Chapter One

Introduction

1.1 Background of the Study

Tuberculosis has been around for millennia, it is still a serious worldwide health issue. It is one among the top ten killers worldwide, causing illness in about 10 million people each year. It has surpassed HIV/AIDS over the past five years to become the most common infectious disease-related cause of mortality. Despite the fact that most persons who have Tuberculosis (TB) disease are curable with prompt diagnosis and appropriate treatmentthis still occurs[1].

The World Health Organization (WHO) has issued a severe warning to its members about the issue with tuberculosis treatment as the disease is re-emerging in a significant portion around the world[1].

Tuberculosis (TB) is an ancient communicable disease that has been in existence over the past millennia and up to date remains a major global public health challenge. It is caused by closely related acid-fast bacteria known as Mycobacterium tuberculosis complex (MTBC) with seven human-adopted MTBC lineages (designated lineage 1 through lineage 7) and two lineages adapted to various wild and domestic animal species but capable of causing human infection[2-5].

The prevalence of infectious diseases, chronic diseases, blood- borne viruses, and sexually transmitted diseases are much higher in the prison population than in the general population[6-12]. Pulmonary Tuberculosis (PTB) has been reported up to 100 times more in the prison population than the civilian population[13]. The number one reason being that higher rate is attributed to prisoners who are from population groups already at high risk of TB infection and disease (addicted to alcohol, drug users, homelessness, living in unfavourable social conditions such as

poverty and misery).Another reason is that like HIV in prison predisposes them to higher chances of TB transmission[14].

TB is a serious cause of morbidity and mortality in prison among the inmates. The prison system often faces various problems that hinder TB control, lack of laboratory facility and other diagnostic tools, interrupted supply and stock out of drugs, inadequate control measures, lack of coordination between civilian and to prison health services, and low priority for health care inmates.

TB remains a major public health problem in Nigeria. The Country ranks third among the 22 High burden Countries (HBCs) in the world (WHO: Estimated epidemiological burden of TB.2014), with an estimated 564,460 new cases of TB occurring annually and the sixth leading cause of death in the country, with a populationof 215.6 million.

Prisons are longer-term facilities operated under the legal authority of state departments of corrections or the Federal Bureau of Prisons. Prison typically hold individual with sentences of more than 1 year. Whereas a local jail detention might range from hours to months, state imprisonment averages approximately 3 years and federal imprisonment approximately 5 years [16,17]. Prison systems worldwide have been known as focal points for the concentration and dissemination of tuberculosis (TB) amongst inmates. Prisons are increasingly becomingbreeding grounds from which infection is transmitted to thegeneral population and accounts for up to 25% of a country'sTB burden[18]. An estimated 360,000 inmates move throughthe South African correctional centre system annually; this movement worsens the spread of communicable diseasesespecially TB[19]. The spread of TB between prisoners,staff, and visitors and the emergence of drug-resistant TBin prisons now threatens the control efforts of nationaltuberculosis program in sub-Saharan Africa (SSA) TB among prisoners in this region is not well documenteddespite its high endemicity reporting mechanisms are poor or non-existent.

While there have been many comprehensive literature reports of TB in prisons from USA and Europe, very little research hasbeen done in developing countries including SSA on the riskfactors associated with the incidence and spread of TB in the correctional facility settings[20]. Although precise information on prison conditions and the burden of the disease in prisons in Africa remains scarce, available data suggest that many of these facilities haveout-dated infrastructure and are overpopulated. The situation is even more disturbing given the difficult living conditions, extreme overcrowding, poor ventilation, poor sanitation andhygiene, poor nutrition, and substandard health care. These conditions contribute to the high prevalence and transmission of TB in prison settings[21]. According to WHO, the prevalence of TB in prisons is up to 100 (range: 10–100) times higher than that of the civilian population, in both low and high-TB burden countries[18]. Studies carried out in Ivory Coast, Malawi, Botswana, California, and Bangladesh state that prisons found TB prevalence of 10 to 35 times higher in prisoners than in the general population[21]. A 3- to 1000-fold increase in the prevalence of TB in prisons compared to the general population has also been documented in a recent systematic review[22].

World over, prisoners are increasing in number. Two per cent above world population growth, since the year 2000, the world prisoner population has grown by approximately 20%, causing a significant financial burden on government and having huge social and health consequences[23-24]. Over 11million people are held in custody globally, either as remand or convicted prisoners. Currently, 17countries in the world have an incarceration rate of more than 400 per 100,000 population in prison. USA is the largest incarcerator, accounting for approximately 20% of the world prisoners with an incarceration rate of 655 per 100,000 population. A rising prison population without a corresponding expansion of infrastructure has raised health concerns in many countries over prison crowding making custodial centers an important public health issue[25].

It is a known fact that the Substandard care offered to TB patient in prisons result in under-diagnosis and underreporting of case, continuous transmission, poor treatment outcome and development of drug resistance.

Interestingly, an upsurge in the positivity rate of MTB/GXPERT test for investigation from Agodi Custodial Center was observed, which makes it imperative for me to investigate further, including the risk factors responsible for the demography.

1.2 Statement of the Problem

Tuberculosis in prison is a major arena where the disease is re-emerging and this reflected in the theory of TB for one is TB for all.

1.3 Justification of the Study

The improvement of health care service delivery in prison is germane because, the opportunity for effective TB control in prisons is an opportunity to contribute to an effective TB control in a wider community. Therefore, more light need to be shed on the inadequate judicial and health policies of the prison system.

1.4 Aim and Objectives of the Study

The aim of the study is to determine the prevalence of pulmonary mycobacterial infections and its risk factors among inmates of Agodi Custodial Centre, Ibadan, Oyo state, Nigeria.

The specific objectives are to:

I. Detect pulmonary mycobacterial infection among the inmates.

II. Characterize the Mycobacterial isolates from the inmates.

III. Identify risk factors for the outbreak and spread of infection among the inmates.

IV. Investigate possible strains/diversities in the most prevalent pulmonary mycobacterial species isolated from the inmates.

1.5 Research Questions

1. What is the prevalence of pulmonary mycobacterial infections among the inmates?
2. What are the mycobacterial isolates from the inmates?
3. What are the risk factors associated with the outbreak and spread of mycobacterial infections among inmates?
4. What are the circulating Mycobacterial strains among the inmates?

1.6 Significance of the Study

The findings of this study will help to develop prevention and control strategies for Mycobacterial infections and its associated factors in prisons. This intervention will go a long way in eradicating TB and other mycobacterial infections by the year 2030 as proposed by the World Health Organization (WHO).

1.7 Scope of the Study

It spelt out the National Tuberculosis and Leprosy Control Programme. (NTBLCP) algorithm of tests on mycobacterial infections for inmates right from admission to release from prison and as well investigating the diversity of isolates from the inmates.

1.8 Limitation of the Study

Access to the inmates was a challenge due to heavy security personnel attached to the correctional center despite the study approval granted by the management of the center. Also, some inmates refused to participate in the study, probably for security reasons or other reasons best known to them.

1.9 Operational Definition of Terms

Prevalence: The total numbers of individual in a population who have a disease at a specific period of time, usually expressed as percentage of the population.

Incidence: It is a measure of the number of the new cases of a characteristics that develop in a population in a specified period.

Millennium: Is a period of a thousand year, especially when calculated from the traditional date of the birth of Christ.

Disease: Is a disorder of structure or function in a human, animal or plant, especially one that has a known cause and a distinction group of symptoms signs or anatomical changes.

Infection: The invasion and multiplication of microorganism such as bacteria, viruses and parasite that are not normally present within the body.

Pulmonary Tuberculosis: Is a serious infection caused by *Mycobaterium tuberculosis* that involves the lungs but may spread to other organs.

Extra Pulmonary Tuberculosis: A serious infection caused by *Mycobaterium tuberculosis*outside or apart from the lungs. E.g. Pott Diseases (spine) Genito-urinary (renal) Tuberculosis.

Lineages: Are sequences of biological entities connected by ancestors' descent relationship.

Mortality: The quality or state of being mortal. It is the number of death in a population

Morbidity: Is the state of being symptomatic or unhealthy for a disease or condition.

Multi Drug Resistance (MDR): Tuberculosis resistance to at least Rifampicin and Isoniazid.

Pre-XDR: Tuberculosis caused by *Mycobaterium tuberculosis*strains that fulfil the definition of MDR/RR Tuberculosis that are also resistant to any fluoroquinolone.

Extensively Drug Resistance (XDR): Tuberculosis caused by *Mycobaterium tuberculosis*strains that fulfil the definition of MDR/RR TB and also resistant to fluoroquinolone and at least one additional group A drugs (Bedaquilin, Linezolid, Moxifloxacin, Levofloxacin) New definition. In the old definition, there is addition of one of the injectables).

Mono-Resistance: Tuberculosis resistant to one first line drug.

Poly-Resistance: Tuberculosis resistant more than one first line drug other than Rifampicinand Isoniazid.

Rifampicin Resistance (RR-TB): Rifampicin resistant tuberculosis with or without resistant to other drugs, MDR-TB and XDR TB are form of RR TB.

Extremely Drug Resistance (XXDR): Tuberculosis caused by Mycobacterium tuberculosis strains that is resistant to all available first-line and second-line drugs. (There is yet no official nomenclature).

Endnotes

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Chapter Two

Literature Review

2.1 Great White Plague Review

M. tuberculosis infection has been known throughout human history. The bacterium is believed to have originated from East Africa. As early humans moved out of East Africa, settling in Europe and Asia, TB infection moved with them and continued to wreak devastation for centuries throughout the known worl[1]. Evidence of tubercular decay was seen on the spines of mummies from the Egyptian pre-dynastic era and the Peruvian pre-Columbian era, around 2400 B.C[2]. Ancient Greeks termed the illness "phthisis." Later, the "Great White Plague" of TB infection raged across Europe for over a century. Throughout this time, the disease was considered almost inevitably fatal, and no effective treatment or cure existed[1].

A milestone occurred when Hermann Heinrich Robert Koch discovered and expounded the etiology of tuberculosis in his presentation "Die Aetiologie der Tuberculose" to the Berlin physiological society. He presented his discoveries on March 24th, 1882, and later received the Nobel Prize in 1905. This was the start of an era of unprecedented advances in the treatment and prevention of this deadlydisease[3]. In 1943 another milestone was marked: the first known effective cure for the infection, antibiotic called streptomycin, was discovered in a laboratory at Rutgers University in New Jersey. The first large-scale clinical trial of streptomycin took place at the British Medical Research Council in 1948 and was the first published drug trialto randomize participants. This study set the methodological standard for modern-day randomized, controlled trials. It also was the first time patients showed resistance to streptomycin. Also in 1948, two new anti-tuberculosis agents, thiacetazone and paraaminosalicylic acid, entered the market.

When either of these agents was administered with streptomycin, cure rates dramatically increased and acquired resistance in the bacteria decreased[4].

Isoniazid was successfully tested and added to the TB regimen in 1951. This was followed by the development of a plethora of new drugs: pyrazinamide and cycloserine in 1952, ethionamide in 1956, rifampin in 1957, and ethambutol in 1962. Rifampicin was highly effective and easy to administer, and marked a significant advance in the fight against TB[5]. Unfortunately, the wide spread use of the new drugs led to the development of bacterial mutations conferring resistance.Resistance to rifampicin was observed in M.tuberculosis shortly after its initiation as a TB treatment standard. Bacterial resistance to isoniazid also quickly developed, but when other drugs like streptomycin or para-amino salicylic acid were discovered, the resistance was successfully suppressed. These observations led to the regular use of multidrug treatment regimens, an approach that is still considered the standard in treatment of tuberculosis as well as in other diseases[6].

After a series of international clinical trials led by the British Medical Research Council, a four-drug regimen was recommended for use in newly diagnosed tuberculosis patients. The pillars of this regimen were isoniazid and rifampicin, the most effective and well tolerated oral agents available, given for a period of 6 to 8 months. This short-course chemotherapy remains the first-line treatment regimen of non-resistant TB today[7].

