PATTERN OF FIRST-LINE ANTI-TUBERCULOSIS DRUG RESISTANCE AND ASSOCIATED FACTORS IN PATIENTS ATTENDING NATIONAL TUBERCULOSIS AND LEPROSY TRAINING CENTRE AND REFERRAL HOSPITAL ZARIA

BY

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DECLARATION

I declare that the work in the dissertation entitled **"Pattern of First-line Anti-Tuberculosis Drug Resistance and associated Factors among Patients Attending National Tuberculosis and Leprosy Training Centre and Referral Hospital, Zaria"** was performed by me in the Department of Community Medicine, Ahmadu Bello University, Zaria under the supervision of Prof. Adebola T. Olayinka. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____ _____

JOSHUA AYUBA RIKOTO **DATE**

CERTIFICATION

I certify that the work for this dissertation entitled **"Pattern of First-line Anti-Tuberculosis Drug Resistance and associated Factors among Patients Attending National Tuberculosis and Leprosy Training Centre and Referral Hospital, Zaria"** by Joshua Ayuba Rikoto meets the regulations governing the award of the degree of Masters of Public Health in Field Epidemiology and Laboratory Management of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation

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DEDICATION

This work is dedicated to my beloved Wife and Children for their patience and understanding throughout the course of the training.

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LIST OF ACRONYMS

AFB	-	Acid-fast bacilli
BCG	-	Bacille-Calmette-Guérin
DOTS	-	Directly observed therapy short course
DR-TB	-	Drug-resistant T tuberculosis
DST	-	Drug susceptibility testing
EPTB	-	Extrapulmonary tuberculosis
LED	-	Light-emitting diode
LPA	-	Line-probe assay
MDR-TB	-	Multidrug-resistant tuberculosis
MDR	-	Multidrug resistant
MTBC	-	Mycobacterium tuberculosis complex
NTBLTC	-	National Tuberculosis and Leprosy Training Centre
NTM	-	Non tuberculosis mycobacteria
PCR	-	Polymerase chain reaction
PTB	-	Pulmonary tuberculosis
PPD	-	Purified protein derivative
TB	-	Tuberculosis
WHO	-	World Health Organization
XDR-TB	-	Extensively drug-resistant tuberculosis
ZN	-	Ziehl Neelsen

SUMMARY

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis.* Despite the availability of Short-course regimens of first-line drugs that can cure around 90% of cases, TB remains a major global health problem causing ill-health among millions of people each year. It ranks as the second leading cause of death from an infectious disease worldwide. In 2012, nearly 8.6 million people developed TB and 1.3 million died from the disease worldwide. Among these deaths, there was an estimated 450,000 who developed multi-drug resistant TB (MDR-TB) with an estimated 170,000 deaths. The African Region has 24% of the world's cases and the highest rates of cases and deaths per capita. This study was therefore carried out to determine the pattern of first-line anti-Tb drug resistance and associated factors in patients attending National Tuberculosis and Leprosy Training Centre/Referral Hospital Zaria.

Sputum samples were collected from 200 DR-TB suspects median age 32 years (range 15 – 75 years) of which 138 (69%) were males. Among these, 156 (78%) and 44(22%) were new and retreatment cases respectively. Also, 59.5 % of the patients were in the age group 21-40 years and 90.6% of them were either unemployed or self employed, and 68.9% either have no formal education or terminated at secondary school level. The sputum samples were first screened for *M. Tuberculosis* complex and rifampicin resistance using GeneXpert(MTB/Rif) and confirmed with Hain line probe aassay(LPA).

Of the 200 samples, 81(40.5%) were positive for *Mycobacterium tuberculosis*, out of which 55(67.9%) were rifampicin (RIF) resistant. More males (74.1%) were positive for MTBC and for MDR-TB (76.2%) than females. The highest resistance to any one drug alone and in combination with other drugs was found in rifampicin (67.9%). However, rifampicin mono

resistance was 13.6%, Isoniazid mono resistance was 1.2% while mono resistance to streptomycin and ethambutol were not seen. Furthermore, 6 (7.4%) were resistant to all the 4 first line drugs while MDR-TB was (51.8%). On bivariate analysis, six factors were found to be associated with development of MDR-TB, 4 of which are patient related while 2 are health care related. Among these, being a retreatment case was the only statistically significant factor (OR=8.2, P-value <0.01). However, on logistic regression only two of the factors - being a retreatment case (OR=9.7) and male sex (OR=2.2) remained associated with MDR-TB. The study found being admitted at a hospital during TB treatment to be a protective factor against development of MDR-TB (OR=0.48).

The study concluded that there was a high rate of rifampicin resistance and MDR-TB among patients attending NTBLTC/Referral Hospital Zaria and recommends that health care providers should adequately educate TB patients on the need for treatment adherence in order to prevent development of anti-TB drug resistance. The TB-DOTS strategy should also be reinforced to ensure patient compliance. Availability of drugs should also be ensured at all times and non-fixed drug combinations should be discouraged.

Key words: MDR-TB, Drug resistance pattern, Drug sensitivity testing, Rifampicin resistance, LPA.

CHAPTER ONE – INTRODUCTION

1.1 Background

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis.* Despite the availability of Short-course regimens of first-line drugs that can cure around 90% of cases since the 1980s, TB remains a major global health problem causing ill-health among millions of people each year. It ranks as the second leading cause of death from an infectious disease worldwide.[1] In 2012 for example, nearly 8.6 million people developed TB and 1.3 million died from the disease worldwide. Among these deaths, there was an estimated 450,000 people reported to have developed multi-drug resistant TB(MDR-TB) with an estimated 170,000 deaths due to MDR-TB.[2]

Geographically, the burden of TB is highest in Asia and Africa, India and China together account for almost 40% of the world's TB cases, while about 60% of cases are in the South-East Asia and Western Pacific regions. The African Region has 24% of the world's cases, and the highest rates of cases and deaths per capita.[1] Although there has been considerable progress in reducing TB cases and deaths globally, the emergence of anti-Tuberculosis drug resistance has become a major problem confronting the global control of tuberculosis.[2] For this reason, knowledge of the estimate and pattern of drug resistance is extremely important in the epidemiology and control of TB.[3]

Recently, anti-TB drug resistance, particularly multi-drug resistant TB has received increased attention worldwide and it is said to occur in the majority of cases due to failure or inadequacy of TB control programs,[4] and in some cases due to inadequate

treatment regimens prescribed by providers, nosocomial transmission as well as non-compliance by patients.[5]

MDR-TB is caused by strains of *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin the two most potent anti-TB drugs.[6] In 2011, global estimate of MDR-TB cases notified to WHO mostly by European countries and South Africa was 60,000. Of the notified cases globally, 3.7% were new cases while 20% had been previously treated. Extensively resistant TB (XDR-TB) was also identified in 84 countries and the average proportion of MDR-TB cases with XDR-TB was 9.0%.[1] It is also reported that each year, about 440,000 MDR-TB cases are estimated to emerge, and 150,000 persons with MDR-TB die globally.[7]

Regionally, Africa ranks 4th (32,000) while the Americas have the least (6,200) estimated number of MDR-TB cases.[8] However, MDR-TB in Africa may be more prevalent than is thought as such, factors associated with TB drug resistance need to be understood so as to develop appropriate control strategies for national programs.[1]

1.2 Problem Statement

In 2012, there was an estimated 450,000 people reported to have developed MDR-TB resulting to about 170,000 deaths globally.[2] Of the total number of estimated MDR-TB cases, Africa has 32,000 cases.[8] In Nigeria, there is no standardized data regarding MDR-TB even though a few studies show prevalence ranging from 5.5% in Lagos,[9] 76.4% in South-Western Nigeria,[10] 5.2% in Cross Rivers state,[11] 13% in Abuja[12] and 44% in Jos.[13] Some of these estimates could however be under-estimates because only a few hospitals have the complex and expensive facilities required for culture and drug sensitivity testing (DST) for the diagnosis of MDR-TB. Consequently, few patients are diagnosed with drug resistant TB (DR-TB)

and even fewer receive adequate treatment and such undiagnosed and untreated drug resistant tuberculosis creates the potential for epidemics.

1.3 Justification

Laboratory records at the TB Directly Observed Therapy Short-course (TB-DOTS) Unit of the NTBLTC & Referral Hospital Zaria indicate a rise in incidence of DR-TB especially with introduction of molecular diagnostic techniques at the facility. However, in this facility patients are being diagnosed with drug resistant TB but the pattern, and factors associated with anti-TB drug resistance have not been analyzed and consequently, have not been documented.

The introduction of the TB-DOTS program was intended to prevent drug resistance by ensuring that patients take their recommended treatment under the watch of the Health worker. However, this did not yield the desired result due to both service and patient related factors such as poor compliance, inadequate supervision, inadequate dosing, incorrect drug combination and duration of treatment.

