# Prevalence and Risk Factors of Pulmonary Tuberculosis among Antenatal Patients in Adeoyo Maternity Teaching Hospital Ibadan, Oyo State

**Theresa Olaitan OGUNDELE**
**LCU/PG/001280**

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## Certification

This thesis titled "Prevalence and Risk factors of Pulmonary tuberculosis among ante natal patients in Adeoyo Maternity Teaching Hospital, Ibadan, Oyo state was carried out by Ogundele Theresa Olaitan with Matric. No. LCU/PG/001280 in the Department of Biological Sciences, Faculty of Applied Sciences, Lead City University Ibadan, Oyo State, Nigeria under my supervision.

**Dr. T. Y. Raheem** **Date**
**Supervisor**

**Dr. Felicia Adesina** **Date**
**Head of Department**

## Dedication

This research work is dedicated to God Almighty for his mercy, wisdom and guidance at all times.

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“Even though the above mentioned institutions and persons have assisted in the process of this research work, I alone stand responsible for the errors, if any found in the work.

## Abstract

*Mycobacterium tuberculosis* infection is a public health concern, World Health Organization declared tuberculosis as a public health emergency in 2005. The infection is a significant contributor to maternal mortality, spontaneous abortion, preterm labour, low birth weight and increased neonatal mortality. Tuberculosis is among the 3 leading causes of death in pregnancy among age 15-45 years in high burden areas (Nigeria). Diagnosis of tuberculosis in pregnancy may be challenging as the initial symptoms may be linked to the pregnancy, the normal weight gain in pregnancy may further make up for weight loss. 150 pregnant women attending ante natal clinic at Adeoyo Maternity Teaching Hospital, Ibadan, Oyo State were screened for *Mycobacterium tuberculosis* using Lowenstein Jensen media for culture, slide microscopy using Ziehl Neelsen stain for Acid fast bacilli, and Molecular method using GeneXpert machine. Antimicrobial susceptibility test was performed on Lowenstein Jensen media using proportion method according to CLSI guidelines. The subjects were screened for diabetes using Randox reagent for glucose estimation alongside control and standard. HIV screening using combined kits of Determine and Unigold were also performed. Out of 150 respondents screened for diabetes, 7(4.7%) were diabetic, 150 clients were screened for HIV/AIDS, 2(1.3) were reactive, culture media results show 2(1.3%) yielded growth of MTB, 3(2.0%) grew bacilli, 2(1.3%) yielded growth of yeast cells. Slide microscopy results revealed that 2(1.3%) were AFB positive. Molecular detection of MTB (GeneXpert) showed that 7(4.7%) were positive (RIF resistant not detected). Antimicrobial susceptibility test revealed that the isolates were sensitive to the first line anti-tuberculosis drugs (Isoniazid, Rifampicin, Ethambutol). The prevalence of tuberculosis in pregnancy was found to be 4.7% and the likely risk factors are Diabetes, HIV/AIDS and accommodation congestion. This calls for prompt and immediate forms of intervention. All the circulating strain isolated were sensitive to the first line Anti-TB drugs.



**Keywords:** Microbacteria, GeneXpert, Acid Fast Bacilli, Tuberculosis, Antimicrobial susceptibility test, Lowenstein Jensen media

**Wordcount:** 299

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## List of Acronyms

| | |
|---|---|
| DST: | Drug Susceptibility Test |
| DS-TB: | Drug Sensitive Tuberculosis |
| DR-TB: | Drug Resistant Tuberculosis |
| MDR-TB: | Multi Drug Resistant Tuberculosis |
| TB: | Tuberculosis |
| PG: | Peptidoglycan |
| MA: | Mycolic Acid |
| AG: | Arabinogalactan |
| ITR: | Individualized Treatment Regimen |
| IUATLD: | International Union against Tuberculosis and Lung diseases |
| DOTS: | Directly Observed Therapy Short Course |
| ART: | Antiretroviral Treatment |
| EPTB: | Extra Pulmonary Tuberculosis |
| AFB: | Acid Fast Bacilli |
| NGS: | Next Generation Sequencer |
| MTB: | *Mycobacterium Tuberculosis* |
| INH: | Isoniazid |
| RIF: | Rifampicin |
| NAAT: | Nucleic Acid Amplification Test |
| WGS: | Whole Genome Sequencer |
| FIND: | Foundation for Innovative New Diagnostics |
| PCR: | Polymerase Chain Reaction |
| ZN: | Ziehl Neelsen |
| LJ: | Lowenstein Jensen |
| RTI: | Respiratory Tract Infection |
| URTI: | Upper Respiratory Tract Infection |

| | |
|---|---|
| LRTI: | Lower Respiratory Tract Infection |
| MTBC: | *Mycobacterium Tuberculosis* Complex |
| TST: | Tuberculin Skin Test |
| NTM: | Non Tuberculosis Mycobacteria |
| CDC: | Centers for Disease Control |
| LED-FM: | Light Emitting Diode Fluorescence Microscopy |
| BCG: | Bacille Calmette Guerin |
| PLWHA's: | People Living with HIV/AIDS |
| HIV: | Human Immunodeficiency Virus |
| AIDS: | Acquired Immunodeficiency Syndrome |
| LTBI: | Latent Tuberculosis Infections |
| HCW: | Health Care Workers |

# Chapter One

# Introduction

## 1.1 Background to the Study

Tuberculosis (TB) is a bacterial infection that is spread through the inhaling of tiny droplets from the coughs or sneezes of an infected person. It mainly affects the lungs, but any other part of the body e.g the abdomen, glands, bones and nervous system can be affected[1]. Tuberculosis is curable if it is treated with the right antibiotics[2]. Pulmonary TB (also known as TB that affects the lungs) is the most contagious type, but usually spreads after prolonged exposure to someone with the illness. The body's natural defence against infection and illness (the immune system) kills the bacteria in most healthy people, and they are asymptomatic[3]. The immune system cannot destroy the bacteria sometimes, but can prevent its spread in the body. The body won't show any symptoms, but the bacteria will still be there, this is known as latent TB. People with latent TB do not spread the infection to others. If the immune system does not destroy the bacteria, it can spread within the lungs or other parts of the body and symptoms can develop within a few weeks or months. This is referred to as active TB[4]. Latent TB could eventually develop into an active TB disease when the immune system becomes weakened[4]. The main tuberculosis bacterium is *Mycobacterium tuberculosis* and people infected with this bacterium most times do not develop active TB but remain in the latent (inactive) TB stage. The organisms can overcome the body's defenses, multiply, and cause an active disease in people who are immunocompromised eg those with HIV (human immunodeficiency virus) or those taking drugs that suppress the immune system[5]. When an infected person coughs, speaks, sneezes, sings, or laughs the TB bacterium is spread through the air[6]. The spread of TB is not likeliy through personal items, such as clothing, bedding and

eating utensils or through a handshake, a toilet, or other items that a person with TB has touched. To prevent the transmission of TB, good ventilation is of utmost importance[7]. All ages, races, genders, and income levels, are affected by TB. People at higher risk are those who live or work with people who have TB, those who cannot access health care, people from other countries where TB is prevalent, those who abuse alcohol, those who use intravenous drugs, those with HIV/AIDS, diabetics, the immunocompromised, the elderly and healthcare workers who come in contact with high risk populations[8]. The discovery of *Mycobacterium tuberculosis* was announced on March 24, 1882 by Dr. Robert Koch. TB killed one out of every seven people living in the United States and Europe at that time. This discovery by Dr. Koch was the vital step taken in the control and elimination of this disease[8]. A day to educate the public about the impact of TB around the world (World TB Day) was slated for March 24, a century later. World TB Day won't be a celebration if TB is not eliminated and so it is of utmost importance to educate the public about the devastating effect of TB and how it can be eliminated[9]. The disease tuberculosis has been known as consumption, phthisis and the White Plague down the history[8]. *Mycobacterium tuberculosis* originated from more primitive organisms of the same genus *Mycobacterium*. The results of a new DNA study of a tuberculosis genome reconstructed from remains in Southern Peru in 2014 suggested that human tuberculosis is less than 6,000 years old[10]. Its spread was from humans to other humans along trade routes and to domesticated animals in Africa, such as goats and cows. Seals and sea lions that bred on African beaches were believed to have acquired the disease and carried it across the Atlantic to South America. It was also believed that hunters were the first humans to contract the disease there[10].

*Mycobacterium tuberculosis* (MTB) is a small, aerobic, nonmotile bacillus. It's high lipid content accounts for many of its unique clinical characteristics. Every 16 to 20 hours it undergoes division which is an extremely slow rate compared with other bacteria, which usually divide in less than an hour. It has an outer membrane lipid bilayer[12]. When a Gram stain is done, MTB either stains very weakly "Gram-positive" or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall. It can withstand weak disinfectants and can also survive in a dry state for weeks. The bacterium can grow within the cells of a host organism and can be also be cultured in the laboratory[13]. Scientists can identify MTB under a microscope by using histological stains on expectorated samples from sputum. MTB is classified as an acid-fast bacillus because it retains certain stains even after being treated with acidic solution. The most common acid fast staining techniques are the Ziehl Neelsen stain and the Kinyoun stain, the acid-fast bacilli is bright red that stands out against a blue background. Auramine rhodamine staining and fluorescence microscopy are also used for its identification[14]. There are four other TB causing mycobacteria, also known as *Mycobacterium tuberculosis* complex (MTBC) which are: *M. bovis, M. africanum, M. canetti,* and *M. microti.*

*Mycobacterium africanum* is not widespread, but it is a significant cause of tuberculosis in parts of Africa[15]. *M. bovis* was once a common cause of tuberculosis, but the introduction of pasteurized milk has almost completely eliminated this as a public health problem in developed countries. *M. canetti* is rare and seems to be limited to the Horn of Africa, although a few cases have been seen in African emigrants. *M. microti* is also rare and is seen almost only in immunodeficient people, although its prevalence may be significantly underestimated. Other known pathogenic mycobacteria include *M. leprae, M. avium,* and *M. kansasii*. The latter two

species are classified as nontuberculous mycobacteria (NTM). They neither cause TB nor leprosy, but can cause lung diseases that resemble TB[16].

Pulmonary tuberculosis primarily affects the lungs and can also develop outside the lungs (Extrapulmonary TB), extrapulmonary TB may coexist with pulmonary TB. Fever, cough which last up to 3weeks, chills, night sweats, loss of appetite, weight loss, and fatigue are the general signs and symptoms. Significant nail clubbing may also occur[17]. About 90% of those infected with *M. tuberculosis* are asymptomatic, with only a 10% lifetime chance that the latent infection will progress to overt, active tuberculous disease. The risk of developing active TB increases to nearly 10% a year in those with HIV. When there is ineffective treatment, the death rate for active TB cases increases up to 66%[18]. The diagnosis of TB should be considered in those with signs of lung disease or people with general symptoms lasting longer than two weeks[19]. An initial evaluation of a chest X-ray and multiple sputum cultures for acid-fast bacilli are very important. A definitive diagnosis of TB is made by identifying *M. tuberculosis* in a clinical sample (e.g sputum, pus, or a tissue biopsy). The culture process for this organism can take two to six weeks for blood or sputum. Treatment is often begun before cultures are confirmed, therefore nucleic acid amplification tests and adenosine deaminase testing which allow rapid diagnosis of TB is an option even though they are not routinely recommended[20].

Blood tests used in detecting antibodies are neither specific nor sensitive and so are not recommended[21]. The prevention and control of tuberculosis rely primarily on the vaccination of infants and the detection and appropriate treatment of active cases. The World Health Organization (WHO) has achieved some success with improved treatment regimens, and a decrease in number of cases[22].

## 1.2 Statement of the Problem

There is existing paucity of documents regarding the prevalence and risk factors of Pulmonary tuberculosis among pregnant women in Oyo state and there is need for empirical studies on the predisposing risk factors and prevalence of tuberculosis.

## 1.3 Justification of the Study

Pregnancy has been documented as a vulnerability group to infectious diseases[16]. Generally cases of Pulmonary tuberculosis has been established among pregnant women, however document supporting this in Oyo state is scarce[22]. Moreso the distribution of the circulatory strains of *Mycobacterium tuberculosis* in this population is still not well documented.

## 1.4 Aim and Objectives of the Study

The aim of the research is to generate data that will contribute to TB disease reduction among pregnant women in the study area (Adeoyo Maternity Teaching Hospital, Yemetu, Ibadan, Oyo State). The objectives are;

i. to determine occurrence of pulmonary tuberculosis among antenatal patients in the hospital.

ii. to identify the different species of *Mycobacterium tuberculosis* complex in pregnant women in the study site.

iii. to access the drug susceptibility pattern of *Mycobacterium tuberculosis* isolates.

iv. to investigate possible risk factors that could be responsible e.g Occupation, HIV/AIDS, diabetes, nature of accommodation (congestion).

## 1.5 Significance of the Study

The study will provide an empirical data that will assist and improve the health care delivery of the respondents/subjects thereby improving the treatment pattern. Also the information gotten will be used for epidemiological surveillance towards eradicating and reducing the epidemic.

The research community will also leverage on the outcome of the work for further investigations and researches.

## 1.6 Scope of the Study

The research work is an experimental, analytical study that was carried out at Adeoyo Maternity Teaching Hospital, Ibadan, Oyo State South West Nigeria. One Hundred and fifty pregnant women attending ante-natal clinic were recruited into the study after giving their consent over a period of six months.

## 1.7 Limitations of the Study

Some of the sputum samples were not fit for investigation as a result of inability of the respondents due to their condition to demonstrate deep cough in order to produce sputum.

Interruption of health care delivery as a result of industrial unrest of health care workers and unavailability of basic amenities thereby elongating the period of sample collection.

## 1.8 Operational Definition of Terms

Prevalence: Is the proportion of a population who have a specific characteristic in a given time period.

Risk factors: Something that increases the chance of developing a disease.

Antenatal period: Is the period of time from conception to before birth.

Tuberculosis: Is a potentially serious infectious disease that mainly affects the lungs.

Mortality: Is the condition of one day having to die or rate of loss.

Diabetes: Is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.

Antibiotics: They are medications that destroy or slow down the growth of bacteria.

Immune system: They defend the body against invaders such as viruses, bacteria and foreign bodies.

Symptoms: Is a physical or mental problem a person experiences that may indicate a disease or condition.

Contagious: This is being able to pass an infectious disease from one individual to another through contact.

Asymptomatic: This is having no signs or symptoms of disease.

Disease: This is any harmful deviation from the normal structural or functional state of an organism, generally associated with certain signs and symptoms and differing in nature from physical injury.

Treatment: Is a medical care given to a patient for an illness or injury.

Fatigue: This is an overall feeling of tiredness or lack of energy.

Hemoptysis: It is the spitting of blood that originated in the lungs or bronchial tubes.

Pneumonia: This is an infection that inflames the air sacs in one or both lungs.

Endemic: This describes a disease that is present permanently in a region or population, it affects many people at one time.

Pericarditis: It is the swelling and irritation of the thin saclke tissue surrounding the heart.

Infection: This occurs when a microorganism enters a person's body and causes harm.

## Endnotes


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## Chapter Two

## Literature Review

Respiratory Tract Infections (RTIs) are infectious diseases involving the respiratory tract[1]. An infection of this type usually is further classified as an Upper Respiratory Tract Infection (URI or URTI) or a Lower Respiratory Tract Infection (LRI or LRTI). Lower respiratory infections, such as pneumonia, tend to be far more severe than upper respiratory infections, such as the common cold.

The upper respiratory tract is considered the airway above the glottis or vocal cords; sometimes, it is taken as the tract above the cricoid cartilage. This part of the tract includes the nose, sinuses, pharynx, and larynx. Typical infections of the upper respiratory tract include tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, certain influenza types, and the common cold[2]. Symptoms of URIs can include cough, sore throat, runny nose, nasal congestion, headache, low grade fever, facial pressure, and sneezing.

The lower respiratory tract consists of the trachea (windpipe), bronchial tubes, bronchioles, and the lungs. Lower respiratory tract infections are generally more severe than upper respiratory infections. LRIs are the leading cause of death among all infectious diseases[3]. The two most common LRIs are bronchitis and pneumonia. Influenza affects both the upper and lower respiratory tracts, but more dangerous strains such as the highly pernicious H5N1 tend to bind to receptors deep in the lungs[4].

Tuberculosis (TB) is an infectious disease usually caused by *Mycobacterium tuberculosis* (MTB) bacteria. Tuberculosis generally affects the lungs, but can also affect other parts of the body (disseminated TB) like the kidney, cerebrospinal system lymphoid cells and so on. Most infections show no symptoms, in which case it is

known as latent tuberculosis. About 10% of latent infections progress to active disease which, if left untreated, kills about half of those affected[7]. Typical symptoms of active TB are a chronic cough with blood containing mucus, fever, night sweats, and weight loss[5]. It was historically called consumption due to the weight loss. Infection of other organs can cause a wide range of symptoms. Tuberculosis is spread from one person to the next through the air when people who have active TB in their lungs cough, spit, speak, or sneeze. People with latent TB do not spread the disease. Active infection occurs more often in people with HIV/AIDS and in those who smoke. Diagnosis of active TB is based on chest X-rays, as well as microscopic examination and culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests[10].

## 2.1 History of Pulmonary Tuberculosis Infection

Tuberculosis is a highly contagious disease caused by the bacteria *Mycobacterium tuberculosis* (*M. tuberculosis*), which is believed to be present in the nature for at least 15,000 years. *Mycobacterium tuberculosis* is a pathogenic bacterial species in the family Mycobacteriaceae and the causative agent of most cases of tuberculosis. Tuberculosis has been known to mankind since ancient times. It is believed that the genus *Mycobacterium* was present in the environment about 150 million years ago, and an early variant of *M. tuberculosis* was originated in East Africa about 3 million years ago. A growing pool of evidence suggests that the current strains of *M. tuberculosis* is originated from a common ancestor around 20,000 – 15,000 years ago[6].

Studies on Egyptian mummies (2400 - 3400 B.C) revealed the presence of skeletal deformities related to tuberculosis, such as characteristic Pott's deformities. However, no evidence on tuberculosis was found in Egyptian papyri. The description of

tuberculosis was initially found in India and China as early as 3300 and 2300 years ago, respectively. Moreover, tuberculosis was mentioned in the Biblical books using the Hebrew word 'schachepheth' to describe tuberculosis. In the Andean states, the first pre-Columbian evidence of tuberculosis was observed in Peruvian mummies, indicating the presence of the disease before the European colonization in South America. Tuberculosis was well documented in the Ancient Greece as 'Phthisis' or 'Consumption'. Hippocrates described the symptoms of Phthisis, which are very much similar to the common characteristics of tubercular lung lesions. A Greek physician, Clarissimus Galen, who became the physician of the Roman Emperor Marcus Aurelius in 174 AD, described the symptoms of tuberculosis as fever, sweating, coughing and blood-stained sputum. He also suggested that an effective treatment of tuberculosis should include fresh air, milk, and soy beverages[6].

In Roman times, tuberculosis was mentioned by Celso, Aretaeus of Cappadocia, and Caelius Aurelianus. However, it remained unrecognized at that time. After the decline of the Roman Empire in the 5th century, a vast pool of archeologic evidence of tuberculosis was found throughout Europe, indicating that the disease was widespread in Europe during this time. In the Middle Ages, a new clinical form of tuberculosis was described as scrofula, which is a disease of cervical lymph nodes. In England and France, the disease was known as 'king's evil', and there was a popular believe that the disease can be treated with the 'royal touch'. The practice of the 'royal touch' established by English and French kings continued for several years. Queen Anne was the last British monarch to employ this method for healing[6].

The first medical intervention for treating tuberculosis was proposed by a French surgeon, Guy de Chauliac. He advised the removal of scrofulous gland as a treatment option. In the 16th century, a clear description about the contagious nature of

tuberculosis was first provided by an Italian physician, Girolamo Fracastoro. In 1679, Francis Sylvius provided the exact pathological and anatomical description of tuberculosis in his book 'Opera Medica'. In 1720, a British physician, Benjamin Marten, first described the infectious origin of tuberculosis in his publication entitled 'A new theory of Consumption. In the 17th and 18th centuries, the terms 'Consumption' and 'phthisis' were used to describe tuberculosis. In 1819, a French physician, Theophile Laennec, identified the pathological signs of tuberculosis, including consolidation, pleurisy, and pulmonary cavitation. He also identified that *M. tuberculosis* can infect the gastrointestinal tract, bones, joints, nervous systems, lymph nodes, genital and urinary tracts, and skin (extra pulmonary tuberculosis), in addition to the respiratory tract (pulmonary tuberculosis). In 1834, Johann Schonlein first coined the term 'tuberculosis'. At the beginning of the 19th century, there was a scientific debate about the exact etiology of tuberculosis. Many theories existed that time, describing the disease as an infectious disease, a hereditary disease, or a type of cancer. In 1843, Philipp Friedrich Hermann Klencke, a German physician, experimentally produced the human and bovine forms of tuberculosis for the first time by inoculating extracts from a military tubercle into the liver and lungs. In 1854, sanatorium cure for tuberculosis was introduced by Hermann Brehmer, a tuberculosis patient, in his doctoral thesis. He mentioned that a long-term stay in the Himalayan mountains helped cure his tuberculosis. A French military surgeon, Jean-Antoine Villemin, experimentally proved the infectious nature of tuberculosis in 1865. He inoculated a rabbit with fluid taken from a tuberculous cavity of a person who died of tuberculosis. A German physician and microbiologist, Robert Koch, successfully identified, isolated, and cultured the tubercle bacillus in animal serum. Afterward, he

produced animal models of tuberculosis by inoculating the bacillus. In 1882, his groundbreaking work was published in the Society of Physiology in Berlin[7].

## 2.2 Causative Agent of Pulmonary Tuberculosis

The main cause of Tuberculosis is *Mycobacterium tuberculosis* (MTB), a small, aerobic, nonmotile bacillus[8]. The high lipid content of this pathogen accounts for many of its unique clinical characteristics. It divides every 16 to 20 hours, which is an extremely slow rate compared with other bacteria, which usually divide in less than an hour[9]. Mycobacteria have an outer membrane lipid bilayer. If a Gram stain is performed, MTB either stains very weakly "Gram-positive" or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall. MTB can withstand weak disinfectants and survive in a dry state for weeks. In nature, the bacterium can grow only within the cells of a host organism, but *M. tuberculosis* can be cultured in the laboratory. Using histological stains on expectorated samples from phlegm (also called "sputum"), scientists can identify MTB under a microscope. Since MTB retains certain stains even after being treated with acidic solution, it is classified as an acid-fast bacillus[10]. The most common acid fast staining techniques are the Ziehl Neelsen stain and the Kinyoun stain, which dye acid fast bacilli a bright red that stands out against a blue background[12]. Auramine rhodamine staining and fluorescence microscopy are also used[16]. The *Mycobacterium tuberculosis* complex (MTBC) includes four other TB-causing mycobacteria: *M. bovis, M. africanum, M. canetti,* and *M. microti. M. africanum* is not widespread, but it is a significant cause of tuberculosis in parts of Africa[34]. *M. bovis* was once a common cause of tuberculosis, but the introduction of pasteurized milk has almost completely eliminated this as a public health problem in developed countries[15]. *M. canetti* is rare and seems to be limited to the Horn of Africa, although a few cases have been seen in African

emigrants[38]. *M. microti* is also rare and is seen almost only in immunodeficient people, although its prevalence may be significantly underestimated[16].

Other known pathogenic mycobacteria include *M. leprae*, *M. avium*, and *M. kansasii*. The latter two species are classified as "nontuberculous mycobacteria" (NTM). NTM cause neither TB nor leprosy, but they do cause lung diseases that resemble TB[40].