Pulmonary Tuberculosis has been reported up to 100 times more in prison population than the civilian population[8]. Prison systems worldwide have been known as focal points for the concentration and dissemination of tuberculosis (TB) amongst inmates. Prisons are increasingly becoming breeding grounds from which infection is transmitted to the general population and accounts for up to 25% of a country's TB burden[9]. TB is a serious cause of morbidity and

mortality in prison among the inmates. The prison system often faces various problems that hinder TB control lack of laboratory facility and other diagnostic tools, interrupted supply and stock out of drugs, inadequate infection control measures, lack of coordination between civilian and prison health services, and low priority for health care to inmates[10]. Although precise information on prison conditions and the burden of the disease in prisons in Africa remains scarce, available data suggest that many of these facilities have outdated infrastructure and are over populated. The situation is even more disturbing given the difficult living conditions, extreme overcrowding, poor ventilation, poor sanitation andhygiene, poor nutrition, and substandard health care. These conditions contribute to the high prevalence and transmission of TB in prison settings[11]. According to WHO, the prevalence of TB in prisons is up to 100 (range: 10–100) times higher than that of the civilian population, in both low and high-TB burden countries[9]. Studies carried out in Ivory Coast, Malawi, Botswana, California, and Bangladesh statethat prisons found TB prevalence of 10 to 35 times higher in prisoners than in the general population[11]. A 3- to 1000- fold increase in the prevalence of TB in prisons compared to the general population has also been documented in a recent systematic review[12].

A prospective study of TB in Cameroonian prisons showed an annual incidence of active TB to be 1700 cases per 100,000 person-years[13]. In a Ugandan study, the reported TB incidence was 955 per 100,000 person-years, which is nearly 20 times higher (505 per 100,000) than that of the general population reported in Columbia. Several factors have been implicated as determinants of TB infection in prisons and these include: younger age, compared to older age groups, malnutrition (defined as body mass index (BMI) <18.5 kg/m2), high risk behavior such as substance abuse and injection drug usage and smoking[14-18]. Additionally, previously diagnosed/treated TB, exposure to TB patients, previous imprisonment, and longer duration of

imprisonment were associated with development of TB in prisons[8,18-21]. The architecture of prisons and the general living conditions in these facilities increase the risk of TB disease.

Prison construction plans invariably focused on security as higher priority than adequate ventilation. Various studies showed that in situations where inmates with active TB live in poorly ventilated custodial settings the potential for rapid transmission of TB was high. The overcrowded conditions of most prisons and poor ventilated close quarters in which inmates are housed facilitate the transmission of TB. These factors may often lead to TB outbreaks in the prison systems[22].

2.2 Pathogenesis

Mycobacterium tuberculosis is an airborne pathogen. Once inhaled, droplets bearing the mycobacteria settle throughout the airways. Most of the bacilli are trapped in the upper parts of the airways where the mucus secreting goblet cells are located. The mucus catches the invading bacilli, and the cilia on the surface of the cells constantly undulate to move the mucus and trapped foreign particles upward for removal. This system provides the body with an initial physical defense that prevents infection in most persons exposed to tuberculosi[23].

The bacteria that are able to pass the mucociliary system and reach the alveoli are quickly engulfed by alveolar macrophages. This next line of defense is the innate immune system, and it provides an opportunity for the body to destroy the invading mycobacteria and prevent the infection. Many mechanisms and macrophage receptors are involved in uptake of the mycobacteria. The complement system plays a key role in the phagocytosis of the bacteria. The Complement protein C3 binds to the cell wall and enhances recognition of the mycobacteria by macrophages.Opsonization by C3 is fast, even in the airspaces of a host with no previous exposure to *M. tuberculosis*[24]. The phagocytosis by macrophages initiates a cascade of events

that results in either effective control of the infection (which may be followed by latent tuberculosis) or progression to active disease, called primary progressive tuberculosis. The result is mainly determined by the strength of the host defenses and the balance that occurs between host defenses and the invading mycobacteria.After being swallowed by macrophages, the mycobacteria continue to multiply slowly, with bacterial cell division occurring every 25 to 32 hours. The initial development involves the production of proteolytic enzymes and cytokines by macrophages in order to try to degrade the bacteria. The cytokines that are released attract T lymphocytes to the site; T cells now lead the cell-mediated immunity. Macrophages present mycobacterial antigens on their surface to the T cells. This immune process continues for 2 to 12 weeks; the microorganisms continue to grow until they reach adequate numbers to fully provoke the cell-mediated immune response, which can be detected by a skin test called Tuberculin purified protein derivative (PPD)[25].

For those individuals with intact cell-mediated immunity, the next defensive step is the formation of granulomas around the *M. tuberculosis* organisms. These nodular type lesions, called Ghon complexes, form from an accumulation of activated T cells and macrophages that limits replication and the spread of the mycobacteria. This destroys the macrophages and produces necrosis at the center of the lesion, yet the bacteria are able to survive since *M. tuberculosis* can change their phenotypic expression in order to enhance survival. By 2 to 3 weeks, the necrotic environment resembles soft cheese, often referred to as caseous necrosis[26]. The conditions for this necrosis include low pH and limited nutrients. These conditions restrict further growth and the lesions undergo fibrosis and calcification, successfully controlling the infection and causing the bacteria to enter a dormant form. For immune compromised individuals, granuloma formation is initiated but ultimately is unsuccessful in containing the bacteria. The necrotic tissue

undergoes liquefaction and the fibrous wall of the granuloma loses structure. The liquefied necrotic material may then move into a bronchus or nearby blood vessel. If *M. tuberculosis* discharges into a vessel, extra-pulmonary tuberculosis is most likely to occur. Bacilli can also drain into the lymphatic system and collect in the trachea-bronchial lymph nodes of the affected lung, where the organism can form new caseous granulomas[27].

2.3 The Bacteriology

Mycobacterium tuberculosis is a large, non-motile, slow-growing obligate aerobic bacterium. As an obligate aerobe, it has a predilection for the oxygenated environment of the upper lobes of the lungs. *M.tuberculosis* has a doubling time of 18 hours and clinical cultures can take approximately 6–8 weeks. It is resistant to dehydration and so can survive in expectorated sputum. Morphologically the bacterial cell wall contains an array of complex lipids such as mycolic acids, long-chain fatty acids facilitating the acid-fast characteristics; Wax D; and Phosphatides, which contribute to the clinically relevant feature of caseating necrosis[28].

Cord factor, also known as trehalosedimycolate, is a glycolipid found in the cell wall, getting its name from the "serpentine" or cord-like pattern demonstrated by virulent strains[28]. On the surface of *M. tuberculosis*, cord factor is protective and non-toxic, typically preventing phagocytosis by macrophages during primary infection. In a secondary reaction, cord factor and host lipids in the alveoli may be an initiating factor in caseating necrosis development in lung tissue[29]. Traditionally cord formation has been related to virulence since avirulent *M. tuberculosis* strains do not form cords. However recent findings suggest similar cord formation in non-pathogenic, opportunistic Mycobacterium species, i.e. *M. abscessus, M. chubuense,M. gilvum, M. haemophilum, M. marinum, M. obuense, M. parafortuitum*, and *M. vaccae*. The cord patterns among species are not easily distinguished by light microscopy, an important diagnostic

implication in minimizing thepotential for false negatives and unnecessary exposure to an ineffective and arduous drug regimen[30]. Phthiocerol dimycocerosate, a lipid located in the bacterial cell wall, is also necessary for TB pathogenesis in the lungs. Its early involvement in infection occurs when bacilli encounter host macrophages. Current research suggests that phthiocerol dimycocerosate controls the bacterial invasion of macrophages by targeting lipid organization in the host cell membrane, altering its biophysical properties. These changes in lipid ordering facilitate receptor-mediated phagocytosis of *M. tuberculosis*, contributing to the control of phagosomal pH and protection of the bacteria from further immune response. The pathogenesis of *M.tuberculosis* hinges on this ability to invade macrophages and use them to evade host cell bactericidal activity[31].

In 1998 the complete gene sequence of *M. tuberculosis* variant (H37Rv) was determined, comprising of 4,411,529 base pairs and 4000 genes[32].The genome has an extremely high guanine + cytosinecontent and is remarkably different than most bacteriain that it possesses dedicated enzymes specialized forlipogenesis and lipolysis. It has been suggested that these fatty acid-utilizing enzymes are potentially associated with the ability of *M. tuberculosis* to survive in host tissues, using fatty acids as a carbon source[33].

2.4 Diagnosis

2.4.1 Culture and Sensitivity

The permeability of the aforementioned barrier enables the bacterium to resist conventional gram staining, causing gram stains to show a weak positive, or to show up white; so typically an alternative (acidfast) stain is used instead. Acid-fast, also known as the Ziehl-Neelsen stain refers to the ability of *M.tuberculosis* to retain carbolfuchsin stain, despite decolorization treatment with ethanol-hydrochloric acid[34]. Preparation prior to staining involves NaOH treatment, which

destroys unwanted bacteria, human cells, and fluid, followed by centrifugation. This is followed by culture on Lowenstein-Jensen media for up to 8 weeks. Lowenstein-Jensen media contains complex nutrients and dyes, i.e. egg yolk and malachite green dyes; dyes inhibit normal flora present in sputum samples[28].

Bacteriological cultures can provide a definitive diagnosis of tuberculosis. The primary advantage of culture tests over sputum microscopy is their higher sensitivity, allowing for the detection of very low numbers of bacilli (approximately 10 bacilli/ml of sputum compared with at least 5000 bacilli/ml of sputum for microscopy). The use of cultures increases the potential of diagnosing TB at early stages of the disease. Culture tests are also used for the detection of treatment failures and for diagnosing extrapulmonary TB. The use of culture tests increases thenumber of TB cases found by 30–50%. Moreover, cultures are used for species identification and drug susceptibility testing[36]. As the EU has adopted the culture-based case definition, the main distinction in TB cases is between culturepositive and -negative, and not sputum smear status. The first section of the European Standards for Tuberculosis Care (ESTC) 2012 dedicated to the Standards for Diagnosis (ESTC 1 through ESTC 6) specifies the minimum requirements for obtaining a valid TB diagnosis in different settings or with various suspect types, both microbiological and clinical[36]. If this is not feasible, culture tests should at least be performed for:

- Diagnosis of cases with clinical and radiological signs of pulmonary TB where smears are repeatedly negative.
- Diagnosis of extrapulmonary TB.
- Diagnosis of childhood TB.
- Diagnosis of TB among HIV-positive adults and children; and
- Diagnosis and monitoring of MDR- and XDR-TB.

Tuberculosis although, mainly a pulmonary disease can affect any organ of the body.The isolation of the aetiological agent for effective microbiological diagnosis is dependent on:

- Selection of the correct type of specimen.
- The quality of the sample; and
- Adequate use of storage and transportation procedures.

Processing of inappropriate clinical specimens for mycobacterial cultures is a waste of both financial and human resources[37]. Clinical staff should be properly trained and accept only suitable specimens. Because Mycobacteria are usually slow growing and require a long incubation time, a variety of other microorganisms can overgrow the cultures of specimens obtained from non-sterile sites. Appropriate pre-treatment and processing of samples, as well as the use of selective culture media is critical for eliminating contaminants while not seriously affecting the viability of Mycobacteria[38].

2.4.2 Culture Media

As *M. tuberculosis* grows slowly, with a generation time of 18–24 hours (other bacteria reproduce within minutes), usual bacteriology techniques are not applicable to mycobacterial cultures. Moreover, growth requirements are such that M. tuberculosis will not grow in primary isolation on simple, chemically-defined media. The only media that allows for abundant growth are egg-enriched media containing glycerol and asparagine, and agar or liquid media supplemented with serum or bovine albumin. Many different media have been developed for *M. tuberculosis* growth and are generally classified into two main groups: solid media (egg- and agar-based) and liquid media.Antibiotics can be added to culture media in order to prevent the growth of non-specific flora.Both solid and liquid media are recommended for *M. tuberculosis*

isolation from biological samples. An advantageof solid over liquid media is that colonies of mixed cultures and contaminants can be observed while liquid mediapromotes a faster growth of Mycobacteria.The choice of media depends primarily on the type of specimen. Non-selective media are recommended for use with samples from normally sterile sites (bone marrow, tissue biopsy samples, cerebrospinal fluid and other bodyfluids etc.), while selective media, that contain antimicrobial agents to prevent growth by contaminating bacteria and fungi, are recommended for use with contaminated (or potentially contaminated) specimens (sputum, abscesscontents, bronchial washings, gastric lavage fluid, urine, etc.)[39].

The most commonly used non-selective media are:

- Egg-based media: Löwenstein-Jensen (LJ) medium and Ogawa medium.
- Agar-based media: Middlebrook 7H10 and Middlebrook 7H11; and
- Liquid media: Middlebrook 7H9 broth.

Other commonly used selective media available in some countries are:

- Egg-based media: Gruft modification of LJ (containing malachite green, penicillin and Nalidixic acid as selective agents, and Mycobactosel LJ (containing malachite green, Cycloheximide, lincomycin and Nalidixicacid as selective agents).
- Agar-based media: selective 7H11 (Mitchison's medium), containing Carbenicillin, Amphotericin B, Polimixin B and Trimethoprim as selective agents; and
- Liquid media: in general they contain a modified Middlebrook 7H9 broth plus a mixture of antimicrobialagents. Several automated systems have been commercially developed for rapid detection of mycobacteriain liquid medium:
- BACTEC MGIT 960 system (BD [Becton, Dickinson and Company] Diagnostic Systems)
- ESP Culture System II (Trek Diagnostic Systems).
- MB/BacT (bioMérieux).

