). Branch migration inhibition (BMI), is suitable for the detection of drug resistance in *M. tuberculosis*, which is frequently associated with multiple mutations within known genes (Liu *et al.*, 2010). Whole-genome sequencing of rifampicin-resistant *M. tuberculosis* strains identifies compensatory mutations in RNA polymerase genes (Iñaki, 2011; *Liu et al.*, 2014). PCR line probe assay (LPA) the Genotype MTBDR (Hains Lifescience, Nehren, Germany) is used for the detection of RMP and/or INH resistance in *M. tuberculosis*. The assay is based on reverse hybridization between amplicons derived from a multiplex PCR and nitrocellulose-bound probes covering wild-type sequences and, in this first version of the assay, includes the most frequent mutations in the 81 base-pair region of *rpoB* and mutations at codon 315 in *katG (*Hains, 2009*)*. This method is cheaper than the methods mentioned above and is less complicated and affordable in poor resource settings (FMOHN, 2010).

2.4 Prevention

Five priorities are needed to address the M*DR-TB* epidemic, these are:

1) High-quality treatment of drug-susceptible TB to prevent M*DR-TB.*

2) Expansion of rapid testing and detection of M*DR-TB* cases.

3) Immediate access to quality care.

4) Infection control.

5) Increased political commitment, including adequate funding for current interventions as well as research to develop new diagnostics, drugs and treatment regimens (WHO, 2014).

CHAPTER THREE: METHODOLOGY

3.0 Introduction

3.1 Study area and population

The study was carried out in Aminu Kano Teaching Hospital, Kano, Nigeria. Kano state is the most densely populated state in Nigeria according to national census (2006). It is the largest city in northern Nigeria and is located in the north-west Nigeria. The state has a total population of 12,284, 335 (national population census, 2006), fourty four (44) local government areas, the residents are mostly Hausa / Fulani. It has one thousand three hundred and ninety six (1396) health facilities (Public and Private). There are four referral hospitals (including the university teaching hospital and national orthopaedic hospital), three hundred and seventy nine (379) DOTS centers, eighty four microscopy sites (84), four GeneXpert distributed across the state, one MDR ward and a north-west zonal tuberculosis reference laboratory in the hospital. Aminu Kano Teaching Hospital is the largest tertiary hospital in the state, based in Tarauni local government area, in Kano metropolis (NTBLCP, 2014). It has various, wards, clinics including HIV, DOTS, ICU, isolation ward, theatres and TB north-west zonal reference laboratory. About 1000 patients attend the hospital daily with an average of 20-25 follow-up TB patients (with exception of weekends), and the hospital. The state boarders with Niger republic to the north, Kaduna state south, Jigawa state to the west and Bauchi state to the east.

3.2 Sample size

The Slovene (1961) was used to calculate the sample size as shown below.

$n = \frac{z^2pq}{r^2}$, since the entire TB population is far above 10,000 in Kano state

Where n = desired sample size (of the population greater than 10,000)

z = standard normal deviation (usually set at 1.96 or more sample at 2.0) which correspond to 95% confidence.

P = proportion of target population (used as 0.50 if no reasonable population estimate)

q = 1.0 – p

r = degree of accuracy desired (usually 0.05 or occasionally 0.02).

From the above formula;$n = \frac{z^2pq}{r^2}$

Then, $n = \frac{(1.96)^2\,(0.5)(1.0-0.5)}{(0.05)^2}$

Therefore n = 384

3.3 Sampling procedure

This was achieved by simple random sampling; every individual in the target population had an equal chance of being part of the sample.

3.4 Materials

Sterile applicator sticks, staining rack, slides rack, slide box, sterile sputum containers, sterile slides, sterile absorbent cotton wool, sterile falcon tubes, forceps and cold box were used for samples collection, transportation and processing (smear preparation and Zeil Nielsen stain). While sterile plastic pipettes tip, bench mats, liquid gum, solid tape, biohazard waste containers, were used to support the PCR (line probe assay).

3.5 Equipment

List of equipment used for Zeil Nielsen(smearing, staining and microscopy) are: Biosafety cabinets, refrigerated centrifuge, refrigerator, microscope and timer were used to Micro centrifuge, heating block, ultrasonic water bath, and positive-displacement pipettes, filter tips, 1.5 ml O-ring screw-top tubes, plastic loops, and 3ml Pasteur pipettes were used for DNA extraction. Sonicator, water bath, automatic pipettes, reagents preparation hood, mini centrifuge, thermocyler and twin cubator were used for PCR (line probe assay).

3.6 Reagents

Strong carbolfuschin, methylene blue, 3% acid alcohol, 5% sodium hypochlorite, emersion oil and sterile distilled water were used for Zeil Nielsen staining and microscopy. While $Mgcl_2$ or Mn, primer nucleotide mix (PNM) , Buffer, Taq polymerase and Molecular grade water were used to prepared master mix as described by the manufacturers (Hains, 2009). MTBDRplus kit Hains Life Science, buffer solution and PCR tubes were used to support PCR (line probe assay).