***Mycobacterium bovis***

*Mycobacterium bovis* (*M. bovis*) is a slow growing (16 to 20 hour generation time) aerobic bacterium and the causative agent of tuberculosis in cattle (known as bovine TB). It is related to *Mycobacterium tuberculosis*, the bacterium which causes tuberculosis in humans. *M. bovis* can jump the species barrier and cause tuberculosis-like infection in humans and other mammals. *Mycobacterium bovis* is a facultative intracellular parasite. For in vitro growth, special culture media are required, for example Dorset's egg medium incorporates egg yolk, phosphate buffer, magnesium salts and sodium pyruvate; amino acids may be added but glycerol is not included as it is inhibitory. It is inhibited by glycerine. Culture generally requires several weeks at 37 °C to reach colonies visible to the unaided human eye. It is strictly aerobic, grows at 37 °C and not at 25 °C. Optimum growth is at 37-38 °C. It does not reduce nitrate and niacin, resistant to pyrazineamide and sensitive to thiophene-2-carboxylic acid hydrazide[22].

Initially (after 3–4 weeks) there is minute dull flakes, thickening to form dry irregular masses standing high above the culture medium surface with eventual confluent growth over the whole culture surface, forming a rough waxy blanket, becoming thick and wrinkled and reaching up the sides of the container. It appears yellow when they are first visible, darkening to deep yellow and eventually brick red, if exposed to light.

In fluid media, growth is only on the surface, unless a wetting agent (e.g Tween 80) is added to the medium. *M. bovis* is similar in structure and metabolism to *M. tuberculosis*. It is a Gram positive, acid fast, rod shaped, aerobic bacteria[23]. Unlike *M. tuberculosis*, *M. bovis* lacks pyruvate kinase activity, due to pykA containing a point mutation that affects binding of Mg2+ cofactor. Pyruvate kinase catalyses the final step of glycolysis, the dephosphorylation of phosphorenolpyruvate to pyruvate. Therefore, in *M. bovis*, glycolytic intermediates are unable to enter into oxidative metabolism. Although no specific studies have been performed, it seems that *M. bovis* must rely on amino acids or fatty acids as an alternative carbon source for energy metabolism. During the first half of the 20th century, *M. bovis* is estimated to have been responsible for more losses among farm animals than all other infectious diseases combined. The infection occurs if the bacterium is ingested or inhaled[24].

*M. bovis* is usually transmitted to humans by consuming raw milk from infected cows, although it can also spread via aerosol droplets. Actual infections in humans are nowadays rare in developed countries, mainly because pasteurisation kills *M. bovis* bacteria in infected milk. In the UK, cattle are tested for the disease as part of an eradication program and culled if they test positive. Such cattle can still enter the human food chain, but only after a meat inspector or a government veterinary surgeon has inspected the carcass and certified that it is fit for human consumption. However, in areas of the developing world where pasteurisation is not routine, *M. bovis* is a relatively common cause of human tuberculosis[22]. Bovine tuberculosis is a chronic infectious disease which affects a broad range of mammalian hosts, including humans, cattle, deer, llamas, pigs, domestic cats, wild carnivores (foxes, coyotes) and omnivores (common brushtail possum, mustelids and rodents); it rarely affects equids or sheep. The disease can be transmitted in several ways; for example, it can be

spread in exhaled air, sputum, urine, faeces, and pus, so the disease can be transmitted by direct contact, contact with the excreta of an infected animal, or inhalation of aerosols, depending on the species involved. The infection of humans with *M. bovis* is referred to as zoonotic tuberculosis. In 2017, the World Health Organization (WHO), World Organization for Animal Health (OIE), Food and Agriculture Organization (FAO), and The International Union against Tuberculosis and Lung Disease (The Union), published the first Roadmap for Zoonotic Tuberculosis, recognizing zoonotic tuberculosis as a prominent global health problem[23]. The main route of transmission is through the consumption of unpasteurized milk or other dairy products, although transmission via inhalation and via consumption of poorly cooked meat has also been reported. In 2018, based on the most recent Global Tuberculosis Report, an estimated 142,000 new cases of zoonotic tuberculosis, and 12,500 deaths due to the disease occurred. Cases of zoonotic tuberculosis have been reported in Africa, the Americas, Europe, the Eastern Mediterranean, and the Western Pacific. Human zoonotic `tuberculosis cases are linked to the presence of bovine tuberculosis in cattle, and regions without adequate disease control measures and/or disease surveillance are at higher risk[34]. It is difficult to clinically distinguish zoonotic tuberculosis from tuberculosis caused by *Mycobacterium tuberculosis* in people, and the current most commonly used diagnostics cannot effectively distinguish between *M. bovis* and *M. tuberculosis*, which contributes to an underestimation of total cases worldwide. Controlling this disease requires animal health, food safety, and human health sectors to work together under a One Health approach (multi-disciplinary collaborations to improve the health of animals, people, and the environment).

***M. africanum***

*Mycobacterium africanum* is a species of *Mycobacterium* that is most commonly found in West African countries, where it is estimated to cause up to 40% of pulmonary tuberculosis. The symptoms of infection resemble those of *M. tuberculosis*. There are seven major lineages in the *Mycobacterium tuberculosis* complex (MTBC), with lineages 5 and 6 classified as *Mycobacterium* africanum. MTBC lineage 5 is *M. africanum* type 1, West African 1 (MAF1), and is classified based on a characteristic deletion of Region of Differentiation (RD) 711. MAF1 is commonly found around the Gulf of Guinea. MTBC lineage 6 is also known as *M. africanum* type 1, West African 2 (MAF2), and is classified based on a deletion of RD702. MAF2 is prevalent in Western Africa. *M. africanum* type 2, East African, was previously recognized as a strain of *Mycobacterium africanum*; it was recently reclassified as *Mycobacterium tuberculosis* genotype "Uganda" in a sublineage of MTBC lineage 4. *M. africanum* was first described as a subspecies within the MTBC, with phenotypic characteristics intermediate between *M. tuberculosis* and *M. bovis*, based on biochemical testing by Castets in 1968. Early genetic analysis showed that it was distinct from *M. tuberculosis* due to a genomic RD9 deletion and distinct GyrB nucleotide sequence, and distinct from *M. bovis* due to an intact RD12 and RD4[33].

*M. africanum* is grown in pyruvate containing media under low oxygen conditions, and forms characteristic "dysgonic" colonies. Unlike *M. tuberculosis*, *M. africanum* shows catalase activity, is nitrate negative, and is susceptible to thiopene-2-carboxylic acid hydrazide (TCH) and pyrazinamide (PZA). *M. africanum* is also slower growing than *M. tuberculosis*, typically taking 10 weeks to develop colonies rather than 3 to 4 for *M. tuberculosis*. *M. africanum* is most commonly found in West African countries. It is an infection of humans only and is spread by an airborne route from individuals

with open cases of disease. It is not fully understood why the distribution of *M. africanum* is limited to West Africa, with only sporadic cases found in other regions. Phylogenetic evidence shows that *M. africanum* branched at an early stage from modern Mtb lineages in America, Europe and Asia. Some research suggests that *M. africanum* is adapted to West African populations. *M. africanum* may be being outcompeted by other Mtb lineages in other regions; however, genetic studies have found no difference in the number of virulence genes or genetic diversity between *M. tuberculosis* and *M. africanum*. No animal reservoir has been identified for *Mycobacterium* africanum despite having been found various wild animals[34].

It has a similar degree of infectivity to the regular *M. tuberculosis* organism but is less likely to progress to clinical disease in an immunocompetent individual. However, *M. africanum* is more likely to progress from infection to causing disease in an HIV positive patient. In countries where *M. africanum* is endemic, it represents an important opportunistic infection of the later stages of HIV disease. It is not fully understood how the genetic differences between *M. africanum* and *M. tuberculosis* give rise to the lower pathogenicity of the former[31]. However, it is known that the Region of Difference 9 (RD9) is lacking in *M. africanum* but present in *M. tuberculosis*. *M. africanum* also has notable differences in lipid catabolism and metabolism. Additionally, virulence pathways such as the dosR/Rv0081 regulon or ESAT-6 regulation are disrupted in *M. africanum*. Because of the similar symptoms and different growth conditions between *Mycobacterium tuberculosis* and africanum, culture methods are unreliable for diagnosis. Molecular biology based genotyping has improved identification. In particular, “spoligotyping” or “spacer oligonucleotide typing”, is a rapid polymerase chain reaction based method for genotyping strains in the MTBC. Recently, lateral flow rapid tests have been developed based on the mpt64

antigen found in all members of the MTBC. *M. africanum* has a lower rate of progression from latency to active disease than *M. tuberculosis*. *M. africanum* tuberculosis is treated with an identical regime to tuberculosis caused by *M. tuberculosis*. The overall rate of cure is similar, but as more *M. africanum* patients are likely to be HIV positive, they may have higher mortality from other HIV-related disease[34].

***Mycobacterium microti***

*M. microti* is also known as the 'Vole bacillus'. Microtus is a genus that includes small field rodents such as the vole. This *Mycobacterium* species was first described as a pathogen of field voles in England. *M. microti* has a slow growth on glycerol free egg media at 37 °C often requiring incubation for 28–60 days. It may adapt tolerance to glycerol and may fail to grow in liquid media. It is usually susceptible to the first line anti tuberculosis antibiotics isoniazid, ethambutol, rifampin, streptomycin and pyrazinamide[42]. Commercially available nucleic acid hybridisation assays are widely used to identify members of the *M. tuberculosis* complex. Differentiation between individual members of the M tuberculosis complex is possible using a variety of molecular techniques, and individual strains within a species may be further distinguished using a variety of molecular typing methods. It is the cause of naturally acquired generalized tuberculosis in voles and other mammals, including cats and new world camelids such as llamas[44]. Human infections are rare, but do occur in both immunocompromised and apparently immunocompetent patients.

***Mycobacterium leprae***

*Mycobacterium leprae* is a bacterium that causes leprosy, also known as "Hansen's disease", which is a chronic infectious disease that damages the peripheral nerves and

targets the skin, eyes, nose, and muscles. Leprosy can occur at all ages from infancy to elderly, but is curable in which treatments can avert disabilities. It was discovered in 1873 by the Norwegian physician Gerhard Armauer Hansen, who was searching for the bacteria in the skin nodules of patients with leprosy. It was the first bacterium to be identified as causing disease in humans46.It is an intracellular, pleomorphic, acid fast, pathogenic bacterium. *M. leprae* is an aerobic bacillus with parallel sides and round ends, surrounded by the characteristic waxy coating unique to mycobacteria. In size and shape, it closely resembles *Mycobacterium tuberculosis*. This bacterium often occurs in large numbers within the lesions of lepromatous leprosy that are usually grouped together like bundles of cigars or arranged in a palisade. Due to its thick waxy coating, *M. leprae* stains with a carbol fuchsin rather than with the traditional Gram stain. Efforts to culture the bacteria in vivo are still unsuccessful[40].

Optical microscopy shows *M. leprae* in clumps, rounded masses, or in groups of bacilli side by side, and ranging from 1–8 μm in length and 0.2–0.5 μm in diameter. The organism has been successfully grown on an artificial cell culture medium on a very limited basis. This can be used as a diagnostic test for the presence of bacilli in body lesions of suspected leprosy patients. The difficulty in culturing the organism appears to be because it is an obligate intracellular parasite that lacks many necessary genes for independent survival. The complex and unique cell wall that makes members of the genus *Mycobacterium* difficult to destroy is apparently also the reason for the extremely slow replication rate. Virulence factors include a waxy exterior coating, formed by the production of mycolic acids unique to *Mycobacterium*. Since in vitro cultivation is not generally possible, it has instead been grown in mouse foot pads and more recently in nine-banded armadillos because they, like humans, are

susceptible to leprosy[38]. Since armadillos have a much lower body temperature than most mammals this allows the bacterium to often grow in their lungs, liver, and spleen. Armadillos have been known to have infected humans in the southeastern United States, although the geographic range of the disease and its complexity has been spreading. The incubation period of *M. leprae* can range between 9 months and 20 years. It replicates intracellularly inside histiocytes and nerve cells and has two forms. One form is "tuberculoid," which induces a cell-mediated response that limits its growth. Through this form, *M. leprae* multiplies at the site of entry, usually the skin, invading and colonizing Schwann cells. The microbe then induces T-helper lymphocytes, epithelioid cells, and giant cell infiltration of the skin, causing infected individuals to exhibit large flattened patches with raised and elevated red edges on their skin. These patches have dry, pale, hairless centers, accompanied by a loss of sensation on the skin. The loss of sensation may develop as a result of invasion of the peripheral sensory nerves. The macule at the cutaneous site of entry and the loss of pain sensation are key clinical indications that an individual has a tuberculoid form of leprosy[32].

The second form of leprosy is the "lepromatous" form, in which the microbes proliferate within the macrophages at the site of entry. They also grow within the epithelial tissues of the face and ear lobes. The suppressor T-cells that are induced are numerous, but the epithelioid and giant cells are rare or absent. With cell-mediated immunity impaired, large numbers of *M. leprae* appear in the macrophages and the infected patients develop papules at the entry site, marked by a folding of the skin. Gradual destruction of cutaneous nerves lead to what is referred to as "classic lion face." Extensive penetration of this microbe may lead to severe body damage; for example the loss of bones, fingers, and toes. *Mycobacterium* leprae has the longest

doubling time (at 13 days of doubling time in the mouse footpad) of all known bacteria and has thwarted every effort at culture in the laboratory[32]. Comparing the genome sequence of *M. leprae* with that of *M. tuberculosis* provides clear explanations for these properties, and reveals an extreme case of reductive evolution. Less than half of the genome contains functional genes. Gene deletion and decay appear to have eliminated many important metabolic activities, including siderophore production, part of the oxidative and most of the microaerophilic and anaerobic respiratory chains, and numerous catabolic systems and their regulatory circuits[39]. *Mycobacterium* leprae has undergone a dramatic reduction in genome size with the loss of many genes. This genome reduction is not complete and numerous genes are still present as nonfunctional pseudogenes. Downsizing from a genome of 4.42 Mbp, such as that of *M. tuberculosis*, to one of 3.27 Mbp would account for the loss of some 1200 protein-coding sequences. Some evidence shows that many of the genes that were present in the genome of the common ancestor of *M. leprae* and *M. tuberculosis* have been lost in the *M. leprae* genome. 1500 genes are still common to both *M. leprae* and *M. tuberculosis*. Information from the completed genome can be useful to develop diagnostic skin tests, to understand the mechanisms of nerve damage and drug resistance, and to identify novel drug targets for rational design of new therapeutic regimens and drugs to treat leprosy and its complications[32]. Almost complete sequences of *M. leprae* from medieval skeletons with osteological lesions suggestive of leprosy from different Europe geographic origins were obtained using DNA capture techniques and high-throughput sequencing. Ancient sequences were compared with those of modern strains from biopsies of leprosy patients representing diverse genotypes and geographic origins, giving new insights in the understanding of its evolution and course through history, phylogeography of the leprosy bacillus, and

the disappearance of leprosy from Europe. The closest relative to *M. leprae* is M. lepromatosis[34]. The symptoms of *M. leprae*, also known as leprosy, are unattractive skin sores that are pale in color, lumps or bumps that do not go away after several weeks or months, nerve damage which can lead to complications with the ability to sense feeling in the arms and legs as well as muscle weakness. Symptoms usually take 3–5 years from being exposed to manifest within the body. However, some individuals do not begin to show symptoms until 20 years after exposure to the disease. This long incubation period makes the ability to properly be able to diagnose when an individual came into contact with the disease very difficult.

The diagnosis of leprosy is primarily a clinical one. In one Ethiopian study, the following criteria had a sensitivity of 94% with a positive predictive value of 98% in diagnosing leprosy. Diagnosis was based on one or more of three signs: Hypopigmented or reddish skin patches with definite loss of sensation, thickened peripheral nerves, and acid-fast bacilli on skin smears or biopsy material[31]. *Mycobacterium* leprae was sensitive to dapsone (diaminodiphenylsulfone, the first effective treatment which was discovered for leprosy in the 1940s), but resistance against this antibiotic began to develop in the 1960s. Therapy with dapsone alone is now strongly contraindicated. Currently, a multidrug treatment (MDT) is recommended by the World Health Organization, including dapsone, rifampicin, and clofazimine (The two later were both discovered in the 1960s). In patients receiving the MDT, a high proportion of the bacilli die within a short amount of time without immediate relief of symptoms. This suggests many symptoms of leprosy must be due in part to the presence of dead cells. Multidrug therapy (MDT) uses combinations of antibiotics that kill *M. leprae* including: dapsone, rifampin, clofazamine, fluoroquinolones, azithromycin, and minocycline. Antibiotics must be taken regularly

until treatment is complete due to the fact *M. leprae* has the ability to grow back. A preventive measure of *M. leprae* is to avoid close contact with infectious people who are untreated. Blindness, crippling of the hands and feet, and paralysis are all effects of nerve damage done by untreated *M. leprae*. Treatment does not reverse the nerve damage done, which is why it is recommended to get treated as soon as possible. The Bacillus Calmette Guerin vaccine offers a variable amount of protection against leprosy in addition to its main target of tuberculosis[33].

***Mycobacterium avium***

*Mycobacterium avium* complex is a group of mycobacteria comprising *Mycobacterium* intracellulare and *Mycobacterium avium* that are commonly grouped because they infect humans together; this group, in turn, is part of the group of nontuberculous mycobacteria. These bacteria cause disease in humans called *Mycobacterium avium* intracellulare infection or *Mycobacterium avium* complex infection. These bacteria are common and are found in fresh and salt water, in household dust and in soil. MAC bacteria usually cause infection in those who are immunocompromised or those with severe lung disease. In the Runyon classification, both bacteria are nonchromogens. They can be differentiated from *M. tuberculosis* and each other by commercially available DNA probes. They are characterized as Gram positive, nonmotile, acid fast, short to long rods. Usually, colonies are smooth, rarely rough, and not pigmented colonies. Older colonies may become yellow[37].

Growth on Lowenstein Jensen medium and Middlebrook 7H10 agar occurs at 37°C after seven or more days. The complex can be (but is not often) resistant to isoniazid, ethambutol, rifampin, and streptomycin. M. intracellulare and *M. avium* form the *M. avium* complex (MAC). MAC bacteria enter most people's body when inhaled into the

lungs or swallowed, but only cause infection in those who are immunocompromised or who have severe lung disease such as those with cystic fibrosis or chronic obstructive lung disease (COPD). MAC infection can cause COPD and lymphadenitis, and can cause disseminated disease, especially in people with immunodeficiency[32].

***Mycobacterium Kansasii***

*Mycobacterium kansasii* is a slow growing, non-tuberculosis *Mycobacterium* (NTM) that, like other mycobacterial species, tends to cause six clinical patterns of infection: pulmonary disease, skin and soft tissue disease, musculoskeletal infections including monoarticular septic arthritis and tenosynovitis, disseminated disease, catheter-associated disease, and lymphadenitis. Chronic pulmonary cavitary disease in the upper lobe is the most common presentation of *M. kansasii* infections, and patients may be initially be misdiagnosed with pulmonary tuberculosis. *Mycobacterium kansasii* is a non-tuberculosis *Mycobacterium* (NTM) that is readily recognized based on its characteristic photochromogenicity; it produces a yellow pigment when exposed to light. Under light microscopy, *M. kansasii* appears as thick rectangular, beaded, gram positive rods which are longer than those of *M. tuberculosis*[34]. Clinically, *M. kansasii* causes a chronic, upper lobe cavitary disease, resembling that from *M. tuberculosis*. The prevalence of NTM infections has steadily increased when compared to tuberculosis whose prevalence has decreased in the last few decades. There is no clear data regarding the prevalence of *M. kansasii*, although some studies have in fact shown decreasing prevalence[31].

*Mycobacterium Kansasii* grows best at 32 degrees Celcius, but can be cultured at 37 degrees. Its invitro and chemical characteristics are similar to those of *M. marinum* and *M. szulgai*. It produces mature colonies in greater then 7 days. Like other

mycobacteria in the family, *M. kansasii* is strictly a gram positive, non-motile and non spore-forming organism. Colony morphology ranges from flat to raised and smooth to rough. When grown in the dark *M. kansasii* colonies are at first non pigmented but turn yellow after exposure to light due to deposition of beta-carotene crystals. Microscopic examinations show that when compared to *M. tuberculosis*, *M. kansasii* appears longer and broader and are often beaded or cross-barred in appearance when stained with Ziehl Neelsen or Kinyoun stain. In Runyon classification, *M. kansasii* belongs to the group photochromogens. Biochemical characteristics include Catalase positive, nitrate reduction, tween hydrolysis, and urea hydrolysis. Identification using traditional methods could take as long as two months. Although *M. kansasii* was long thought to be homogenous species, genetic studies have shown that there are at least seven subtypes, with subtype one being most frequently isolated in human infections. Pulsed-field gel electrophoresis techniques demonstrate that the clinical isolates are very closely related and may be clonal worldwide. Pathogenic strains are highly Catalase positive[40]. *M. kansasii* is widely prevalent in the environment but has seldom been isolated from soil. Some documented sources include city tap water, swimming pools, fish tanks, fish bites, brackish water, and seawater. Tap water appears to be the major reservoir. Infection is mostly acquired through the aerosol route. Infectivity is low in regions of endemicity. Transmission from humans to humans is not thought to occur, except in two cases where familial clustering was noted. Clustering was thought to be due to a shared environment, susceptibility or genetic predisposition rather than true human to human transmission. *M. kansasii* infection mostly occurs in males, at an average patient age of 45–62 years. Lung infections caused by *M. kansasii* occur in geographic clusters. *M. kansasii* infections are more likely to occur in urban areas than rural areas, and several studies have reported an association with

mining practices. In the United Kingdom, *M. kansasii* infections are most frequent in Wales. Of all countries in Europe, Poland has the highest *M. kansasii* isolation rate (35% of all NTM compared to 5% in Europe). *M. kansasii* infections are also prevalent in areas where HIV infection is common due to the susceptibility of the hosts[41].

*M. kansasii* infections were the most common non-tuberculosis mycobacterial infections during the 1960s and 70s, before being surpassed by *M. avium* intracellulare. In the 80s with the rise of HIV infections, there was a resurgence of *M. kansasii*, which has now decreased in the antiretroviral therapy (AR) era. *M. kansasii* infections can occur in both immunocompetent and immunosuppressed patients. In the 1980s the overall prevalence of *M. kansasii* infections was estimated to be 0.5 cases per 100,000 persons. Since mycobacterial infections are not reported, there is no reliable data for *M. kansasii* infections in transplant patients. Generally in transplant patients mycobacterial infections present as disseminated infections. Fifty percent of patients with pulmonary disease also have dissemination. Renal transplant patients have been the most frequently reported to have disseminated disease. M. abscesses, M. chelonae, and M.kansasii are the mycobacteria most commonly present with dissemination[42]. Even in HIV and late-stage AIDS patients, *M. kansasii* presents with pulmonary disease.

Mycobacterial infections, including *M. kansasii* infections, can be categorized into six clinical patterns: pulmonary disease, skin, and soft tissues, musculoskeletal infections (monoarticular septic arthritis and tenosynovitis), disseminated disease, catheter-associated disease, and lymphadenitis. Chronic pulmonary cavitary disease in the upper lobe is the most common presentation of *M. kansasii* infections. Not surprisingly these patients may be initially diagnosed with pulmonary tuberculosis[34].

