MOLECULAR DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* AND RIFAMPICIN RESISTANCE AMONG PATIENTS ATTENDING DIRECTLY OBSERVED TREATMENT SHORTCOURSE(DOTS) CLINIC IN AMINU KANO TEACHING HOSPITAL KANO, NIGERIA.

BY

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SUPERVISED BY

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October, 2015

DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Dr. Dalha Wada Taura and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write up by (Tukur Wada Panda, SPS/12/MMB/00029) were carried out under my supervisor.

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DEDICATION

This work is dedicated to my late father Mal. Wada Ibrahim Panda may his soul rest in peace, Ameen.

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LIST OF ABBREVIATIONS

AFB	-	Acid Fast Bacilli
BSC	-	Biological Safety Cabinet
BSL II	-	Biosafety Level II
DOTS	-	Directly observed treatment, short-course.
DNA	-	Deoxy-ribonucleic acid
DST	-	Drug Susceptibility test
FM	-	Fluorescent microscopy
LJ	-	Lowenstein Jensen
LPA	-	Line Probe Assay
MTBC	-	*Mycobacterium tuberculosis* complex
MGIT	-	*Mycobacterium*growth indicator tube
NTM	-	Non-tuberculosis *Mycobacterium*
NALC	-	n-acetly-l-cysteine
NaOH	-	Sodium hydroxide
$NaNO_3$	-	Sodium Nitrate
NRA	-	Nitrate reduction assay

PBS	-	Phosphate Buffered Saline
PCR	-	Polymerase Chain Reaction
PPE	-	Personal Protective Equipment
PCC	-	Probe Check Control
QA	-	Quality Assurance
QC	-	Quality Control
RIF	-	Rifampicin
RNA	-	Ribonucleic Acid
SIRE	-	Streptomycin, Isoniazid, Rifampicin, Ethambutol
SPC	-	sample Processing Control
SOP	-	Standard Operating Procedure
TB	-	Tuberculosis
UC	-	Universal control
ZN	-	Zeihl-Neelson

ABSTRACT

A total of three hundred (300) sputum samples were examinedfrom patients attending Directly Observed Treatment Shortcourse(DOTS) Clinic of Aminu Kano Teaching Hospital, Kano with presumptive case of pulmonary tuberculosis between September, 2013 to October, 2014 with aim of detecting the incidence rate of *Mycobacterium tuberculosis*complex(MTBC) and identifying Rifampicin resistant, using GeneXpert machine which simultaneously detected MTBC and Rifampicin resistance in a single run, Culture and drug susceptibility test (DST) method that also detected MTBC and Rifampicin resistant respectively. Out of 300 sputum sample examined 104 yielded positive giving an overall incidence rate of 34.7%. Of these 15 were found to be resistance to Rifampicin with 14.4% incidence rate.In addition, out of the 300 samples examined among the three subject groups, out of which 85(28.3%), 132(44.0%) and 83(27.7%) were DR-TB suspect, HIV positive and smear negative subject groups respectively. Of which the highest incidence rate of *Mycobacterium tuberculosis* among the three subject groups were HIV positive DR-TB suspect, and smear negative subject groups with 46(15.3%), 34(11.4%) and 24 (8.0%) respectively. For Rifampicin resistance the incidence rate were found to be 12(11.5%), 2(1.92%) and 1(0.96) in DR-TB suspects, smear negative and HIV positive subject groups. With regards to sexes the incidence of *Mycobacterium tuberculosis*was higher in males with 61(20.3%) than females with 43(14.4%), while Rifampicin resistance in males was said to be higher than in females as 10(9.6%) and 5(4.8%) respectively. However with the regards to the age groups the incidence of *Mycobacterium tuberculosis*was found to be higher between (28 – 35) years of age groups with 34(11.33%) detection rates and lower at > 46 years of age groups with detection rates of 17(5.66%), while the incidence rate Rifampicin resistance was higher among > 46 years of age groups with 4(3.85%) and lower between the age groups of (15 – 27) years and (36 – 45) years with 2(1.92%). This study gives the latest incidence rate of *Mycobacterium tuberculosis*and Rifampicin resistance among patients attending DOTS clinic in AKTH and it also concludes that MTB/RIF assay provides sensitive detection of tuberculosis and Rifampicin resistance directly from the sputum sample in less than 2 hours with minimal hand-on time, therefore it is best recommended to be used for diagnosis of TB.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study:

Mycobacterium tuberculosis is an organism that causes tuberculosis. Worldwide, *M. tuberculosis* causes more death than any other single microbial agent. Approximately one-third of the world's population is infected with this organism (Warren, 2010). Each year, it is estimated that 1.7 million people die of tuberculosis and that 9 million new cases occur (Warren, 2010). An estimated 500,000 people are infected with a multidrug resistant strain of *Mycobacterium tuberculosis*(Warren, 2010).Tuberculosis is also one of the deadliest health threats today (Small and Pai, 2010). Traditionally, tuberculosis is mostly being diagnosed by a combination of chest X-rays, the staining of sputum with special dyes, followed by microscopy, the growth of *Mycobacterium tuberculosis* in culture and montoux test (Small and Pai, 2010). Tuberculosis (TB) is a major global health crisis. The world health organization (WHO) estimates the incidence rate for all forms of TB at 133 per 100,000 populations and a prevalence rate of 199 per 100,000 populations (WHO, 2009a). Nigeria ranked 10^{th} among the 22 high burden countries in the world (WHO, 2013). In 2011, Nigeria detected a total of 93,050 cases of TB, which translated into a 43% cases detection rate (WHO, 2013).Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect oter sites as well (extra pulmonary TB). The disease is spread in the air when people who are sick with pulmonary TB expel bacteria, for example by coughing (WHO, 2009b). In general, a relatively small proportion of

people infected with *M. tuberculosis* will develop TB disease; however, the probability of developing TB is much higher among people infected with HIV. Tuberculosis is also more common among men than women, and affects mostly adults in the economically productive age groups (WHO 2009b). Tuberculosis remains the leading cause of death from a curable infectious disease, despite the availability of short-course therapy that can be both inexpensive and effective discovered 50 years ago (WHO, 2014).

The most common method for diagnosing TB worldwide is sputum smear microscopy (developed more than 100 years ago), in which bacteria are observed in sputum samples examined under a microscope. Following recent breakthroughs in TB diagnostics, the use of rapid molecular tests for the diagnosis of TB and drug-resistant TB is increasing. In countries with more developed laboratory capacity, cases of TB are also diagnosed via culture methods, the current reference standard (WHO, 2013).Without treatment, TB mortality rates are high. In studies of the natural history of the disease among sputum smear positive/HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture-positive (but smear negative) cases, 20% died within 10 years (WHO, 2013) . Effective drug treatments were first developed in 1940s. The most effective first-line anti TB drug, Rifampicin became available in the 1960s. The currently recommended treatment for new cases of drug-susceptible TB is a regimen of four first line drugs: Isoniazid (INH), Rifampicin(RMP), Ethambutol (EMB) and Pyrazinamide. Treatment for multidrug- resistant TB (MDRTB), defined as resistant to Isoniazid (INH) and Rifampicin (RMP) (the two most powerful anti-TB drugs) is longer, and requires more expensive and more toxic drugs. For the first time in four decades new TB drugs are starting to emerge from the pipeline and combination regiment that include new

compounds are being tested in clinical trials. There are several TB vaccines in Phase I or Phase II trial. For the time been however, a vaccine that is effective in preventing TB in adults remains elusive (WHO, 2014).

Tuberculosis incidence is falling at a rate of 2% per year and TB death decrease from 1.4 million in 2011 to 1.3 million death in 2012 (WHO, 2013). However further gains are under serious threat due to two urgent challenges. Effort must be intensified to reach the three million new TB cases that are "missed" every year by the health systems. Seventy five of these missed cases are in India, South Africa, Bangladesh, Pakistan, Indonesia, China, DR Congo, Mozambique, Nigeria, Ethiopia,Philippines, and Myanmar(WHO, 2013). In 2012 the large number of new TB cases occurs in Asia accounting for 60% of new cases globally. However, sub-Saharan Africa carries the greatest proportion of new cases per population with over 255 cases pe100, 00 populations (WHO, 2013). One of the key challenges addressed in the new strategy is the changing landscape of tuberculosis care and prevention caused by the global epidemiological and demographic transitions. Increases in the burden of non-communicable diseases and ageing populations are changing the importance of different risk factors for tuberculosis, and the profile of co-morbidities and clinical challenges for people with tuberculosis(WHO, 2013).Although classic risk factors and co morbidities such as overcrowding, under nutrition, silicosis, and HIV infection are crucial to address, chronic conditions that impair host defences against tuberculosis, such as diabetes, are important factors in many settings. (Knut, 2014,Gambo*et.al;* 2013; Creswell*et.al;*2014).There are many new challenges to the control and prevention of TB. Similarly, the emergence of multi resistance TB (MDR-TB) with an estimated 2.2% among new smear positive cases and 9.4% among treatment

cases, has set back some of the progress made in TB control (NTBLCP 2009).An alarming increase in the global incidence of drug resistant *Mycobacterium tuberculosis* infection has created a critical need for methods that can rapidly detect *Mycobacterium tuberculosis* and identify resistant cases (Verma-Basil *et.al*;2004). Failure to quickly and effectively recognise and treat patients with drug resistant tuberculosis (DR-TB), particularly Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis drug (Gebre-Selassie, 2003).

1.2 Justification of the study:

Studies have being conducted to identify the incidence rate of *Mycobacterium tuberculosis* among patients with presumptive tuberculosis using sputum AFB smear microscopy but less known about the incidencerate using GeneXpert technology, thereby the actual rate of *Mycobacterium tuberculosis* was known due the fact that AFB microscopy have a lot of limitations in term of sensitivity and specificity to the *Mycobacterium tuberculosis.* The emergence of resistant TB has been reported worldwide, but there is no such reportat Aminu Kano Teaching Hospital because of the inadequate facility to conduct such research, since the use of AFB smear microscopy cannot be used to detect the resistant to tuberculosis. Surveillance for Rifampicin resistance tuberculosis among HIV-positive, HIV-negative patients and drug resistance suspect would provide physician with information needed to prescribe proper anti-TB drugs for treating Tuberculosis (TB). This is essential for improved patient's management.

1.3.0 Aim and Objectives

1.3.1 Aim:

Theaim of this research is to determine *Mycobacterium tuberculosis* andRifampicin resistance among patients attendingDirectly Observed Treatment Shortcourse(DOTS) Clinic inAminu Kano Teaching Hospital, Kano.

1.3.2 Objectives of the research:

1. To determine the incidence rates of *Mycobacterium tuberculosis*using molecular and culture method.
2. To identify resistance to Rifampicin among the MTBC positive patients using molecular and Drug susceptibility testing.
3. To determine the incidence rates of *Mycobacterium tuberculosis* among HIV positive, DR-TB suspect and sputum AFB smears negative patients using molecular and culture method.
4. To identify resistance to Rifampicin in HIV positive patients and drug resistance suspects as well as in sputum AFB smears negative patients using molecular and drug susceptibility testing method.

CHAPTER TWO

2.0 LITERATURE REVIEW:

Mycobacterium tuberculosis is pathogenic bacterial specie in the family mycobacteriaceae and the causative agent of tuberculosis (Ishmael, 2004). First discovered in 1882 by Robert Koch, *Mycobacterium tuberculosis,* known as "tubercle bacillus" was first described on 24th March, 1882, by Robert Koch (Robert, 2008) who subsequently received the Nobel prize in physiology and medicine for this recovery in 1905, the bacterium is also known as "Koch bacillus" (Robert, 2008). Tuberculosis has existed throughout history, but the name has changed frequently over time. In 1720, though the history of tuberculosis started to take shape into what is known today; as the physician Benjamin Marten describe in his theory of consumption, tuberculosis may be caused by small living creature transmitted through air to other patient (Cole, 2008).*Mycobacterium tuberculosis*has an unusual coating, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which make the cell impervious to gram staining. The Ziehl-Neelson or acid fast stain is used instead (Cole, 2008).

Mycobacterium tuberculosis are non-motile, non-sporulation, weakly gram positive, acid-fast bacilli that appear microscopically as straight or slightly curved rods, 1 to 4um in length and 0.3 to 0.6um wide. *Mycobacterium tuberculosis* is within the order *Actinomyceteles* which it shares with bacteria such as *Corynobacterium specie, Norcadia specie,* and *Rhodococcus specie.* These bacteria also express unique mycolic acid in the

cell wall envelop that play critical role in the structure and function of the cell wall that confers many characteristics in the genus, this includes acid fastness, extreme hydrophobicity, resistance to drying, acidity/alkalinity as well as distinctive immunostimulatory properties (Ishamael,2004).

Scientific classification of *Mycobacterium tuberculosis*complex

Kingdom	-	Bacteria
Phylum	-	Actinobacteria
Class	-	Actinobacteria
Subclass	-	Actinobacteridae
Order	-	Actinomycetales
Suborder	-	Corynobaterianeae
Family	-	Mycobacteriaceae
Genus	-	Mycobacteria
Specie	-	*Mycobacterium tuberculosis*
	-	*M. bovis*
	-	*M. africanum*
	-	*M. microti*
	-	*M. canetti*
	-	*M. caprae*
	-	*M. pinnipedii*

(Ishmael, 2004).

2.1 Evolution and history of *Mycobacterium tuberculosis*

The *Mycobacterium tuberculosis* complex evolved in Africa and most probably in the Horn of Africa (Blouin *et.al;*2012). The *Mycobacterium tuberculosis* group has a number of members that include *Mycobacterium africanum, Mycobacterium bovis*(Dassies's bacillus), *Mycobacterium caprae, Mycobacterium microti, Mycobacterium pinnipediii* and *Mycobacterium orygis.* This group may also include *Mycobacterium canetti clade*(Wooldridge, 2009).The *Mycobacterium tuberculosis* which includes *Mycobacterium protuberculosis*is a group of smooth colony of*Mycobacterium specie.* Unlike the established members of *Mycobacterium tuberculosis* group, they undergo recombination with other species. The majority of the known strains of this group have been isolated from the Horn of Africa. The ancestor of *Mycobacterium tuberculosis* appears to be specie of *Mycobacterium canetti,* first described in 1969 (Blouin, 2014).The bacterium is also known as "Koch bacillus" (Robert, 2008). Tuberculosis has existed throughout history, but the name has changed frequently over time. In 1720, though the history of tuberculosis started to take shape into what is known today; as the physician Benjamin Marten describe in his theory of consumption, tuberculosis may be caused by small living creatures transmitted through air to other patients (Blouin*et.al;* 2014).

2.2 Pathophysiology of *Mycobacterium tuberculosis.*

Mycobacterium tuberculosis requires oxygen to grow. It does not retain any bacteriological stain due to high lipid content in its wall; hence ZiehlNeelson staining or acid fast staining is used. Despite this it considered a gram positive bacterium. While *Mycobacterium* do not seem to fit the gram positive category from an empirical standpoint (i.e. they do not retain the crystal violet stain), they are also classified as acid fast gram positive bacteria due to their lack of an outer cell membrane (Ishmael, 2004).*Mycobacterium tuberculosis* divides every 15-20 hours, which is extremely slow compared to other bacteria, which tend to have division times measured in minutes (*Escherichia coli* can divide roughly every 20 minutes). It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall rich in lipids (e.g. mycolic acid) is likely responsible for this resistance and a key virulence factor (Murray *et.al;*2005).When in lungs, *Mycobacterium tuberculosis* is taken up by alveolar macrophages, but they are unable to digest and eradicate the bacterium. Its cell wall prevents the fusion of the phagosome with the lysosome, which contain a host of antimycobacterial factors. Specifically, Mycobacterium tuberculosis blocks the bridging molecule, early endosomal autoantigen 1 (EEA 1); however, this blockage does not prevent fusion of vesicles fill with nutrients. Consequently, the bacteria multiply unchecked within the macrophage. The bacteria also carry the gene which prevents acidification of phagosome (Bell, 2005). In addition, production of the diterpeneisotuberculosinol prevents maturation of phagosome (Mann *et.al;*2009). The bacteria also evade macrophage-killing by neutralizing reaction nitrogen intermediates (Flynn and Chan, 2003).

2.3 The disease tuberculosis:

Tuberculosis is a widespread and in many cases fatal infectious disease caused by various strains of Mycobacteria, usually *Mycobacterium tuberculosis* (Kumar *et.al;*2007). Tuberculosis typically infects the lungs and can also affect other parts of the body. It is spread through the air when people who have active TB infection cough, sneezes or otherwise transmits respiratory fluids through the air (Konstantinos, 2010). Most infection does not have symptoms, known as latent tuberculosis. About one in ten latent infection eventually progress to active disease which, if left untreated kills more than 50% of those so infected (WHO, 2011).Tuberculosis has been present in human since antiquity (Lawn, and Zumla, 2011).The earliest unambiguous detection of *Mycobacterium tuberculosis* involves evidence of the disease in the remains of bison in Wyoming dated to around 17,000 years ago (Rothschild *et.al;* 2011). However, whether tuberculosis is originated in bovine, then was transferred to humans, or whether it diverged from a common ancestor, is currently unclear (Pearce,2006). A comparison of the genes of *Mycobacterium tuberculosis* complex (MTBC) in human to MTBC in animals suggests human did not acquire MTBC from animals during animal domestication, as was previously believed. Both strains of the tuberculosis bacteria share common ancestor, which could have infected humans as early as the Neolithic revolution (WHO, 2011).Roughly one-third of the world's population has been infected with *Mycobacterium tuberculosis* (WHO, 2011) with new infections occurring in about 1% of the population each year (WHO, 2002). However, most infection remains asymptomatic (Skolnic, 2011). In 2013, an estimated 9 million cases and 1.5 million associated deaths occurred mostly in developing countries (WHO, 2013). In 2012, an estimated 8.6 million chronic cases were active (WHO, 2013). In 2010, 8.8 million new cases of TB were

diagnosed and 1.20 – 1.45 million deaths occurred, most of this occurring in developing countries. (WHO, 2011). Of this 1.45 million deaths, about 0.35 million occur in those also infected with HIV. The absolute number of tuberculosis cases has been decreasing since 2006, new cases have decreased since 2002 (WHO, 2011). Tuberculosis is more common in developing countries, about 80% of the population in many Asian and African countries test positive in tuberculin test, while 5 – 10% of the US population test positive (Kumar*et.al;* 2007).