3.7 Algorithm for diagnosis of TB and DR-TB (Adapted from FMOHN, 2011).

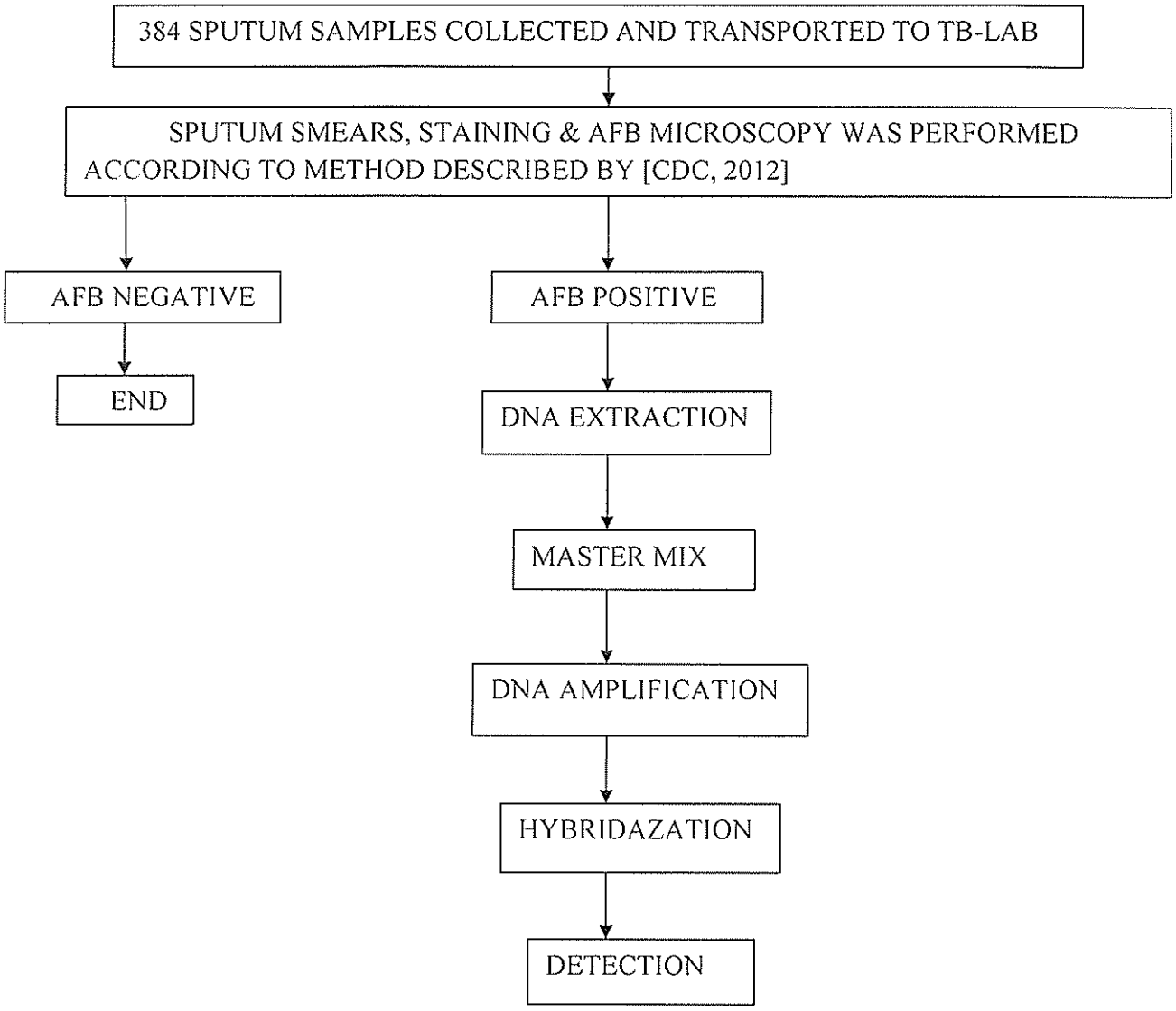

Figure 1 : Algorithm for diagnosis of MTB and DR-TB

3.8 Experimental Procedures

3.8.1 Sample collection

Early morning deep expectorated purulent sputum samples were collected in a sterile, wide mouth, screw cap, labelled and transparent container, from consented participants as described by CDC, (2012). Samples were transported from clinic in a cold box to the laboratory for analysis.

3.8.2 Samples analysis

Samples analysis was carried out according to method described by (CDC, 2012; ASM, 2012).Sputum smears and staining were performed within 24 hrs of receiving the specimens and results recorded. Positive (AFB) samples were analysed using PCR(line probe assay)*MTB*DR Plus (Hains, 2009).

3.8.2.1 Staining and Microscopic Examination

Smears were prepared directly from sputum specimens and standard ZN staining procedure was used. The smears were examined under the microscope using oil emersion objective. AFB detected were quantified and results recorded as acid fast bacilli positive (AFB + ve) or acid fast bacilli negative (AFB -ve).

3.9Determination the prevalence of DR-TB

The prevalence of DR-TB was determined using line probe assay as described by Hains, (2009) (DNA extraction, amplification, hybridization and detection).

3.9.1 DNA extraction

DNA extraction method was carried out on AFB positive specimens. The specimens were sonificated after partial cell lysis through a heat-killing step, using the GenoLyse kit (Hains Life science, Germany) (Hains, 2009).

Five hundred microliter (500ul) of decontaminated sputum sample was transferred into a 1.5ml screwcap tube and centrifuged for 15 min at 10,000 rpm at 2 - 4°C in a refrigerated centrifuge.

The pellets were resuspended (after decanting supernatant) in 100µl of an alkaliclysis buffer and incubated for 5 min at 95°C in a water bath.

Subsequently, 100µlof neutralization buffer was added to lysate. The mixture was vortexed and centrifuged for 5 min at 14,000rpm.

The extracted DNA was kept in the freezer at (-20 or -80°C).

Five microliter (5µl) of the supernatant (extracted DNA) was used for Line probe assay.

3.9.2 PCR (Line probe assay) analysis

Master Mix preparation

Thirty five microliter (35µl) of Primer Nucleotide Mix (PNM) was pipetted and dispensed into a sterile Appendof tube. Then, five microliter (5µl) of 10XPCR buffer, two microliter (2µl) of $Mgcl_2$, three microliter (3µl)of Molecular grade water, and0.2µlTaq polymerase were added. The solution was mixed.

3.9.3Procedure for preparation of DNA amplification mixture

The protocol was carried out according to Hains, (2009) and it involved the following steps:

Twelve specimens including negative and positive controls.

Forty five microliters (45µl) of the prepared master mix was pipetted and dispensed into each sterile PCR tube.

Five microliters (5µl) of extracted DNA was added into each of the tube above with exception of the controls.

Five microliters (5µl) of the control positive and negative each) was added in to the control tubes respectively.

3.9.4 DNA Amplification

Hot 40 programme was selected (for sputum sample) in the thermocyler.

The PCR was runned for (2-3) hours.

Before the PCR tubes were placed into the thermal cycler, they were mixed slightly and spun down for 5 - 10 seconds in a mini-centrifuge at 10,000rpm.