The most common symptoms of pulmonary *M. kansasii* infection include a cough, sputum production, weight loss, breathlessness, chest pain, hemoptysis, and fever or sweats. Of all the NTM infections, *M. kansasii* infections most often resemble Mycobacterial tuberculosis infections. Risk factors for *M. kansasii* infections are the same as those for other mycobacteria, namely, smoking, pneumoconiosis, silicosis, chronic obstructive pulmonary disease, malignancy, immunosuppressed state, chronic kidney disease, alcoholism and concurrent or prior *M. tuberculosis* infection. While cavitary disease occurs in 90% *M. kansasii*I infections, nodular and brochiectatic disease can also occur. If left untreated pulmonary infections (both cavitary and nodular) are characterized by the persistence of AFB in the sputum and progressive destruction of the lung architecture[32]. Pulmonary disease may be present in AIDS patients but there is less likelihood of cavitations; hilar lymphadenopathy and interstitial infiltrates are more common. However, if the CD4 count is high there is an increased chance of cavitary disease. The second most frequent organ involved in *M. kansasii* infection is the skin. Cutaneous infection resembles secondary to local lymphatic spread. Cutaneous lesions may include nodules, pustules, verrucous lesions, erythematous plaques, abscesses, and ulcers. In HIV and immunocompromised patients, the presentation can be atypical and include bacteremia, osteomyelitis, abscesses, and cellulitis. Pericarditis with cardiac tamponade has been reported in HIV patients. In disseminated disease, the symptoms are vague and nonspecific. In a series of 49 patients, fever was present in 60%, hepatosplenomegaly in 40%; pulmonary infiltrates in 25% and lymphadenopathy in 10%. Bone involvement, such as vertebral osteomyelitis and sacroiliitis, is common with *M. kansasii* disseminated diseases. Psoas abscess, bone marrow granuloma, liver granuloma, and possible spleen abscesses have been described. Since the symptoms are not specific and the

differential diagnosis is broad, microbiologic isolation is required to make the diagnosis. *M. kansasii* rarely represents colonization or environmental contamination. Therefore any isolation of the organism needs to be evaluated for therapy. Highly sensitive and specific PCR tests are available for *M. kansasii*[31].

Culture results along with clinical and radiological diagnosis are recommended for accurate diagnosis of *M. kansasii*. As per the guidelines from ATS (American Thoracic Society) and IDSA (Infectious Disease Society of America) diagnosis of NTM should include a minimal radiological evaluation which includes a chest X-ray combined with positive sputum cultures and exclusion of other diagnoses clinically. Cultures are considered positive when two consecutive positive sputum cultures, one positive culture from bronchoscopy specimens, or one positive sputum culture with compatible pathology is present. Cultures are considered to be positive when a) 2 sputum cultures are consecutively positive, b) bronchoscopy specimens with one positive culture or c) one sputum culture is positive and a compatible pathology is present[43].

Rifampin is the cornerstone of M kansasii therapy. The first line therapy recommended by IDSA-ATS guidelines for *M. kansasii* is rifampin, ethambutol, and isoniazid plus pyridoxine. The recommended duration of therapy is at least 12 months or more with the goal to have culture negative results for 12 months on therapy. Based on *M. kansasii* susceptibilities in vitro, patients with rifampin-resistant *M. kansasii* disease should be treated with a 3-drug regimen, which should include clarithromycin or azithromycin. The other two drugs should be chosen from moxifloxacin, ethambutol, sulfamethoxazole, or streptomycin. The Clinical and Laboratory Standards Institute (CLSI) recommends that all initial isolates of *M. kansasii* be tested only for clarithromycin and rifampin susceptibility. INH and streptomycin

susceptibility should be tested as secondary agents. One strategy to treat *M. kansasii* infection in HIV-positive patients is to use an NRTI-based regimen, allowing for a full dose of rifampin, which is the cornerstone of therapy for *M. kansasii*[44]. If a protease inhibitor (PI) such as darunavir or atazanavir or non-nucleoside reverse transcriptase inhibitor [NNRTI] such as efavirenz or nevirapine is included in HIV treatment regimens, rifabutin can be used instead of rifampin. Clarithromycin can be added to improve efficacy, but rifabutin-related toxicity can increase when combined with clarithromycin. Serial sputum samples for AFB should be obtained regularly so patients failing therapy are recognized early and susceptibility testing for additional drugs can be done. Serial CXR can be administered, although lung disease is likely to evolve at a slow pace. Routine monitoring for adverse effects of medications and drug-drug interactions is recommended. With appropriate treatment, the prognosis is usually good. Mortality is higher and can go up to 50% in patients with HIV who have *M. kansasii* infection. In HIV patients who have a pulmonary infection with *M. kansasii*, survival predictors include higher CD4 cell count, negative smear microscopy, antiretroviral therapy and adequate treatment for *M. kansasii*. Complications can include disseminated disease. Bone involvement, such as vertebral osteomyelitis and sacroiliitis are common with disseminated diseases[42]. Pneumothorax, Psoas abscess, bone marrow granuloma, liver granuloma, and possible spleen abscesses have also been described in the literature. CNS complications like meningoencephalitis are very rare and are usually fatal. Prognosis of *M. kansasii* is usually good when recognized and treated early. Treatment duration is long and the patients should adhere to the duration of the treatment as relapse rates are high if treatment is stopped early. Treatment is prolonged and requires meticulous monitoring, but *M. kansasii* is a treatable infection. With rifampin-based regimens,

the relapse rate is very low. Ethambutol can cause visual disturbances[43]. Rifampin is a strong CYP3A4 Inducer and may decrease the serum concentrations or decrease the therapeutic effect of most multiple medications.

Tuberculosis may infect any part of the body, but most commonly occurs in the lungs (known as pulmonary tuberculosis)[12]. Extrapulmonary TB occurs when tuberculosis develops outside of the lungs, although extrapulmonary TB may coexist with pulmonary TB[9].

### 2.2.1 Pulmonary

If a tuberculosis infection does become active, it most commonly involves the lungs (in about 90% of cases)[18]. Symptoms may include chest pain and a prolonged cough producing sputum. About 25% of people may not have any symptoms (i.e. they remain "asymptomatic")[19]. Occasionally, people may cough up blood in small amounts, and in very rare cases, the infection may erode into the pulmonary artery or a Rasmussen's aneurysm, resulting in massive bleeding[20]. Tuberculosis may become a chronic illness and cause extensive scarring in the upper lobes of the lungs. The upper lung lobes are more frequently affected by tuberculosis than the lower ones[21]. The reason for this difference is not clear. It may be due to either better air flow, or poor lymph drainage within the upper lungs[41].

### 2.2.2 Extrapulmonary

In 15-20% of active cases, the infection spreads outside the lungs, causing other kinds of TB[42]. These are collectively denoted as "extrapulmonary tuberculosis"[43]. Extrapulmonary TB occurs more commonly in people with a weakened immune system and young children. In those with HIV, this occurs in more than 50% of

cases[44]. Notable extrapulmonary infection sites include the pleura (in tuberculous pleurisy), the central nervous system (in tuberculous meningitis), the lymphatic system (in scrofula of the neck), the genitourinary system (in urogenital tuberculosis), and the bones and joints (in Pott disease of the spine), among others. A potentially more serious, widespread form of TB is called "disseminated tuberculosis", it is also known as miliary tuberculosis. Miliary TB currently makes up about 10% of extrapulmonary cases[45].

### 2.3 Structure of *Mycobacterium tuberculosis*

Mycobacteria belong to the diverse family of Actinobacteria. The main components of the mycobacterial cell wall are the peptidoglycan layer (PG), mycolic acid (MA) and arabinogalactan (AG)[46]. The mycobacterial cell wall resembles both the Gram-positive and Gram-negative cell envelope by having a PG layer nearly as thick as the former and an outer, waxy layer mimicking the outer membrane of the latter. The cell wall of mycobacteria plays a key role in intrinsic antibiotic resistance and virulence but it is unclear why and how its complex structure evolved. It was recently proposed that the 'mycomembrane' evolved by successive horizontal acquisition of genes; explaining the narrow distribution of the AG and MA biosynthetic genes in some lineages of the Actinobacteria that evolved later, while the PG genes are broadly distributed[47]. PG is a dynamic structure but the factors that regulate its composition and biogenesis in the slow growing intracellular pathogen *M. tuberculosis* are not well understood[47]. The mycobacterial PG plays a key role in the cell's growth, cell to cell communication and in the initiation of the host immune response. The cell envelope of some model bacteria such as Escherichia coli, have long been the focus of extensive research; however, these organisms do not serve as appropriate models when studying the unique physiology and biochemistry of *M. tuberculosis*[48].

## 2.4 Pathophysiology of *Mycobacterium tuberculosis* Infection

### 2.4.1 Mode of Transmission

*Mycobacterium tuberculosis* is spread from person to person through the air by droplet nuclei, particles 1 to 5 mm in diameter that contain *M. tuberculosis* complex. Droplet nuclei are produced when persons with pulmonary or laryngeal TB cough, sneeze, speak, or sing. They also may be produced by aerosol treatments, sputum induction, aerosolization during bronchoscopy, and through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory[49]. Droplet nuclei, containing two to three *M. tuberculosis* organisms, are so small that air currents normally present in any indoor space can keep them airborne for long periods of time. Droplet nuclei are small enough to reach the alveoli within the lungs, where the organisms replicate. Although patients with TB also generate larger particles containing numerous bacilli, these particles do not serve as effective vehicles for transmission of infection because they do not remain airborne, and if inhaled, do not reach alveoli[50]. Organisms deposited on intact mucosa or skin do 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 expecto- rated[48]. Four factors determine the likelihood of transmission of *M. tuberculosis*: (i) the number of organisms being expelled into the air, (ii) the concentration of organisms in the air, determined by the volume of the space and its ventilation, (iii) the length of time an exposed person breathes the contaminated air, and (iv) presumably the immune status of the exposed individual[51].

### 2.4.2 Risk Factors

The risk of progression from exposure to the tuberculosis bacilli to the development of active disease is a two stage process governed by both exogenous and endogenous

risk factors[52]. Exogenous factors play a key role in accentuating the progression from exposure to infection among which the bacillary load in the sputum and the proximity of an individual to an infectious TB case are key factors. Similarly endogenous factors lead in progression from infection to active TB disease[53]. Along with well established risk factors (such as human immunodeficiency virus (HIV), malnutrition, and young age), emerging variables such as diabetes, indoor air pollution, alcohol, use of immunosuppressive drugs, pregnancy and tobacco smoke play a significant role at both the individual and population level[56]. Socioeconomic and behavioral factors are also shown to increase the susceptibility to infection. Specific groups such as health care workers and indigenous population are also at an increased risk of TB infection and disease[58].

**A) Tuberculosis in Pregnancy**

Tuberculosis (TB) is believed to be nearly as old as human history. Traces of it in Egyptian mummies date back to about 7000 years ago, when it was described as phthisis by Hippocrates[103]. It was declared a public health emergency in the African Region in 2005[105]. It has since continued to be a major cause of disability and death. About 9.4 million new cases of tuberculosis were diagnosed in 2009 alone and 1.7 million people reportedly died from the disease in the same year, translating to about 4700 deaths per day[105]. About one third of the world's population (estimated to be about 1.75 billion) is infected with the tubercule bacillus[106]. As much as 75% of individuals with TB are within the economically productive age group of 15 to 54 years. This significantly impairs socioeconomic development, thereby perpetuating the poverty cycle[107]. Tuberculosis has been on the rise in tandem with HIV/AIDS. This is because people with HIV/AIDS, whose immune systems are weakened have with a 20-37 times the risk of developing a progressive disease compared with HIV

negative individuals.108 Tuberculosis (TB) was declared a public health emergency by WHO in 2005. The disease is a significant contributor to maternal mortality and is among the three leading causes of death among women aged 15-45 years in high burden areas. Rifampicin, Isoniazid (INH) and Ethambutol are the first line anti tuberculosis drugs while Pyrazinamide use in pregnancy is gaining popularity. Isoniazid preventive therapy is a WHO innovation aimed at reducing the infection in HIV positive pregnant women. Babies born to this mother should be commenced on INH prophylaxis for six months, after which they are vaccinated with BCG if they test negative. Successful control of TB demands improved living conditions, public enlightenment, primary prevention of HIV/AIDS and BCG vaccination. The wide array of opinion of Medical practitioners on tuberculosis in pregnancy simply reflects the Public Health significance of the condition. It is best described as a doubled edged sword, one blade being the effect of tuberculosis on pregnancy and the pattern of growth of the newborn, while the other is the effect of pregnancy on the progression of tuberculosis. Tuberculosis not only accounts for a significant proportion of the global burden of disease, it is also a significant contributor to maternal mortality, with the disease being among the three leading causes of death among women aged 15-45 years[109]. The exact incidence of tuberculosis in pregnancy is not readily available in many countries due to a lot of confounding factors. It is, however, expected that the incidence of tuberculosis among pregnant women would be as high as in the general population, with possibly higher incidence in developing countries. Earlier study by Schaefer reported a new case rate of 18-29/100,000 in pregnancy, which was similar to the 19-39/100,000 reported for the city of New York[110]. A recent United Kingdom study, however, quoted an incidence of 4.2 per 100,000 maternities, which may be a reflection the current global fall in the incidence of the disease[112]. Researchers from

the days of Hippocrates have expressed their worries about the untoward effects that pregnancy may have on preexisting tuberculosis. Pulmonary cavities resulting from tuberculosis were believed to collapse as a result of the increased intra abdominal pressure associated with pregnancy. This belief was widely held till the beginning of the fourteenth century. A German physician recommended that young women with TB should get married and become pregnant to slow the progression of the disease. This was practiced in many areas till the 19th century, while in the early 20th century, induced abortion was recommended for these women[113]. Researchers like Hedvall and Schaefer, however demonstrated no net benefit or adverse effect of pregnancy on the progression of TB[114]. Frequent, consecutive pregnancies may, however, have a negative effect, as they may promote recrudescence or reactivation of latent tuberculosis[115]. It is, however, important to note that the diagnosis of tuberculosis in pregnancy may be more challenging, as the symptoms may initially be ascribed to the pregnancy. The weight loss associated with the disease may also be temporarily masked by the normal weight gain in pregnancy[116]. The effects of TB on pregnancy may be influenced by many factors, including the severity of the disease, how advanced the pregnancy has gone at the time of diagnosis, the presence of extrapulmonary spread, and HIV coinfection and the treatment instituted. The worst prognosis is recorded in women in whom a diagnosis of advanced disease is made in the puerperium as well as those with HIV coinfection. Failure to comply with treatment also worsens the prognosis[119]. Other obstetric complications that have been reported in these women include a higher rate of spontaneous abortion, small for date uterus, and suboptimal weight gain in pregnancy[120]. Others include preterm labour, low birth weight and increased neonatal mortality[121]. Late diagnosis is an independent factor, which may increase obstetric morbidity about fourfolds, while the risk of

preterm labour may be increased ninefolds[122]. Congenital tuberculosis is a rare complication of in utero tuberculosis infection while the risk of postnatal transmission is significantly higher[123]. Congenital tuberculosis may be as a result of haematogenous spread through the umbilical vein to the foetal liver or by ingestion and aspiration of infected amniotic fluid[124]. A primary focus subsequently develops in the liver, with involvement of the periportal lymph nodes. The tubercle bacilli infect the lungs secondarily, unlike in adults where over 80% of the primary infections occur in the lungs[125]. Congenital tuberculosis may be difficult to distinguish from other neonatal or congenital infections from which similar symptoms may arise in the second to the third week of life. These symptoms include hepatosplenomegaly, respiratory distress, fever, and lymphadenopathy. Radiographic abnormalities may also be present but these generally appear later[126]. The diagnosis of neonatal tuberculosis may, however, be facilitated by employing a set of diagnostic criteria including the demonstration of primary hepatic complex/caseating granuloma on percutaneous liver biopsy at birth, tuberculous infection of the placenta, or maternal genital tract tuberculosis, and the demonstration of lesions during the first week of life. The possibility of postnatal transmission must be excluded by a thorough investigation of all contacts, including hospital staffs and attendants. As much as half of the neonates delivered with congenital tuberculosis may eventually die, especially in the absence of treatment[127]. Tuberculosis (TB) has been recognized as a serious cause of morbidity and mortality in pregnancy for over a century. Efforts to research and roll out TB screening and treatment at antenatal clinics in high-burden populations has lagged behind similar efforts for HIV. Microbiological screening is important in pregnancy due to overlapping symptoms. There is a need for more data

on optimal treatment strategies during pregnancy and the effects of infection and treatment on maternal and neonatal morbidity and mortality.

**Epidemiology of TB in Pregnancy**

The recently published WHO Tuberculosis Report for 2014 states that in 2013 there were an estimated 3.3 million cases among women, with 510 000 deaths; a third of these women were co infected with HIV.128 The report does not mention the word 'pregnancy' or 'pregnant', indicative of the fact that most countries do not screen routinely for TB in pregnancy nor do they report the pregnancy status of female TB cases.129 The symptoms of TB overlap considerably with those of pregnancy, and one South African study has shown that the sensitivity of clinical screening for TB among pregnant women is as low as 28%.6 This shows that without active screening and case finding programmes among pregnant women, we can never hope to reach sufficient numbers of cases diagnosed and treated. A review of the available data suggested that the prevalence of active TB among pregnant women ranges from 0.06% to 0.25% in low burden countries. In high burden countries, rates of between 0.07% and 0.5% were found among HIV negative women, and between 0.7% and 11% among HIV positive women[130]. In an epidemiological modelling study, Sugarman et al. estimated that there may have been 216 500 (95% uncertainty range 192 000–247 000) active TB cases among pregnant women globally in 2011, with the highest case burden (41.3% of cases) in the WHO African region[131]. Large birthing centres represent a great opportunity for active case finding. A recent evaluation of the Xpert MTB/RIF assay among both obstetric and gynaecological admissions with suspected TB found culture proven TB in 27.7% of suspected cases[132]. This TB burden among maternal admissions with suspected TB was comparable to that among all adult admissions at the same centre, where active TB was diagnosed in 10% of

adult admissions able to produce a sputum specimen in whom TB was not suspected on admission, making inpatients potentially a higher risk group for missed TB infections than prisoners, refugees, and other well publicized groups[133]. In the context of maternal HIV infection, and also HIV negative women from high TB burden communities, the WHO prudently recommends standardized TB screening in antenatal clinics[11]. However, there is a general consensus that the uptake of this guidance has been poor[134].

**Effects on Immunity**

Women are at increased risk of TB during pregnancy and it is commonly assumed that immunological changes associated with pregnancy present an opportunity for mycobacterial infection or reactivation. In the late stages of pregnancy, a whole range of modifications in immune correlates have been observed. With respect to cellular immunity, increased levels and activity of phagocytes and plasmacytoid dendritic cells have been reported, with down regulated natural killer (NK) cell cytotoxicity by progesterone induced blocking factor and interleukin (IL)-10, and also a decrease in interferon gamma (IFN-γ) production, indicting a generally suppressed innate cellular response[135]. Adaptive cellular responses have also been evaluated, showing that Th1 cytokines (IFN-γ and IL-12) are down regulated; however, reports on whether Th2 responses are modulated in pregnancy have been conflicting[136]. Observed changes in humoral immunity include increased levels of complement proteins and acute phase reactants, and also increased T-cell-dependent immunoglobulin production[137]. Pregnant women who are HIV positive and have LTBI are more likely to progress to active TB disease.

**TB as a Cause of Maternal Deaths**

Maternal mortality is high among women co-infected with HIV and TB, and TB is associated with increased mortality both during pregnancy and postpartum[138]. Over 50% of pregnant women who die of TB during pregnancy and postpartum are HIV positive. A medical record review of maternal mortality in Zambia dating to the pre nevirapine era, documented TB as a cause of death in 25% of HIV negative versus 32% of HIV positive mothers[139]. More recent reviews or verbal autopsies of maternal deaths in Kenya and South Africa have recorded death as being directly attributable to TB in up to 20% of HIV positive pregnant women[140]. However, post mortem studies among the general population have shown that medical record reviews and verbal autopsies grossly underestimate the burden of TB as a cause of death[141]. There has been just one post mortem study undertaken in Africa on maternal deaths, and this study identified TB as a cause of death in 12.9% of deaths overall and 27.7% of deaths among HIV infected women[142]. Such studies are the exception not the rule, and so for the most part we must rely on comprehensive record reviews to monitor changes in the burden of TB deaths over time. In South Africa the prevalence of non obstetric infectious causes of death has increased steadily over the last two decades, concurrently with the HIV pandemic, so that as of 2007, infections such as TB, pneumonia, meningitis, and malaria accounted for 47% of all maternal deaths as reviewed[143]. Many record reviews have highlighted serious shortcomings in how we record causes of death, with 'HIV/AIDS' often being treated as a mutually exclusive cause of death, with separate categories for TB and other opportunistic infections. Consistent recording of maternal deaths is challenging due to the varied obstetric and non obstetric causes that may present as comorbidities.

**Effects of Maternal TB Infection and Treatment on the Neonate**

It has long been known that a few neonates born to mothers who have active TB disease will contract TB congenitally. Congenital TB may be subclinical or associated with a range of birth defects[145]. This has been little studied at the centre of the HIV/TB pandemic in Sub Saharan Africa, but one study of 107 pregnant South African women diagnosed with active TB (50% with extrapulmonary or disseminated infection) documented seven perinatal deaths, with an adjusted perinatal mortality rate attributable to TB of 65.2/1000 among HIV infected women[146]. Sixty-six percent of neonates were of low birth weight, compared to a hospital population rate of 22%[147]. Another South African study detected TB in 9% of infants born to mothers with active TB, and found maternal TB to be strongly associated with both maternal and neonatal mortality[148]. Epidemiological methods suggest a lower rate of vertical transmission, but these rely on routine culture returns and it is highly likely that many neonates born to mothers with TB are either not screened or may develop symptoms after discharge[149]. A Mexican study found maternal TB to be associated with increased morbidity, lower birth weight, and an increased risk of death. Studies from South Asia have linked TB during pregnancy to poor perinatal outcomes, including low birth weight, small for gestational age, perinatal mortality, and also maternal morbidity and mortality[150]. Even in low burden countries such as the UK, where TB during pregnancy is much less prevalent (62/100 000 pregnancies), it is still associated with low birth weight[151]. A recent South African study of 97 HIV infected women, half of whom had TB, found just one case of mother-to-child-transmission of HIV, and so could not prove that in the context of HIV, maternal TB has any influence on mother to child transmission of HIV, although women with TB had a higher HIV viral load at birth and had on average initiated Anti retroviral treatment (ART) earlier[156].

## Extrapulmonary TB and Pregnancy

The literature is abound with case series and individual reports of extrapulmonary TB (EPTB) in pregnancy, so much so that, taken with the proven burden of pulmonary TB in pregnancy, it is reasonable to suggest that EPTB will account for a significant minority of cases of TB in pregnancy. Reported outcomes are frequently better in high income populations, but many cases are diagnosed late, as the symptoms may be confused with those of other possible conditions; some cases may even be diagnosed retrospectively after identification of neonatal TB[157].

Reported presentations include, but are not restricted to, papulonecrotic tuberculids, TB spine, meningitis, primitive caeco-appendicular TB, genital TB as a possible cause of ectopic pregnancy, pericarditis, hemoptysis, and peritoneal TB[159]. Foetal outcomes range from being completely asymptomatic, to serious congenital abnormalities, and even a spontaneous abortion with TB histology in both placenta and foetus[160].

## Treatment of Tuberculosis in Pregnancy

Untreated tuberculosis represents a far greater hazard to a pregnant woman and her fetus than those treatment of the disease[161]. The management of tuberculosis in pregnancy is a multidisciplinary approach, with the team comprising the obstetrician, communicable disease specialty personnel, neonatologists, counselling unit, and public health officials. Treatment is achieved through the use of Directly Observed Therapy, Short Course (DOTS). This therapy entails the use of combination therapy for at least 6 months, depending on the combination of antituberculous agents that are available. This combination includes isoniazide and rifampicin compulsorily, supported by ethambutol and pyrazinamide[148]. For patients with drug susceptible TB and good drug adherence, these regimens will cure around 90% of TB cases.