2.4.2.1 Solid Media

2.4.2.1.1 Egg-based Media

LJ medium, which contains malachite green as an inhibitor of non-mycobacterial organism, is the most commonly used egg-based medium, especially for sputum culture. LJ is user-prepared or commercially prepared in slant tubes. LJ containing glycerol favours *M. tuberculosis* growth, while LJ without glycerol but containing sodium pyruvate enhances *M. bovis* growth. Both media should be used in geographical regions where patients may beinfected with either organisms[40]. Ogawa medium is LJ without asparagine. Non-selective egg-based media canbe stored in the refrigerator for several months provided that the tube caps are tightly closed to minimize evaporation.

A disadvantage of egg-based media it that when contamination does occur it may involve the entire slant surface, so the culture is generally lost. If specimens contain few bacilli it may take three to eight weeks before cultures become positive.

2.4.2.1.2 Agar-based Media

These media are prepared in slant tubes or plates and are less likely than egg-based media to become contaminated. Middlebrook 7H10 and 7H11 media are usually prepared in the laboratory from commerciallyavailable agar-powdered bases, with the addition of Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment. Because of the transparency of 7H10 and 7H11 plates, *M. tuberculosis* micro colonies with typical cordformation can be detected and counted using a microscope as early as one week after incubation. Moreover, visibility of colonial morphology on agar plates is better than on egg-containing slants, aiding the identification of Mycobacteria. Middlebrook 7H11 is preferable to 7H10 because it contains 0.1% casein hydrolysate, a substance favouring the recovery of isoniazid-resistant mycobacteria.

Furthermore, 7H11 is also better for growing multi-drug resistant (MDR) strains as these may not grow at all on 7H10 agar plates.

A disadvantage of Middlebrook media is that the surface dries more rapidly than egg-based media. It is important to know that daylight, heating and storage at 4°C for more than four weeks may cause the release of formaldehyde in a sufficient concentration to inhibit the growth of Mycobacteria.

2.4.2.2 Liquid Media

Liquid media offer a considerable time advantage over solid media: 7–14 days in Middlebrook 7H9 liquid medium, compared with 18–28 days in Middlebrook 7H11 agar, or 21–42 days in LJ medium [41]. One of the most widely used automated systems for rapid detection of mycobacteria in liquid medium is the BACTECMGIT 960 system[42]. The system's culture tubes consist of modified Middlebrook 7H9 broth, a growth supplement,and an antimicrobial agent mixture. A similar principle is used in the ESP Culture System II and the MB/BacT system.

In the BACTEC 960 system and ESP Culture System II, M. tuberculosis growth is detected by the rate of oxygen consumption within the headspace of the cultures; in the MB/BacT system, a colorimetric sensor detects theproduction of CO2 dissolved in the culture medium.

Clinically, tuberculosis can be diagnosed by signs and symptoms, characteristics on chest radiography, and positive skin reactivity findings from the tuberculin (Mantoux) skin test[43]. Sign and symptoms suggestive of TB include: significant cough that lasts 3 weeks or longer, chest pain, hemoptysis, coughing up sputum (productive cough), fatigue, weight loss, anorexia, chills, pyrexia, night sweats. On chestradiograph, TB disease activity is evidenced by any parenchymal, nodal, or pleural abnormality with or without associated calcification[44]. Confirmation of these

findings and tests are supported by the microscopic identification of acid-fast rods and the culture of the bacteria.

A second approach, known as interferon-gamma release assay (IGRA) measures the body's immune reaction to TB by testing blood in the laboratory. More specifically the level of interferon-gamma is measured upon exposure of blood cells to an antigen from *M. tuberculosis*. A positive test indicates infection with TB; however additional tests are required to determine whether a latent infection or active TB disease is present. A negative test showing a lack of reaction indicates a lower likelihood of TB infections or disease. This test is not influenced by BCG immunization.Laboratory diagnoses may also be obtained within 2 weeks by detecting radioactive carbon dioxide using a liquid BACTEC medium. If growth occurs, further biochemical tests can be performed, including niacin detection, which is almost exclusively seen in *M. tuberculosis*[28]. During treatment, it may be necessary to determine the presence of drug resistance. Detection of mutations in the catalase gene confirms resistance to isoniazid and RNA polymerase gene mutations confirm resistance torifampin. The luciferase assay can expediently detect drug-resistant organisms as well[28].

2.5 Treatment and Prognosis

Patients with latent tuberculosis infection have the bacteria in their bodies but do not typically present with symptoms because the bacteria are not active. If the bacteria become active and multiply, then the symptoms of TB will become evident in the patient. For this reason, patients with known latent TB are prescribed preventative pharmacological interventions. The current medications that are used for the treatment of latent tuberculosis are isoniazid, rifampicin, and rifapentine. Non-resistant TB is usually treated by a regimen of several drugs taken for a period of 6 to 9 months. Currently, there are 10 drugs that are approved by the FDA for the treatment of

active TB, of these approved drugs, the first-line pharmacological intervention that forms the core treatment regimen includes isoniazid, rifampicin, ethambutol, and pyrazinamide. Treatment regimens for non-resistant TB have an initial phase of 2 months, followed by a continuation phase of usually 4-7 months. The 6-month regimen consists of isoniazid, rifampicin, and pyrazinamide given for 2 months followed by isoniazid and rifampicin for 4 months. Ethambutol or streptomycin is added in the first 2 months in patients with advanced disease. The success rate with the 6-month regimen in sputum conversion (conversion defined as a negative culture in 3 consecutive samples taken 1 day apart) is far beyond 90% within the first two months of therapy. The relapse rate after 3-5 years is *about 0-3%). It is very important to complete the treatment regimen* because bacteria could still be active and become resistant to these first-line drugs if the treatment is stopped prematurely. For this reason, physician supervision and follow-up become important to ensure patient compliance[45]. Shortening anti-tuberculosis treatment regimens is one strategy expected to improve patient adherence to treatment, resulting in better case management and disease control and minimizing the risk of drug resistance. Gatifloxacin, normally a second-line drug, was selected for a recent phase 3 trial to evaluate the efficacy of a 4-month regimen compared to the standard 6 months. This trial assessed the effect of shortening rifampin-sensitive TB treatment by using a fluoroquinolone-based approach. The results of the study failed to show that 4-month treatment with gatifloxacin, which was substituted for ethambutol, was non-inferior to the standard 6-month regimen. There was a higher recurrence rate observed with the 4 month regimen[46]. MDR-TB and XDR-TB are inherently more difficult to treat because the treatment must be individualized and closely monitored. Depending on the susceptibility of the infection, treatment regimens for resistant strains can last up to 36 months or more. An additional complication of this treatment is that, while first-line TB drugs are relatively nontoxic, second-

line treatments like fluoroquinolones carry the risk of more serious side effects. Second-line treatments also tend to be more expensive than first-line drugs, and they may not be available in areas where access to health care is limited. These factors all complicate the treatment of an illness that is already difficult to defeat[47]. An important strategy to address MDR-TB and XDRTB is the development of novel pharmacological approaches to circumvent bacterial resistance. These research foci vary widely, as does their effectiveness. One recent study examined the addition of efflux inhibitors including verapamil, chlorpromazine, farnezol, reserpine, and others, as adjuvants to increase theeffectiveness of antibiotic regimens already used in the treatment of TB. The efflux inhibitors reduce the bacterium's ability to expel the antibiotic before it achieves its intended effect. So while efflux inhibitors are not themselves toxic to these microorganisms, their use in a TB regimen may prolong the intracellular presence and subsequent damage of the concurrently administeredantibiotics[48]. Other approaches currently being explored include the re-purposing of drugs currently in use for other conditions, in which anti-TB activity has been observed. Some of these drugs are not currently in use as antibiotics at all. One example of this is the antipsychotic thioridazine, a member of the phenothiazine class of neuroleptics which exhibits anti-mycobacterial action and has been shown to be effective in the treatment of some XDR-TB cases. Trials for the use of thioridazine in this capacity are expected to begin soon[47]. There are also a few new drugs in development with novel mechanisms of action against TB. These include bedaquiline, a diarylquinoline which inhibits ATP synthesis in mycobacteria, and delamanid, which inhibits bacterial mycolic acid (an important cell-wall component) synthesis. As these drugs and others proceed through trials, their efficacy and safety for patients will become more evident[49].

2.5.1 DOTS Assisted Treatment for Prison Inmates

The effectiveness of any anti-TB therapy mainly involves, appropriate medicine being taken in appropriate doses and ingested correctly for appropriate durations. Inmate's compliance is essential to achieve cure. All factors that can cause non-compliance must be addressed and the services providing TB care in prisons must offer support to inmates to ensure that treatment will be completed. Collaboration between the prisons and NTBLCP is essential to ensure that TB infected inmates complete their treatment after release. The NGOs must be involved working in communities and with hard-to-reach populations to facilitate this process, they play an active role in tracing released prisoners, delivering DOTS and counseling them.A relationship of trust and confidence between the infected inmates and prison health staff promotes compliance to treatment. Respecting inmates and being considerateat every contact is vital for prison health official. Inmate's compliance to treatment requires that they understand the disease and what is necessary for successful treatment and cure. Time must be set apart during registration for treatment to really meet the inmates, this initial meeting is a prime opportunity to advice, counsel and educate them on the following:

- The importance of cough hygiene (covering of mouth when coughing and sneezing).
- Directly Observed Treatment Short-course (DOTS).
- How to recognize potential side effects.
- The need for follow-up, through sputum smear microscopy monitoring.
- The use of isolation measures.

A new meeting with the inmates on treatment at the end of the initial phase of treatment will allow for explanation of progress and the need for the continuation phase.

Many TB patients receiving self-administered treatment will not adhere to treatment. Some patients stop treatment once they feel better, once they have taken their medicines for the first few weeks, if unpleasant side effects occur. Prisoners, in particular, may find the motivation to complete treatment difficult; they have more immediate worries than the dangers of not receiving a full course of TB treatment.Since the prediction of who will or not comply with prescription is impossible, DOTS is necessary to ensure compliance. DOT has been used in TB control programs worldwide to facilitate adherence and improve treatment outcomes.DOTS also allow early identification of toxic side effects of medicines and other problems that may affect compliance. In this intervention, the swallowing of medicines is directly observed by another person accountable to or supervised by health services (a nurse or prison guard).Unfortunately this might not always be possible, hence the making use of prison volunteers which may be trained and sensitized prison security staff, so also the prisoners who were selected based on their willingness to assist the program, good behavior, and being highly regarded by the other inmates. Close supervision of the process of DOTS by prisoners is crucial, especially in the beginning, to prevent them corrupting the system (selling anti-TB drugs. Conclusively, prison program volunteers need to be continuously sensitized, educated, and motivated to ensure their support to the program.

2.6 Epidemiology

2.6.1 Current Global Distribution

The latest trends in the global distribution of tuberculosis were published in 2018 by the World Health Organization in their annual report on tuberculosis[50]. Globally, TB is one of the top 10 causes of death and the leading cause of death in HIV infection / AIDS. Many people continue to fall sick each year from TB infection. TB caused an estimated 1.3 million deaths among HIV-

negative individuals and an estimated 300000 death among HIV-positive individuals in 2017. Worldwide, an estimated 10.0 million people developed TB disease in 2017 with a breakdown of 5.8 million men, 3.2 million women and 1.0 million children. Cases were reported in all countries and age groups; overall 90% were adults (aged ≥15 years), 9% were individuals living with HIV (72% in Africa) and two thirds were from eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). The listed 8 countries and 22 other countries in WHO's list of 30 high TB burden countries made up for 87% of the world's cases; while 6% of global cases were in the WHO European Region (3%) and WHO Region of the Americas (3%)[50].

In 2017, fewer than 10 new cases per 100 000 individuals were reported in most high-income countries, 150–400 in the majority of the 30 high TB burden countries, and over 500 in a few countries including Mozambique, the Philippines and South Africa[50]. Globally in 2017, an estimated 558 000 individuals developed TB that had resistant to rifampicin (RR-TB), considered to be the most effective first-line drug, andout of these, 82% had multidrug-resistant TB (MDR-TB). India (24%), China (13%) and the Russian Federation (10%) accounted for almost half of the world's cases of MDR/RR-TB[50]. Globally prevalence of MDR/RR-TB in new TB cases is 3.5% while that of previously treated cases is 18% with the highest proportions (>50% in previously treated cases) found in countries of the former Soviet Union. In 2017, among cases of MDR-TB, 8.5% were estimated to have extensively drug-resistant TB (XDRTB)[50]. Latent TB infection is estimated to be present globally in about 1.7 billion people (23% of the world's population). These individuals are at risk of developing active TB disease during their lifetime[50]. TB incidence rate is falling globally at about 2% per year. The fastest regional declines from 2013 to 2017 were reported in the WHO European Region (5% per year)

and the WHO African Region (4% per year). Similarly, TB deaths among HIV-negative individual has fallen globally by an estimated 29% since the year 2000 while that of the HIV-positive individual has also fallen by 44% since the same year. Among WHO regions, the fastest declines in the 5 years 2013–2017 werereported in the WHO European Region (11% per year)and the WHO South-East Asia Region (4% per year)[50].