The PCR machine was heated up to 95°C, for 15 minutes, to denatured the DNA and at 95°C respectively for 30 seconds

2 minutes at 58°C for 30 cycles, for the primers to annealed

40 seconds at 70°C, for extension and elongation

25seconds at 95°C for 20 cycles, the DNA was further denatured

40seconds at 53°C, for the primers to reannealed

40 seconds at 70°C, for extension and elongation

3.9.5 Hybridization

The hybridization procedure was performed directly after the amplicons were taken out of the thermal cycler. The procedure described by Hains, (2009) employed and it involved the steps below:

Hybridization and stringent washing buffers were pre-heated to 45°C.

Twenty microliters (20µl) of denaturation buffer was mixed thoroughly in a plastic well tray with twenty microliters (20µl) of amplified sample and incubated at room temperature for five minutes (5min).

One millilitre (1ml) of hybridization buffer was added to each well and mixed.

Pre labelled test strips were added into each well, and the wells were incubated for 30 minutes at 45°C after labelling.

Solution was completely aspirated following incubation.

One ml of stringent buffer was added to each strip and incubated at 45°C for 15 minutes, and then one millilitre (1ml) of rinse buffer was also added to each strip and incubated at room temperature for 1 min.

The rinse buffer was removed.

One millilitre (1ml) of diluted conjugate buffer was added to each strip, and incubated for 30 minutes at room temperature.

After incubation, solution was removed and the test strips were rinsed twice with rinse buffer solution for 1 min, the strips were also washed with distilled water for 1 min.

One ml of substrate buffer was added to each strip, and incubated for 5 to 8 minutes at room temperature.

Solution was removed, and the reaction stopped by rinsing twice with distilled water.

The test strips were dried and then taped to the *MTB*DRplus assay worksheet for interpretation.

Reference standard for result interpretation

The results of the PCR (Line probe assay) obtained in this study was interpreted according to the method described by Hains, (2009); using the reference picture shown below.

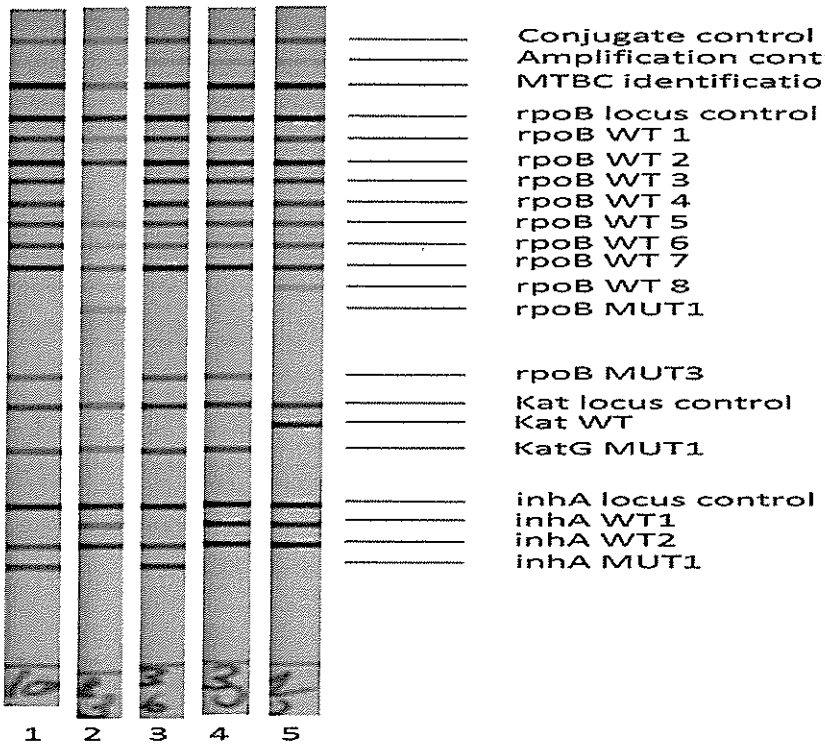

Figure 2 : Reference standard for result interpretation

Twenty-two probes (for the identification of *MTB*) located on the DNA strip. The zone hybridized the amplicons generated from *MTB.* Five controls [conjugate control (CC), amplification control (AC), the *rpoB*, *katG*, and *inhA* loci control zones] were set on the DNA strip. The *rpoB*, *katG*, and *inhA* loci control zones detect a region specific for the respective loci and stained positive. The CC area showed the efficiency of conjugate binding and the development of substrate reaction. Then, TUB (specific region of the 23S rRNA gene) zone was expressed and this indicated the presence of *M. tuberculosis*. Eight *rpoB* wild-type probes (WT1 to WT8) encompass the region of the *rpoB* gene coding for amino acids 505 to 534. Four other probes (*rpoB* MUT1, MUT2, MUT3, and MUT4) were specific for the most common mutations D516V, H526Y, H526D, and S531L, respectively. Three probes were specific for the codon 315 region of *katG*: *katG* WT (the wild-type probe), *katG* MUT1, and katG MUT2. *katG* WT was designed for the AGC-to-ACC (S315T1) while *katG* MUT1 and katG MUT2 were designed for

AGC-to-ACA (S315T2) mutations respectively. Six probes were designed for the promoter region (−8, −15, and −16) of the *inhA* gene. Two wild-type probes, *inhA* WT1 and WT2, cover −15, −16, and −8 nucleic acid positions, respectively; four others (*inhA* MUT1, MUT2, MUT3A, and MUT3B) detect mutations of C15T, A16G, T8C, and T8A, respectively. The MTBDRplus results were interpreted as described by Hains (2009).Then, the prevalence of DR-TB was determined.

3.10 Determination of *Mycobacterium drug-resistance tuberculosis* patterns

The patterns of DR-TB were determined using line probe assay as described by Hains, (2009) (DNA extraction, amplification, hybridization and detection) mono drug resistant was determined by the presence of katG, inhA or rpoB gene. While the presence of rpoB and katG or inhA genes indicate MDR-TB.

3.11 Determination of genes associated with MTB resistance

The genes associated with drug resistance in *M. Tuberculosis* were determined as describe by line probe assay (DNA extraction, amplification, hybridization and detection) (Hains, 2009).

3.13 Data quality control

The data quality control was achieved by; pre-test, monitoring and evaluation.

3.14 Ethical consideration

Ethical approval to carry out in this study was obtained from institutional review boards of Aminu Kano Teaching Hospital, Kano, Nigeria. To ascertain the practice of ethics in this study, The following activities were implemented: Participants understudy signed the consent form voluntarily. The importance of the research was explained to the participants, citations quoted were fully recognized through referencing. Respondents were coded instead of reflecting

names.