Treatment is done on outpatient basis, unless otherwise indicated[149]. The use of these first line antituberculous drugs in pregnancy are considered safe for the mother and the baby by The British Thoracic Society, International Union Against Tuberculosis and Lung Disease, and the World Health Organisation[162].

**Isoniazide (INH)**

Isoniazide is safe during pregnancy even in the first trimester, though it can cross the placenta[146]. The women must, however, be followed up because of the possibility of INH induced hepatotoxicity. Pyridoxine supplementation is recommended for all pregnant women taking INH at a dose of 50 mg daily[165].

**Rifampicin**

This is also believed to be safe in pregnancy, though in an unknown proportion of cases, there may be an increased risk of haemorrhagic disorders in the newborn (some authorities prescribe supplemental vitamin K (10 mg/day) for the last four to eight weeks of pregnancy.) while some other researchers reported the possibility of limb deformity but none of these are in excess of what is obtained in the normal population.

**Ethambutol**

The retrobulbar neuritis that may complicate the use of this drug in adults generated the fear that it may interfere with ophthalmological development when used in pregnancy but this has not been demonstrated when the standard dose is used. This was also confirmed in experimental studies on some abortuses[167].

**Pyrazinamide**

The use of pyrazinamide in pregnancy was avoided by many physicians for a long time due to unavailability of adequate data on its teratogenicity. Presently, many international organizations now recommend its use, including the International Union Against Tuberculosis and Lung diseases (IUATLD), British Thoracic Society, American Thoracic Society, the World Health Organisation as well as the Revised National Tuberculosis Control Programme of India. There are no reports of significant adverse events from the use of this drug in the treatment of TB in pregnant women despite its use as part of the standard regimen in many countries[150]. Its use is particularly indicated in women with tuberculous meningitis in pregnancy, HIV coinfection, and suspected INH resistance[151]. Breastfed infants of mothers on antituberculous therapy should, however, be monitored for jaundice, which may suggest drug induced hepatitis, as well as joint pains resulting from drug induced hyperuricaemia[163].

**Streptomycin**

The drug has been proven to be potentially teratogenic throughout pregnancy. It causes fetal malformations and eighth-nerve paralysis, with deficits ranging from mild hearing loss to bilateral deafness. Many centres are against the use of this drug in pregnancy[168].

**Multidrug-Resistant Tuberculosis in Pregnancy (MDR-TB)**

Pregnant women with MDR-TB have a less favourable prognosis[169]. They may sometimes require treatment with second-line drugs, including cycloserine, ofloxacin, amikacin, kanamycin, capreomycin, and ethionamide. The safety of these drugs is

unfortunately not well established in pregnancy[170]. Para-amino salicylic acid had been used as combination therapy with INH in pregnancy in the past without any significant teratogenic side effects, though maternal gastrointestinal side effects may be pronounced. Ethionamide is associated with growth retardation, central nervous system and skeletal abnormalities in animal studies involving rats and rabbits[171]. Human studies also demonstrated increased central nervous system defects following its use in early pregnancy[172]. Its use is, therefore, not recommended in pregnancy. Therapeutic abortion has been proposed as an option of management for these women, as MDR-TB poses more risk to the woman and the society at large. Another option is to delay initiating treatment to the second trimester where possible[173]. Individualised Treatment Regimen (ITR) using various combinations of the 2nd line antituberculous agents based on their susceptibility profile had, however, been tried in some pregnant women with no adverse obstetric outcome[174]. The outlook for those patients is expected to improve as experience and knowledge in the management of the condition increases.

**B) HIV/AIDS as a Risk Factor of Tuberculosis**

The world health organization (WHO) has projected that tuberculosis (TB) and human immunodeficiency virus (HIV/AIDS) infections will be among the top 20 causes of death in 2030[1]. In 2010, there were 8.8 million (range, 8.5-9.2 million) incident cases of TB and 1.1 million (range 0.9-1.2 million) deaths from TB among HIV negative people, in addition, there was an additional 0.35 million (range 0.32-0.39 million) deaths from HIV associated TB[2]. Prevalent and incident TB cases are significantly associated with mortality in an HIV program[3]. According to the WHO, TB is the leading infectious killer of people living with HIV/AIDS (PLWHAs)[4]. Patients who are HIV positive and infected with TB are 20 to 40 times more likely to

develop active TB than people not infected with HIV living in the same country[5]. Thus, TB has become the commonest HIV associated opportunistic disease in the world and it affects people living with HIV/AIDS (PLWHAs) by accelerating HIV disease progression, by showing increased infectivity, and by reducing HIV treatment efficacy. While some risk factors are known, there is still a need to investigate TB risk factors among PLWHAs. Such a list of risk factors cannot be exhaustive and new risk factors are added on a continuous basis and even in specific settings.

### C) Diabetes as a Risk Factor of Tuberculosis

People with diabetes are at a higher risk of developing tuberculosis (TB) than those without diabetes. The association between diabetes and tuberculosis in developed countries was around 2.5 times more likely to develop tuberculosis. These findings were also true of developing regions where the prevalence of diabetes was twice as high in people with tuberculosis than in people without tuberculosis[172]. Not only does diabetes contribute to a person's risk of developing tuberculosis, but it also makes it more difficult to treat those who have both diseases. People with diabetes are more likely to fail treatment and more likely to die during treatment compared to those without diabetes.

### D) Overcrowding as a Risk Factor of Tuberculosis

Overcrowding contributes to the spread of tuberculosis. Close proximity to infected individuals is a significant issue in any closed institution and for healthcare workers. Many countries have specific guidelines for tuberculosis control in institutions. In prisons, the situation is complicated by the fact that inmates have an increased incidence of HIV, are frequently moved to other prisons or back into the community with little warning and may be poorly managed in terms of health services. Since

release of prisoners is often into poor circumstances and crowded hostels, the consequence of undetected or inadequately treated tuberculosis may be rapid spread of disease. Mass incarceration can lead to an increase in cases of tuberculosis and a rise in drug resistant disease[144]. An effective public health program with an active community care component can overcome such problems. Approximately 25-60% of household contacts of an index case may acquire infection although the extent to which individual genetic predisposition or immunologic impairment contribute to this is uncertain. Development of disease occurs in up to 10% of infected persons and is significantly affected by impaired cell-mediated immunity[123].

**E) Occupation as a Risk Factor**

Tuberculosis is a known occupational hazard for healthcare workers (HCWs), especially in countries with a high burden of tuberculosis. It is estimated that HCWs have a 2 to 3 fold increased risk of developing tuberculosis compared with the general population[173].

### 2.4.3 Pathogenesis

About 90% of those infected with *M. tuberculosis* have asymptomatic, latent TB infections (sometimes called LTBI), with only a 10% lifetime chance that the latent infection will progress to overt, active tuberculous disease[59]. In those with HIV, the risk of developing active TB increases to nearly 10% a year[60]. If effective treatment is not given, the death rate for active TB cases is up to 66%[61]. TB infection begins when the mycobacteria reach the alveolar air sacs of the lungs, where they invade and replicate within endosomes of alveolar macrophages[58]. Macrophages identify the bacterium as foreign and attempt to eliminate it by phagocytosis. During this process, the bacterium is enveloped by the macrophage and stored temporarily in a membrane

bound vesicle called a phagosome. The phagosome then combines with a lysosome to create a phagolysosome. In the phagolysosome, the cell attempts to use reactive oxygen species and acid to kill the bacterium. However, *M. tuberculosis* has a thick, waxy mycolic acid capsule that protects it from these toxic substances. *M. tuberculosis* is able to reproduce inside the macrophage and will eventually kill the immune cell. The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe[59]. Tuberculosis of the lungs may also occur via infection from the blood stream. This is known as a Simon focus and is typically found in the top of the lung[60]. This hematogenous transmission can also spread infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and the bones[61]. All parts of the body can be affected by the disease, though for unknown reasons it rarely affects the heart, skeletal muscles, pancreas, or thyroid[62]. Tuberculosis is classified as one of the granulomatous inflammatory diseases. Macrophages, epithelioid cells, T lymphocytes, B lymphocytes, and fibroblasts aggregate to form granulomas, with lymphocytes surrounding the infected macrophages. When other macrophages attack the infected macrophage, they fuse together to form a giant multinucleated cell in the alveolar lumen. The granuloma may prevent dissemination of the mycobacteria and provide a local environment for interaction of cells of the immune system[64]. However, more recent evidence suggests that the bacteria use the granulomas to avoid destruction by the host's immune system. Macrophages and dendritic cells in the granulomas are unable to present antigen to lymphocytes; thus the immune response is suppressed[65]. Bacteria inside the granuloma can become dormant, resulting in latent infection. Another feature of the granulomas is the development of abnormal cell death (necrosis) in the center of tubercles. To the naked eye, this has the texture of soft,

white cheese and is termed caseous necrosis[69]. If TB bacteria gain entry to the blood stream from an area of damaged tissue, they can spread throughout the body and set up many foci of infection, all appearing as tiny, white tubercles in the tissues[66]. This severe form of TB disease, most common in young children and those with HIV, is called miliary tuberculosis[67]. People with this disseminated TB have a high fatality rate even with treatment (about 30%)[68]. In many people, the infection waxes and wanes. Tissue destruction and necrosis are often balanced by healing and fibrosis[70]. Affected tissue is replaced by scarring and cavities filled with caseous necrotic material. During active disease, some of these cavities are joined to the air passages (bronchi) and this material can be coughed up. It contains living bacteria and thus can spread the infection. Treatment with appropriate antibiotics kills bacteria and allows healing to take place. Upon cure, affected areas are eventually replaced by scar tissue[74].

## 2.5 Clinical Manifestations of Tuberculosis

Cough is the commonest presentation. Initially it may be nonproductive, but as inflammation and tissue necrosis ensue, sputum is produced. Haemoptysis is occasionally a presenting symptom but usually results from previous disease and may not indicate active tuberculosis[75]. It may arise from tuberculous bronchiectasis, rupture of a dilated vessel in the wall of a cavity (Rasmussen's aneurysm), bacterial or fungal infection (especially Aspergillus mycetoma) in a cavity or erosion into an airway (broncholithiasis). Inflammation of the lung parenchyma adjacent to a pleural surface may cause pleuritic pain. Dyspnoea is unusual unless there is extensive disease and may result in respiratory failure[76]. Rales or crackles may be heard in the area of involvement and bronchial breathing indicating consolidation. In general prolonged cough (greater than 2 weeks) with or without sputum production and or

haemoptysis, prolonged fever, night sweats, anorexia, weight loss, loss of apetite, breathlessness, chest pain and fatigue are the signs and symptoms of tuberculosis[77].

Most tuberculosis occurs as pulmonary disease with 17% occurring at an extrapulmonary site only. However, as much as 70% of HIV-1 infected patients will have evidence of extrapulmonary disease or mycobacteremia once the CD4 count is below 100 cells µl/ml. Co-infected persons are more likely to present atypically, potentially delaying the diagnosis of tuberculosis[8].

**Central Nervous System Tuberculosis**

Tuberculosis of the central nervous system can present as tuberculous meningitis, tuberculomas, or tuberculous spinal meningitis. It accounts for approximately 5% of cases of extrapulmonary tuberculosis. It is most often seen in young children. Rupture of a subependymal tubercle into the subarachnoid space rather than direct hematogenous seeding is believed to be the main precipitating cause. The base of the brain is the most pronounced site. Aneurysm, thrombosis, and focal hemorrhage infarction may occur due to vasculitis of local arteries or veins by *M. tuberculosis*. Involvement of perforating vessels to the basal ganglia and pons may lead to movement disorders. Vasculitis of branches of the middle cerebral artery may cause hemiparesis[9]. The clinical spectrum of tuberculous meningitis ranges from chronic headache and subtle mental status changes to sudden, severe meningitis progressing to coma. A prodrome of malaise, intermittent headache, and low grade fever can be followed by protracted headache, vomiting, confusion, meningismus, and focal neurologic signs within 2 to 3 weeks. If untreated stupor, coma, seizures, and hemiparesis and death can occur within five to eight weeks after the onset of illness. Fever is not always present, and the peripheral white blood cell count is usually

normal. Patients may have mild anemia or hyponatremia due to inappropriate antidiuretic hormone secretion [10]. Paresis of cranial nerves, especially ocular nerves, is a frequent finding. Approximately three fourths of cases have evidence of concomitant extrameningeal tuberculosis, and 50% have abnormalities on chest X-ray. Tuberculomas are space occupying lesions in the brain. They are usually multiple but can be single. Patients may present with seizures or other focal neurologic symptoms without evidence of systemic illness or meningeal inflammation. The meninges can become involved with encasement of the spinal cord by a gelatinous or fibrous exudates in advanced cases. Patients may have bladder or rectal sphincter weakness, hypesthesia, anesthesia, paresthesias in the distribution of a nerve root, or paralysis and pain resulting from nerve root or cord compression[11].

**Pleural Tuberculosis**

Tuberculous pleurisy can occur within weeks to months after primary infection (early postprimary pleurisy), complicate chronic pulmonary tuberculosis, or develop concurrently in 10%-30% of cases with military tuberculosis. Early postprimary pleurisy usually affects adolescents and young adults. The effusion can resolve within several months in as many as 90% of cases; however, without treatment, 65% will relapse with chronic organ tuberculosis within 5 years[12]. Elderly patients with chronic pulmonary tuberculosis may have cirrhosis or congestive heart failure, so tuberculous pleurisy may be easily mistakenly attributed to underlying co-morbidities. Military tuberculosis may present with tuberculous polyserositis with bilateral pleural, peritoneal, and pericardial tuberculosis. The clinical course of tuberculous pleurisy may be low grade and subtle or abrupt and severe and can be confused with acute bacterial pneumonia. Patients usually have cough, pleuritic chest pain, and occasionally high fever. Night sweats, chills, weakness, dyspnea, and weight loss can

also occur[12].The effusion is usually minimal to moderate in volume and almost always unilateral (unless military tuberculosis exists concurrently). Empyema can occur when a cavity ruptures into the pleural space; empyema is associated with bronchopleural fistula formation and frank pus. It is rapidly fatal if antituberculosis therapy is not given expeditiously.

**Lymphadenitis**

Lymphadenitis is the most common form of extrapulmonary tuberculosis. In HIV-negative persons, it is usually unilateral and located in the cervical or supraclavicular area. The most common site is the upper border of the sternocleidomastoid muscle. Patients usually present with a painless, red, firm mass without systemic symptoms[13]. It is most often seen in young adult females. Children often have an ongoing primary infection, but other age groups seldom have concurrent extranodal tuberculosis. Mediastinal adenopathy is often seen in children with primary infection, but it is uncommon in young adults and elderly persons. Differential diagnosis of mediastinal adenopathy includes histoplasmosis, lymphoma, and cardinoma. Less commonly, tuberculosis can also cause fibrosing mediastinitis, and patients can present with dyspnea on exertion due to compression of pulmonary veins and arteries or superior vena cava syndrome. In individuals with AIDS, peripheral lymph node tuberculosis is always multifocal and associated with systemic symptoms, such as fever and weight loss. Mediastinal lymphadenopathy is frequent, and CT scan reveals multiple coalescing mediastinal masses with low density centers, peripheral contrast enhancement, and no calcification. Abdominal lymphadenopathy in the intra-abdominal cavity is also common in AIDS patients. Lymph nodes can obstruct the biliary tract, ureters, or bowel. Abscesses in the liver, spleen, pancreas, or kidney can exist concurrently[14].

**Pericardial Tuberculosis**

Pericardial tuberculosis is usually caused by extension from a contiguous focus of infection, such as mediastinal or hilar nodes, the lung, spine, or sternum. Dissemination to the pericardium can occur with military tuberculosis. The onset may be abrupt or insidious. Patients may present with dyspnea, orthopnea, dull retrosternal pain, a pericardial friction rub, or symptoms and signs of cardiac tamponade. Fever, weight loss and night sweats usually occur before cardiopulmonary complaints. A few patients present with findings of chronic constrictive pericarditis. A pleural effusion can be found in as many as 39% of cases with pericardial tuberculosis, and radiographic evidence of concurrent pulmonary tuberculosis in 32%-72% of cases[12].

**Bone and Joint Tuberculosis**

One third of cases with skeletal tuberculosis involve the spine (Pott's disease or tuberculous spondylitis). The disease can occur via hematogenous, contiguous, or lymphatic spread. The anterior superior or inferior angle of the vertebral body is infected first, and later the intervertebral disk and adjacent vertebra. The lower thoracic spine is involved most frequently followed by the lumbar, the cervical, and the sacral spine. In countries with endemic tuberculosis, Pott's disease usually develops in older children and young adults within one year after primary lung infection. However, in industrialized countries, it is a disease of older persons due to late reactivation of tuberculosis. Evidence of other foci of tuberculosis and systemic symptoms are often absent. In the early stage, only back pain or stiffness is present. Diagnosis may be delayed with the sequelae of paralysis, deformity, or sinus formation[14].

In 50% of cases, weakness or paralysis of lower extremities (Pott's paraplegia) may present initially or develop after treatment has begun. It may be due to arachnoiditis, vasculitis, or compression of the cord. In addition, paraspinal cold abscesses develop in 50% or more of the cases. These abscesses may appear after treatment has been initiated, or it may only be visible by CT or MRI. The pus, confined by tight ligmentous investments, can dissect along tissue planes to present as a mass or a draining sinus in the supraclavicular space, above the posterior iliac crest in the Petit triangle, or in the groin, the buttock, or even the popliteal fossa. The abscess can also spread to distant vertebral bodies without affecting the intervening vertebrae, or perforate into the bowel forming a gas-filled psoas abscess[13].

Systemic symptoms or extra-skeletal tuberculosis are occasionally absent. The hip or knee is most frequently involved. Indolent progressive inflammation develops weeks or months later. This population had more systemic symptoms, multiple joint involvement, and periarticular abscess formation. The earliest manifestation of tuberculous arthritis is pain, and the symptom may precede signs of inflammation and radiographic changes by weeks or months. Tenosynovitis of the hand, arthritis of the wrist, and carpal tunnel syndrome can also be caused by tuberculosis. Tuberculous osteomyelitis can affect all bones including the ribs, skull, phalanx, pelvis, and long bones[14]. Tuberculosis is the most common infectious cause for single of multiple osteomyelitic rib lesions since other causes of osteomyelitis of the rib are rare.

**Disseminated (Miliary) Tuberculosis**

The term, miliary tuberculosis, initially was used to describe the resemblance of the pathologic lesions to millet seeds. Now this term denotes any progressive disseminated tuberculosis spread hematogenously. It can be divided into three groups

according to clinical presentations and histologic findings: 1) acute miliary tuberculosis, 2) cryptic miliary tuberculosis, and 3) nonreactive tuberculosis[15].

Acute miliary tuberculosis is associated with a brisk and histologically typical tissue reaction. In the prechemotherapy era, it occurred either soon after primary infection in children or young adults or as a terminal event in untreated chronic tuberculosis. Children have acute or subacute onset, high intermittent fevers, night sweats, and occasional rigors. Pleural effusion, peritonitis, or meningitis occurs in as many as two thirds of cases. The clinical course of young adults is usually more chronic and initially less severe. However, now older individuals are affected by miliary tuberculosis more frequently, and their underlying illnesses may obscure the diagnosis. Patients usually have nonspecific constitutional symptoms, such as fever, anorexia, weakness, and weight loss. They may have headache due to meningitis, abdominal pain resulting from peritonitis, or pleural pain caused by pleuritis. Patients may have normal white cell count, anemia, hyponatremia, elevation of alkaline phosphatase and transaminases. Fulminant miliary tuberculosis may be associated with severe refractory hypoxemia (adult respiratory distress syndrome) and disseminated intravascular coagulation[16].

Cryptic miliary tuberculosis occurs in older patients with miliary tuberculosis; chest X-rays are normal and tuberculin test results are negative. Patients have a chronic clinical course characterized by mild intermittent fever, anemia, and, ultimately, meningeal involvement preceding death.

Nonreactive tuberculosis is very rare and characterized by massive hematogenous dissemination of tubercle bacilli, "nongranulomatous" (nonreactive) tissue lesions, and often a septic presentation. Patients present with overwhelming sepsis,

splenomegaly and subtle diffuse mottling on the chest X-ray. Hematologic abnormalities include leukopenia, thrombocytopenia, anemia, pancytopenia, leukemoid reactions, myelofibrosis, or polycythemia. Disseminated tuberculosis should be considered when pancytopenia is associated with fever and weight loss[15].

Miliary tuberculosis develops in 10% of AIDS patients with tuberculosis and 38% of AIDS patients with extrapulmonary tuberculosis. AIDS patients usually have constitutional symptoms and hectic fevers. The chest X-ray is abnormal in 80% of cases and includes typical miliary mottling. The tuberculin skin test is positive in only 10% of patients, and sputum smears are positive in 25%. However, blood culture can be positive in 50%-60% of cases. Patients can also have abscesses of various soft tissue and organs, including the liver, spleen, pancreas, psoas muscle, mediastinum, neck, chest wall, abdominal wall, and prostate[14].

**Genitourinary Tuberculosis**

This is mostly a disease of middle aged adults, and the onset is usually insidious. Asymptomatic renal cortical foci may occur in all forms of tuberculosis. Unsuspected renal foci were noted in 73% of cases with pulmonary tuberculosis in an autopsy study. In normal hosts, the interval between infection and active renal disease is usually years and sometimes decades[12].

In two large series of renal tuberculosis comprising 78 and 102 cases, respectively, 61%-71% had primarily genitourinary symptoms; dysuria and gross hematuria are most common. Constitutional symptoms occurred in only 14%-33% of cases. Skin tuberculin tests were positive in 88%-95%. 66%-75% had abnormal chest X-ray while only 7-38% had active pulmonary tuberculosis. Abnormal urinalysis was noted in 66%-93% of cases, and abnormal intravenous pyelogram (IVP) in 68%-93%. Pyuria,

albuminuria, and hematuria were the most common laboratory abnormalities. Sterile pyuria is a typical finding of renal tuberculosis, but 12%-45% of patients had positive cultures for bacterial pathogens at the same time, which can mask the true diagnosis[11].

Renal tuberculosis may spread to the prostate, seminal vesicles, epididymis, and testis in that order. In cases with male genital tuberculosis, 80% had coexistent renal disease and most advanced renal tuberculosis is associated with some male genital foci. The usual clinical manifestations are a tender scrotal mass associated with a draining sinus or oligospermia unresponsive to treatment. Genital foci can also result from lymphatic or hematogenous spread and present as a painful testicular or scrotal mass. The presence of epididymal or prostatic calcification suggests the diagnosis, but nontuberculous chronic prostatitis may also have a similar presentation[12].

Female genital tuberculosis starts with a hematogenous focus in the endosalpinx, and may spread to the endometrium, ovaries, cervix, and vagina. A granulomatous ulcerating mass in the cervix may resemble carcinoma. Abdominal pain, menstrual disorders, or infertility are common complaints. Patients may present with the pictures of pelvic inflammatory disease with unresponsiveness to therapy. It is uncommon for patients to have systemic symptoms, and signs of old tuberculosis are often absent. Pregnancies are often ectopic in the presence of pelvic tuberculosis[14].

**Abdominal Tuberculosis**

Abdominal tuberculosis can affect the gastrointestinal tract, the peritoneum, the liver, and the pancreas. Before effective antituberculous agents were available, 70% of patients with advanced pulmonary disease acquired gastrointestinal tuberculosis by swallowing infected secretions. However, fewer than 25% of cases with gastrointestinal tuberculosis have radiographic evidence of pulmonary tuberculosis.