2.6.2 Vulnerable Populations

Some populations are particularly vulnerable to tuberculosis infection. This population comprised of HIV-positive patients and other immunosuppressed patients including alcoholics and intravenous drug users, as well as people in very crowded living conditions such as prisoners or those in densely populated urban areas[51]. People from countries with a high incidence & prevalence of tuberculosis, especially those living in poverty, are also more susceptible. In developed countries, immigrants from areas with a high TB prevalence are more vulnerable than the general population.Children are a statistically significant portion of these vulnerable populations because their symptoms may go undetected and they may suffer for a long time before finally being diagnosed and treated[52]. Many tuberculosis control programs have neglected this population even while faced with a rising number of infections. Children have a higher rate of primary disease progression after infection, which means they may spread the infection faster than other patients. International TB control units may not be accounting for the increase in incidence among juveniles, and thus disregarding an important factor for the spread of infection[53].TB infection is very common among alcoholics and intravenous drug users, especially those infected with HIV. These patients may be unwilling or unable to get timely treatment for symptoms, so their infections may go undetected until they are severe and difficult to treat. Preventative care and supervision are important factors in controlling TB in these patients.

Another worrying vulnerability in HIV-positive patients is the possibility of reactivation of latent TB infection. As mentioned previously, TB infection can sometimes persist in a latent, non-pathogenic state. Corbett and associates have shown a pattern in which the presence of HIV in these patients actually awakens the latent TB infection and they become likely to develop active TB. Globally, tuberculosis is the leading cause of death of HIV positive patients[55]. Residents of urban areas, where there is an estimated incidence of infection up to 3-5 times higher than in rural areas, are also vulnerable. This may be due to the fact that people in urban areas often live in densely packed conditions and are in contact with a large number of people, and thus more likely to be exposed to pathogens[56]. Additionally, an overall decrease in baseline health and an increase in respiratory complaints and asthma due to pollution and other factors may prevent these patients from seeking care in a timely manner after the onset of symptoms[57]. These factors also apply to other conditions of the dense population, including prisons and shelters. In order to decrease the rate of infection in this population, control units need to aim at increasing the awareness of TB, its signs and symptoms, and the importance of care across urban and other densely populated areas[58]. An interesting, perhaps somewhat counterintuitive fact is the phenomenon of genetic resistance to TB infection found in some urban populations. There is evidence that since the onset of urbanization in ancient times, the increased prevalence of TB in crowded conditions led to the development of genetic resistance in some families, particularly those who continued to reside in these areas for many generations. Research by the University of London and the University of Oxford determined the frequency of an allele (SLC11A1 1729 +55del4) associated with a natural resistance to TB and Leprosy[59]. This allele showed a significant correlation with the long-term urban settlement. This resistance is likely due to natural selection, especiallyconsidering the astronomical mortality rate of TB before the

development of antibiotics in the 20th century. This is good news for an ever more denselypopulated world, as the persistence of this resistantpopulation may eventually increase the resistance of the general population to TB over time.Most vulnerable populations have in common that they are lower on the socio-economic spectrum. In fact, there is a clear correlation between poverty and vulnerability to TB infection. Ignorance or denial of the significance of symptoms, delayed or inaccurate diagnosis and lack of access to care and treatment are all factors that contribute to this vulnerability[60]. Addressing these issues through targeted campaigns to increase patients' awareness, access to accurate diagnostic ability and effective treatment should be the strategies for success of TB control units worldwide.

2.7 Global Initiatives

While tuberculosis infection spans the globe, the fight against it has been spearheaded by a few key organizations. The WHO has implemented far-reaching programs that work with local governments, other international aid organizations, NGOs, and other stakeholders to develop research and provide equipment and services to improve community management of this epidemic. In 1995, the WHO first began to standardize the collection of regular reports of global TB incidence and other statistics. The year 2015 marked the twentieth year of TB data collection, and the fifteenth year since the adoption of Millennial Development Goals (MDGs) signed by all 191 United Nations member states in 2000. These goals proposed specific metrics to track progress on a variety of issues, including reducing the incidence and improving treatment of infectious diseases. One of the declared MDGs was to reverse the then-increasing incidence of TB, a goal which has been met by its evaluation date of 2015. Another was to increase the TB cure rate, which has risen from less than 80% in 1990 to around 86% since 2013. However, as the time period encompassed by the MDGs comes to an end in 2015, the global fight against TB

is far from over[42]. For more than two decades, the WHO's anti-TB efforts have been based on the Directly Observed Treatment, Short Course or "DOTS" treatment strategy as the global standard of care. This strategy includes five elements necessary for effective disease management. The DOTS principles illustrate the many facets of the struggle to control infectious diseases. This system has provided a basis for global TB care, including the WHO "Stop TB" program, initiated in 2006, and it continues to guide the "End TB" program that began in 2015. Clearly, difficulties in any one element of this strategy may compromise the success of a control program as a whole. Given the myriad uncertainties in many areas of endemic TB, it is easy to see how challenging this effort really is. Despite this, the WHO has set ambitious goals and progress metrics to guide its many programs for the next two decades[42].

In order to achieve the End TB Strategy milestones for 2020 and 2025, TB diagnosis, treatment and prevention services should be provided within the context of progress towards universal health coverage (UHC), as well as the presence of a multi-sectoral action to address the social and economic factors that drive TB epidemics[50]. SDG Target 3.8 is aimed to achieve UHC by 2030. WHO estimation published in 2017 believed that most middle-income countries could mobilize the funding required to achieve UHC by 2030 from domestic resources, while this is not likely to be achieved in lowincome countries[50].

2.8 Progress in Research and Development

Breakthroughs in technology are required to accelerate the annual decline in the global TB incidence rate to an average of 17% per year. Area of research focus include a vaccine to lower the risk of infection, a vaccine or new drug treatment to reduce the risk in latently infected people, rapid diagnostics for use at the point of care and simpler, shorter drug regimens for

treatment. There is slow progress in the development pipelines with few diagnostic technologies emerging in 2017 in spite of recent increase funding for TB research and development.

Despite vast improvements in research and technology and the development of multiple drug regimens to battle this insidious killer, *M. tuberculosis* continues to be a major health concern worldwide. As TB infection continues to be the most prevalent fatal infectious disease in theworld, funding for research and program implementation has trailed global investment in other diseases. Advances in diagnostic tools, active patient supervision, and a global focus in detecting and mapping strains of drug-resistant pathogens are proven strategies for controlling this disease. However, integral to these clinically important strategies is a solid commitment by the international community to finally eradicate this devastating infection[50].

2.9 Antibiotic Resistance

One of the biggest obstacles in the global fight against tuberculosis is bacterial development of resistance to the essential drugs used to treat it. After centuries of rampant pathogenesis and devastating mortality rates, a series of incredible discoveries in the 20th century led to the development of effective treatments for this formerly invulnerable infection. However, over time, as these treatments were massively and sometimes inappropriately administered, and unsupervised patients failed to rigorously complete their prescribed regimens, the highly versatile *M.tuberculosis*developed a variety of mutations to subvert the cellular mechanisms of these drugs. Treatment of MDR-TB and XDR-TB takes significantly longer (up to 36 months or more) than treatment of non-resistant TB, and the second-line and third-line drugs required are typically more expensive and far more toxic than firstline drugs. These factors may preclude patient compliance without financial assistance and vigilant supervision and follow-up by healthcare professionals.This has led to the persistence of TB as the most common cause of death

from infectious disease in the world and continues to challenge healthcare practitioners globally every day[61]. Multiple-drug resistant strains of tuberculosis are resistant to both of the most commonly used first-line drugs, isoniazid, and rifampin. These drugs have been in use for over 50 years and their mechanisms of action have been extensively studied. This has enabled the elucidation of *M. tuberculosis*' ability to evade them through genetic mutation. As the cellular mechanisms of TB drugs differ, so do the bacterial mutations that confer resistance to these drugs.It is well known that the catalase-peroxidase enzyme, encoded by the katG gene of *M. tuberculosis*, is responsible for activating the pro-drug isoniazid. The activation of isoniazid leads to an isonicotinoyl acylradical that then combines with NAD+/NADH to form the isoniazid-NADH adduct (INH-NADH)[62]. Theisoniazid-NADH adduct is a potent inhibitor of inhA, a gene which produces an enoyl acyl-carrier protein reductase involved in the production of mycolic acids, which are the main structural components of themycobacterial cell wall[63]. Mutations in katG can lead to isoniazid-resistance due to their inability to activate the isoniazid prodrug, preventing its effect[64]. In contrast, rifampicin acts by inhibiting bacterial DNA-dependent RNA synthesis. It accomplishes this by inhibiting bacterial DNA-dependent RNA polymerase. Rifampicin binds to RNA polymerase at a site adjacent to the RNA polymerase active center and blocks RNA synthesis by physically blocking the formation of the phosphodiester bond in the RNA backbone, preventing extension of RNA products beyond a length of 2-3 nucleotides[65]. Resistance to rifampicin arises from mutations that alter residues of the rifampicin binding site on RNA polymerase, resulting in decreased affinity for rifampicin and prevention of its effect[66]. Extensive drug resistant (XDR-TB) strains of bacteria were first reported in 2006. In addition to isoniazid and rifampicin, XDR strains show resistance to any of the fluoroquinolones (e.g. ofloxacin, moxifloxacin, and others) and to at least one of three

injectable second-line drugs (amikacin, capreomycin or kanamycin). Extensive drug-resistant tuberculosis cases are rare; however, 77 countries worldwide had reported at least one case by the end of 2011.Information from countries with reliable data suggests that approximately 9.7% of MDR-TB cases worldwide have XDR tuberculosis[67]. Not all of the cellular mechanisms of extensive drug resistance have been described. However, the drugs involved have been in use in various capacities for some time, so some insights into bacterial resistance have been observed. Fluoroquinolones are derivatives of nalidixic acid, a substance that was discovered as a byproduct of an antimalarial compound. This acid's antibiotic effects were first observed in 1965, but its action against TB was not evident until much later. Derivatives of this acid interfere with bacterial DNA replication by inhibiting a protein called topoisomerase II (also called DNA gyrase), which is responsible for initiating super coiling of the cell's DNA. A similar protein, topoisomerase IV, is also inhibited by these drugs, but only type II is found in *M. tuberculosis*. Resistance to fluoroquinolones occurs when mutations develop in bacterial genes called gyrA and gyrB, whichare responsible for the production of this protein[68]. The injectable second-line drugs kanamycin and amikacin both inhibit bacterial protein synthesis at 16sRNA. Bacterial mutations in the rrs and eis genes have been shown to confer resistance to kanamycin, but only rrs mutations show evidence of resistance to amikacin. In contrast, capreomycin, another injectable, is able to bind to bacterial ribosomes at the interface between small and large ribosomal subunits. Resistance to capreomycin is conferred by mutations at both rrs and eis, as well as an additional location called tlyA[68]. Resistance to other common second-line TB drugs including ethionamide, cycloserine, macrolides like clarithromycin, and others has been observed as well. In 2009Velayati reported an observation of patients in Iran with XDR-TB whose infections were also resistant to all second-line drugs available at the time. The authors

called this new level of resistance totally drug resistant, or TDR-TB. Cultures of *M. tuberculosis* from these patients were not identical, however. The bacterial strains cultured from these individuals were found to originate from at least four different known MDR-TB superfamilies. This was not the detection of an entirely new, super-resistant strain of bacteria. Instead, it was evident that these patients had been intermittently and ineffectively treated for less resistant TB infection prior to developing this extreeme level of drug-resistance. If these patients had been treated appropriately for their original TB infections, it is unlikely that any further drug resistance would have developed[69]. This report was not the first time TB patients with extreme resistance were reported. In 2007, two patients in Italy were found to be resistant to all available first-line and second-line drugs available. The authors reporting those cases called the new level of resistance XXDR, or extremely-drug resistant TB. There is as yet no official nomenclature or standard definition of any higher resistance than XDR-TB, but if treatment inconsistencies continue in areas where MDR-TB and XDR-TB are already endemic, then cases like this may become more common[70]. The WHO estimates that there were around 480,000 MDR-TB cases worldwide in 2014. Unfortunately, only asmall proportion of these cases are detected and treated appropriately. This is due to a variety of factors, including lack of diagnostic equipment or training, lack of access to appropriate treatment, and lack of patientcompliance. Another, perhaps more worrying factor is inconsistencies in the training of physicians in endemic TB areas. Reports on TDR-TB patients in India in 2012 confirmed that these patients had been treated for TB by an average of four different physicians over a period of 18 months, and that the treatments they received were inconsistent and often inappropriate. This shows that standardizing effective treatment protocols is only one part of the solution to resistance; physician education, especially in high-burden MDR-TB and XDRTB areas, is an essential part of this as well. Timely detection

and appropriate treatment of resistant TB strains is extremely important to prevent further evolution and spread of "untreatable" TDR-TB strains[71].