The classic symptoms of active TB infection are a chronic cough with blood stained sputum, fever,night sweat, and weight loss. Infection of other organs causes a wide range of symptoms. Diagnosis of active TB relies on radiology as well as microscopic examination of sputum and microbiological cultures of body fluids as well as molecular and non-molecular methods. Diagnosis of latent TB relies on the tuberculin skin tests. Treatment is difficult and requires administration of multiple antibiotics over a long period of time. Social contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in multidrug resistant tuberculosis (MDR-TB) infections. Prevention relies on screening programs and vaccination with the Bacillus calmette Guerin vaccine (McShane, 2011),

2.4 Transmission of *Mycobacterium tuberculosis*

Tuberculosis is spread from person to person through the air by droplet nuclei, particles 1 to 5 um in diameter that contain *Mycobacterium tuberculosis complex* (Edwards*et.al;*2006). Droplet nuclei are produced when persons with pulmonary or laryngeal tuberculosis cough, sneeze, speak, or sing. They also maybe produced by

aerosol treatments, sputum induction, and aerosolization during bronchoscopy, and through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory. Droplet nuclei containing two to three *Mycobacterium tuberculosis* organisms and are so small that air currents normally present in any indoor space can keep them airborne for long periods of time (Riley, 2003). Droplet nuclei are small enough to reach the alveoli within the lungs, where the organisms replicate. Although patientswigth tuberculosis also generate larger particles containing numerous bacilli, these particles do not reach alveoli. Organisms deposited on intact mucosa or skin does not invade tissue. When large particles are inhaled, they impact on the wall of the upper airways, where they are trapped in the mucous blanket, carried to the oropharynx, and swallowed or expectorated (CDC, 2004).

Four factors determine the likelihood of transmission of *Mycobacterium tuberculosis*the number of organisms being expelled into the air (Sudre, 2002), the concentration of organisms in the air which is determined by the volume of the space and its ventilation (Statke,2002) the length of time an exposed person breathes the contaminated air (Edwards, 2006) and the immune status of the exposed individual. HIV infected persons and others with impaired cell-mediated immunity are thought to be more likely to become infected with *Mycobacterium tuberculosis* after exposure(Horsburgh, 2006).

Techniques that reduce the number of droplet nuclei in a given spaces are effective in limiting the airborne transmission of tuberculosis. Ventilation with fresh air is especially important, particularly in health care settings, where six or more room-air changes an hour is desirable (CDC, 2004). The number of viable airborne tubercle bacilli can be reduced by ultraviolet irradiation of air in the upper part of the room (Riley, 2004). The

most important means to reduce the number of bacilli released into the air is by treating the patient with effective anti-tuberculosis chemotherapy (Jindani*et.al;*2008). If mask are to be used on coughing patients with infectious tuberculosis, they should be fabricated to filter droplet nuclei and moulded to fit tightly around the nose and mouth. Measures such as disposing of such personal items such as clothes and bedding, sterilizing fomites, used caps, gowns and gauze or paper masks, boiling dishes, and washing walls are unnecessary because they have no bearing on airborne transmission (Jindani*et.al;*2008).

There are five closely related mycobacteria grouped in the *Mycobacterium tuberculosis* complex: *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum*, *Mycobacterium microti, Mycobacterium canetti*(Van Soolingen*et.al;*2007). *Mycobacterium tuberculosis* is transmitted through the airborne route and there are no known animal reservoirs. *Mycobacterium bovis*may penetrate the gastrointestinal mucosa or invade the lymphatic tissue of the oropharynx when ingested in milk containing large numbers of organisms. Human infection with *Mycobacterium bovis* has decreased significantly in developed countries as result of the pasteurization of milk and effective tuberculosis control programs for cattle (O'Relly*et.al;*1995). Airborne transmission of both *Mycobacterium bovis* and *Mycobacterium africanum*can also occur (Danker*et.al;* 2003). *Mycobacterium bovis* BCG is a live-attenuated strains of *Mycobacterium bovis*is widely used as a vaccine for tuberculosis. It may also be used as an agent to enhance immunity against transitional-cell carcinoma of the bladder. When used in this manner, adverse reactions such as dissemination may be encountered, and in such cases *Mycobacterium bovis* BCG may be cultured from non-urinary tract system specimens, i.e. blood, sputum, bone marrow, etc. (Grange *et.al;*2009).

2.5. Pathogenesis of tuberculosis.

After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. Whether or not an inhaled tubercle bacillus establishes an infection in the lung depends on both bacterial virulence and the inherent microbicidal ability of the alveolar macrophage that ingests it (Edwards, 2006). If the bacillus is able to survive initial defences, it can multiply within the alveolar macrophages. The tubercle bacillus grows slowly, dividing approximately every 25 to 32 hours within the macrophage. *Mycobacterium tuberculosis* has no known endotoxins or exotoxins; therefore, there is no immediate host response to infection. The organisms grow for 2 to 12 weeks, until they reach 1000to 10,000 cellsin number, which is sufficient to elicit a cellular immune response that can be detected by a reaction to the tuberculin skin test (Dannenberg, 2002).

Before the development of cellular immunity, tubercle bacilli spread via the lymphatic to the hilar lymph nodes and thence through the bloodstream to more distant sites. Certain organs and tissues are notably resistant to subsequent multiplication of these bacilli. The bone marrow, liver, and spleen are almost always seeded with mycobacteria, but uncontrolled multiplication of the bacteria in these sites is exceptional. Organisms deposited in the upper lung zones, kidneys, bones, and brain may find environments that favour their growth, and numerous bacterial divisions may occur before specific cellular immunity develops and limit multiplication. (Dannenberg, 2002).

In persons with intact cell-mediated immunity, collections of activated T cells and macrophages from formation of granulomas that limit multiplication and spread of the

organism. Antibodies against *Mycobacterium tuberculosis* are formed but do not appear to be protective (Reggiardo, and Middlebrook, 2004). The organism tends to be localized in the centre of the granuloma, which is often necrotic (Cannetti, 2005). For the majority of individuals with normal immune function, proliferation of *Mycobacterium tuberculosis* is arrested once cell-mediated immunity develops, even though small numbers of viable bacilli may remain within the granuloma. Although a primary complex can sometimes be seen on chest radiograph, the majority of pulmonary tuberculosis infections are clinically and radiographically in apparent (Dannenberg, 2002). Most commonly, a positive tuberculosis skin test result is the only indication that infection with *Mycobacterium tuberculosis*has taken place. Individuals with latent tuberculosis infection but not active disease are not infectious and thus cannot transmit the organism (Styblo, 2001).

2.6. Epidemiology of *Mycobacterium tuberculosis.*

Tuberculosis more commonly known as TB, is a bacterial infection that usually affects the lungs but can affect any part of the body except hair, teeth and nail. Tuberculosis remains one of the deadliest diseases in the world. The World Health Organization (WHO, 2006) estimates that each year more than 8 million new cases of tuberculosis occur and approximately 3 million persons die from the disease (WHO, 2006). Ninety-five per cent of tuberculosis cases occur in developing countries where few resources are available to ensure proper treatment and where human immunodeficiency virus (HIV) infection is common. It is estimated that between 19 and 43% of the world's population is infected with *Mycobacterium tuberculosis* (Sudre, 2002).Tuberculosis is a social disease with medical implications. It has always occurred disproportionately among disadvantaged populations such as the homeless, malnourished, and overcrowded. Within

the past decade it also has become clear that the spread of HIV infection and the immigration of persons from areas of high incidence have resulted in increased numbers of tuberculosis cases.Unlike HIV, TB is completely preventable and treatment is a fraction of the cost of medication use to treat HIV (Mayer, 2010). If a person has TB and HIV co-infection, it means they have both HIV and either latent TB or active TB disease. When person have both HIV and TB, each disease speeds up the progress of the other. In addition to HIV infection speeding up the progression from latent to active TB, TB also accelerates the progress of HIV (Mayer, 2010).

In recent decades, the dramatic spread of HIV epidemic in sub-Sahara Africa has result in notification rates of TB increasing up to 10 times in some countries (Dye, 2003). The incidence of TB is also increasing in other high HIV prevalence countries, where the population with HIV infection and TB overlap. Even all the countries with well-organized national tuberculosis programmes have seen an increase in TB cases. This is the underlying factor that suggests that TB control will not make much head way n HIV prevalent settings unless HIV control is also achieved. Tubrculosis is the most treatable HIV related disease and a leading killer of people living with HIV/AIDs control programmes, is an imperative requirement (Cobert, 2003).About one-third of the HIV positive population worldwide is co-infected with *Mycobacterium tuberculosis*. This accounts to about 14 million people worldwide. Globally, it is estimated that about 33% of all people infected with HIV are co-infected with both HIV and TB (between 12 and 15 million people) (WHO, 2011). In part of sub-Sahara Africa, up to 70% of TB patients are co-infected with HIV. It is estimated that up to 33% of all AIDS deaths worldwide can be directly attributed to TB, in sub-Sahara Africa this increase to 50% (WHO,

2003).Tuberculosis and HIV together are responsible for the death of over 4 million people annually. Tuberculosis is one of the most common infections that threaten people living with HIV in the developing countries. Of the 1.7 million deaths from TB in 2008, almost one-third were people co-infected with HIV or AIDS (WHO, 2009). In Asia, where the HIV epidemic is still at early stage, the rate of HIV infection in tuberculosis patients has been lower. A HIV-positive infected with *Mycobacterium tuberculosis* have a 50-60% lifetime risk of developing disease as compared to a HIV negative patient who has only 10% risk (WHO, 2009). This is especially important in India where it is estimated that 40% of the adult population harbour *Mycobacterium tuberculosis*. Hospital based seroprevalence patient studies among tuberculosis patients from different region of India have shown a great variation, the prevalence rate varying from 0.4% - 28.1% have been reported (WHO, 2009). The prevalence of HIV infection among patients of tuberculosis is rising at an alarming rate in the western part of the country like Mumbai (2.56 – 10.15%), Pune (10 – 25.75%) and South India (0.59 – 8.89%) but at much slower pace in north India. A rising trend of HIV infection in patients with pulmonary tuberculosis has also been seen in Lucknow (1.25% in 1996 to 4.28 in 2001). (WHO, 2009). In India, there were an estimated 5.1 million people living with HIV at the end of the year 2002 (WHO, 2009).

2.7.0 Clinical manifestations of tuberculosis.

The clinic manifestations of tuberculosis are quite variable and depend on a number of factors. Before the beginning of the epidemic of infection with HIV, approximately 85% of reported tuberculosis cases were limited to the lungs, with the remaining 15% involving only non-pulmonary or both pulmonary and non-pulmonary sites (Farer *et.al;*

2009). This proportional distribution is substantially different among persons with HIV infection. Although there are no nationaldata in Nigeria that describe the sites of involvement in HIV infected persons with tuberculosis, one large retrospective study of tuberculosis in patients with advanced HIV infection reported that 38% had only pulmonary and non-pulmonary involvement, 30% had only extra-pulmonary sites, and 32% had both pulmonary and non-pulmonary involvement (Small *et.al;*1991). Moreover, extra-pulmonary involvement tends to increase in frequency with worsening immune (Jones, 1993).

2.7.1 Systemic effects of Tuberculosis.

Tuberculosis involving any site may produce symptoms and findings that are not specifically related to the organ or tissues involved but, rather, are systemic in nature. Of the systemic effects, fever is the most easily quantified. The frequency with which fever has been observed in patients with tuberculosis varies from approximately 37 to 80% (Arango, 2008). The medium duration of fever after beginning treatment was 10 days, with a range of 1 to 109 days. Loss of appetite, weight loss, weakness, night sweats, and malaise are also common but are more difficult to quantify and may relate to co-existing disease.

The most common haematological manifestations of tuberculosis are increases in the peripheral blood leukocyte count and anaemia, each of which occurs in approximately 10% of patients with apparently localized tuberculosis (Cameron, 2004). The increase in

white blood cell counts is usually slight, but leukemoid reactions may occur. Leukopenia has also been reported. An increase in peripheral blood monocyte and eosinophil counts also may occur with tuberculosis. Anaemia is common when the infection is disseminated. In some instances, anaemia or pancytopenia may result from direct involvement of the bone marrow and, thus, be a local, rather than a remote, effect.

Hyponatremia, which in one series was found to occur in 11% of patients (Chung, and Hubbard, 1969), has been determined to be caused by production of an antidiuretic hormone-like substance found within affected lung tissue (Cameron, 2004).

In many patients, tuberculosis is associated with other serious disorders. These include HIV infection, alcoholism, chronic renal failure, diabetes mellitus, neoplastic diseases, and drug abuse. The signs and symptoms of these diseases and their complications can easily obscure or modify those of tuberculosis and result in considerable delays in diagnosis or misdiagnoses for an extended periods of time, especially in patients with HIV infection (Kramer, 2000).

2.7.2 Pulmonary Tuberculosis

Sign and Symptoms: Cough is the most common symptom of pulmonary tuberculosis. Early in the course of the illness it may be non-productive, but subsequently, as inflammation and tissue necrosis ensure, sputum is usually produced and is key to most of the diagnostic methods. Haemoptysis may rarely be a presenting symptom but usually is the result of previous disease and does notnecessarily indicate active tuberculosis. Haemoptysis may result from residual tuberculous bronchiectasis, rapture of a dilated vessel in the wall of a cavity (Rasmussen's aneurysm), bacterial or fungal infection

(especially *Aspergillus* in the form of a mycetoma) in a residual cavity or from erosion of calcified lesion into the lumen of an airway (broncholithiasis). Inflammation of the lung parenchyma adjacent to a pleural surface may cause pleuritic pain. Dyspnea is unusual unless there is extensive disease. Tuberculosis may, however, cause severe respiratory failure (Huseby, 2006).

Physical findings in pulmonary tuberculosis are not generally helpful in defining the disease. Rales may be heard in the area of involvement as well as bronchial breath sounds if there is lung consolidation (Daley, 2002).

Pulmonary tuberculosis nearly always causes abnormalities on the chest film, although an endobronchial lesion may not be associated with a radiographic finding. In addition, in patients with pulmonary tuberculosis disease and HIV infection.In primary tuberculosis occurring as a result of recent infection, the process is generally seen as middle or lower lung zone infiltrate, often associated with ipsillateral hilar adenopathy. Atelectasis may result from compression of airways by enlarged lymph nodes. This manifestation is more common in children. If the primary process persist beyond the time when specific cell-mediated immunity develops, cavitation may occur (so-called “progressive primary” tuberculosis) (Huseby, 2006).

Tuberculosis that develops as a result of endogenous reactivation of latent infection usually causes abnormalities in the upper lobes of one or both lungs. Cavitation is common in this form of tuberculosis. The most frequent sited are the apical and posterior segment of the left upper lobe. Healing of the tuberculous lesions usually results in development of a scar with loss of lung parenchymal volume and, often, calcification. In

the immunocompetent adult with tuberculosis, intrathoracic adenopathy is uncommon but may occur, especially with primary infection. In contrast, intrathoracic or extrathoracic lymphatic involvement is quite common in children. As tuberculosis progresses, infected material may be spread via the airways into other parts of the lungs, causing a patchy bronchopneumonia. Erosion of a parenchymal focus of tuberculosis into a blood or lymph vessel may lead to dissemination of the organism and a "miliary" (evenly distributed small nodules) pattern on the chest film. Disseminated tuberculosis can occur in primary disease and may be an early complication of tuberculosis in children (both immunocompetent and immunocompromised). When it occurs in children, it is most common in infants and the very young (< 5years) (Markowit*et.al;* 1993)

Old healed tuberculosis presents a different radiologic appearance from active tuberculosis. Dense pulmonary nodules, with or without visible calcification, may be seen in the hilar area or upper lobes. Smaller nodules, with or without fibrotic scars, are often seen in the upper lobes, and upper lobe volume loss often accompanies these scars. Nodules and fibrotic lesions of old healed tuberculosis have well-demarcated, sharp margins and are often described as "hard". Bronchiectasis of the upper lobes is a nonspecific finding that sometimes occurs from previous pulmonary tuberculosis, pleural scarring may be caused by trauma or other infections. Nodules and fibrotic scars may contain slowly multiplying tubercle bacilli with significant potential for future progression to active tuberculosis. Person who have nodular or fibrotic lesions consistent with findings of old tuberculosis on chest radiograph and a positive tuberculin skin test reaction should be considered high-priority candidates for treatment of latent infection regardless of age. Conversely, calcified nodular lesions (calcified granuloma) or apical

pleural thickening poses a much lower risk for future progression to active tuberculosis (Grzybowski, 2001).

In patients with HIV infection, the nature of the radiographic findings depends to a certain extent on the degree of immunocompromise produced by the HIV infection. Tuberculosis that occurs relatively early in the course of HIV infection tends to have the typical radiographic findings described above (Pitchenik and Rubinson,2005). With more advanced HIV disease the radiographic findings become more "atypical": cavitation is uncommon, and lower lung zone or diffuse infiltrates and intrathoracic adenopathy are frequent.

2.7.3 Extrapulmonary Tuberculosis.

Extrapulmonary tuberculosis usually presents more of a diagnostic problem than pulmonary tuberculosis. In part this relates to its being less common and, therefore, less familiar to most clinicians (Alvarez, and McCabe, 2004). In addition, extrapulmonary tuberculosis involves relatively inaccessible sites and, because of the nature of the sites involved, fewer bacilli can cause much greater damage. The combination of small numbers of bacilli and inaccessible sites causes bacteriologic confirmation of a diagnosis to be more difficult, and invasive procedures are frequently required to establish a diagnosis (Alvarez and McCabe, 2004).Extrapulmonary tuberculosis in HIV-infected patientspresumably are the basis for the high frequency of extrapulmonary tuberculosis among patients with HIV infection is the failure of the immune response to contain *Mycobacterium tuberculosis*, thereby enabling haematogenous dissemination and subsequent involvement of single or multiple non-pulmonary sites. Because of the

frequency of extrapulmonary tuberculosis among HIV-infected patients, diagnostic specimens from any suspected site of disease should be examined for mycobacteria. Moreover, cultures of blood and bone marrow may reveal *Mycobacterium tuberculosis* in patients who do not have an obvious localized site of disease but who are being evaluated because of fever (Alvarez and McCabe, 2004).