3.15Limitation / delimitation

i. Language barrier was the main problem in this study. Language barrier was solved by interpreting the questionnaire in to Hausa language.

ii. Culture and tradition: some people were resistant to self–disclose information on tuberculosis, because the disease is associated to poverty and stigma. This issue was solved by educating the participants.

iii. Under medical conditions, some patients cannot produce purulent sputum sample. Non purulent sample was collected.

CHAPTER FOUR

4.0 RESULTS

Table 1 : Demographic characteristics of the participants

Demographic characteristics	Frequency	Percentage (%)
Gender		
Male	165	42.97
Female	219	57.03
Total	**384**	**100**
Ethnicity		
Hausa	259	67.45
Fulani	105	27.34
Igbo	10	2.64
Yoruba	05	1.30
Igala	05	1.30
Total	**384**	**100**
HIV status		
Positive	24	6.25
Negative	360	93.75
Total	**384**	**100**
Marital status		
Single	67	16.75
Married	305	79.43
Widow	12	3.12
Total	**384**	**100**
Occupational status		
Civil servant	50	13.02
Self employed	184	47.92
Private employed	50	13.02
Unemployed	100	26.04
Total	**384**	**100**
Educational status		
Primary education	50	13.02
Secondary education	254	66.15
Tertiary education	80	20.83
Total	**384**	**100**
Medication		
At home	359	93.49
At hospital	25	6.51
Total	**384**	**100**

Table 1 shows a total of three hundred and eighty four (384) sputum samples from participants (follow-up) (those that completed two months intensive phase of anti-TB treatment of first line cat-one regimen) attending chest clinic (DOTS) in Aminu Kano Teaching Hospital, Kano, Nigeria, studied. Out of 384 samples 165(42.97%) were from males and 219(57.03%) from females participants.

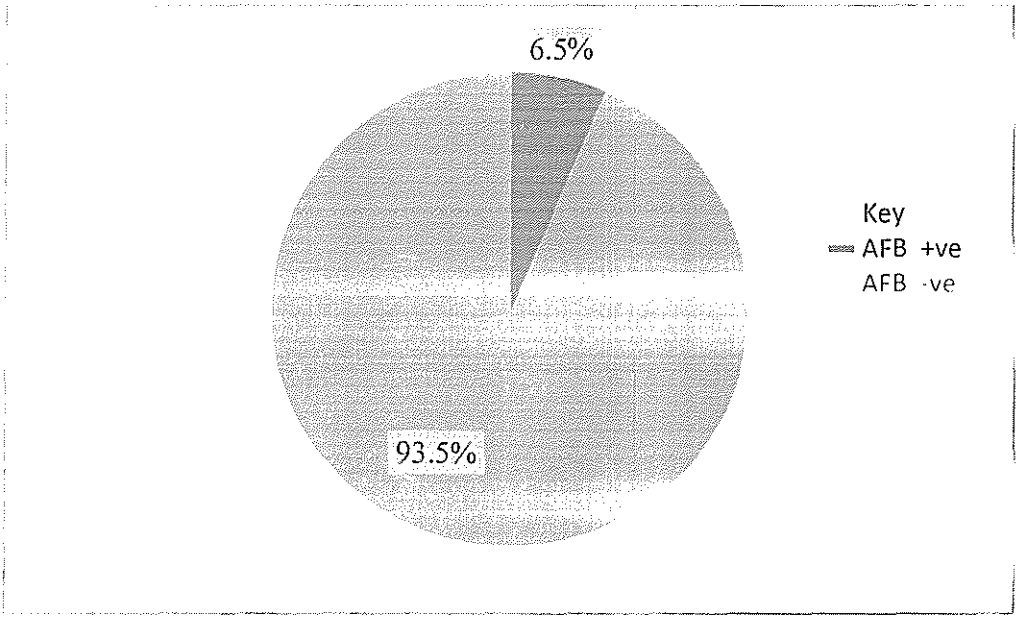

Figure 3 : Acid fast bacilli (AFB) positive and negative among the participant

Zeil- Nielsen staining and microscopy was carried out on three hundred and eighty four (384) sputum samples from follow-up tuberculosis patients. 25(6.5%) samples were AFB positive while 359(93.5%) were AFB negative. The positive AFB samples were analysed using PCR.