2.10 Prevention and Control

The major health-care interventions for preventing new infections and progression to TB disease are the treatment of latent TB infection and vaccination of children with the Bacille Calmette-Guérin (BCG) vaccine. While preventive treatment for a latent TB infection is expanding, accessibility of care is still a problem to those that require it; this is unlike BCG vaccination coverage which is high[50]. WHO has strongly recommended treatment for latent TB infection in two important groups' i.e. individuals living with HIV, and children aged less than 5 years who are household contacts of an individual with bacteriological confirmed pulmonary TB[50].

Progress is being made in the fight against TB infection on many fronts. One important strategy to control this disease is the development of tools to provide a quick, accurate diagnosis of drug-resistant strains of bacteria in the field. Another, equally critical element is coordination between healthcare providers. Infrastructure, up-to-date hospitals and clinics, and community education all play essential roles in the struggle to control this disease[42].

A serious concern in modern TB treatment is the delay in diagnosis of drug-resistant strains of the bacteria, which require specialized treatment regimens. This is especially important because the areas where MDR-TB and XDR-TB are endemic are also largely underdeveloped areas with limited access to modern healthcare and laboratory equipment. In cases where TB is suspected but the bacteria's susceptibility is unknown, patients may receive ineffective treatment before the resistance of their infection is identified. This can increase the patient's risk of morbidity and mortality from TB, as well as encourage bacterial development of more extensive resistance

mechanisms to a broader range of antibiotics. This delay may also prolong the window for transmission of the infection, perpetuating the reservoir of resistant microorganisms.

It is clear that access to timely diagnosis is essential to providing appropriate treatment and reducing the prevalence of resistant organisms[72]. Currently, a widely-used standard for drug susceptibility testing (DST) of TB is the BACTEC MGIT960, a fully-automated system created for the culture and identification of Mycobacterial strains through DNA analysis which has been in use since 1998.In a study by Catanzaro and associates, the BACTEC MGIT960 was compared with the three rapid diagnostic kits for performance and accuracy. The study included the Line Probe Assay (LPA) and Pyrosequencing (PSQ), which both produced results in an average of 1.1 days, and Microscopic Observation of Drug Susceptibility (MODS), which produced results in an average of 14.3 days. In contrast, the BACTEC MGIT960 took an average of 24.7 days to produce results. All three rapid test kits had very high specificity for detection of the most common types of drug resistance in MDR-TB and XDRTB, ranging from 97-100%. The sensitivity of the kits was somewhat lower but still significantly high: for resistance to isoniazid, rifampicin, moxifloxacin, and ofloxacin, sensitivity was found to be 94-100%, for amikacin and capreomycin, it was 84-90%, and for kanamycin, it was 48-62%. This means that all three rapid test kits were able to identify bacterial strains with 6 out of the 7 most common types of drug resistance in almost 100% of patients, in a fraction of the time necessary for other methods[72]. Due to a lack of data on the effectiveness and accuracy of these rapid test kits, only one of them (a version of the Line Probe Assay called MTBDRplus) is currently WHO-approved and has been in use since 2008. Increasing the financial and human resources necessary to obtain and analyze data on the use of this technology could have a hugely positive impact on the timely diagnosis of MDR-TB and XDR-TB[72]. Another problematic aspect of diagnosis in the field is the

storage and transport of samples to testinglocations. A recent study evaluated the safety and efficiency of four different storage techniques inlocations where samples have to be transported (or even mailed) to labs where they can be analyzed. The labs involved were in Madagascar, India, Argentina, and Brazil, all relatively high-burden countries with challenging environments. The four storage methods were traditional Ziehl-Neelsen (ZN) slides, storage of samples in ethanol, and two commercially-produced card systems called the FTA card and the GenoCard. All four methods were tested with susceptible *M. tuberculosis* strains and MDR-TB strains, as well as with non-tubercular Mycobacterial strains for reference. The four methods all showed excellent results; 100% specificity and 100% sensitivity at 3 out of the 4 participating labs, and 88-100% specificity and 80-84% sensitivity at the fourth lab. Out of 200 samples tested with each method i.e. 100 susceptible TB and 100 MDRTB, no less than 194 samples were correctly identified by any method. The FTA card resulted in 200 correctly identified samples, ethanol storage resulted in 197 correct identifications, the ZN slides produced 195 correct results, and the GenoCard resulted in 194 samples being correctly identified. These results are hugely positive for areas where one or more storage method may be impractical or unavailable. However, this study, which tested the efficacy of these methods using isolated Mycobacterial strains, was only the beginning; more testing will have to be performed using sputum samples before these methods can become accepted protocols[73]. Another important strategy to control TB infection is an investment in the structure and design of hospitals and community healthcare centers in areas of endemic XDR-TB. In many underdeveloped areas, hospitals are crowded, sanitation is sub par, and the structure of buildings may prevent the necessary level of isolation of contagious patients. This is especially imperative in areas with a high prevalence of HIV and AIDS, as these patients

are particularly vulnerable to the pathogenesis of TB. Nosocomial spread of extremely virulent XDR-TB can be devastating, as seen in Tugela Ferry, South Africa in 2005 and 2006[74].

Community and patient awareness of TB and the implications of antibiotic resistance are also important for controlling this disease. Globally, one of the most active XDR-TB endemic areas is found in the underdeveloped regions of Eastern Europe[75]. A recent survey of the relevant knowledge of school and other institutional staff in the Republic of Georgia revealed a large amount of misinformation about antibiotics. 55% of respondents believed that antibiotics were effective against both bacterial and viral infections, and 55% believed that antibiotics would improve the common cold[76]. This is concerning since antibiotics are not regulated by prescription in Georgia; instead, they are available over the counter for anyone to purchase at any time. 91% of survey respondents had used antibiotics, but their other responses show that this use is likely inappropriate in around 55% of cases. Such rampantly inappropriate use of antibiotics has certainly contributed to the development and prevalence of XDR-TB in this region.This illustrates howimportant it is for TB control programs to increase awareness of these issues, especially in MDR-TB and XDR-TB endemic areas[76]. In order to effectively implement these principles, investments are necessary for equipment, staff, community outreach, and transport and delivery of supplies. Yet anti-TB efforts continue to be underfunded in comparison to less prevalent diseases such as HIV. In 2014, TB caused 1.5 million deaths, whereas HIV caused around 1.2 million. This difference in mortality rates is not especially large, but according to the WHO, in 2014 only 6.6 billion dollars were invested in the fight against TB, whereas investment in HIV programs worldwide was nearly 20.2 billion dollars. This huge disparity in funding prevents effective implementation of programs that have been proven to be successful

for TB control. Clearly, the lack of funding is directly related to the continued prevalence of TB[42].

2.11 TB Control in Prison

Prisons are indeed ideal environments for Tuberculosis, Mycobacterial infections and other infections control. In planning and implementing TB control, prison health services therefore, must take advantage of the special features of the prison environment. Having prisoners all in one place facilitated identification of prisoners with Mycobacterial infections, promotion of adherence treatment, and accurate recording and reporting. Some inmates had little access to health care in the community. For these people, a prison with effective health care services could provide an opportunity for access to health care, including TB. Hence, the opportunity for effective TB control in prisons is an opportunity to contribute to effective TB control in the general population[77].

2.12 Case Finding and Screening in Prisons

Case detection and treatment success as a core element of TB control, must be conducted promptly, effectively, and systematically, the failure of which would bring the reversal of a growing TB incidence and to the reduction of TB prevalence and mortality. As a result, DOT, the Stop TB Strategy, and other earlier formed programme like the Global Plan to Stop TB are all geared towards Passive Case finding and Active Case finding[78] . DOT, traditionally relies on the passive case finding for case detection. Passive case finding examines TB suspects (those with cough for two or more weeks) among persons who spontaneously visit health centers seeking relief for respiratory symptoms. Passive case finding assumes the complete access to TB diagnostic services, otherwise it may result in delayed case finding because of patient's health seeking behaviors, like failing to recognize the symptoms of TB, using traditional and non-TB

medicine to merely relieve symptoms and because of providers failure to recognize TB. In some prisons, passive case finding is further compounded by corrupt practices that may limit a prisoners' ability to seek care. Furthermore, prisoners in most countries tend to be heavy smokers, so that using ordinary "coughing" to judge may be confusing[78]. In populations at increased risk of developing TB (people living with HIV, other immune-suppressed individuals, and mostly prisoners), case finding has to be conducted actively to avoid gaps and delays in diagnosis and commencement of treatment. Active case finding involves screening of prisoners at different points during incarceration and using other methods, which includes symptom-based screening, chest radiography, tuberculin skin testing (TST), immunoglobulin gamma interferon assay (IGRA), or combination of this methods[78]. In prisons, passive and active case finding should be implemented simultaneously and systematically. When combined will increase case detection greatly. Some advantages of passive case finding include; Identifies cases missed through other case finding measures (entry-screening, contact investigation, point mass screening, or surveys). Identifjes incidence cases, those who develop TB after entry. It is relatively less expensive and simpler for programs to implement. The disadvantages of passive case finding are follows: Relies on patients' readiness to attend medical services for evaluation. It may result in delayed case finding and treatment initiation, with prolonged transmission to others. It may result in advanced disease that can be more difficult to treat. It may be biased by internal regulating mechanisms among prisoners, leading to denial of medicals to the strong prisoners[78]. The advantages of active case finding includes: It increase case notification, linking the prison health system to the national program and feeds data into the system. It reduces delays more quickly and, consequence reduction in transmission through the immediate removal of infectious cases by separating them from the general prison population, giving correct treatment. It is likely to

give higher prevalence rate than the one outside in the general population, which serves as useful tool for advocacy in obtaining funds to tackle challenges encountered in prison. It avoid bias in triage by internal prisoner procedure. The disadvantages of active case finding are; It increases duties and workload of health staff in prison, which are not sufficiently motivated. It constitutes a burden on the penal and public health care system, which needs to support active case finding activities; high cost deems these activities unsustainable. It increases the work load of health centers and hospital laboratories. It diverts funds from DOTS activities. It leads to potential over-diagnosis of TB, for any diagnosis that is based on radiography only[78].

2.13 TB Co-Infections among Prison Inmates

Tuberculosis and HIV remain serious challenges for public health programmes globally, owing to the sustained high rates of morbidity and mortality associated with these diseases. WHO has advocated for greater control efforts, including ambitious targets for both HIV and tuberculosis that aim to reach, test, and treat high-risk populations, particularly those that are incarcerated[79].

