EVALUATION OF SENSITIVITY OF GENEXPERTS AND CULTURE IN THE DIAGNOSIS OF*MYCOBACTERIUM TUBERCULOSIS*IN PATIENTS ATTENDINGSELECTED HOSPITALS IN ABUJA, NIGERIA.

BY

CHARITYMAKOLO DANLADI
M.Sc./SCI/9821/2011/2012

DEPARTMENT OF MICROBIOLOGY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA

JULY, 2016

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CHARITYMAKOLODANLADI
M.Sc./SC/9821/2011/2012

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DEPARTMENT OF MICROBIOLOGY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

JULY, 2016

DECLARATION

I declare that the work in this dissertation entitled**Evaluation of Sensitivity of Genexperts and Culture in the Diagnosis of *Mycobacterium tuberculosis* in Patients Attending Selected Hospitals in Abuja, Nigeria**has been performed by me in the Department of Microbiology, Ahmadu Bello University, Zaria under the supervision of Professor H.I. Inabo and Professor O.S. Olonitola.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 this or any other Institution.

Charity Makolo DANLADI_____ _____
Signature Date

CERTIFICATION

This dissertation titled**Evaluation of Sensitivity of Genexperts and Culture in the Diagnosis of *Mycobacterium tuberculosis* in Patients Attending Selected Hospitals in Abuja, Nigeria** meets the regulations governing the award of aMaster of Science in Microbiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. .H.I. Inabo _____ _____
Chairman, Supervisory Committee (Signature) (Date)
Department of Microbiology
Ahmadu Bello University, Zaria

Prof .O. S. Olonitola _____ _____
Member, Supervisory Committee (Signature) (Date)
Department of Microbiology
Ahmadu Bello University, Zaria

Prof. I.O. Abdullahi _____ _____
Head of Department (Signature) (Date)
Department of Microbiology
Ahmadu Bello University, Zaria

Prof. K. Bala _____ _____
Dean School of Postgraduate Studies (Signature) (Date)
Ahmadu Bello University, Zaria

DEDICATION

This work is dedicated to GOD Almighty for sustaining me with His Grace through this programme and my son Master Danladi David.

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I want to thank God Almighty for seeing me through this programme. His grace has been very sufficient.

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ABSTRACT

Tuberculosis is a disease caused by *Mycobacterium tuberculosis*. A cross sectionalstudy to evaluate the Sensitivityof Genexperts and culture in the diagnosis of *M tuberculosis* was conducted duringthe period of February,2014 to February, 2015 usingsputum samples among selected out-patients attending Hospitals in FCT Abuja, Nigeria. A total of 671 sputa were collected and analyzed using microscopy, Genexpertsand culture. The occurrence of the isolates using microscopy was 21.9%, (147/671) whileGenexpertsshowed 21.5% (140/671) and culture shows23.4% (157/671) withsignificant difference at P<0.05). Tubercle bacilli identification with Rapid Standard Diagnostic Bioline test was 78.1% (121/671) and Non tuberculous *Mycobacterium* (NTM) was 21.9% (34/671). There was a high statistically significant difference betweentuberculosis occurrence obtained by microscopy, Genexperts and culture at p<0.05.AlsoStandard diagnostic bioline for *Mycobacteriumtuberculosis* identification showed a statistically significant difference at P<0.05. The sensitivity of microscopy was 72.1%while that of Genexpertswas 68.8%.While the Specificity were 90.3% and 93.0% respectively. Ofall the four first line drugs used for antibacterial susceptibility testingthe culture isolatesshowed a susceptibility of 92.4% and resistanceof 7.6% to Rifampicin as compared to other drugs while Genexpertsshowed Rifampicin resistance of 0.6% and showed a highly statistical significant difference at p<0.05Cigarette smoking and sex was found to be highly associated to tuberculosis as a risk factor but there was no association in other socio demographic factors consideredFrom this work, culture was found to be still better than microscopy and Genexpertsin diagnosing tuberculosis and detecting the rifampicin resistant tuberculosis than genexperts system.

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ABBREVIATIONS USED

AFB = Acid Fast Bacilli

BCG = Bacille Calmette Guerin

DNA = Deoxyribonucleic Acid

TB = Tuberculosis

MAC = *Mycobacterium Avium Complex*

MTBC = *Mycobacterium tuberculosis Complex*

MDR-TB = Multi Drugs Resistance Tuberculosis.

PPD = Purified Protein Derivatives

DST = Drug Susceptibility Test

CDC = Centre for Disease Control

WHO = World Health Organizations

TST = Tuberculin Skin Test

L J M = Lowenstein Jensen Medium

LTBI = Latent Tuberculosis Infection

RACGP = Royal American College of General Practitioners

HIV = Human Immunodeficiency Virus

IGRA = Interferon Gamma Release Assay

PCR = Polymerase Chain Reaction.

FCT = Federal Capital Territory

DHQ = Defence Headquarters

RIF = Rifampicin

B.C = Before Christ

sSNP = synonymous Single-Nucleotide Polymorphisms

NTM = Nontuberculous Mycobacteria

MAI = *Mycobacterium Avium* and *MycobacteriumIntracellulare*

GC = Guanine and Cytosine

PFGE = Pulse Field Gel Electrophoresis

VNTR = Variable Numbers of Tandem Repeats

sSRNA = Synonymous single Ribonucleic Acids

NIAID = National Institute of Allergy and Infectious Disease

TNF = Tumor Necrosis Factor

AIDS = Acquire Immune Deficiency Syndrome

PA = Posterior-Anterior

DOTS = Directly Observed Treatment, Short-course

CPT = Cotrimoxazole

INH = Isoniazid

ARV = Anti-Retro Viral

BMRC = British Medical Research Council

PASAT = Para-Amino SalicylicAcid

NALC/NaOH = N-acetyl L-cysteine–sodium hydroxide

BSC = Biosafety Cabinet

ZN = Ziehl Neelson

NTBLC = National Tuberculosis and Leprosy Control

PCC = Probe Check Control

SPSS = Statistical Package for Social Sciences

DSM =Strptomycin dihydro- Streptomycin

EMB = Ethambutol

PE = Proline Glutamate genes of *Mycobacterium tuberculosis*

PPE = Proline-Proline-glutamate

MAS = Mycolic Acids

PG = Peptidoglycan

AG = Arabinogalactan

GL = Glycolipids

PL = Phospholipids

TDM = Trehalose 6,6- dimycolate

TMM = Trehalose Mono-Mycolate

CHAPTER ONE

1.0 Introduction

1.1 Backgroundof the Study

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis.* The bacterium infects both humans and animals. The bacteria most often affect the lungs, but can also infect any part of the body such as bladder, kidney, spine, and brain. If not treated tuberculosis can be fatal (Ismael and Ray, 2004;CDC, 2012;WHO, 2012, Amir *et al*., 2014).

Approximately one third of the world's population has been infected with tuberculosis, and new infections occur at a rate of one person per second (Barryand Konstantinos,2009; CDC, 2012). Though it is not all infections with *M. tuberculosis* that cause tuberculosis, some infections are asymptomatic and are called latent tuberculosis infection(Haileyesus, 2015). Infection is acquired by inhalation of *M. tuberculosis* in aerosols and dust. Airborne transmission is efficient since infected people cough up enormous number of *Mycobacterium*. In 2007, about 13.7 million new chronic active cases were estimated (WHO, 2009). Also in 2010, 8.8 million new cases and 1.45 million deaths were recorded mostly in the developing countries. This has occurred especially among HIV infected persons resulting in about 350,000deaths and equal to 3,800 deaths a day (WHO, 2011).

Thereare two tuberculosis related conditions that exists: latent infection and active tuberculosis. In latent tuberculosis an individual lives with the bacterium without becoming ill, most people who inhale *Mycobacterium tuberculosis* in aerosols become infected but their bodies are able to fight the bacteria and halt further bacterial

growth thus they are asymptomatic. The latent form of infection can be detected by Tuberculin Skin Test (TST) and are not infectious or cannot spread the *Mycobacterium tuberculosis* to others (CDC, 2012). However, if the *Mycobacterium tuberculosis* becomes active in the body for any reasons and multiplies, the individual now becomes ill leading to tuberculosis (CDC, 2012).

In active tuberculosis the immune system cannot halt their growth resulting in tuberculosis and an individual can transmit the bacteria to people they spend time with every day such as family members, co-workers and health workers (CDC, 2012).

Tuberculosis can be transmitted through the air, from one person to another through coughing, sneezing, speaking or singing and when people nearby inhale these bacteria they become infected, this is known as primary infection which sometimes manifest as pneumonia or pleural effusion (CDC, 2011; RACGP, 2012). People with weakimmune system especially those with HIV infection are at risk of developing tuberculosis than normal subjects (CDC, 2012).

Overall, 5-10% of infected persons who do not receive treatment for Latent Tuberculosis Infection (LTBI) will develop tuberculosis at sometimes in their lives with the greatest risk being two years of infection (RACGP, 2012). People with medical conditions, a weakened immune system such as HIV infection, substance abuse, silicosis, diabetes mellitus, kidney disease, are at high risk.Others include: organ transplant recipients, head and neck cancer patients, those on medical treatment with corticosteroids and specialized treatment for rheumatoid arthritis or Crohn's disease are at high risk of tuberculosis disease also (CDC, 2012).

The symptoms of tuberculosis depend on the part of the body affected, usually they grow in the lung (pulmonary tuberculosis) with symptoms of intense cough that last three weeks or longer, pain in the chest, coughing up blood or sputum , weakness or fatigue, weight loss, loss of appetite, chills, fever and sweating at night (CDC, 2012). In 2007, Australians reported 39% extra pulmonary infections from lymphnodes, pleural space, bone and joints, genitourinary and meninges (RACGP, 2012).

Mycobacterium tuberculosis can be diagnosed by several methods which include; microscopy (Acid fast or Ziehl-Neelsen staining), chest X-ray, Tuberculin skin tests (TST), molecular assays (Genexpert), immune assays (Interferon Gamma Release Assay <IGRA>), Culture and drug susceptibility testing. Pulmonary tuberculosis can be histopathologically identified by observing typical changes (necrotizing granulomas, and acid fast bacilli). Tuberculosis is preventable, treatments are available and cure is possible.

The work of Okuonghae and Aihei, (2008) has identified early case detection, proper case management advocacy, communication and social mobilization as keys to the effective functioning of Direct Observation Treatment (DOT) in Nigeria.

1.2 Statement of the Problem

Tuberculosis is a medical burden affecting one third of the world population especially developing countries. Tuberculosis is also the second most common cause of death from infectious diseases. Bacille Calmette Guerin (BCG) vaccination programmes and improved living conditions have caused a steady decline in tuberculosis cases in industrialized countries from 200/100,000 in 1900to less than 10/100,000 in 1980 (Kalkut,2000). However, re-emergence of tuberculoses has been reported with a higher percentage of drug resistant isolates since 1985 and it is

associated with drug abuse, HIV infection, young patients and origin of patients from developing countries of Africa (South-East Asia, Latin America and the Caribbean's) where tuberculosis cases are high. Over 95% of tuberculosis deaths occur in low and middle income countries(ATS, 1992.;Grange, 1999;CDC, 2000). Also this re-emergence may be due to worsening socio-economic conditions in industrialized world and low public health infrastructure which prevent proper tuberculosis control. In Nigeria, the prevalence of tuberculosis by WHO estimation in 2010 was 320,000 (0.32%) with deaths of 33,000 cases, Indiahave 3,100,000(0.31%) prevalenceand 320,000 (0.32%) deaths cases also China has prevalence of 1,500,000 (0.15%)and 54,000 deaths while Pakistan have 630,000 (0.63%) prevalence and 58,000 deaths.Case detection rate remains as low as 21% even though treatment rate has increase to 60% (Okuonghae and Aihie, 2008).

In 2010, WHO reportedabout 290,000 cases of Multi-Drug Resistance tuberculosis (MDR-TB) and 27 high burdens MDR-TB with at least 4,000 death cases yearly.In the developing countries, tuberculosis remains an immense health and economic problem, causing approximately 8 million new cases and three (3) million deaths annually which make it the leading cause of mortality due to an infectious disease in those countries (Schluger and Rom, 1998; North and Yu-Jin, 2004; Magombedze*et al*.,2006). Also co-infection with HIV further complicates management of tuberculosis and increases the mortality rates (Rastogi and McFadden 1992; Chakraborty, 2004).The incidence of tuberculosis ranges from less than 10 per 100,000 cases in North America to 100 to 300 per 100,000 cases in Asia and western Russia to over 300 per 100,000 cases in Southern and Central Africa. There is one death from tuberculosis every 15seconds (over two million per year), also without treatment, up to 60% of people with the disease will die (Kaye and Frieden,1996).

Essentially, all these cases are in the Third World (WHO, 2002) reflecting the poverty and the lack of healthy living conditions and adequate medical care (Waaler,2002). This global crisis is compounded by the emergence of multidrug resistance in countries like the former Soviet Union, South Africa, and India, where some antibiotics are available but are of inferior quality or are not used for a sufficient time to control the disease according to recommended regimens **(**Iseman, 1994;O'Brien, 2001).

Similar studies were carried out amongprison inmates in Aba, Nigeria and it was discovered that out of the 168 inmates tested, 91 inmates had tuberculosis (Lawrence and Christian, 2010).In Osun State, Nigeria, the prevalence of *Mycobacterium tuberculosis* complex was 25% with Ziehl Neelsen microscopy and 15% by using PCR identification (Ajiboye, 2014). About half a million children (0-14 years) fell ill with tuberculosis and 64,000 died from the tuberculosis (WHO, 2011). While 84,263 new infection and 27,000 death cases was reported in Nigeria annually. This is due to drug abuse, worsening socio-economic conditions and low public health infrastructure which prevent proper tuberculosis control.Thirteen (13) other States including F.C.T form 75% of the total Tuberculosis burden in Nigeria Tobacco use has greatly increased the risk of tuberculosis and death more than 20% of tuberculosis cases worldwide are attributed to smoking (Vyl Smit *et al*., 2010).

Most of the patients presenting in hospitals within the Abuja municipalityundergo treatment/management of tuberculosis and HIV infection thus it become necessary to determine the occurrence of tuberculosis using microscopy, culture and Genexpertsparticularly its co-existence with HIV infection, its susceptibility and

resistance rate within the Federal Capital Territory and ways to combats with the scourge of tuberculosis.

1.3 Justification for the Study

Tuberculosis has a high burden and its distribution is worldwide, WHO declared Tuberculosis a global emergency in 1993 due to increase in morbidity and mortality (Brewer and Heymaan, 2004). India has been classified with other sub-Saharan countries as among those with a high burden oftuberculosis (Charkraborty, 2004). The incidence of tuberculosis has increased steadily due to HIV epidemic which leads to increase risk of tuberculosis in HIV infected population (Panther, 1992).

Another confronting obstacle is drug resistance with the present threat due to emergence of strains resistant to most potent drugs- Isoniazid and Rifampicin (Venkataraman and Paramasivian, 2003).It is therefore imperative to detect drug resistant strains at the earliest stage so that appropriate therapy can be initiated to reduce both morbidity and mortality in infected patients and also prevent the spread of multi-drug resistant strains of *M.tuberculosis* to the community.

This makes drug susceptibility testing and Genexpertsso important in treatment and control of tuberculosis. Conventional drug susceptibility testing though takes longer time (up to 6-8 weeks) before results are obtained leading to loss of time before treatment commence and thus encourage transmission of these strains, Genexpertson the other hand detect the infection early so that treatment and control can commence early. Advances in technology have led to development of newer modalities of susceptibility testing based on automated system and/molecular diagnostic methods though expensive. Hence in low resource poor settings like Nigeria there is an urgent

need for a rapid method of diagnostic testing asperformed in this work using Genexperts system.

1. 4 Aim of the Study

The aim of this study is to compare Genexpertsand culture in the diagnosis of *Mycobacterium tuberculosis*amongst outpatients in Abuja,Nigeria. This is with the view to identifying infected individuals as well as doing sensitivities of the isolates towards effective therapy.

1.5 Specific Objectives of the Study

The specific objectives of the study are:

1. To determine the occurrence,isolate and identify *Mycobacterium tuberculosis*infection from sputum samples of patients attendingsome hospitals in Abuja,
2. To determine the antibacterial susceptibility and Rifampicin (RIF) resistant *Mycobacterium tuberculosis* using automated Genexperts system.
3. To identify socio-demographic factors associated with tuberculosis.
4 To examine the Sensitivities of Genexperts and culture in the diagnosis of the infection, this is with a view to determine current occurrence of the infections among outpatients in some selected hospitals in Abuja, Nigeria.

CHAPTER TWO

2.0 Literature Review

2.1 Tuberculosis

Tuberculosis is a disease caused by *Mycobacterium tuberculosis.* It is also known as Consumption, phthisis, scrofula, Pott's disease, and the White Plague.Also, King's Evils, lupus vulgaris, are other colourful names for tuberculosis (TB) used in the last several centuries.For thousands of years;tuberculosis (TB) has afflicted humans. Definitiveevidence of tuberculosis (TB) has been found in the spines of Egyptian mummies dating from 5000 B.C. and mummy Inca child dated 700BC (Dubos and Dubos, 1952). In 460 B.C, Hippocrates wrote of tuberculosis (phthisis orconsumption), as the most prevalent disease and one that killed almost everyone it infected. Archeological findingsfrom a numbers of these Neolithic sites in Europe, ancientEgypt to the Greek and Roman empires show evidence of adisease consistent with modern tuberculosis (Hershkovitz*et al*., 2008).

It was described by Hippocrates(400 B.C.) and was clearly documentedby Claudius Galen during the Roman Empire. Likewise,tuberculosis has been more recently immortalized by artists such as JohnKeats, Anton Chekhov, Emily Bronte, CharlotteBronte, Franz Kafka, Amedeo Modigliani, and FrederickChopin, all of whom were afflicted by the disease, ***c***elebrities who have suffered and died from tuberculosis during pre-treatment times include musicians (Henry Purcell, Niccolo Paganini, Edvard Grieg), writers (Voltaire, Walter Scott, , Robert Louis Stevenson, George Orwell), and poets (Elizabeth Barrett Browning). Tuberculosis seems to have claimed more than its fair share of novelists, composers, artists and poets (Davies*et al*.,1999;Mathema *et al.,* 2006;Donohue, 2011).It is generally accepted that the microorganism originated from other, more primitive organisms of the same genus

Mycobacterium. Contrary to previous findingsthat tuberculosis is passed from other animals to humans, scientific research has revealed that tuberculosis can be transmitted from humans to other animals instead. Specifically, it has been hypothesized that *M. bovis*, which causes a tuberculosis-like disease in cattle, was the hypothetical evolutionary precursor of *M. tuberculosis* (Stead, 1997). This hypothesis is now considered doubtful in the light of new data, since the genomes in the *M. tuberculosis* complex, including the human and animal pathogens *M. africanum*, *M. microti*, and *M. canetti*, as well as *M. tuberculosis* and *M. bovis*, were characterized by DNA sequencing and related methods. These studies have shown a greater than 99.9% similarity of DNA sequence among the members of the *M. tuberculosis* complex (Brosch *et al.*, 2002), but the existence of rare synonymous single-nucleotide polymorphisms (sSNP) allows discrimination between these closely related bacteria. sSNP analyses suggest that *M. bovis* evolved at the same time as *M. tuberculosis*(Sreevatsan *et al.*,1997), and a study of the distribution of deletions and insertions in the genomes of the *M. tuberculosis* complex provides strong evidence for the independent evolution of both *M. tuberculosis* and *M. bovis* from another precursor species, possibly related to *M. canetti*(Brosch *et al.*, 2002).

Scientific work investigating the evolutionary origins of the *Mycobacterium tuberculosis* complex has concluded that the most recent common ancestor of the complex was a human-specific pathogen, which encountered an evolutionary diversification(Davies, 1998). Although relatively little was known about its frequency before the 19th century,in Europe the epidemic of tuberculosis started in the 17th century and lasted two hundred years, known as the Great White Plague. Being the principal cause of death in 1650,its incidence is thought to have peaked between the end of the 18th and 19th centuryand it was estimated that one-quarter

Europeans died of tuberculosisdue to high population density and poor sanitary conditions that characterized most European and North American cities which created a perfect environment for the propagation (Dubos and Dubos,1952*)*.Later in 19th century, mortality decreased, due to improved sanitation and housing, whichallowed better control of the tuberculosis,presumably public health measures also, played a role in these declining mortality rates **(**Daniel*et al.*,1994).

During this time, tuberculosis was socommon, and so little was knownabout it, death was accepted as inevitable end. In 1720, the English physician BenjaminMarten hypothesized that tuberculosis could be caused by "wonderfully minute livingcreatures." In 1882, these minute creatures were finally seen by Robert Koch, who had discovered a staining technique that identified the assaulting microbe,*Mycobacterium tuberculosis* had at last been identified by Koch in 1882 (Kassim and Ray, 2004). Hemade the landmark discovery that tuberculosis is caused by an infectious agent, *Mycobacterium tuberculosis*or‘Koch's bacillus’. In his findings introduced “the possibilitythat antimicrobial agents could be developed to combatthis age-old scourge’.

Treatment, however remained unchanged and at its best was one of good nutrition, rest, fresh air and social isolation in sanatoriums likethe “little red” cottage as” the first tuberculosis sanatorium inthe United States which was opened in 1884,by Edward Livingston Trudeau whohimself had recovered from tuberculosis.In 1943, Selman Wakesmandiscovered Streptomycin, the firstantibiotic to treat tuberculosis. This wasfollowed by a rapid succession of anti-tuberculosis drugs that were so effective and almostall sanatoriums closed for good in the1960s. Tuberculosis rates dropped steadily untilthe 1980s, at which time they began to increase slightly. This

increase hasbeen attributed to the rise in HIVinfection, immigration and theemergence of multi-drug resistant tuberculosis (*http://www.cdc.gov/nchstp/tb/notes/)*.

Tuberculosis was usually transmitted through direct contact or discharged fluids(fomes) from infected patients. He noted that phthisis could be contracted without either direct contact but was unsure of the process by which the disease propagated across distance (Francis, 1958; Keers,1977;Ferlinz, 1995).

Robert (1768) gave the first clinical description of tuberculosis meningitis. While Percivall(1779) an English surgeon, described the vertebral lesions that carry his name(Dobson, 1979).William Stark proposed that ordinary lung tubercles could eventually evolve into ulcers and cavities, that the different forms of tuberculosis were simply different manifestations of the same disease. Today, despite the availability ofeffective anti-tuberculosis chemotherapy for over 50 years, tuberculosis remains a major public health threat.