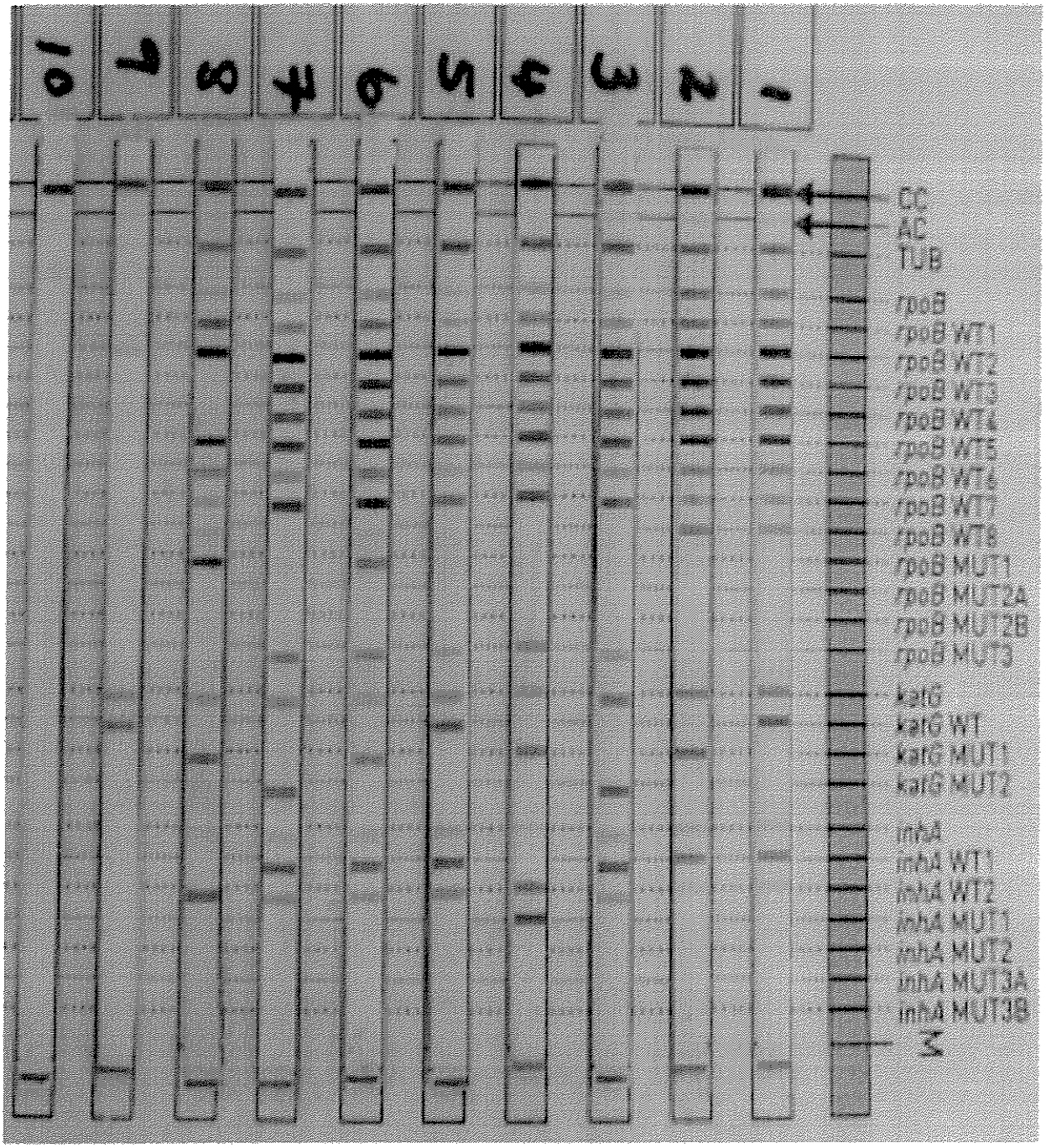

Figure 4 : PCR (line probe assay) results

4.1 Prevalence of DR-TB among the participants (n = 25)

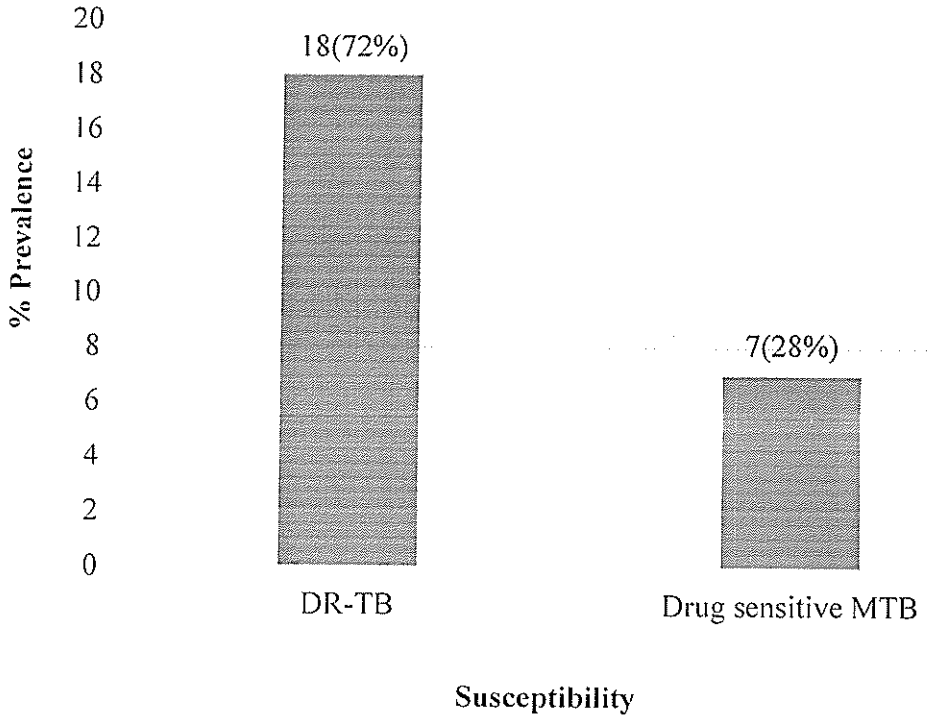

Figure 5 : Drug susceptibility in Mycobacterium tuberculosis among the study population (n=25).

Drug resistance tuberculosis 18(72%) and drug sensitive 7(28%) were obtained from AFB positive samples after the PCR (Line probe assay) as shown in figure 5 above.

4.2 Pattern of DR-TB among the participants (n = 25)

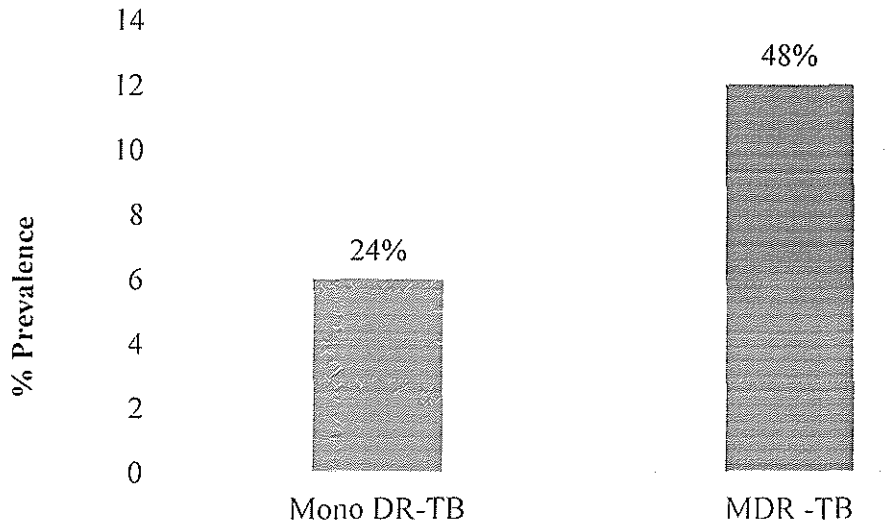

Figure 6 : Patterns of drug resistance in Mycobacterium tuberculosis among the participants

Monodrug resistance 6(24%) and multidrug resistance tuberculosis 12(48%) were obtained after the PCR (Line probe assay) as shown in figure 6 above.