Disseminated tuberculosis occurs because of the inadequacy of host defences in containing tuberculous infection. This failure of containment may occur in either latent or recently acquired tuberculous infection. Because of HIV or other causes of immunosuppression, the organism proliferates and disseminates throughout the body (Munt, 2001). Multiorgan involvement is probably much more common than is recognised because, generally, once *Mycobacterium tuberculosis* is identified in any specimen, other sites are not evaluated. The term “miliary” is derived from the visual similarity of some disseminated lesions to millet seeds. Grossly, these lesions are 1 similarity of some disseminated lesions to millet seeds. Grossly, these lesions are 1 to 2mm yellowish nodules that, histologically, are granulomas. Thus disseminated tuberculosis is sometimes called “miliary” tuberculosis. When these small nodules occur in the lung, the resulting radiographic pattern is also termed “miliary” (Massaro, 2004).

Because of the multisystem involvement in disseminated tuberculosis, the clinical manifestations are protean. The presenting symptoms and signs are generally nonspecific and are dominated by systemic effects, particularly fever, weight loss, night sweats, anorexia, and weakness (Grieco, and Chmel, 1974). Other symptoms depend on the relative severity of disease in the organs involved. A productive cough is common because most patients with disseminated disease also have pulmonary involvement.

Headache and mental status changes are less frequent and are usually associated with meningeal involvement (Munt, 2001). Physical findings likewise are variable. Fever, wasting, hepatomegaly, pulmonary findings, lymphadenopathy, and splenomegaly occur in descending order of frequency (Massaro, 2004).

The chest film is abnormal in most but not all patients with disseminated tuberculosis. In the series reported by Grieco and Chmel (1974), only 14 of 28 patients (50%) had a miliary pattern on chest film, whereas 90% of 69 patients reported by Munt (2001) had a miliary pattern (Munt, 2001). Overall, it appears that at the time of diagnosis approximately 85% of patients have the characteristic radiographic findings of miliary tuberculosis (Munt, 2001). Other radiographic abnormalities may be present as well. These include upper lobe infiltrates with or without cavitation, pleural effusion, and pericardial effusion. In patients with HIV infection the radiographic pattern is usually one of diffuse infiltration rather than discrete nodules (Munt, 2001).

2.7.4 Lymph node tuberculosis:

Tuberculous lymphadenitis usually presents as painless swelling of one or more lymph nodes. The nodes involved most commonly are those of the posterior or anterior cervical chain or those in the supraclavicular fossa. Frequently the process is bilateral and other non-contiguous groups ofnodes can be involved (Kent, 2007). At least initially the nodes are discrete and the overlying skin is normal. With continuing disease the nodes may become matted and the overlying skin inflamed. Rupture of the node can result in formation of a sinus tract, which may be slow to heal. Intrathoracic adenopathy may compress bronchi, causing atelectasis leading to lung infection and perhaps

bronchiectasis. This manifestation is particularly common in children. Needless biopsy or surgical resection of the node may be needed to obtain diagnostic material if the chest radiograph is normal and the sputum smear and culture are negative (Kent, 2007).

In persons not infected with HIV but with tuberculous lymphadenitis, systemic symptoms are not common unless there is concomitant tuberculosis elsewhere. The frequency of pulmonary involvement in reported series of patients with tuberculous lymphadenitis is quite variable, ranging from approximately 5 to 70% (Kent, 2007). In HIV-infected persons lymphadenitis is commonly associated with multiple organ involvement (Kent, 2007).

2.7.5 Pleural Tuberculosis:

There are two mechanisms by which the pleural space becomes involved in tuberculosis. The difference in pathogenesis results in different clinical presentations approaches to diagnosis, treatment, and sequelae. Early in the course of a tuberculous infection a few organisms may gain access to the pleural space and, in the presence of cell-mediated immunity, cause a hypersensitivity response (Berger, and Mejia, 2003). Commonly this form of tuberculous pleuritic goes unnoticed, and the process resolves spontaneously. In some patients, however, tuberculous involvement of the pleura is manifested as an acute illness with fever and pleuritic pain. If the effusion is large enough, dyspnea may occur, although the effusion generally is small and rarely are bilateral. In approximately 30% of patients there is no radiographic evidence of involvement of the lung parenchyma; however, parachymal disease is nearly always present, as evidenced by findings of lung dissection (Berger, and Mejia, 2003).

The second variety of tuberculous involvement of the pleura is empyema. This is much less common than tuberculous pleurisy with effusion and results from a large number of organisms spilling into the pleural space, usually from rupture of a cavity or an adjacent parenchymal focus via a bronchopleural fistula (Johnson, 2003). A tuberculous empyema is usually associated with evident pulmonary parenchymal disease on chest films and air may be seen in the pleural space. In the absence of concurrent pulmonary tuberculosis, diagnosis of pleural tuberculosis requires thoracentesis and, usually, pleural biopsy (Johnson, 2003).

2.7.6 Genitourinary tuberculosis:

In patients with genitourinary tuberculosis, local symptoms predominate and systemic symptoms are less common (Christensen, 2004). Dysuria, haematuria, and frequent urination are common, and flank pain may also be noted. However, the symptoms may be subtle, and, often, there is advanced destruction of the kidneys by the time a diagnosis is established (Lattimer, 2005). In women genital involvement is more common without renal tuberculosis than in men and may cause pelvic pain, menstrual irregularities, and infertility as presenting complaints (Christensen, 2004). In men a painless or only slightly painful scrotal mass is probably the most common presenting symptom of genital involvement, but symptoms of prostatitis, orchitis, or epididymitis may also occur (Christensen, 2004). A substantial number of patients with any form of genitourinary tuberculosis are asymptomatic and are detected because of an evaluation for an abnormal routine urinalysis. In patients with renal or genital tuberculosis, urinalyses are abnormal in more than 90%, the main finding being pyuria, and/or haematuria (Christensen, 2004). The finding of pyuria in acid urine with no routine bacterial organisms isolated from a

urine culture should prompt an evaluation for tuberculosis by culturing the urine for mycobacteria. Acid-fast bacilli (AFB) smears of the urine should be done, but the yield is low. The suspicion of genitourinary tuberculosis should be heightened by the presence of abnormalities on the chest film. In most series, approximately 40 to 75% of patients with genitourinary tuberculosis have chest radiographic abnormalities; although in many these may be the result of previous, not current, tuberculosis (Christensen, 2004).

2.7.7 Skeletal tuberculosis:

The usual presenting symptom of skeletal tuberculosis is pain (Berney, 2002). Swelling of the involved joint may be noted, as may limitation of motion and, occasionally, sinus tracts. Systemic symptoms of infection are not common. Since the epiphyseal region of bones is highly vascularized in infants and young children, bone involvement with tuberculosis is much more common in children than adults. Approximately 1% of young children with tuberculosis disease will develop a bony focus (Gutman, 2003). Because of the subtle nature of the symptoms, diagnostic evaluations often are not undertaken until the process is advanced. Delay in diagnosis can be especially catastrophic in vertebral tuberculosis, where compression of the spinal cord may cause severe and irreversible neurologic sequelae, including paraplegia.

Fortunately, such neurological sequelae represent the more severe end of the spectrum (Gutman, 2003). Early in the process the only abnormality noted may be soft tissue swelling. Subsequently, subchondral osteoporosis, cystic changes, and sclerosis may be noted before joint space is actually narrowed. The early changes of spinal tuberculosis may be particularly difficult to detect by standard films of the spine. Computed

tomographic scans and magnetic resonance imaging of the spine are considerably more sensitive than routine films and should be obtained when there is a high index of suspicion of tuberculosis. Bone biopsy may be needed to obtain diagnosis materials if the chest radiograph in normal and the sputum smear and culture are negative (Gutman, 2003).

Central nervous system tuberculosis: Tuberculous meningitis is a particularly devastating disease. Meningitis can result from direct meningeal seeding and proliferation during a tuberculous bacillemia either at the time of initial infection or at the time of breakdown of an old pulmonary focus, or can result from breakdown of an old parameningeal focus with rupture into the subarachnoid space. The consequences of subarachnoid space contamination can be diffuse meningitis or localized arteritis. In tuberculous meningitis the process is localized primarily at the base of the brain (Gutman,2003). Symptoms, therefore, include those related to cranial nerve involvement as well as headache, decreased level of consciousness, and neck stiffness. The duration of illness before diagnosis is quite variable and relates in part to the presence or absence of other sites of involvement. In most series more than 50% of patients with meningitis have abnormalities on chest film, consistent with an old or current tuberculous process, often military tuberculosis (Gutman, 2003).

Physical findings and screening laboratory studies are not particularly helpful in establishing a diagnosis. In the presence of meningeal signs on physical examination, lumbar puncture is usually the next step in the diagnostic sequence. If there are focal findings on physical examination or if there are suggestionsof increased intracranial pressure, a computerized tomographic scan of the head. If it can be obtained

expeditiously, should be performing before the lumbar puncture (Gutman, 2003). With meningitis, the scan may be normal but can also show diffuse oedema or obstructive hydrocephalus. Tuberculomas are generally seen as ring-enhancing mass lesions.

To other major central nervous system form of tuberculosis, the tuberculoma. Present a more subtle clinical picture than tuberculous meningitis (Gutman, 2003). The usual presentation is that of a slowly growing focal lesion, although a few patients have increased intracranial pressure and no focal findings. The cerebrospinal fluid is usually normal, and the diagnosis is established by computed tomographic or magnetic resonance scanning and subsequent resection, biopsy, or aspiration of any ring-enhancing lesion.

2.7.8 Abdominal tuberculosis:

Tuberculosis can involve any intra-abdominal organ as well as the peritoneum, and the clinical manifestations depend on the areas of involvement. In the gut itself tuberculosis may occur in any location from the mouth to the anus, although lesionsproximal to the terminal ileum are unusual (Gutman, 2003). The most common sites of involvement are the terminal ileum and caecum, with other portions of the colon and the rectum involved less frequently. In the terminal ileum or caecum the most common manifestations are pain, which may be misdiagnosed as appendicitis, and intestinal obstruction (Gutman, 2003). A palpable mass may be noted that, together with the appearance of the abnormality on barium enema or small bowel films can easily be mistaken for a carcinoma. Rectal lesions usually present as anal fissures, fistulae, or perirectal abscesses. Because of the concern with carcinoma, the diagnosis often is made at surgery. However, laparoscopy or colonoscopy with biopsy may be sufficient to obtain diagnostic material.

Tuberculosis peritonitis frequently causes pain as its presenting manifestation, often accompanied by abdominal swelling (Gutman, 2003). Fever, weight loss, and anorexia are also common. Active pulmonary tuberculosis is uncommon in patients with tuberculous peritonitis. Because the process frequently coexists with other disorders, especially hepatic cirrhosis with ascites, the symptoms of tuberculosis may be obscured. The combination of fever and abdominal tenderness in a person with ascites should always prompt an evaluation for intra-abdominal infection, and a paracentesis should be performed (Gutman, 2003). However, this is often not diagnostic, and laparoscopy with biopsy is recommended if tuberculosis is suspected.

Pericardial tuberculosis: The symptoms, physical findings, and laboratory abnormalities associated with tuberculous pericarditis may be the result of either the infectious process itself or the pericardial inflammation causing pain, effusion, and eventually hemodynamic effects. The systemic symptoms produced by the infection are quite nonspecific. Fever, weight loss, and night sweats are common in reported series (Dye, 2003). Symptoms of cardiopulmonary origin tend to occur later and include cough, dyspnea, orthopnea, ankle swelling, and chest pain. The chest pain may occasionally mimic angina but usually is described as being dull, aching, and often affected by position and by inspiration.

Apart from fever, the most common physical findings are those caused by the pericardial fluid or fibrosis cardiac tamponade or constriction (Dye, 2003). Varying proportions of patients in reported series have signs of full blown cardiac constriction when first evaluated. It is assumed that in these patients the acute phase of the process was unnoticed. In the absence of concurrent extracardiac tuberculosis, diagnosis of pericardial

tuberculosis requires aspiration of pericardial fluid or, usually, pericardial biopsy (Dye, 2003).

2.8. Drug resistant*Mycobacterium tuberculosis*

Drug resistance in tuberculosis (TB) is a matter of great concern for TB control programme since there is no cure for some multidrug resistance TB (MDR-TB) strains of *Mycobacterium tuberculosis.* There is concern that these strains could spread around the world, stressing the need for additional control measures, such as new diagnostic methods, better drugs for treatment, and a more effective vaccines. MDR-TB, defined as resistance to at least rifampicin (RIF) and isoniazid (INH), is a compounding factor for the control of the disease since patients harbouring MDR strains of *Mycobacterium tuberculosis* need to be entered into alternative treatment regimens involving second line drugs that are more costly, more toxic and less effective (CDC, 2006).

Rifampicin inhibits gene transcription, by interacting with the beta subunit of the ribonucleic acid (RNA) polymerase enzymes. It is a bactericidal against dividing *Mycobacterium tuberculosis* and also has some activity against non- ividing bacilli. *Mycobacterium tuberculosis*strains are normally susceptible to 0.1 -2mg/l. The introduction of Rifampicin thus, allowed reduction of the duration of standard antituberculosis treatment from one year to nine month. (CDC, 2006).

Pharmacokinetics: This drug is readily absorbed from the gastrointestinal tract (food may delay or decrease Rifampicin absorption); within 2 to 4 hours after ingestion of a dose of 600mg peak plasma concentrations may reach 7 – 10mg/l. It can also be given intravenously. In blood, Rifampicin is bound to plasma proteins and distributes into body

tissues and fluids, including cerebrospinal fluid and breast milk, and crosses the placenta. The half -life of Rifampicin ranges from 2 to 5 hours, Rifampicin in metabolized in the liver and excreated in the bile, faeces and urine (Ghandi, 2006).

Toxicity: Rifampicin is well tolerated, although adverse effect may arise during intermediate therapy or when restarting an interrupted treatment. Adverse effect includes diverse alteration in the gastrointestinal tract, skin, kidney and nervous system. It may also produce thermobocytopenia.

2.9. HIV/TB Coinfection.

People living with HIV are 26 – 31 times more likely to develop TB than persons with HIV. TB is the most common presenting illness among people with HIV, including those taking antiretroviral treatment and is major cause of HIV related death. (WHO, 2013). Tuberculosis is the most common opportunistic infection and a major among cause of mortality among HIV positive persons. It is the first manifestation of AIDS in more than 50% of cases in developing countries (WHO, 2010). HIV by itself does not cause multi-drug resistant tuberculosis, but fuels the spread of this dangerous condition by increasing susceptibility to tuberculosis infection and also accelerating the progress from infection to disease (WHO, 2010). In person with AIDS, factors such as increased vulnerability to tuberculosis, increased opportunity to acquire tuberculosis due to overcrowding, exposure to patients with multidrug resistant tuberculosis, increased hospital visits and mal-absorption of antitubercula drugs resulting in sub optimal therapeutic blood vessel in spite of strict adherence to treatment regimen have all been postulated as the possible cause for increased risk of acquired multidrug resistance tuberculosis (Hakem, 2009).

2.10. Laboratory examination of *Mycobacterium tuberculosis*

The contribution of microbiology laboratory to the diagnosis and management of tuberculosis involves the detection and isolation of mycobacteria, the identification of the mycobacterial species or complex isolated, and the determination of susceptibilities of the organisms to antimycobacterial drugs. Only laboratories having a sufficient volume of work and assured competence should provide clinicalmycobacteriology services. Such procedures are time-consuming and employ re-agents and special techniques not used routinely in the study of bacteria in other genera. Furthermore, handling of mycobacterial specimens requires special safety precautions and suitable isolation areas that may place a burden on some laboratories.

2.10.1 Specimen collection, storage and transport.

Pre-analytic processes are the most important aspects in the TB laboratory, as inappropriately collected specimens will not produce reliable results for proper management of patients. It is therefore necessary that adequate care can be taken to ensure that the specimens received are of the best quality, suited for AFB smear microscopy, GeneXpert, LPA, culture and DST where necessary. (Banda, 2000).

Specimen:Sputum, other specimen such as urine, aspirate, CSF, pleural fluids, gastric washes and tissues.

2.10.2 Special safety precautions

Always wear personal protective equipment (PPE) such as al laboratory coat and gloves when collecting and handling specimens. Ensure that the specimen cup is leak-proof and that its cover is secured properly.Use appropriate disinfectants to wipe the outside of specimen cups if leakage is suspected. Cotton wool or absorbent gauze soaked in freshly prepared mycobactericidal disinfectant can be used for this. The disinfectant-soaked cotton wool or absorbent gauze is use wipe bench tops frequently to ensure that any spill from specimen does not cause contamination.Ensure that there is no eating or drinking in the specimen collection area. (Banda, 2000).

2.10.3 Quality control and quality assurance

Ensure that patients are properly educated on how to collect appropriate specimens and adequate amounts of specimens are collected. About 3.0 – 10.0ml of specimen should be collected into the sputum cup; a mark can be made on the cup to guide the patient.Ensure that specimen cups are properly labelled and label the slide of the container and the cover.

Specimens for culture should be processed within 1 hour of receipt. If there is delay in processing, specimens should be stored at 2 - 8^0C for up to 5 days. Make sure that specimens are not more than 24 hours old if they are to be cultured but if specimens have been kept cool (between 4 - 8^0C), they could be up to 5 days old when submitted.Ensure that adequate information is obtained with the specimens such as name and basic demographic e.g. sex, age, address, telephone number, hospital number etc. Date and time of collection, HIV status and reason for examination (Banda, 2000).

Procedure

Take the labelled specimen container to an open space.Open the lid of specimen container and take a deep breath and cough deeply.Spit out material into the specimen container.Continue this process until the required amount of specimen is collected into the container.Replace the lid on the container, ensuring that it is secure.Bring container with specimen to the laboratory. (Banda, 2000).

Patients are then given another labelled container to use to bring in an early morning specimen the following day, at which time they will be given the third container for the second spot specimen. This is applicable to AFB smear microscopy, but for other TB diagnosis one early morning sputum is needed (Banda, 2000).

2.10.4 Receipt of specimen and Transport.

Specimens are received by the laboratory personnel at the reception area, on the receipt of the specimen; it is checked for name or identifier, amount of specimen, condition of specimen etc. as outline in the acceptance and rejection criteria.

All specimens are recorded in the logbook in the reception area. If the specimen is from a DOTS patient, it would already have a TB lab number. If it is a specimen from another lab or clinic, a lab number would be generated. The specimens are then placed in the specimen receipt window to be collected by the laboratory staff.(Banda, 2000).Tightly close specimen container and seal cover paraffin or masking tape.Wrap each specimen individually in absorbent paper towel or cotton wool.Place each specimen in a sealable biohazard or plastic resealable bag.Place the sealed bags of specimens in a transport box with ice packs at the bottom.Place the completed request forms in plastic resealable bags

and seal.Tape the bag with request forms to the top or side of the transport box. (Banda, 2000).