2.2 Classification and General Features of *Mycobacterium tuberculosis*

Kingdom: Bacteria

Phylum: Actinobacteria

Class: Actinobacteria

Order: Actinomycetales

Sub-Order: Corynebacterineae

Family: Mycobacteriaceae

Genus: *Mycobacterium*a genus of gram-positive,acid-fastbacteria(familyMycobacteriaceae),including*M. avium-intracellulare,* a complexthatcausesopportunisticinfections in patientswithHIVinfection,*M. balnei(M.marinum),*thecause of swimming pool granuloma; *M. bovis,*thecause of

cattletuberculosis, transmitted to humansthroughmilk;*M. kansasii*,thecause of a tuberculosis-likedisease;*M. leprae*,thecause of leprosy; and*M. tuberculosis*(thetuberclebacillus),thecause of tuberculosis, usually of thelungs.(SaunderDorland's Medical Dictionary,2007).

Species*: M. tuberculosis* Complex and *Nontuberculosismycobacteria*Mycobacteria belong to the single Genus *Mycobacterium* and Family Mycobacteriaceae and in the Order Actinomycetales (Stackebrandt *et al.,* 1997). They are known to syntheses mycolic acids. There are over ninety species which can be phenotypically differentiated (Rastogi *et al.*, 2001). Actinomycetales include diverse micro-organisms, but Mycobateria and related organisms allied taxa are easily distinguished on the basis of their ability to synthesize mycolic acids. *Mycobacterium* species can be differentiated based on their phenotypic characteristics, biochemical tests, cultural properties even though with the limitation of not precisely identifying *Mycobacterium* species, more and accurate ways of classifying the species are now in use which includes Molecular and phylogeny. The over 90 species can be classified into the following three principal groups: strict pathogens, including the human pathogens *M. tuberculosis, M. leprae*, and the animal pathogens *M. bovis*, Opportunistic pathogens which include *M.simiae, M.avium, M.xenopi*.Rare pathogens including saprophytes such as *M.smegmatis and M.phlei*.The genus *Mycobacterium* contains a number of strict and opportunistic pathogens that afflict humans and animals. The strict pathogens of human are*Mycobacterium tuberculosis*which causes tuberculosis and *Mycobacterium leprae* which cause leprosy in humans and have ability to grow inside phagosomes and phagolysosomes of infected host macrophages. Other opportunistic pathogens comprise of a variety of *Mycobacterium* species examples *Mycobacterium avium, Mycobacterium simae,Mycobacteriun kansasii,Mycobacterium haemophillum*

which is common among immunocompromised patients. Also *Mycobacteruim ulcerans* which cause skin chronic ulcers white necrotic centres (Buruli ulcer) and others. The *Mycobacterium* cell is enveloped, has a tripartite structure containing a high proportion of lipids which is approximately about forty percent (40%) of the total weight, this gives the Mycobacteria adaptation to the intracellular growth and survival, immune modulation and drug resistance(Rastogi *et al*., 2001).

2.2.1 Species of *Mycobacterium*

The Runyon classification of Nontuberculous Mycobacteria was based on the rate of growth, production of yellow pigment and whether this pigment was produced in the dark or only after exposure to light (Rogall*et al.*,1990). It was introduced by Ernest Runyon (Runyon, 1959).On these bases, the nontuberculous mycobacteria are divided into four groups:

A. Slowly growing Mycobacteria

The first three groups are classified as "Slowly growing Mycobacteria".

I. PhotochromogensMycobacteria (photochromogens) are slow growing, and produce a yellow-orange pigment when exposed to light. The group includes *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium asiaticum*, and *Mycobacterium simiae*. *Mycobacterium szulgai*is a photochromogen when grown at 24 degrees, and a scotochromogen at 37 degrees.

II. Scotochromogens Mycobacteria" (scotochromogens) is slow-growing and produces a yellow-orange pigment regardless of whether they are grown in the dark or the light. The group includes *Mycobacterium gordonae*and *Mycobacterium scrofulaceum*, among others. *Mycobacterium szulgai* is a scotochromogen when grown at 37 degrees.

III. Nonchromogens Mycobacteria (nonchromogens) are slow-growing and never produce pigment, regardless of culture conditions. The group includes *Mycobacterium avium* and *Mycobacterium intracellulare* (together known as the MAI complex),*Mycobacterium ulcerans*and numerous other organisms.

IV. Rapid GrowersMycobacteria: grow rapidly,(colonies in 5 days). They do not produce pigment. *Mycobacterium fortuitum, Mycobacterium peregrinum, Mycobacterium abscessus, Mycobacterium chelonae, Mycobacterium thermoresistibile*.Some rapidly growing mycobacteria are considered "late-pigmenting" (Brown-Elliott and Wallace, 2002).*M. chelonae* is atypical fast-growing mycobacteria that are a rare cause of human infection.

Noted clinically important ofnontuberculous mycobacteria (NTMB) including *M.kansasii, M.genavense, M.marinum, M.simiae, M.scrofulaceum, M.szulgai, M.avium, M.haemophilum, M.intracellulare, M.malmoense, M. ulcerans, M.xenopi, M. abscessus, M.chelonae, M. fortuitum,* and (rarely) *M. smegmatis*(Horsburgh,1996).

V Mycolactone-producing Mycobacteria example: *M.ulcerans*-causes Bairmsdale ulcer,*M.pseudoshottsii, M. shottsii.*

Mycobacteriumsimiae, group-*M.triplex, M.genaverse, M.florentinum, M.lentiflarium* and *M.parascroflaaceum.*Four clinical syndromes comprise nearly all cases: pulmonary disease, lymphadenitis, skin or soft tissue disease, and disseminated disease in AIDS. *M.avium* and *M.intracellulare* (known together as *M.avium-intracellulare* complex) are the most common causes of pulmonary disease, lymphadenitis, and disseminated disease. All four clinical syndromes seem to be increasing in frequency, particularly in immune suppressed hosts. Specific reservoirs of these organisms leading to human disease are still being found (Runyon, 1959).

Nontubercuosis mycubacteria (NTMB) are acquired from the environment, but *M abscessus* is a rapidly growing *Mycobacterium* found in soil and water throughout the world. Disease in patients who are immunocompetent usually consists of localized skin and soft tissue infections.*M.kansasii* occur most commonly Kansas, Texas, Illinois, England, and urban settings.

M. tuberculosis* groupMTCM. tuberculosis,M. bovis, M. africanum,M. microti,M. canetti,M. caprae, M. pinnipedii*MPM (Mycolactone producing mycobacteria)

R1P(Photochromogens Mycobacteria) *M. marinum*

R2S ScotochromogensMycobacteria),*M. pseudoshottsii*

R3N(Nonchromogens Mycobacteria) *M. ulceran*sin,*M. shottsii,M. liflandii*, other *M. lacus,M. kumamotonense*

Leprosy,*M. leprae,M. lepraemurium,M. lepromatosis.*

MAC (*Mycobacterium avium*complex)

R3N (Nonchromogens Mycobacteria) *M. intracellulare*/*M. avium,M. avium subspecies paratuberculosis,M. chimaera*

R2S (Scotochromogens Mycobacteria) *M. bohemicum.*

***GK kansasi* group:** R1P (Photochromogens Mycobacteria) *M. kansasii*

R3N (Nonchromogens Mycobacteria)*M. gastri, M. seoulense*

R2S Scotochromogens Mycobacteria (*M. nebraskense,*

***M. haemophilum* group:** *M. haemophilum,* R2S(Scotochromogens Mycobacteria) *M. szulgai.*

R3N (Nonchromogens Mycobacteria)*M. malmoense*

***M. gordonae* group** : RIP (Photochromogens Mycobacteria) *M asiaticum*

R2S (Scotochromogens Mycobacteria) *M. gordonae*

***M. conspicuum* group**:R2S (Scotochromogens Mycobatceria) *M. conspicuum*

M. xenopi group:*M. shimoidei/, M. xenopi, M. heckeshornense, M. hassiacum*

***M. celatum* group**R2S(Scotochromogens Mycobacteria) *M. cookii*

R3N(Nonchromogens Mycobacteria)*M. branderi, M. celatum*

M. hiberniae* group:M. terrae, M. hiberniaeM. nonchromogenicum/M. arupense*

***M. simiae* clade**: R3N (Nonchromogens Mycobacteria*M. genavense/M. triplex,M. florentinum/M. montefiorense,M. heidelbergense/M.parmense,M. simiae*

R2S (Scotochromogens Mycobacteria),*M. lentiflavum,M. kubicae* group

R3N (Nonchromogens Mycobacteria)*M. parascrofulaceum*

R2S (Scotochromogens Mycobacteria) *M. palustre/M. kubicae*

M. interjectum group (*M. interjectum,M. saskatchewanense.*

M. intermedium group**:***M. intermedium*

Ungrouped:*M. triviale, M. doricum, M. tusciae, M. arosiense*

Rapidly growing/Runyon IV

M. neoaurum group

M. mageritense,M. wolinskyi, M. canariasense, M. cosmeticum, M. diernhoferi, M. hodleri, M. frederiksbergense,M. neoaurum, M. brisbanense, M. fluoroanthenivorans

***M. fortuitum* group** :*M. chitae/M. fallax/M. gadium, M. rhodesiae, M. houstonense, M. neworleansense/M. boenickei/M. fortuitum/M. porcinum/M. senegalense, M. septicum/M. peregrinum/M. alvei, M. farcinogenes*

***Fortuitum/T* groups**

M. vaccae group: *M. obuense/M. gilvum/M. parafortuitum, M. chlorophenolicum/M. chubuense, M. psychrotolerans/M. sphagni, M. aubagnense/M. mucogenicum/M. phocaicum,*

Aurum /Vanbaalenii: M. aurum, M. vanbaalenii, M. vaccae, M. austroafricanum, M. pyrenivorans,M. poriferae

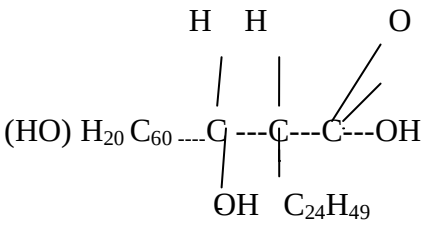
M. smegmatis* group:M. agri*/*M. thermoresistibile*, *M. duvalii*/*M. flavescens*, *Mmonacense, M. pulveris*/*M. conceptionense*/*M. moriokaense, M. novocastrense*/*M. brumae*/*M. phlei, M. confluentis*/*M. madagascarienseM. smegmatis*/*M. goodii*

***M. chelonae* group.** *M. komossense*, *M. murale*/*M. tokaiense*, *M. aichiense*, *M. chelonae*, *M. abscessus,M. immunogenum, M. massiliense*, *M. bolletii*

M. elephantis* groupM. elephantis, M. holsaticum*(Runyon, 1959).

2.2.2 Morphology of *Mycobacterium tuberculosis*

Mycobacteria are aerobic, acid –alcohol fast, rod-shaped and approximately 1-10 m long, slender bacilli which may be curved or bent. These may be granular, isolated, in pairs or in groups. Stained bacilli may present a beaded appearance, actinomycetes with occasional branching which lack aerial hyphae, are non –motile, non- sporulating organisms that contain arabinose, galactose and meso-diaminopimelic in their cell wall. The Guanine and Cytosine (GC) deoxyribonucleic acid (DNA) base ratio are in the range of 62 % to 70%. Have mycolic acids of high molecular weight about 60 – 90 carbons β – hydroxyl-fatty acids with a long α – side chain with more than two point of unsaturation in the molecule, (Goodfellow and Wayen,1982). Capsule – like structure and so were no longer un- encapsulated organisms (Rastogi, 1993). Though always considered as obligate aerobes initially, some species are presently micro aerophilic whose growth is enhanced by 10% $Co_2$and they grow as a narrow band under the surface of a semi-solid medium **(**Goodfellow and Wayne,1982). *Mycobacterium*is defined by the length of carbon backbone, the number ofunsaturated links, the presence of supplementary oxygenated functions and the esters produced on pyrolysist.



Mycolic Fatty acid
mycoside

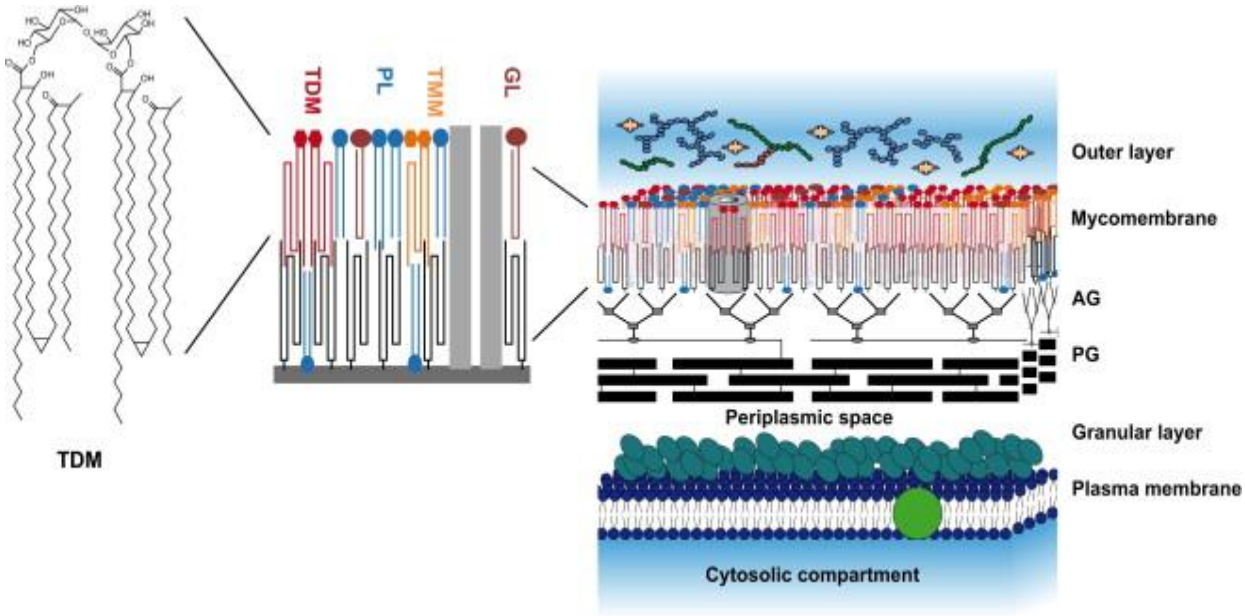


Figure 1

AMycobacterial Cell Wall

The outer layer is mainly composed of glucan and proteins, with only a tiny amount of lipid. The mycomembrane corresponds to the permeability barrier. Its inner leaflet is formed by a parallel arrangement of Mycolic acid (MA) chains (in black) linked to arabinogalactan (AG) that in turn is covalently attached to peptidoglycan (PG); the inner leaflet of the mycomembrane is presumably composed of free lipids that include Trehalose 6,6-dimycolate (TDM in red), TMM (in orange), various glycolipids (GL, in brown), and phospholipid (PL, in blue)Zuber *et al*. (2008). A representation of TDM shows the very-long chain of Mycolic Acids that have to pack after folding at the site of the motifs (here mycolic unit, cyclopropane, and keto group) to fit in a conventional membrane of 7–8 nm in thickness. The granular layer above the plasma membrane is composed of proteins; these proteins may precipitate upon treatments of

bacteria before the observation by transmission electron microscopy and yield a thicker appearance to the outer leaflet of the plasma membrane (Zuber *et al*., 2008).

2.2.3Virulence factors in Mycobacterium tuberculosis

Virulence is the ability of a microbe to cause disease in a host (Pathogenicity).Virulence is caused by several factors that are encoded by the bacteria. The composition and amounts of mycolic acids have been shown to affect the virulence, growth rate, colony morphology and permeability of M. tuberculosis (Dubnau *et al.*, 2000; Glickman *et al*., 2000; Liu *et al*., 1996; Yuan *et al*., 1998).Theydo this indifferent ways; first, the virulence factors can induce cell adhesion to the host cell. Second, they can increase the colonization of the host body and the persistence. Third, they cause invasion into host cells. Fourth, thebacteriacan express inhibitors of the immune response. Fifth, some bacteria can expresstoxins.ThoughIt has often been noted that there are no classical bacterial virulence factors for *M.tuberculosis*.

Models for Measuring Virulence

Nevertheless, TB virulence can be quantified, with the help of two models: the animal model and the cellular model. The animal model utilises mice, guinea pigs, and non-human primates. Typically, the animals are infected either via the aerosol route, or intranasal, like the natural infection. The animals usually receive 100 CFU ((colony forming unit, or number of living bacteria) at day zero. Then, typically, following a month, there is a three (3) log increase of the CFU. Then, the number of bacteria remains constant for two or three months..(Pricile, 2002)

The Mouse Model

There are typically two target organs in the mouse model- the lungs and the spleen.

The CFU, lesions areexamined to determine the severity of the strain. The parameters associated with virulence in the mouse model are colonization, persistence, and pathogenicity. It alsodepends on the route of entry and infecting dose, morbidity and mortality which can be examine.

The Cellular Model

The second model used to quantify virulence is the cellular model. Mycobacteria can infect the macrophages and dendritic cells, the pneumocytes and adipocytescells.The first feature examine is invasion, survival, and replication. Thus to quantify the bacterial burden, and obtain a precise measureof the invasion parameters,survival and multiplication inside the cells. This method can be applied to several hundreds of strains.A second feature examine inside the cells is trafficking. The bacterium has the ability toblock phagosome maturation. One of the critical features of Mycobacteria is that they residein endosomes that cannot be acidified into lysosomes. Though thefactorsresponsible for these phenomena is unknown,it helps the bacteria survive andmultiply within the cells.Examining cellular models also allows thestudy of cytokines release and profiles example, the release of TNFα, IL-2, IL-12, and IFNγ.

The biophysical properties of the bacterium are suspected to play a key role in its persistence. All the mycobacteria, including *M tuberculosis*, have a dense outer envelope consisting of large fatty acids whose structure confers considerable

Impermeability to antibiotic agents as well as, for certain pathogens, biochemical means of manipulating host immune responses (.Barry, *et.al* 1998; Brennan and Nikaido,1995)

The glycolipid trehalose 6, 6-dimycolate (TDM; cord factor) occurs in the outer envelope of all Mycobacteria and is the most abundant extractable lipid at the surface of virulent *M. tuberculosis* (Minnikin, 1982) the impact of TDM on organismshas

been studied widely. TDM is known, for example, to be capable of inducing granulomas in animals; these granulomas are similar to those that are characteristic of tuberculosis infection (Hunter, *et, al.* 2006). TDM also is capable of triggering increased chemokine and cytokine production (Ryll *et,al.,*2001), inhibiting trafficking of phagocytosed bacteria to acidic compartments in macrophages (Indrigo, *et.al,*2003), and influencing the morphology of mycobacterial colonies (Glickman *et, al*2000;Hunte,*et,al.* 2006). In liposomes, TDMinhibits vesicle fusion, potentially explaining its role in mediatingthe above-mentioned phagosome-lysosome fusion invivo (Spargo *et, al* 1991).These virulence factors are separated into four groups;

Secreted Factors

The first category is secreted factors, which are products that are exported outside the bacteria. They include culture filtrate proteins, such as HspX, Esat6/CFP-10, and 19-kD.These proteins have been studied thoroughly, due to their potential for vaccine and diagnostic applications. More recently, the protein Kinase G was shown to be involved in phagosome maturation arrest.

Cell Surface Components

The cell surface components include proteins involved in synthesis of the cell wall. Among them is the LAM, which has multiple functions. First, it is animmune modulator. In addition, LAM hasbeen able to inhibit the release ofcalcium.

Enzymes Involved in Metabolism

The third category includes enzymes involved in general or cellular metabolism. Some ofthese enzymes are involved in lipid and fatty acid metabolism, which allows

bacteria to growon fatty acids. It was also discovered that when introducing knock-out mutants to the aminoacid and purine biosynthesis pathway, the Mycobacteria has a different phenotype in virulence.A similar phenomenon was observed when disrupting the genes involved in metal uptake, aswell as factors involved in anaerobic respiration and oxidative stress proteins.

Transcriptional Regulators

The final category of virulence factors includes the transcriptional regulators. Following thesystematic knock-out of all the sigma factors, four have been found to account for change invirulence in the mouse model. The PhoP/PhoR response regulatory is also an importantvirulence factor, and a knock-out mutant of PhoP/PhoR is considered a promising vaccinecandidate(Priscille, 2002)

2.2.4Strain variation

Mycobacterium tuberculosis is genetically diverse, which results in significant phenotypic differences between clinical isolates. Different strains of *M. tuberculosis* are associated with different geographic regions. However, phenotypic studies suggest the strain variation which never has implications for the development of new diagnostics and vaccines. Micro evolutionary variation does affect the relative fitness and transmission dynamics of antibiotic-resistant strains (Gagneux, 2009).

Typing of strains is useful in the investigation of tuberculosis outbreaks, because it gives the investigator evidence for-or-against transmission from person to person. Until the early 2000s, *M. tuberculosis* strains were typed by pulsed field gel electrophoresis (PFGE) (Zhang *et al.*,1992). This has now been superseded by variable numbers of tandem repeats (VNTR), which is technically easier to perform

and allows better discrimination between strains. This method makes use of the presence of repeated DNA sequences within the *M. tuberculosis* genome.

Three generations of VNTR typing for *M. tuberculosis* are noted. The first scheme, called exact tandem repeat, used only five loci (Frothingham *et al*., 1998). But the resolution afforded by these five loci was not as good as PFGE. The second scheme, called Mycobacterial interspersed repetitive unit, had discrimination as good as PFGE (Mazars *et al*.,2001; Hawkey *et al*., 2003). The third generation (Mycobacterial interspersed repetitive unit - 2) added a further nine loci to bring the total to 24. This provides a degree of resolution greater than PFGE and is currently the standard for typing *M. tuberculosis*(Supply *et al.*,2006).

2.2.5 Hyper virulent strain

Tuberculosisoutbreaks are often caused by hyper virulent strains of *M. tuberculosis*. In laboratory experiments, these clinical isolates elicit unusual immunopathology, and may be either hyper inflammatory or hypo inflammatory. The majority of hyper virulent mutants have deletions in their cell wall-modifying enzymes or regulators that respond to environmental stimuli. The mechanisms that enable *M. tuberculosis* to mask its full pathogenic potential, inducing a granuloma that provides a protective niche, enable the bacilli to sustain a long-term, persistent infection (Casali, 2009).