Tuberculosis can involve any gastrointestinal site from the oropharynx to the anus. Patients can present with nonhealing ulcers of the tongue or oropharynx, or nonhealing sockets after tooth extraction. An adjacent caseous node might result in esophageal stricture with obstruction, tracheoesophageal fistula formation, and rare fatal hematemesis from an aortoesophageal fistula. Patients might have ulcerative or hyperplastic lesions in the stomach or gastric outlet obstruction. Duodenal involvement may lead to symptoms of peptic ulcer or obstruction[9]. Perforation, obstruction, enteroenteric and enterocutaneous fistula, massive hemorrhage, and severe malabsorption may follow small bowel involvement. The most typical site of enteric tuberculosis is the ileocecal area producing symptoms of pain, anorexia, diarrhea, obstruction, hemorrhage, and a palpable mass. Patients with anal tuberculosis might have ulcers, perianal warty growths, and fistulas.

Tuberculous peritonitis results from either spread of adjacent tuberculous disease or military tuberculosis. The clinical picture has two types: plastic and serous. The plastic type is less common; characterized by tender abdominal masses and a "doughy abdomen". The serous type presents with ascites often without signs of peritonitis. Patients usually have symptoms of fever, abdominal pain, and weight loss. The onset may be insidious or acute. Some cases were diagnosed at routine hernia repair, or during surgery for an unknown mass or an acute abdomen. Tuberculous peritonitis was often undiagnosed in patients having cirrhosis with ascites. Peritoneal dialysis patients may present with the clinical pictures of bacterial peritonitis unresponsive to routine antibiotics. Tuberculosis peritonitis in women may mimic advanced ovarian cancer. Of 22 women with tuberculosis peritonitis, 90.9% had elevated CA-125 levels (mean 564.95 U/mL; range 3-2021 U/mL) and 77.3% had detectable pelvic masses[14].

Tuberculosis is a frequent cause of granulomatous hepatitis with elevated alkaline phosphatase and gamma-glutamyl transpeptidase levels that are out of proportion to bilirubin levels with normal or mildly elevated transaminase levels. Very rarely, tuberculosis granulomatous hepatitis causes jaundice without evidence of extrahepatic tuberculosis (primary tuberculosis of the liver). Focal hepatic tuberculosis describes single or multiple tuberculous abscesses occurring in patients with little natural immunity to tuberculosis and in children. Pancreatic tuberculosis may present with an abscess or a mass involving local nodes and resembling carcinoma[21]. Abdominal lymph nodes may obstruct the biliary tract causing tuberculous ascending cholangitis.

## 2.6 Global Epidemiology of *Mycobacterium tuberculosis*

Tuberculosis (TB) remains an enormous public health concern causing disease in an estimated 10 million people globally in 2017[81]. In addition, TB is one of the top 10 causes of death worldwide, and has been the leading cause of death from a single infectious agent for the past 5 years, surpassing even that of HIV/AIDS[82]. Such statistics are disconcerting given the reality that TB is not a new disease but rather one that has plagued populations for millennia, and especially since the majority of people who develop TB can be cured with a timely diagnosis and appropriate management[82]. The WHO's End TB Strategy and the United Nations' Sustainable Development Goals share a common aim to end the global TB epidemic, targeting an 80% reduction in TB incidence by 2030 compared with 2012[83]. At the current global decline in TB incidence of just 2% per annum, these targets will almost certainly be unattainable[1]. India and South Africa rank among the top 10 high TB burden countries. With 2.7 million new cases in 2018, India has the highest absolute burden of TB[81]. With 567 new cases per 100 000 population, South Africa has the second highest rate

of TB incidence.84 Despite these top ranks, and collectively accounting for 31% of the world's burden of TB, and 37% of TB deaths, the nature of the epidemic in South Africa and India is considerably different. A total of 1.4 million people died from TB in 2019 (including 208 000 people with HIV). Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS) [85]. In 2019, an estimated 10 million people fell ill with tuberculosis (TB) worldwide. 5.6 million men, 3.2 million women and 1.2 million children[86]. TB is present in all countries and age groups. But TB is curable and preventable. About 1.2 million children fell ill with TB globally. Child and adolescent TB is often overlooked by health providers and can be difficult to diagnose and treat, the 30 high TB burden countries accounted for 87% of new TB cases. Eight countries account for two thirds of the total, with India leading the count, followed by Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa[85]. Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat. A global total of 206 030 people with multidrug- or rifampicin-resistant TB (MDR/RR-TB) were detected and notified in 2019, a 10% increase from 186 883 in 2018. Globally, TB incidence is falling at about 2% per year and between 2015 and 2019 the cumulative reduction was 9%. This was less than half way to the End TB Strategy milestone of 20% reduction between 2015 and 2020. An estimated 60 million lives were saved through TB diagnosis and treatment between 2000 and 2019[86].

### 2.6.1 Epidemiology of *Mycobacterium tuberculosis* in Nigeria

Tuberculosis is a global treat and Nigeria is among the high TB, TB/HIV and DR-TB countries globally. The country ranks 7th among the 30 high TB burden countries globally and 2nd in Africa, accounting for 4% of the estimated incidence cases globally.81 In 2015, the global estimate for new (incident) TB cases was estimated at

10.4 million. People living with HIV accounted for 1.2 million (11%) of the incident cases[87]. An estimated 460,000 cases of tuberculosis occur in Nigeria annually. TB prevalence among HIV/AIDS patients rose up to 27% due to increased association of TB with HIV/AIDS.82 The TB incidence and mortality rates for the country is 219/100,000 and 39/100,000 population respectively. The treatment coverage for the country in 2016 was 24% making it the lowest TB treatment coverage rate globally. Nigeria ranks 8th among the 30 high Multidrug Resistance (MDR) TB burden countries with a drug resistance TB prevalence of 4.3% among new cases and 25% among previously treated cases. A recent national survey by the National Tuberculosis, Leprosy and Bruli ulcer Control Programme on catastrophic cost experienced by TB patients in Nigeria in 2017 reports that 51% of TB patients experienced catastrophic cost with a higher proportion (93%) among drug resistance TB patients by the human capital catastrophic cost estimation threshold of 20% indicating the severity of TB in the country.88 According to the 2018 World Health Organization Global Tuberculosis Report, Nigeria has a TB incidence rate of 219 per 100,000 population while the estimated incidence of Multidrug Resistant, rifampicin resistant TB (MDR/RR-TB) was 12 per 100,000. Nigeria was listed as one of the top 20 countries with the highest incident TB cases among people living with HIV and in the general populations[89].

## 2.7 Diagnosis

Most tuberculosis programmes use direct smear examination of sputum but, if resources permit, culture is desirable. Reliable susceptibility testing is a luxury few developing countries can afford, although it is especially desirable for purposes of retreatment. Rapid methods of culture and susceptibility testing are widely available in the wealthier nations. Molecular techniques have provided quick, sensitive, and

specific tests for *Mycobacterium tuberculosis* such as polymerase chain reaction, DNA and RNA probes, and γ interferon tests, but these are expensive and technically demanding[90]. They are most useful in diagnosing multidrug resistant organisms quickly and in differentiating M tuberculosis from other, non-infectious mycobacterial species. Tuberculin testing is helpful in ranking tuberculosis among the differential diagnoses of conditions with symptoms, signs, and radiological changes that would be compatible with pulmonary tuberculosis but where sputum is negative on direct smear or culture. A strongly positive tuberculin test in such a patient who has not previously had BCG vaccination or tuberculosis increases the probability that tuberculosis is the diagnosis. In those who have previously received BCG vaccination, γ interferon tests will differentiate between that and M tuberculosis as a cause of the strongly positive tuberculin test[91]. Chest radiography is a more expensive test than examination of sputum by direct smear, but when available and reliable it is an important investigation, especially when clinical suspicion of tuberculosis exists but the sputum is negative[92].

### 2.7.1 Smear Microscopy of *Mycobacterium tuberculosis* using Ziehl Nelseen Stain

This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus *Mycobacterium*, are resistant and can only be visualized by acid-fast staining. When the smear is stained with carbol fuchsin, it solubilizes the lipoidal material present in the Mycobacterial cell wall but by the application of heat, carbol fuchsin further penetrates through lipoidal wall and enters into cytoplasm. Then after all cell appears red. Then the smear is decolorized with decolorizing agent (3% HCL in 95% alcohol) but the acid fast cells are resistant due to the presence of large amount of lipoidal material in their cell wall

which prevents the penetration of decolorizing solution. The non acid fast organism lack the lipoidal material in their cell wall due to which they are easily decolorized, leaving the cells colourless. Then the smear is stained with counterstain, methylene blue. Only decolorized cells absorb the counter stain and take its colour and appears blue while acid-fast cells retain the red colour. The smear is microscopically examined using the 100 X oil immersion objective for Acid fast bacilli.

### 2.7.2 Light Emitting Diode Fluorescence Microscopy (LED-FM) of *Mycobacterium tuberculosis*

Smear microscopy remains the cornerstone frontline diagnostic test in the majority of primary health centres among countries with a high burden and limited resources. Based on its increased sensitivity and reduced reading time compared with conventional Ziehl Neelsen (ZN) light microscopy, the World Health Organization (WHO) recommended the introduction of light-emitting diode fluorescence microscopy (LED-FM) as an alternative to ZN microscopy in both high and low volume laboratories[163]. The WHO recommendation was mostly based on results from accuracy studies in reference or research laboratory centres presented in a meta analysis[164]. However, in 2013, only 6% of microscopy centres had reportedly switched to LED-FM[165]. One barrier to the uptake of LED-FM is a shared concern, among technologists, of LED-FM's lower specificity compared with ZN microscopy and the lack of clear quality control procedures[165]. Mycobacteria retain the primary stain (freshly filtered auramine phenol) even after exposure to decolorizing with acid alcohol, hence the term ‘acid fast’. A counter stain is employed to highlight the stained organisms for easier recognition. Potassium permanganate is used as counterstain and it helps prevent non specific fluorescence. With auramine staining, the bacilli appear as slender bright yellow luminous rods, standing out clearly against

a dark background. The identification of the mycobacteria with auramine O is due to the affinity of themycolic acid in the cell walls for the fluorochromes. In fluorescent microscopy, light rays of shorter wave length pass through smear stained by a fluorescent dye, such as auramine O, which have the property of absorbing light rays of shorter wave length and emitting light rays of longer wave length. A mercury vapour lamp is used as a source of light and by means of suitable filter only light rays of shorter wave lengths are allowed to emerge and these rays are used for microscopy. The condenser of the microscope is made of quartz which will not absorb ultra-violet rays[164].

### 2.7.3 Cultural Method for Detection of *Mycobacterium tuberculosis*

Storage of sputum specimens at -20°c preserves 100% viability of M tuberculosis. N-acetyl-cystein 2% NaoH(NALC-NAOH) is used for decontamination of this specimen. Decontaminated specimens can be inoculated in solid and liquid media with WHO advocating parallel inoculation in both media in order to combine the higher specificity of solid media with the higher sensitivity of liquid. Incubation at 37°C is optimal and it shows that *M. tuberculosis* is a microaerophilic organism which is a characteristic put into account to develop the culture media[169]. A 5–10% CO2 enrichment of atmosphere stimulates primary isolation[170]. In 1903, the first egg yolk agar medium was reported, further complemented by glycerol and malachite green to inhibit contaminants. This Lowenstein Jensen (LJ) medium is solidified by coagulation at 83°C for 40 min. It remains the most commonly used culture medium worldwide[170]. The LJ medium was complemented with sodium pyruvate, gelatin, sodium glutamate, activated carbon and oligonucleotides and the concentration of glycerol lowered. These modifications optimized the isolation of *Mycobacterium* bovis, *Mycobacterium* africanum, and mycobacteria[169]. In both media, eggs buffer

harmful effects of quaternary ammonium compounds against *M. tuberculosis*. The advantages of these inexpensive media are high sensitivity and the characteristic morphology of colonies. However, they are sensitive to the quality of organic compounds (eggs), long to prepare and rapidly perishable. Solid culture media ease the detection of contaminants, allowing to pick up the right colony for further analyses, the morphology of colonies facilitates differentiation between *Mycobacterium* species in mixed infections. Among liquid media, the Dubos broth contains inorganic salts, enzymatic digest of caseine and acid L-asparagine as a source of nutrient, polysorbate 80 and oleic acid ester as a source of essential fatty acids and bovine albumin as a protective agent from the binding of free fatty acids toxic to mycobacteria[170]. Low levels of penicillin and nalidixic acid are also present in LJ medium to inhibit growth of Gram-positive and Gram-negative bacteria, to limit growth to *Mycobacterium* species only. Presence of malachite green in the medium inhibits most other bacteria. It is disinfected and solidified by a process of inspissation. Presence of glycerol enhances the growth of *M. tuberculosis*. For cultivation of *M. bovis*, glycerol is omitted and sodium pyruvate is added. The medium appears green, opaque, and opalescent. LJ medium is used for diagnosis of mycobacterial infections, testing antibiotic susceptibility of isolates and for differentiating different species of *Mycobacterium* (by colony morphology, growth rate, biochemical characteristics, and microscopy) [169].

### 2.7.4 Molecular Detection of *Mycobacterium tuberculosis*

The processing of clinical specimens in the mycobacterial diagnostic laboratory has undergone remarkable improvements during the last decade. While microscopy and culture are still the major backbone for laboratory diagnosis of tuberculosis on a worldwide basis, new methods including molecular diagnostic tests have evolved over

the last two decades. The majority of molecular tests have been focused on (i) detection of nucleic acids, both DNA and RNA, that are specific to *Mycobacterium tuberculosis*, by amplification techniques such as polymerase chain reaction (PCR); and (ii) detection of mutations in the genes that are associated with resistance to antituberculosis drugs by sequencing or nucleic acid hybridization. Recent developments in direct and rapid detection of mycobacteria, with emphasis on *M. tuberculosis* species identification by 16S rRNA gene sequence analysis or oligohybridization and strain typing contribute to these advances. Advancements in molecular methods for MTB detection has shortened the time to diagnosis to a few days, whereas diagnosis by conventional culture systems needs several weeks[174]. The majority of molecular tests have been aimed at the detection of MTB specific nucleic acids, both in DNA and RNA, by using amplification techniques such as polymerase chain reaction (PCR), and detection of genes mutation that are related with the resistance to anti-TB drugs by sequencing or nucleic acid hybridization[175]. Moreover, the WHO has announced the need for diagnostic options that are a sputum based replacement test for smear microscopy, a non sputum based biomarker test that is resource adjusted at facilities below microscopy laboratories, a simple initial test for first contact care providers as a rule out test, and a fast drug sensitivity test at the microscopy laboratory level. In middle and high income countries, development continued with innovations in microscopy (for example, light emitting diode [LED] microscopes), MTB culture systems (for example, rapid automated liquid culture systems, like the Becton Dickinson MGIT 960), nucleic acid amplification systems line probe assays and automated systems, such as the Cepheid Xpert MTB/RIF system (Cepheid, Inc., Sunnyvale, CA, USA) [176].

### 2.7.4.1 Nucliec Acid Amplification Test (NAAT)

The conventional bacteriological diagnosis of TB has several limitations therefore the NAAT has emerged as a potential alternative[177]. The NAAT systems, with rapid turn around times, facilitate testing and treatment initiation in the same visit and, therefore, loss to follow up cases can be reduced. Most NAAT assays detect the mycobacterial insertion element IS6110 for the identification of the MTB complex organisms. NAAT detects MTB ribosomal RNA or DNA directly from sputum specimens, both the acid fast bacilli (AFB) smear positive and AFB smear negative[177]. The NAAT shows very high sensitivity in sputum smear positive patients and around 61 to 76% sensitivity in patients with smear negative sputum[178]. Currently, the NAAT that is endorsed by the WHO is the Xpert/RIF MTB assay[178]. The other two NAATs, the Amplified *Mycobacterium tuberculosis* Direct (MTD) Test (Gen-Probe, Inc) and Amplicor *Mycobacterium tuberculosis* Test (Roche Molecular Systems, Inc), have also been approved by the Food and Drug Administration (FDA) for testing respiratory AFB smear positive specimens[176]. Other commercial NAATs are also available, including the loop-mediated isothermal amplification based MTB detection system,the cross priming amplification based TB diagnostic system and the Genedrive *Mycobacterium tuberculosis* iD[178]. In multi-bacillary diseases with a high mycobacterial load, a positive AFB smear with a positive NAAT would indicate active tuberculosis whereas a positive AFB smear with a negative NAAT in the absence of inhibitors would indicate nontuberculous mycobacterial (NTM) disease[177]. If the culture was positive in the above case, the physician could consider the patient as a bacteriologically confirmed case of TB. A NAAT could determine whether AFB smear positive patients had TB or not. Moreover, if the NAAT result is positive but the AFB smear result is negative, the decision to begin anti-TB treatment would rely

on the clinical judgment while awaiting culture results. According to Centers for Disease Control (CDC), if the sputum is smear negative and the NAAT also negative, an additional specimen should be tested with NAAT. However, if the culture results detected MTB bacteria growth, then the patient could also be classified as bacteriologically confirmed for having pulmonary TB. Finally, if the AFB smear, the NAAT, and the MTB culture showed negative results, the physician could classify the patients as a clinically diagnosed case of TB or consider another diagnosis. In locations where the rate of cultures positive for TB is low, it might be more efficient to limit the NAAT to cases with positive smear results; on the other hand, in locations where TB cases are high, a NAAT can be used in cases with negative smear results.

#### 2.7.4.2 Expert MTB/RIF Assay

The Xpert MTB/RIF assay is a nucleic acid amplification based test using a cartridge based on the GeneXpert Instrument System[155]. The basis of the Xpert MTB/RIF assay is a real time PCR that can be used to detect DNA specific to the MTB in sputum samples. A single Xpert MTB/RIF test directly from sputum can detect 99% of smear positive patients and more than 80% of smear negative cases[157]. According to the WHO in 2013, a Xpert MTB/RIF assay could be used for: an add on test following microscopic TB examination; a replacement examination for AFB smear microscopy; detection of MTB in both AFB smear positive and smear negative culture positive cases; detection of MTB in pleural fluid; detection of MTB in lymph node in samples from biopsy or fine needle aspiration; detection of MTB in gastric fluid; detection of MTB in samples of cerebrospinal fluid; and detection of MTB in tissue samples[155]. In 2014, the WHO stated that the Xpert MTB/RIF assay could be used as the initial diagnostic test in all subjects suspected on having pulmonary TB[156]. The Xpert MTB/RIF assay detects rifampicin resistance by PCR amplification of the 81-bp

fragment of the MTB rpoB gene and subsequent probing of this region for rifampicin resistant associated mutations and the results can be obtained within 2 hours. The WHO recommends subjects who are at high risk of MDR-TB should always have their sputum checked using the Xpert MTB/RIF test[155]. The Xpert MTB/RIF can be used as an initial test and as an add-on test after a negative AFB smear microscopy result. The use of Xpert MTB/RIF test has shortened the median time to treatment for AFB smear-negative TB from 56 days (range 39-81 days) to 5 days (range 2-8 days). However, Xpert MTB is more expensive than conventional sputum microscopy. Nevertheless, in one analysis, if a rapid sputum based test unit cost is of US$ 2-4, it would be lower to a similar cost of the conventional sputum smear microscopy. In order to facilitate access, the Foundation for Innovative New Diagnostics (FIND) has negotiated significant price reductions. Nevertheless, implementation of Xpert MTB/RIF would not be able to improve the control of drug sensitive TB without improvement to the health system, especially as to reducing the initial loss to follow up and reducing the time to treatment initiation[156].

#### 2.7.4.3 Whole Genome Sequencing (WGS)

Microbial genomics has allowed the investigation of the organisms' genetic markers that may impact treatment and infection prognosis. Whole genome sequencing (WGS) is becoming an affordable and accessible method that can identify microevolution within MTB lineages as they are transmitted between hosts[177]. There are two classes of sequencers that exist: the first generation sequencer and the second generation (widely known as the next generation sequencer [NGS]). The first generation sequencer is relatively slow, but has a high throughput and low cost (approximately $65 per bacterial genome). The second generation has a lower throughput, higher cost

(approximately $150 per genome in the case of the Illumina MiSeq) and is able to sequence multiple genomes in less than a day[178].

## 2.8 Treatment

Treatment depends on whether a person has active or latent TB. For people with latent TB, preventive therapy is recommended which typically involves taking an antibiotic called isoniazid daily for 6-9 months. People with active TB usually need to take a combination of antibiotics for 6-12 months[93]. First-line treatment options include isoniazid, rifampin, ethambutol, and pyrazinamide. While some people with active TB require a short hospital stay, many can receive treatment at home. Most people start feeling better and are no longer able to pass on the infection after a few weeks of treatment[94]. However, it is essential to complete the full course of treatment exactly as the physician directs to keep the disease from recurring and to prevent the bacteria from becoming resistant to the drugs. Drug resistant TB is much more difficult to treat and can be very dangerous if a person passes it on to other people[95].

## 2.9 Prevention and Control

As with all health conditions, prevention of TB is always better than a cure. Although there is no sure fire way to completely prevent the spread of TB at a point in time, there are a number of measures that can be put in place to reduce the spread of the illness.

A) The BCG (Bacille Calmette-Guérin) is a live vaccine against tuberculosis. The vaccine is prepared from a strain of the weakened bovine tuberculosis bacillus, *Mycobacterium* bovis.The BCG is currently the only licensed vaccine against TB, and has been in use since 1921[96]. It is one of the most widely used vaccines worldwide,

yet we still see around 9 million new cases of TB annually a testament to the BCG's limited effectiveness. The BCG is 80% effective in preventing TB for 15 years, more effective against complex forms of TB in children and has limited effectiveness in people over the age of 35. It is less effective when given in equatorial regions (due to high levels of naturally occurring environmental mycobacteria) [97].

B) Also early diagnosis and treatment is the most effective way to prevent the spread of tuberculosis[98]. A person with infectious tuberculosis can infect up to 10-15 other people per year[99]. But once diagnosed with TB, and started on treatment, the majority of patients are no longer infectious after just two weeks of taking the medication. Limiting the spread of TB depends on successfully finding and treating people with the illness, to prevent them from passing it on to others. This can be done through raising awareness of TB, so people with TB symptoms know to seek help[100]. Outreach workers and volunteers also work within communities with high rates of TB to find people with symptoms and refer them for testing. When someone is diagnosed with infectious TB, their close contacts are screened for the illness, this is known as contact tracing[101]. As TB is an airborne infection, TB bacteria are released into the air when someone with infectious TB coughs or sneezes. The risk of infection can be reduced by using a few simple precautions such as:

C) Good ventilation: TB can remain suspended in the air for several hours with no ventilation.

D) Natural light: UV light kills off TB bacteria.

E) Good hygiene: covering the mouth and nose when coughing or sneezing reduces the spread of TB bacteria. In healthcare settings, the spread of TB is reduced through the use of protective masks, ventilation systems, keeping potentially infectious

patients separate from other patients, and the regular screening of healthcare workers for TB.

F) Having a healthy immune system: This is the best form of defence against TB. 60% of adults with a healthy immune system can completely kill TB bacteria[102].

## Endnotes


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# Chapter Three

# Methodology

## 3.1 Research Design

Quantitative experimental design targeting pregnant women.

## 3.2. Study Area

The study was carried out in Adeoyo Maternity Teaching Hospital, Ibadan, Oyo state, South West Nigeria.

## 3.3. Sample and Sampling Technique

This was be obtained using the statistical formula for population > 10,000 as stated below;

$n=z^2pq/d^2$

where n = the desired sample size when population is >10,000

Z = the standard normal deviate set at 1.96 which corresponds to the 95% confidence level[1].

P = prevalence of 8.5% *Mycobacterium tuberculosis* among pregnant women

q= 1- p

= 1- 0.085 = 0.915

d= the desired degree of accuracy set at 0.05

from $n= z^2pq/d^2$

$n= (1.96)^2 (0.085)(0.92)/(0.05)^2 = (3.84)(0.0782)/0.0025 = 120$

n = 120

Add 10% (Attrition rate) =120 × 1.11 = 133.2 patients

Therefore approximate sample size of 150 samples were needed for the project.