2.10.5 Acceptance criteria

All specimens being sent for laboratory investigation must be clearly labelled with the following information on the container.

Patient identification or laboratory serial number.

Date and time of collection.

All specimens must be accompanied by patient documentation (specimen request form). Information required for each specimen is as follows:

Patient identification or laboratory serial number.

Patient age or date of birth, gender of patient.

Physician's name (most specimens comes from DOTS clinic, so this is optional).

HIV status.

Reason for examination.

Date and time of specimen collection.

Limitations

Some patients are unable to produce specimens that are purulent or mucu-purulent, and, as such, salivary specimens are submitted.

In such cases these specimens should be accepted, but it should be documented on the patient form and the register that a salivary specimen was received.(Banda, 2000).

2.10.6 Preparation of smear, staining and microscopy (ZN and FM)

Principle

Most mycobacteria grow at a relatively slow rate; therefore the acid-fast smear plays an important role in the early diagnosis and treatment of mycobacterial infections. Acid-fast bacilli are difficult to stain because of the lipid content of the cell wall. Heating during staining dissolves the lipid sufficiently to allow penetration of the primary stain. The cell wall retains the primary stain even after exposure to the decolourizing agent: acid alcohol. This resistance to decolourization by acid alcohol is required for an organism to be termed acid fast. A counter stain is then employed to highlight the stain organism (WHO, 2009).

2.10.7 Safety Considerations

Always prepare smears and allow to air dry thoroughly. Smears from cultured materials are to be prepared in a certified BSC, and always wear appropriate PPE suited to the type of specimens being manipulated.Use appropriate disinfectants for discard of materials used for smear preparation.

2.10.8 Procedure for smear preparation: Direct

Arrange specimens and prepare worksheet.Label the slides on frosted end with pencil according to the worksheet.Using applicator stick, transfer a portion of the specimen to the centre of the slide and use the applicator stick to make a thin smear on slide. Use

small, concentric circular movements to make smear of about 2 X 3 cm in diameter.Place the smear on a flat surface with the smear facing upward and allow the smear to air dry for atleast 1 hour.After thorough drying, fix the smears by passing 3 times through a blue flame, ensuring that the smear face upward.The slides are ready for staining with either ZN or FM. (Bench manual, 1998).

2.10.9 Procedure for staining with ZiehlNeelsen stains.

Arrange the fixed slides (including positive and negative controls) to be stained on a staining rack and ensure that slides do not touch each other.

Flood the slides with carbolfuchsin working solution and heat the slides until it start to steam. Allow slides to stain for 5minutes. Do not allow stain to dry or boil.Wash the slides gently with running tap water to remove excess carbolfuchsin (until no more colour runs off).Drain off excess rising water.Flood the slides with acid alcohol for 3 minutes to decolourize. If the red colour has not completely disappeared, repeat until the red colour disappears.Wash the slides for 1 minute with running tap water, and drain off excess rising water.Flood the slides with methylene blue, and counterstain for 1 minute.Rinse with tap water, drain and dry. **Do not blot.**(Bench manual, 1998).

2.10.10 Procedure for staining with Fluorochrome stain (FM).

Place the slides to be stained smear up on a staining rack; including positive and negative control slides.Flood with Auramine O working solution, and allow to stain for 15 minutes.Rinse the slide with tap water and drain.Flood the slides with acid alcohol, and allow to de-colourize for 2 minutes.Rinse the slides with tap water and drain.Flood the slides with potassium permanganate to counterstain for 2 minutes. Time is critical here

because counterstaining for a longer amount of time may quench the fluorescence of the AFB.Rinse with tap water, drain and allow smear to air dry.Examine the smear under a fluorescence microscope as soon as possible (within 24 hours). (Mycobacteriology bench manual, 1998).

2.10.11 Reading of ZN and FM Smears.

Put on the microscope.Place the dry smear on the stage of the microscope and place a drop of immersion oil on the smear.Switch to oil immersion (X100), and examine the smears for the presence of acid-fast bacilli.

A minimum of 100 fields should be examined in a systematic fashion before a smear is reported as negative. (Mycobacteriology bench manual, 1998).

Turn on UV microscope at least 10 minute before use to warm up.Place the dry smear on the stage of the microscope. Use x10 objective and examine at low power for the presence of fluorescence. Use x40 objective to examine any fluorescence seen and to confirm presence of AFB.

A minimum of 100 fields before a smear is reported as negative. (Mycobacteriology bench manual, 1998).

2.10.12 Interpretation of ZN results (x100)

NUMBER OF AFB FOUND	RECORDS	REPORT AS
No AFB in at least 100 field	0	Negative
1 – 9 AFB in 100 field	Actual AFB counts	Actual AFB counts

10 – 99 AFB in 100 field	1+	1+
1 – 10 AFB per field in at least 50 fields	2+	2+
>10 AFB per field in at least 20 field	3+	3+

KEY: AFB = Acid fast Bacilli, 1+ = Low, 2+ = Moderate, 3+ = High.

2.10.13 Interpretation of FM results (x40)

NUMBER OF AFB FOUND	RECORDS	REPORT AS
No AFB in at least 100 field	0	Negative
1 – 19 AFB in 100 field	Actual AFB counts	Actual AFB counts
20 – 199 AFB in 100 field	1+	1+
5 – 50 AFB per field in at least 50 fields	2+	2+
>50 AFB per field in at least 20 field	3+	3+

KEY: AFB = Acid fast Bacilli, 1+ = Low, 2+ = Moderate, 3+ = High.

2.10.14 Quality control and Quality assurance

Always use a positive and negative control smear when staining a batch of smears. Positive control smears can be prepared from a positive specimen, fixed and stored. Negative smears can be prepared from a confirmed negative specimen.

Stains should be filtered regularly, at least weekly, to prevent deposits that can give false positive result.

Limitations

Smears should only be examined by experienced personnel.

At least 100 HPF should be examining before a smear is reported as negative.

2.11.0 Identification of MTBC and drug resistance to RIF using GeneXpertDx system

Principle

The GeneXpert MTB/RIF system is a fully automated, nested real-time PCR system, which detects MTB complex DNA in smear positive and negative sputum samples. It simultaneously identifies mutations in the *rpoB* gene, which are associated with rifampicin resistance. The primers in the Xpert MTB/RIF amplify a portion of the *rpoB* 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 Rifampicin resistance. Furthermore, the assay includes a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. A probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability (Cepheid, 2010).

2.11.1 Storage and handling of Equipment.

Store the Xpert MTB/RIF cartridges and reagents at 2 - 8^0C.Do not use expired reagents and cartridges and do not open a cartridge until you are ready to perform testing.Use the

cartridge within 30 minutes of opening the lid.The cartridge is stable for up to 7 days after opening the package.(Cepheid, 2010).

2.11.2 Start-Up of GeneXpert Instrument

Note: Before start processing of specimen, the following steps were checked,that the GeneXpert instrument was functioning and the modules were available.

The computerwas turn on, and then the GeneXpertDx instrument.On the windows desktop, double click the GeneXpertDx shortcut icon.Log on to the GeneXpertDx System software using your user name and password.Click on check status, and check if modules are available. If not, proceed to trouble shooting in user manual (Cepheid, 2010).

2.11.3 Preparation of Sample

Put on appropriate PPE (gloves, lab coat and N95 respirators, optional).

Disinfect the BSC and bring out the number of cartridges needed.Open the pouch and label each cartridge with the sample identification.Write on the side of the cartridge that does not have the 2D barcode.Leave the specimen in leak-proof sputum collection container.Carefully unscrew the lid of the sputum container and add 2 volume of sample reagent to 1 volume of sputum sample, and close the lid. Shake vigorously 10 - 20 times and incubate for 10 minutes at room temperature.Shake the specimen again vigorously 10 – 20 times and continue incubation for another 5 minutes (Cepheid, 2010).

Note: Samples should be liquefied with no visible clumps of sputum. If there are still clumps of sputum, shake again vigorously and incubate for another 3 – 5 minutes.

2.11.4 Addition of Sample to the Cartridge

Note: Start the test within 30 minutes of adding the sample to the cartridge.

Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark (2.0ml).Open the cartridge lid.Transfer sample into the open port of the Xpert MTB/RIF cartridge and dispense slowly to minimize the risk of aerosol formation.Close the cartridge lid. Make sure the lid snaps firmly into place (Cepheid, 2010).

Note: Remaining liquefied sample may be kept for up to 12 hours at 2 - 8^0C should repeat testing be required.

2.11.5 Start the test on the GeneXpert Instrument

In the GeneXpertDx System window, click create test. The scan cartridge barcode dialog box appears.Scan the barcode test window appears.Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge S/N and Expiration date.In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the View Results window and all the reports.

Click Start Test.If a dialog box appears, type your password.Open the instrument module door with the blinking green light and load the cartridge.Close the door.The test starts and the green light stops blinking.When the test is finished, the light turns off.Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.Dispose of used cartridges in a biohazard discard bag; autoclave before discard (Cepheid, 2010).

2.11.6 Reading and Recording of results.

Viewing Results on GeneXpert Software

In the GeneXpertDx System window, click view results on the menu bar. The view results window appears.If the software reports Error, invalid or No result, repeat the test using the already prepared specimen and a new cartridge.If the test again shows Error, invalid or No result, proceed to troubleshooting section to exudate technical problems before requesting a new specimen (Cepheid-GeneXpert, 2010).

2.11.7 Reporting of Results.

Note: Results must be reported in a special register of TB laboratory examination. Use red ink for positive results.

Report “MTB not detected” or “MTB detected”.

For rifampicin resistance results, report “Rif resistance not detected” or Rif resistance detected.

Report “Please submit a new specimen” if the system repeatedly did not produce a result and you have excluded and/or fixed a technical problem. (Cepheid-GeneXpert, 2010).

2.11.8 Quality Control

Internal quality control using a known MDR sample is performed each time a new lot or batch of reagent is received or every quarter.

2.11.9 Safety precautions

Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents, because it is often impossible to know which might be infectious, all biological specimens should be treated with universal precaution.

Wear protective disposable gloves, laboratory coats or any other PPE that may be necessary when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents. (Cepheid GeneXpert, 2010).

2.12.0 Processing of clinical specimen for TB culture

Principle

Specimen processing involves 3 steps that place prior to smear preparation and inoculation into growth media: digestion (to loosen mucoid material in sputum sample), decontamination (to limit contamination of cultures by fast-growing nonmycobacterial commensal organisms) and concentration (to increase the sensitivity of smear microscopy and culture). N-acetyl-l-cysteine (NALC) is used to digest the mucus. Sodium hydroxide (NaOH) is used to decontaminate the specimen, while sodium citrate acts as a chelating agent (modified Petroffs method). (Isenberg, 1992).

2.12.1 Safety Consideration

Appropriate personal protective equipment must be worn at all times when handling specimen containers and during manipulation of specimen.Laboratory procedures that give rise to infectious aerosols must be conducted in a certified BSC. Therefore, all specimens must be processed in a BSC within the BSL2 or BSL3 facility.Safety centrifuge buckets must be used for centrifugation. Buckets must be loaded and unloaded after centrifugation within the BSC and all items are be removed under the BSC and must first be sprayed with 0.5% sodium hydrochloride or other mycobactericidaldisinfectant and dried before removal. The Use of glass items should be minimized in the BSL2. Plastic transfer pipettes and disposable loops are strongly recommended.Frosted slides that can be marked with a pencil are preferred over diamond markers. Avoid creation of aerosol and separate dry and liquid waste materials.Do not discard the remaining liquid in the pipette; instead, discard the entire pipette with the liquid inside. Pipettes can be discarded in empty media containers, which can be easily autoclaved and discarded.Examine each centrifuge tube for possible leaks. If you discover a cracked centrifuge tube, wipe the outside of that tube with a disinfectant prior to further handling.Decontaminate the centrifuge buckets by disinfecting with 0.5% sodium hypochlorite solution or other suitable disinfectant. (U. S. Dept. of Health 1999).

2.12.2 Quality control and Quality assurance

Document use and preventive maintenance of all equipment used for this procedure.

Always check the expiration of all reagents before using them and add NALC to the NaOH solution just before starting processing, and never use the NALC-NaOH solution if more than 24 hours old.Ensure that all media and reagents used are checked for sterility

after preparation and include blanks (buffer) at the beginning and end of each batch of specimens processed.If possible, subculture eachsediment on blood agar plates after inoculating on LJs.Use only distilled water in reagent preparation to avoid the introduction of AFB. Additionally, reagents should be autoclaved at 120^0C for 15 minutes and sterility checked before use. The presence of AFB in the tap water may result in false positive acid fast smear and/or culture. (Murray, 1995).

2.12.3 Procedure for digestion and decontamination

Arrange equipment, reagents, supplies and specimens in a BSC.Cover the working area with bench pads and spray it with suitable disinfectant.Work in sets of 8 specimens at a time with no more than 16 specimens or one full centrifuge load. This depends on the type of centrifuge available.Label each 50.0mL of sputum (3.0 – 10.0 mL are recommended) into the labelled 50.0mL sterile screw-capped conical centrifuge tube. Handle only one specimen at a time. In case the specimen cannot be transferred into the tube, add an equal amount of buffer to the specimen, mix and then pour into the 50mL tube. **Note:** For blank or negative control, use buffer in place of the specimen and process.Minimize aerosol production by opening the specimen container slowly, avoiding vigorous shaking. Do not leave open specimen containers or tubes in the BSC.Using a sterile universal tube, add NaOH-NALC equal to the amount of specimen in the tube.Recap each tube tightly. Vortex for 20 seconds at a moderate speed and invert each tube 5 times to ensure that NALC-NaOH solution comes into contact with the entire surface of the tube.Avoid extreme agitation or shaking, which can increase the oxidation and inactivation of the NACL.Let the tubes stand for 15 min. Timing is very critical here to ensure that mycobacteria are not over killed.After this time add sterile 0.067 M

phosphate buffer (pH 6.8) to the digested decontaminated specimen up to the 45.0mL mark to reduce the continued action of NaOH and lower the viscosity of the mixture.Recap the tubes tightly, and invert several times to mix the contests. Load tubes in centrifuge buckets inside the BSC. **Note:** Load and unload tubes in centrifuge bucket in BSC.After centrifugation, unload tubes from centrifuge buckets in BSC. Carefully pour off the supernatant into a discard container (with funnel to avoid splash) with suitable disinfectant.Resuspend the sediment with 2.0mL of buffer and is now ready for inoculation to LJ slants, preparation of DNA and smear.If there is a delay in inoculation, store sediment in refrigerator (2 - 8^0C) for not more than 4 hours. (Isenberg, 1992).

2.12.4 Culturing of Mycobacteria using LJ medium

Principle

LJ medium is an egg-based medium containing malachite green. The medium adequately supports the growth of mycobacteria, while the malachite green serves as an inhibitor for the growth of contaminants as well as acid-base indicator. (Isenberg, 1992).

2.12.5 Procedure for inoculation into LJ medium

Remove slants from the refrigerator before start processing to attain room temperature.Label the LJ slants with the specimen number, date, name of patient and test request.

Note: Do not inoculate a slant with a wet surface (contamination source). Remove the excess fluid in the LJ slants prior to inoculation with a sterile pipette or by inverting the tube over sterile gauze.

Inoculate only one medium at a time. Keep caps of centrifuge tubes or culture media closed until ready to inoculate and always minimize the aerosol production by opening the caps of centrifuge tubes slowly.In a BSC and using a sterile plastic pipette, add 2 – 4 drops of sediment to each LJ. Use a new pipette to inoculate each slant.Spread the sediment over the surface of the slant by gently rolling the liquid over the slant. Loose the tube caps, and place in a slanted rack.Incubate in a slanted position with screw caps loose for 1 week to ensure even distribution and absorption of inoculum.After 1 week the tubes are incubated upright for up to 8 weeks.Incubate all cultures at 35 - 37^0C. (Isenberg, 1992).

2.12.6 Examination of Culture medium

Examine the slants after 3 and 7 days of incubation to allow early detection of contaminated cultures (or rapidly growing mycobacteria). After 7 days tighten the caps on the tubes.Thereafter, examine the cultures weekly for growth.Decontaminate and discard negative cultures after 8 weeks of incubation. (Isenberg, 1992).

2.12.7 Interpretation of Results

Appearance of Mycobacteria Growth on LJ Media

Note: Any growth suspected of being mycobacteria must be subject to ZN smear to confirm AFB

Mycobacterium tuberculosis (MTB): Pigment is pale cream. Colonies may be granular, rough and dry.Mucoidforms existbut are rare. Growth is slow.

Mycobacterium avium complex (MAC): Pigment is pale cream to yellow. Colonies are usually smooth or confluent. Growth is slow.

Other Non-tuberculosis mycobacterium (NTM):Pigment may be yellow/orange or non-pigmented. Growth may be rapid or slow. Morphology varies.(Murray, 2005).

Note: Mycobacteria cannot be speciated by their appearance alone in culture since morphology may be varied and may be similar between species. (WHO, 2004).

2.12.8 Reporting of Results

When growth from solid media is seen and confirmed by AFB, results are recorded in the worksheet and reported as follows:

NUMBER OF COLONIES	QUANTIFICATION CODE
None	**0**
Contamination	**C**
<50 colonies	Actual count
50 – 99 colonies	1+
100 – 200 colonies	2+
>200, almost confluent	3+
Confluent growth	4+

(WHO, 2004).

KEY: 1+ = Low, 2+ = Moderate, 3+ = High 4+ = Very High.

2.13.0 IDENTIFICATION AND DST NITRATE REDUCTION ASSAY

PRINCIPLE

Nitrate reduction assay is based on the ability of *Mycobacteria tuberculosis* to reduce nitrate to nitrite, which is routinely used for biochemical identification of mycobacterial species. Briefly, conventional LJ slants containing $NaNO_3$ and an antibiotic are inoculated, incubated and then examined by addition of a reagent that produces a colour change in the presence of mycobacterial growth. Nitrate reduction assay uses the detection of nitrate as indication of growth when it is used as a drug susceptibility test. Susceptibility to RIF and INH can be determined by the GREISS method in 21 – 28 days when applied to smear – positive sputum sample.(Eichbaum and Rubin, 2002).

2.13.1 Antibiotic Preparation

Rifampicin (RMP):950ug/mg (95%)

Weigh 0.021g or (0.0084g) of RIF using analytical balance and dissolve in 5 mL (2mL) of methanol. The Solution is be used immediately (no shelf life). (Eichbaum and Rubin, 2002).