2.2.6Genome *of M. tuberculosis*

The genome of the H37Rv strain was published in 1998 (Cole *et al.*, 1998; Camus *et al*., 2012). Its size is 4 million base pairs, with 3959 genes; 40% of these genes have had their function characterized, with possible function postulated for another 44%. Within the genome are also six pseudogenes.The genome contains 250 genes involved

in fatty acid metabolism, with 39 of these involved in the polyketide metabolism generating the waxy coat. Such large numbers of conserved genes show the evolutionary importance of the waxy coat to pathogen survival. Furthermore, experimental studies have since validated the importance of a lipid metabolism for *M. tuberculosis*, consisting entirely of host-derived lipids such as fats and cholesterol. Bacteria isolated from the lungs of infected mice were shown to preferentially use fatty acids over carbohydrate substrates (Bloch and Segal,1956).*M. tuberculosis* can also grow on lipid cholesterol as a sole source of carbon, and genes involved in the cholesterol use pathway(s) have been validated as important during various stages of the infection lifecycle of *M. tuberculosis*, especially during the chronic phase of infection when other nutrients are likely not available (Wipperman *et al.*, 2013). About 10% of the coding capacity is taken up by the *PE*/*PPE* gene families that encode acidic, glycine-rich proteins. These proteins have a conserved N-terminal motif, deletion of which impairs growth in macrophages and granulomas (Glickman and Jacobs,2001). Nine noncoding sRNA have been characterized in *M. tuberculosis* (Amvig and Young, 2008) with a further 56 predicted in a bioinformatics screen (Living *et al.*,2006).

In 2013, a study on the genome of several sensitive, ultra resistant, and multi resistant *M. tuberculosis* strains was made on antibiotic resistance mechanisms. Results reveal new relationships and drug resistance genes not previously associated and suggest some genes and intergenic regions associated with drug resistance may be in the development of this involved in the resistance to more than one drug (Gagneux*et al.*,2006;Matthias,2015).

2.2.7 Mode of transmission

Tuberculosis is primarily an airborne disease, transmission of tuberculosis usually occurs only after prolonged exposure to someone with active tuberculosis. On average, people have a 50 percent chance of becoming infected with tuberculosis if they spend eight hours a day for six months or 24 hours a day for two months working or living with someone with active tuberculosis, researchers have estimated that people are most likely to be contagious when their sputum contains bacilli, also when they cough frequently and when the extent of their lung disease, as revealed by a chest X-ray, is great. Theyexpelled bacilli from the lungs when a tuberculosis patient coughs, sneezes, speak, sings, or laughs. Only people with active disease are contagious. Droplet nuclei are tiny and may remain in the air for prolonged periods, ready to be inhaled. They are small enough to bypass the natural defenses of upper respiratory passages, such as hairs in the nose or the hair like cilia in the bronchial tubes. Infection begins when the bacilli reach the tiny air sacs of the lungs known as alveoli, where they multiply within macrophages. People who have been treated with appropriate drugs for at least two weeks usually are not infectious. The disease is not likely to be transmitted through personal items belonging to those with tuberculosis, such as clothing, bedding, or other items they have touched. Adequate ventilation is the most important measure to prevent the transmission of tuberculosis, because most infected people expel relatively few bacilli(NIAID, 2009).

2.2.8 *Mycobacterium tuberculosis*infection

The site of initial infection is usually the alveoli, the balloon like sacs at the ends of the small air passages in the lungs known as bronchioles. In the alveoli, white blood cells called macrophages ingest the inhaled *M. tuberculosis* bacilli. Some of the bacilli may be killed immediately; others may multiply within the macrophages.

Infrequently, but especially in HIV-infected people and in children, the bacilli spread to other sites in the body. This dissemination sometimes results in life-threatening meningitis and other problems.

During the two to eight weeks after initial infection in people with intact immune systems, macrophages present pieces of the bacilli, displayed on their cell surfaces, to another type of white blood cell, the T cell. When stimulated, T cells release an elaborate array of chemical signals. Once this response, called cell-mediated reaction is established, a person's T cells usually will respond to the tuberculin skin test (PPD test) and produce a characteristic red welt. Some of the T-cell signals produce inflammatory reactions; other recruit and activate specialized cells to kill the bacilli and wall-off infected macrophages in tiny, hard grayish nodules known as tubercles. From then on the body's immune system maintains a standoff with the infection, sometimes for years. In the tubercles, tubercle bacilli may persist within macrophages, but further multiplication and spread of *M. tuberculosis* are confined. Most people undergo complete healing of their initial infection, and the tubercles calcify and lose their viability. A positive tubercle skin test, and in some cases a chest x-ray, may provide the only evidence of the infection. If however, the body's resistance is low because of aging, infections such as HIV, malnutrition, or other factors, the bacilli may break out of the tubercles in the alveoli and cause active disease. (*http://tuberculosis.emedtv.com/active-tuberculosis/infection/html*).

2.2.9 People at risk for tuberculosis

These include, persons infected with HIV, children younger than 5 years of age,persons who were recently infected with *M. tuberculosis* (within the past 2 years),persons with a history of untreated or inadequately treated tuberculosis,

including persons with fibrotic changes on chest radiograph consistent with prior tuberculosis,persons who are receiving immunosuppressive therapy such as tumor necrosis factor-alpha (TNF) antagonists, systemic corticosteroids equivalent to/greater than 15 mg of prednisone per day, or immunosuppressive drug therapy following organ transplantation; persons with silicosis, diabetes mellitus, chronic renal failure, leukemia, or cancer of the head, neck, or lung; persons who have had a gastrectomy or jejunoileal bypass; persons who weigh less than 90% of their ideal body weight; cigarette smokers and persons who abuse drugs and/or alcohol; and populations defined locally as having an increased incidence of disease due to *M. tuberculosis*, including medically underserved, low-income populations (Thomas, 1996; Chris ,2012).

2. 2. 10 Clinical manifestations

Latent: People with latent tuberculosis do not have symptoms, are not contagious, and cannot spread the disease to others. However, anything that stresses the immune system, such as the development of a chronic disease or HIV/AIDS, can allow the bacteria to become active and begin to multiply in the body, which now becomeactive tuberculosis.(WHO, 2011)

Active:Symptoms of active tuberculosis may be mild and include an ongoing cough for three or more weeks, fever, weight loss, night sweats, poor appetite, and fatigue. A person with tuberculosis can also cough up blood (hemoptysis) and chest pain.

The list of signs and symptoms mentioned in various sources for tuberculosis includes;

Early infection symptoms: Fever, Chills ,Sweating, Night sweats,Flu-like symptoms

Gastrointestinal symptoms: weight loss,No appetite,weakness,Fatigue.

Symptoms of chronic lung infection (pulmonary tuberculosis): Persistent cough,Chest pain,Coughing up bloody sputum,Shortness of breath,Breathing difficulty,Recurring of fever,Weight loss,Progressive shortness of breath,Urine, discoloration, Cloudy urine,Reddish urine(Scott Kahan *et al.*,2004).

Complications of tuberculosis can occur, especially if the infection spreads throughout thelungs and to other parts of the body. Complications include disseminated tuberculosis, tuberculous meningitis, and kidney disease. If untreated or incompletely treated, tuberculosis can be fatal(Scott Kahan *et al.*, 2004).

2.3 Active Tuberculosis

On the average, people infected with *M. tuberculosis* have a 10 percent chance of developing active tuberculosis at some time in their lives. The risk of developing active disease is greatest in the first year after infection, but active disease often does not occur until many years later. Active tuberculosis usually results from the spread of bacilli from the alveoli through the bloodstream or lymphatic system to other sites, usually elsewhere in the lungs or local lymph nodes. In 15 percent of cases, the bacilli causing disease in other regions, such as the skin, kidneys, bones, or reproductive and urinary systems. At the new sites, the body's immune defenses kill many bacilli, but immune cells and local tissue die as well. The dead cells and tissue, along with live immune cells, which form granulomas whose centers, have the consistency of soft cheese, where the bacilli survive but do not flourish. As more lung tissue is destroyed and the granulomas expand, cavities in the lungs develop, and sometimes break into larger airways called bronchi. This allows large numbers of bacilli to spread when patients cough. As the disease progresses, the granulomas may liquefy, perhaps as a result of enzymes secreted by the body’s own immune cells. This creates a rich

medium in which the bacilli multiply rapidly and spread (http://tuberculosis.emedtv.com; Chris,2012)

2.3.1 Sites of Tuberculosis

I. Pulmonary tuberculosis (TB):

This is a contagious bacterial infection that involves the lungs, but may spread to other organs, caused by the bacteria *Mycobacterium tuberculosis* (Fitzgerald, 2009; Ellner, 2015).

II. II. Extra pulmonary Tuberculosis

This infection occurs in sites other than the lungs, including; the larynx, the lymph nodes, the pleura, the brain, the kidneys, or the bones and joints. In HIV-infected persons, it is often accompanied by pulmonary case. Usually are notinfectious unless they have pulmonary disease in addition. Those located in the oral cavity or the larynx; an open abscess or lesion in which the concentration of organisms is high, especially if drainage from the abscess or lesion is extensive or fluid is aerosolized. Persons with tuberculosis pleural effusions may have underlying pulmonary tuberculosis that is masked on chest radiograph because the effusion fluid compresses the lung. These patients should be considered infectious until pulmonary tuberculosis is excluded. The incidence of extra pulmonary forms of tuberculosis varies from country to another, such that between 1964 and 1989, 5-10% of the approximately seven million new cases each year in the developing countries were extra pulmonary. This distribution also can be affected by origin of the individuals within a country (Talavera *et al.*, 2001). Also Australian reported 39% cases of extra pulmonary infections in 2007 (Barry and Konstantinos, 2009).

Miliary Tuberculosis

Miliary tuberculosis occurs when tubercle bacilli enter the bloodstream and disseminated to all parts of the body, where they grow and cause diseases in multiple sites. This condition is rare but serious. “Miliary” refers to the radiograph appearance of millet seeds scattered throughout the lung. It is most common in infants and children younger than 5 years of age, and in severely immunocompromised persons. Miliary tuberculosis may be detected in an individual organ, including the brain; in several organs; or throughout the whole body. The condition is characterized by a large amount of tubercle bacilli, although it may easily be missed, and is fatal if untreated. Up to 25% of patients with miliary tuberculosis may have meningeal involvement (CDC, 2000).

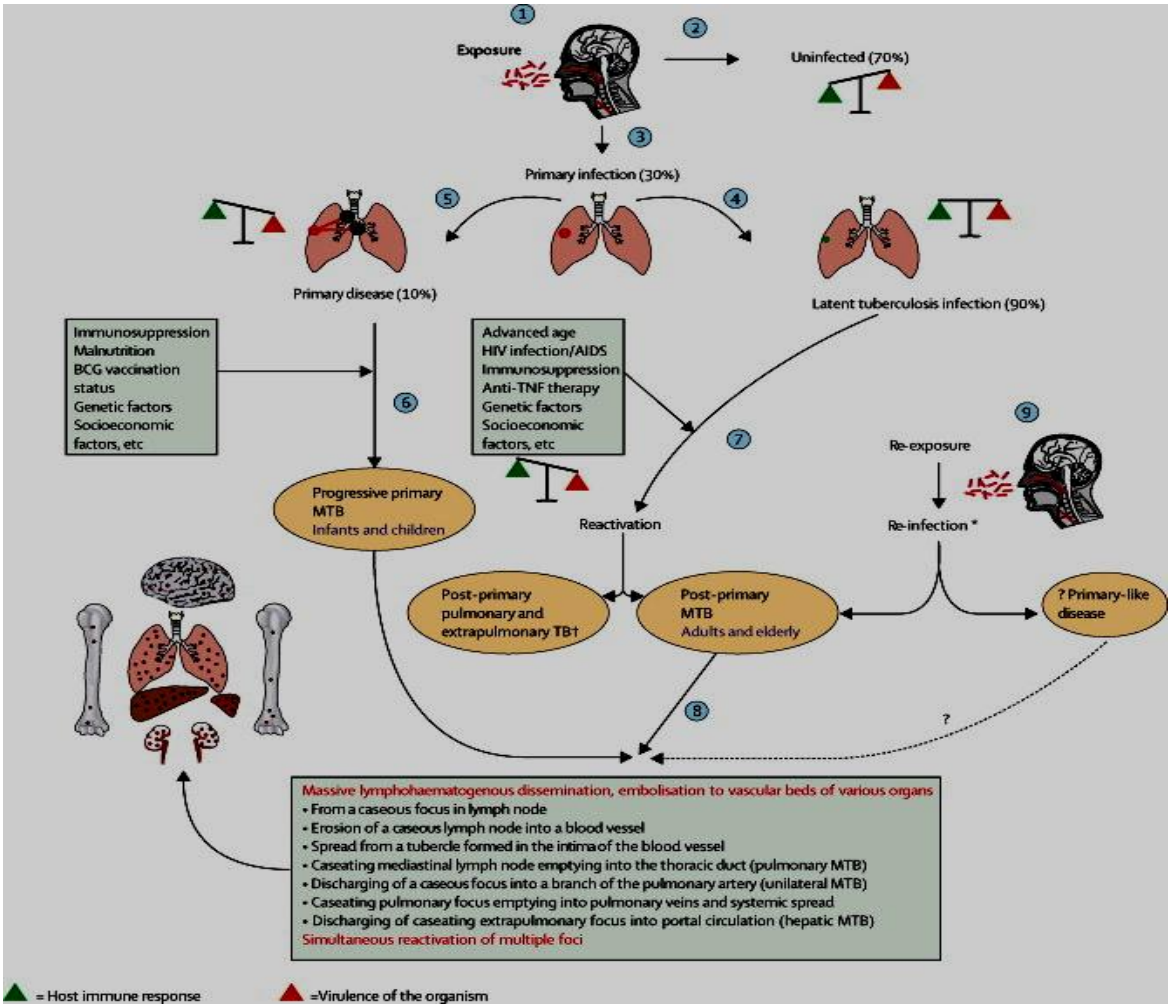


Figure 2 Life Cycle of *M. tuberculosis*

III. Central Nervous System

When tuberculosis occurs in the tissue surrounding the brain or spinal cord, it is called tuberculous meningitis. Tuberculous meningitis is often seen at the base of the brain on imaging studies. Symptoms include headache, decreased level of consciousness, and neck stiffness. The duration of illness before diagnosis is variable and relates in part to the presence or absence of other sites of involvement. In many cases, patients with meningitis have abnormalities on–a chest radiograph consistent with old or current tuberculosis, often have miliary tuberculosis (CDC, 2000).

2.3.2 Risk of latent tuberculosis infection progressing to tuberculosis (LTBI)

Anyone who has LTBI can develop tuberculosis, but some people are at higher risk than others. HIV infection is the greatest risk factor for the development of tuberculosis in persons with LTBI, due to a weakened immune system. The risk of developing the disease is 7% to 10% each yearfor persons who are infected with both *M. tuberculosis* and HIV and who are not receiving highly active treatment for HIV; it is 10% over a lifetime for persons infected only with *M. tuberculosis*.Children younger than 5 years of age are also at increased risk for progression of LTBI to tuberculosis (Thomas, 1996; CDC, 2006).

2.3.3 Diagnosis of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis can be diagnosed by several methods which include microscopy (Acid fast Ziehl-Neelsen stain) and other staining methods, chest X-ray, Tuberculin skin tests (TST), molecular assays DNA genotyping, immune assays (Interferon Gamma Release Assay <IGRA>), culture and drug susceptibility testing. Histopathological staining helps to observe typical changes (necrotizing granulomas and acid fast bacilli) and Serological testing even though not commonly performed

in some overseas countries, because of its insensitivity and non-specificity (WHO, 2011).

2.3.3.1 Detection of Mycobacterium tuberculosis by Ziehl-Neelsen

Acid-fast microscopy is rapid and inexpensive. Approximately 10^3-10^5 cfu/mlbacilli per mL of sputum are required for a positive smear. Microscopy detects acid-fast bacilli in ~50% of pulmonary tuberculosis. The Ziehl-Neelsen stain is standard, and utilises the particular staining properties of the waxy coats of the Mycobacterial cell walls. Culture or molecular assays are then required to confirm if acid-fast bacilli are *Mycobacterium tuberculosis* complex (Chris, 2012)

2.3.3.2 Culture of Mycobacterium tuberculosis

Culture is the most sensitive procedure that can detect as low as 10 cells/ml. Automated liquid cultures are preferred as they are more rapid and sensitive than solid culture media and can also test drug susceptibility, example*Mycobacterium* Growth Indicator Tube (MGIT)and Lowenstein-Jensen medium (WHO , 2011; NTBLC, 2013)

2.3.3.3 Molecular assays

Molecular assays detect DNA specific for *Mycobacterium tuberculosis* complex. In smear-positive samples they are highly sensitive, but in smear-negative specimens, sensitivity drops to 50%. A new rapid polymerase chain reaction (PCR) test (Xpert® *Mycobacterium tuberculosis*/Rifampicin assay ((MTB/RIF), Cepheid, Sunnyvale CA.) that is not prone to contamination is use by some experts(Johnson,2011). Sensitivity is equivalent to solid culture and it simultaneously detects rifampicin resistance (RACGP, 2012).

2.3.3.4Antibacterial susceptibility testing

The cultured isolatesare tested against first line treatment agents (Isoniazid, Rifampicin, Pyrazinamide Ethambutol, and Streptomycin). Resistant strains are then tested against second line drugs. Multidrug resistant cases (MDR) are defined by resistance to both isoniazid and rifampicin. Rifampicin resistant strains are usually also isoniazid resistant. Drug resistant strains require longer treatment; for Multi Drugs Resistance tuberculosis (MDR-TB), World Health Organization guidelines recommend at least 20 months of therapy (WHO,2011).

2.3.3.5 Diagnosis of latent tuberculosis

Diagnosis of latent tuberculosis infection is dependent on tests of immunerecognition of *Mycobacterium tuberculosis* (antigens) with tuberculin skin testing (TST) or interferon gamma release assays (IGRA). These tests become positive 4–6 weeks after infection or interferon gamma release assays (IGRA) (Johnson, 2011).

TreatingLTBI decreases the risk of progression to active tuberculosis by 60–90% and eliminates the potential for transmission. Preventive chemotherapy is typically with isoniazid alone (9 months on average) rather than requiringcombinationtherapy. The risk of progression to disease is greatest within the first 2–3 years, and especially within 1 year (Johnson, 2011).

2.3.3.6 Tuberculin skin test (TST)

The TST, or Mantoux test, assesses inflammation in the dermis following intradermal injection of tuberculin protein (purified protein derivative PPD). The test needs to be read 48–72 hours after administration. The diameter (≥10mm) of in duration gives a semi quantitative assessment of the likelihood of latent tuberculosis infection. False

positives can result from previous bacilli Calmette-Guérin (BCG) vaccination and exposure to environmental *Mycobacterium* species(CDC,2005).

2.3.3.7Heaf test

Skin testing for tuberculosis (TB) utilizes a form of the diagnostic reagent tuberculin. The multiple puncture technique is referred to as the Heaf, or tine testperformed to determine whether or not children had been exposed to tuberculosis infection. Also known as the Sterneedle test (Thomas,1996) it was administered by a Heaf gun (trademarked "Sterneedle") a spring-loaded instrument with six needles arranged in a circular formation. Patients who exhibited a negative reaction to the testwere considered for BCG vaccination.The Heaf test was used to test for tuberculosis in adolescents aged around 13–14.A Heaf gun was used to inject multiple samples of testing serum under the skin at once. The needle points were dipped in tuberculin purified protein derivative (PPD) and pricked into the skin.The gun injected PPD equivalent to 100,000 units per ml to the skin over the flexor surface of the left forearm in a circular pattern of six. The test was read between two and seven days later. The injection could not be into sites containing superficial veins.

The reading of the Heaf test was defined by a scale:

Negative - No indurations, maybe six minute puncture scars,

Grade 1 - four to six papules (also considered negative)

Grade 2 - Confluent papules form indurated ring (positive)

Grade 3 - Central filling to form disc (positive)

Grade 4 - Disc >10 mm with or without blistering (strongly positive)

Grades 1 and 2 could result from previous BCG or avian tuberculosis, rather than human TB infection.Children who were found to have a grade 3 or 4 reaction were referred for X-ray and follow-up (Galbraith, *et. al*, 1972)

2.3.3.8 Interferon gamma release assays (IGRA)

Interferon gamma release assays utilize the ability of human lymphocytes to survive for a short period in a test tube. If primed by previous tuberculosis infection, lymphocytes will produce detectable amounts of gamma interferon. There are two commercially available tests: the Quantiferon Gold In-Tube™ assay (Celestis Australia) and the T-spot™ test (Oxford Immunotech). Interferon gamma release assays are unaffected by previous BCG vaccination. The venous sample does not require special timing or preparation by the patient. A Medicare rebate is available only if the patient is immunosuppressed. A positive IGRA suggests that the patient's immune system recognises tuberculosis antigens. This may be due to either current infection or a remote past infection. Interferon gamma release assays do not diagnose active tuberculosis thusmicrobiological tests are needed. Similarly a negative test cannot exclude active tuberculosis because sensitivity is not 100% (Mazurek *et al*., 2005).

2.3.3.9 Chest X-ray

Both posterior-anterior (PA) and lateral films are important with clear notes to the radiologist. Typical changes include air space consolidation, cavitation and fibrous contraction in one or both upper lobes or superior parts of the lower lobes. In human immunodeficiency virus (HIV) patients, atypical findings are more common, including [rarely] no changes despite active disease. It can be difficult to distinguish active from latent infection on chest X-ray in these patients so microbiology is also important CDC, 2005)

2.3.4 Epidemiology of *M. tuberculosis* complex

The incidence of tuberculosis grew progressively during the middle Ages and Renaissance, displacing leprosy, peaking between the 18th and 19th century as field workers moved to the cities looking for work.*M. tuberculosis* as a member of the *Mycobacterium tuberculosis* complex; other members include *Mycobacterium africanum* and *Mycobacterium bovis*. *M. africanum* is most commonly found in West African countries; it causes up to a quarter of cases of tuberculosis in the Gambia (Jong*et al.,* 2010). The symptoms of infection resemble those of *M. tuberculosis*. The infectivity is similar to *M. tuberculosis,* and it is an important opportunistic pathogen in the setting of advanced immunosuppression due to HIV or other causes. Management is identical to management for disease due to *M. tuberculosis*.