### 3.4 Inclusion Criteria

Pregnant women attending Adeoyo Maternity Teaching Hospital Ibadan were included. Those that gave their informed consent.

### 3.5 Exclusion Criteria

Pregnant women with critical health challenge. Those who do not give their informed consent.

### 3.6 Ethical Approval

Ethical clearance was obtained from the Oyo state research ethical review committee.

### 3.7 Laboratory Analysis

#### 3.7.1 Data Collection

Questionnaires were administered to the patients so as to collect their demographic information which includes age, gender, trimester, occupation, residential address, number in the household, medical history, among others.

#### 3.7.2 Sample Collection

One hundred and fifty (150) samples were obtained from pregnant women attending ante natal clinic at Adeoyo Maternity Teaching hospital over a period of six months. Sputum samples were collected into well labelled sterile wide mouth screw capped falcon tubes. The patients were instructed on how to expel the sputum which was done through a deep cough to avoid the expulsion of saliva. These samples were collected in duplicate at different times of the

day. 5ml of venous blood samples was also collected aseptically from the pregnant women into well labelled sample bottles (fluoride oxalate and EDTA bottles).

### 3.7.3 Sample Transportation

Sputum and blood samples were well packed and corked to avoid leakages, they were kept in a Giostyle box with icepack to maintain 2 - 4°c temperature for onward transportation to the laboratory for processing.

**Culture Media**

Lowenstein Jensen media are the main solid media used to culture Mycobacteria. *M. tuberculosis* needs a protein enriched medium and also requires aerobic conditions for culture[2]. The constituents of LJ media are malachite green, inspissated eggs, and glycerol or pyruvate. LJ medium that contains glycerol favors the growth of *M. tuberculosis* while LJ medium without glycerol but contains pyruvate encourages the growth of *M. bovis*[3]. *M. tuberculosis* grows slowly with a generation time of about 16 to 24 hours and takes 3 to 6 weeks or longer to give visible colonies[4]. It is prepared according to the manufacturer's instructions.

**Chemical Reagents**

Ziehl Nelseen stain: Carbol fuchsin, Acid alcohol, Methylene blue, immersion oil, and distilled water[5].

**Selected Antibiotics for Susceptibility Test**

Antibiotic susceptibility testing was carried out on the isolates using first line and second line anti TB drugs by using CLSI guidelines: Isoniazid, Rifampicin,

Ethambutol, Kanamycin, Amikacin, Capreomycin, Ofloxacin were used. All are reconstituted according to the manufacturer's instructions.

### 3.7.4 Sample Processing

Sample was processed in BSL3 laboratory, the South West TB Reference Laboratory UCH Ibadan under a biosafety cabinet 2 and Jericho chest hospital Ibadan.

#### 3.7.4.1 Glucose Estimation

Venous blood sample was collected asceptically into well labelled Fluoride oxalate bottles, samples were spun at 1500 rpm for 5mins and the plasma is separated into clean sterile bottles. One ml glucose reagent (Randox) was pipetted and ten ul of sample was added. This was done along side with the blank and standard. This was incubated at 37°c for 10 mins. The blank, standard, and sample was aliquot into a cuvette and read using a spectrophotometer at 515nm wavelength to determine its optical density[6].

#### 3.7.4.2 HIV Screening

Venous blood sample was collected asceptically into well labelled EDTA bottles, the samples were spun at 1500 rpm for 5 mins and plasma separated into clean sterile bottles. 50ul of sample was placed on the Rapid kits (Determine and Unigold)[7]. The results was read under 5mins and the reactivity determined.

#### 3.7 4.3 Smear Microscopy:

The smear from the sputum were prepared on a clean slide and stained with Ziehl Nelsen stain and then observed under oil immersion lens to check for Acid fast bacilli (AFB) following WHO guidelines[8]. Sputum smear was prepared on a clean and

grease free slide using sterile loop. The smear was allowed to air dry and then fixed with gentle heat. The sputum smear was then covered with strong carbol fuchsin stain and heated until vapour just begins to rise (about 60° C). Only a small flame was applied under the slides using an ignited swab previously dampened with a few drops of acid alcohol or 70% v/v ethanol. The heated stain was allowed to remain on the slide for 5 minutes and then washed off with clean water. The smear was then covered with 3% v/v acid alcohol for 5 minutes until the smear was well decolorized, i.e gave a pale pink colour[9]. It was then washed well with water. The smear was then covered with methylene blue stain for 1-2 minutes and the stain washed off with water. The back of the slide was then wiped clean using a damp cotton wool and placed in a draining rack for the smear to air dry. The smear was then examined microscopically using a 100 X oil immersion objective.

#### 3.7.4.4 Cultural Method

The cultural method is the gold standard even though it takes time. The samples were inoculated into slopes of Lowenstein Jensen (LJ) medium and incubated at 37°C for a maximum period of eight weeks. The media was inspected on a daily basis for growth of mycobacteria species. The specimen was inoculated into the two slopes of LJ media, one with glycerol and the other with pyruvate[10]. NaoH-NALC equal to the amount of specimen is added to the sputum specimen, this is used for digesting and decontaminating the organism and centrifuged at 4°c for 15min at 3000xg[10]. The supernatant is discarded and sediment resuspended with 2ml of buffer. 0.2-0.4ml of the sediment is put into each LJ slope and distributed over the surface. The culture is incubated at 35-37°C until growth is observed or discarded as negative after eight weeks. A known *Mycobacterium tuberculosis* isolate is also used to control the process. *Mycobacterium tuberculosis* produces rough (having the appearance of bread

crumbs or cauliflower), raised, dry, non-pigmented (cream/buff colored) colonies. This was verified by staining with Ziehl-Nelsen (ZN). The colony characteristics can be used to identify *Mycobacterium tuberculosis*[11].

### 3.7.4.5 Antibiotic Susceptibility Test

Proportion method which involves macroscopic observation of growth in drug free and drug-containing media was used. Bacterial suspension was prepared by scrapping up colonies from the surface of the media and emulsified in sterile distilled water and compared visually with Mcfarland turbidity standard No 1[12]. The bacterial suspension dilutions were inoculated into drug free and drug-containing culture media (LJ medium). For growth control, the drug-free culture media were inoculated. Tubes were labelled for growth control as C1, C2, C3, and drug containing culture labelled as INH, RIF, EMB, KAN, AK, CAP and OFL and incubated at 37°c[13]. It was read after 4weeks as a provisional result and after 6weeks for the definitive interpretation of result. A strain is considered to be susceptible if there are no colonies or considerably less than 1% growth on the drug-containing medium compared with growth control and a strain is considered to be resistant if the number of colonies on the drug-containing medium exceeds 1% of that in the growth control medium[14].

### 3.7.4.6 Molecular (Genexpert Testing of TB)

The Xpert MTB/RIF assay is a nucleic acid amplification test that uses a cartridge which is based on the GeneXpert Instrument System[15]. The Xpert MTB/RIF assay is a real time PCR which can be used to detect DNA specific to the MTB in sputum samples[16]. Xpert MTB/RIF test can detect 99% of smear positive patients and more than 80% of smear negative patients directly from sputum. The GeneXpert is a molecular TB test which detects the DNA in TB. Sputum sample is used and the

result is out in less than 2 hours. Expert MTB/RIF is a cartridge based nucleic acid amplification test for simultaneous rapid tuberculosis diagnosis and rapid antibiotic sensitivity (can rapidly identify possible multi drug resistant TB)[17].The sputum sample was mixed with the reagent provided with assay, transferred using a sterile pipette into the cartridge(Expert MTB/RIF cartridge) and placed in the Genexpert machine. All processing from this point on was fully automated. Results from the Expert MTB/RIF assay indicates whether or not MTB was detected. If MTB was detected, the results will also state whether resistance to RIF was detected, not detected, or indeterminate.

### 3.8 Data Analysis

The data analysis was done using statistical analysis system (SAS). Data cleaning consist of examining frequency distribution for all variables in order to detect those values which were not logical or outliers.

## Endnotes


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# Chapter Four

# Results and Discussion of Findings

## 4.1 Blood Glucose Estimation

Out of 150 respondents screened for blood glucose estimation using Randox kit, 7(4.7%) were found to be diabetic (high glucose level) as shown in Table 4.1.

**Table 4.1: Blood Glucose Estimation Results**

| Respondents | Value (mg/dl) | Interpretation |
|---|---|---|
| Std | 200mg/dl | |
| Control | High 130mg/dl | |
| Reference range | 70 – 120mg/dl | |
| 1. | 52.0 | Low |
| 2. | 55.0 | Low |
| 3. | 56.0 | Low |
| 4. | 60.0 | Low |
| 5. | 70.0 | Normal |
| 6. | 75.0 | Normal |
| 7. | 70.0 | Normal |
| 8. | 72.0 | Normal |
| 9. | 77.0 | Normal |
| 10. | 72.0 | Normal |
| 11. | 75.0 | Normal |
| 12. | 60.0 | Low |
| 13. | 85.0 | Normal |
| 14. | 70.0 | Normal |
| 15. | 90.0 | Normal |
| 16. | 62.0 | Low |
| 17. | 72.0 | Normal |
| 18. | 88.0 | Normal |
| 19. | 60.0 | Low |
| 20. | 78.0 | Normal |
| 21. | 85.0 | Normal |
| 22. | 70.0 | Normal |
| 23. | 72.0 | Normal |
| 24. | 85.0 | Normal |
| 25. | 85.0 | Normal |
| 26. | 70.0 | Normal |
| 27. | 72.0 | Normal |
| 28. | 60.0 | Low |
| 29. | 85.0 | Normal |
| 30. | 72.0 | Normal |
| 31. | 78.0 | Normal |
| 32. | 70.0 | Normal |
| 33. | 62.0 | Low |
| 34. | 60.0 | Low |
| 35. | 75.0 | Normal |
| 36. | 81.0 | Normal |
| 37. | 84.0 | Normal |
| 38. | 65.0 | Low |
| 39. | 91.0 | Normal |
| 40. | 170.0 | High |
| 41. | 84.0 | Normal |
| 42. | 60.0 | Low |
| 43. | 175.0 | High |
| 44. | 92.0 | Normal |

| | | |
|---|---|---|
| 45. | 93.0 | Normal |
| 46. | 65.0 | Low |
| 47. | 84.0 | Normal |
| 48. | 81.0 | Normal |
| 49. | 65.0 | Low |
| 50. | 91.0 | Normal |
| 51. | 72.0 | Normal |
| 52. | 205.0 | High |
| 53. | 80.0 | Normal |
| 54. | 62.0 | Low |
| 55. | 60.0 | Low |
| 56. | 74.0 | Normal |
| 57. | 84.0 | Normal |
| 58. | 75.0 | Normal |
| 59. | 81.0 | Normal |
| 60. | 65.0 | Low |
| 61. | 74.0 | Normal |
| 62. | 75.0 | Normal |
| 63. | 60.0 | Low |
| 64. | 75.0 | Normal |
| 65. | 65.0 | Low |
| 66. | 74.0 | Normal |
| 67. | 75.0 | Normal |
| 68. | 64.0 | Low |
| 69. | 280.0 | High |
| 70. | 65.0 | Low |
| 71. | 81.0 | Normal |
| 72. | 62.0 | Low |
| 73. | 82.0 | Normal |
| 74. | 80.0 | Normal |
| 75. | 91.0 | Normal |
| 76. | 65.0 | Low |
| 77. | 92.0 | Normal |
| 78. | 84.0 | Normal |
| 79. | 100.0 | Normal |
| 80. | 65.0 | Low |
| 81. | 91.0 | Normal |
| 82. | 80.0 | Normal |
| 83. | 110.0 | Normal |
| 84. | 62.0 | Low |
| 85. | 95.0 | Normal |
| 86. | 82.0 | Normal |
| 87. | 79.0 | Normal |
| 88. | 62.0 | Low |
| 89. | 140.0 | High |
| 90. | 65.0 | Low |
| 91. | 91.0 | Normal |
| 92. | 65.0 | Low |
| 93. | 84.0 | Normal |
| 94. | 82.0 | Normal |

| | | |
|---|---|---|
| 95. | 80.0 | Normal |
| 96. | 65.0 | Low |
| 97. | 83.0 | Normal |
| 98. | 80.0 | Normal |
| 99. | 60.0 | Low |
| 100. | 85.0 | Normal |
| 101. | 82.0 | Normal |
| 102. | 65.0 | Low |
| 103. | 82.0 | Normal |
| 104. | 80.0 | Normal |
| 105. | 82.0 | Normal |
| 106. | 83.0 | Normal |
| 107. | 79.0 | Normal |
| 108. | 65.0 | Low |
| 109. | 72.0 | Normal |
| 110. | 60.0 | Low |
| 111. | 75.0 | Normal |
| 112. | 85.0 | Normal |
| 113. | 88.0 | Normal |
| 114. | 60.0 | Low |
| 115. | 90.0 | Normal |
| 116. | 75.0 | Normal |
| 117. | 150.0 | High |
| 118. | 62.0 | Low |
| 119. | 85.0 | Normal |
| 120. | 170.0 | High |
| 121 | 70.0 | Normal |
| 122 | 85.0 | Normal |
| 123 | 90.0 | Normal |
| 124 | 60.0 | Low |
| 125 | 62.0 | Low |
| 126 | 85.0 | Normal |
| 127 | 92.0 | Normal |
| 128 | 70.0 | Normal |
| 129 | 75.0 | Normal |
| 130 | 84.0 | Normal |
| 131 | 62.0 | Low |
| 132 | 85.0 | Normal |
| 133 | 88.0 | Normal |
| 134 | 72.0 | Normal |
| 135 | 70.0 | Normal |
| 136 | 85.0 | Normal |
| 137 | 88.0 | Normal |
| 138 | 72.0 | Normal |
| 139 | 60.0 | Low |
| 140 | 75.0 | Normal |
| 141 | 88.0 | Normal |
| 142 | 72.0 | Normal |
| 143 | 65.0 | Low |
| 144 | 84.0 | Normal |

| | | |
|---|---|---|
| 145 | 70.0 | Normal |
| 146 | 65.0 | Low |
| 147 | 72.0 | Normal |
| 148 | 72.0 | Normal |
| 149 | 70.0 | Normal |
| 150 | 60.0 | Low |

**Source:** *Researcher`s Field Study (2021)*

**Table 4.1: Distribution of Random Blood Glucose Estimation**

| RBG Estimation (mg/dl) | Frequency | Percentage (%) |
|---|---|---|
| 52.00 | 1 | .7 |
| 55.00 | 2 | 1.3 |
| 56.00 | 1 | .7 |
| 60.00 | 13 | 8.7 |
| 62.00 | 9 | 6.0 |
| 64.00 | 1 | .7 |
| 65.00 | 15 | 10.0 |
| 70.00 | 11 | 7.3 |
| 72.00 | 13 | 8.7 |
| 74.00 | 3 | 2.0 |
| 75.00 | 11 | 7.3 |
| 77.00 | 1 | .7 |
| 78.00 | 2 | 1.3 |
| 79.00 | 2 | 1.3 |
| 80.00 | 4 | 2.7 |
| 81.00 | 4 | 2.7 |
| 82.00 | 6 | 4.0 |
| 83.00 | 2 | 1.3 |
| 84.00 | 9 | 6.0 |
| 85.00 | 11 | 7.3 |
| 88.00 | 5 | 3.3 |
| 90.00 | 3 | 2.0 |
| 91.00 | 5 | 3.3 |
| 92.00 | 2 | 1.3 |
| 93.00 | 1 | .7 |
| 95.00 | 1 | .7 |
| 100.00 | 4 | 2.7 |
| 110.00 | 1 | .7 |
| **140.00** | 1 | .7 |
| **150.00** | 1 | .7 |
| **168.00** | 1 | .7 |
| **170.00** | 1 | .7 |
| **175.00** | 1 | .7 |
| **205.00** | 1 | .7 |
| **280.00** | 1 | .7 |
| **Total** | **150** | **100.0** |

***Source:*** *Researcher's Field Survey (2021)*

## 4.2 HIV Screening Results of the Respondents

Out of 150 clients screened using both Determine and Unigold kits, 2(1.3%) were reactive as seen in Table 4.3. Figure 4.0 and Figure 4.1 show Determine and Unigold Reactive and Non reactive strips.

**Table 4.2: HIV Screening Results of the Respondents**

| HIV screening | Frequency | Percent |
|---|---|---|
| **Reactive** | 2 | 1.3 |
| **Non-reactive** | 148 | 98.7 |
| **Total** | **150** | **100** |

***Source:*** *Researcher's Field Survey (2021)*

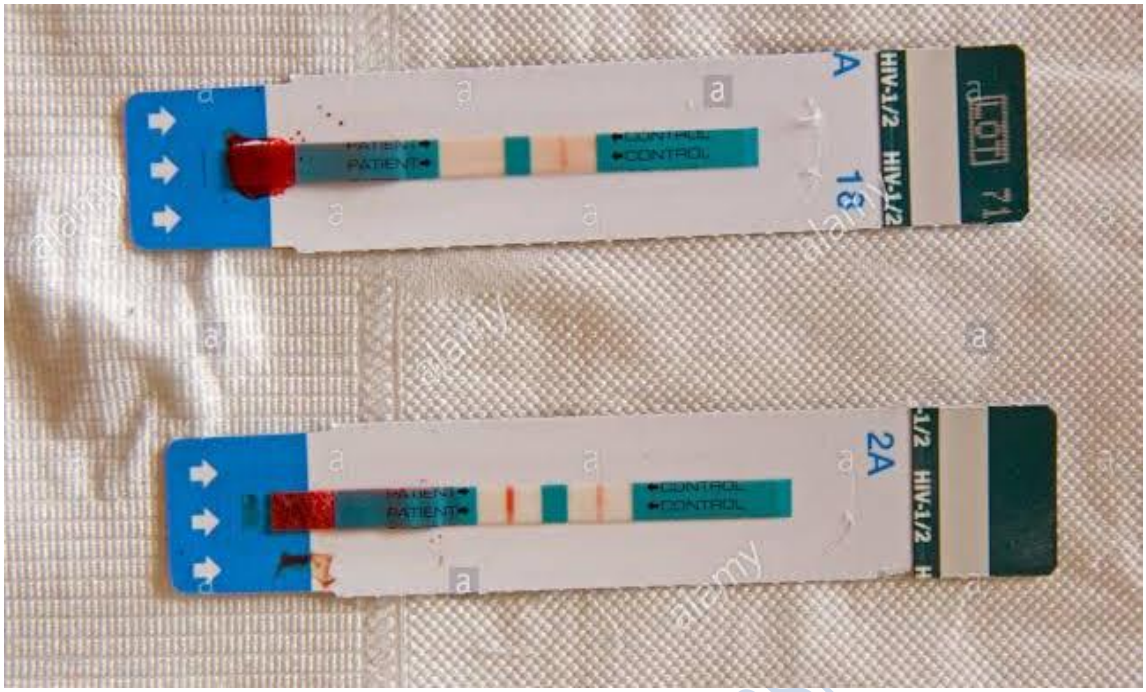



**Plate 4.1: Positive and Negative HIV Screening Result of the Respondents using Determine.**

***Source:*** *Researcher's Field Survey (2021)*

**Plate 4.1: Positive and Negative HIV Screening Result using Unigold**
***Source:*** *Researcher's Field Survey (2021)*

### 4.4 Specimen Culture on Lowenstein Jensen Media

Table 4.3 shows that out of 150 specimen cultured, 2(1.3%) yielded growth of MTB, 3(2.0%) grew bacilli, while 2(1.3%) yielded growth of yeast cells. A typical characteristic Positive and Negative culture media are shown in Figure 4.2 and Figure 4.3.

**Table 4.3: Culture Results of Respondents using LJ Media**

| Respondents Sample | Bacilli | Yeast cells | MTB | No Growth |
|---|---|---|---|---|
| 1. | - | - | - | - |
| 2. | - | - | - | - |
| 3. | - | - | - | - |
| 4. | + | - | - | |
| 5. | - | - | - | - |
| 6. | - | - | - | - |
| 7. | - | - | - | - |
| 8. | - | - | - | - |
| 9. | - | - | - | - |
| 10. | - | - | - | - |
| 11. | - | - | - | - |
| 12. | - | - | - | - |
| 13. | - | - | - | - |
| 14. | - | - | - | - |
| 15. | - | - | - | - |
| 16. | - | - | - | - |
| 17. | - | + | - | |
| 18. | - | - | - | - |
| 19. | - | - | - | - |
| 20. | - | - | - | - |
| 21. | - | - | - | - |
| 22. | - | - | - | - |
| 23. | - | - | - | - |
| 24. | - | - | - | - |
| 25. | - | - | - | - |
| 26. | - | - | - | - |
| 27. | - | - | - | - |
| 28. | - | - | - | - |
| 29. | - | - | - | - |
| 30. | - | - | - | - |
| 31. | - | - | - | - |
| 32. | - | - | - | - |
| 33. | - | + | - | |
| 34. | - | - | - | - |
| 35. | - | - | - | - |
| 36. | - | - | - | - |
| 37. | - | - | - | - |
| 38. | - | - | - | - |
| 39. | - | - | - | - |
| 40. | - | - | - | - |
| 41. | - | - | - | - |
| 42. | - | - | - | - |
| 43. | - | - | - | - |
| 44. | - | - | - | - |
| 45. | - | - | + | |
| 46. | - | - | - | - |
| 47. | - | - | - | - |
| 48. | - | - | - | - |
| 49. | - | - | - | - |
| 50. | - | - | - | - |
| 51. | - | - | - | - |
| 52. | - | - | - | - |

| | | | | |
|---|---|---|---|---|
| 53. | - | - | - | - |
| 54. | - | - | - | - |
| 55. | - | - | - | - |
| 56. | - | - | - | - |
| 57. | - | - | - | - |
| 58. | - | - | - | - |
| 59. | - | - | - | - |
| 60. | - | - | - | - |
| 61. | - | - | - | - |
| 62. | - | - | - | - |
| 63. | - | - | - | - |
| 64. | - | - | - | - |
| 65. | - | - | - | - |
| 66. | + | - | - | |
| 67. | - | - | - | - |
| 68. | - | - | - | - |
| 69. | - | - | - | - |
| 70. | - | - | - | - |
| 71. | - | - | + | |
| 72. | - | - | - | - |
| 73. | - | - | - | - |
| 74. | - | - | - | - |
| 75. | - | - | - | - |
| 76. | - | - | - | - |
| 77. | - | - | - | - |
| 78. | - | - | - | - |
| 79. | - | - | - | - |
| 80. | - | - | - | - |
| 81. | - | - | - | - |
| 82. | - | - | - | - |
| 83. | - | - | - | - |
| 84. | - | - | - | - |
| 85. | - | - | - | - |
| 86. | - | - | - | - |
| 87. | - | - | - | - |
| 88. | - | - | - | - |
| 89. | - | - | - | - |
| 90. | - | - | - | - |
| 91. | - | - | - | - |
| 92. | - | - | - | - |
| 93. | - | - | - | - |
| 94. | - | - | - | - |
| 95. | - | - | - | - |
| 96. | - | - | - | - |
| 97. | - | - | - | - |
| 98. | - | - | - | - |
| 99. | - | - | - | - |
| 100. | - | - | - | - |
| 101. | - | - | - | - |
| 102. | - | - | - | - |
| 103. | - | - | - | - |
| 104. | - | - | - | - |
| 105. | + | - | - | |
| 106. | - | - | - | - |
| 107. | - | - | - | - |

| | | | | |
|---|---|---|---|---|
| 108. | - | - | - | - |
| 109. | - | - | - | - |
| 110. | - | - | - | - |
| 111. | - | - | - | - |
| 112. | - | - | - | - |
| 113. | - | - | - | - |
| 114. | - | - | - | - |
| 115. | - | - | - | - |
| 116. | - | - | - | - |
| 117. | - | - | - | - |
| 118. | - | - | - | - |
| 119. | - | - | - | - |
| 120. | - | - | - | - |
| 121. | - | - | - | - |
| 122. | - | - | - | - |
| 123. | - | - | - | - |
| 124. | - | - | - | - |
| 125. | - | - | - | - |
| 126. | - | - | - | - |
| 127. | - | - | - | - |
| 128. | - | - | - | - |
| 129. | - | - | - | - |
| 130. | - | - | - | - |
| 131. | - | - | - | - |
| 132. | - | - | - | - |
| 133. | - | - | - | - |
| 134. | - | - | - | - |
| 135. | - | - | - | - |
| 136. | - | - | - | - |
| 137. | - | - | - | - |
| 138. | - | - | - | - |
| 139. | - | - | - | - |
| 140. | - | - | - | - |
| 141. | - | - | - | - |
| 142. | - | - | - | - |
| 143. | - | - | - | - |
| 144. | - | - | - | - |
| 145. | - | - | - | - |
| 146. | - | - | - | - |
| 147. | - | - | - | - |
| 148. | - | - | - | - |
| 149. | - | - | - | - |
| 150. | - | - | - | - |