Furthermore, insufficient knowledge of drug resistance and infection control on the part of the health workers may contribute to the spread of MDR-TB;[14] hence knowing the factors associated with MDR-TB at population level can help in the identification of high-risk groups and in the planning of effective public health control measures. Moreover, because MDR-TB is a disease that threatens life; the recognition of factors associated with its development and the identification of populations at risk will assist in focusing case-finding efforts.[12] Finally, because data on TB drug resistance in Nigeria are scanty, this study is not only timely but necessary; as the information obtained will aid data based decision making and adequate planning for infection control by the facility management and the National TB control Program.

1.4 Research Questions

i. What is the pattern of anti-TB drug resistance in patients attending National TB and Leprosy Training Centre/ Referral Hospital Zaria?

ii. What are the factors associated with anti-TB drug resistance in patients attending National TB and Leprosy Training Centre/ Referral Hospital Zaria?

1.5 General and Specific Objectives

1.5.1 General Objective

To determine the pattern and factors associated with anti-TB drug resistance in patients attending the National TB and Leprosy Training Centre/referral Hospital Zaria.

1.5.2 Specific Objectives

- To determine the pattern of resistance to first-line anti-TB drugs in patients attending the National TB and Leprosy Training Centre / Referral Hospital Zaria.
- To identify patient-related factors associated with DR-TB at the National TB and Leprosy Training Centre/Referral Hospital Zaria.
- To identify health care-related factors that may be associated with DR-TB in patients attending National TB and Leprosy Training Centre / Referral Hospital Zaria

CHAPTER 2: LITERATURE REVIEW

2.1 History of TB

Mycobacterium tuberculosis, the bacteria that causes tuberculosis has been present in the human population since antiquity as definite signs of tuberculosis were found in fragments of the spinal column from Egyptian mummies since 2400 BC. However, exact pathological and anatomical descriptions of the disease began to appear in the seventeenth century.[15] In 1720, the English physician Benjamin Marten was the first to propose that TB could be caused by "wonderfully minute living creatures," and also suggested its mode of transmission. He stated that:

> "*It may be very likely that by an habitual lying in the same bed with a consumptive patient, constantly eating and drinking with him, or by very frequently conversing so nearly as to draw in part of the breath he emits from the lungs, a consumption may be caught by a sound person...I imagine that slightly conversing with consumptive patients is seldom or never sufficient to catch the disease.*"[15]

In 1865, the French military doctor, Jean-Antoine Villemin demonstrated that TB – then referred to as "consumption" could be passed from humans to cattle and from cattle to rabbits. On the basis of this revolutionary evidence, he postulated a specific microorganism as the cause of the disease, finally putting to rest the centuries-old belief that consumption arose spontaneously in each affected organism.[15]

In 1882, Robert Koch discovered a staining technique that enabled him to see *Mycobacterium tuberculosis* and in 1895, Wilhelm Konrad von Rontgen discovered

the radiation which enabled the progress and severity of a patient's disease to be accurately followed and reviewed. Another important development was provided by the French bacteriologist Calmette, who, together with Guerin, used specific culture media to lower the virulence of the bovine TB bacterium, creating the basis for the BCG vaccine but the greatest breakthrough came in the middle of World War II, with the discovery of chemotherapy.[15]

2.2 Causative Organisms

TB is caused by a bacterium called *Mycobacterium tuberculosis*. The bacteria usually attack the lungs, but can also attack any part of the body such as the kidney, spine, and brain.[16] TB is spread from person to person through the air and a person needs to inhale only a few of these germs to become infected. *M. tuberculosis* and seven very closely related mycobacterial species (*M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti and M. mungi*) together comprise what is known as the *M. tuberculosis* complex (MTBC). Most, but not all, of these species have been found to cause disease in humans.[17]

2.3 Epidemiology of Tuberculosis

Despite the global effort to control tuberculosis, TB still remains a major global health problem, causing ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide.[2] Tuberculosis mostly affects young adults, in their most productive years even though all age groups are at risk.[18] Over 95% of cases and deaths are in developing countries and people who are co-infected with HIV and TB are 21 to 34 times more likely to become sick with TB.[18] Tobacco use greatly increases the risk of TB disease and death as more than 20% of TB cases worldwide are attributable to smoking.[19]

In 2012, there were 8.6 million new TB cases and 1.3 million TB deaths. Among these cases, about 2.9 million cases and 410 000 TB deaths were among women. Similarly, an estimated 530 000 cases and 74 000 deaths occurred among children. It was also estimated that 58% of cases in 2012 were in Asia, followed by the African Region with 27%.[2] In Nigeria, despite the implementation of the recommended DOTS strategy nationwide, the estimated burden of TB continued to rise and placing always the country between 4th to 5th among the high burden countries in the world.[20]

The impact of TB on both individual and population health is enormous. *Mycobacterium tuberculosis* (MTB) was the cause of the "White Plague" of the 17th and 18th centuries in Europe. During this period nearly 100 percent of the European population was infected with MTB, and 25 percent of all adult deaths were caused by MTB.[21] Presently, TB is the leading infectious cause of adult mortality in the world accounting for 1.5 - 2 million deaths per year.[23] One third of the world's population is infected and is especially prevalent in regions of HIV prevalence, thus providing a large pool of patients with the disease capable of spreading infection and less than half of all TB cases world-wide are ever diagnosed, and fewer than 60% of those diagnosed are cured.[22] TB is therefore projected to remain one of the world's top 10 causes of adult mortality by the year 2020; as one estimate suggests 171 million new cases and 60 million deaths over this period in the "best-case scenario", and 249 million new cases and 90 million deaths in the "worse-case scenario".[23]

2.4 Pathogenesis of the Infection

TB infection begins when the tubercle bacilli inhaled through the air multiplies in the small air sacs of the lungs. A small number enter the bloodstream and spread throughout the body, but the body's immune system usually keeps the bacilli under control. Such People are said to have latent TB infection but not TB disease They do not have symptoms of TB, and they cannot spread TB to others.[19] However, if for any reason the immune system cannot keep the tubercle bacilli under control and the bacilli begin to multiply rapidly, causing TB disease. Patients with pulmonary tuberculosis (PTB) are the most important source of infection and the bacilli are spread into the air by coughing, sneezing, talking, spitting, and singing, and they can remain suspended in the air for long periods of time. A single cough can produce 3,000 infectious droplet nuclei which are so small that they avoid the defenses of the bronchi and penetrate into the terminal alveoli of the lungs, where multiplication and infection begins.[23]

The infecting dose of tubercle bacilli and the immune status of the host usually determine the risk of progression from infection to disease. When infection progresses to disease, it manifests as infiltrates and lesions within the lung tissue, enlarged lymph nodes within the chest, pleural effusion, or disease disseminated in other parts of the body.[23] If the primary infection resolves, small numbers of tubercle bacilli can remain dormant in scarred areas of the body for many years. Factors that enhance the risk of developing TB following infection include age, (infants and children below 5 years and the elderly being particularly at high risk), under nutrition, tobacco, alcohol, corticosteroids, immunosuppressive drugs and other diseases such as diabetes

mellitus, silicosis, leukemia, measles, and whooping cough in children. Of all these factors however, none is as important as HIV.[17] [23]

2.5 Molecular Techniques for TB Diagnosis

A variety of molecular assays are available for TB identification and DST. These include the GeneXpert, Line probe assays such MTBC, CM and AS for identification, resistance testing for Isoniazid and Rifampicin from clinical specimen or culture – using MTBDRplus and also MTBDRsl – now available for second line drugs such as fluoroquinolones, aminoglycosides or ethambutol.

i. XpertMTB/RIF

This newly developed Xpert MTB/RIF assay utilizes real-time PCR (rt-PCR) technology to both diagnose TB and detect rifampicin resistance concurrently using unprocessed sputum samples regardless of their smear status as well as clinical specimens from extrapulmonary sites.[24] The assay is conducted within a simple, almost fully automated cartridge-based system and can be used close to the point of care by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 hours.[24]

ii. Line Probe Assays

Line probe assays (LPA) were endorsed by the WHO in 2008 for molecular detection of drug resistance from smear-positive patients at risk of MDR-TB. Two commercial LPAs are currently available: the INNO-LiPA Rif.TB test (Innogenetics NV, Gent, Belgium) and the GenoType MTBDR*plus* test and MTBDR*sl* (Hain Lifescience GmbH, Nehren, Germany). LPAs use a PCR/hybridization technique to identify members of the MTBC while simultaneously identifying drug-resistant strains by detecting the most common single nucleotide polymorphisms (SNPs) associated with resistance.[25]

LPAs are highly accurate for the detection of first-line drug resistance, especially in smear-positive sputum specimens, with ≥97% sensitivity and specificity of ≥99% for the detection of RIF resistance, alone or in combination with INH (sensitivity ≥90%; specificity ≥99%), on isolates of *M. tuberculosis* and on smear-positive sputum specimens.[26]

The major advantage of LPAs is that they can be performed directly on smear-positive sputum samples, giving rapid (approximately 5 h) drug susceptibility results without the need for culture. They however have disadvantages in that they are labour intensive and require highly trained personnel and dedicated laboratory space and equipment.[25]

2.6 TB drugs and the development of resistance

Fifty years after the introduction of effective specific TB chemotherapy, the number of cases is higher worldwide and, more threateningly, there is an increasing number of cases of infections with organisms resistant to the major anti-tuberculosis agents.[26] This resistance to anti-TB drugs, which was recognized in the very early days of the chemotherapeutic era, has emerged as a serious problem and is often due to widespread and sometimes incorrect use of anti-tuberculosis treatment and failure to implement proper TB control programs and correctly manage TB cases.[27]

TB drug resistance is characterized by both the types of drugs to which the bacteria lack susceptibility and the manner in which resistance was acquired. Clinically, drug resistance is divided into 2 types: primary resistance and acquired resistance. Primary resistance occurs in a patient newly diagnosed with TB who has not previously been treated with TB drugs or had therapy of less than one month duration. These patients are likely to have been infected with a drug resistant strain of *M. tuberculosis*.