2.3.5 *Global burden of tuberculosis*

More than two billion people (about one-third of the world population) are estimated to be infected with *Mycobacterium tuberculosis* (Lonnroth and Raviglione,2008). The global incidence of tuberculosis (TB) peaked around 2003 and appears to be declining slowly (WHO, 2011). In 2012, 8.6 million individuals became ill with tuberculosis and 1.4 million died (Corbett*et al.*,2003;WHO, 2013)both these statistics reflect a decline compared with prior years. The number of individuals infected with tuberculosis also was peaked in 2005, when nine million individuals became ill (Corbett *etal*., 2003).The death peaked at 1.8 million in 2003.Then new infections occur at a rate of one per second (Dolin, 2010)**.** However, not all infections with *M. tuberculosis* cause tuberculosis since many infections are asymptomatic.

In 2007, there was an estimated 13.7 million chronic active cases, and in 2010 there about 8.8 million new cases, and 1.45 million deaths, mostly in developing countries. 350,000 of these deaths occur in those co-infected with HIV. Tuberculosis is the second most common cause of death from infectious disease (afterHIV)(Dolin,2010). China has achieved particularly dramatic progress, withabout 80 percent decline inits tuberculosis mortality rate. The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries tested positive for tuberculosis while only 5–10% of the United State population test positive (Kumar *et al.*,2007). In 2007, the country with the highest estimated incidence rate of tuberculosis was Swaziland, with 1200 cases per 100,000 people. India had the largest total incidence, with an estimated 2.0 million new cases (WHO, 2009). In developed countries, tuberculosis is less common. In the United Kingdom, the national average was 15 per 100,000 in 2007, and the highest incidence rates in Western Europe were 30 per 100,000 in Portugal and Spain. These rates compared with 98 per 100,000 in China and 48 per 100,000 in Brazil. In the United States, the overall tuberculosis case rate was 4 per 100,000 persons in 2007(WHO, 2009).

2.3.6Tuberculosis age and sex distribution

The incidence of tuberculosis varies with age. In Africa, tuberculosis primarily affects adolescents and young adults of 25-34 age groups accounting for 33.6% (15,303) of the smear positive cases registered in 2010 (WHO, 2006;WHO, 2011). However, in countries where tuberculosis has gone from high to low incidence, like the United States, tuberculosis is mainly a disease of older people, or of the immuno compromised(Kumar*et al.*, 2007; CDC, 2006). Tuberculosis incidence is seasonal, with peaks occurring every spring/summer (Douglas*etal.*, 1996; Martineau*et al.*,2011; Parrinello*et al.*, 2012;Korthals *et al.*, 2012). "The reasons for this are unclear, but may

be related to vitamin D deficiency during the winter (Korthals *et al*., 2012;Koh *et al*., 2013).In Europe, deaths from tuberculosis fell from 500 out of 100,000 in 1850to 50 out of 100,000 by 1950 due to improvements in public health, even before the arrival of antibiotics, in 2012, anestimated 530,000 children became ill with tuberculosis and 74,000 HIV-negative children died of tuberculosis.

Tobacco use has greatly increases the risk of tuberculosis and death. More than 20% of tuberculosis cases worldwide are attributable to smoking(WHO,2014). Theprevalence of tuberculosis in Nigeria was estimated to be 320,000 with deaths of 33,000. India has 3,100,000 prevalence and 320,000 death cases and so China has prevalence of 1,500,000 and 54,000 death cases while Pakistan has 630,000 prevalence and 58,000 death cases(WHO,2009; WHO,2010;CDC,2011;)WHO,1999; MMWR, 1999)report the incidence, prevalence and deaths of tuberculosis by countries and their States.Nigeria is ranks 10th among the 22 high-burden tuberculosis countries in the world. A case detection rate of 40% and treatment cases of 83% was reported in 2009.WHO, (2010) estimated 210,000 new cases of all forms of tuberculosis in the countryequivalent to 133 per 100,000 cases inpopulation. There were an estimated 320,000 prevalence cases of tuberculosis in 2010, equivalent to 199 per 100,000 cases. There were 90,447 tuberculosis cases notified in 2010 with 41,416 (58%) cases as new smear positives. The main goal of Nigeria's tuberculosis program is to halve the tuberculosis prevalenceand death rates by 2015. Tuberculosis death rates have declined from 11% in 2006 to 5% in 2010. Lagos, Kano and Oyo have the highest tuberculosis prevalence rate. Other states experienced a drop in cases notified, resulting in a 4% overall decline in 2010. Oyo increased by 46.5% from 2008 to 2010. Benue has a high tuberculosis burden which is attributable to a high HIV prevalence (WHO, 2014). DOTS require health workers to monitor patients for complete course

of treatment. The five key components of DOTS are: Political commitment with increased and sustained financing. Case detection through quality assurance bacteriology, Standardized treatment with supervision and patient support, An effective drug supply and management system, and. Monitoring and evaluation system and impact measurement

The success rate and the cost effectiveness of this program have been proven around the world. Epidemics in New York City, Tanzania, Peru, and China in the early 1990s were brought under control using DOTS (WHO, 2013). DOTS and the recently Stop TB strategy .in Nigeria has been implemented in all States and local government areas in the country and 3,000 DOTS canters have been operating across the country since 2006 to observed a significant improvement in TB detection and treatment. WHO,(2006), DOTS programmers detected 2,496,478 new smear positive cases (99% of all new smear positive cases that were notified) out of an estimated 4.1 million new smear-positive cases, giving a case detection rate of 61%. Though is still below the 70% target set for 2005. New smear-positive case detection rates by DOTS programmes in 2006 were lowest in the African (46%) and European (52%) regions and highest in the Western Pacific Region (77%), the South-East Asia Region (67%) and the Region of the Americas (69%). The Western Pacific is still the only region to have exceeded the 70% target, although the Americas (69%) and the South-East Asia regions (67%) fall just short on 2006 estimates (Ojieabu and Erah 2009) Between (1995- 2008) 50 million patients with TB were cured using DOTS and this averted up to 7 million deaths (Marais, 2013). In China TB detection rate rose from 835 in 1990 to about 130,000 in 1995 through DOTS program with about 91% cure for treatment patients in 1993 while Bangladesh had 80% success by DOTS (Ahmed, 2011). Dusumu (2001) reported a 100% compliance and cure rate of up to 90%.

By 2005, 187 countries had started implementing DOTS with 4.9 million cases of tuberculosis being treated using DOTS in that year.Among 107 cases of smear-positive pulmonary TB detected, 73% were new, 14% were treated for TB in the past and 11% were on treatment at the time of the survey. This shows that 89% of the detected confirmed smear-positive TB patients were not on anti-TB treatment during the survey.(FNTBPS) This may be a result of the under-coverage of DOTS treatment and microscopy centers and may also be attributable to low community awareness about TB services (NTBLC, 2011). Globally,5.7 million TB cases were notified through TB DOTS programmes in 2010, the percentage of people successfully treated reached its highest level at 87% in 2009. Since 1995, 46 million people have been successfully treated and up to 6.8 million lives saved through DOTS and the Stop TB Strategy (WHO, 2011)

Among the 22 highest TB burden countries, Brazil and China show a sustained decline in TB burden over the past 20 years. TB burden started to decline during the early to mid 2000s in Tanzania and Kenya, after the peak in the HIV epidemic. China, in particular, has made dramatic progress through domestic investment and international collaboration on TB. Between 1990 and 2010, the TB death rate fell by almost 80%, with deaths falling from 216,000 to 55,000, and the TB prevalence rate was halved

2.3.7Global spread of tuberculosis

As the incidence of tuberculosis continued to decline in the early 1980s, most medical experts expected that the disease would be completely eliminated in industrialized

nations by the year 2010. But by the mid-1980s, the number of tuberculosis cases began to increase between 1985 and 1991; the number of reported cases in the United States increased 20 percent. Worldwide the incidence also skyrocketed in this period, and by the year 2000, the tuberculosis had infected more than one-third of the world'spopulation. Multiple factors contributed to the global increase in tuberculosis. Infection with the human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS), is the single greatest risk for progression of tuberculosis. A second factor contributing to tuberculosis resurgence is the failure of patients to complete the full six to nine months of antibiotic therapy required to cure the tuberculosis. Many people stop taking antibiotics when they begin to feel healthier, but successful treatment of tuberculosis requires therapy beyond the period of obvious symptoms.

Failure to complywith the prescribed treatment willresult in active infections, spreading the disease to others. An infected person may infect as many as 10 to 15 other people in a single year., also it can cause the emergence of tuberculosis bacterial strains with acquired drug resistance, which further complicate treatment by increasing the length and cost of therapy (WHO,2013).

The emergence of strains of bacteria that are resistant to multiple drug therapy is a serious problem, particularly because no ready drug treatment is available to combat newly emerging strains. To improve compliance, the WHO strongly recommends that all countries, especially those in Africa and Asia, adopt a program called directly observed treatment, short-course (DOTS

Migration, international air travel, and tourism also have contributed to the global spread of tuberculosis. Theextreme difficulty of screening immigrants and travellers

for tuberculosis allows the disease to cross international borders easily. The substantial increase in homelessness, and the related circumstances of poverty, crowding, and malnutrition, also contributed to the increased incidence of tuberculosis in the United States and other industrialized countries during the early 1990s (WHO, 2013).While industrialized nations with good public health systems have been able to control the recent tuberculosis resurgence, curbing the spread of tuberculosis on a global scale will require ongoing international efforts.

2.3.8TuberculosisandHIV

The tuberculosis burden is compounded by a high prevalence of HIV in the country which stands at about 4.1% in general population. The prevalence of HIV among tuberculosis patients increased from 2.2% in 1991 to 19.1% in 2001 and 25% in 2010. This indicates that the tuberculosis situation in the country is HIV-driven. The proportion of tuberculosis patients tested for HIV was 79% in 2010, with a 25% TB-HIV co-infection rate. 59% of these patients were started on Cotrimoxazole (CPT) prophylaxis and 1.8% provided with Isoniazid (IPT) prophylaxis (WHO, 2014).

The proportion of tuberculosis/HIV co-infected patients on anti-retro viral (ARV) therapy was 33% in 2010 .The proportion of HIV-registered cases screened for tuberculosis was 57% in 2010.The proportion of HIV cases that developed tuberculosis was 4% in 2010 and 3% in 2011. At least one-third of people living with HIV worldwide in 2012 are infected with tuberculosis, although not yet ill with active tuberculosis. People living with HIV and infected with tuberculosis are 30 times more likely to develop active tuberculosis than people without HIV.Tuberculosis and HIV form a lethal combination, each speeding the other's progress. In 2012 about 320,000 people died of HIVassociatedtuberculosis. Approximately 20% of deaths among

people with HIV are due to tuberculosis. Also there was an estimated 1.1 million new cases of HIV-positive new tuberculosis cases, 75% of whom were living in Africa (WHO, 2014). MDR-TB in people co-infected with HIV appears to have a more rapid and deadly disease course than seen in patients with MDR-TB who are otherwise healthy.

Diagnosing tuberculosis in HIV-infected people is often difficult. These patients frequently have conditions that produce symptoms similar to those of tuberculosis, and may not react to the standard tuberculin skin test because their immune systems are suppressed, X-rays, sputum smears, and physical examination may also fail to provide an indication of tuberculosis infection in HIV-infected individuals.

2.3.9Multi Drug Resistant-Tuberculosis in Nigeria (MDR-TB)

Multi-drug resistant (MDR) tuberculosis is tuberculosis that is resistant to any of the first-line drugs, specifically Rifampicin and Isoniazid.The emergence of MDR-tuberculosis also poses a threat,which if not effectively addressed, may wipe out the achievements of previous efforts in controlling tuberculosis.The estimated number of MDR-tuberculosis cases among notified tuberculosis cases was 2,400, of which 21 cases were notified in the country and 23 cases were undergoing treatment in 2010.There are only four Reference Laboratories providing services for drug resistant treatment of tuberculosis in Nigeria (WHO,2014).

2.4 Directly Observed Treatment (DOTS) Expansion

The Directly observed treatment short course (DOTS) is an internationally recommended strategy for controlling tuberculosis. It was adopted by Nigeria in 2003. The tuberculosis DOTS service centers expanded from 2,780 in 2008 to 3,931 in

2009, with tuberculosis microscopy laboratory services increasing from 900 to 1,025 in 2010. More than 179 communities have well-established community tuberculosis care activities in 2010 (WHO, 2014).

2.5 Strategy and Control

DOTS strategy aims to decrease tuberculosis-related morbidity, prevent tuberculosis death, and decrease transmission.DOTS strategy comprises of five components: political commitment; case detection through microscopy; adequate treatment of cases; uninterrupted supply of drugs; and recording and reporting of cases.

2.6Treatment

The first specific treatment intervention for tuberculosis was the introduction of sanatoria in the mid-1800's. A regime of bed rest followed by graded exercise, fresh air and sunlight exposure was believed to provide some level of treatment.Followed by nurse visiting homesin an attempt to provide advice on disease curtailment, such as getting the affected patient to sleep in a separate room from the rest of the family if possible.

In 1944 the first specific anti-tuberculosis drug used in humans was discovered in America (Streptomycin).First introduced in trials by the newly formed British Medical Research Council (BMRC) in UK, after initial success, acquired resistance developed as a result of the bacteria being subjected to a single drug, leading to many resistances.A second specific drug was developed in Europe: para-amino salicylic acid (PAS). When used in combination with Streptomycin cure of over 90% of patients could be achieved but best results were only obtained if treatment was continued for two years. A few years later the most effective drug used for killing the

tubercle bacillus was developed - isoniazid. When the three drugs were combined treatment time could be reduced to eighteen months. At last the tuberculosis sanatoria were emptying (Davies *et al*., 1999).

Chemical structures of drugs for treatment of Tuberculosis

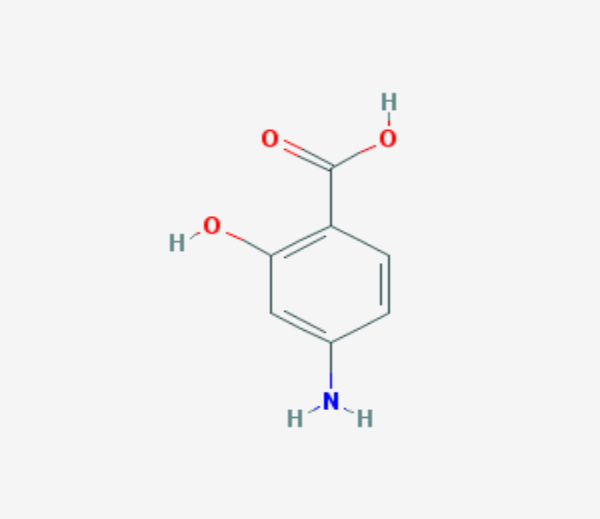


Figure 3: 4-Aminosalicylic acid; /4-Amino-2-hydroxybenzoic acid;

Molecular Formula**:** $C_7H_7NO_3$

4-Aminosalicylic acid is an antitubercular agent often administered in association with Isoniazid. The sodium salt of the drug is better tolerated than the free acid.

Aminosalicylic Acid is an analog of para-aminobenzoic acid (PABA) with antitubercular activity. Aminosalicylic acid exerts its bacteriostatic activity against *Mycobacterium tuberculosis* by competing with PABA for enzymes involved in folatesynthesis, thereby suppressing growth and reproduction of *M. tuberculosis*, eventually leading to cell death.

Two decades later, anti-tuberculosis (47 e discovered which could be added to the treatment regimen (pyrazinamide),others, such as Ethambutol, though not

particularly effective in killing bacteria, were useful in combination with other drugs in preventing the emergence of resistance to tuberculosis (Brooks *et. al,* 2007)

In the late 1960s a new, and perhaps the most important, drug in the treatment of tuberculosis was discovered - Rifampicin. This drug was able to kill the very slowly dividing bacteria, the so-called "persisters" which the other drugs could not. It was found that by combining Rifampicin with at least two others initially, the length of treatment time could be reduced to as little as six months. So the new standard of treatment of tuberculosis became isoniazid (H), rifampicin (R), and pyrazinamide (Z) for two months followed by isoniazid and rifampicin for four months. This is conveniently abbreviated as 2HRZ/4HR.

Tuberculosis is a treatable and curable disease. Active, drug-sensitive tuberculosisis treated with a standard six-month course of four antimicrobial drugs that are provided with information, supervision and support to the patient by a health worker or trained volunteer. Without such supervision and support, treatment adherence can be difficult and the disease can spread. The vast majority of tuberculosis cases can be cured when medicines are provided and taken properly. Wallace Fox of the BMRC reported that home treatment of tuberculosis was as successful as hospital treatment. This meant that the great expense of hospitalization could largely be avoided, a very important saving in resource poor countries.

Unfortunately the very success of the drug treatment of tuberculosis has been the catalyst for the emergence of a new wave of drug resistance. Patients were allowed to take their medication at home in a completely unsupervised way. The experience of the early single use of streptomycin shows that taking one drug on its own for tuberculosis would lead to drug resistance. Using the specialist tuberculosis nurse

visitors in a new role as treatment supervisors, the UK had been fortunate in avoiding the numbers of drug resistant cases but poor medical practice and patient co-operation has resulted in a slight increase. Across the world it remains a very great concern (Davies *et al*., 1999).

Since 1995, over 56 million people have been successfully treated and an estimated 22 million lives saved through the use of DOTS and the Stop tuberculosis Strategy recommended by (WHO, 2012).Treatment of tuberculosis starts with prevention. In countries where tuberculosis is common, vaccination with the BCG vaccine is often recommended.Preventing the spread of tuberculosis and othercontagious diseases also includes covering the mouth and nose with an elbow or a tissue when sneezing or coughing. Others include:

Isoniazid (INH),rifampicin combination,Pyrazinamide,,Ethambutol,RifampicinStreptomycin, Short-course chemotherapy.Longer treatment courses for multi-drug resistant tuberculosis (MDR-TB). Surgery - sometimes part or all of an affected lung is removed after initial therapy with anti-tuberculosis medications.Smoking cessation.

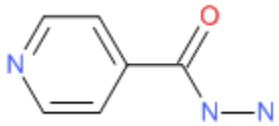


Figure 4 Isoniazide Mol formular C6-H7-N3-O

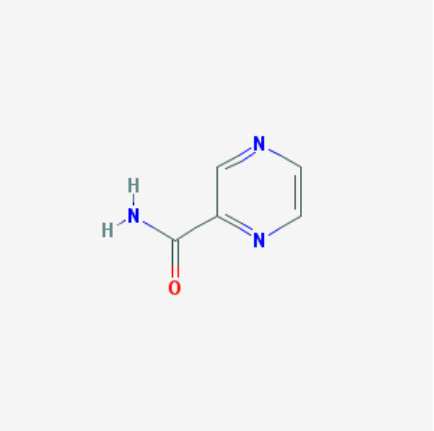


Figure 5 Pyrazinamide Mol. Formular C5-H5-N3-O

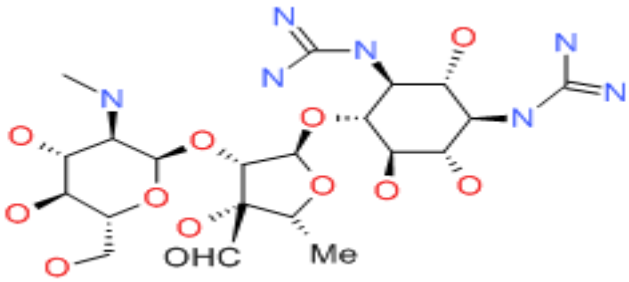


Figure 6Streptomycin Mol. Formular C21-H39-N7-O12

50

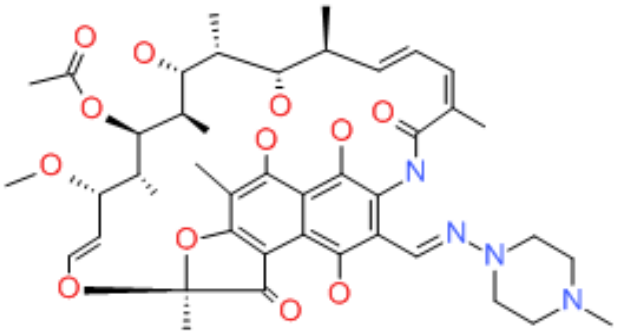


Figure 7 Rifampicin Mol formular C43-H58-N4-O12

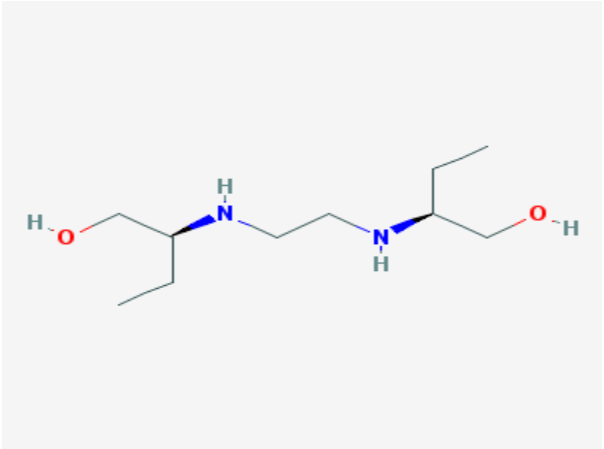
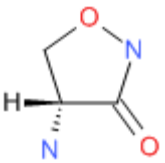


Figure 8Ethambutol Mol. structure $C_{10}H_{24}N_2$



51

Figure 9Cycloserine Mol formular C3-H6-N2-O2

2.7 Therapyfor Multidrug Resistant Tuberculosis (MDR-TB)

Treatment for MDR-TB often requires the use of second line anti-tuberculosis drugs and all can produce serious side effects. Therapy for 18 months to two years may be necessary, and patients receive at least three drugs to which the bacteria are susceptible to. Drugs include; the Kanamycin, Capreomycin, Amikacin Sulfate, andthe Fluoroquinolones (Ofloxacin and Levofloxacin

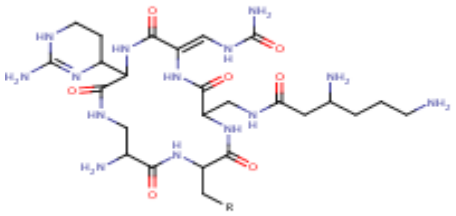


Figure 10 Capreomycin structures)