2.13.2 Griess Reagent Preparation

The following reagents are mixed together shortly before use in a glass tube:

- One part 50% (vol/vol) concentrated hydrochloric acid.
- Two parts 0.2% (wt/vol) sulphanilamide.

- Two parts 0.1% (wt/vol) n-1-napthylethylenediamine dihydrochloride.

2.13.3 Inoculation of Processed material

Label 4 LJ tubes per specimen (three controls, one containing RIF).In a BSC, inoculate 0.2 mL (4 drops) of processed specimen to each of the 4 LJ slopes and incubate at 37^0C for 28 days. (Eichbaum and Rubin, 2002).

2.13.4 Reading of Slants and Addition of Greiss Reagent

After 28 days of incubation remove the slopes from incubator and place in BSC.Examine the control slopes for growth of organisms.Using all safety precautions and wearing PPE (N95 masks, gown and gloves), open one control slope slowly and add 0.5 mL GRIESS reagent to the slope.Observe for development of red colour (development of red colour indicates that there was growth on the slope and that the nitrate was converted to nitrite.If red colour was observed in the control, add an equal amount of GREISS reagent to the slope containing RIF.Observe for development of red colour.If there is no growth or colour in the control slope, re-incubate the other control slope with the drug containing slopes for further two weeks and then perform the GREISS test. (Eichbaum and Rubin, 2002).

2.13.5 Interpretation of results

Red colour observed in the control slope indicates that there was mycobacterial growth and that nitrate was converted to nitrite.

No red colour in the control slope indicates that there was no mycobacterial growth; report as no growth seen.

Red colour observed in the control and drug slopes indicate that there was growth in the drug slopes despite the presence of drugs. This indicates that the isolate is resistance to the drugs.

Red colour observed in the control slope and no colour in the drug slopes indicate that the growth of mycobacteria was inhibited by the presence of the drugs and, in this case, the isolate is sensitive to the drug. (Eichbaum and Rubin, 2002).

Limitations

This test is used only with smear positive specimens from culture positive. Smear-negative specimens from culture may need to be inoculated for up to 6 weeks before testing with the GRIESS method and the result of this test is considered preliminary and should be confirmed by other methods such as the LPA's or conventional DST (if available) because occasionally, (1%) *M. tuberculosis* complex fails to produce colour change because they lack nitrate-reductase activity. *M bovis*is also nitrate-reductase negative, so this test cannot be used to detect *M. bovis*. In addition to *M. tuberculosis,* other species of mycobacteria produces nitrates, and if there are NTMs in the population, then there results should be interpreted with caution. (Eichbaum and Rubin, 2002).

2.13.6 Quality control and Quality assurance

Use appropriate positive and negative controls with each batch of specimens inoculated and perform sterility checks on LJs prepared in house.

2.14. Chest X – Ray

This is another diagnostic tools used for the diagnosis of TB patients. In active tuberculosis, infiltrates or consolidations and or cavities are often seen in the upper lungs with or without mediastinal or hilar lymphadenopathy or pleural effusions (tuberculosis pleurisy). However, lesions may appear anywhere in the lungs. In disseminated TB a pattern of much tuberculosis are tiny nodules throughout the lung field and is commonly called milliary TB. In HIV patients, any abnormality may indicate TB or the chest X-ray may even appear entirely normal.

Abnormalities in chest X-ray may not be suggestive, but are not necessarily diagnostic of TB. However, chest radiograph may be used to rule out the possibility of pulmonary tuberculosis in person who has a positive reaction of the tuberculin test and no symptoms to the disease.

2.15 Tuberculin skin test

The tuberculin skin test is currently the only widely used method for identifying infection with *M. tuberculosis* in person who does not have tuberculosis disease. Although currently the tuberculin skin test antigens that are available are substantially less than 100% sensitive and specific for detection of infection with *M. tuberculosis*, no better diagnostic method is widely available. Proper use of the tuberculin skin test requires a knowledge of the antigen used (tuberculin), the immunologic basis for the reaction to this antigen, the immunologic basis for the reaction to this antigen, the technique(s) of administering and reading the test, and the results of epidemiologic and clinic experience with the test (Gutman, 1993).

The tuberculin test, like all medical tests, is subject to variability, but many of the inherent variations in administration and reading of test can be avoided by careful attention to details. The test is administered by injecting 0.1ml of 5 TU PPD intradermally (Mantoux method) into the vesioolar or dorsal surface of the forearm. Other areas may be used, but the forearm is preferred. The use of a skin area free of lesions and away from vein is recommended. The injection is made using a one- quarter to one-half inch, 27 gauge needles and a tuberculin syringe. The tuberculin should be injected just beneath the surface of the skin, with the needle bevel upward or downward (Gutman, 1993). A discrete, pale elevation of the skin (a wheal) 6 t0 10mm in diameter should be produced when the injection is done correctly. If it is recognized that the first test was improperly administered, another test dose can be given at once, selecting a site several centimetres away from the original injection. A note in the record should indicate the site chosen for the second test.

Test is to be read between 48 and 72 hours after injection, when the induration is maximum. Tests read after 72 hours tend to underestimate the true size of induration. Reading is performed in a good light, with the forearm slightly flexed at the elbow. The basic of reading is the presence or absence of induration, which may be determined by inspection (from a side view against the light as well as by direct light) and by palpation. For standardization, the diameter of induration should be measured transversely to the long axis of the forearm and recorded in millimetres (Gutman, 1993). The absence is induration should be recorded as “0 mm,” negative. Interobserver variability may be decreased by using the ball-point pen method of sokal to measure induration (Horsburgh, 1996).

2.16 Treatment of tuberculosis.

The history of tuberculosis (TB) change dramatically after the introduction of anti-mycobacterial agents. Drug treatment is fundamental for controlling TB, promoting the cure of the patients and breaking the chain of transmission when the anti-tuberculosis drug regimen is completely and correctly followed.

Anti-tuberculosis drug treatment started in 1944, when streptomycin (SM) and Paraminosalicylic acid (PAS) were discovered (Horsburgh, 1996). In 1950, the first trial was performed comparing the efficacy of Streptomycin (SM) and Paraminosalicylic acid (PAS) both as monotherapy or combined. The study demonstrated that combined therapy was more effective and resulted in the first multidrug anti-tuberculosis treatment that consisted of a long course of both drugs (Horsburgh, 1996). In 1952, a third drug, Isoniazid (INH) was added to previous combination, greatly improving the efficacy of treatment, but which still had to be administered for 18 – 24 months (Shampate, 2006). In 1960, Ethambutol (EMB) substituted Paraminosalicylic acid (PAS), and the treatment course was reduced to 18 months (Shampate, 2006). In the '70s, with the introduction of rifampicin (RIF) into the combination, treatment was shortened to just 9 months (Shampate, 2006). Finally in 1980, pyrazinamide (PZA) was introduced into the anti-tuberculosis treatment, which could be reduced further to only 6 month (Shampate, 2006).

Two biological features explain why combined drug therapy is more effective at curing TB than monotherapy. One is that treatment of active TB with a single drug results in the selection of drug resistance bacilli and failure to eliminate the disease. The other is that

different populations of tubercle bacilli each of them showing a distinct pattern of susceptibility for antituberculosis drugs may co-exist in a TB patient (Shampate, 2006).

Antituberculosis treatment has two main objectives. First, there is a need to rapidly kill those bacilli living extracellularly in lung cavities, which are metabolically active and are dividing continuously; this is required in order to attain the negativization of sputum and therefore to prevent further transmission of the disease. Second, it is necessary to achieve complete sterilization and elimination of those bacilli replicating less actively in acidic and anoxic closed lesions, and to kill semi-tissues, otherwise these bacilli may persist and will be responsible for subsequent TB relapse.Isoniazid (INH) is the drug with the highest activity against rapidly dividing bacilli, whereas Rifampicin(RIF) and Pyrazinamide(PZA) have the greatest sterilizing activity against bacteria that are not dividing. These reasons, along with the prevention of drug resistance, support the use of a combination therapy for the treatment of TB (Onyebiyoh, 2005).

Drug for treating TB are usually classified as first and second line drugs. Traditionally, there are five first-line drugs; Isoniazid(INH), Rifampicin(RIF), Streptomycin(SM), Ethambutol(EMB) and Pyrazinamide(PZA). Second-line drugs include the aminoglycoside, kanamycin and amikacin, the polypeptide capreomycin, PAS, cycloserine, the thioamideethionamide and prothionamide and several fluoroquinolones such as maxifloxacin, levofloxacin and gatifloxacin. Some reports, however, include SM among the second-line drugs, since its use has declined in recent years, due to the high rates of resistance, and also because other more effective drugs have been incorporated into the first lineantituberculosis treatment (Onyebiyoh, 2005).

The current short-course treatment for the complete elimination of active and dormant bacilli involves two phase;

i. Initial phase: three or more drugs (usually isoniazid, rifampicin, PZA, ethambutol, or SM) are used for two months, and allow rapid killing of actively dividing bacteria, resulting in the negativization of sputum.
ii. Continuation phase: fewer drugs (usually INH and RIF) are used for four to seven months, aimed at killing any remaining or dormant bacilli and preventing recurrence (Onyebiyoh, 2005).

The WHO has established four TB diagnostic categories, assuming from a public health perspective that the highest priority of national TB programs is to identify and cure those patients with sputum smear-positive pulmonary TB, i.e. infectious TB patients (WHO, 2003).

In addition to these guidelines for TB treatment, there are otheralternatives. For example the CDC in the United State also suggests continuation phase consisting of INH and rifampicinonce per week for four month for patient in category I (CDC, 2003). This treatment can be used when sputum is negative for AFB after the first two months of treatment but should be extended to 9 months if the result of the culture at that time is still positive. This guideline applies only to HIV-negative patients (CDC, 2003).

In general, the duration of the continuation phase must be estimated once the first 2 months of treatment (initial phase) have been completed. If the patient had cavitation on initial chest radiography and culture still positive after 2 months of treatment the continuation phase should be extended to 7 months (CDC, 2003).

When drug resistance develops, patients are to be treated with a new combination containing at least three drugs that they had never received before (or that do not show cross-resistance with those to which resistance is suspected). In this conditionthe treatment is longer, more toxic, more expensive and less effective than regimen containing first-line drugs, and are directly observed (CDC, 2003).

In Children, drug regimens similar to those described above for adults can be given, although EMB is not recommended because of its ocular toxicity. Rifampicin is not been approves for paediatric use (CDC, 2003).

On case of pregnancy, similar drug regimens can be prescribed, although SM and other second-line aminoglycosides are not be given because they are ototoxic for the foetus. Also, there has been concern about the use of PZA. A drug regimen of 9 months of INH and RIF supplemented with EMB during the first month has been proposed. All antituberculosis drugs are compatible with breast feeding, although babies should be given chemoprophylaxis for at least 3 months after the mother is considered non-infectious (Staut, 2004).

Since HIV/AIDS patients have a higher probability of acquiring TB (either PTB or EPTB) or other mycobacterial opportunistic infections, a particularly drug regimens have been designed for treating active TB disease in them (CDC, 2003).Although, in general, HIV-positive patients respond well to a standard short-course treatment of TB, treatment failure due to malabsorption of antimycobacterial drugs has been reported. The WHO recommends not using SM in HIV-positive patient in order to prevent the adverse effects

of these drugs, often enhanced byanti-retroviral drugs; EMB can be used instead(Staut, 2004).

Chemoprophylaxis of TB is indicated for asymptomatic patients having positive tuberculin skin test (TST) but not showing active disease (Latent TB infection), especially when they are at risk of developing the disease (for example, HIV positive patients) (Balcell, 2006).

It is of prime importance to ensure the patient adherence to the antituberculosis treatment in order to achieve complete elimination of the bacilli (and hence avoid disease relapse), and also to prevent the emergence of drug resistance. For this reason the antituberculosis treatment has to be supervised (CDC, 2003).

2.17.0 Prevention and control of TB.

TB prevention consists of two main parts. The first part of TB prevention is to stop the transmission of TB from one individual to another. This is done by identifying people with active TB and then curing them through the provision of drug treatment. With proper TB treatment, patient with TB disease will very quickly not be infectious and so can no longer spread the disease to others (CDC, 2003). The second main part of TB prevention is to prevent people with latent TB from developing active and infectious TB. Any factor that increases the number of infectious people, such as presence of TB and HIV infection together, or which increase the number of people infected by each

infectious person, such as ineffective treatment because of drug resistance TB, reduce the overall effective of the main TB prevention effort. As a result, it is more likely that the number of people globally developing TB will increase rather than decrease.

2.17.1 TB Prevention – the BCG Vaccine

TB vaccine called Bacillus Calmette Guerin (BCG) was first developed in 1920s. it is one of the most widely used of all current vaccines, and it reaches more than 80% of new born children and infants in countries where it is part of national childhood immunization programme .However, it is also one of the most variable vaccines in routine used (WHO, 2011).

TB BCG vaccine has been shown to provide children with excellent protection against disseminated TB, however protection against pulmonary TB in adult is variable. Since most transmission originates from adult cases of pulmonary TB. The BCG vaccine is generally used to protect children, rather than to interrupt transmission amongst adult. The BCG vaccine will often result in the person vaccinated having a positive result to a TB skin test (WHO, 2011).

2.17.2 TB Treatment as part of TB Prevention

TB drug treatment for the prevention of TB, also known as chemoprophylaxis, can reduce the risk of first episode of active TB occurring in people either exposed to infection, or with latent TB. It can also reduce the risk of a recurrent TB episode. (WHO, 2009).

For TB prevention the world health organization (WHO, 2011) recommends the drug isoniazid should be taken daily for at least six months and preferably nine months. The main target groups for TB treatment and prevention are those mostly at risk of progressing from latent TB to active TB. These include:

i. Infant and children aged less than 4 years old.
ii. People infected within the previous two years.
iii. People infected with HIV.
iv. People who have certain clinical conditions, or condition which compromise their immune system such as people with diabetes, and people with chronic renal failure (WHO, 2011)

Isoniazid is a cheap drug, but in a smaller way to use than the BCG vaccine, it is mainly used to protect individuals rather than to interrupt transmission between adults. This is because children rarely have infectious TB and it is hard to administer isoniazid on a large scale to adult who do not have any symptoms. Taking isoniazid daily for six months is difficult in respect to adherence, and as a result, many individuals who could benefit from the treatment, stop taking the drug before the end of the six months period (WHO, 2011)

At paediatric HIV/TB conference in Kampala, doctors were unable to agree as to whether children infected with HIV should be given isoniazid as preventive treatment for TB. Those arguing for drug treatment as prevention, claimed that in children co-infected with HIV and TB, up to 50% of exposedchildren ended up developing the disease (WHO, 2011)

There have also been concerns about possible impact of TB treatment for prevention programmes on the emergence of drug resistance. However, a review of the scientific evidence has now shown that there is no need for this to be concern (WHO, 2011). The benefit of isoniazid preventive therapy for people with HIV, and who have had latent TB, has also recently been emphasized.

2.17.3 Preventing TB transmission in households

In order to reduce exposure in household where someone has infectious TB, the following action should be taken where ever possible:

i. House should be adequately ventilated.
ii. Anyone who coughs should be educated cough etiquette and respiratory hygiene, and should follow such practice at all times.
iii. While smear positive TB patients should spend as much time as possible outdoors, if possible sleep in a separate, adequately ventilated room.

Cough etiquette and respiratory hygiene means covering nose and mouth when coughing or sneezing. This can be done with a tissue, or cough in upper sleeve or elbow, but should not cough or sneeze into hands. The tissue should then be safely disposed (CDC, 2012)

2.17.4 Physical measure for TB prevention

Before drug treatment for TB becomes available, removing TB patients from their homes and putting them in isolation in sanatoria was the main way of reducing transmission. However,the policy changed in the vast majority of countries, after studies showed that, if patients stayed at home and were treated as an outpatient basis, this did not increase the

rate of TB amongst household contacts of the people with TB. This is because drug treatment makes TB patient uninfectious and most household contacts that do become infected, will have already become infected before the diagnosis of TB has been made(WHO, 2008).So generally there is now need for people to leave their homes because they have TB patient. The only exception to this is, as described above. This measures describe above also mainly apply to resource poor settings, and the recommendations can be different where more resources are available. (WHO, 2008).

2.17.5 TB prevention in health care facility

Doctors and other health care workers, who provide care for patients with TB, must follow infection control procedures to ensure that TB infection is not passed from one person to another. Every country should have infection control guidance which clearly needs to take into account localfacilities and resources, as well as the number of people being provided with care. However, infectious control guidance must not only be written but also implemented. It is not just in resource poor countries that TB transmission occurs in hospital (Haley, 1989). In 2012 it is reported that a patient in UK had become infected with TB and died, as result of receiving kidney dialysis when sitting next to another patient with infectious TB (WHO, 2012).

2.17.6 Control of TB in HIV patients

Multiple TB outbreaks occurred in health care facilities in the late 1980s and early 1990s (WHO, 2006). In response, guidelines and regulations were developed and implemented to help ensure safe TB control practices. Investigation for these outbreaks found lapses in administrative, environmental and respiratory control measures (WHO, 2006).

For the administrative controls, treatment of TB uses antibiotics to kill the bacteria. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which hinders the entry of drugs and makes many antibiotics ineffective. (Brennan, 1995). The two antibiotics commonly used are isoniazid and rifampicin and treatment can be prolonged, taking several months. Latent TB treatment usually employs a single dose antibiotic while active TB is best treated with combinations of several antibiotics to reduce the risk of the bacteria developing antibiotic resistance (Lawn, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS:

3.1 Study design:

This is a prospective descriptive cross sectional study.

3.2 Study area:

The study was carried out at North-west TB reference laboratory, which is a specialized Tuberculosis diagnostic laboratory located atAminu Kano teaching hospital, Kano.

Aminu Kano teaching hospital is the only tertiary health institution under the Federal ministry of Health in Kano state, Nigeria. The hospital was established on 24th of August, 1988 by the then minister of health Professor OlikoyeRansomeKuti (National health policy, 1986). Prior to its movement to the permanent site along Zaria road in Tarauni local government in January, 1996. The hospital was operating at a temporary site in Murtala Mohammed specialist hospital, kano.

This 600 beds tertiary hospital provides highly specialized services and general care for specific disease conditions for different categories of patients it receives from and within Kano, the neighbouring state of Jigawa, Katsina, Kaduna, Bauchi, Gombe, and Zamfara state (AKTH annual report., 2013).