Despite the importance of targeting high-risk populations, prisoners are not sufficiently prioritised in major policy documents, nor are prison activities integrated into national programmes. Additionally, international reporting of tuberculosis and HIV cases among prisoners remains sporadic[80]. In *The Lancet Public Health*, Olivia Cords and colleagues did an updated systematic review and meta-analysis of studies of tuberculosis among incarcerated individuals, assessing three outcomes: incidence of tuberculosis, prevalence of tuberculosis, and incidence of *Mycobacterium tuberculosis* infection. This study provides the largest compilation of data on tuberculosis among prisoners so far, and includes the period in which rapid nucleic acid amplification tests were introduced (2010 onwards). The study shows that incidence rates of tuberculosis are consistently much higher in prisons than in the general population, with

incidence rate ratios ranging from around four in North America to around 27 in South America, and averaging higher than ten globally. The prevalence of tuberculosis per 100 000 prisoners was greater than 1000 in all regions except for North America. Among only a few available studies on *M tuberculosis* infection, Cords and colleagues found extremely high annual risks of *M tuberculosis* infection among prisoners[81]. Among studies done in Iran, Colombia, Nigeria, and Brazil, the annual risk of *M tuberculosis* infection in incarcerated populations was greater than 15%. In four studies from Brazil, the annual risk was greater than 25%, among the highest rates of *M tuberculosis* infection recorded in any population[81].These results show that incarcerated populations have some of the highest tuberculosis incidence rates in the world and should therefore be targeted for tuberculosis control efforts. The revolving door effect, a term used to describe the experiences of individuals who cycle in and out of correctional facilities and the community, might increase tuberculosis transmission particularly if tuberculosis therapy is interrupted and individuals return to conditions of poverty and deprivation, which propagate tuberculosis[81]. Routine screening for tuberculosis at entry into facilities and periodically, isolation of presumptive and confirmed cases, infection prevention and control measures, and appropriatediagnosis and treatment for tuberculosis are a few interventions that should be prioritised. Routine screening will still require that optimised algorithms are implemented that combine sensitive screening approaches with rapid nucleic acid amplification tests to make active case finding more effective[82].Efforts to control *M tuberculosis* infection require particular attention; these measures are more difficult to implement as they require infrastructural changes or policy reforms to reduce overcrowding and to ensure that facilities are built with sufficient ventilation.Cords and colleagues, also reported that, in incarcerated individuals living with HIV, the odds of tuberculosis were more than three times higher than in those living withoutHIV (odds

ratio 3·6; 95% CI 2·0–5·9)[83]. This finding highlights the importance of HIV prevention and treatment interventions in incarcerated populations, as these populations have a higher prevalence of HIV than the general population.[81]. With greater prominence given to implementing tuberculosis preventive therapy among high-risk populations, including the introduction of shorter and more tolerable regimens, tuberculosis preventive therapy should be prioritised in all incarcerated individuals with HIV infection and in other high-risk groups within incarcerated populations. Further research is required to explore how to effectively implement interventions for tuberculosis and HIV treatment in incarcerated populations. Furthermore, logistics within prisons and the impact of stigma on incarcerated individuals, and how these factors influence the uptake, acceptability, and feasibility of interventions should be considered[84]. Strategies to promote continued engagement in care for incarcerated individuals following their release from correctional facilities are required to ensure sustainability of efforts.[85]The ongoing COVID-19 pandemic and subsequent development of effective vaccines have demonstrated what can be done when there is a collective political will and resources are made available. These aspects still remain key barriers for tuberculosis and HIV control in correctional highest level for tangible progress to be made towards elimination targets[86]. The burden of tuberculosis in incarcerated populations also raises substantial concerns about the spread and control of other infectious diseases—particularly SARS-CoV-2—in such settings. Although incarceration restricts personal liberties, this should not be done at the expense of reducing the minimum standard of health care, which should remain at least equivalent to that in the general population[87]. Tuberculosis control among incarcerated populations has inherent challenges, but the controlled and confined environment also provides an opportunity to implement effective strategies[88]. We therefore call for incarcerated populations to be included in

all national efforts and advocate for better synergies with existing and broader infectious disease control programmes to meet the specific needs of prisoners. Tuberculosis elimination can only be achieved if all populations and chains of transmission are sufficiently addressed; incarcerated populations should not become the weakest link that undermines the progress being made elsewhere[88]. Taking a peep on a study published in PLOS ONE journal by Nilaramba Adhikari in 2022, titled "prevalence and associated risk factors for tuberculosis among people living with HIV in Nepal" National Tuberculosis Control Center carried out thisstudy in anti-retroviral therapy (ART) sites to estimate the prevalence of TB and identify theassociated risk factors for TB among the people living with Human Immunodeficiency Virus(PLHIVs) in Nepal.The author carried out a cross-sectional institution-based study. He explained that, achieving the global tuberculosis control, early and accurate diagnosis of drug sensitive anddrug resistant TB is crucial. However, underdiagnosis still remains as a pertaining obstacle especially in the countries where patient face substantial geographical and socio-economic challenges while accessing health care. Similarly, the scenario of many countries depicts that case detection relies on patients reporting symptoms to health care facility. In such conditionof delays in case identification and management, there remains a higher probability of transmissionand continuation of epidemic[89]. Transmission of tuberculosis depends on numerous factors, which includes the index case, the susceptibility of the exposed host, behavior of bio-aerosols, pathogen-inherent factors, and environment in which transmission occurs. Therefore, elimination of transmission relies on the ability to identify and treat the infected individuals at risk of becoming infectious, utilizingprognostic and diagnostic biomarkers to target preventive and curative therapies. Moreover, tuberculosis and HIV co-infection has fuelled the TB epidemic, presenting programmatic and treatment challenges across the world[90]. Co-morbidity of Human

Immunodeficiency Virus (HIV) and tuberculosis, in the individual host, potentiate pathogens of one another, which eventually deteriorate the immunological functions. HIV co-infection is the prime risk factor for developing active TB in the high-burden settings like prison. This in turn increases the susceptibility to primary infection or re-infection and the risk of TB reactivation for patients with latent TB. Similarly, infection of *Mycobacterium tuberculosis* also has a negative effect on the immune response to HIV, accelerating the progression from HIV infections to AIDS[91]. Globally HIV infection attributed 0.76 million incident TB cases along with an estimated 208,000 TB deaths among HIV positive people in 2019. Furthermore, the risk of developing TB among people living with HIV (PLHIVs) was 18 (range, 15–21) times higher than the general population[89]. Besides, estimation shows that one in every four deaths among PLHIVs was attributed to TB[92]. World Health Organization (WHO) estimates around 68,000 incident TB cases in Nepal. However, only around 27,232 new and relapse TB cases were reported in the national health management information system (HMIS) in 2019/20, resulting around 40,768 incident TB cases missing every year in Nepal[93]. Missing TB cases has posed significantchallenges for the prevention, and control of TB in Nepal. Such undiagnosed TB cases has intensified transmission of TB in communities, putting vulnerable groups especially PLHIVs at higher risk of TB[94]. In 2019, the estimated number of PLHIVs in Nepal was 29,503 resultingadult (15–49 years) HIV prevalence of 0.13%. HIV epidemic in Nepal is concentratedlargely among key population, notably among client of sex workers (9%), men who have sexwith men and transgender (9%), male sex workers (3%), and people who inject drugs (3%). Onthe other hand, routine program data as of July 2020 shows only 19,410 PLHIVs on were antiretroviraltherapy (ART) along with 12% lost to follow up[95]. It reflects the urgent need tointensify the diagnosis of TB and HIV in health care settings to address the burden of HIV

andTB co-morbidity in Nepal.The study concluded thatPrevalence of TB among PLHIVs in Nepal was found 9.9%. Risk of developing TB was higheramong PLHIVs who were male, Dalit, with HIV stage progressed to WHO stage 3 and 4 andwith family history of TB. Hence, targeted interventions are needed to prevent the risk ofdeveloping TB among PLHIVs. Similarly, integrated, and comprehensive TB and HIV diagnosisand treatment services are needed for the management of TB/HIV co-infection in Nepal.

3.14 A Systematic Review and Meta-Analysis

In another comprehensive study on prevalent and risk factors of pulmonary Tuberculosis Habtamus Belew Mera and co-author used searched from 2006-2020 for articles published in adata based and electronic engine strategy by reporting with PRISMA (Preferred reporting Items for Systematic Reviews and Meta-Analysis).They extensively extracted a total of 3,479 articles from the database. In 2017, more than 10 million new cases of tuberculosis were reported worldwide, with one-third ofthese cases going unreported due to gaps in the healthcaresystem[97-99]. Although the World Health Organization(WHO) End Tuberculosis Global Strategy sets patronizingtargets for 2020–2035, it aims to detect an estimated 90%of TB cases and reduce TB deaths by 95% in 2035 as comparedto 2015, particularly among TB key populations whoare most at risk of TB infection but have limited access toquality TB healthcare service[100–103]. To that end, WHO has prioritized the most vulnerable TB patients, includingthe poor, refugees, HIV-positive people, and prisoners,who are TB key populations[104]. Prison inmates arethought to be reservoirs for MTB transmission within theirwalls and in the community as a whole, but they are ignoredfor a variety of known reasons, including a lack of quality TBdiagnostic services, overcrowding or stressful surroundings,a lack of perioding active case screening, comorbid illness,poor nutrition, and poor wall ventilation, particularly inSSA prisons[105–107]. Facts suggest

that the risk of developingtuberculosis in prison is 6–30 times higher than in thegeneral population, but 200 times higher in SSA, particularlyin overcrowded prisons[103,105]. In 2016, for example, areview of 24 SSA countries' prisons revealed TB prevalenceranging from 0.4 to 16.3%[108]. Another study publishedin the same year found that regional variation in TB prevalencewas 5.3% in Southern and East Africa and 2.9% inCentral and West Africa[108]. In SSA prisons, overcrowdingand poor ventilation are severe, with data showing that 86%of countries with data had prison occupancy rates above100%, increasing the risk of airborne TB infection[109]. Despite its burden, there is no aggregated data on pulmonaryTB prevalence and its predictors in SSA prison inmates. These studies were published between 2006 and 2019, and the current study included 11 country prisoninmates. The majority of the studies, 14/37 (37.8%), were conducted in various regions of Ethiopia, four in Nigeria, four in South Africa ,two in Malawi, two in Uganda, three in Zambia, two in Cameroon, one in Côte d'Ivoire, two in Ghana , one in Tanzania, and two in Democratic Republic Congo (DRC)[112-137]. Whereas WHO has classified five of them among the thirty high burden country lists for TB, TB/HIV, and MDR-TB[98].The highest prevalence of pulmonary TB among prison inmates (23.08%)was reported in Nigeria from Aba Federal prison[127]. while the lowest prevalence (1.42%) of pulmonary TB wasreported among eighteen prisons in Malawi[134]. In this meta-analysis, 72,844 prison inmates were included in SSA prisons to evaluate the pooled prevalence of pulmonary TB and its predictors. Concerning the study design, almost all 89.2% (33/37) of the studies were cross-sectional study. Study-specific sample size ranged from 52 in Nigeria[127].to 31,547 in South Africa[133]. All the original studies used sputum as a specimen for diagnosis of TB, and different diagnostic techniques were carried out to confirm pulmonary TB in the prisoners such as AFB or direct light microscopy, fluorescent microscopy (FM), culture, and GeneXpert. The quality score of all the 37 studies

rangedsix to nine.Habtamus B.M., discussed that Tuberculosis remains a major public health threat worldwide, particularly in the SSA region[138-142]. The current meta-analysis found a high prevalence of pulmonary TB among SSA prison inmates. TB/HIV co-infection, BMI, long durationof incarceration, and previous TB exposure were predictorsof pulmonary TB infection among prison inmates.The current meta-analysis estimated that the overall pooledprevalence of TB among prison inmates using the 37 studieswas 7.74%. This prevalence is similar with the studyconducted in Malaysia 7.7%[99]. However, the findingof this meta-analysis was higher than studies in SouthThailand (2.1%), Peru (2.51%), Asian countries(4.5%), Brazil, and SSA (2%-3.6%) as reported in a previous systematic review conducted romprison inmates[150-153]. Moreover, the finding of our study was lower than a study conducted in Brazil diagnosed with smear, GeneXpert, and culture 12%-12.9%, Nepal. Sputum smear and GeneXpert test 10%, and SSA10%-13.7% as conducted in previous reports among prison inmates[154,155,120,137]. The possible explanations for theobserved variations might be attributable to the difference in the geographical variation, overcrowding, method of diagnosis, and number of prisoners in a cell with poor ventilation.In this systematic review and meta-analysis, thepooled estimated incidence of pulmonary TB among prisonerswas 10,700/100,000, which is higher than WHO targetfor end TB strategy incidence in 2019, which is approximately130/100, 000[98]. Despite this, high incidenceof TB in prisons could be possibly the fact that they are aforgotten population in the case of early screening, diagnosis, and treatment of TB in a high-risk population like prison inmates.

2.15 Prison Judiciary Aspect

"Prisoners are sent to prison *AS* punishment, and not *FOR* punishment". This often repeated statement by British prison commissioner Paterson implies that the loss of an individual's right

to liberty is enforced by containment in a closed environment. This keeping of the individual in the custody of the State, should not, however, have deleterious effects on the health of those persons. This is unfortunately precisely the case – to some degree or another – in many of the world's prisons. Is it possible then to define a "healthy environment" in a prison, let alone talk about prisoners' rights regarding any health services that are to be provided to them by the detaining authorities? The answer to this question is that prisoners have unalienable rights conferred upon them by international treaties and covenants, have a right to health care, and most certainly have a right ***not*** to contract disease in prison. How these rights apply to the often harmful prison environment and to HIV infection is the subject of this chapter.