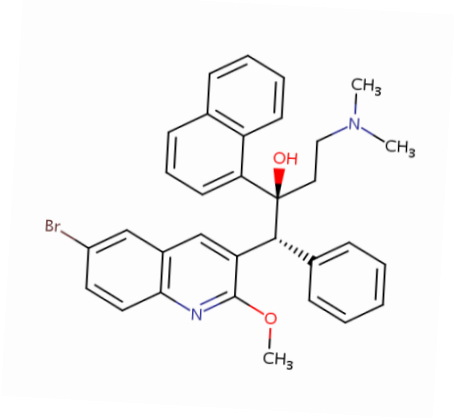


Figure 11Bedaquiline Mol formular C32-H31-Br-N2-O2

52

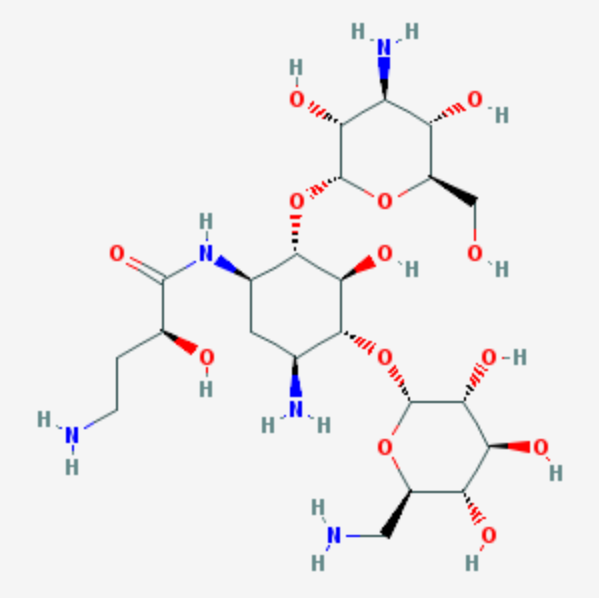


Figure 12 Structural formular of amikacin sulphate ($C_{22}H_{43}N_5O_{13}$)

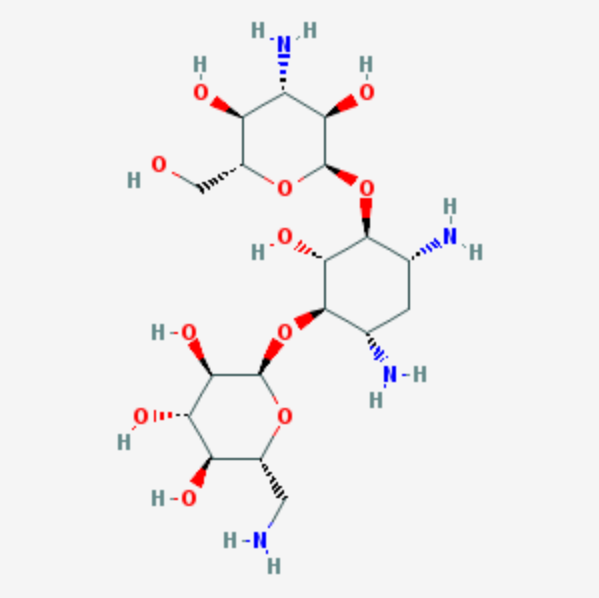


Figure 13 Structure of Kanamycin (mol formular ($C_{12}H_{36}N_4O_{11}$)

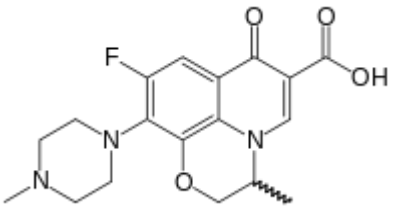


Figure 14Structural formular of Ofloxacin ($C_{18}H_{20}FN_3O_4$)

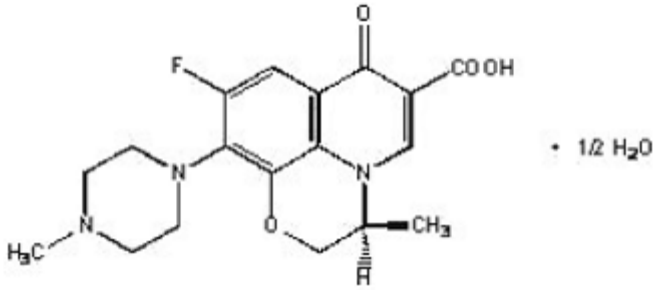


Figure 15Levofloxacin structural (Mol formular $C_{36}H_{42}F_2N_6O_9$)

2.8 Prevention of tuberculosis

Tuberculosis is largely a preventable disease. In the United States, prevention has focused on identifying infected individuals early especially those who run the highest risk of developing active disease - and treating them with drugs in a program of Directly Observed Therapy(DOT)(CDC,2005)

Isoniazid (INH) prevents the disease in most people in close contact with infected people or who are infected with the tubercle bacilli but who do not have active tuberculosis. The drug is given daily for six to 12 months and strict patient compliance in taking medication is essential to prevent drug-resistant strains from emerging. Adverse reactions to Isoniazid(INH) are rare, although a small percentage of patients, especially those older than 35, suffer INH-related hepatitis. Rifampin for

one year is recommended for close contacts of patients with INH-resistant tuberculosis(CDC, 2005)

In the United States, people with any of the following risk factors should be considered for preventive therapy, regardless of age, if they have not been previously treated for tuberculosis: Close contacts of people with newly diagnosed infectious tuberculosis.In addition, children and adolescents who react negatively to the PPD test, but who have been in close contact with infectious people within the past three months, should be considered for preventive therapy. Therapy should continue until a second skin test is done 12 weeks after their first contact with an infectious person.

People with positive tuberculin skin tests and abnormal chest X-rays compatible with inactive tuberculosis (lesions caused by prior disease),people whose skin test results have recently converted from negative to positive, people with positive skin test reactions who also have special medical conditions known to increase the risk of tuberculosis (e.g., HIV infection, diabetes mellitus) or who are on corticosteroid therapy. HIV-positive people or those suspected to be HIV-infected who now have, or had at any time in the past, positive skin test reactions, but who do not have active infection, injection drug users who have positive skin test reactions. In addition, people younger than 35 in the following groups should be consideredfor preventive therapy if they have positive skin test reactions. Foreign-born people from countries where tuberculosis is commonPeople in medically underserved, low-income groups, especially African Americans, Hispanics, and Native Americans.

Residents of long-term care facilities such as prisons, nursing homes,mental institutions and Health care workers in frequent contact with tuberculosis patients or involved with high-risk procedures such as those that induce coughing should have a skin test every six months(CDC, 2003)

Hospitals and clinics caring for high-risk populations can take precautions to prevent the spread of tuberculosis. All patients should be taught to cover their mouths and noses when coughing or sneezing. Ultraviolet light can be used to sterilize the air, and negative pressure rooms and special filters are available, as are special respirators and masks, that filter out the droplet nuclei. Until they are no longer infectious, hospitalized tuberculosis patients should be isolated in rooms with controlled ventilation and air flow (CDC, 2003)

The death rate for untreated tuberculosis patients is between 40 and 60 percent. With appropriate antibiotics, however, people with drug-susceptible cases of tuberculosis can be cured more than 90 percent at the time.

2.8.1 Effective vaccines needed

In those parts of the world where the disease is common, a vaccine composed of live, attenuated (weakened) mycobacteria from cows (*M. bovis*, called Bacillus Calmette-Guerin [BCG]) is given to infants as part of the immunization program recommended by the World Health Organization (WHO). In infants, BCG prevents the spread of *M. tuberculosis* within the body, but does not prevent initial infection (CDC,2005)

In adults, the effectiveness of BCG has varied widely in large-scale studies. In addition, positive skin test reactions occur in people who have received BCG vaccine, thus limiting the effectiveness of the Purified Protein Derivatives skin test to identify new infections. As a result, BCG is not recommended for general use in the United States. Because of BCG's limitations, more effective vaccines are needed (CDC, 2005)

2.8.2 Prognosis

Tuberculosis is treatable and curable, if adherence to instructions given during treatment to patient is complying.Symptoms often improve in 2-3 weeks. A chest X-ray will not show this improvement until weeks or months later. The outlook is excellent if pulmonary tuberculosis is diagnosed early and treatment is begun quickly (Fitzgerald *etal*., 2009;Ellner, 2011).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area

Federal Capital TerritoryAbujais a Cosmopolitan Citywhich is surrounded by four states Niger in the North-West, Kaduna in the north-East, and Nassarawa in the East West with Kogi in the South West. FCT lies between Latitude 8^0 19' 59.97" to 8^0 59' 59.97" N and Longitude 6^0 19' 59.98" to 6^0 59' 59.97"E (Fig. 3.1).The main indigenes are the Gbagyis but known to harbor almost all tribes in Nigeria. It is projected to have a population of about 3,016,493 people as at 2014 based on the 2006 population projection(AGIS,2006n)

3.2 Study Design

This was a Cross sectional study of patients from the following Hospitals: General Hospitals in Wuse, Asokoro, Nyanya, Maitama and Defence Headquarters Medical Center FCT, Abuja, Nigeria.The study was conducted for the period of one year (February, 2013 to February, 2014). It combined the use of questionnaires and laboratory experiment in this study.

3.2.1 Inclusion criteria

Subjects in the study were those who consented and presented with symptoms of tuberculosis (cough, fever, night sweat, loss of weight) for three weeks or more,age, sex,smoking,occupation, Educational level and sociological level were also considered. (Appendix VIII)

3.2.1Exclusion criteria

Healthy subjects who had no symptoms of tuberculosis were excluded from this study.

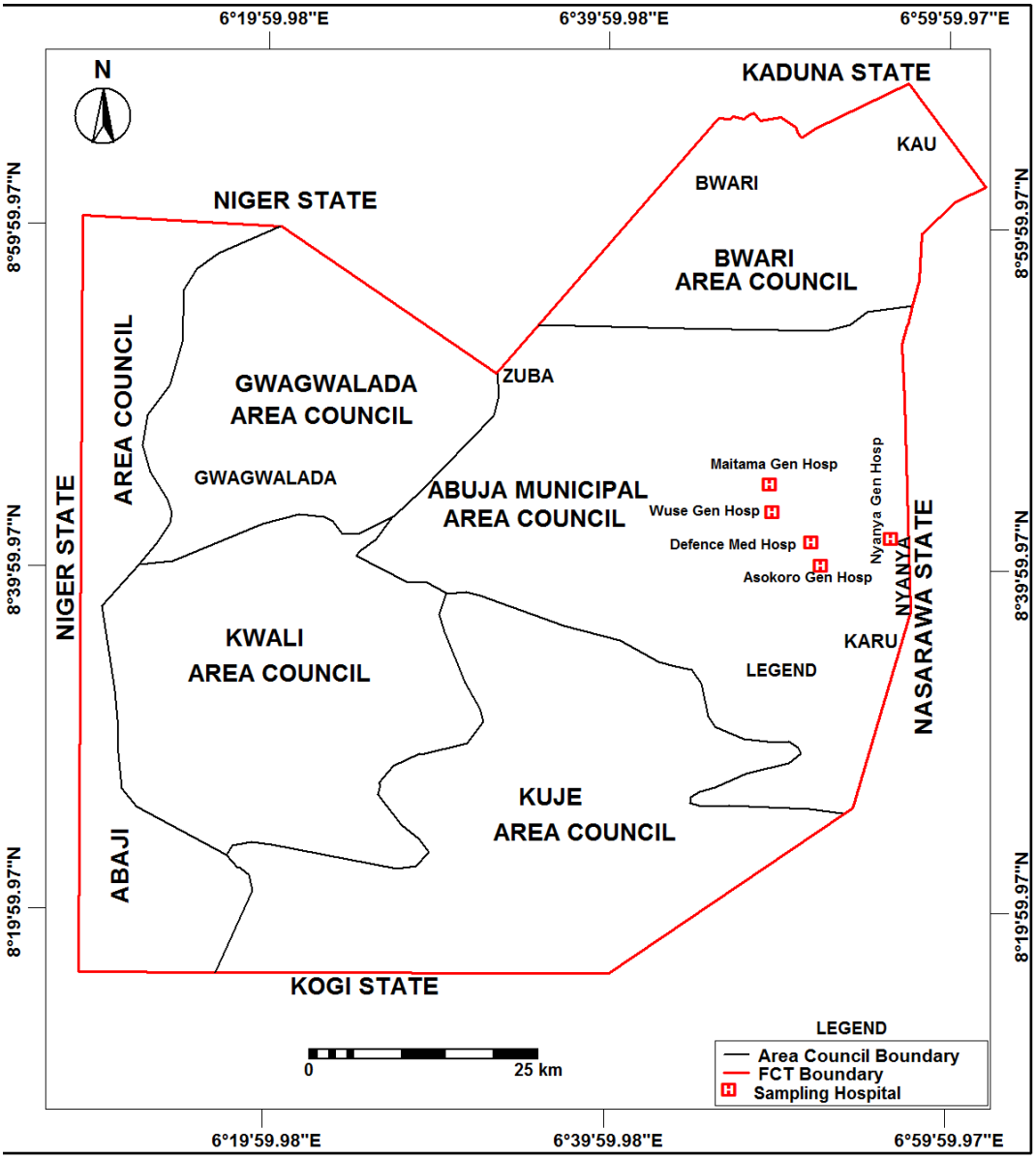


Figure 3.1: **Map of Abuja Showing Sampling Hospitals**
Source: **Modified from Administrative Map of F.C.T** (AGIS)

3.2.2Informed consent

Before the enrolment into this study, informed consent of the participants was obtained. All participants were interviewed to obtain socio-demographic data such as age, sex, address, smoking, antibacterial therapy, educational level and occupation. Socio-economic level were assessed by occupation and level of education for all participants as shown in (Appendix VII)

3.2.3Ethical approval

Ethical approval was obtained from the Federal Capital TerritoryHealth Research and Ethics Committee,Abuja (Ref: FHREC/2014/01/14/24-04-14)as shown in (Appendix XII)

3.2.4Determination of patients Sample size

The sample size for the study was determined using the formula by Thrushfield (2005) at 95% confidence level and a reportedprevalence of 0.3%(326/100,000populations) (WHO, 2013).

Using the formula:

$$N = \frac{Z^2Pq}{d^2}$$

Where,

N = Sample size

Z = statistics for a level of 95% confidence level 1.96.

P = prevalence of Tuberculosis in2013 by WHO =0.3%

d = Level of significance (allowable error) = 5%, 0.05.

q = 1 – p

$$N = \frac{(1.96)^2 \times 0.3 \times (1 - 0.30)}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.30 \times (1 - 0.30)}{0.0025}$$

$$= \frac{3.8416 \times 0.30 \times 0.70}{0.0025}$$

$$= 322.694$$

Therefore, 671 samples werecollected from tuberculosis suspected patients and analyzed for this work and convenience sampling technique was employed to collect sample in the different hospitals.

3.3Collection of Sample and Processing

Two sputa were collected from each participantand processed as shown below.

3.3.1 Collection of Sample

Each subject was provided with a wide mouthed sample container and was asked to take a deep breath three times and held for five seconds, after which the patient expectorate into the container with the supervision of the Nurse and coveredimmediately. This was repeated until about 5ml was produced, the samples were then submitted, this process was repeated and one more samplewas submitted for diagnosis (spot-morning). Laboratory identification numbers, Date, Sex, were givenand recorded.

3.3.2 Microscopy and Genexperts of sputum sample

Two sputa were collected from each participant (spot – morning) in a wide mouthed sample container and registered. Smears were processed and prepared onlabeled,clean, grease-free slides and one sample (5 ml) was used for DNA genotyping.

3.3.3 Decontamination and digestion of sputum for culture

Principle

Isolation of *Mycobacterium* species from sputum requires digestion, decontamination and concentration treatment prior to inoculation of media for growth detection. Digestion and liquefaction of mucous and organic debris allows eradication of rapidly growing contaminants which will overgrow slower growing Mycobacteria especially *Mycobacterium tuberculosis* complex. The N-acetyl L-cysteine–sodium hydroxide (NALC/NaOH) method is the mildest and most widely used decontamination procedure for recovery of *Mycobacterium* species from sputum and other clinical specimens. The starting concentration of NaOH is 4% with a final concentration of 1% in the specimen.The mucolytic agent N-acetyl L-cysteine–sodium hydroxide (NALC/NaOH) enhancesrapid liquefaction and digestion of sputum. Tri-sodium citrate binds heavy metal ions that might be present in the specimen that might inactivate NALC.

3.3.4 Procedure for Decontamination

The biosafety cabinet (BSC) was prepared (BSCL3).Exactly 50ml sterile conical screw cap centrifuge tube waslabeledand 5 ml ofsputum was transferred intotubes.

For thick specimen, equal amount of buffer was added to the specimen, mixed and then transferred into tubes. Buffer was used as blank or negative control,

Equal amount of NALC-NaOH was transferred to specimen in the tube and tightly recappedvortexes for 20 seconds at a moderate speed.Tubes were inverted 5 times for proper mixing of NALC-NaOH solution with the entire surface of the tube.

Extreme agitation or shaking was avoided which could increase the oxidation and inactivation of the NALC. The tubes were allowed to stand for 15 minutes. Timing was critical to ensure that *Mycobacteria* were not over killed.

Then about 35ml ofsterile 0.067M phosphate buffer (pH 6.8) was added to the specimen in the tube to 45ml mark to reduce the continued action of NaOH and lower the viscosity of the mixture. Tubes were recapped tightly, inverted several times and loaded into centrifuge buckets and centrifugedat 4^0C for 15 minutes at 3,000 x g.

The supernatantwascarefully poured offinto a discard container and sediment was re-suspended with 2 ml of phosphate bufferfor inoculation on two L-J medium one containing glycerol and the other pyruvate and the tubes were incubated at 37^0C for 8 weeks.Tubes were observed after four to seven days and then weekly, growth recorded accordingly and after 8 weeks negative cultures were discarded.

3.4 Culture of Mycobacteria Using Lowenstein–Jensen (LJ) Medium

Principle

Lowenstein-Jensen medium is an egg-based medium that is enriched to support growth of Mycobacteria. Prior to inoculation onto LJ medium, clinical specimens was processed (digestion, decontamination and concentrationas described above) to destroy contaminating flora. LJalso contains malachite green which gives it a greenish background that makes colonial identification easier while at the same time inhibiting the growth of other organisms.

3.4.1 Preparation and inoculation of Lowenstein – Jensen (LJ) medium

Approximately 37.3 grams of the LJ medium powder was weighed and dissolved in 600ml of distilled water and 12 ml of glycerol added.Another LJ medium was prepared with pyruvate added and heated with frequent agitation and boiled for 1minute.

The medium was sterilized in an autoclave at 121^0C for 15 minutes cooled to 50^0C.1litre of whole fresh egg was prepared aseptically and added slowly to the medium bubbles avoided.The medium werethen distributed into labelled sterile screw capped tubesas LJ with glycerol and pyruvateand placed in slant positionrespectively. Medium were then tyndallised and inspissated at 85-90^0C for 45 minutes using water bath and then stored in refrigerator.

LJ slants wereremoved from the refrigerator,allowed to attainedroom temperature,labelledwith the specimen number, datedand arranged in the biosafety cabinet. The LJ slants were opened and tilted to discard water of condensation,with a sterile plastic pipette, 2-4 drops of sputum sediment was added to LJ slant andspread over the surface of the slant by gently rolling the liquid over the slant. Caps loosen and placed in a slanted rack and incubated for between 2-8weeks at 37^0C.

Positive control was included (H37Rv Prepared from a McFarland No 1 standard dilution of the control strain and buffer phosphate saline as a negative control.

Examination of cultures: Slants was examinedafter 3 days and 7 days of incubation for contamination or rapidly growing Mycobacteria, thentubes caps were tighten and examined weekly for 8 weeks, negative cultures were decontaminated and discarded after 8 weeks.

*M. tuberculosis complex (MTBC):*Pale cream, may be granular, rough, dry colonies. Mucoid forms exist but are rare, slow growth.

*M. avium complex (MAC)**:*** Pale cream to yellow, usually smooth colonies or confluent andslow growth.

Non-Tuberculosis Mycobacterium (NTM) canbe pigmented yellow/orange or non-pigmented. They growrapidly/slowly andvaried morphology. Any growth suspected of being Mycobacteria was smeared and stained with ZN to confirmed Acid Fast Bacilli (AFB). A Rapid diagnostic test (Standard Diagnostics Bioline) was used to determineif isolates were *M. tuberculosis* complex.

3.5Rapid Diagnostic Test for M.TBC (Standard Diagnostic Bioline) MPT64

3.5.1Procedure for Standard Diagnostic Bioline (SD)

Using a standard sterile loop, Colonies from solid cultures were picked and the AFB was confirmed by ZN staining. The SD Bio line device was removed from refrigerator and allowed to attainroom temperature. The devicewas removed from the foil pouch immediately before testing andapproximately 3-4 of the colonies were suspended in 200 µg of extraction buffer dispensed in sterile screw capped tube prior to testing or condensation fluid from the culture slant agar tubes can also be used.100 µg of suspendedspecimen was taken from the fluid and applied directly to the sample well(S) and placed on a flat, dry surface. It was allowed to flow through the window within fifteen (15) minutes and a purple colour was observed on the window.

A color band will appear at the left section of the result window to show that testis working properly (control band).

The right section of the result window indicates the test results. The appearance of a colourband at the result window, showedthe test band.

- Negative Result: The presence of only control band within the window indicates negative result.
- Positive Result: The presence of two colour bands (that is the T-band and C-band) within the result window indicates a positive result

- Invalid Result: If the control band is not visible within the window after performing the test the result is considered invalid.

3.6 Susceptibility Test (DST proportion method)

Principle:

This monitor growth of *Mycobacterium* strains on drug-containing LJ slants and compared it with growth on drug-free slants. The Critical Proportion for Resistance is 1%. The growth on the 10^{-4} control slant is 1% of the growth on the 10^{-2} control slant. The strain is presumed to be resistant when growth of more than a certain proportion of the inoculums (critical proportion) occurs on culture medium containing a defined concentration, the critical concentration, of the drug. Therefore comparing growth on the drug, containing LJ slant at 10^{-2} dilution against growth on the drug-free control LJ slant 10^{-4} dilution, we expect the growth on the drug slant 10^{-2} to be less than the growth on the 10^{-4} control slant if the *Mycobacterium* strain is susceptible. However if the growth on the drug-containing LJ slant (10^{-2}) is equal to or more than the growth on the drug free control LJ slant (10^{-4}), this is reported as Resistance.