The hospital has the following clinical departments namely; surgery, medicine, community medicine, obstetrics and gynaecology, paediatrics, maxillofacial surgery, radiology, anaesthesia, psychiatry, ophthalmology, otorhinolaryngology, microbiology, histopathology, haematology, chemical pathology, pharmacy nursing dentistry, physiotherapy, medical record, Antenatal clinic and speciality clinic that takes care of out-patients. It also has the following wards namely; male medical ward (MMW), female medical ward (FMW), dental ward (DW), post-natal ward (PNW), Gynae ward (GW), emergency paediatrics ward (EPU), accident and emergency (A&E) and General Out-patient department (GOPD). (AKTH annual report, 2012).

Kano state is located at latitude 9^0 55'N and 8^0 53'E at a latitude of 1300 metres, 4100ft above sea level. The climate condition is characterized by breeze harmattan between October and March and a rainy period between April and September. The temperature in the state is generally cold, and the annual rainfall is about 1300mm, with an estimated large land mass of about 130,913 square kilometres, and a population of 12,178,712 people with population density of 403 per square kilometres, based on the 2006 national population census. Their main occupation is farming while most of the urban populations are civil servants and traders. The adult literacy rate was estimated at 29.1% and 16.5% for male and female respectively (Bureau of statistics, 2008).

3.3.0 Study population:

The study population targets patients that present themselves to directly observable therapy short-course (DOTS) clinic with request form and are presumptive of having pulmonary tuberculosis.

3.3.1 Inclusion criteria:

Adult of both sexes aged fifteen and above with symptom of tuberculosis.

Drug resistance tuberculosis suspect.

All HIV positive patients that are tuberculosis suspect.

Patients that are sputum AFBsmear negative with TB

symptoms.

3.3.2 Exclusion criteria:

Children of both sexes aged below fifteen years.

HIV positive children with tuberculosis.

Children with gastric washout/induced sputum AFB smear negative.

3.4 Sample size

A total of 300 sputum samples were collected and analysed from patients attending directly observed treatment short-course (DOTS) clinic of Aminu Kano teaching hospital with suspected cases of pulmonary tuberculosis between September, 2013 to October, 2014.

Sample size was determined using OpenEpi Version 2.3

The percentage prevalence rate was 24% (National TB and Leprosy control programme, 2010)

Population size(for finite population correction factor or fpc)(*N*):	100
Hypothesized % frequency of outcome factor in the population (*p*):	24%
Confidence limits as % of 100(absolute +/- %)(*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

Equation

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)] = 281$

The samples sizes were approximately rounded up to 300.

3.5. Ethical clearance:

Ethical clearance was obtained from Aminu Kano teaching hospital research ethics committee. However, an informed consent wassought from patients before their enrolment. (See appendix I and II).

3.6.0 Laboratory procedure:

3.6.1 Specimen collection, transportation and processing:

A minimum of 1ml spot sputum sample was collected into a universal transparent, easy label, with screw cap lid container.

The patient was asked to open the labelled container in an open space and take a deep breath and cough deeply.The sputum was spit out into the specimen container and the process was continued until the required amount of the specimen was collected into the container.The lid of the container was replaced and ensured it was secured. (Banda, 2000).

Sample was transported to the laboratory in a tightly closed specimen container and sealed with masking tape, each specimen were wrap individually in absorbent paper towel and place each in a sealable biohazard bag. The sealed bags of specimens were place in a transport box with ice packs at the bottom. All the specimens were accompanied with the specimen request form containing information required for the study (Cepheid, 2010).

3.6.2 Start-up of GeneXpert instrument:

The following steps were performed to check the function of GeneXpert instrument and the availability of their modules, before sample preparation. The computer and the GeneXpert diagnostic instrument were on and the GeneXpert short cut icon on the window desktop was double click, the GeneXpert system software using name and password were log in to check status for availability of the modules (Cepheid GeneXpertDx system, 2010).

3.6.3 Sample preparation:

A sputum container was unscrew carefully and 2 volume of sample reagent was added to 1 volume of sputum sample and the sample was vortex for 20 seconds and incubate at room temperature for 5minutes, the sample was vortex again for 20 second and incubate for another 10 minutes. The number of cartridges needed was brought out and labelled with the sample identification number (Cepheid, 2010).

3.6.4 Addition of sample to the cartridge:

Using sterile transfer pipette 2mls of liquefied sample was aspirated into transfer pipette and dispensed into the open port of the Xpert MTB/RIF cartridge. The lid of the cartridge is closed firmly (Cepheid GeneXpert GX MTB/RIF, 2010).

In the GeneXpert window, create test was clicked and a scan cartridge barcode dialog box appeared, the Xpert MTB/RIF cartridge barcode was scan; and create test window appeared. The software automatically fills the boxes for select assay, reagent lot ID, cartridge S/N and expiration date. Start test wasclick and the instrument module door blink green light and open, the cartridge was loaded. The test will start immediately and

the green light stops blinking and turn off immediately the test finish. The cartridge was removed and disposed into biohazard bags for autoclaving (Cepheid, 2010).

3.7.0 Sputum culture for TB:

3.7.1 Lowenstein Jensen media preparation:

37.4g of commercially Lowenstein Jensen base medium powder was dissolved in 600mls of distilled water and 12.0mls of glycerol was added. It was mixed thoroughly and heated with frequent agitation until the medium boils. It was allowed to cool at approximate temperature of 50^0 C. 500mls of homogenized eggs was added, mixed thoroughly and placed in 7mls amount in sterile falcon tubes. The tubes were arranged in the inspissatorat 85^0 C for 1 hour to coagulate.

3.7.2 Procedure for decontamination and inoculation:

All equipment, reagents, supplies and specimens were arranged in Biological safety cabinet II.The working area was covered with bench pad and sprayed with0.5% bleach.Sterile conical screw capped centrifuge tube (50mls) was labelled with the respective specimen identification number and a maximum of 5mls of sputum sample were transferred into 50mls sterile screw capped conical centrifuge tube slowly avoiding vigorous shaking.Equal volume of NALC-NaOH solution was added to each specimen and vortex for 20seconds at a moderate speed and inverted 5 times to ensure that NALC-NaOH solution mixed with entire surface of the tube.The tubeswere allowed to stand at room temperature (20 - 25^0C) for 15minutes for decontamination and sterile 0.067M phosphate buffer saline (pH 6.8) were added to the digested-decontaminated specimen up to the 45mls mark to reduce the action of NaOH and lower the viscosity of the

mixture.The specimen was inverted several times and centrifuge at 4^0C for 15minutes at 3000g and supernatant were carefully poured off into a discard container containing 5% phenol.The specimens were re-suspended with 2mls of Phosphate buffer and were inoculated into the Lowenstein Jensen (LJ) medium using sterile Pasteur pipette by adding 2-3 drops (Kent, 1985).

3.7.3 Identification of MTBC using rapid test (SD-Bioline) from solid culture.

Sterile screw capped tubes were labelled appropriately with specimen identification number and 100 – 200ul of the extraction buffer were dispensed into the sterile screw capped tubes. A sterile wire loop was carefully used to take a colony out of the solid culture tube.The scrapped bacterial colonies were suspended in the extraction buffer and vortex for 20 seconds. (Khatleen, E., 2010).

3.7.2 Test procedure:

The device (SD-BIOLINE) was removed from the foil pouch immediately before testing, placed SD-BIOLINE cassette on a flat surface and labelled appropriately with sample identification number.The tube containing the properly mixed sample suspension was unscrewed.Using a sterile pipette tip, 100ul of the sample was placed onto the specimen placing area.The cap on sample tube was replaced tightly and the timer was set for 15 minutes.

The reading area of the test was observed after 15 minutes for result interpretation. (SD Bioline kit insert).

3.7.5 Interpretation of result:

Positive test for TB complex: Red- A pink to red band appears at the test "T" position and the control "C" position in the read window. This indicates that MPT64 antigen was detected in the sample. The intensity of the C and T lines may vary. The background area should be white to light pink.

Negative test for TB complex: Green- No pink to red band is visible at the test "T" position of the read window. This indicates that MPT64 antigen was not detected in the sample. A line at the control "C" position read window indicates proper performance of the test procedures. The background area should be white to light pink.

Invalid test: Yellow- The test is invalid if no pink to red band is visible at the control "C" position in the read window or if the background area colour inhibits test interpretation. If invalid, the sample must be retested with a new device. (Eichbaum, and Rubin, 2002).

3.7.6 Drug susceptibility testing for rifampicin using nitrate reduction assay:

Preparation of stock Solution of Active ingredients for Rifampicin (RPM) 950ug (95%)

0.021g of Rifampicin was weighed and dissolved in 5mls of methanol and the solution was used immediately (Eichbaum, and Rubin, 2002).1.0g/l of KNO_3 was added to the prepared LJ medium and completely dissolved by stirring. 1g KNO_3 of prepared stock of KNO_3 was added to 5mls sterile distilled water and then 1.0ml stock was added to 200mls of LJ before inspissation. Rifampicin at a concentration of 40.0mg/L was added to LJ medium and mixed thoroughly.The falcon tubes were labelled with name of drug together with the control containing no drug in the medium.The media were dispensed into the labelled bottles, closed and inspissated at 85^0C for 1 hour.The media were cooled

and sterility check was done and stored in refrigerator before used. (Eichbaum and Rubin, 2002).

3.7.7 Inoculation of processed sample:

Four LJ tubes per specimen were labelled (three controls and one containing Rif).0.2ml (4 drops) of processed specimen to each of the 4 LJ slopes was inoculated in a BSC level II.They were incubated at 37^0C for 28 days. (Eichbaum and Rubin, 2002).

3.7.8 Reading of slants and addition of GREISS reagents

The slopes were removed from the incubator after 7 days of incubation and placed in Biological Safety Cabinet.They were examined for growth of organisms.One of the control slope was open and 0.5mL GRIESS reagent was added, then colour development was observed (development of red colour indicates that there was growth on the slope and that the nitrate was converted to nitrite).Equal amount of GRIESS reagent was added to the slope containing RIF (if red colour was observed in the control).The development of red colour was observed.When there is no growth or colour development in the control slope, the other control slopes were re-incubated with the drug containing slopes for a further three weeks and then perform GRIESS test for each week.(Eichbaum and Rubin, 2002).

3.7.9 Interpretation of results:Red colour observed in the control slope indicated that there was mycobacterial growth and that nitrate was converted to nitrite.No red colour in the control slope indicates that there was no mycobacterial growth; report as no growth seen.

Red colour observed in the control and drug slopes indicate that there was growth in the drug slopes despite the presence of drugs. This indicates that the isolate is resistant to the drugs.

Red colour observed in the control slope and no colour in the drug slopes indicate that growth of mycobacteria was inhibited by the presence of the drugs and, in this case, the isolate is sensitive to the drugs. (Eichbaum and Rubin 2002).

CHAPTER FOUR

4.0 RESULTS:

4.1 Sex Percentage distribution of *Mycobacterium tuberculosis* in comparison with GeneXpert and culture method.

Out of the 300 sputum samples, of which 151 (50.3%) males and 149 (49.7%) females subjected to molecular detection of *Mycobacterium tuberculosis* using GeneXpert machine and Culture in LJ medium.

Table 4.1 shows the sex percentage distribution of *Mycobacterium tuberculosis* using GeneXpert and culture method, in which culture has the highest incidence detection rate of 104 (34.6%) and GeneXpert have 61 (20.3%) in both males and females. In respect to that culture yielded 61 (20.3%) and 43 (14.3%) for male and female respectively, while GeneXpert detects 58 (19.3%) and 38 (12.7%) for male and female respectively.

4.2 Sex Percentage distribution of detection and isolation of *Mycobacterium tuberculosis*in three groups of subjects by GeneXpert and Culture.

In comparison to the sex percentage distribution of detection and isolation of *Mycobacterium tuberculosis* in all the three subjects, the HIV-positive patient has the highest detection and isolation rate of 46 (15.3%) among which 25 (8.3%) were males and 21 (7.0%) were females, then followed by DR-TB suspect with 34 (11.3%) of which 22 (7.3%) were males and 12 (4.1%) were females, and the last group were AFB smear

negative subjects with a total 24 (8.0%) of which 14 (4.7%) were males and 10 (3.3%) were females as in (Table 4.2).

TABLE 4.1: Sex Percentage distribution of *Mycobacterium tuberculosis* in comparison with GeneXpert and culture method.

SEX	**NO EXAMINED** (%)	**GENE EXPERT RESULT NO POS (%)**	**CULTURE RESULT NO POS (%)**
MALES	151(50.3)	58(19.3)	61(20.3)
FEMALES	149(49.7)	38(12.7)	43(14.3)
TOTAL	**300(100)**	**96(32.0)**	**104(34.6)**

KEY:NO POS = number positive, NO NEG = number negative, (%) = Percentage

TABLE 4.2: Sex Percentage distribution of detection and isolation of *Mycobacterium tuberculosis*in three groups of subjects by GeneXpert and Culture.

SEX	NO EXAMINED (%)	DR-TB NO POS (%)	HIV-POSITIVE NO POS (%)	AFB SMEAR NEG. NO POS (%)
MALES	151(50.3)	22(7.3)	25(8.3)	14(4.7)
FEMALES	149(49.7)	12(4.1)	21(7.0)	10(3.3)
TOTAL	**300(100)**	**34(11.3)**	**46(15.3)**	**24(8.0)**

KEY:NO POS = number positive, NO NEG = number negative, (%) = Percentage

4.3 Sex Percentage distribution of Rifampicin resistance in all the three groups of subjects by GeneXpert and NRA

From the result of Table 4.3 show the percentage distribution of rifampicin resistance in all the three subjects, DR-TB suspects has the highest incidence rate of rifampicin resistance of 12 (11.5%) with 8 (7.7%) in males and 4 (3.8%) in females, followed by AFB smear negative group 2 (1.92%) in which 1 (0.96%) from each males and females. The HIV positive subject only 1(0.96) incidence rate were recorded in male.

4.4 Sex Percentage incidence of *Mycobacterium tuberculosis* and Rifampicin resistance in DR-TB subjects by GeneXpert and Culture

The incidence of *Mycobacterium tuberculosis* and rifampicin resistance among DR-TB suspects in relation sex. Out of 85 examined; 53 (62.4%) were males and 32 (37.6%) were females a total of 34 (40%) were positive for *Mycobacterium tuberculosis.* Of the 53 (62.4%) males 22 (25.9%) were found to be positive with *Mycobacterium tuberculosis* and out of 32 (37.6%) females examined 12 (14.1%) were positive for MTB. The highest percentage incidences of rifampicin resistance were found to be in male 8 (23.4%) while females have 4 (11.8%) (Table 4.4)

TABLE 4.3: Sex Percentage distribution of Rifampicin resistance by GeneXpert and NRA in all the three groups of subjects.

SEX	NO EXAMINED (%)	DR-TB suspects NO. POS. (%)	HIV-POSITIVE NO. POS. (%)	AFB-SMEAR NEG NO. POS. (%)
MALES	151(50.3)	8(7.7)	1(0.96)	1(0.96)
FEMALES	149(48.7)	4(3.8)	0(0.00)	1(0.96)
TOTAL	**300(100)**	**12(11.5)**	**1(0.96)**	**2(1.92)**

KEY:NO POS = number positive, NO NEG = number negative, (%) = Percentage

TABLE 4.4: Sex Percentage incidence of *Mycobacterium tuberculosis* and Rifampicin resistance in DR-TB subjects by GeneXpert and Culture.

SEX	**NO EXAMINED (%)**	**NO POS. (%)**	**RIF RES (%)**
MALE	53(62.4)	22(25.9)	8(23.4)
FEMALE	32(37.6)	12(14.1)	4(11.8)
TOTAL	**85(100)**	**34(40)**	**12(35.3)**

KEY:NO POS = Number positive, NO NEG = Number Negative, RIF SEN = RifampicinsensitiveRIF RES = Rifampicin resistance, (%) = Percentage

4.5 Age Percentage distribution of *Mycobacterium tuberculosis* and Rifampicin resistance in DR-TB subjects by GeneXpert and Culture.

In respect to age distribution on incidence rate of *Mycobacterium tuberculosis* and rifampicin in DR-TB subjects (Table 4. 5). The age bracket with the highest incidence of *Mycobacterium tuberculosis* was 12 (14.1%) at the age groups of (28 – 35) years followed by 9 (10.6%) of the age group of (15 – 27) years and 7 (8.2%) with those that are age group greater than 46 years. The rifampicin resistance was found to be higher4 (11.8%) within the age greater than 46 years, followed by 4 (11.8%) with the age group of (25 – 35) years then 2 (5.9) for the age groups of (36 – 45) and (15 – 27) years age bracket.

4.6 Percentage distribution of HIV-Positive subjects co-infection with *Mycobacterium tuberculosis* and Rifampicin resistance among sex group by GeneXpert and Culture.

Sex percentage distribution of HIV- positive co-infection with *Mycobacterium tuberculosis* and rifampicin resistance show that out of 132 HIV positive patients examined 46 (34.8%) were positive for MTB and 1 (2.2%) were rifampicin resistance, out of which 55 (41.7%) male examined 25 (22.7%) were positive with MTB and 1 (2.2%) were resistance to rifampicin, while 77 (58.3%) females examined, 21 (15.9%) were positive for MTB and no rifampicin resistance were detected in female gender (Table 4.6).

TABLE 4.5: Age Percentage distribution of *Mycobacterium tuberculosis* and Rifampicin resistance in DR-TB subjects by GeneXpert and Culture.

AGE	**NO EXAMINED (%)**	**NO POS. (%)**	**RIF RES (%)**
15-27	21(24.7)	9 (10.6)	2(5.9)
28-35	25(29.0)	12(14.1)	4(11.8)
36-45	19(22.4)	6(7.1)	2(5.9)
>46	20(23.5)	7(8.2)	4(11.8)
TOTAL	**85(100)**	**34(40.0)**	**12(35.3)**

KEY:NO POS= number positive, NO NEG = number negative, RIF SEN = Rifampicinsensitive**,** RIF RES = Rifampicin resistance, (%) = Percentage.

TABLE4.6: Percentage distribution of HIV-Positive subjects co-infection with *Mycobacterium tuberculosis* and Rifampicin resistance among sex group.

SEX	**NO EXAMINED (%)**	**NO POS. (%)**	**RIF RES (%)**
MALE	55(41.7)	25(18.9)	1(2.2)
FEMALE	77(58.3)	21(15.9)	0(0)
TOTAL	**132(100)**	**46(34.8)**	**1(2.2)**

KEY:NO POS = number positive, NO NEG = number negative, RIF SEN = Rifampicinsensitive,RIF RES = Rifampicin resistance, (%) = Percentage.