4.3 Genes associated with Mycobacterium tuberculosis resistance.

Table 2 : Genes associated with Mycobacterium tuberculosis resistance.

Identified *M. tuberculosis*	**Genes associated with resistance in identified *M.tuberculosis***		
	rpoB & inhA genes	**inhA gene**	**katG gene**
Mono drug resistance *MTB*0	3(12%)	0(0%)	0(0%)
Mono drug resistance *MTB*	0(0%)	0(0%)	3(12%)
Multidrug resistance *MTB*	12(48%)	0(0%)	0(0%)
Total	**12(48%)**	**3(12%)**	**3(12%)**

Table 2 above shows the genes associated with resistance in identified *M .tuberculosis.* The determined genes associated with *M. tuberculosis* resistance in this study were rpoB, inhA and katG. The presence of rpoB and either inhA or katG in *M. tuberculosis* indicate MDR-TB, while presence of one of this gene (i.e. rpoB, inhA, or katG gene) indicate mono-DR-TB.

CHAPTER FIVE

5.1 Discussion

There is limited or no data on drug resistance tuberculosis among the follow-up tuberculosis patients in the hospitals of north-west Nigeria. There is also very little information on the same subject from the other parts of the country. This was primarily a laboratory based study to address the prevalence of DR-TB, patterns of distribution and determination of the genes associated with resistance in tuberculosis, among the follow-up tuberculosis patients.

5.1.1 Prevalence of drug resistance tuberculosis (DR-TB)

The results of this study have confirmed the presence of *DR-TB* (monodrug-resistance and multi drug resistance) in Kano north western Nigeria. This study also showed that the prevalence of *DR-TB* among the follow-up TB patients is 72%. Mono *DR-TB* rates was 6(24%) and M*DR-TB* 12(48%). The M*DR-TB* was 48% in this study was higher than the findings of Idigbe *et al.*, (2001) who reported 27% of M*DR-TB* among the follow-up TB patients in Lagos south western Nigeria. Our finding on isoniazid resistance 24% was lower than their reported prevalence of 36%; this may have been due to HIV and congestion in Lagos. In Jos north central, Nigeria Ani *et al.*, (2010) found a monoresistance prevalence of 15% and multidrug resistance of 31% among follow-up TB patients, which was lower than our finding, may be due economic factor. Lawson *et al.,* (2010) found higher monoresistance rates of 31% in Abuja north central Nigeria. Akaninyene *et al.*, (2013) reported 42% of DR-TB among the follow-up TB patient in Calabar south southern Nigeria; this finding was lower than our finding. This may be due to HIV infections, socio economic factors and lack of compliance during treatment in the studied area.

In Ethiopia, Desta *et al.*, (2009) found 58.7% DR-TB prevalence among follow-up TB patients; this finding was lower than our finding this may be due to treatment failure. In another study carried out by Berhanu *et al.*, (2014) in Haramaya, Ethiopia reported 23% prevalence among the follow-up tuberculosis patients. This study was also lower than our findings this could be as a result of good intervention plan. Outside of Africa, drug-resistance rates have been similarly

lower than our finding, Borann *et al.,* (2009) in Cambodia reported monoresistance TB of 52% among follow-up TB patients. Dam *et al.,* (2011) in India reported 39.2% of *DR-TB* among follow-up TB patients this was associated with treatment failure, this finding is also lower than our finding, and this may be due to poor compliance to the treatment guideline of the participants.

In this study, females showed more resistant to anti-tuberculosis drugs (48%) than males (24%), due to poor compliance to the treatment plan. This study was contrary to the findings of Deepica *et al.,* (2015) who reported that, male resistant cases were 37.65 % while female were 17.64% and these may be due to HIV and high level of un-employment in India.

5.1.2 Determination of the patterns of drug resistance in *Mycobacterium tuberculosis*

Mono drug resistant, multi-drug resistant tuberculosis (M*DR-TB*) and extensively drug resistant TB (X*DR-TB*) is a global emergency (WHO, 2010). The patterns of drug resistance in this study were monodrug-resistant and multidrug-resistant among the follow-up tuberculosis patients. Monoresistance 6(24%) and multi drugs resistance 12(48%) as shown in figure 6; this is comparable with the studies done in Nigeria by Igdiebe *et al.*, (2001) in Lagos south west, Ani *et al.*, (2010) in Jos north central and Akaninyene *et al.*, (2013) whose findings patterns were mono resistance and multi resistance, this is because the genes associated with resistance are the same. But our findings are contrary to that of Lawson *et al.,* (2010) in Abuja north central Nigeria whose finding was only monoresistant tuberculosis. Our study was similar to that of Desta *et al.*, (2009); Kedir *et al.*, (2015) in Ethiopia.

Outside Africa, Borann *et al.,* (2009) in Cambodia reported monoresistance TB among follow-up patients this finding is contrary to our findings. Dam *et al.,* (2011) in India reported the drug resistance pattern as monoresistance and multidrug resistance tuberculosis among the follow-up patients; these findings are similar to ours. A study by Hazbón *et al.*, (2006) in Brazil reported mono drug-resistance pattern unlike our study that had both monodrug and multi-drug resistance tuberculosis pattern. While Srinivas *et al.*, (2015), in Baylor Plaza, Houston, USA reported monoresistance TB, multidrug resistance TB and extensively drugs-resistant tuberculosis this could be due availability of the modern facilities.

5.1.3 Determination of genes associated with resistance in *Mycobacterium tuberculosis*

In other bacteria, acquired drug resistance is generally mediated through horizontal transfer by mobile genetic elements, such as plasmids, transposons or integrons, in *M. tuberculosis*, acquired drug resistance gene through spontaneous mutations in chromosomal genes. This produces the selection of resistant strains during sub-optimal drug therapy (Pedro *et al.*, 2014).