2.15.1 Prisons can be Bad for Public Health

Public health policies are meant to ensure the best possible conditions for all members of society, so that everyone can be healthy. Prisoners are often forgotten in this equation. Prisoners enter and leave prisons. They are released if found innocent. They come and go from prison during the investigation and for trial. Furthermore they are often transferred, for a variety of reasons, from one prison to another. Prisoners are in contact with many different people who go in and out of the prison every day[143-147]. Prison guards, prison staff, medical personnel, delivery persons, and repairmen, not to mention family visitors and lawyers, come and go every day. Prisoners eventually are released from prison when they have served their time, or occasionally when there is an amnesty. This turnover and constant movement in and out of prison makes it all the more important to control any contagious disease within the prison so that it does not spread into the outside community.

Prisoner turnover is variable from country to country. Often the annual turnover of the prisoner population is 4–6 times the actual number of inmates being held at any given time. For a country

like the Russian Federation, with a prisoner population numbering about just under one million at the present time, the turnover is closer to some 300,000 per year as many prisoners tend to "overstay", particularly in pre-trial prisons. For all these reasons, it is not possible to tackle public health issues, such as tuberculosis or HIV, if the prison populations are not taken into account.

2.15.2Human Rights and Prisoners'Instruments and Mechanisms

All human beings, and this obviously includes prisoners, have certain unalienable rights, which are acknowledged by internationally recognised instruments. Since the Second World War, human rights have been quantified and set down in treaties and conventions. In 1948, the United Nations General Assembly adopted the Universal Declaration of Human Rights. Later, two covenants were adopted, the International Covenant on Civil and Political Rights (ICCPR), and the International Covenant on Economic, Social and Cultural Rights (ICESCR). These state thatprisoners have rights, even when they are deprived of liberty in custody. The ICCPR specifically provides that "all persons deprived of their liberty should be treated with humanity and with respect for the inherent dignity of the human person". In 1955, the United Nations, in its Standard Minimum Rules for the Protection of Prisoners (SMR) set down standards that include principles for providing health care in custody. The 94 rules in the SMR setting down the minimum requirements for prisoners were approved by the United Nations Economic and Social Council, which in 1977 extended their applicability to persons detained without charge, i.e. in places other than prisons. These standard minimum rules for the protection of people in custody have been supplemented over the years by additional instruments. In 1984, the United Nations adopted the Convention against Torture and other Cruel, Inhuman or Degrading Treatment or Punishment. In 1985, the United Nations Standard Minimum Rules for the Administration of

Juvenile Justice, called the "Beijing Rules", were adopted for the protection of young offenders. In 1988 and 1990, respectively, the United Nations adopted the Body of Principles for the Protection of All Persons under any form of Detention or Imprisonment and the Basic Principles for the Treatment of Prisoners. At a regional level, the Council of Europe developed its European Prison Rules, in 1987. Human Rights treaties make states accountable for the way they act, or fail to act. UN bodies, and regional, national and non-governmental agencies are in charge of monitoring human rights. Prisoners of war are protected by international humanitarian law as set down in the Third Geneva Convention of 1949. Respect for even basic human rights has traditionally been a problem in prisons. In Europe particularly, there have been major attempts to protect prisoners from violations of their basic rights, as evidenced for example by the European Convention against Torture. The Council of Europe has created a specific body, the Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment, known as the CPT, to monitor ill treatment and the conditions of prisoners, including health issues. Many other nongovernmental organisations also monitor prisoners' conditions, in particular all aspects of health within prisons.

2.15.3 The Right to Health Care and a Healthy Environment in Prison

With specific reference to health, the right to conditions "adequate for the health and well-being" of all was already recognised in the Universal Declaration of Human Rights. The ICESCR furthermore states that prisoners have a "right to the highest attainable standard of physical and mental health". The standard minimum rules for prisoners regulate the provision of health care for them. These rules, as well as other instruments regulating the rights and regulations for the treatment of prisoners, have been extensively reviewed and commented on in a comprehensive text by Penal Reform International. The CPT issued standards for health services in prisons

(published in their annual report for 1992). Most recently, in 1998, the Committee of Ministers of the Council of Europe promulgated new recommendations on health care in prisons. Apart from the civil and political rights, the so-called "second generation" economic and social human rights, as set down in the ICESCR, also apply to prisoners. The right to the highest attainable standard of health should also apply to prison health conditions and health care. This right to health care and a healthy environment is clearly linked, particularly in the case of HIV, to other "first generation" rights, such as non-discrimination, privacy and confidentiality.

2.15.4 Health Care in Prison: Equivalence Versus Equity

Prisoners cannot fend for themselves in their situation of detention, and it is the responsibility of the State to provide for health services and a healthy environment. Human rights instruments call for prisoners to receive health care at least equivalent to that available for the outside population. On the one hand, "equivalence" rather than "equity" has been called for because a prison is a closed institution with a custodial role that does not always allow for the same provision of care available outside. On the other hand, because prisoners are more likely to already be in a bad state of health when they enter prison, and the unfavourable conditions therein make the health situation even worse, the need for health care and treatments will often be greater in a prison than in an outside community. However, providing even basic health care to prisoners has proved extremely difficult in countries where the overall health systems have collapsed or are chronically insufficient. As regards the specific issue of HIV, there are various areas concerned by this provision. The authorities have a duty to both preserve the health of individual prisoners and to promote the public health of the prison – and outside – population. The above-mentioned treaties and conventions state that prison authorities have a duty to provide:

- safe and healthy living quarters for all prisoners;

- protection of individuals from violence and coercion;
- provision of adequate health care services and medicines, as far as possible free of charge;
- information and education about preventive health measures and healthy lifestyles;
- implementation of elementary preventive health measures;
- means for detecting sexually transmitted infections and for treating them, so as to reduce risk of HIV transmission;
- continuation of medical treatments begun outside (includingthose for drug users) or the possibility of commencing them inside;
- provision of specific protection for vulnerable prisoners, such as those who are HIV-positive, from violence from other prisoners, or from those with infectious diseases which could be extremely dangerous for them, such as tuberculosis;
- Where voluntary testing for HIV is available, it should always be provided together with adequate counselling, before and after testing.In prisons, the human environment is often one of violence and high-risk lifestyles, either engaged in voluntarily by those prisoners with positions of power, or forced upon the weaker prisoners. Prisoners have a right to live in conditions where their individual safety is guaranteed. It is paramount for the prison administration to have a thorough knowledge of how HIV is likely to be transmitted in a given prison. If sexual coercion and/or violence are the main issue, better surveillance and active interventions to protect targeted prisoners must be enforced. If drug injection and sharing of injection equipment is the main problem, active education may not be sufficient. It may be necessary to take measures to stop coercion by drug ringleaders, who may seek to force other prisoners to buy and inject drugs, and make available drug treatment programs and harm reduction measures for drug-addicted prisoners. HIV-positive inmates should not be denied access to recreation, education or normal

access to the outside. From a strictly medical point of view, there is no justification for segregation as long as the prisoner is healthy. Solitary confinement of HIV-positive inmates should be forbidden. Any restrictions should be exceptional, such as mandatory testing for particularly risky situations, such as prisoners working as medical orderlies in hospitals or dental clinics. Prisoners working in other places less obviously posing a risk, such as laundries, kitchens or as cleaners, may also be exposed to injuries and therefore HIV infection (see chapter 10). The protection of HIV-positive prisoners from other prisoners with contagious diseases such as tuberculosis is discussed in Chapter 7. There may also be considerations of personal security where, for example, prisoners known to be HIV-positive request to be kept in a secure unit as they fear for their own safety.Both prison reform and penal reform are crucial elements if the many problems affecting the prisons of Eastern Europe and the countries of the former Soviet Union are to be resolved. Diminishing the overall prison population will allow improvements of the physical and working conditions of the prisons, and help to ensure the security of all individuals in custody. Obviously, financial resources will have to be allotted to the prison systems as well. One effective way to curb the rise in prison populations would be to offer alternatives to imprisonment for non-violent offenders.

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Chapter Three

Methodology

3.1 Study Site

This study was carried out on prison inmates of Agodi Cusodial Center and sample processed at South West Zonal TB Reference Laboratory unit of Medical Microbiology & Parasitology Department, University College Hospital (UCH) Ibadan. It is a reference laboratory which receives samples from all the six states in south-west geopolitical zone of Nigeria. Consecutive collection of two hundred (200) samples from Agodi Custodial Centre between January and June, 2022 (six months) were used for this cross-sectional study.

3.2 Study Materials

Tube racks, Respirator (N-95), Laboratory coats, Sterile gloves, Sterile pipettes,Sterile universal containers, Permanent marker,Discard jars, Bench pads, cotton wool and absorbent gauze, Lowensten Jensen medium (see appendix I),Pyruvic Acid medium(see appendix II) Dextran (a mycobacteriocidal disinfectant), Phosphate buffer (see appendix IX), Sterile disposable loops, Timer, funnel, Incubator, Biological safety cabinet.

3.3 Sample Analysis

All the procedures were carried out in a BSL2 plus laboratory with appropriate safety precautions. Protective Personal Equipment was worn at all times in containment area.

3.4 Microscopy

3.4.1 Direct Smear Preparation

Sputum samples were arranged as documented on the worksheet. The frosted side of the slides were labeled with pencil according to the assigned serial number of each specimen.Applicator sticks were used to pick the purulent or bloody portion of the sputum and a concentric circular movement was made on the slide to obtain a thin smear of 2 x 3cm size.The smears were allowed to dry facing up for one hour. The thoroughly dried smears were fixed by passing it through a blue flame three times within 3 seconds[1].

The slides were arranged with boththe positive and negative controls on a staining rack, spacing them one finger apart.They were flooded with strong carbol fuchsin,and heated to steam by passing spirit lamp flame under the slides and left for 5 minutes,

Running tap water was flushed on the slides to remove the excess stain.Excess water was drained by tilting each slide individually. 3%Acid Alcohol was poured on the slides to decolorize the smear until there was no more red color or faint red color and it was left for 3minutes.

The slides were washed for 1minutes with running water and excess water drained.They were counterstained with 0.3%Methylene blue for 1minute.They were rinsed with water and allowed to dry.Immersion oil was applied on the slide and it was viewed with 100 objective for Acid Fast Bacilli.

3.5 GeneX/Pert/MTb-Rif Assay

3.5.1 Start-up of Gene Xpert Instrument

The Gene Xpert Dx instrument and the computer were turnedon.On the windows desktop, the GeneXpert Ds shortcuticon was double clicked.The instrument software was logged on with username and password createdCheck Status botton was clicked to check for the availability of the module.

3.5.2 Sample Preparation

The screw capped tubes were labeled with the laboratory serial number, 2volumes of sample reagent was added to 1volume of sample in a screw capped tube and the lid closed. The above tubes were vortex for 10seconds and allowed to stand for 5minutes at room temperature.The sample and reagent tubes were vortex again for another 10seconds and left at room temperature, incubation continue for10minutes to allow complete digestion.With the aid of sterile transfer pipettes, 2mls of the well digested mixture of sample and reagent were taken.The Xpert MTB/RIF cartridges were opened and the 2mls mixture was dispensed into its port.The lids were snapped firmly into place.On the instrument system screen, create test botton was clicked.The cartridge barcode was scanned.Using the barcode information,the software automatically fills the boxes for the following fields: Select Assay,Reagent Lot ID,Cartridge SN and Expiration Date. In the Sample ID box,the sample ID was correctly and carefully imputed (which is associated with the test results and would show in the View Result window and all the reports).Start Test botton was then clicked.The instrument module door with the blinking green light was opened and the cartridge loaded.The door of the module was closed for the assay to start and the green light stop blinking.The counting down of 1hour 26minutes began,after which the light turned

off.The module door then opened for the cartridge to be discarded.The Viewed Results on the menu bar window was clicked to show the results.Report"MTB NOT DETECTED" or "MTB DETECTED".For Rifampicin resistance results,it was reported "Rif RESISTANCE NOT DETECED or RIF RESISTANCE DETECED.INVALID,ERROR,INCOMPLETE and INDETERMINATE results could as well be reported[1].

3.6 Decontamination with N-acetyl l-cysteine- Sodium Hydroxide (NALC/NaOH+NaCitrate) Method

Tools, reagents and specimens were arranged in a BSC class 2 and the working area was covered with paper towels and sprayed with 5% phenol.5ml of sputum was transferred into a 50 ml sterile screw-capped conical centrifuge tube. An equal volume of NALC-NaOH/NaCitrate solution was added to specimen. The cap was replaced, tightened and vortex for 20 seconds. The tube was inverted 5 times to ensure that NALC-NaOH solution comes in contacts with the entire surface of the tube. The tubes were allowed to stand at room temperature for 15 minutes for decontamination. Sterile 0.067M phosphate buffer (pH 6.8) was added to 40ml mark of the digested-decontaminated specimen to reduce the continued action of NaOH and lower the viscosity of the mixture. The tube was recapped tightly and inverted several times to mix the contents. It was then centrifuged at 4^0C and 3,000 xg for 15 minutes by using aerosol free centrifuge safety cups. After centrifugation, the supernatant was poured off into a splash proof discard container with 5% phenol inside. The sediment was re-suspended in 2ml of phosphate buffered saline (PBS) and vortex to mix[1].