4.7 Age Percentage distribution of HIV-Positive subjects co-infection with *Mycobacterium tuberculosis* and Rifampicin resistance by GeneXpert and Culture.

The highest incidence rate of *Mycobacterium tuberculosis* were found to be 12 (9.1%) within the age group of (28 – 35) years age group, then 8 (6.1%) and 7 (5.3%) were found to be with age group greater than 46 years and (15 – 27) years age brackets respectively. The rifampicin resistance were found to be 1 (12.5%) in the age group greater than 46 years while all the remaining age group, resistance to rifampicin were not detected (Table 4.7).

4.8 Sex percentage distribution of *Mycobacterium tuberculosis* and Rifampicin resistance pattern in AFB smear negative by GeneXpert and Culture.

The sex incidence of *Mycobacterium tuberculosis* and rifampicin resistance in AFB smear negative patients, of 83 sputum sample examined 43 (51.8%) were male with the highest incidence of *Mycobacterium tuberculosis* 14 (16.8%) and 40 (48.2%) females, of with incidence rate 10 (12.1%). The incidence rates of rifampicin resistance were the same in both males and females 1 (4.2%) (Table 4.8).

4.9 Age percentage distribution of *Mycobacterium tuberculosis* and Rifampicin resistance in AFB smear negative.

The percentage age distribution of *Mycobacterium tuberculosis* and rifampicin resistance in AFB smear negative patients, that the age bracket with highest detection rate of *Mycobacterium tuberculosis* of 13 (15.7%) were (15 – 27) years followed by 6 (7.2%) for the age bracket of (36 -45) years, then 6 (7.2) for the age bracket of (28 – 35) years and the lowest detection rates of 2 (2.4%) were found within the age bracket of greater than 46 years but the incidence with rifampicin resistance were found to be 2 (8.3%) only within the age bracket of greater than 46 years and all the remaining age group rifampicin resistance were not detected (Table 4.9).

TABLE 4.7: Age Percentage distribution of HIV-Positive subjects co-infection with *Mycobacterium tuberculosis* and Rifampicin resistance by GeneXpert and Culture.

AGE	**NO EXAMINED (%)**	**NO POS. (%)**	**RIF RES (%)**
15-27	29(22.0)	7(5.3)	0(0)
28-35	45(34.1)	19 (14.1)	0(0)
36-45	34(25.8)	12(9.1)	0(0)
>46	24(18.1)	8(6.1)	1(2.1)
TOTAL	**132(100)**	**46(34.9)**	**1(2.1)**

KEY:NO POS = number positive, NO NEG = number negative, RIF SEN = Rifampicinsensitive**,**

RIF RES = Rifampicin resistance, (%) = Percentage, Minimum age level is 15 years.

TABLE 4.8: Sex percentage distribution of *Mycobacterium tuberculosis* and Rifampicin resistance pattern in AFB smear negative by GeneXpert and Culture.

SEX	**NO EXAMINED (%)**	**NO POS. (%)**	**RIF RES (%)**
MALE	43(51.8)	14(16.8)	1(4.2)
FEMALE	40(48.2)	10(12.1)	1(4.2)
TOTAL	**83(100)**	**24(28.9)**	**2(8.4)**

KEY:NO POS = number positive, NO NEG = NO Negative, RIF SEN = Rifampicinsensitive**,** RIF RES = Rifampicin resistance, (%) = Percentage.

TABLE 4.9: Age percentage distribution of *Mycobacterium tuberculosis* and Rifampicin resistance in AFB smear negative by GeneXpert and Culture.

AGE	**NO EXAMINED (%)**	**NO POS. (%)**	**RIF RES (%)**
15-27	27(32.5)	13(15.7)	0(0)
28-35	13(15.7)	3(3.6)	0(0)
36-45	16(19.3)	6(7.2)	0(0)
>46	27(32.5)	2(2.4)	2(8.3)
TOTAL	**83(100)**	**24(28.9)**	**2(8.3)**

KEY:NO POS = number positive, NO NEG = number negative, RIF SEN = Rifampicinsensitive**,**

RIF RES = Rifampicin resistance, (%) = Percentage, Minimum age level is 15 year

4.10 Sex percentage incidence of *Mycobacterium tuberculosis* and Rifampicin resistances among the three subjects by GeneXpert and Culture.

The percentage sex detection rate of *Mycobacterium tuberculosis* and rifampicin resistance among all the three groups, in which males has the highest percentage rate of

Mycobacterium tuberculosis detected 61 (20.3%) and in females were 43 (14.4%) with total of 104 (34.7%) of *Mycobacterium tuberculosis* detected from all the sexes, while the highest incidence of rifampicin resistance were found with male 10 (9.6%) and female were 5 (4.8%) with a total resistance of 15 (14.4%) (Table 4.10).

4.11 Age percentage incidence of *Mycobacterium tuberculosis* and Rifampicin resistance among the subjects by GeneXpert and Culture.

The highest incidence distribution of *Mycobacterium tuberculosis* and Rifampicin resistance were found to be 7 (6.7%) within the age bracket of greater than 46 years then 4 (3.9%) within the bracket of (28 – 35) yearsand followed by 2 (1.9%) for the age of (36 – 45) and (15 – 27) years (Table 4.11).

TABLE 4.10: Sex percentage incidence of *Mycobacterium tuberculosis* and Rifampicin resistances among the three groups by GeneXpert and Culture.

SEX	NO EXAMINED (%)	NO POS. (%)	RIF RES (%)
MALE	151(50.3)	61(20.3)	10(9.6)
FEMALE	149(49.7)	43(14.4)	5(4.8)
TOTAL	**300(100)**	**104(34.7)**	**15(14.4)**

KEY**:** NO POS = number positive, NO NEG = number negative, RIF SEN = Rifampicinsensitive**,** RIF RES = Rifampicin resistance, (%) = Percentage.

TABLE 4.11: Age percentage incidence of *Mycobacterium tuberculosis* and Rifampicin resistance among the subjects by GeneXpert and Culture.

AGE	NO. EXAMINED (%)	NO. POS. (%)	RIF RES (%)
15-27	77(25.7)	29(9.7)	2(1.9)
28-35	83(27.7)	34(11.3)	4(3.9)
36-45	69(23.0)	24(8.0)	2(1.9)
>46	71(23.6)	17(5.7)	7(6.7)
TOTAL	**300(100)**	**104(34.7)**	**15(14.4)**

KEY**:** NO POS = number positive, NO NEG = number negative, RIF SEN = Rifampicinsensitive**,** RIF RES = Rifampicin resistance, (%) = Percentage, Minimum age level is 15 years.

Statiscal analysis:

The mean rank of all the three groups were found to be 152.15, 153.36, and 144.26 for DR-TB suspects, HIV-positive patients and AFB smear negative respectively the chi-square value was found to be 0.947 and its significant probability values was 0.023 which is less than the alpha (0.05) i.e. $P < 0.05$. This implies that the incidence rate of *Mycobacterium tuberculosis* among the three different groups differs. (Table 4.12)

The mean rank of all the three groups were found to be 154.73, 152.62, and 142.80 for DR-TB suspects, HIV-positive patients and AFB smear negative respectively the chi-square value was found to be 1.444 and its significant probability values was 0.040 which is less than the alpha (0.05) i.e. $P < 0.05$. This implies that the incidence rate of Rifampicin resistance among the three different groups differs. (Table 4.13).

TABLE 4.12: Statistical Analysis of Mycobacterium tuberculosis detection and isolation within the three groups by GeneXpert and Culture.

Groups	**N value**	**Mean Rank**
DR-TB Suspects	85	152.15
HIV-Positive Patients	132	153.26
AFB smear negative	83	144.26
CHI-Square value	**0.947**	**0.023**

TABLE 4.13: Statistical Analysis of Rifampicin resistance within the three groups by GeneXpert and Culture.

Groups	**N value**	**Mean Rank**
DR-TB Suspects	85	154.73
HIV-Positive Patients	132	152.62
AFB smear negative	83	142.80
CHI-Square value	**1.444**	**0.004**

CHAPTER FIVE

5.0 DISCUSSION

The study was set to isolate and determine the incidence rate of *Mycobacterium tuberculosis* and to assess its resistance to rifampicin in Aminu Kano teaching hospital, Kano Nigeria in order to achieve effective medical management of the patient's condition.

The incidence rate of *Mycobacterium tuberculosis*in this study was 34.7%, this result was found to be higher than the finding by Nevadiola (2014) conducted at Federal medical centre, Makurdi Benue were 21.5% of *Mycobacterium tuberculosis*were recorded. This may be due the fact that this research work was conducted at Zonal reference laboratory which cover seven state from the zone, patients were referred form all this state but for FMC Makurdi the might have covered only the state since it is not a reference laboratory.

Other studies conducted in different part of Nigeria reported that the incidence rate of *Mycobacterium tuberculosis*were between 18 to 35%, but for the Rifampicin

monoresistance the incidence rate was found to 14.4% in this study which corresponds with the finding in Benue State, North central part of the Nigeria were 13.5% and 15.1% were recorded at the Federal medical centre and Airforce Specialist hospital respectively (Nwadiola, 2014). This result was also in agreement with the finding in abuja, the capital of Nigeria were 19.0% of Rifampicin monoresistance was recorded (Lawson, 2010) and that of Akure south west part of Nigeria with finding of 18.8% (Bello, 2014). Other studies conducted in different part of Nigeria reported as Rifampicin resistance of 11.8% to 22% (Nwadiola, 2014, Akaninyene, 2013, Lawson, 2010, Sadgren, 2010). Rifampicin resistant reported in USA, Western pacific region and Europe were 2.1%, 4.9% and 12% respectively. The resistance reported in this study may be due to the fact that some organism become resistant by random mutation in the bacterial chromosome which occur spontaneously in wild type strain, even before the strain come in contact with an anti-TB drug, other reason may be due Invitro resistance to anti-TB drugs was first reported in Nigeria over three decades ago and local health practitioners have the perception that drug resistance has increased in the recent years (Anikwe, 2014). This might be due to the fact that there was no enough funding and interest in the TB control programs.

The prevalence of rifampicin monoresistant in this study was 14.4%, this result corresponds with the finding in Benue, North central part of Nigeria were 13.5% and 15.1% were recorded at Federal medical centre and Airforce specialist hospital respectively both in Benue State (Nwadiola, 2014). The result was also closed with the finding with one in Abuja, the capital of Nigeria were 19.0% of rifampicin monoresistant was recorded (Lawson, 2010) and that of Akure South west part of Nigeria with finding of 18.8% (Bello, 2014). Other studies conducted in different part of Nigeria reported as

rifampicin resistant of 11.8 to 22%. Hence, our finding is in tandem with other findings in Nigeria (Nwadiola, 2014, Akaninyene, 2013, Lawson, 2010). Rifampicin resistant reported from America, Western Pacific region and Europe were 2.1%, 4.9% and 12% respectively (WHO, 2011). The reisistance reported in this study may be due to the poor administration and non- adherence to the drug regimen.

The data presented in this study showed that the age groups with the higher rate of *Mycobacterium tuberculosis* are between 28 – 35 years age group which is similar to the study conducted at Akure by Bello, 2014, this may be due to the fact that 75% of people with tuberculosis are within the economically productive age groups of between 15 – 54 years (Dye, 2006). This also showed a similar finding recorded at Akure which documented the rate of resistant to rifampicin among age groups of 24 – 32 years and also in other regions of the country (Bello, 2014, Dinic, 2012, Akaninyene, 2013, Olusoji, 2013).

The study also decumented that the incidence rate of *Mycobacterium tuberculosis* is more in male with 25.9% incidence than a female with 14.1%. this difference is partly due to the fact that men interact more than women and this correspondedwth work of (Nwadiola, 20014, Liza, 2006), who reported that tuberculosis were found more in men than in women. It was also found that resistant of rifampicin to TB was also common among males with 9.6% incidence than females with 4.8%. A research conducted by Obaidullah, 2014 shows similar finding that 24 patients that are resistant to rifampicin, male have higher incidence cases than the female; this difference may be due to the fact that women have less access to diagnostic facilities in some setting, but the broader

pattern also reflects real epidemiological and physiological differences between men and women both in exposure to infection and susceptibility to disease (Borgdorff, 2000).

In comparison with sex percentage distribution of detection and isolation of *Mycobacterium tuberculosis* among the three subjects; the HIV-positive subject has the highest percentage incidence of 34.8% among which 18.9% were males and 15.9% were females, then followed by DR-TB suspect with 40% of which 25.9% were males and 14.1% were females and the least subject group were AFB smear negative with a percentage incidence of 28.9% of which 16.8% were males and 12.1% were females, this corroborated the work of Sybio, 1986 and Hariris, 1990, who reported that the epidemic of HIV increases considerably when TB infection exist with alteration in the immune system, such as co-infection with Human immunodefeiciency Virus. Human Immunodeficiency Virus promotes progression of *Mycobacterium tuberculosis* latent infection to disease and, in turn, *Mycobacterium tuberculosis* enhanced HIV replication, accelerating the natural evolution of HIV infection (Rosas-Taraco, 2006) which is also similar work with that of (Pennap, 2009) at Nassarawa State of Nigeria.

From this study the percentage distribution of rifampicin resistant in all the three subjects, DR-TB subjects has the highest rate of rifampicin resistance of 35.3% with 23.5% in males and 11.8% in females, followed by AFB smear negative with 8.4% incidence rate in which 4.2% resistance to rifampicin were found in both males and females. For HIV positive subjects only 2.2% incidence rate was recorded in males.

Finally, the study demonstrates that the Xpert MTB/RIF assay can rapidly detect the presence of *Mycobacterium tuberculosis* and identify the mutation most frequently

associated with rifampicin resistance directly from the smear negative, HIV positive subjects as well as DR-TB suspect from clinical sputum sample and also increases the chances of isolation of *Mycobacterium tuberculosis* and rifampicin resistance when culture method and nitrate reduction assay was used.

5.1 CONCLUSION

This study gives the latest incidence rate of *Mycobacterium tuberculosis* and rifampicin resistance among patients attending DOTS clinic in AKTH, Kano which shows that a significant incidence of *Mycobacterium tuberculosis* and Rifampicin resistance among the patient particularly in HIV positive and the smear negative patients which are always difficult to get using smear microscopy and at the same time rifampicin was also obtained which cannot be found using microscopy. Therefore, in order to achieve effective medical management of the condition and concludes that laboratory facilities for rapid detection of *Mycobacterium tuberculosis*and drug resistant MTB should be scaled up across the country. These remains an important step to achieve maximal important in managing drug resistant in Nigeria. MTB/RIF test provides sensitive detection of

tuberculosis and rifampicin resistance directly from the sputum sample in less than 2 hours with minimal hand-on time.

5.2 RECOMMENDATIONS

Government should provideGeneXpert Machine to at least secondary and tertiary hospitals for the diagnosis of TB patients since it has a high sensitivity and specificity in the detection of *Mycobacterium tuberculosis* and Rifampicin resistance and at the same time it can detected TB on HIV positive and smear negative patients that are very difficult to get using microscopy and give result to presumptive DR-TB suspected with instant result within 2 hours.

5.3 LIMITATIONS

1. The performance of the Xpert MTB/RIF was validated using the procedures provided by the manufacture. Modifications to these procedures may alter the performance of the test.

2. Mutations or polymorphisms in primers or probe-binding regions may affect detection of new or unknown rifampicin resistant strain resulting in a false-negative result.

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APPENDIX I

CONSENT FORM

MOLECULAR DETECTION OF *MYCOBATERIUM TUBERCULOSIS*AND RIFAMPICIN RESISTANCE AMONG PATIENTS ATTENDING DOTS CLINIC AT AMINU KANO TEACHING HOSPITAL, KANO.

My name is Tukur Wada Panda, of the department of Microbiology and Parasitology, Aminu Kano Teaching Hospital, Kano. I am undertaking a study on Molecular detection of *Mycobacterium tuberculosis*complex and Rifampicin resistance pattern among patients attending DOTS clinic atAminu Kano Teaching Hospital, Kano. This will be done by using the part of the specimen we would want to obtain from you. The specimen is sputum. There will be no pain or discomfort during collection.

This study will help doctors in your management and other patient in Kano and its environs.

The test will be done free and the result made available to you and the managing physicians within days.

You will not suffer any consequences if you decide not to participate in the study or decide to opt out.

Kindly sign this form below.

I _____ understand the purpose for the investigation, and volunteer to donate my specimen for the study.

Witness _____ Sign & Date_____

Interviewers'_____ Sign & Date_____

APPENDIX II

LETTER OF ETHICAL CLEARANCE

AMINU KANO TEACHING HOSPITAL

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

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

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DIRECTOR OF ADMINISTRATION
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LLB, CIPM, AHAN

AKTH/MAC/SUB/12A/P-3/VI/1244 **14th August, 2014**

Tukur Wada Panda
Department of Microbiology
BUK, Kano

Ufs:

The Head of Department
Microbiology
AKTH, Kano

RE: ETHICAL CLEARANCE

Further to the request for approval in respect of your research proposal titled "Molecular Detection of Mycobacterium and Rifampicin Resistance among Patients Attending Dots Clinics of Aminu Kano Teaching Hospital, Kano", the Committee reviewed your proposal and noted same as a Prospective Study.

However, the Committee advised you to seek cooperation of the Doctors in DOT clinic.

Your response on the above issues raised is being awaited please.