A culture of *M. tuberculosis* strain H37Rv, freshly sub-cultured was used as control strain.

The Biological Safety Cabinet was sets up with 10 specimens at a time to work with. A 3mm sterile loop was used to take loopful primary culture and placed on the side wall of a Bijou bottle containing 1 ml of sterile distilled water and 6 glass beads of (5 mm diameter). The bacterial inoculums was emulsified on the side wall of the Bijou bottle in round rotating movements with inoculation loop, till the bacterial mass completely emulsified and suspension be fully dissolved in the 1 ml of sterile distilled

water. The bottle was vortexes for 20-30 seconds to further break up clumps. Slowly 2-3 ml of distilled water was added to the suspension to allow the

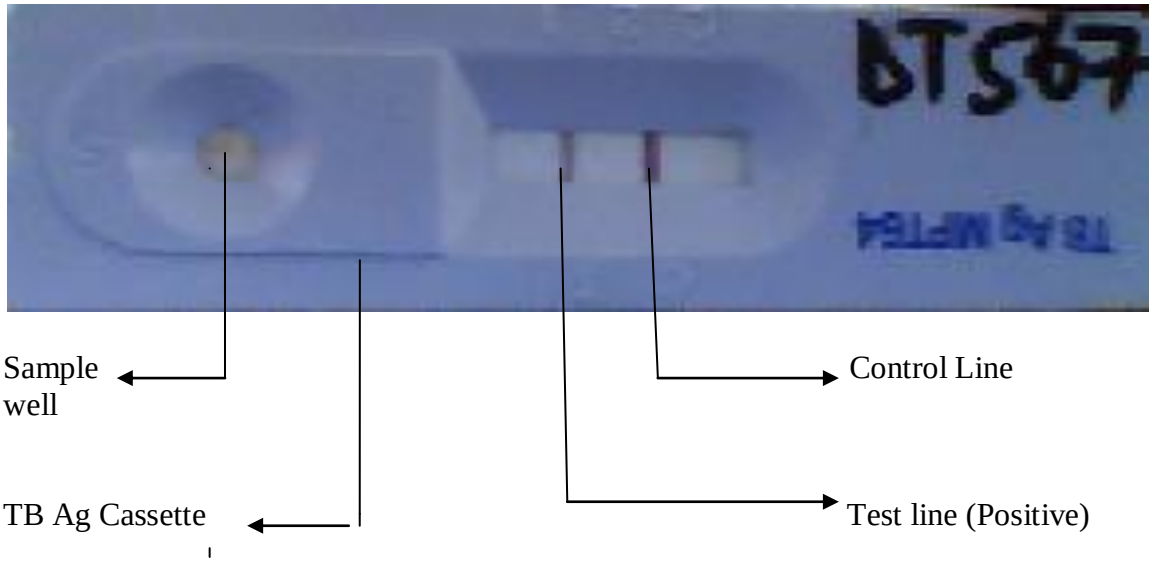


Figure 3.2: Rapid Standard Diagnostic Bioline Positive isolate

coarse particles to settle down standing it for approximately 15 minutes. Using a sterile transfer pipette the supernatant was carefully transferred to another sterile bottle with dimensions similar to that containing the McFarland No 1. Avoiding clumps, the opacity/turbidity of the suspension was matched with McFarland Standard No 1, against a black background. This is the ***neat*** bacterial suspension; standardized at 1 mg/ml, which is equal to 10^6- 10^8 cfu/ml without clumps. Where necessary, the turbidity of the bacterial suspension was adjusted to match the McFarland standard No1. Where the suspension was too turbid, sterile distilled water was added and where the suspension was insufficiently turbid, more cells was not added to the suspension, instead the suspension was allowed to settle and some of the supernatant was discarded to concentrate cells, and the turbidity was adjusted by adding few drops of sterile distilled water. An automatic pipette was used to dilute the calibrated bacterial suspension by transferring 10µl of neat into 0.99ml(100µl/9.9ml sterile distilled water; this is the 10^{-2} dilution. From the 10^{-2} dilution, 10µl was transferred into 0.99ml sterile distilled water to make 10^{-4} dilution (O'Reilly and Daborn, 1995; NTBLC, 2013).

The following drug concentrations were added to LJ Medium and Critical proportion for interpretation of the proportion method.Drugs(Streptomycin dihydro streptomycin (sulphate)4.0µg/ml, Isonized 0.2 µg/ml, Rifampicin40.0 µg/ml and Ethambutol2.0 µg/ml concentration respectively at 1.0% each for Critical proportion to determine resistance.This technique enables a growth of 30–100 colonies on the growth control (drug-free) medium using the most dilute suspension for inoculation(NTBLC,2013).

The LJ slant was removed from the refrigerator and dried. Slants were properly labelled and the condensed moisture was removed before inoculation. A standard inoculating loop of internal diameter 3mm was used to inoculate 10µl (one loopful) of suspension dilutions on the drug-free and drug-containing slants as indicated in (Appendix IX)for culture.For growth control (GC), drug-free slants are inoculated. Two tubes labelled GC1 are inoculated with suspension S1 (dilution 10^{-2}), two tubes labelled GC3 are inoculated with suspension S3 (dilution 10^{-4}). Tubes with test medium containing INH, RMP, DSM and EMB (one tube per drug) – were inoculated with S1 (dilution 10^{-2}) and incubated at 37^{o}cfor 4-6 weeks.First reading was taken at the 28^{th} day and at that time resistant strain to any drug was reported. Strains susceptible to a drug at the 28^{th} day required further incubation up to the 42^{nd} day for a definitive interpretation.

Reading format

No growth = 0, < 50 colonies = Actual count,50- 100 colonies =1+, 100–200 colonies =2+, 200– 500 colonies= 3+, >500 colonies (confluent growth) = 4+. Growth on the GC3 tube was expected to be between 30 and 100 colonies to allow for result interpretation. When there was no growth on drug-free medium after 6 weeks, the test cannot be interpreted and was repeated.

For interpretation of the test, the number of colonies on the drug medium inoculated with S1 was compared with the number of colonies on GC3, (controls) inoculated with a 1% dilution of S1. Similarly, the number of colonies on drug medium inoculated with S2 can be compared with the number of colonies on GC4 (controls) inoculated with 1% dilution of S2.

Resistance occurs when growth on the drug-containing slant is equal to or greater than growth on the corresponding GC tube as explained above.

Strains are susceptible when growth on the drug-containing slant is less than growth on the corresponding GC tube.“Borderline cases” (with about 1% growth on drug-containing medium) was reported as resistant (under reservation) and retested.Fewer than 20 colonies have grown on GC3; a reliable interpretation was possible only for resistant strains.More than 100 colonies have grown on GC3; interpretation was possible only for strains that are sensitive. Test was repeated whenever a definitive interpretation was not possible (O'Reilly and Daborn 1995; Grange, 1999; CDC, 2000; NTBLC, 2013).

3.7GenexpertsFor Detection of MTB/RIF

This is an automated (MTB/RIF) system(Real-time) PCR system for detecting *M.tuberculosis* and rifampicin resistant tuberculosis complex, it uses the principle of polymerase chain reaction (PCR) and it identifies the regions of the target (Mutations in rpoβ) gene associated with RIF resistance when PCR amplified.

The system can amplify and simultaneously quantify a targeted DNA molecule in smear positive and negative sputum samples. The primers in the Genexpert MTB/RIF assay amplify a portion of the rpoβ gene containing the 81 base pair core region. The probes are able to differentiate between the conserved wild–type sequence and mutations in the core region that are associated with RIF’s resistance. The 5 wild type (A-E) sites of the rpoβ gene of *Mycobacterium tuberculosis* are present and can be detected using the Genexpert system.The gene rPoβ encodes the β-subunit of RNA polymerase, an oligomeric enzyme responsible for RNA synthesis. Mutations within a limited region of rPoβ are known to be related to rifampicin resistance in *M.tuberculosis* (Telenti *et. al*, 1993). The assay includes a sample processing control

(SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitors in the PCR reaction. A probe Check Control (PCC) verifies reagent rehydration PCR tube filling in the cartridge, probe integrity, and dye stability.

3.7.1 Test procedure

The computer and the GenexpertDx instrument were turned on. The cartridges were labelled with sample identification number. 2ml of sample buffer reagent wasadded to 1ml of sample v/v (2:1) and the lid firmly closed, shaken for 10-20 times then allowed to stand for 10 minutes. The container were shaken for another 10-20 times and allowed further to stand for 5 minutes. This inactivates the bacilli as it liquefies the sample. Then 2ml of sample was transferred each using a sterile Pasteur pipette to the cartridge port slowly and the lid tightly closed. Genexpert barcode reader scans all the informationrequired and cartridges were loaded to the available module during test run, filtering and washing of sample start automatically, bacilli were concentrated and inhibitors removed. Ultrasonic lyses of filtered captured organisms occurred to release the DNA. The DNA molecules were mixed with the reagent, there was hemi-nested real-time PCR and products were detected. It took two hours to run a sample and obtain results. The instrument automatically printed out the result of test as shown below.

1. “MTB not detected” or “MTB detected”.

2 “Rif resistance not detected” or “Rif resistance detected”.

(Cepheid Genexpert,(2010),Cepheid Genexpert Operator manual).

Primers used for genexpert

The Genexpert MTB/RIF assay uses 3 specific primers and 5 unique molecular probes to ensure a high degree of specificity

Assay targets the *rpoB* gene, which is critical for identifying mutations associated with rifampicin resistance

No cross reactions were observed with many other bacterial species tested, including a comprehensive panel of Mycobacteria *rpoB* gene 81 base pairRif resistance determining region

5^1GCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG – 3^1

3^1- CGTGGTCGGTCGACTCGGTTAAGTACCTGGTCTTGTTGGGCGACAGCCCCAACTGGGTGTTCGCGGCTGACAGCCGCGAC - 5^1

3.8Data Analysis

Data generated from the interview and results obtained from the Laboratory analysis of samples were entered into the Microsoft Excel and reduced to percentages (%) by descriptive statistical tools and presented in tables and figures, Statistical Package for Social Sciences (SPSS) version 21.0 was used to analyzed the data. Chi Square was used to determine association between the various socio-demographic studied and tuberculosis at 95% interval and 0.05 significant levels.

CHAPTER FOUR

4.0 Results

4.1 General overview on Occurrence of Tuberculosis in the study groups

Table 4.1 shows that the occurrence of tuberculosis in the study population was21.9% by Microscopy(147/671), 21.5% by Genexperts(144/671), 23.4% by culture (157/671). Majority of theenrolled subjectswere from Nyanya Hospital (n=163,24.3%),Asokoro Hospital (n=156, 23.2%), Patients from WuseHospital were 152, (22.7%) while those from Maitama Hospital were100, (14.9%),and patients fromDHQ were100, (14.9%)(Table 4.1).The number of females enrolled were more (n = 344, 51.3%) as compared to the males (n = 327, 48.7 %) (Table 4.2) .The majority of the study population fell between the age group of 31-40years (n = 227, 33.8%), while the least population was observed among those subjects who were between 1-10 years of age (n = 17, 2.5%) (Table 4.3)

The number of mucopurulent sputum enrolled were 506,75%, while **bloody** mucopurulent sputum were 4, (0.6%) and mucosalivary were 161, (24.0%).(Table 4.4). The number of the HIV-TB positive enrolled patientswere204, 30.4%, while HIV negative TB positive enrolled patients were406, (60.5%),HIV negative TB positive cases were higher in the study population with case detection by microscopy was24.9% (101/406), Genexperts22.9% (93/406) and byculture 23.4% (95/406)(Table 4.5).

The total number of people who smoke cigarette was (n = 160, 23.8%) with TB occurrence by microscopy 71.3% (114/160), Genexperts61.3% (98/160) and culture 65.6% (105/160) while those that do not smoke (n = 511, 76.2%) had positive

tuberculosis cases by microscopy 6.5% (33/511), Genexperts9.0% (46/511) and by culture 10.2% (52/511) (Table 4.7) .

Occupation as a risk factor shows that patients doing business/trading/commercial transportation had the highest enrolled subjects (n = 249, 37.1%) with occurrence by microscopy as 20.9% (52/249), 18.9% by Genexperts(47/249) and 24.1% by culture (60/249), while farmers were least (n = 60, 8.9%) with occurrence by microscopy 21.7%, (13/60), Genexperts18.4% (11/60) while culture was 18.3% (11/60) (Table 4.6).

Socio-economic status as a risk factor shows that the low enrolled subjects were (n = 286, 42.6%) and had occurrence by microscopy 23.8% (68/286), Genexperts 23.7% (68/286) and culture 25.9% (74/286) while high enrolled subjects (n = 1, (0.1%) with occurrence as 0.0% by microscopy and culture while Genexpertswas 100% (1/1) in the study populations (Table 4.8).

4.2Detection rate of Tuberculosis among the Enrolled Patients by Microscopy, Genexpertsand Culture

The detection of TB by microscopy was (21.9%), Genexperts(21.5%) and culture (23.4%) at 95% C.I. and (P value < 0.05) this show a high significant difference between the techniques used for diagnosis tuberculosis(Table 4.1).

The occurrenceof TB among patients from Nyanya Hospital by microscopy was 36.2%,Genexpertswas 31.3% and culture 38.1%.

Table 4.1 Occurrence rate of Tuberculosisamong theEnrolled subjects from theFive Hospitals Using Microscopy, Genexpertsand Culture

General Hospital	Total No (%)	Microscopy	Genexperts	Culture
		No (%) Positive	**No (%) MTB Detected**	**No (%) Positive TB**
Asokoro	**156 (23.2)**	29 (18.6)	36 (23.1)	32 (20.5)
Nyanya	**163 (24.3)**	**59 (36.2)**	**51 (31.3)**	**49 (30.1**)
Maitama	**100 (14.9)**	19 (19.0)	18 (18.0)	28 (28.0)
Wuse	**152 (22.7)**	**22 (14.5)**	23 **(15.1)**	**34 (22.4)**
DHQ	**100 (14.9)**	18 (18.0)	16 (16.0)	14 (14.0)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**

	Chi square	**df**	**P value**
Microscopy	26.753	4	0.000**
Genexperts	15.674	4	0.003**
Culture	10.962	4	0.027*

Key:* = significant,** = highly significant, No= Number, (%) = percentage,

Patients attending Asokoro Hospital had TB occurrence of 18.6% by microscopy, Genexpertswas 23.1% and culture was 20.5%.While those attending Maitama Hospital was 19.0% by microscopy, Genexpertswas 18.0% and culture was 28.0%. (Table 4.1)

Patients attending Wuse Hospital hadoccurrence of 14.5% by microscopy,Genexpertswas 15.1% and culture was 22.4%.Patients from DHQ Hospitalby microscopy was 18.0%, Genexpertswas 16% and culture was 14% at p< (0.05) (Table 4.1). There was a statistically significant difference in all the techniques at p-value < 0.05.

4.3Implication of Gender in the Detection rate of Tuberculosis byMicroscopy, Genexpertsand Culture

The detection rate of TB among males by microscopy was 26.6 %, Genexperts26.6%, and culture was 28.1%. Thedetection of tuberculosis among females by microscopy was 17.4%, Genexperts16.6% and culture 18.9%. (Table 4.2) there was a statistical difference in association indicating that males had higher TB detection than females at p <0.05, Odd ratio 1.716 (Table 4.2a).

4.4Gender-Related Occurrence of Tuberculosis in the Five Hospitals

The detection of TB among patients attending Asokoro Hospital by microscopy was 21.3% among males and16.0% among females,Genexpertswas 30.7% among males and16.0% among femaleswhile by culture it was 26.7% among males and 14.8% femalesrespectively. Genexpertsshows a significant difference between males and females at P –value of 0.030(Table4.2b).

Therewas a highly significant difference between males and females at P= 0.008 by microscopy and 0.005 by Genexpertswhile culture was not significant among patients in Wuse Hospital (Table 4.2b).

Table 4.2a:Implication of Gender in the detection rate of Tuberculosisby microscopy, Genexpertsand culture

Gender	Total no (%)	Microscopy	Genexperts	Culture
		No (%) Positive	No (%) MTB Detected	No (%) Positive TB
Male	**327 (48.7)**	87 (26.6)	87 (26.6)	92 (28.1)
Female	**344 (51.3)**	60 (17.4)	57 (16.6)	65 (18.9)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**

	Chi square	Df	P- value	Odd ratio	C.I.
Microscopy	8.228	1	0.004**	1.716	1.184 - 2.487
Genexperts	10.017	1	0.002**	0.548	0.376 - 0.797
Culture	7.984	1	0.005**	1.68	1.170 - 2.413

Key**= highly significant,.

%= percentage, No=Number,

Table 4.2b: Implication of Gender in the detection rate of Tuberculosis in the Five Hospitals by microscopy, Genexpertsand culture

			Microscopy	Genexperts	Culture
Hospital	**Gender**	**Total no (%)**	**No (%) Positive**	**No (%) MTB Detected**	**No (%) Positive for TB**
Asokoro	Male	75 (48.1)	16 (21.3)	**23 (30.7)**	20 (26.7)
	Female	81 (51.9)	13 (16.0)	**13 (16.0)**	12 (14.8)
		156 (23.2)	**29 (18.6)**	**36 (23.1)**	**32 (20.5)**
		Chi square	0.718	4.687	3.355
		Df	1	1	1
		P value	0.397ns	**0.030***	0.067ns
Nyanya	Male	85 (52.1)	35 (41.2)	29 (34.1)	29 (34.1)
	Female	78 (47.9)	24 (30.8)	22 (28.2)	20 (25.6)
		163 (24.3)	**59 (36.2)**	**51 (31.3)**	**49 (30.1)**
		Chi square	1.908	0.661	1.39
		Df	1	1	1
		P value	0.167ns	0.416ns	0.238ns
Maitama	Male	46 (46.0)	11 (23.9)	10 (21.7)	15 (32.6)
	Female	54 (54.0)	8 (14.8)	8 (14.8)	13 (24.1)
		100 (14.9)	**19 (19.0)**	**18 (18.0)**	**28 (28.0)**
		Chi square	1.336	0.807	0.897
		Df	1	1	1
		P value	0.248ns	0.369ns	0.343ns
Wuse	Male	71 (46.7)	**16 (22.5)**	**17 (23.9)**	20 (28.2)
	Female	81 (53.3)	**6 (7.4)**	**6 (7.4)**	14 (17.3)
		152 (22.7)	**22 (14.5)**	**23 (15.1)**	**34 (22.4)**
		Chi square	6.995	8.056	2.582
		Df	1	1	1
		P value	**0.008****	**0.005****	0.108ns
	Male	50 (50.0)	9 (18.0)	8 (16.0)	8 (16.0)
DHQ	Female	50 (50.0)	9 (18.0)	8 (16.0)	6 (12.0)
		100 (14.9)	**18 (18.0)**	**16 (16.0)**	**14 (14.0)**
Total		**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**
		Chi square	0.000	0.000	0.332
		Df	1	1	1
		P value	**1.000ns**	**1.000ns**	00.564ns

4.5Sensitivities of Microscopy, Genexperts and Culture in the detection of TB in different Age Groups

The overall TB detection in all the ages for microscopy was 21.9%, Genexperts(21.5%) and culture (23.5%). The patients in the age group 60 years showed the highest TB detection of 42.9% by microscopy, Genexperts (14.3%) and culture had no positive case 0.0% followed by theage bracket 41-50 years with the detection rate of 25.5% by microscopy, Genexperts(24.5%) and culture (32.7%). While age bracket 1-10 years showed the least detection of 0.0% by microscopy, 5.9% byGenexpertsand 0.0% by culture (Table 4.3). There was an association within the same age bracket between 31-40 years showing a statistical significant difference with culture giving a higher case detection rate oftuberculosis than microscopy and Genexperts(P-value 0.007). (Table 4.3)

4.6Efficiency of Sputum Appearances in the detection of TBby Microscopy, Genexperts and Culturein the detection of Tuberculosis.

The detection of TB in all the bloody mucopurulent sputum samples from patients by microscopy was 100%, Genexperts(50.0%) and culture (100%).While the detection rate of TB in mucosalivary sputum by microscopy was (5.0%), Genexperts, (5.0%) and culture (11.2%). The detection of TB in mucopurulent sputum by microscopy was 26.7%, Genexperts(26.5%) and culture (26.7%) respectively at ($p< 0.05$). There was a highly statistical significant difference between culture, microscopy and Genexpertsparticularly among bloody muco purulent sputum in the diagnostic techniques used ($p<0.05$). (Table 4.4)

Table 4 3: Sensitivities of Microscopy, Genexperts and Culture in the detection of TB in different Age Groups

Age (years)	Total no (%)	Microscopy	Genexperts	Culture
		No (%) positive	No (%) MTB Detected	No (%) TB Positive
1 - 10	17 (2.5)	0 (0.0)	1 (5.9)	0 (0.0)
11 - 20	57 (8.5)	13 (22.8)	14 (24.6)	12 (21.1)
21 - 30	196 (29.2)	44 (22.4)	47 (24.0)	47 (24.0)
31 - 40	227 (33.8)	48 (21.1)	41 (18.1)	49 (21.6)
41 - 50	110 (16.4)	28 (25.5)	27 (24.5)	36 (32.7)
51 - 60	36 (5.4)	8 (22.2)	10 (27.8)	12 (33.3)
> 60	28 (4.1)	6 (21.43)	4 (14.29)	1 (3.57)
Total	671 (**100.0)**	**147 (21.9)**	**144(21.5)**	**157(23.4)**

	Chi square	**Df**	**P value**
Microscopy	5.721	6	0.455ns
Genexperts	7.395	6	0.286ns
Culture	19.286	6	0.004**

Key: ns =no significant ** = highly significant,

No= Number, (%) = percentage

Table 4.4.Efficiency of Sputum Appearances in the detection of TB by Microscopy, Genexperts and Culture

Appearance of Sputum	Total no (%)	Microscopy	Genexperts	Culture
		No (%) Positive	**No (%) MTB Detected**	**No (%) TB Positive**
Mucopurulent	**506 (75.4)**	135 (26.7)	134 (26.5)	135 (26.7)
Mucosalivary	**161 (24.0)**	8 (5.0)	8 (5.0)	18 (11.2)
Bloody Mucopurulent	**4 (0.6)**	4 (100.0)	2 (50.0)	4 (100.0)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.40)**

	Chi square	**df**	**P value**
Microscopy	47.995	2	0.000**
Genexperts	35.482	2	0.000**
Culture	29.545	2	0.000**

Key: ** = highly significant,

No= Number, (%) = percentage

4.7Occurrence of TB–HIV co-Infection among the study group

The occurrence of TB-HIV co-infections was 17.6% by microscopy, Genexperts(19.6%), and culture (23.5%). While the occurrence of TB-HIV negative cases was 23.8% by microscopy, Genexperts(22.3%) and culture TB positive HIV negative cases was (23.3%). No significant difference in the three diagnostic techniques at $p>0.05$ (Table 4.5).