**Source**: *Researcher`s Field Survey (2021)*

**Table 4.3: Culture Results of Respondents**

| Culture | Frequency | Percent |
|---|---|---|
| **No growth** | 143 | 95.4 |
| **Bacilli** | 3 | 2.0 |
| **Yeast cells** | 2 | 1.3 |
| **+MTB** | 2 | 1.3 |
| **Total** | **150** | **100** |

***Source:*** *Researcher's Field Survey (2021)*

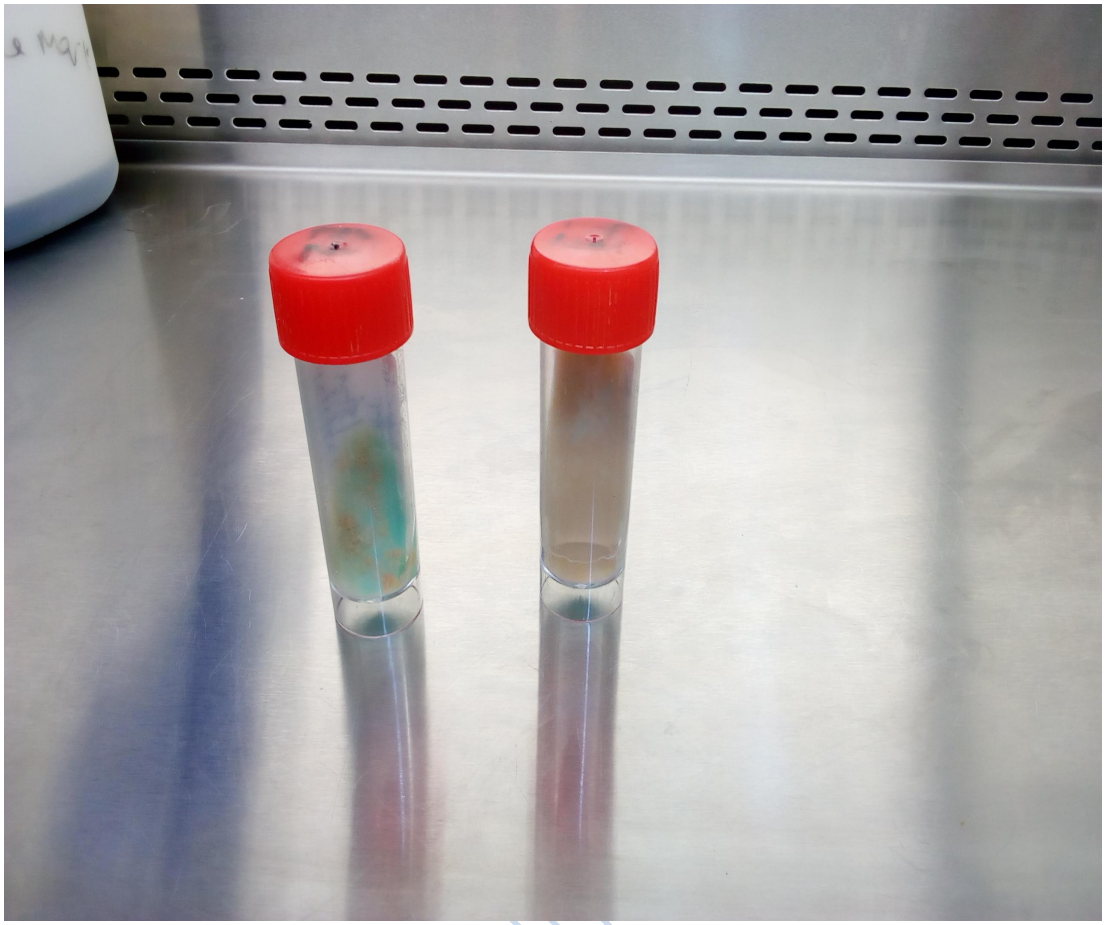


**Plate 4.3: Lowenstein Jensen MTB Positive Media**

***Source:*** *Researcher's Field Survey (2021)*

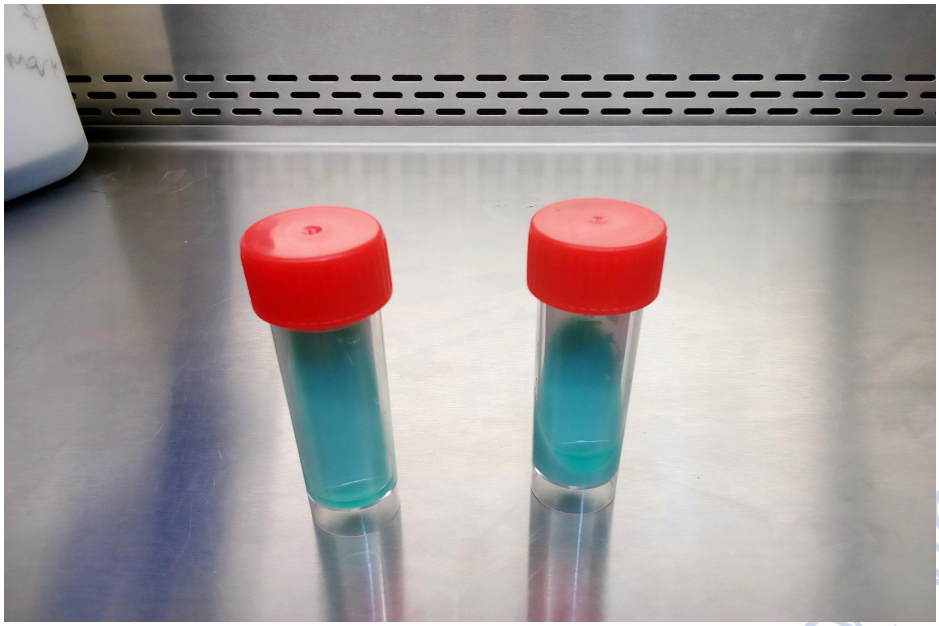


**Plate 4.4: Lowenstein Jensen MTB Negative Media**

***Source:*** *Researcher's Field Survey (2021)*

### 4.4 Slide Microscopy for AFB(Acid Fast Bacilli)

The distribution of slide microscopy results has shown in Table 4.4 revealed that 2(1.3%) was AFB positive. Figure 4.4 showed AFB positive slide.

**Table 4.4: Slide Microscopy Results of Respondents**

| Slide Microscopy | Frequency | Percent |
|---|---|---|
| **AFB Negative** | 148 | 98.7 |
| **AFB positive** | 2 | 1.3 |
| **Total** | **150** | **100** |

***Source:*** *Researcher's Field Survey (2021)*

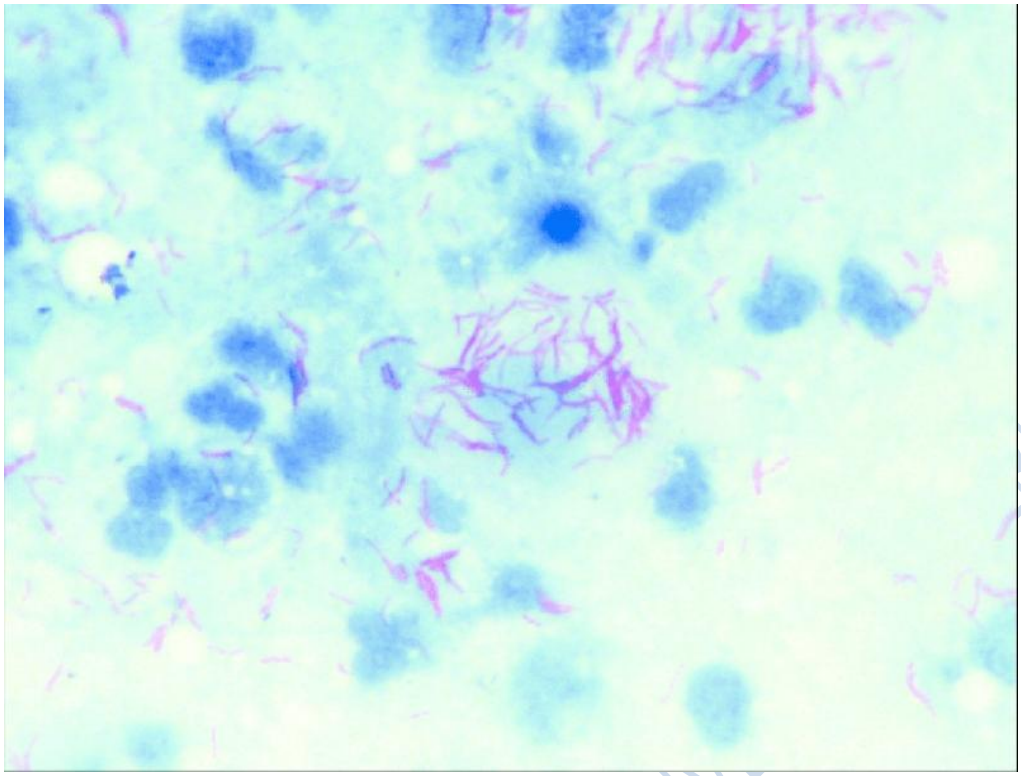


**Plate 4.5: Positive Slide Microscopy Result of Respondents**

***Source:*** *Researcher's Field Survey (2021)*

## 4.5 Antimicrobial Susceptibility Pattern of *Mycobacteria tuberculosis*

Antimicrobial susceptibility pattern of MTB to First line anti-TB drugs (Isoniazid, Rifampicin, and Ethambutol) and Kanamycin, Amikacin, Capremycin and Ofloxacin is shown in Figure 4.5. The MTB were found susceptible to all the anti-TB drugs used.

**Table 4.5: Antimicrobial Susceptibility Test Results of *Mycobacteria tuberculosis***

| | Control sample | | Sample A | | Sample B | |
|---|---|---|---|---|---|---|
| **Antibiotics** | **Susceptibility** | **Resistance** | **Susceptibility** | **Resistance** | **Susceptibility** | **Resistance** |
| INH | + | - | + | - | + | - |
| RIF | + | - | + | - | + | - |
| ETH | + | - | + | - | + | - |
| KAN | + | - | + | - | + | - |
| AM | + | - | + | - | + | - |
| CAP | + | - | + | - | + | - |
| OFL | + | - | + | - | + | - |

**Source:** *Researcher`s Field Survey (2021)*

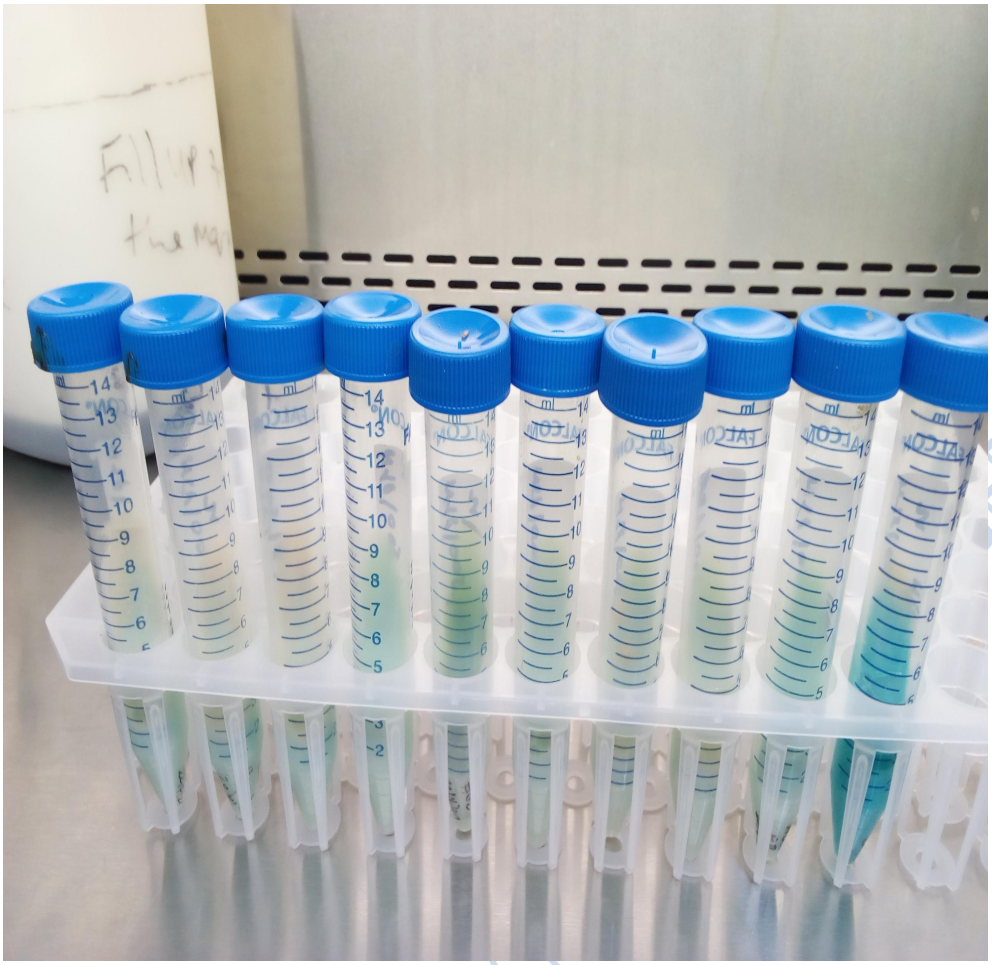


**Plate 4.6: Antimicrobial Susceptibility Test of Respondents using Proportion Method**
***Source:*** *Researcher's Field Survey (2021).*

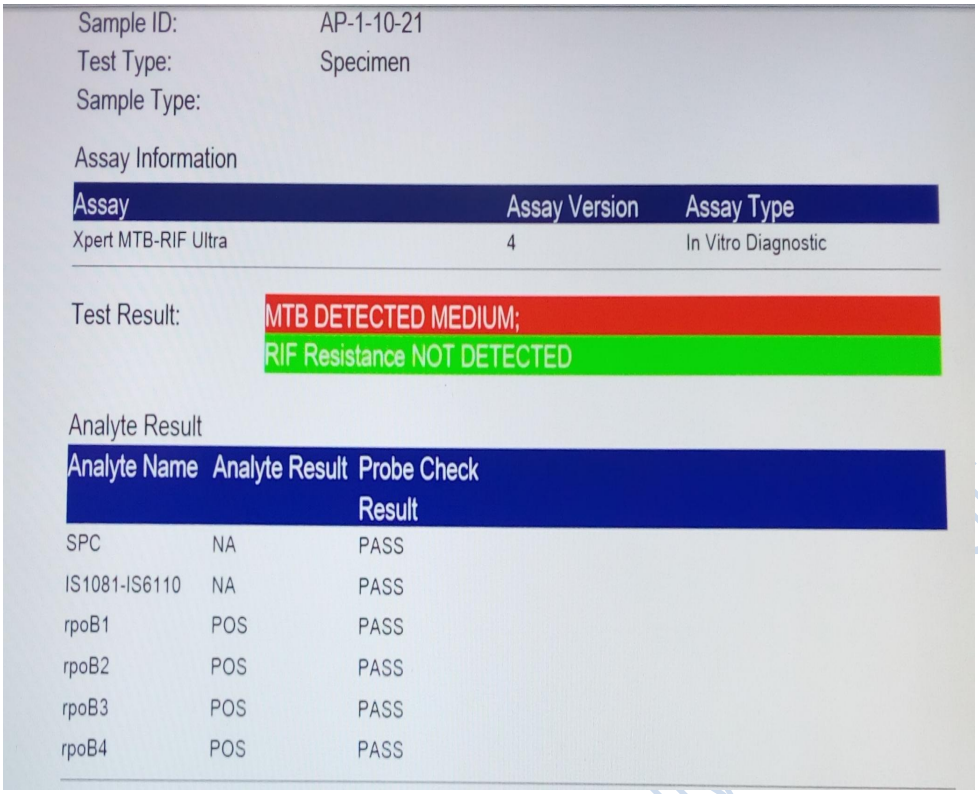
## 4.6 Molecular Detection of MTB (GeneXpert) of Respondents

Molecular detection of MTB showed that 7(4.7%) out of 150 respondents were positive (RIF resistant not detected) as seen in Table 4.5 and Figure 4.6.

**Table 4.5: Distribution of Molecular GenXpert Results**

| Molecular GenXpert | Frequency | Percent |
|---|---|---|
| **MTB not detected** | 143 | 95.3 |
| **RIF resist not detected** | 7 | 4.7 |
| **Total** | **150** | **100** |

***Source:*** *Researcher's Field Survey (2021)*



Sample ID: AP-1-10-21
Test Type: Specimen
Sample Type:

Assay Information

| Assay | Assay Version | Assay Type |
|---|---|---|
| Xpert MTB-RIF Ultra | 4 | In Vitro Diagnostic |

Test Result: MTB DETECTED MEDIUM;
RIF Resistance NOT DETECTED

Analyte Result

| Analyte Name | Analyte Result | Probe Check Result |
|---|---|---|
| SPC | NA | PASS |
| IS1081-IS6110 | NA | PASS |
| rpoB1 | POS | PASS |
| rpoB2 | POS | PASS |
| rpoB3 | POS | PASS |
| rpoB4 | POS | PASS |

**Figure 4.6: Molecular Detection of MTB**
***Source:*** *Researcher's Field Survey (2021)*

## 4.7. Comparison of the 3 Diagnostic Methods used

Figure 4.7 shows that Molecular method (GeneXpert) is the most sensitive 4.7% while both Slide microscopy and Culture are 1.3%.

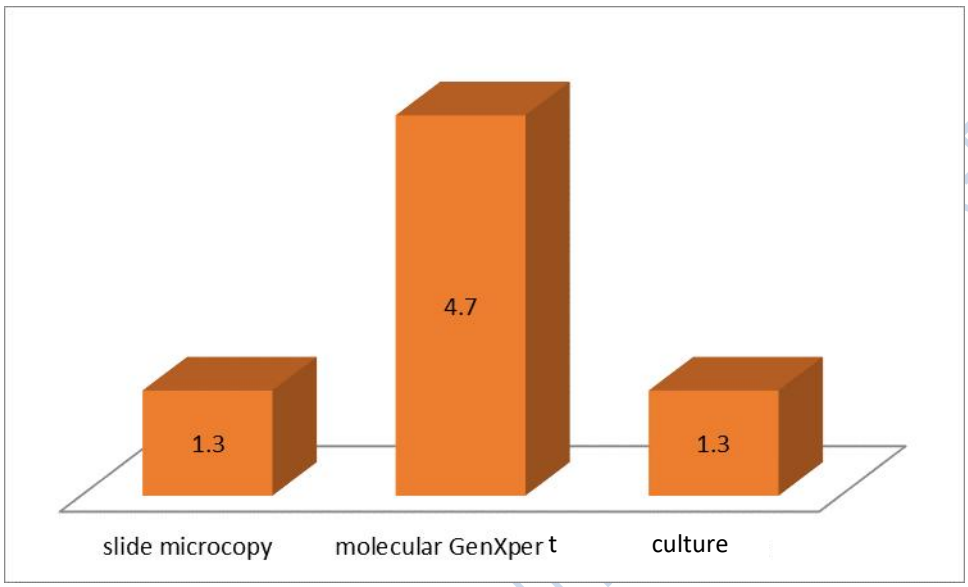



**Figure 4.7: Comparison of the Three Diagnostic Methods used.**
***Source:*** *Researcher's Field Survey (2021)*

### 4.8. MTB Risk Factors among the Respondents

Table 4.6 shows that 2(28.5%) out of 7 that are diabetic were equally found to be MTB positive. 1(14.3%) out of 2 that are HIV positive were MTB positive. 2(28.5%) out of 7 household had 5 people living per room.

**Table 4.6: MTB Positive Risk Factors among the Respondents**

| S/N | Occupation | No of people per room | Age (years) | HIV status | Glucose estimation (Mg/dl) | Address |
|---|---|---|---|---|---|---|
| **1.** | Trading | 5 | 41 | Reactive | 84 (Normal ) | Agbowo |
| **2.** | Trading | 2 | 24 | Non-reactive | 168 (High) | Yemetu |
| **3.** | Trading | 3 | 29 | | 175 (High) | Olunde |
| **4.** | Fashion Designer | 2 | 27 | Non-reactive | 79 (Normal ) | Bodija |
| **5.** | | 5 | 30 | Non-reactive | 77 (Normal ) | Yemetu |
| **6.** | Teacher | 2 | 25 | | 100 (Normal ) | Academy |
| **7.** | Fashion Designer | 3 | 25 | Non-reactive | 62 (Normal) | Bako |
| | Trading | | | Non-reactive | | |
| | | | | Non-reactive | | |

***Source:*** *Researcher's Field Survey (2021)*

## 4.9 Discussion of Findings

The findings revealed the prevalence of tuberculosis in pregnancy to be 4.7%, which can be leverage upon by the research community, stakeholders in the health industry and assist in the epidemiological survey, hereby leading to better management, control and prevention of this deadly disease. This prevalence is a bit higher than that previously documented in high burden countries (0.5%)[1]. This situation therefore calls for more awareness in the path of the populace, prompt and collaborative response from all health care gladiators so as to mitigate the effect of this on the expectant mother and the consequential effect on the unborn child.

The prevalence of 4.7% in tuberculosis in pregnancy from this research work provide the data which is a current and empirical situation in this part of the country. The most sensitive method of screening for MTB according to this findings is the Molecular expert which detected 7 MTB out of 150 respondents, this method of screening for MTB according to the findings is considered the most sensitive which is in tandem with the existing document[2]. Both culture and slide (smear) microscopy according to this research has the same sensitivity (1.3%). This is contrary to existing document which says that culture method in more sensitive than smear microscopy[3]. This disparity could be as a result of the circulating strain (*M. tuberculosis*) in the respondents used for this research work.

According to this research work, the diabetes is one of the risk factors to having MTB as it was discovered that 2 out of 7(28.6%) respondents that were TB positive were also diabetic as this condition depletes the cells of immune response thereby making the subjects susceptible to TB infection. This is in tandem with the existing document[4].

Also HIV/AIDS is considered as a risk factor based on the fact that 1 out 7(14.3%) patients that were TB positive were equally reactive to HIV screening. The reason for this could be attributed to the clinical presentation of HIV/AIDS infection as a generalized Acquired Immuno Deficiency Syndrome. This is in an agreement with existing document[5].

Among the risk factors according to the findings is also accommodation congestion which indicated that 2 households that were 5 in number in a room were positive for MTB 28%. This is one of the factors that aids the transmission and spread of MTB infection as the route of transmission is through aerosols and it is airborne. This is affirmed in the existing document[6].