Acquired or secondary resistance on the other hand refers to the presence of a resistant strain in a TB patient who has previously received at least one month of TB therapy. These cases are likely to have been initially infected with a drug-susceptible M. tuberculosis strain, but during the course of anti-tuberculosis treatment, drug resistance emerged.[28]

The phenomenon of acquired resistance was first observed with the introduction of streptomycin in 1940s as monotherapy for TB. After initial bacteriologic and radiographic response, resistance to streptomycin emerged in 85% of patients tested, and monotherapy with streptomycin afforded no long-term survival benefit. When a single drug is used to treat a large load of TB organisms, the susceptible organisms are killed, leaving the resistant strains which gradually, multiply and constitute a greater percentage of the population and subsequently results in treatment failure.[29] Combination therapy was therefore introduced to prevent the development of resistance and remains one of the cornerstones of anti-TB therapy, which usually comprises four drugs, including INH and RIF.[30]

Drug-resistant strains of *Mycobacterium tuberculosis* pose a serious threat to global TB prevention and control efforts.[25] Of particular concern is the emergence of multi-drug resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin.[31] At any point in time, about 630 000 people worldwide are thought to be infected with strains of *M. tuberculosis* showing resistance to isoniazid and rifampicin; the two drugs that are currently the most effective against TB.[32] WHO reported that 3.7% of all new notified TB cases and 20% of re-treatment cases in 2011 have MDR-TB and about 9% of these cases were XDR-TB[33]

Inadequate chemotherapy is the commonest cause of MDR-TB.[34] Most people with tuberculosis are cured by a strictly followed, six-month drug regimen that is provided to patients with support and supervision.[35] However, inappropriate or incorrect use of antimicrobial drugs, or use of ineffective formulations of drugs, can cause drug resistance. In some countries, it is becoming increasingly difficult to treat MDR-TB because treatment options are limited and recommended medicines are not always available. In some cases even a more severe form of drug resistant TB called extensively drug-resistant TB, (XDR-TB) which is a form of multi-drug resistant tuberculosis that respond to even fewer available medicines develops.[17]

2.7 Mechanism of TB drug resistance

Major advances in molecular biology tools and the availability of new information generated after deciphering the complete genome sequence of *M. tuberculosis has shown that* specific gene mutations were associated with drug resistance.[36] *M. tuberculosis* is often acquired early in life with acute infection and with developing immunity, granuloma formation, and calcification. This is followed by a long latent period, which continues until reactivation occurs in a proportion of the individuals. This means that unlike organisms that colonize the nasopharynx or the gastrointestinal tract, individual strains of *M. tuberculosis* have little opportunity to interact and exchange genetic information with other strains.[26] In these locations, other bacteria may transmit antibiotic resistance determinants through transmissible genetic elements, transposons, integrons, and plasmids, by transduction or trans- formation. This option is not available for *M. tuberculosis*, so resistance can only occur through chromosomal mutation as naturally occurring mutations that confer resistance to anti-tuberculosis drugs occur spontaneously and independently.[26]

a. Rifampicin (Rif) Resistance

Rifampicin is a lipophylic ansamycin introduced in 1972. The target of rifampicin in *M. tuberculosis* is the β-subunit of RNA polymerase, where it binds and inhibits the elongation of messenger RNA. The great majority of *M. tuberculosis* clinical isolates resistant to rifampicin show mutations in the gene *rpoB* that encodes the β-subunit of RNA polymerase. This results in conformational changes that determine a low affinity for the drug and consequently the development of resistance. Almost all rifampicin-resistant strains also show resistance to other drugs, particularly to isoniazid. For this reason, detection of rifampicin resistance has been proposed as a surrogate molecular marker for MDR.[36]

b. Isoniazid (INH) Resistance

Isoniazid is one of the main drugs for the treatment of TB. It has a simple structure containing a pyridine ring and a hydrazide group, with both components being essential for the high activity against *M. tuberculosis*. Two molecular mechanisms have been shown to be the main cause for isoniazid resistance; mutations in *katG* and mutations in *inhA*, or more frequently in its promoter region.[36] Isoniazid is a pro-drug requiring activation by the catalase/peroxidase enzyme encoded by *katG*. Mutations in *katG*, the most commonly found in *M. tuberculosis* clinical isolates, give high levels of resistance. Mutations in *inhA* cause not only resistance to isoniazid, but also resistance to the structurally related second-line drug ethionamide, with the most common *inhA* mutation occurring in its promoter region which has been found more frequently associated with mono-resistant strains.[36]

c. Streptomycin (STR) Resistance

Streptomycin is an aminocyclitol glycoside antibiotic that was the first antibiotic used in the treatment of TB. Unfortunately, resistance to streptomycin emerged quite

rapidly due to its use as mono-therapy.[36] In prokaryotes, its mechanism of action is to inhibit the initiation of translation by binding to the 16S rRNA. Streptomycin resistance emerges through mutations in *rrs* and *rpsL* that produce an alteration in the streptomycin binding site.[26] Mutations in rpsL that result in a high level of resistance to streptomycin fall into two categories, restrictive and non restrictive. Restrictive mutations are associated with an attenuation of virulence, whereas nonrestrictive mutations are not.[26]

d. Ethambutol (ETH) Resistance

Ethambutol is a major component of the first-line therapy of tuberculosis. It is active against multiplying bacilli, where it interferes in the biosynthesis of cell wall arabinogalactan.[36] Mutations in codon 306 of *embB* (*embB*306) are suggested as a major resistance mechanism in clinical isolates.[37][38]

2.8 Global Burden of Drug Resistant TB

Accurate data regarding rates of drug resistance are not universally available. Different prevalence rates have however been reported from different parts of the world. In Eastern Europe for instance, the proportions of new TB cases with MDR-TB at country level ranged from 32.3% in Belarus, 22.9%, in Estonia, 30.3% in Kazakhstan, 26.4% in Kyrgyzstan, 19.4% in the Republic of Moldova, 23.2% in Uzbekistan and 35.1% in the Russian Federation.[6] Similarly, 15% prevalence of MDR-TB was reported in Georgia[39] and 35.3% in Minsk, Belarus.[40] For previously treated TB cases with MDR-TB, countries with the highest reported proportions were Belarus 75.6%, Moldova 63.5%, Uzbekistan 62.0%, Estonia 57.7%, Azerbaijan 55.8%, Tajikistan 53.6% Kyrgyzstan 51.6%, and Kazakhstan 51.3%. These data indicate that eastern European and central Asian countries continue to represent hot spots for MDR-TB, with nearly one third of new and two thirds of previously treated

TB cases affected by MDR-TB in some settings.[41] The very high frequency of drug-resistant forms of TB indicates the existence of serious problems in the organization of TB treatment,[40] and the high level of MDR-TB among new TB cases especially indicates enormous on-going transmission of resistant strains of *M. tuberculosis* in the community[32].

In Asia, rates ranging from 12%[42] to 32.7% were reported in Eastern China, while 17.4% and 47.5% prevalence of MDR-TB were reported in India majority (66.9%) of the patients being from a lower socioeconomic background.[41][3] Elsewhere, 32.7% prevalence was found in Korea[44] while in Abu Dhabi, it was 16.2%.[45] However, the highest prevalence of 73.5% was reported in Bangladesh.[46]

In Africa, the lowest rate of MDR-TB was reported in Nairobi, Kenya with 0.7%.[47] Other rates reported are 1.1% [48] and 12.7% [49] in Kampala Uganda, 6.7% in western region of Cameroon,[50] and 46.3% in Ethiopia.[51] In South Africa, there are an estimated 13,000 cases of multidrug-resistant tuberculosis (MDR-TB) emerging each year, in addition to a growing epidemic of extensively drug-resistant tuberculosis (XDR-TB) associated with high mortality especially among HIV infected individuals. The prevalence found in 2 different studies are 55%[52] and 84.4%[27] respectively.