Regards

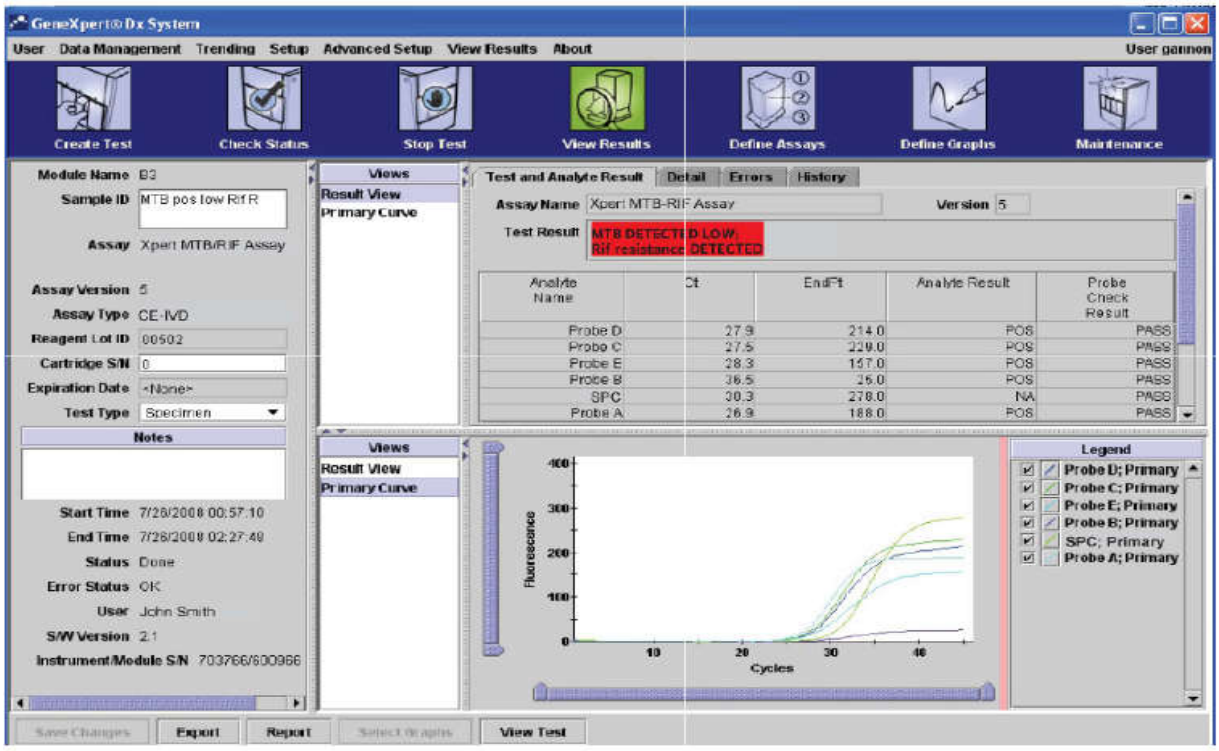
Barrister Bara'atu Aliyu-Datti
Secretary, Research Ethical Committee
For: Chairman

APPENDIX III

Results: MTB detected, RIF resistance not detected

APPENDIX IV

Results: MTB detected, RIF resistance detected



APPENDIXV

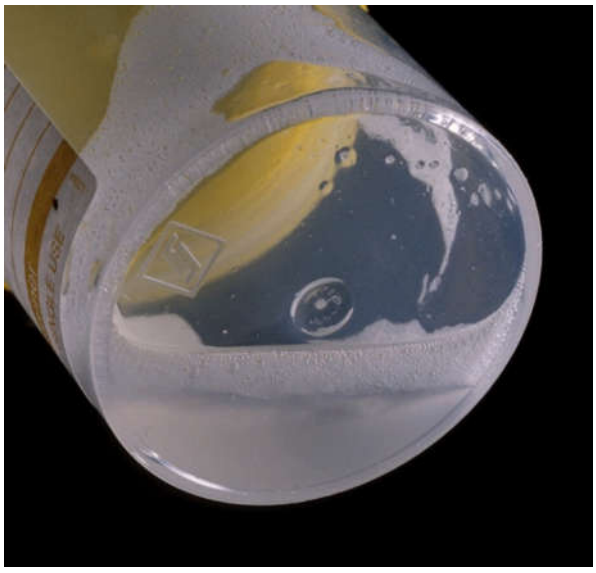
Results: MTB not detected

APPENDIX VI

Specimen Quality

Mucoid sputum

Purulent sputum



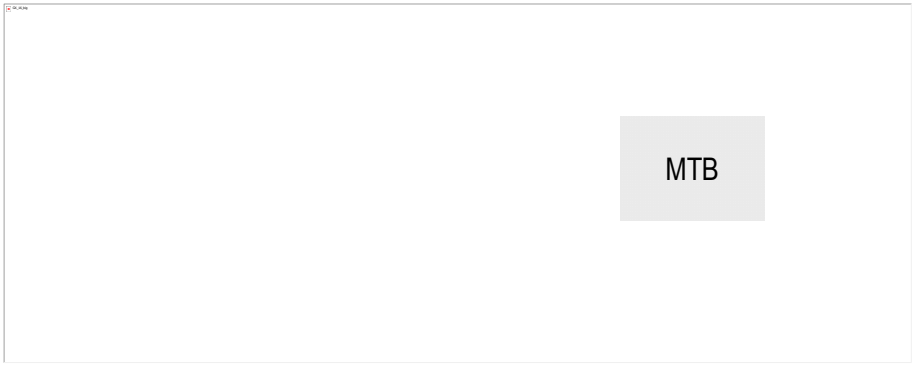
Salivary or Induced sputum **Blood stain sputum**

Salivary or blood sputum is not acceptable for Xpert processing

APPENDIX VII

GENEXPERT DX SYSTEM MACHINE WITH FOUR MODULES

CARTRIDGES USED FOR TB DETECTION.



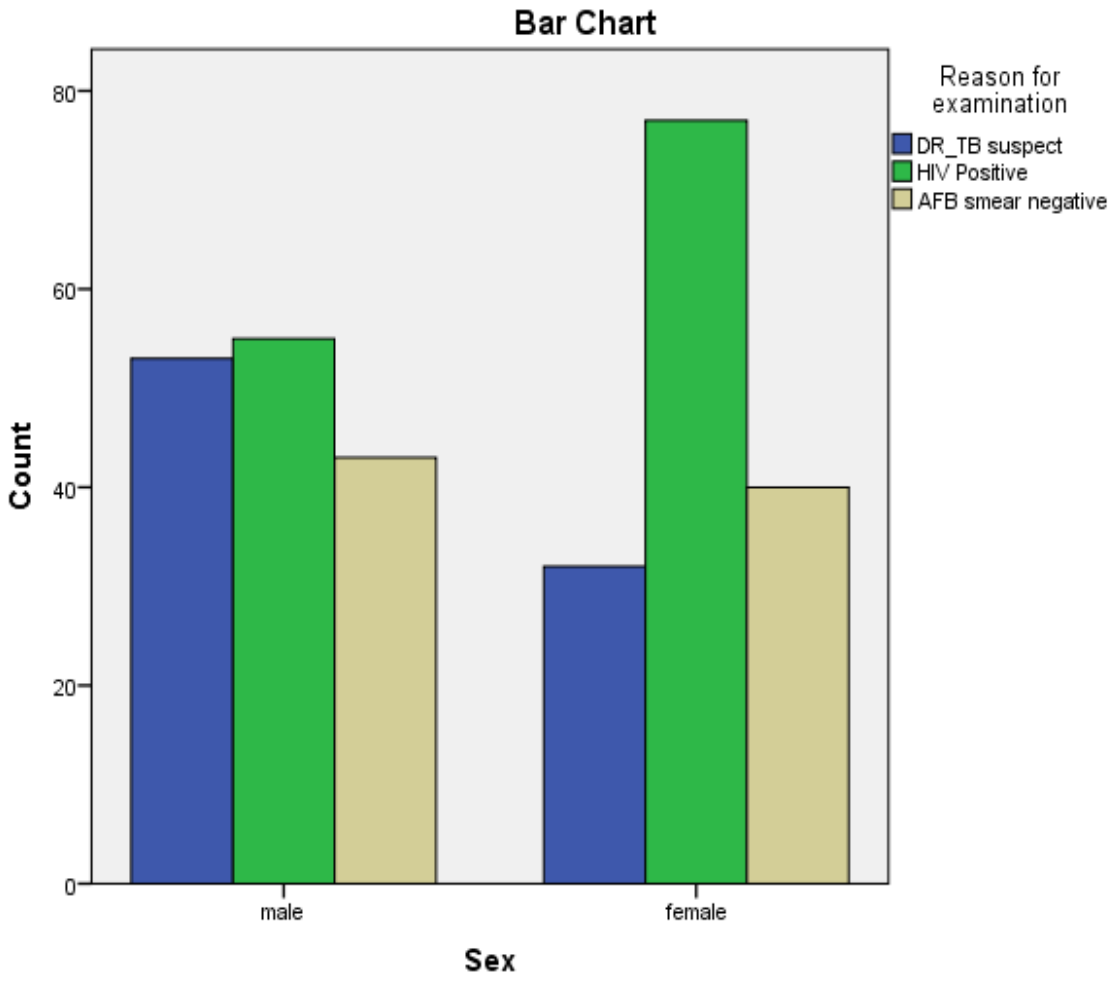


FIGURE 1: Detection and isolation of *Mycobacterium tuberculosis* using GeneXpert and culture method base on sex.

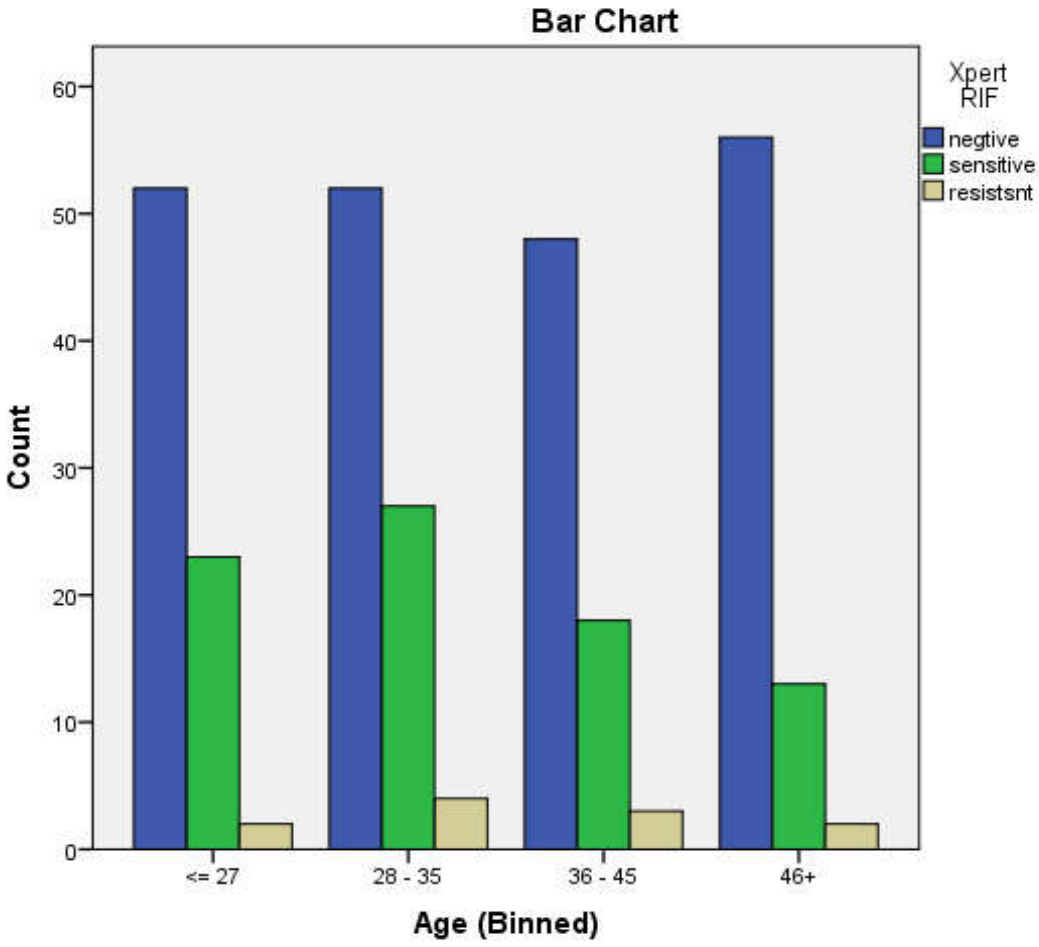


FIGURE 2: Detection rate of resistance to rifampicin among age groups.

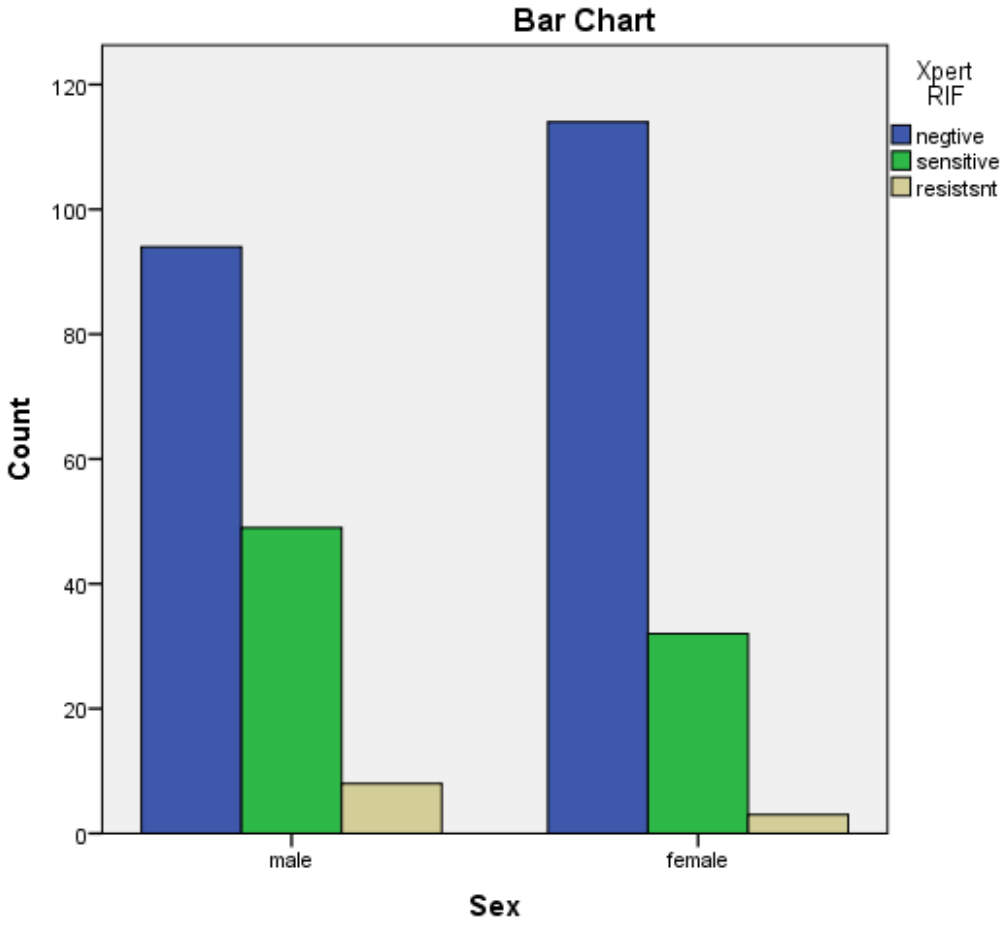


FIGURE 3: Detection rate of resistance to rifampicin among sex groups.

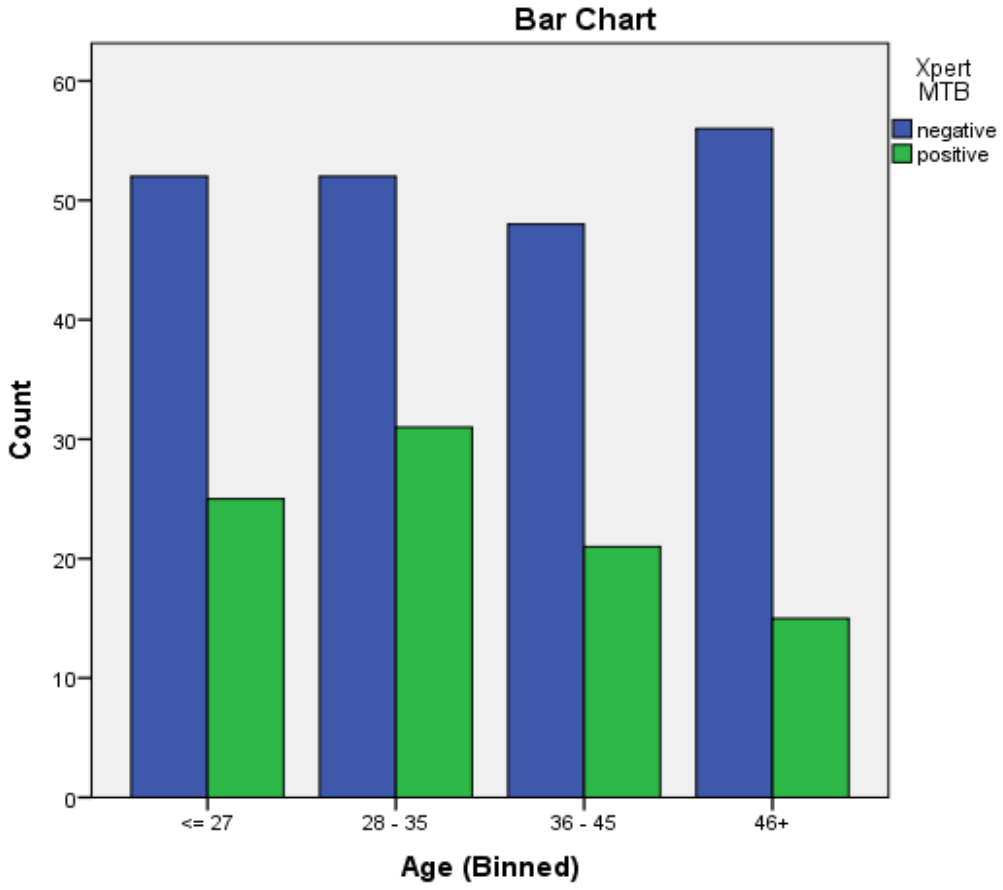


FIGURE 4: Detection and isolation of *Mycobacterium tuberculosis* using GeneXpert and culture among age groups.

NPar Tests

Kruskal-Wallis Test for MTB

Ranks

	Reason for examination	N	Mean Rank
Xpert MTB	DR_TB suspect	85	152.15
	HIV Positive	132	153.36
	AFB smear negative	83	144.26
	Total	300	

Test Statistics[a,b]

	Xpert MTB
Chi-Square	.947
Df	2
Asymp. Sig.	.023

a. Kruskal Wallis Test

b. Grouping Variable: Reason for examination

FIGURE 5. Compared the detection rate of *Mycobacterium tuberculosis* among the three groups.

NPar Tests

Kruskal-Wallis Test for RF

Ranks

	Reason for examination	N	Mean Rank
Xpert RIF	DR_TB suspect	85	154.73
	HIV Positive	132	152.62
	AFB smear negative	83	142.80
	Total	300	

Test Statistics[a,b]

	Xpert RIF
Chi-Square	1.444
Df	2
Asymp. Sig.	.040

a. Kruskal Wallis Test

b. Grouping Variable: Reason for examination

FIGURE 6: Compared the incidence rate of Rifampicin resistance among the three groups.