However there is no correlation between occupations and TB infection in this study, as very few health care workers recruited in this study were all empty being negative. The majority of respondents that were MTB positive were traders which probably contracted the infection through their interaction with customers.

The research established that all the TB isolates were sensitive to first line anti-TB drugs (Isoniazid, Rifampicin and Ethambutol). This means non of the isolates were multi-drug resistant. This might be as a result of the circulating strain among the respondents and their level of exposure to indiscriminate use of prophylactic drugs due to their physiological conditions.

## Endnotes


1. World Health Organization. Global tuberculosis report 2017. Geneva: World Health Organization, 2017.

2. R. Prasad, A. Singh, & N. Gupta. *Tuberculosis and COVID-19 in India: Challenges and opportunities.* **Lung India: Official Organ of Indian Chest Society.** 2020 Jul; 37(4):292.

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# Chapter Five

# Conclusion

## 5.1 Summary of Findings

The research outcome reveals the prevalence of tuberculosis in pregnancy to be 4.7%, which is slightly higher than that previously documented in high burden countries (0.5%). This therefore calls for prompt and wholistic intervention involving all the stakeholders and adoption of an effective one health approach so as to curtail the menance and reduce the burden on the subjects, the unborn baby and the community in general.

## 5.2 Conclusion

The result of this study have provided empirical data regarding the prevalence of tuberculosis in pregnancy (4.7%) in Oyo state and the possible risk factors i.e (Diabetes, HIV/AIDS, accommodation congestion) that can predispose pregnant women to tuberculosis infection. This can be leverage upon by the research community and assist in their epidemiological survey leading to control and prevention of this deadly disease. It has also shown drug susceptibility pattern of *M. tuberculosis* among the pregnant women in the study area. This however, makes the intervention easier and reduce cost of treatment if properly and promptly initiated. This research has provided empirical data which when fully implemented will help in TB reduction in the community and better management of pregnant women and consequently improve the health condition of their babies.

## 5.3 Recommendations

i. Routine screening for tuberculosis should be incorporated into the array of tests for antenatal clients in state.

ii. Molecular method (GeneXpert) should be made available for routine screening of tuberculosis due to its high sensitivity.

iii. Further studies are needed to determine the TB lineages or clades of the strains detected in pregnant women in this study.

## 5.4 Contribution to Knowledge

The study has provided the empirical data representing the current prevalence (4.7%) of *Mycobacterium tuberculosis*, the types of circulating strains and the contributing factors of acquiring MTB among pregnant women in Oyo State, Nigeria.

These data will assist the research community, improve the treatment and management of MTB among these subjects and form the basis for epidemiological survey towards controlling and preventing of the spread of this deadly disease.

## 5.5 Area of Further Research

i. Further studies involving relatively larger subjects will be needed.

ii. Genomic studies such as genetic sequencing of TB isolates from pregnant women to determine lineages or clades that can impact on severity, diagnosis, treatment and prognosis of infection.

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## Appendices

## Appendix I

**Distribution of Respondents by Age**

| Age | Frequency | Percent | Average |
|---|---|---|---|
| 17-22 years | 16 | 10.5 | |
| 23-28 years | 53 | 38.7 | |
| 29-34 years | 39 | 26.1 | 29.33 |
| 35-40 years | 34 | 22.8 | |
| 41 years and above | 3 | 2.0 | |
| Total | **150** | **100** | |

## Appendix II

**Distribution by Trimester**

| Trimester | Frequency | Percent |
|---|---|---|
| 1st | 46 | 30.7 |
| 2nd | 92 | 61.3 |
| 3rd | 12 | 8.0 |
| Total | **150** | **100** |

## Appendix III

**Distribution of Respondents by Occupation**

| Occupation | Frequency | Percentage |
|---|---|---|
| **Trading** | 55 | 36.7 |
| **Hairdresser** | 19 | 12.7 |
| **Nurse** | 5 | 3.3 |
| **Tailor** | 8 | 5.3 |
| **Student** | 4 | 2.7 |
| **Fashion Designer** | 19 | 12.7 |
| **Chemist** | 12 | 8.0 |
| **Decorator** | 1 | .7 |
| **Catering** | 2 | 1.3 |
| **Makeup Artist** | 1 | .7 |
| **Civil Servant** | 3 | 2.0 |
| **Surveyor** | 1 | .7 |
| **Teacher** | 17 | 11.3 |
| **Computer Analyst** | 1 | .7 |
| **Microfinance** | 1 | .7 |
| **Banker** | 1 | .7 |
| **Total** | 150 | 100 |

## Appendix IV

**Distribution of Respondents by Family Size**

| No living together in the family | Frequency | Percentage |
|---|---|---|
| 2.00 | 43 | 28.7 |
| 3.00 | 34 | 22.7 |
| 4.00 | 38 | 25.3 |
| 5.00 | 14 | 9.3 |
| 6.00 | 9 | 6.0 |
| 7.00 | 2 | 1.3 |
| 8.00 | 6 | 4.0 |
| 9.00 | 2 | 1.3 |
| 10.00 | 2 | 1.3 |
| Total | **150** | **100.0** |

## Appendix V

**Distribution of Respondents by no per Room**

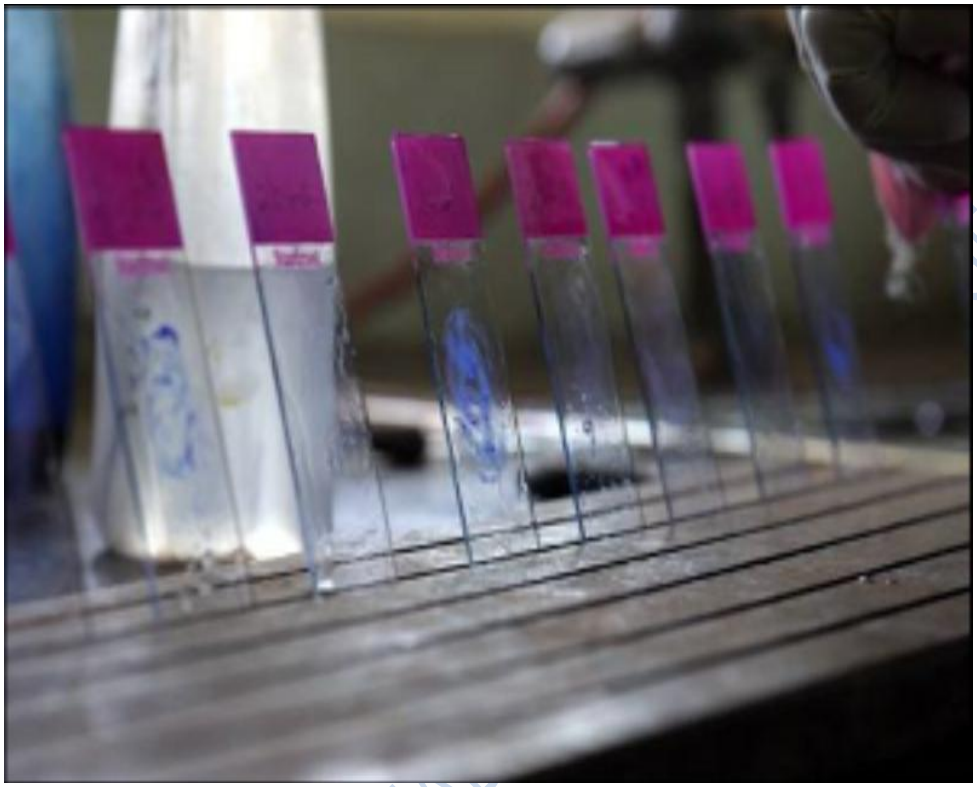
| No staying per room | Frequency | Percentage (%) |
|---|---|---|
| **1.00** | 7 | 4.7 |
| **2.00** | 94 | 62.7 |
| **3.00** | 28 | 18.7 |
| **4.00** | 16 | 10.7 |
| **5.00** | 4 | 2.7 |
| **6.00** | 1 | .7 |
| **Total** | **150** | **100.0** |

## Appendix VI

**Prepared Lowenstein Jensen Media**

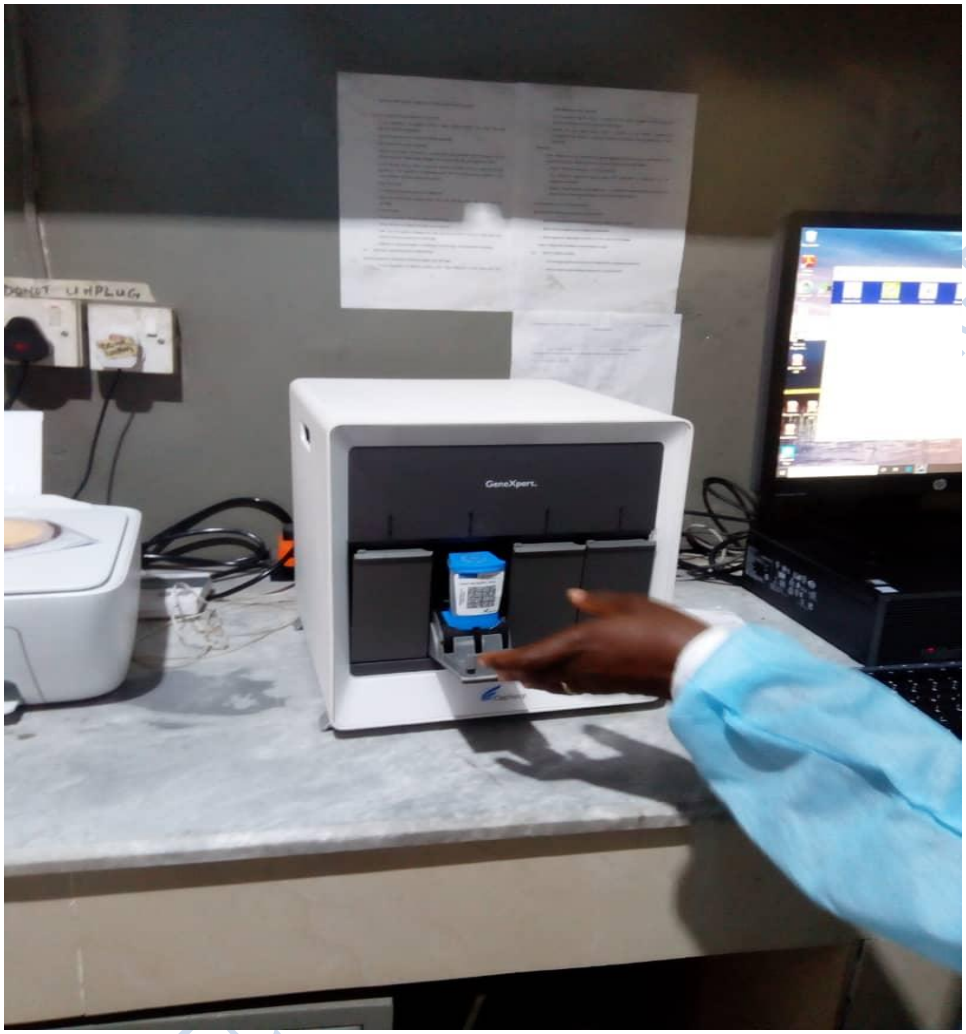
## Appendix VII

### Stained AFB Slides with Ziehl Neelsen



## Appendix VIII

**Loading of GeneXpert Machine with a Catridge**



## Appendix IX

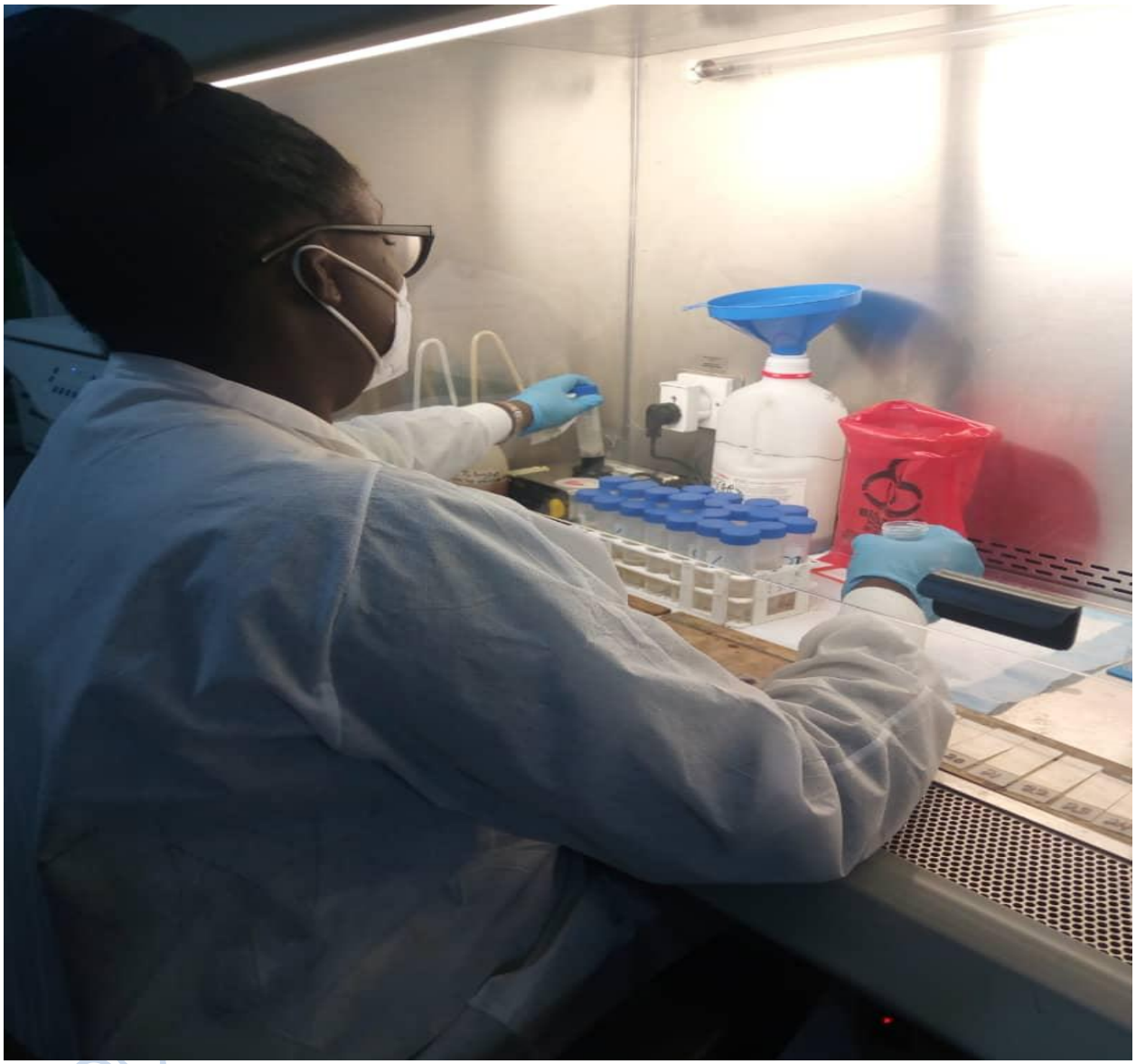
**Researcher Discussing with the Respondents**

## Appendix X

**Researcher in the TB Reference Laboratory**

## Appendix XI

### Researcher Processing Sample in the Laboratory



## Appendix XII

## Questionnaire

**Survey Questionnaires Designed to Obtain Informations on Prevalence and Risk Factors of Pulmonary Tuberculosis among Ante natal Patients in Adeoyo Maternity Teaching Hospital Ibadan, Oyo State, Nigeria.**

Dear Respondents,

This questionnaire is designed to obtain information on demographic characteristics and predisposing factors of pregnant women to Pulmonary tuberculosis in Adeoyo Maternity Teaching Hospital Ibadan, Oyo State, Nigeria.

By: Ogundele Theresa Olaitan, Lead City University, Ibadan, Oyo State.

Please tick appropriate options. All information supplied will be treated as confidential and used for research purpose only.

Thank you.

1. Name/ Serial number:

2. Age (years) 16-20....., 21-25.....,

26-30....., 31-35....., 36-40.....,41-45....., 46-50....., 51-55.....

3. Educational Qualification/Background: Secondary....., Post Secondary.....

University....., Not educated.....

4. Occupation: i. Health care worker..... ii. Artisan..... iii. Civil servant.....

iv. Students....., v. Retiree.....

5. Trimester: 1st(1 - 3 months) ....., 2nd(4 - 6 months).....,3rd(7 - 9 months).....

6. Number of parity.....

7. State of Origin:

8. LGA:

9. Number living in a household:

10. Number living per room.....

11. Any symptom: i. Fever....., ii. Headache....., iii. Cough.....

iv. Weight loss.....,  v. Fatigue: .....  vi. Shortness of breath..... vii. Chest pain.....

viii. Night sweats.....  ix. Loss of apetite .....

12. Any treatment:  Yes.....,  No.....

13. If yes, which one  i. Malaria drug....., ii. Antibiotics....., iii. Pain killer.....

iv. Cough Syrup.....,  v. Others.....

14. Comorbidity: i. Obesity....., ii. Hypertension....., iii. Diabetes mellitus.....

iv. Cardiovascular disease....., v. Atrial fibrillation....., vi. Venous thromboembolism....., vii. Chronic renal failure....., viii. Respiratory disease.....,ix. Immunocompromised.....

15. Do you smoke  yes.....,  no.....

16. Sample collected: i. Sputum....., ii. Venous blood.....

iii. Others specify..... iv. Others specify.....

TELEGRAMS.....  TELEPHONE.....

# MINISTRY OF HEALTH

**DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION**

**PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA**

*Your Ref. No.* .....
*All communications should be addressed to*
*the Honorable Commissioner quoting*
Our Ref. No. AD 13/479/4406

31st August, 2021

The Principal Investigator,
Department of Biological Sciences,
Faculty of Basic and Applied Sciences,
Lead City University,
Ibadan, Nigeria.

**Attention: Ogundele Theresa**

ETHICS APPROVAL FOR THE IMPLEMENTATION
OF YOUR RESEARCH PROPOSAL IN OYO STATE

This is to acknowledge that your Research Proposal titled: "Prevalence and Risk Factors of Pulmonary Tuberculosis among Antenatal Patients in Adeoyo Maternity Teaching Hospital, Ibadan, Oyo State, Nigeria." has been reviewed by the Oyo State Ethics Review Committee.

2. The committee has noted your compliance. In the light of this, I am pleased to convey to you the full approval by the committee for the implementation of the Research Proposal in Oyo State, Nigeria.

3. Please note that the National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations, in line with this, the Committee will monitor closely and follow up the implementation of the research study. However, the Ministry of Health would like to have a copy of the results and conclusions of findings as this will help in policy making in the health sector.

4. Wishing you all the best.

Dr. Abbas Gbolahan
Director, Planning, Research & Statistics
Secretary, Oyo State, Research Ethics Review Committee

**Informed Consent Form**

Prevalence and Risk factors of Pulmonary Tuberculosis among antenatal patients in Adeoyo Maternity Teaching Hospital, Ibadan, Oyo state, Nigeria.

I am Ogundele T .O, a M.Sc. student from the Department of Biological sciences, Faculty of Basic and Applied science, Lead city university Ibadan. The purpose of the research is to investigate the prevalence and risk factors of Pulmonary tuberculosis among antenatal patients in Adeoyo Maternity Teaching Hospital, Ibadan, Oyo State, Nigeria.

Information on their age, gender, trimester, occupation, residential address, medical history e.t.c will be obtained through the administration of questionnaires and analysis in the Laboratory. Interaction with each participant will not last more than 30 minutes.

All information you share with me will be treated confidentially as your name will not be indicated in any of the study documents. Your participation in this research is entirely voluntary and will not affect you in any way.

You may choose to withdraw from this study any time, therefore a note, letter, text message, email or phone call informing me of your withdrawal as soon as possible would be appreciated.

Do you agree to participate in this study? Yes No

**Signature**

## Composition and Preparation of Media

### Preparation of Lowenstein Jensen Media

Ingredients

A. Mineral salt solution

Potassium dihydrogen phosphate anhydrous (KH2PO4): 2.4 g

Magnesium sulphate anhydrous: (MgSo4.7H20): 0.24 g

Magnesium citrate: 0.6 g

Asparagine: 3.6 g

Glycerol (reagent grade): 12 ml

Distilled water: 600 ml

Dissolve the ingredients in order in the distilled water by heating. Autoclave at 121°C for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and may be stored in suitable amounts in the refrigerator.

B. Malachite green solution 2%

Malachite green dye: 2.0 g

Distilled water: 100 ml

Dissolve the dye in the distilled water completely. Filter and store in the refrigerator.

C. Homogenized whole eggs

Get a fresh (those are not more than seven days old), hen's eggs

Clean the eggs by scrubbing thoroughly with a hand brush in water and soap.

Let the eggs soak for 30 minutes in a soap solution.

Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes.

Before handling the clean dry eggs scrub and wash the hands with a disinfectant.

Crack the eggs with the edge of the beaker into a sterile flask and beat them in a sterile blender for 30 seconds to one minute.

**Preparation of Complete Medium**

Aseptically pool the following reagents in a large, sterile flask and mixed well:

Mineral salt solution: 600ml

Malachite green: 20 ml

Homogenised eggs (25-30 eggs, depending on size): 1000ml

The complete egg medium is distributed in 6-8ml volumes in sterile universal containers or culture bottles (14 ml or 28 ml) and the caps tightly closed and inspissated without delay to prevent sedimentation of heavier ingredients.

Cultures are usually made in bottles rather than in Petri dishes because of the long incubation time required. Use of bottle limits both chances of contamination and drying of the culture media (if the caps are tightly closed).

**Coagulation of Medium**

Heat the inspissator to 85°C to quicken the build-up of the temperature before loading.

Place the bottles in a slanted position in the inspissator and coagulate the medium for 50 minutes at 85°C (since the medium has been prepared with sterile precautions this heating is to solidify the medium, not to sterilize it).

The quality of egg media deteriorates when coagulation is done at too high a temperature or for too long. Discoloration of the coagulated medium may be due to excessive temperature. The appearance of little holes or bubbles on the surface of the medium also indicates faulty coagulation procedures.

**Sterility Check for Lowenstein Jensen Medium**

After inspissation, the whole media batch of the media bottles should be incubated at 35°C-37°C for 24 hours as a check for bacterial sterility.

After 24 hours 5% of the slopes should be picked up randomly and continued for incubation for 14 days to check for fungal sterility.

In both cases the contamination rate should not be > 10 %.

**Storage**

The LJ medium should be dated and stored with the batch number in the refrigerator and can keep for up to 4 weeks if the caps are tightly closed to prevent drying of the medium.

## Biodata

| | |
|---|---|
| Name: | Ogundele Theresa Olaitan |
| Matrix number: | LCU/PG/001280 |
| Bachelor Degree's Qualification: | Bachelor of Medical Laboratory Science (BMLS) |
| Date: | September, 2005 |
| Qualification in view: | M.Sc. Medical Microbiology |
| Session: | 2019/2020 |
| Faculty: | Basic Medical and Applied Sciences |
| Course of study: | Medical Microbiology |
| Nationality: | Nigerian |
| State of origin: | Oyo |
| Local Government Area: | Ibadan North |
| Date of Birth: | 16th August 1979 |
| Religion: | Christianity |
| E-mail: | ogundeletheresa@gmail.com |
| Telephone number: | +2348052213933 |
| Next of kin: | Mr Ogundele Matthew Toxin |
| Telephone number of next of kin: | +2348038084226 |

**Signature** **Date**

**University Compliance Certification**

This is to certify that the thesis by Ogundele, Theresa Olaitan with Matric. Number LCU/PG/001280 in the department of Biological Sciences, Faculty of Basic Medical and Applied Sciences, Lead City University, is in full compliance with the approved University format and style.



**Signature** **Date**