In Nigeria, DR-TB is an emerging phenomenon but different prevalence rates ranging from as low as 5.2% in Cross Rivers state[11] to 44% in Jos[13] to as high as 76.4% in South-Western Nigeria.[53]

2.9 Pattern of Anti-TB Drug Resistance

Anti-TB drug resistance patterns have been reported from different parts of the world. In Mexico for instance, 23.3% resistance to Isoniazid (INH), 11.1% for Rifampicin (Rif) and 11.1% for both INH and Rif was reported. In this study, resistance to INH alone or in combination with other anti-TB drugs was most prevalent and previous anti- TB treatment was identified as a risk factor for developing resistance to *M. tuberculosis.*[54] Similarly, in Turkey, 14.4% resistance for INH, 21.1%STR, 10.6% Rif and 14.2 for EMB have been reported.[5]

In Nigeria, the pattern of drug resistance in Abuja was found to be Rif 4.1%, INH 5.1% STR 7.1% and EMB 9.4%.[55] Similarly, in Calabar, resistance to INH was reported to be 2%; ETH 8%, streptomycin 7% but found no rifampicin mono resistance. Combined resistance was however reported as 3% for Rif + INH; 1% for Rif + ETH; 2% for INH + STR; 7% to INH + ETH; and 9% were resistant to STR + ETH. Resistance to three drugs H+E+S was seen in 1% and 1% was resistant to all four drugs. MDR-TB was seen in 4% of the cases.[56] There was also a high prevalence of Rifampicin resistant *M. tuberculosis* in Benue state especially in the young adults.[57] In another study, a high rate of transmitted drug-resistant TB (5.5%) exceeding the upper limit of the WHO MDR-TB model (4.3%) was reported. This high rate was inferred by rates of rifampicin resistance in treatment-naive patients. Furthermore, 1.4% INH monoresistance and 2.8% RIF monoresistance was also observed, highlighting the importance of evaluating both drug susceptibilities[58]

2.10 Factors Associated with anti-TB Drug Resistance

Several factors have been adduced to be associated with development of anti-TB drug resistance. Some of these factors include inappropriate treatment which has been described as the most important influencing factor of MDR-TB in China.[42] Also previous treatment with an inadequate initial drug regimen may be associated with the development of XDR-TB.[59][60] Other factors which are said to be associated with TB drug resistance include poorly organized or poorly funded National TB Control programs, lack of or inadequate guidelines, poor training on the part of health workers, lack of or inadequate treatment monitoring, non-standardized treatment, history of frequent shortages of drug supplies, poor quality anti-TB drugs and wrong dose or combination.[39]

Some of these factors associated with development of DR-TB are said to be patient related while others are health care related. Patient related factors include history of previous TB treatment, inadequate adherence to treatment, adverse effects and mal-absorption, social barriers, substance dependency, lack of money, infection with highly virulent MDR-TB strains and HIV infection in some regions.[61] Some factors related to health care include dominant private sector, poor infection control, prescription of an inadequate TB regimen, and delay in commencement of treatment after diagnosis, health care worker ignorance, lack of laboratory standardization and delay in laboratory results all contribute to the emergence of drug resistance.[59]

CHAPTER THREE - METHODOLOGY

3.1 Study Area

The study area is situated in Kaduna State North Central Nigeria. Kaduna State has a total area of 46,000,053 square kilometers and is bordered by Zamfara, Katsina and Kano States to the north, Bauchi and Plateau States to the East Nasarawa State to the south, Niger State to the West and the Federal Capital Territory Abuja to the South-West. Norhern Kaduna is inhabited predominantly by Hausa/Fulani while Southern Kaduna is inhabited by about 30 other ethnic groups.

Zaria is one of the 23 Local Governments that make up Kaduna State. It is located in the northern part of Kaduna and is home to the famous Ahmadu Bello University and several other Federal and state owned institutions including the study site. The study site is the National Tuberculosis and Leprosy Training Centre/ Referral Hospital Zaria which was established in 1991. The centre is engaged in training, provision of TB, TB/HIV and leprosy services and operational research relating to TB, TB/HIV and leprosy. Within the centre is situated the National TB Reference Laboratory which is responsible for TB diagnosis and surveillance. The TB Laboratory is also actively involved in TB/HIV collaboration activities. In 2012 laboratory records show that 4589 patients were seen at the laboratory, 12,343 sputum samples analyzed, and 618 patients tested positive for *M. tuberculosis.*

3.2 Study Design

The study design was cross sectional

3.3 Study Population

All patients suspected of having drug resistant tuberculosis who present at the TB-DOTS Unit of the NTBLTC/Referral Hospital Zaria from 19th December 2013 to 20th April, 2014.

3.3.1 Inclusion Criteria

Patients included in the study were both new and retreatment TB cases aged 15 years and above whom either the Doctors suspected of having drug resistant TB and or are found to be Rifampicin resistant by GeneXpert.

3.3.2 Exclusion Criteria

Patients infected with Non-Tuberculosis Mycobacteria (NTMs) were excluded.

3.4 Sample Size Determination

Sample size was calculated based on 13% prevalence of MDR-TB reported in a study conducted at Zankli Medical Centre Abuja [12].

Sample size was calculated using the formula of Lwanga & Lemeshow:

$$n = \frac{Z^2pq}{d^2}$$

where:

n = required sample size

$Z = _{(1-a/2)} = 1.96$ - value of the standard distribution corresponding to a significance level of α (1.96 for a 2-sided test at the 0.05 level)

p = expected proportion in the population = 13% (0.13)

q = 1-p (1-0.13) = 0.87

d = absolute precision = 5% (0.05)

Using the above information, the sample size was:

$$n = \frac{(1.96)^2 \times 0.13 \times 0.87}{(0.05)^2} \qquad n = \frac{0.4345}{0.0025} \qquad n = 174$$

However, 10% non- response rate was envisaged and was calculated by:

R% = calculated sample size x 1/1-R%

10% = 174 x 1/1- 0.10 10% = 1/0.9 =1.1

10% = 174 x 1.1 = 191, which was rounded up to 200.

3.5 Sampling Technique

Non- probability sampling technique was used where participants were recruited consecutively from 19th December 2013 to 20th April 2014 until the calculated sample size was attained. This technique was adopted due to the rare nature of the event under study which necessitated the inclusion of all DR-TB suspects presenting at the facility within the study period. This includes patients presenting primarily at the facility as well as those that came on referral.

3.6 Study Instruments

3.6.1 Data Collection

A semi-structured questionnaire was used to obtain data on socio-demographic characteristics and factors associated with DR-TB in the participants. The major factors investigated were previous TB treatment, contact with a TB case, Treatment interruption, treatment delay, cigarette smoking, drinking alcohol and whether

patients were adequately informed on the need for treatment adherence, home treatment and sex.

3.6.2 Laboratory Analysis

1. Molecular techniques - GeneXpert (MTB/Rif), for identification of Mycobacteria Tuberculosis Complex as well as rifampicin resistance in sputa.
2. PCR based Hain Line Probe Assay (LPA) GenoType MTBDR*plus* 2.0 (Hain Lifescience GmbH, Nehren, Germany) for confirmation MDR-TB by detection of Rifampicin and isoniazid resistance.
3. BACTEC MGIT 960 (BD Bioscience, Sparks, MD, USA) to test for Ethambutol and Streptomycin resistance.

3.7 Data Collection Methods

Questionnaires were administered by interviewer and by telephone. For patients who do not understand English Language, an interpreter was provided who explained to them what the study was all about before obtaining consent. For those interviewed by telephone, consent was obtained verbally. Of the 55 participants who were resistant to at least one drug and were considered eligible for interview, 8 had no contact information, 7 decline consent, 4 were critically ill, 2 died before interview while 2 had hearing defect due to drug side effects. Consequently, only 32 of the 55 participants were interviewed.