4.8Relationship of Socio-Demographic Characteristicsand Occupation with Occurrence of Tuberculosis

Table 4.6 shows the occurrence of tuberculosis among civil servants to be 18.5% by microscopy, Genexperts19.0% and 23.7% by culture.

The occurrence of tuberculosis among Business/Trading/commercial transportation patients was 20.9% by microscopy, 18.9% Genexperts,and 24.1% by culture. While the occurrence of tuberculosis among farmers was 21.7% by microscopy, 18.4% by Genexperts and 18.3% by culture.

The occurrence of tuberculosis among others was 30.0% by microscopy, (10.0%) by Genexpertsand 0.0% by culture respectively. Only Genexpertshad a significant difference ($p<0.05$). (Table 4.6)

4.9Correlation of Smoking with Tuberculosis infection in the study group

The occurrence of tuberculosis among patients who smoke was (71.0%) by microscopy, 61.3% byGenexpertsand 65.6% by culture. While occurrence of tuberculosis among Non-smokers' was 6.5% by microscopy,Genexperts9.0%,

Table 4.5 Occurrence of TB –HIV Co-infection among the study group

HIV Status	Total no (%)	Microscopy	Genexperts	Culture
		No (%) Positive	**No (%) MTB Detected**	**No (%) TB Positive**
Positive	**204 (30.4)**	36 (17.6)	40 (19.6)	48 (23.5)
Negative	**467 (69.6)**	111 (23.8)	104 (22.3)	109 (23.3)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**

	Chi square	**df**	**P value**
Microscopy	3.110	1	0.078ns
Genexperts	0.597	1	0.440ns
Culture	0.003	1	0.958ns

Key: ns =no significant, No= Number, (%) = percentage

Table.4.6Relationship of Socio-Demographic Characteristics and Occupation with occurrence of Tuberculosis

Occupation	Total no (%)	Microscopy	Genexperts	Culture
		No (%) Positive	No (%) MTB Detected	No (%) TB Positive
Civil Servant	**211 (31.4)**	39 (18.5)	40 (19.0)	50 (23.7)
Business/Trading/commercial transportation	**249 (37.1)**	52 (20.9)	47 (18.9)	**60 (24.1)**
Farmer	**60 (8.9)**	13 (21.7)	11 (18.3)	11 (18.3)
Others (Medical staff,students)	**151 (22.50)**	**43 (28.48)**	**46 (30.46)**	36 (23.84)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**

	Chi square	Df	P value
Microscopy	5.410	3	0.144ns
Genexperts	9.381	3	0.025*
Culture	0.953	3	0.813ns

Key:ns =non significant,
No= Number,
(%) = percentage

culture 10.2%. There was statistically significant association between smokers and non-smokers who had tuberculoses at P<0.05(Table 4.7)

4.10 Occurrence of Tuberculosis in Relation to Socio-Economic Status

The occurrence of tuberculosis amongthe low socio economic patients was microscopy 23.8%,Genexperts(23.8%) andculture (25.9%). The occurrence of tuberculosis among the middle socio economic patients was 20.6% by microscopy, 19.5% Genexperts, and 21.6% by culture.While the occurrenceof tuberculosis among high socio-economic patients was0.0% by microscopy, Genexperts100.0%, culture 0.0% respectively. There was no statistical significant difference in the socio-status among patients using the diagnostic techniques in the study populations (Table 4.8).

4.11Rate of Isolation of Mycobacterium tuberculosisamong the Five Hospitals Using Rapid Standard Diagnostic Bioline test

Majority of the enrolled subjects were from Nyanya Hospital (n=163, 24.3%) with occurrence of 89.8% (44/49) followed by those from Asokoro Hospital (n=156, 23.2%) with occurrence of 78.1% (25/32), Patients from Wuse Hospital were (n=152, 22.7%) with occurrence of 68.6% (24/34) and patients from Maitama Hospital (n=100, 14.9%) had occurrence of 78.6% (22/28), while patients from DHQ (n=100, 14.9%) had occurrence of 54.5% (6/14) (Table 4.9).

4.12Antibacterial Susceptibility of Tuberculosis Using the First Line Drugs

Among the drugsused for susceptibility test,*M. tuberculosis* showedsusceptibility of 92.4% and resistance of 7.6% to Rifampicin while susceptibility to Streptomycin was 58% and 42% resistance. There was a statistical association between the organismsusceptible to Rifampicin andthose that are resistantto Streptomycin at P-value 0.000 as shown in Table 4.10.

Table 4.7: Correlation of Smoking with Tuberculosis Infection in the study group

Smoking	Total no (%)	Microscopy	Genexperts	Culture
		No (%) **Positive**	**No** (%) **MTB Detected**	**No** (%) **TB Positive**
Yes	**160 (23.8)**	114 (71.3)	98 (61.3)	105 (65.6)
No	**511 (76.2)**	33 (6.5)	46 (9.0)	52 (10.2)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**

	Chi square	**Df**	**P value**	**Odd ratio**	**C.I.**
Microscopy	298.991	1	0.000**	35.897	21.957 -58.588
Genexperts	197.347	1	0.000**	0.063	0.040 - 0.097
Culture	209.019	1	0.000**	16.851	10.914 - 26.018

Key: ** = highly significant,
No= Number,
(%) = percentage

Table 4.8Relationships of Socio-economic status with Occurrence of Tuberculosis in the study group.

Socio-Economic Level	Total no (%)	Microscopy No (%) Positive	Genexperts No (%) MTB Detected	Culture No (%)TB Positive
Low	**286 (42.6)**	68 (23.8)	68 (23.8)	74 (25.9)
Medium	**384 (57.2)**	70 (20.6)	75 (19.5)	83 (21.6)
High	**1 (0.1)**	0 (0.0)	1 (100.0)	0 (0.00)
Total	**671 (100.0)**	**147 (21.9)**	**144 (21.5)**	**157 (23.4)**

	Chi square	Df	P value
Microscopy	1.264	2	0.532ns
Genexperts	5.418	2	0.067ns
Culture	1.965	2	0.374ns

Key: ns-= not significant,
No = Number, (%) = percentage

Table 4.9 Rate of Isolation of *Mycobacterium tuberculosis*among the Five Hospitals using Rapid Standard Diagnostic Bioline

General Hospitals	Total no (%)	Culture	TB ID
		No (%) TB Positive	No (%) TB Detected
Asokoro	154 (23.2)	32 (20.5)	25 (78.1)
Nyanya	163 (24.3)	49 (30.1)	44 (89.8)
Maitama	100 (14.9)	28 (28.0)	22 (78.6)
Wuse	152 (22.7)	34 (22.4)	24 (68.6)
DHQ	100 (14.9)	14 (14.0)	6 (54.5)
Total	**671 (100.0)**	**157 (23.4)**	**121(78.1)**

	Chi square	df	P –value
Culture	10.962	4	0.027*
TB ID	9.338	4	0.053*

Key *= Significant,
No= Number, (%) = percentage

Table 4.10 Antibacterial Susceptibility of Tuberculosis Isolated using the first line Drugs

Drug Tested	Total no (%)	Subceptibility Test		Chi square	df	P value
		No (%) Susceptible	No (%) Resistant			
Rifampicin	**119 (100.0)**	110 (92.4)	9 (7.6)	39.123	3	0.000**
Isoniazid	**119 (100.0)**	90 (75.6)	29 (24.4)			
Entambutol	**119 (100.0)**	80 (67.2)	39 (32.8)			
Streptomycin	**119 (100.0)**	69 (58.0)	50 (42.0)			

Key: ** = highly significant, No= Number, (%) = percentage.

Table 4.11 A two by two contingency table for Sensitivity and Specificity.

		Culture		Total
		Positive	**Negative**	
Microscopy	Positive	106	41	**147**
	Negative	51	473	**524**
	Total	**157**	**514**	**671**

Sensitivity and Specificity for Microscopy was Calculated usingCulture as the Gold Standard

Microscopy

Sensitivity = $\frac{\text{True positive (106)}}{\text{True positive (106) + false negative (41)}}$ X 100 = 72.1%

= 72.1%

Specificity= $\frac{\text{True negative (473)}}{\text{True negative (473) + false positive (51)}}$ X 100 = 90.3%

= 90.3% Specificity

Table 4 12: Two by Two Contingency table for calculating Sensitivity and Specificityof Genexperts

		Culture		Total
		Positive	**Negative**	
Genexpert	Positive	108	36	**144**
	Negative	49	478	**527**
	Total	**157**	**514**	**671**

Genexperts

Sensitivity= True positive (108) / True positive (108) + false negative (49) X 100 = 68.8%

Specificity = True negative (478) / True negative (478) + false positive (36) X 100 = 93.0%

CHAPTER FIVE

5.0 DISCUSSION

5.1 Occurrence of Tuberculosis among the Study Population

The results analyzed from the study showed occurrence of tuberculosisamong the selected population and accounted tocase detection by microscopy 21.9%, Genexperts (21.5%) and culture (23.4 %.).Sensitivity and specificity of72.1% and 90.3% respectively were obtained for microscopy and 68.8% and 93.0% was obtained for Genexperts .

The TB detection of 21.9% obtained by microscopy in this study agreed with the 22%obtained with Led microscopyas reported byAjiboye (2014) which may be dueto the entire quality system in place in the facility where the two studies were carried out.

This report is also similar to the findings of Lawrence and Christian, (2010) with a prevalence of 25% in Aba. Also Lockman *et al.,* (2001) reported 42% cases of cluster tuberculosis in Botswana which is higher than the result obtained from the study which may be due to their high HIV infection.The result obtainedis lower than the 76% prevalence detected in 2005 and 63% in 2009 by (Dim and Dim, 2013) in Enugu state. Also 66% prevalence reported in (1998) and 48% detected in (2009) (Kingsley *et al*,2011) and Awe, *et.al*, (2011) reported 55% cases in the Northern part of Nigeria after the DOTS implementation. These are in close agreement with the case detection reported by WHO (2009) (40%), WHO (2010) (56%), WHO (2011) (51%) and NTBLCP, (2012) (59%).

Further, this result shows that culture remains the gold standard in the diagnosis of tuberculosis having a high tuberculosis occurrence as compared to microscopy and

Genexperts. This means, that it required the use of culture medium that is selective for Mycobacteria and suppresses other bacteria.

The sensitivity and specificity of 68.8% and 93.0% obtained forGenexpertsis in consonance withthe WHO (2014)Policy and Validation report from a single–centre evaluation studiesof sensitivity ranging from 70%-100% in culture positive patients and about 60% in those with smear-negative and specificity ranged from 91%-100% with an average rifampicin sensitivity and specificity of 98% and 99%.

Automated instrumentation the (Genexpert)used in the study isa rapid means of diagnosing tuberculosis since results can be achieved inless than two hours.Prompt result lead to early tuberculosis detection, treatment and reduction in the transmission.The occurrence of 21.5% by Genexpertsis similar to the report of RACGP, (2012) which saysGenexpertssensitivity is equivalent to solid culture (LJ)which agreed with the result obtained 21.5% for Genexperts and 23.4% for bacteriological culturedetection of *M. tuberculosis*TheRifampicin resistance tuberculosis could be detected easily and even the negative resultby microscopy can be detected using genexperts.

Patients presenting to Nyanya hospitals had the highest tuberculosis cases. This may be dueto proximity to over populatedMararaba-Nyanya communitythus patients have closed proximity to the hospital than Abuja City.Another contributing factor could be cross infection among the patients visitingthe facility since tuberculosis is air borne. Population growthdue to problem of insurgency leading to overcrowding and migration of people from the North East to this area may also have beena factor in high occurrence of tuberculosisobserved. Whilepatients attending Wuse Hospital had

least TB occurrence due to its location far away fromthe massesEjeta *et al.,* (2013),reported that overcrowding, poor living condition,limited access to treatment and migration are among factors predisposing people to tuberculosis.

Children less than five years are highly susceptible to tuberculosis due to their weak immunity. The age bracket of 5–14 years with upper age limits being the onset of puberty are resistant to infection.The most productive age groups of the population were seriously affectedbytuberculosis(31-60 years)with cases between 33% and 42.9% as obtained from the study. This also agreed with Dim and Dim (2013) report of the highest TB detection in the age-group between 25-44years

This is similar tothe infection rateof 50% reportedby(Styblo *et al*. 1996).However, increase in tuberculosis cases has been observed in many countriesmainly due tounrest and civil strife, resulting in shifting ofpriorities, breakdown of drug supply and health caresystem,population migration to urban city has led to increase tuberculosis (WHO, 1994).

Results obtained from the study population showed that males were more infected with tuberculosis than females. This is in consonance with another study that males were more infected than females (Shrestha-Kuwahara *et al.,* 1999; Awe, *et al* 2011) but contrary to the report of (Dim and Dim, 2013)This might be attributed to their outdoor activities exposing them to risk of contracting tuberculosis.

Holmes *et al*.(1998) observed that male gender was associated with TB-HIV co-infection. This observation might further be explained by the X chromosome being asusceptibility gene contributing to more of males with tuberculosis in someAfrican population as observed by Bellamy *et al*. (2000).

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TB-HIV Co-infection in Abuja

The result obtained showed theoccurrence of 23.5%TB-HIVCo-infectionwhich ishigher than TB case reported by WHO (2011),Agbaji *et al*. (2013) also reported the prevalence of TB-HIV Co-infection to be 9.6%among TB –HIV naïve patients in one of the study in Jos and attributed to impairment of the immune system of the patients. This meansHIV infections may exacerbate or re-activate a 'silent' tuberculosis case and other opportunistic infections. About 75%of adults and children with tuberculosis are co-infected with HIV. The global prevalence of TB-HIV was reported to be 0.18% (Dye *et al.,* 1999), Bassett *et al.* (2010) reported TB-HIV Co-infection prevalence of 19% in Durban, South Africa, which is similar to occurrence with TB obtained in this study for TB-HIV Co-infection. NTBLCP (2011) reported 25% as the proportion of HIV positive in tuberculosis positive patients in Nigeria.

Genexpertsand culture were able to detect TB in HIV negative patients more than microscopy from a single-centre evaluation studies**.** Thesensitivity ranged from 70% to 100% in culture-positive patients and around 60% in those with smear-negative microscopy[Specificity ranged from 91% to 100%. According to (WHO,2014) the average rifampicin sensitivity and specificity were around 98% and 99%, results obtained from the study showed that microscopy detected up to 23.8% while culture detected 23.3% and Genexperts22.3%.though it was not statistically significant. Genexpertsvalidated suspected TB or MDR-TB reportshowed also that 92.2% of culture-positive patients were detected by a single direct genexpert MTB/RIF test. Sensitivity of a single genexpert MTB/RIF test in smear-negative culture-positive patients was 72.5% genexpert MTB/RIF with specificity of 99% genexpert MTB/RIF detected rifampicin resistance with 99.1% sensitivity and excluded resistance with

100% specificity respectively.From the results obtained in the study, the occurrenceof Rifampicin resistant isolates detected was 0.6% which agreed with the report of (WHO, 2011; WHO, 2014).

The specificity of genexpert MTB/RIF, confirmed by clinical and microbiological follow-up of TB suspects, is up to 99% (Genexperts policy report, 2011; WHO, 2014).The specificity obtained in this study was 93.0%.

Socio-demographic Characteristics

In this study, various risk factors wereconsidered like; HIV-AIDS, poverty, poor health facilities,occupation, also patient's non compliance to drugs as prescribed attributes to TB resistance strainsas also observed by (Ejeta *et al*, 2013).

Other pre-disposing factors associated with tuberculosis as seen in the study includes cigarette smoking. Smoke gets into the underlining wall of the lungs predisposing the area of the lungs to support the survival of *Mycobacterium tuberculosis*.20% of death was attributed to tobacco smoking according to Vyl Smit *etal*. (2010). Also medical conditions like organ transplant could lower the immunocompetence of a subject and predispose him/her to tuberculosis.Socio economic status which leads to poor living condition can also be a risk factor.

Detection of rifampicin resistance

Genexpert MTB/RIF detected rifampicin resistance (99.1% sensitivity) and rifampicin susceptibility 100% specificity as stated by (Genexperts policy report (2011).

Test accuracy was retained, with a single genexpert MTB/RIF test directly from sputum detecting 99% of smear-positive patients and 80% of patients with smear-

negative microscopy in comparison, the sensitivity of a single direct smear was 59.5%. HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), but did not significantly affect genexpert MTB/RIF performance.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Tuberculosis exists among the study population around FCT Abuja. Thedetection of tuberculosis was shown to be between (21.5%) to (23.4%) cases.

The Socio-demographic factors that were found to be statistically significant were gender (males), occupations and overcrowding while smoking was the only risk factor statistically associated.

The population studied has been bacteriologically confirmed by Standard diagnostic test as presenting with up to 78% of pulmonary tuberculosis.

The *Mycobacterium tuberculosis*isolates showed 92.4% susceptibility to Rifampicin,75.6% to isoniazid,67.2% for Ethambutol and 58.0% for Streptomycin While 7.6% of the isolates were resistant to Rifampicin, 24.4% to Isoniazid,32.8% to Ethambutol and 42.0% to Streptomycin respectively.And 0.6% of rifampicin resistance tuberculosis was detected byGenexperts.

Culture is still remainthe gold standard in tuberculosis diagnosis.

Genexpert results are reliable for effective and rapid diagnosis of MTB/Rifampicin resistance detection especially among negative microscopy and in TB-HIV infected patients.

Microscopy on the other hand is good should be encourage since it is less expensive and can be carried out in most facilities.

6.2RECOMMENDATIONS

1. Provision of proper public health care facilities which should includes more Laboratories and Equipment by the Government at all levels to support tuberculosis programme in Nigeria.
2. For quality and adequate sputum expectoration, the Hospital should have an assigned Nurse for supervision of patients during sample collection.
3. Tuberculosis was observed to be predominant among the low and middle income earners so anti tuberculosis drugs should be made available to reach the target populations.
4. The current work observed drug resistant *M.tuberculosis*so advocacy should be intensify toeducate patients for adherence treatment to avoid developing resistant and the effect on the general populace.
5. Future researchers are to develops new vaccine that will tackle the resistant strains of *M. tuberculosis*and alsofacilities for care and proper monitoring of many MDR patients that are busy spreading the infections.
6. The engagement of all public, private, voluntary and corporate care providers in tuberculosisprevention, diagnosis and treatment needs to be substantially increased.

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APPENDICES

Appendix I

Reagents used forZiehl Neelsen stain

1% carbol fuchsin

3% of Acid Alcohol or 25% sulphuric Acid

0.3% methylene blue

Appendix Ia: Preparation for 1% carbol fuchsin stain per liter

Basic fuchsin dye	10grams
Phenol	50grams
95% ethanol or methanol	100ml
Water	up to 1litre

Preparation

1. Melted phenol (50g) was added to distilled water while stirring; warming to dissolve completely.
2. The basic fuchsin (10g) was dissolved completely in 95% ethanol and mixed completely.
3. The 5% phenol and alcohol fuchsin were mixed while stirring.
4. Then this was filtered to remove fuchsin crystals or particle.

Appendix Ib: Methylene blue stain per liter

Methylene blue powder	3 grams
Water	1 litre

Preparation for methylene blue per liter

1. The dye were dissolved in half of the water
2. The remaining water, were added and mixed well

Appendix 1c: Decolorizing solution 25% sulfuric Acid contains per liter

Sulphuric acid min. 95%	250ml
Water	up to 1litre

Preparation

1. The cold water were filled in a too large flask
2. Part of the acid were poured along the sides and slowly
3. And then were stopper and swirl for cooling (under a running tap)

4. This was repeated till all acid were used up.

Decolorizing solution (3% Acid Alcohol)

or

Appendix 1d: Preparation for Hydrochloric Acid

Hydrochloric Acid	30ml
Ethanol 95%	950

Acid is always added to water and alcohol and not water or alcohol to acid**.**

Appendix II: Reagents Used for Processing Sputum for Culture

4% sodium hydroxide pellets (4g)

Distilled water 100ml

The NaOH was dissolved in distilled water, and distributed in conical flask and sterilized by autoclaving at 121^0C for 20 minutes.

NALC- 1g just added before use Reagents and solutions

- NaOH/Na citrate (Sodium Hydroxide/Sodium Citrate)
- NALC powder (N-acetyl L-cysteine–sodium hydroxide) (NALC/NaOH)
- Sterile phosphate buffer
    - Working NALC-NaOH solution

This working solution was prepared fresh each day of use, and was used within 24 hours. The working solution was determined based on the number of samples. Required equal amounts of specimen to solution for 18 specimens (5-10 ml for each sample) we used approximately 120-180 ml of working solution.

Appendix III: Composition of Lowenstein-Jensen (L-J) medium

Formula in grams per liter

Potato flour	30.00
Monopotassium phosphate	2.50
Malachite green	0.40
Asparagines	3.60
Magnesium citrate	0.60
Magnesium sulfate	0.24

Final PH 7.0 ± 0.2 at 25^0C

Appendix IV: Materials for Genexpert

Disinfectant, genexpert MTB/RIF Kit cartridges, Pasteur pipette, Lysol, laboratory coat, gloves, wipes, sample reagent buffer, timer, 1 sputum sample and marker for labelling cartridges and sample.

NOTE*:*Heat fixing does not kill *Mycobacterium species*. Slides were handled carefully prior to staining.