The genes associated with drug resistance in this study were rpoB, inhA and katG. The presence of rpoB and either inhA or katG in *M. tuberculosis* indicate MDR-TB, while the presence of one of this gene (i.e. rpoB, inhA, or katG gene) indicate mono DR-TB. The mutations found in this study were katG MUT1, inhA MUT1, inhA MUT2 and rpoB (MUT1, MUT2, MUT3 or MUT4). katG MUT1, inhA MUT1 or inhA MUT2 the presence of any indicate monoresistance, presence of rpoB (MUT1, MUT2, MUT3 or MUT4) and any one mentioned above indicate multidrug resistance tuberculosis. The absences of mutation indicate sensitive *M. tuberculosis* to anti-tuberculosis drugs. The findings in this study were similar to that of Idiegbe *et al.,* (2001) in Lagos Nigeria, Ani *et al.,* (2010) in Jos Nigeria and Akaninyene *et al.*, (2013) in Calabar Nigeria, because the genes associated with resistance were rpoB, inhA and katG gene.

In other part of Africa and outside Africa, the genes in this study were similar [(to those found by Desta *et al.*, (2009) in Ethiopia; Borann *et al.*, (2009) in Cambodia and Dam *et al.*, (2011)in India]. A study conducted in Britain by Pedro *et al.*, (2014), shows that mutations in a 'hot-spot' region of 81bp of *rpoB* were found in about 96% of rifampicin-resistant. This region, spanning codons 507–533, is also known as the rifampicin resistance-determining region (RRDR), this findings was also similar to our findings. But a study reported by Vanshakidge *et al.*, (2010) in India different from our finding as they formed mutations outside of the hot-spot region of *rpoB* in rifampicin-resistant *M. Tuberculosis* were found. Resistance to isoniazid involves mutations in several genes, including *katG*, *ahpC*, *inhA*, *kasA* and *ndh*, resistance (Pedro *et al.*, 2014). A study by Hazbón *et al.*, (2006) in Brazil found that mutations in *katG*, *inhA* and *ahpC* were associated with isoniazid resistance; this was similar to our findings. The reported genes associated with DR-TB by Srinivas *et al.*, (2015), in One Baylor Plaza, Houston, USA were similar to our findings.

5.2 Conclusion

5.2.1 Prevalence of drug resistance tuberculosis (objective no 1)

The prevalence drug resistance tuberculosis among the follow-up patients attending chest clinic in Aminu Kano Teaching Hospital, Kano Nigeria was 72%. Females had higher prevalence of 48% (DR-TB) than males 24%, education and medication plays a vital role in the treatment of tuberculosis because males had high level of education, they also adhere to treatment guide lines than females in this study.

Patterns of drug resistance in tuberculosis (objective no 2)

The patterns of drug resistance among the participants were Monodrug resistance tuberculosis and multidrug-resistance tuberculosis.

Determination of the genes associated with drug resistance in identified *Mycobacterium tuberculosis* (objective no 3).

The identified genes coding for resistance in this study were katG MUT1, inhA MUT1 or inhA MUT2, the presence of either indicate monoresistance. Presence of rpoB (MUT1, MUT2, MUT3 or MUT4) and any one mentioned above indicate multidrug resistance tuberculosis. But their absences indicate drug sensitive *M. tuberculosis.*

5.3 Recommendation

5.3.1 Prevalence of drug resistance tuberculosis (objective no 1)

Adherence to the TB treatment

Federal ministry of health in collaboration with nongovernmental organizations should give more emphasis on adherence to the TB treatment under direct observation in accordance to DOTS strategy and continuing education on TB transmission, adherence to treatment algorithms and patients isolation.

Behaviour change

Using dissemination of knowledge of health, channel of transmission the disease from infected person to another, emphasis should also be made on prevention and control of the disease.

5.3.2 Patterns of drug resistance in tuberculosis (objective no 2)

Those diagnosed with DR-TB should be admitted in MDR-TB ward, while those with drug sensitive tuberculosis should be place on category two anti TB regiments.

5.3.3 The genes associated with resistance in identified *Mycobacterium tuberculosis* (objective no 3)

More research should be conducted to identify other genes associated with resistance and other factors associated to the genes coding for drug resistance and the information be disseminated.

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Appendices

Appendix 1 : Ethical approval

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Microbiology
AKTH, Kano.

RE: ETHICS APPROVAL

Further to your application in respect of your research proposal titled "Genetic Basis of Drug-Resistance in Mycobacterium Tuberculosis Among Patients Attending Chest Clinic in Aminu Kano Teaching Hospital Kano, 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

Appendix 2 : Transmittal letter for the respondents

Transmittal letter for the respondents

Dear respondents,

As part of my degree course requirements for the award of Masters Degree in Microbiology, I am carrying out a study on **"Genetic determinant of drug-resistance in *Mycobacterium tuberculosis* among follow-up tuberculosis patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria".**

As a patient attending above clinic in the above facility, you are selected to take part in this study. You are kindly to answer the questions about how administering of anti TB drugs makes *M. tuberculosis* to developed resistant.

The information you will provide through this questionnaire will be used on for academic purpose and will be treated with confidentiality.

Thank you for your cooperation.

Mohammad Aminu Bashir: MSc/MM/0001/132/DF

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Kampala International University (KIU), western campus Ishaka – Bushenyi, Uganda.

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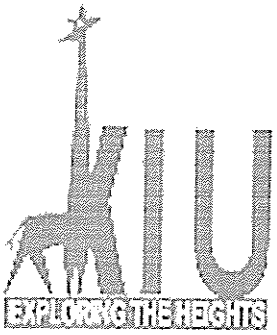
Appendix 3 : Consent form



KAMPALA INTERNATIONAL UNIVERSITY

WESTERN CAMPUS

SCHOOL OF POST GRADUATE STUDIES

RESEARCH AND ETHICS COMMITTEE

CONSENT FORM

STUDY TITLE:"Genetic determinant of drug-resistance in *Mycobacterium tuberculosis* among follow-up tuberculosis patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria".

RESEARCHER: **Mohammad Aminu Bashir**

Your consent is being sought to participate in this study. Please read the following information carefully before you decide whether or not to consent and participate.

This consent form is composed of the following two parts:

PART I: INFORMATION SHEET:

This part contains the information which will assist you to make the decision to either participate or not to participate.