3.7.1 Sample Collection and Processing

1. GeneXpert Testing

Sputum samples from 200 DR-TB suspects were collected in wide mouthed screw capped sputum containers, labeled appropriately and given a unique lab identification

number. The samples were then screened using the GeneXpert (MTB/Rif) technology, which simultaneously detects *M. tuberculosis* (MTB) and Rifampicin (Rif) resistance in positive sputa. Since rifampicin resistance is a marker for multidrug resistant TB, all samples that were MTB positive and showed rifampicin resistance were processed further using the PCR based Hain's Line Probe Assay (LPA)

2. LPA

GenoType MTBDR*plus* 2.0 (Hain Lifescience GmbH, Nehren, Germany) for confirmation of isoniazid (INH) and Rif resistance. LPA uses a PCR/hybridization technique to identify members of the *Mycobacteruim tuberculosis* complex (MTBC) and simultaneously identifies drug-resistant strains by detecting the most common single nucleotide polymorphisms associated with resistance.[25]

3. Bactec MGIT 960

Further testing was performed by indirect proportion method using the BACTEC MGIT 960 system (BD Bioscience, Sparks, MD, USA) to test for resistance to Ethambutol (ETH) and streptomycin (STR)

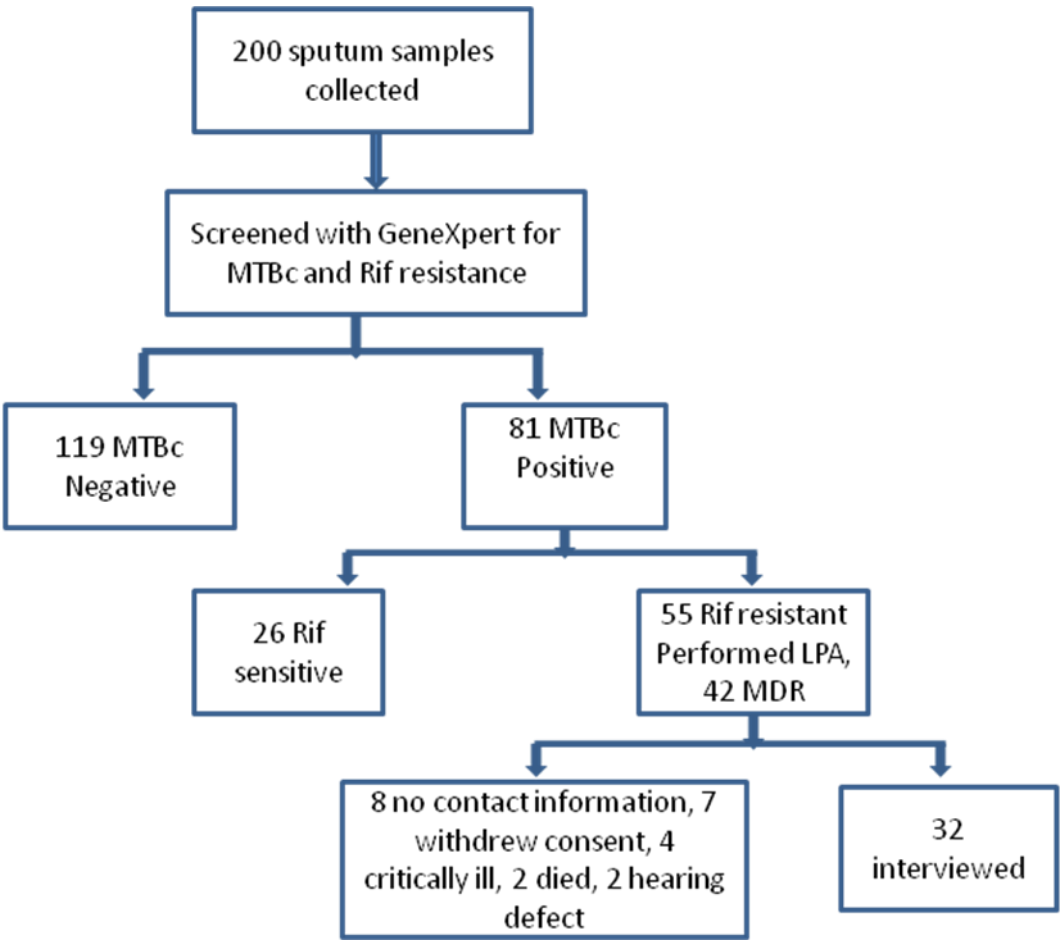


Figure 1: Flowchart of patient enrolment for determination of First-line anti-TB drug resistance pattern in patients attending NTBLTC/Referral Hospital, Zaria; Dec 2013-April 2014.

3.8 Data Management

3.8.1 Quality Assurance and Quality Control

Research assistants were trained and closely supervised to ensure validity and reliability of the data collected. The questionnaires were pre-tested and validated before administration. The data was then cleaned and entered in Excel spreadsheet and imported into Epi Info 7 for analysis. For laboratory assays, all samples were run along with positive and negative controls according to manufacturers' instructions.

3.8.2 Statistical Analysis

Uni-variate analysis was performed to obtain frequencies and proportions while bi-variate analysis was done for measures of association – odds ratio (OR) and 95% confidence intervals (CI) were calculated. Logistic regression was also performed to rule out possible confounding. All statistical analyses were based on samples that tested positive to *M. tuberculosis* and those that showed resistance to at least any of the 4 drugs.

3.9 Ethical Considerations

Ethical clearance (*NHREC Approval No. NHREC/01/02/2007-19/12/2013*) was obtained from the Ethical and Research Committee of NTBLTC and Referral Hospital Zaria. Written informed consent was obtained from all participants and they were adequately informed on the risks and benefits of participation. Confidentiality of the participants was also ensured by entering data on a password protected computer accessed only by the researchers.

Participants were also informed that participation was voluntary and that they were free to withdraw at anytime without consequences. An interpreter was also provided for participants who do not understand English language before they gave consent. All MDR-TB positive patients were referred immediately to the clinic for management.

3.10 Limitations

The major limitations of this study are the small sample size and the use of non probability sampling technique for patient enrollment. These may have affected the final results and therefore limits generalization of the findings.

CHAPTER FOUR- RESULTS

4.1 Socio-demographic Characteristics

Table 4.1: Baseline socio-demographic characteristics of all participants

Variables	Frequency	Percent (%)
Sex (n =200)		
Male	138	69.0
Female	62	31.0
Age group (years) (n= 200)		
11-20	24	12.0
21-30	68	34.0
31-40	51	25.5
41-50	33	16.5
51-60	15	7.5
61-70	6	3.0
70 and above	3	1.5
Treatment status (n=200)		
Retreatment patients	44	22.0
New patients	156	78.0

Sputum samples were collected from 200 DR-TB suspects median age 32 years (range 15 – 75 years) of which 138 (69%) were males. Among these, 156 (78%) and 44(22%) were new and retreatment cases respectively.

Table 4.1.2: Socio-demographic characteristics of participants that were interviewed

Variable (n=32)	Frequency	Percent (%)
Sex		
Male	23	71.9
Female	9	28.1
Marital status		
Single	15	46.9
Married	15	46.9
Divorced	1	3.1
Widowed	1	3.1
Occupation		
Civil servant	3	9.4
Self employed	16	50.0
Unemployed	13	40.6
Personal income per month		
Less than N20,000	26	81.3
More than N20,000	6	18.7
Educational level		
- No formal education	2	6.3
- Primary	6	18.8
- Secondary	14	43.8
- Tertiary	7	21.9
- Graduate	3	9.4

Of the 32 participants interviewed, 16 (50%) were self employed and 13 (40.6%) were unemployed. Also, 26 (81.2%) of the respondents earn less than N20, 000 per month. On education, only 10 (31.3%) had education beyond secondary school level.

4.2 Identification of MTB and Drug Resistance

Table 4.2: Identification of MTB and drug resistance using GeneXpert and LPA

Variables	Frequency	Percent (%)
***M. tuberculosis* identification by GeneXpert (n=200)**		
Positive	81	40.5
Negative	119	59.5
Rifampicin resistance by GeneXpert (n=81)		
Resistant	55	67.9
Sensitive	26	32.1
Rif and INH resistance with LPA (n=55)		
Rif resistance	54	98.2
INH resistance	39	70.9
Rif+INH resistance	38	69.1