Appendix V: Environmental and safety controls in Tuberculosis Laboratory

- Appropriate personal protective equipment was worn at all times when handling specimen containers and during manipulation of specimens.
- The first layer of gloves was removed inside the BSC before transferring inoculated slants to the incubator.
- Laboratory procedures that give rise to infectious aerosols were all conducted in a certified Biological Safety Cabinet (BSC). All specimens were processed in a BSC within the BSL2.
- Safety centrifuge buckets were used for centrifugation. Buckets were loaded and unloaded after centrifugation within the BSC.
- All items to be removed from the BSC were first sprayed with 0.5% sodium hypochlorite disinfectant and dried before removal.
- Use of glass items was minimized in the BSL2. Plastic transfer pipettes and disposable loops were strongly recommended. .
- Pipette with the remaining liquid inside were discarded in appropriate containers which was autoclaved and discarded.

  Each centrifuge tube was examined for possible leaks.

The centrifuge buckets were decontaminated by disinfecting with 0.5% sodium hypochlorite solution.

Appendix VI: Rapid diagnostic test for confirmation of suspected isolates of Mycobacterium tuberculosis complex

PrincipleofStandard Diagnostic (SD)Bioline

- Tuberculosis Ag MPT64 Rapid test is a simple and rapid immuno chromatographic.
- Assay based on the reaction of monoclonal antibodies against MPT64, one of the predominant proteins excreted by *M. tuberculosis* Complex, with the exception of some sub strains of *M. bovis* Bacille Calmette-Guerin (BCG).
- This test cassette consists of a sample pad, a gold conjugate pad, a nitrocellulose membrane, and an absorbent pad. Mouse monoclonal anti-MPT64 was immobilized on a nitrocellulose membrane as the capture material (test line).Another antibodies, which recognize another epitome of MPT64, conjugated with colloidal gold particles were used for antigen capture and detection in a sandwich type assay.
- Standard Diagnostic bioline tuberculosis Ag MPT 64 device has a test and control lines on the surface of the cassette. The control line is used for procedural control (control line should always appear if the test procedure is performed properly and the test reagents of control line are working. As the test sample applied in the sample well it flow laterally through the membrane, the antibody-colloidal gold conjugate binds to the MPT64 Ag in a sample, liquid media. The complex then flows further and bind to the mouse monoclonal anti- MPT64 on the solid phase in the test line, producing red to purple color band seen within 15 minutes. In the absence of MPT64, there was no line in the test band region. This test has a very high sensitivity (98.6%) and specificity (100%) compared with biochemical tests.

- Specimen, suspected colonies of *M. tuberculosis* complex grown on solid media
- 1 Test device foil pouched with a desiccant.
- Extraction buffer (for sample preparation from solid cultures)
- Instruction for use
- Active ingredient of main components
- 1. A test strip includes; Gold conjugate Mouse monoclonal anti-MPT64-gold colloid (0.24 ± 0.48ug).Test line: Mouse monoclonal anti-MPT64 (0.32 ± 0.064ug), Control column goat anti-mouse Immunoglobulin (0.64 ± 0.128ug)
- Assay diluents: 100mM Phosphate Buffer (q.s), Sodium azide (q.s).

Quality Control OF SD Bioline

The device has both positive and negative controls. Appearance of a control band in the read window at the control C position provides an internal positive control that validates the proper reagent function and assures that the correct best procedure was followed. The membrane area surrounding the test and control bands is the internal negative control for the device. A background area that is light pink indicates that the test has been performed correctly.

Limitations;

- The test result must be interpreted within 60 minutes of placing specimen on the test plate. Drying of plates can change the result after the time has elapsed, test was repeated when this occurred.
- A positive result strongly suggests the presence of *M. tuberculosis* complex. However there could be mixed infection with NTM.
- Negative result does not always rule out a possible infection with *M. tuberculosis.* Test is unable to detect MPT64 if the concentration in the

specimen is below the detection limit or if mutation has occurred in the MPT64 gene of *M. tuberculosis*.

- Some organism example *Staphylococcus aureus that* produce protein A could give false positive results. Due precaution should be taken not to contaminate sample with such organism (SD Bioline Kit insert).

Appendix VIIINFORMED CONSENT FORM

Research title

DNA grnotyping (Genexperts) and Culture in the Diagnosis of *Mycobacterium tuberculosis complex* isolated from sputum samples of outpatients in Wuse, Asokoro, Maitama, Nyanya Hospital and Dhq Medical Centre in FCT, Abuja, Nigeria.

Introduction:

You are invited to take part in a research study. Before you decide whether to participate, you need to understand why the research is being carried out and what it would involve. Take time to read or listen as I read the following information, please ask me if there is anything that is not clear or if you would like more information. If you understand everything about the study and you wish to participate in the study, you will sign this Informed Consent form. You would be given a signed copy to keep.

Purpose of the study and study Requirements

What is the purpose of the study? To determine rapid methods of drugs susceptibilities to *Mycobacterium species* isolate from sputum samples of outpatients in some selected FCT Hospitals Abuja using first line Drugs with Lowenstein Jensen medium(L-J) and Genexpert Dx instrument We are interested in devising a rapid means to isolate and treat *Mycobacterium tuberculosis* to avoid the risk of communal developing the resistant tuberculosis.

Why have I been invited to take part? You have been invited to take part in this study because you have shown signs of tuberculosis (had cough for three weeks or more) and we want to rule out tuberculosis infection from the your signs.

How long will my involvement in the study last? The study will take 10 months. But you will be involved in 2-3 days during sample collection.

What will happen if I take part? If you agree to take part in the study, we will ask you to sign this form.

RISKS

What are the risks of the study? There are no risks involve in your participating in this study except for the inconveniences of producing three samples for the study.

Benefits

What are my benefits of participating? You will have direct benefits of detecting if you have tuberculosis and enroll for treatment immediately and avoiding the risk of developing resistant tuberculosis

Confidentiality

Will my participation in the study be kept confidential? The information that is collected during the study will be kept private. No one will be told that you have participated in the study. Every one involve in the study will protect your privacy and maintain the confidentiality of all the information that you provide. Your name or other identifier will not be included in reports from the study.

Voluntariness

What are my rights as a research participant? Your participation in this study is completely voluntary. If you agree to participate in the study or not there is no penalty.

Participant statement: I have read the Informed Consent for this study. I have received an explanation of the planned research, procedures, risks, benefits and privacy of my personal information; I agree to take part in this study. I understand that my participation in this study is voluntary.

Your Name……..

Your signature……Date…….

Investigator or person who conducted Informed Consent discussion: I confirm that I have personally explained the nature and extent of the planned research, study procedures, potential risks, benefits and confidentiality of personal information

Name of person obtaining Consent……

Signature of person obtaining Consent……Date…….

Please keep a copy of this document for your records

AppendixVIIIQUESTIONNIARES FOR SOCIO-DEMOGRAPHIC STUDIES

1. Name----------

2. Sex: Male---------- Female----------

3. Age----------

4. Address----------

   ----

5. Smoking Yes----------, No----------

   How Long ----------

6. a Coughing Yes----------, No ----------

6. b If Yes above for how long 1wk-----,2wks-----, 3wks……

7. Have you visited any Hospital? Yes--------, No------

8. Are you on any antimicrobial therapy? Yes----, No--- Which --------

   How Long ----------

9. What type? ----------

10 Occupation----------

11. Educational Level----------

12. Socio-economic Level----------

Appendix IX: Inoculation table for drug and drug free l j medium

Suspension dilution	Drug-free tubes (growth control, GC)	INH 0.2 µg/ml	RMP 40 µg/ml	DSM 4 µg/ml	EMB 2 µg/ml
S1; 10^{-2}	**x x (GC1)**	**x**	**x**	**x**	**x**
S2; 10^{-3}	**x (GC2)**	**x**	**x**	**x**	**x**
S3; 10^{-4}	**x x (GC3)**	–	–	–	–
S4; 10^{-5}	**x x (GC4)**	–	–	–	–

x = inoculation of one tube;

– = no inoculation

Incubation

1. Inoculated slants were incubated at 37^0C ($35\text{-}38^0$C) with caps slightly loosen.
2. Slants were examined after one week for possible contamination.

Limitations

a. The performance of the Genexpert MTB/RIF was validated using the procedures provided in the insert. Modifications of these procedures may alter the performance of the test.

b. A positive test result does not necessarily indicate the presence of viable organisms but a presumptive for the presence of *Mycobacteriumtuberculosis* and rifampicin resistance.

c. Test results might be affected by antecedent or concurrent antibiotic therapy. Therefore, therapeutic success or failure cannot be assessed using this test because DNA might persist following antimicrobial therapy.

d. Mutations or polymorphisms in primer or probe –binding regions may affect detection of new or unknown MDR-MTB or rifampicin-resistant strains resulting in a false-negative result.

Waste Management

1 All used sputum containers, pipettes and cartridges were collected in an autoclave bag, kept safe, closed in a bin until it was incinerated.

2 All infectious waste were collected in an autoclavable bag, autoclaved and incinerated.

Appendix X

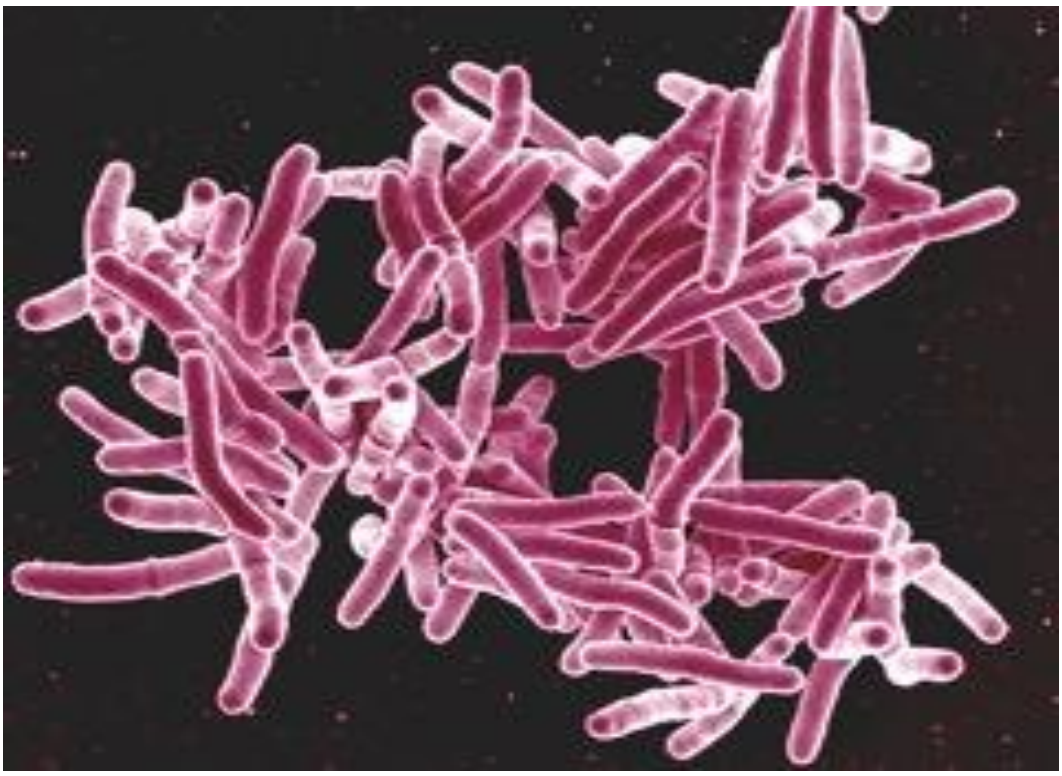

Plate I:*Mycobacterium tuberculosis*, (causes of Tuberculosis)

Source: National Institute of Allergy and Infectious Disease

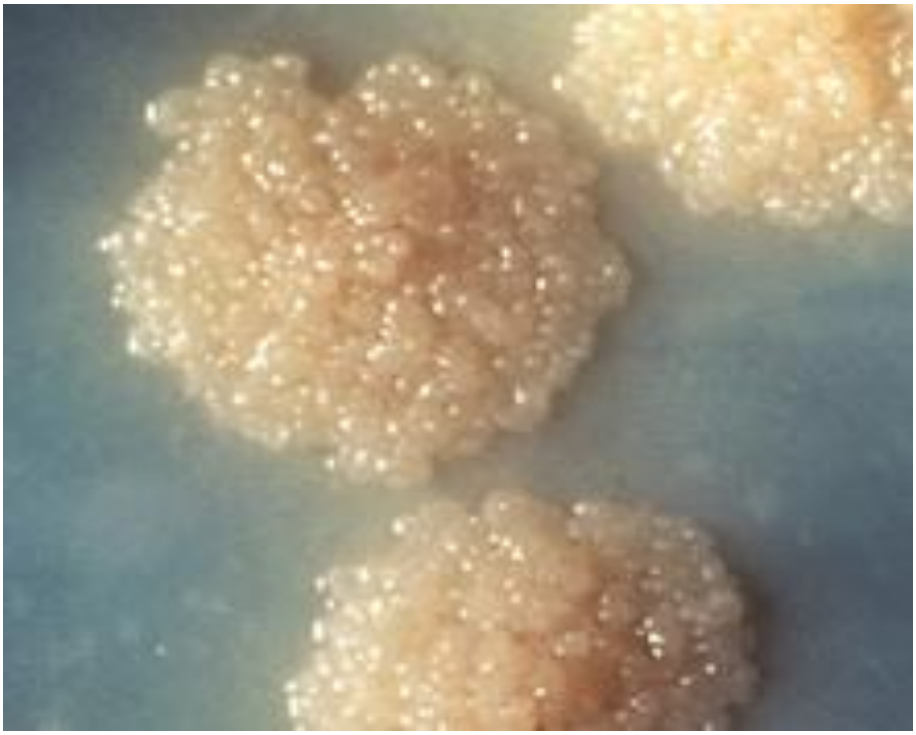

Plate II: Colonies of *Mycobacterium tuberculosis* on a culture plate

Source: Center for Disease Control and Prevention's Public Health Image Library(PHIL), with identification number **#4428**.by Dr George Kubica 1976

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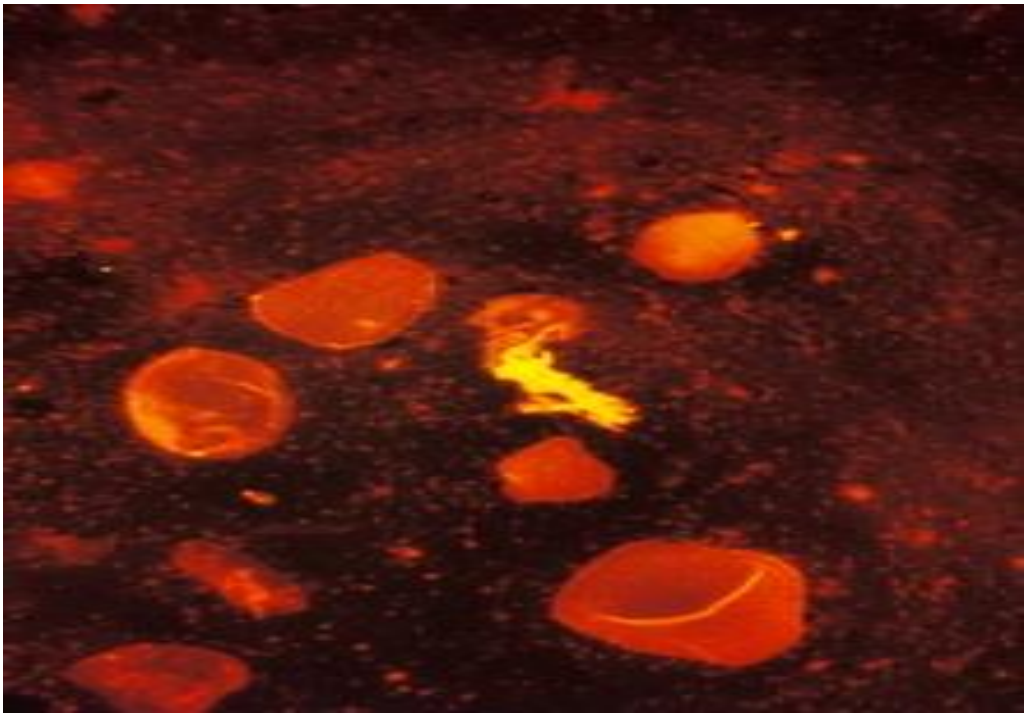

Plate III: A sputum smear stained using fluorescent acid fast stain

Source: Center for Disease Control by/R W Smithwick

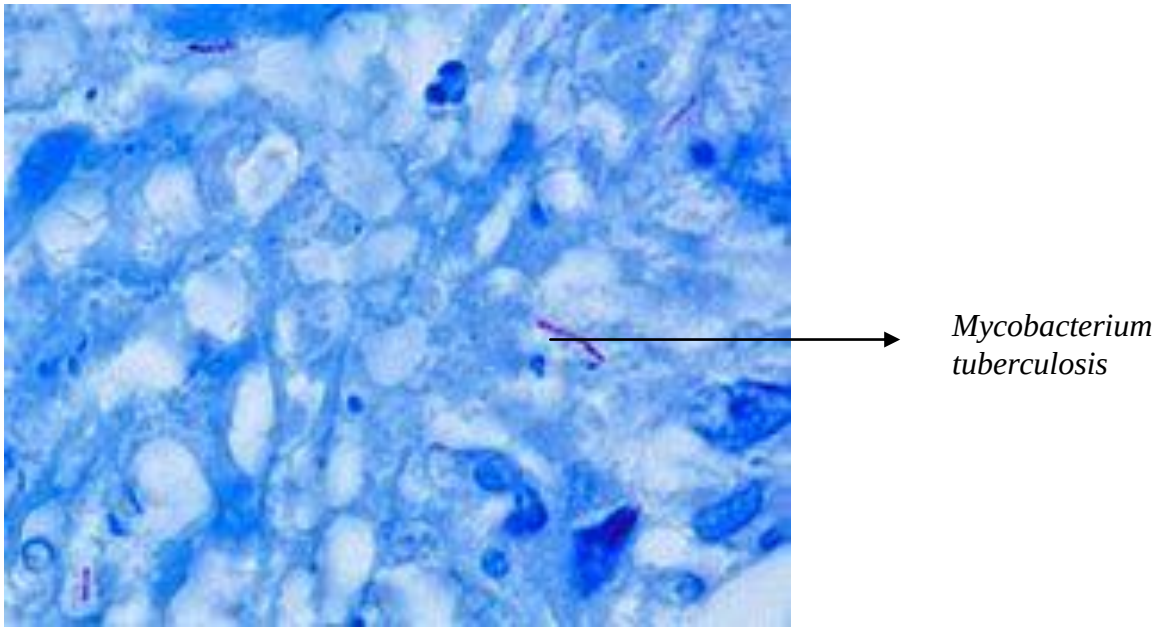

Plate V*: M. tuberculosis* (stained red) in sputum/s tissue (blue)

Source: From the Centre for Disease and Prevention's Control Public Health Image Library by Dr. Edwin P. Ewing, Jr. (PHIL #28), 1994.

Appendix XI: Occurrence of Rimfapicin Resistance Tuberculosis among the Enrolled subjects from the Five Hospitals Using Microscopy, Genexpertsand Culture

General Hospitals	Total Number (%)	Microscopy	Genexperts			Culture	TB ID	
		Number Positive (%)	Number Not Detected, no rifampicin (%)	Number MTB Detected, No Rifampicin (%)	Number MTB Detected, Rifampicin (%)	Number Positive for TB (%)	Number of TB Detected (%)	Number of TB Not Detected (%)
Asokoro	**156 (23.2)**	29 (18.6)	120 (76.9)	34 (21.8)	2 (1.3)	32 (20.5)	25 (78.1)	7 (21.9)
Nyanya	**163 (24.3)**	59 (36.2)	112 (68.7)	51 (31.3)	0 (0.0)	49 (30.1)	44 (89.8)	5 (10.2)
Maitama	**100 (14.9)**	19 (19.0)	82 (82.0)	18 (18.0)	0 (0.0)	28 (28.0)	22 (78.6)	6 (21.4)
Wuse	**152 (22.7)**	22 (14.5)	129 (84.9)	21 (13.8)	2 (1.3)	34 (22.4)	24 (68.6)	11 (31.4)
DHQ	**100 (14.9)**	18 (18.0)	84 (84.0)	16 (16.0)	0 (0.0)	14 (14.0)	6 (54.5)	5 (45.5)
Total	**671 (100.0)**	**147 (21.9)**	**527 (78.5)**	**140 (20.9)**	**4 (0.6)**	**157 (23.4)**	**121 (78.1)**	**34 (21.9)**

	Chi-square	df	P value
Microscopy	26.753	4	0.000**
DNA Genotyping	21.78	4	0.005**
Culture	15.736	4	0.046*
TB ID	9.338	4	0.053*

Key: * - significant, ** = highly significant,
No= Number,
(%) = percentage

Appendix XVII
Ethical Approval

FEDERAL CAPITAL TERRITORY
HEALTH RESEARCH ETHICS COMMITTEE

Research Unit, Room 10, Block A Annex, HHSS
FCT Secretarial No. 1 Kapital Street Area 11, Garki, Abuja - Nigeria

Name of Principal Investigator: Danladi Charity Makolo
Address of Principal Investigator: DHQ Medical Centre, Mogadishu Cantonment Asokoro Abuja.
Date of receipt of valid application: 18/03/2014

NOTICE OF APPROVAL AFTER COMMITTEE REVIEW
Protocol Approval Number: FHREC/2014/01/14/24-04-14

TITLE: **Anti-bacterial Susceptibilities of Mycobacterium Species Isolated From Sputum Samples Of Outpatients In Selected FCT Hospitals Abuja**

The research described in the submitted protocol has been reviewed.

Documents Reviewed:
(i)Application form
(ii)Curriculum Vitae of the Investigator
(iii)Research Protocol:
* Questionnaires
* Participant Information Sheet/ Informed Consent Form

On the basis of the review, this research has been approved by the Committee (FHREC). Subsequent changes are not permitted in this research without prior approval by the FHREC.

This approval dates from **24/04/2014 to 23/04/2015.** 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 FHREC assigned protocol approval number and duration of FHREC approval of the study.

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. The FHREC reserves the right to conduct compliance visit to your research site without previous notification.

In multiyear research, endeavor to submit your annual report to the FHREC early in order to obtain renewal of your approval and avoid disruption of your research. At the end of the research, a copy of the final report of the research should be forwarded to FHREC for record purposes.

Ikwubiela S. Adom
Secretary, FHREC
April 24, 2014

24 APR 2014
APPROVED
Federal Capital Territory Health Research Ethics Committee