PART II: CONSENT FORM:

This part contains a certificate which you can sign if you accept to give your consent to participate and get interviewed. If you do not agree to participate you are not supposed to sign.

PART I: INFORMATION SHEET

Purpose of the research:"To determine the drug resistance in *Mycobacterium tuberculosis* among category-one follow-up tuberculosis patients attending chest clinic in Aminu Kano Teaching Hospital, Kano, Nigeria".

.**Participant selection:** A total of 384 consented participants from follow-up (Those completed two months category one intensive phase of anti TB first line regimens) tuberculosis patients attending chest clinic in Aminu Kano Teaching Hospital Kano, Nigeria.

Discomforts/Risks: There is no risk in this study and you can decide to participate or not.

Incentives/benefits for participation: There are benefits to you for choosing to participate in this study. However, you will help yourself to know whether you have drug resistant, susceptible or no tuberculosis after your two months intensive phase treatment within 24 hours.

Time duration of participation: Your participation in the study will not exceed 30 minutes.

Confidentiality: Records will be kept confidential and will be available only to professional researchers, and the facility. If the results of this study are published, the data will be presented in group form and individual participants will not be identified.

Voluntary participation: Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, there is no penalty in doing so. You may change your mind later and stop participating even if you agreed earlier.

Termination of participation: You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your participation in this clinic in any way. You will still have all the benefits from the research findings. You may stop participating in the research at any time you may wish.

Who to contact: If you have any question (s) you can get me through these contacts below:

Mohammad Aminu Bashir.

MSc./MM/0001/132/DF

bmohammadaminu@yahoo.com or abmkyarama@gmail.com

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Respondent initials……..

Date:……..

Witness initial:……..

Date:……

Babinauku

Takardarsanarwa

Wannan takarda tana bayani ne a game da binciken dana ke so inyi akan kwayoyin cutar dasuke bijirewa tarin fuka. Yin wannan aikin zaikaini gasamun digirina biyu a bangaren nazarin kwayoyin halitta. Yin wannan bincike zai sa agano yadda kwayoyin wannan halitta kebijirewa maganin tarin fuka karona farko da kuma dalilan dasuke sawa maras lafiya yagamu da wadannan matsaloli.Yin wannan biciken zaisa a gano kwayar cutar da take haddasa tarin fuka mai bijirewa magani akaron farko dakuma wacceke bijirewa magani ayayin da maraslafiya kekan magani akarona farko.Wanda keda shaawa zai iya shiga cikin wannan gwajin (bincike). Zaka kozaki iyajanyewa aduk lokacin da kaga kokika ga dama. Idan akwai wani bayani dabaka kobaki ganeba kayi kokiyi tambaya koka kira kokikira ta wannan lambar wayar mai binciken.

Mohammad Aminu Bashir MSC/MM/0001/132

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Sahannunwandazaiyigwaji.....

Kwananwata.....

Sahannunshaida.....

Kwananwata.....

Appendix 4 : Questionnaire for the participants

Questionnaire for the participants

Dear respondent your participation is voluntary and the information you give is confidential. You may also stop the interview at any time you wish, hoping that this information will be used to improve the welfare of the patient.

Ticks the correct answers were necessary.

Section A

1. **Age.....**
2. **Marital status**
   (a) Single
   (b) Married
   (c) Widow
   (d) Separated /divorced
3. **Sex**
   (a) Male
   (b) Female
4. **Educational level**
   (a) Primary
   (b) Secondary
   (c) Tertiary institution
   (d) University
5. **Occupation**
   (a) Civil servant
   (b) Farmer
   (c) Businessman/Businesswoman
   (d) House wife
   (e) Student
   (f) Unemployed
6. **Religion**
   (a) Islam
   (b) Christian
   (c) Others

Section B: Knowledge about tuberculosis (TB)

7. **Have you heard of tuberculosis**
   (a) Yes
   (b) No

8. **If yes, from what source?**
   (a) Media (c) Healthcare workers
9. **How long are you on anti-tuberculosis drugs?**
   (a) For two months
   (b) Six months
10. **Do you know your HIV status?**
   (a) Yes
   (b) No

Medication

11. **Are you taking your anti-TB drugs at DOTS clinic or at home?**
   (a) At DOTS clinic
   (b) At home
12. **If the answer is (b) how?**
   (a) At exact the time prescribe to me in the clinic
   (b) At the time I wishes
   (c) Atimes I forgot to take them until when I remembered

Babinahudu

Tambayoyi

Da fatan alheri, ina me sanarda kaikokecewarshigawannangwajinsai in kayiniyasabodahakaakoda wane lokacizakaiyakozakiiyafita in kayikokinyiniya, kumadukkanninbayanenkakobayanenkizaizamantosirri ne. Dafatanwananaikinzaikawogyaragarayuwarmarasalafiyadakefama da cutartarinfuka.

Kasakakokisakaansar data dace.

Kashina (A)

1. **Shekaru.....**
2. Aurekorashinsa

   (a) Marasaure

   (b) Mai aure

   (c) Waccetayitakaba

   (d) Bazawara

3. **Mace ko namiji**

   (a) Namiji

   (b) Mace

4. **Matshayinkaratu**

(a) Firamare
(b) Sakandare
(c) Makarantargaba da sakandare
(d) Dan jami'akoyarjami'a

5. **Sana'a**

(a) Ma'aikacingwabnati
(b) Manoma
(c) Dan kasuwakoyarkasuwa
(d) Mataraure
(e) Dalibikodaliba (f) Babuaiki

Kashina(B): Masaniya game da tarinfuka

13. Kasamilabarintarinfuka

(a) Eh (b) A'a

14. In hakane ta waccehanya?

(a) Ta kafafunwatsalabarai (b) Ta hanyarma'aikatanlafiya
(c) Ta jarida (d) ta hanyarabokai

15. Tarinfukayanasakacuta ne?

(a) Eh
(b) A'a

16. Kana shanmaganintarinfuka?

(a) Eh
(b) A`a

17. In hakaneharzuwayaushe?

(a) Tsawonwatabiyu
(b) Watashida
(c) Sauranyibayanai.....

18. Kataba yin gwajincutarsida

(c) Eh
(d) A`a

19. Yaddakakeko kike shanmaganintarinfukakaronafarko

(c) A asibiti
(d) A gida

Nagode.