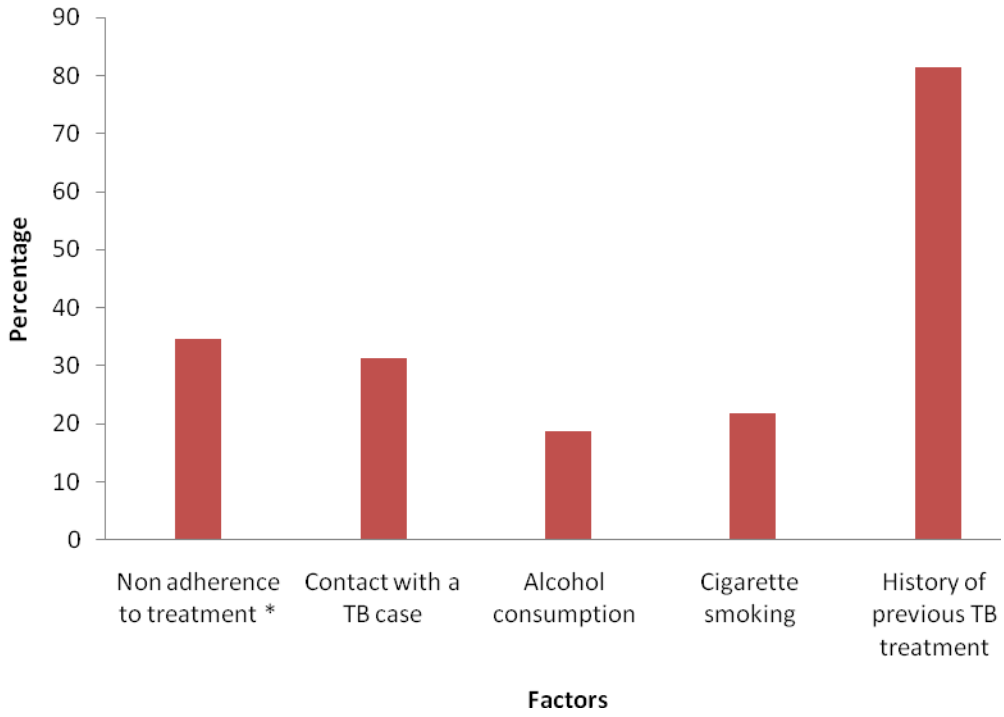
Screening with GeneXpert showed 81 (40.5%) of the 200 cases to be positive for *Mycobacterium tuberculosis*, out of which 55 (67.9%) were rifampicin (RIF) resistant. When tested further with LPA, 54 (98.2%) of the 55 samples still remained rifampicin resistant, 39 (70.9%) were resistant to isoniazid (INH) only, while 38 (69.1%) were resistant to both rifampicin and isoniazid. The 119 cases that were negative for M. Tuberculosis were excluded from any further analysis.

Table 4.2.1: Overall drug resistance pattern shown by MTB positive samples

Resistance profile (n=81)	Frequency	Percent (%)
Resistant to at least one drug	55	67.9
Mono resistance		
RIF only	11	13.6
INH only	1	1.2
ETH only	0	0.0
STR only	0	0.0
Poly resistance		
INH+ETH+STR	1	1.2
MDR-TB		
Rif+INH	32	39.5
RIF+INH+ETH	2	2.5
RIF+INH+STR	9	4.5
RIF+INH+ETH+STR	6	7.4
Total MDR-TB	42	51.8

***Mono resistance**: resistance to at least one first line anti-TB drug*
***Polyresistance:** resistance to two or more drugs excluding Rif and INH*
***MDR-TB:** Resistance to at least Rif and INH with or without other drugs*

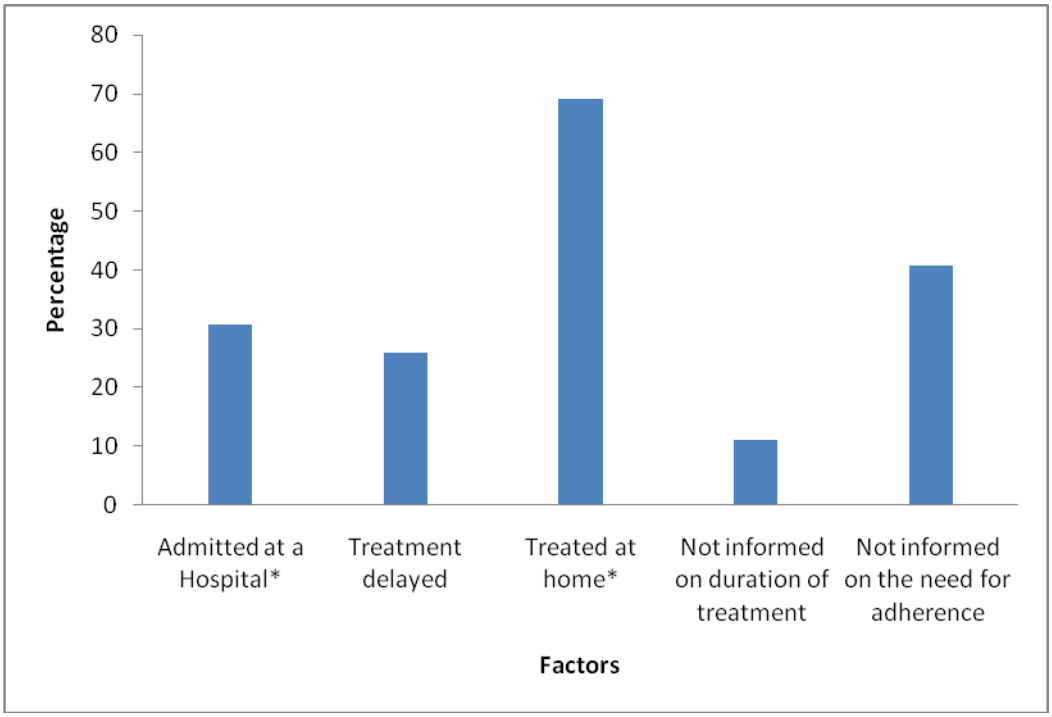
Looking at the drug resistance pattern of the of the 81 MTBC positive samples to the 4 drugs individually, 11 (13.6) were resistant to RIF alone, 1 (1.2%) was resistant to INH alone while mono resistance to ETH or STR was not observed. Furthermore, 55 (67.9%) were resistant to Rifampicin alone and in combination with at least one other drug. On further sub analysis, 32 (39.5%) showed resistance to RIF+INH, 8 (9.9%) were resistant to RIF+INH+STR, 2 (2.5%) were resistant to RIF+INH+ETH, 1 (1.2%) was resistant to INH+ETH+STR, 6 (7.4%) were resistant to all 4 first line drugs (RIF+INH+ETH+STR) while 42 (51.8%) were multidrug resistant.



*(n=26); others (n=32)

Figure 4.1: Frequency of patient related factors

Of the 32 respondents interviewed regarding patient related factors that may be associated with anti-TB drug resistance, 9 (34.6%) said they did not adhere to their first TB treatment regimen, 6 (18.7%) drink alcohol, 7 (21.9%) smoke cigarettes, 10 (38.5%) had contact with a known TB case while 26 (81.3%) had history of previous TB treatment.



*(n=26); others (n=27)

Figure 4.2: Frequency of health care related factors

Health care related factors assessed include hospital admission, delay in commencement of treatment, whether or not patients are adequately informed of the need for treatment adherence. Of these, 8 (30.8%) admitted to have been previously admitted in a hospital while 18 (69.2) received initial TB treatment at home, 7 (25.9%) had their commencement of treatment delayed. Also, 3 (11.1%) were not informed by the care providers on the duration of treatment while 11(40.7) were not informed on the need for treatment adherence.

Table 4.3: Bivariate Analysis of Patient and Health Care Related Factors

Variables	MDR-TB		OR	95% CI	p-value
	Positive	Negative			
Retreatment case (n=81)					
Yes	32(74.4)	11(25.6)	**8.2**	**3.01-22.04**	**<0.01**
No	10(26.3)	28(73.7)			
Contact with a TB case(n= 32)					
Yes	9(37.5)	1(12.5)	4.2	0.44-39.94	0.38*
No	15(62.5)	7(87.5)			
Delayed treatment(n=27)					
Yes	6(31.6)	1(12.5)	3.2	0.32-32.48	0.63*
No	13(68.4)	7(87.5)			
Home treatment (n=26)					
Yes	14(73.7)	4(57.1)	2.1	0.32-12.86	0.64*
No	5(26.3)	3(42.9)			
Interrupted treatment(n=26)					
Yes	8(42.1)	2(28.6)	1.8	0.28-11.87	0.67*
No	11(57.9)	5(71.4)			
Sex (n=81)					
Male	32(76.2)	28(71.6)	1.3	0.46-3.40	0.84
Female	10(23.8)	11(28.2)			
New TB case(n=81)					
Yes	10(23.8)	28(71.8)	**0.12**	**0.05-0.33**	**<0.01**
No	32(76.2)	11(28.2)			
Adhered to treatment(n=26)					
Yes	12(63.2)	5(71.4)	0.69	0.10-4.52	1.00*
No	7(36.8)	2(28.6)			
Admitted at a hospital(n=26)					
Yes	5(26.3)	3(42.9)	0.48	0.08-2.92)	0.64*
No	14(73.7)	4(57.1)			

*Fisher exact

On bivariate analysis, 6 factors were found to be associated with development of MDR-TB but only 1(being a retreatment case) was statistically significant OR 8.2. The risk of development of MDR-TB was also found to be reduced by being a new TB case (OR=0.12, being admitted at a hospital (OR=0.48, and adhering to treatment (OR=0.69).

Table 4.4: Unconditional Logistic Regression Analysis

Variable	OR	95% CI	p-value
Retreatment	9.7	3.3-27.8	<0.01
Sex	2.2	0.7-7.3	0.19
New case	0.1	0.05-0.33	<0.01

After fitting the variables into logistic regression model, being a retreatment case still remained significantly associated with development of MDR-TB (OR= 9.7) while being a new case was still a statistically significant protective factor (OR=0.1, Sex was also found to be associated but was not statistically significant (OR= 2.2 all at a significant level of $p<0.05$.

CHAPTER FIVE

5.1 Discussion

The data presented in this study showed the pattern of first-line anti-tuberculosis drug resistance as well as factors associated with development of such resistance. The study found more males (74.1%) to be positive for MTBC and for MDR-TB (76.2%) than females. This is in line with previous studies in Kenya,[47] Cameroon[50] and Ethiopia[51] in which majority of TB patients were reported to be males and MDR-TB was found to be significantly higher in males than in females. It however differ from a study in Georgia in which being a female was associated with development of drug resistance because according to the researchers, women are more likely to be care providers to patients with MDR-TB thus placing them at increased risk for exposure to and the subsequent development of MDR-TB.[39] In this study however, the result does not mean that *M. tuberculosis* has preference for males, but could be attributed to behavioural factors such as smoking which has been known to be a predisposing factor for TB, with more males generally being smokers than females.[47] This is not however conclusive as another study found that smoking and alcohol may be associated with a lower risk of MDR-TB, and alcohol use may be associated with a lower risk of XDR-TB[59].

It could also be due to socio-cultural factors that seek to protect women, hence men are almost always outdoors and therefore more susceptible to community-acquired resistant strains.[62] The higher prevalence of MDR-TB in men over women may also be explained by the fact that women are more likely to comply with treatment and therefore less likely to receive inadequate treatment than men.

With respect to age, 59.5 % of the patients were in the age group 21-40. This finding corroborates earlier findings that TB affects mostly adults in the economically productive age group.[2][57][63] The findings however differ from studies in Ethiopia in which factors such as age and educational status were not significantly associated with the development of drug resistance.[64]

The study also found 90.6% of the respondents to be either unemployed or self employed, and 68.9% either have no formal education or terminated at secondary school level. This is in line with findings in Uganda in which 77.2% of TB cases were self employed and 81.8% of them had only primary and secondary education;[48] and also in Kazakhstan where 52% were found to be unemployed[65]. These reports suggests that people who have low education and no formal employment are mostly at risk of being infected with TB.[47][48] This is so because the poor are more prone to disease because their low income causes them to have poor nutrition and to be chronically exposed to unsanitary conditions, and also limits their access to health care resulting in chronic disability from treatable diseases and conditions. In addition, the poor also often lack access to education and are more likely to be illiterate, thus it is difficult to reach them with health messages.[66]

This study also found higher levels of DR-TB among retreatment cases than in new TB cases. This is consistent with several other findings[67][64][39][60][49] in which statistically significant association was found between history of previous anti-TB treatment and the development of drug resistance. The findings however differ from a study in Peru in which high MDR rates were found among new smear positive pulmonary TB patients without known risk factors for MDR, suggesting that patients are at risk of infection with a MDR strain by living in a high incidence area where ongoing transmission takes place.[68]

The overall drug resistance rate found in this study for at least one anti-TB drug was 67.9%. This finding is comparable to results found in Belarus (63.8%)[40]Cameroon (63.9%)[50] and 72.9% in Addis Ababa Ethiopia.[51] It is however much higher than results obtained from other studies conducted in Ibadan,(17.4%)[69] Calabar (42%)[56] Abu Dhabi (23%)[45] and 30.1% in Nairobi, Kenya.[47] The differences observed may be attributed to the fact that studies with higher rates were performed in referral hospitals, where people from all over the country are seen. The high number of retreatment cases among the subjects which are more likely to develop resistance may also be a contributing factor.

The rate of MDR-TB found in this study (51.8%) is much higher than 4% and 13% reported in Calabar[56] and Abuja[12] respectively. It is also much higher than the WHO global estimate of 3.7% in new cases and 20% of previously treated cases.[41] It is however lower than 76.4% found in South-Western Nigeria,[53] 73.5% in Bangladesh[46] and 84.4% in South Africa.[27] Reason for this variation may be due to differences in study population and sample size used in the various studies.

The highest resistance to any one drug either alone or in combination with other drugs was found in rifampicin, (67.9%). This compares with 59.8% reported in South western Nigeria[63] but much more higher than 13.2% and 1.9% reported in Uganda,[49][70]. Furthermore, looking at the individual drugs, the 13.6% rifampicin monoresistance reported in this study differs from findings in Calabar where rifampicin mono resistance was not demonstrated at all;[56] but compares to 13.9% recorded in Benue[57] and much lower than 77.2%, 74.4%, and 49.1% reported in Bangladesh,[46] UAE and India,[45][3] and Belarus[40] respectively.

Isoniazid mono resistance was however very low (1.2%) when compared with 14.4% found in Turkey[5] and 12% reported in Kenya.[47] This however is comparable to 2% found in calabar.[56] Furthermore, mono resistance to streptomycin and ethambutol were not found in this study, which is consistent with findings in Cameroon[50] and South-Western Nigeria.[56] The observed low resistance to isoniazid and streptomycin may not be unconnected with the infrequent or controlled use of the drugs.

This study also reported six factors found to be associated with development of MDR-TB. Among these, being a retreatment case was the only statistically significant factor (OR=8.2, P-value <0.01). To address possible confounding, all variables analyzed in the bivariate analysis were included in the multivariate logistic regression model. Two variables, being a retreatment case (OR=9.7) and male sex (OR=2.2) still remained associated with MDR-TB. This is in line with several other studies that identified previous TB treatment to be associated with MDR-TB.[53][62][39][32][40] Previous TB treatment may potentiate drug resistance through improper drug regimens, inadequate or irregular drug supply, unsatisfactory compliance by patients or clinicians, lack of supervision of treatment, and absence of infection control measures in hospitals.[62]

Of the 6 factors identified in the study to be associated with MDR-TB, 4 are patient related which include being a retreatment case, contact with a TB case, interruption of treatment and sex. The other 2 are health care related and include delay in commencement of treatment and encouraging home treatment through non admission of TB patients in the hospital. The study found being admitted at a hospital during TB treatment to be a protective factor against development of MDR-TB (OR=0.48), in that patients admitted at a hospital were more likely to comply with treatment than those treated at home because of close observation by care providers which is an important component of the TB-DOTS strategy. This corroborates earlier findings

indicating that adherence to the TB-DOTS strategy significantly prevent acquired MDR-TB,[28] but differ from findings in China suggesting that without regulated treatment for drug-resistant TB, DOTS itself might not be able to prevent the risk of community-level MDR-TB transmission.[31]

These findings have implications for TB control and public health in general. Rifampicin resistance which has been associated with a previous history of TB represent a first step toward MDR-TB,[71] and is a pointer to weaknesses in the primary TB management services. Usually patients with cough present at the nearest clinic, and if they have TB that is not promptly diagnosed and treated, they will spread the disease. Consequently, if the patient is infected with tuberculosis isolates which are not tested for drug susceptibility from the outset, resistance may be detected too late to permit a cure.[66]

The high rifampicin resistance and MDR-TB therefore seen in this study suggest that non-compliance to primary TB treatment is a major issue that needs to be addressed promptly as it contributed 34.6% of the resistance observed among the participants, and the main reasons that led to their non-compliance was home treatment (69.2%) and not being adequately informed on the need for full compliance to TB treatment (40.7%). This indicates failure in some treatment centres to adhere strictly to the DOTS policy as 69.2% of MDR-TB cases admitted to have been given their drugs to take at home without being observed by a health care provider. This practice could encourage treatment default especially for patients who begin to feel they have recovered when in actual sense they still have the disease.

Furthermore, because rifampicin is the cornerstone of first line TB treatment and since it is considered a marker for MDR-TB, high rate of rifampicin resistance seen in this study has serious implications for TB control as this suggests the likelihood of the

drug becoming useless in the near future as a first-line drug for the treatment of TB. The fact that rifampicin resistance occurred more among retreatment cases, and in combination with other drugs suggests that non compliance could be an issue that needs to be addressed seriously in order to control drug resistant TB.

CHAPTER SIX – CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The pattern of first-line anti-TB drug resistance observed in this study showed a high rate of rifampicin resistance alone or in combination with other drugs. MDR-TB was also very high. Isoniazid mono resistance was however low and mono resistance to ethambutol and streptomycin were not found. The study also found 2 patient related factors (being a retreatment case and male sex) to be associated with development of MDR-TB, while being a new TB case was a statistically significant protective factor. Two health care related factors (delay in commencement of treatment and treatment at home) were found to be associated with MDR-TB but were not statistically significant.

6.2 Recommendations

A high rate of rifampicin resistance - a strategic first-line anti-Tb drug as well as MDR-TB found in this study suggests a serious problem with compliance to first TB treatment. It is also a very serious threat to TB control efforts which needs to be addressed quickly. In the light of this, the following recommendations are made:

i. Health care givers should adequately be trained on standard TB treatment guidelines and should ensure that new TB patients are adequately informed on the duration of treatment and the need for compliance as this will help reduce treatment interruption which may lead to drug resistance. Fixed drug combinations in which patients either take all or none should also be encouraged considering that monotherapy is known to facilitate drug resistance.

ii. The FMoH and SMoH should also strengthen and reinforce compliance to the DOTS policy by ensuring that new TB patients take their drugs at the treatment

centre at least for the first 2 months under supervision of a care provider after which those who cannot be admitted can continue treatment at home.

iii. The FMoH should also ensure the availability of drugs at all times in all treatment centres to avoid the out of stock syndrome which consequently lead to treatment interruption, which may result in development of resistance.

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APPENDIX 1

ETHICAL APPROVAL

FEDERAL MINISTRY OF HEALTH
NATIONAL TUBERCULOSIS AND LEPROSY TRAINING CENTRE
AND REFERRAL HOSPITAL
DEPARTMENT OF PUBLIC HEALTH
Saye Village, Old Zaria - Kaduna Road,
P. M. B. 1089 Zaria, Kaduna State, Nigeria.

OFFICE
Telephone: 08037043838,
08099926347
e-mail: drlabaran@yahoo.com
Web Site: www.ntbltc.org

Your Ref: .....
Our Ref: .....
Date: .....

NHREC Protocol Number NHREC/01/02/2007-21/11/2013
NHREC Approval Number NHREC/01/02/2007-19/12/2013
Date: December 19, 2013

RE: PATTERN OF ANTI-TUBERCULOSIS DRUG RESISTANCE AND ASSOCIATED FACTORS IN PATIENTS ATTENDING NATIONAL TUBERCULOSIS & LEPROSY TRAINING CENTRE & REFERRAL HOSPITAL

NTBLTC Health Research Ethics Committee (NHREC) assigned number: NHREC/02/12/2013
Name of Principal Investigator: Joshua Ayuba Rikoto
Address of Principal Investigator: Nigeria Field Epidemiology & Laboratory Training Program
Date of receipt of valid application: 21 11 2013
Date when final determination of research was made: 19-12-2013

Notice of Expedited Review and Approval

This is to inform you that the research described in the submitted protocol, the consent forms, other participant information materials have been reviewed and given expedited committee approval by the NTBLTC Health Research Ethics Committee.

This approval dates remain as in the initial approval from 19/12/2013 to 19/12/2014. If there is delay in starting the research, please inform the HREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the NHREC assigned number and duration of NHREC approval of the study.* **If this is a multi-year research, endeavour to submit your annual report to the NHREC early in order to obtain renewal of your approval and avoid disruption of your research.**

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the NHREC. No changes are permitted in the research without prior approval by the NHREC except in circumstances outlined in the Code. The NHREC reserves the right to conduct compliance visit to your research site without previous notification.

19/12/13

Dr Kenneth O. ADAGBA *OCC, FASS, MPH*
Chairman, NTBLTC Health Research Ethics Committee

APPENDIX 2

Informed Consent Form

NHREC Protocol No. NHREC/01/02/2007-21/11/2013

NHREC Approval No. NHREC/01/02/2007-19/12/2013 Approved Duration: 19/12/2013 – 19/12/2014

Principal Investigator: Joshua Ayuba Rikoto

Organization: Nigeria Field Epidemiology and Laboratory Training Program (NFELTP) Abuja

Collaborating Institution: Dept. of Community Medicine, ABU Zaria

Title of study: "Pattern of Anti-TB drug resistance and associated factors in patients attending National Tuberculosis and Leprosy Training Centre & Referral Hospital Zaria."

Introduction

This research is conducted by Mr. Joshua Ayuba Rikoto a resident of NFELTP Abuja in partial fulfillment of the requirement for the award of MPH in the Dept. of Community Medicine, ABU Zaria. Please read this form carefully and ask any questions you may have before agreeing to take part in the study.

The purpose of this study is to determine the pattern of anti-Tb drug resistance and also investigate patient and service related factors associated with drug resistant tuberculosis among patients attending N TBLTC and Referral Hospital Zaria. If you agree to participate, you will be required to fill out a survey questionnaire. You may answer the questionnaire yourself, or it can be read to you and you can say out loud the answer you want to be written down.

I do not anticipate any risks participating in this study and there are no direct benefits to you. However, information you provide might help us discover better ways to prevent and treat tuberculosis in your community. The Research records will be kept in a locked file; only the researchers will have access to the records and in any sort of report we make public we will not include any information that will make it possible to identify you.

Taking part in this study is completely voluntary and you may skip any questions that you do not want to answer and that will not affect any services provided to you by the Hospital. You are also free to withdraw at any time without any consequences.

Statement of Consent:

I have read the above information, and have received answers to any questions I asked. I consent to take part in the study.

Name _____ Signature or thump print _____Date _____

Name/ Signature of person obtaining consent _____ Date _____

APPENDIX 3

QUESTIONNAIRE

PATTERN OF ANTI-TUBERCULOSIS DRUG RESISTANCE AND ASSOCIATED FACTORS IN PATIENTS ATTENDING NATIONAL TB AND LEPROSY TRAINING CENTRE AND REFERRAL HOSPITAL ZARIA

SECTION A: DEMOGRAPHIC DATA

QUESTIONNAIRE NO:…

1. Gender: Male ☐ Female ☐
2. Age in years at last birthday……
3. Tribe……
4. Local Govt. of residence: ……..……
5. State: …….
6. Marital status: (tick whichever applies)

   Single ☐ Married ☐ Separated ☐ Divorced ☐ Widowed ☐
7. Occupation:
   a. Employed for wages
   b. Self employed
   c. Retired
   d. Unemployed
   e. Student
8. If employed what do you do? ……
9. What is your personal income level per month?
   a. Less than N20,000 ☐
   b. N20,000 – N50,000 ☐
   c. N51,000 – N100,000 ☐
   d. Above N100,000 ☐
10. Educational level:

    No formal education ☐

    Primary ☐

Secondary ☐

Tertiary ☐

Graduate ☐

Post graduate ☐

SECTION B: Patient related factors

1. Do you smoke cigarettes? Yes ☐ No ☐
2. Do you take alcoholic drinks or any intoxicating substance? Yes ☐ No ☐
3. Have you ever lived with or had close contact with someone who was diagnosed with TB? Yes ☐ No ☐
4. If yes, how long have you lived or had close contact with the person?

   ……..
5. Have you ever been treated for TB before Yes ☐ No ☐
6. If yes, where you admitted at the hospital or treated at home?

   ……
7. If at a hospital what is the name of the hospital?.....
8. Did you take the drugs daily as you were told for the entire treatment duration?

   Yes ☐ No ☐
9. Was there any time that you skipped some days for any reason(s)? Yes ☐ No ☐
10. While you were on treatment, was there any time that you stopped taking the drugs before the treatment duration was completed? Yes ☐ No ☐
11. If yes, why did you stop? (tick as many reasons as are applicable to you)

    i. Lack of money to go to the hospital every day ☐

    ii. No drugs in the hospital ☐

    iii. Got tired of taking the drugs ☐

    iv. Went to a traditional healer ☐

    v. Was ashamed that I had TB ☐

vi. Had no support from my family ☐

vii. Did not believe in the diagnosis ☐

viii. Was having side effects ☐

ix. The drugs were too many ☐

SECTION C: Health care related factors

1. When you were diagnosed with TB, how long did it take before you started treatment? ----------
   ---

2. Anytime you go to the hospital for your TB drugs, how does the staff treat you? ------
   ---

   ---

3. Are you normally attended to promptly? Yes ☐ No ☐

4. If no, for how long do you have to wait before you are attended to? ----------

5. After collecting the drugs were you told for how long you were supposed to take the drugs? Yes ☐ No ☐

6. Were you told why you must take the drugs for such a long time? Yes ☐ No ☐

7. How many types of drugs were you given? ----------

8. What do you think the hospital can do to serve you better?----------
   ---

Thank you for your time.

APPENDIX 4

Protocol for Genexpert MTB/RIF

1. Double click Genexpert Dx
2. Type the user name and password and click OK
3. In the Genexpert Dx system window, click “CREATE TEST”. The scan cartridge barcode dialogue box appears
4. Scan the barcode on the Xpert MTB/RIF cartridge
5. The create test window appears
6. Using the bracode information, the software automatically fills the boxes for the following fields: Select assay, Reagent Lot ID, Cartrideg serial number, and expiration date.
7. In the patient ID box, type patient ID in the sample ID box. The sample ID is associated with the test results and is shown in the “view Results” window and all the reports.
8. Type sample type i.e. sputum in the other sample type window
9. Click” start test”
10. In the dialogue box that appears, type your password
11. Open the instrument module door with the green blinking light and load the cartridge
12. Close the door, the test starts and the green light stops blinking
13. When the test is finished, the lights are turned off
14. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.
15. Dispose off used cartridge in the appropriate specimen waste container according to standard practice.

APPENDIX 5:

Map of Kaduna State showing the study area