3.6.1 Inoculation

LJ and Pyruvic acid medium(PRVT) slopes were brought to room temperature and appropriately labeled.The excess condensate carefully removed by inversion.With the aid of sterile plastic disposable pipette,4drops(0.2mls)ofNaOH/NALC treated sputum were inoculated into LJ and PRVT slopes, allowing the drops to run from the mouth of the tube to the bottom.The inoculated slopes were loosely capped and incubated in a slant position.After one week,the caps were tightened and the slopes straightened to continue incubation for 8 weeks.Every week, the cultures were checked for the presence of growth.(See Appendix xii).

3.7 Identification

The noticeable colonies which may be raised,dry,cream(buffy) termed eugenic growth for Mycobacterium tuberculosis,but flat, and yellowish in case of Non Tuberculous Mycobacteria were smeared on a grease free slide and stained using hot ZN to identify AFB. Slopes with colonies positive for AFB were further tested for**:**

3.7.1 Rate of Growth

Which had been observed right from the beginning of cultural incubation (fast grower).The slow growers colonies came up at about week four (4).

3.7.2 Temperature at which Growth Occurs

The two distinct types colonies were sub-cultured in fresh LJ medium and incubated at differenttemperatures **o**f 25, 32 & 36 degree Celcius.See table in the next chapter for result.

3.7.3 Pigment Production

The positive cultures were deliberately left in the light for 2hours,after which they were re-incubated at 37 degree Celcius overnight.The colonies were examined for the development of a yellow pigment.

3.7.4 Niacin Accumulation Test

This test was performed by adding 1ml of sterile distilled water to the slant of a mature BA, Ogawa and LJ culture media. The culture was placed in a slanting position so that the fluid covers the mycobacterial growth. 0.5 ml of the fliud was removed and added to a clean, screw-capped test tube. 0.5 ml each of 4% alcoholic aniline and 10% aqueous cynogen bromide solutions. It was mixed gently. Yellow colour indicates presence of niacin. Positive and negative controls were run along with the test. The results were read and recorded[2].

3.7.5 68^0C Catalase Test

Several loopful of growth was suspended into the screw cap tube containing 0.5ml of buffer solution (pH 7.0).The suspension was incubated in a 68^0C water bath (Gallicamp,model 112) for 20 minutes and then cooled to room temperature. 0.5ml of the freshly-prepared Tween-peroxide mixture was added and the tube recapped loosely.The tube was observed for the formation of bubbles appearing on the surface of the liquid. Negative tube was held for 20 minutes before discarding. Negative and positive controls were run along with the test. The results were read and recorded[3].

3.7.6 Growth on PNB Medium

One loopful of growth was picked with 3mm diameter-loop and mixed with 5ml of sterile distilled water (4mg/ml-suspension). Then, 0.1ml of the suspension was inoculated onto two slants of PNB containing and PNB free media. The slants were incubated at 37^0C for 4 weeks. The cultures were observed for growth at 4^{th} day and 4^{th} week of incubation. Negative and positive controls were run along with the test. The results were read and recorded[3].

3.7.7 Arysulphatase Test

Arysulphatase test was performed as described by Arora and Arora (2008). The organisms were grown in a medium containing 0.001 M tripotassium phenolphthalein disulphate. Arysulphatase production was detected by adding 2N NaOH dropwise to the culture. A pink colour indicates a positive reaction. Negative and positive controls were run along with the test. The results were read and recorded.

3.7.8 Nitrate Reduction Test

The test organism was suspended in a buffer solution containing nitrate and incubated at 37^0C for 2 hours. Then sulphanilamide and n-naphthylethylenediamine dihydrochloride solution was added. Positive reaction was indicated by development of pink or red colour within 30- 60 seconds[2].

3.8 SD Bioline Test; from Solid Culture

Using a sterile wire loop,pick a loopful of colony of the AFB positive culture andemulsified into 100 micro liter extraction buffer.The tubes were tightly closed and vortexfor the sample suspension to mix properly.SD Bioline Testing Kit was allowed to attain ambient room

temperature prior to testing.The foil pouch were removed and placed on a flat surface inside the BSC. They were labeled with the sample identification number.Tubes containing the sample suspension were unscrewed.With the aid of sterile pipettes,100micro liter of sample was dropped into the specimen placing area and the sample tubes were recapped.

It was timed for 15 minutes with Stop watch.Positive result showed two red bands while negative result showed a single band.

3.9 Drug Sensitivity Testing (DST)

3.9.1 Proportion Method

Antibiotic susceptibility test was performed by proportion method as described by Standard methods[3,1]. The *Mycobacterium tuberculosis* isolates were tested against Isoniazid (Fisher), rifampicin (Sigma), streptomycin (Sigma) and ethambutol (Sigma). Pure drug crystals of isoniazid, rifampicin, Kanamycin,Amikacin,Ofloxacin,Capreomycin and ethambutol were produced by Sigma scientific laboratories and Fisher Laboratory, USA.Two loopfull of the pure isolate were picked and emulsified in 2ml of sterile distilled water containing six beeds in a sterile tubes, this was tightly capped and vortex to have turbidity of 1 McFarland standard.4drops of 1 in 100 suspension each of this isolate suspension were inoculated into LJ DST slopes containing the two of the first line and five second line anti-tuberculous drugs,Three control slopes of LJ DST without anti-tuberculous drugs were inoculated with lowestsuspension(10^{-4}) of isolate into C^3 slope,1 in 1000(10^{-3}) into C^2 slope and 1 in 100(10^{-2}) into C^1slope and the drug slopes as stated earlier.The inoculated slopes were loosely capped and incubated at 37 degree Celcius in a slant position.After one week,the caps were tightened and the slopes straightened to continue incubation for 6 weeks.The slopes were read for growth at 28days and again at 42days

for sensitive strains.The growth were recorded as follows: Confluent growth: 3+ More than 100 colonies: 2+1-100 colonies:Actual number was recorded. All the results were appropriately documented and SPSS analysis was carried out.

3.10 Preparation of Drug Solutions

3.10.1 Drug Potencies

The true potency of a drug is the number of micrograms of active drug per milligram total weight of the product. Thus desired activity (mg/ml) = (weight of drug)x(potency)/(volume of solvent)[4].

3.10.2 Isoniazid

Isoniazid potency is 1g to 1g substance. 20.0mg of isoniazid was dissolved in 40.0mls of sterile distilled water. This gives 500mg/ml solution. 2.0mls of this stock solution was aseptically diluted with 50.0mls of sterile distilled water to give 20.0mg/ml stock solution. It was sterilized by membrane filteration. 10mls of stock solution were added to 100mls of LJ medium, mixed gently, dispensed in 6mls volumes and inspissated at 90^0C for 50 minutes[4].

3.10.3 Rifampicin

Rifampicin potency is generally > 980.0mg/ml. 80.0 mg/potency of rifampicin was dissolved in 5.0mls of absolute methanol. The solution was further diluted with 5.0ml of 95% ethanol to give 8000.0mg/ml stock solution (rifampicin is self sterilizing and does not require further sterilization). 5.0mls of stock solution were added to 1000mls of LJ medium, mixed gently, dispensed in 6mls volumes and inspissated at 90^0C for 50 minutes[4].

3.10.4 Streptomycin

Potency may vary from 667mg to 800mg/mg.40.0mg/potency of streptomycin sulphate was dissolved in 50.0mls of sterile distilled water to give 800.0mg/ml stock solution. 10mls of stock solution was added to 1000mls LJ medium. It was mixed gently, dispensed in 6mls volumes and inspissated at 50^0C for 50 minutes[4].

3.10.5 Ethambutol

Ethambutol potency is 1g to 1g substance. 20.0mg of ethambutol powder was dissolved in 100.0mlsof sterile distilled water to give 200.0mg/ml stock solution. 5mls of stock solution was added to 500mls of LJ medium. It was mixed gently, dispensed in 6mls volumes and inspissated at 900C for 50 minutes[4].

3.11 The Proportion Method

A representative sample of 5.0mg to 10.0mg from the subculture within 1 to 2 weeks after appearance of growth using an inoculation loop was placed into a sterile McCartney bottle(14mls screw capped bottle) containing 1.0ml distilled water and 10 glass beads. The mixture was homogenized on a vortex mixer for up to 1 minute and the opacity of the suspension was adjusted by the addition of sterile distilled water to a standard suspension containing 1mg/ml of tubercle bacilli.

Two serial dilutions were made from the suspension, 102 and 10^4 using the calibrated inoculating loop and sterile McCartney vials containing 1.0ml of distilled water. 0.1ml of 10^{-2} and 10^{-4} suspensions were inoculated onto 2 slants of drug free (control) medium. Then 10^{-2} dilution of the suspension was inoculated onto a series of drug containing media. The suspensions were spread over on the whole surface of the medium and kept at slanting position

with loosen caps. The slants were incubated at 37^0C. When the growth appears on the control media, cap was tightened and incubation continued for 4 weeks.

When enough growth, more than 100 colonies for 10^{-2} suspension and more than 50 colonies for 10^{-4} suspension, was observed on the drug free medium at 4 weeks of incubation, the growth on all media was read. Strains showing drug susceptibility at 4 weeks, further reading at 6 weeks was done before reporting susceptibility. Quality control strains-H37RV was included in each batch of testing.

3.11.1 Reading, Interpreting and Reporting

The seeded media were examined for contamination after 1 week. The first reading of drug susceptibility test result was done at 4 weeks (28 days) of incubation at 37^0C. The growth on the drug containing medium was compared with the growth on the drug free medium at 10^{-4} dilution. When the growth on the drug containing medium was none or less than that of a drug free medium at 10^{-4} dilution, it was classified as susceptible/sensitive.

The following formula was used to calculate the % of resistant:

Number of colonies on drug containing medium = % resistance

Number of colonies on the drug free medium at 10^{-4} dilution

Resistance**:** The criteria for resistanceis 1% of growth for all the drugs.

Susceptible**:** No growth or less than 1% of colonies growing compared to the controls[4-3].

Multi-drug resistance *M.tuberculosis*was defined as resistance to at least isoniazid and rifampicin[5].

Poly-drug resistance *M.tuberculosis*was defined as resistance to more than one drug but not isoniazid and rifampicin at the same time[6].

Mono-drug resistant *M.tuberculosis* was defined as resistance to only one drug[6].

3.12 Line Probe Assay (LPA) For First & Second Line Anti TB Drugs

3.12.1 DNA Extraction

Each micro centrifuge tube was labeled with appropriate specimen identification number. 300 MicroLitre of Molecular Grade Water(MGW) were added into the labeled micro centrifuge tubes above with the aid of automatic pipette.A loopfull of each isolate was picked and transferred into the MGW in the label micro centrifuge.They were closed and vortex for 30 seconds until the clump is suspended. The micro centrifuge tubes with the Mycobacteria suspension were arranged in a floater and incubated in a water bath with a temperature of 95 degree Celcius for 20 minutes.The tubes were removed and incubated for 15minutes in a Sonicator which is an altrasonic water bath (to break cell walls to release DNA material).They were centrifuged in a Micro-centrifuge at 10000 rpms (to remove cell debris).The supernatant were transferred into clean label centrifuge tubes using sterile disposable pipettes. This was stored in -20^{0}c freezer to continue amplification next day[7].

3.12.2 PCR Amplification (Master Mix Preparation, DNA Addition, Amplification in Thermocycler)

Master Mix Preparation

The PCR reaction mix was made up according to the table supplied (see Appendix xii) and procedure was carried out inside Dead Box.

Master reagent were brought out from -20^{0}c freezer to thaw and Hot Star Taq immediately before use.A 2ml micro-centrifuge label “master mix” was placed in a rack.

Since 30 samples plus 3 controls (master mix, positive and negative) with allowance of 2 more needed to be analysed, 35 was multiplied by each volume listed below.

PNM mix 35.0microliter x 35=1225 microliter

PCR buffer 5.0 micro liter x 35 =175 micro liter

Magnesiumchloride 2.0 micro liter x 35 =70 microliter

MGW 2.8 micro liter x 35 =93 micro liter

HotStarTaq(R) polymerase enzyme 0.2 micro liter x 35 =7.0 micro liter

The mixture in the tube was vortex at low setting.0.2ml of micro-centrifuge tubes with cover were arranged in a PCR rack (sequence number noted 1, 2, 3, 4……33)

45 micro liter of the master mix were pipette into each PCR tubes above, beginning from tube 1 to 33.5ml of MGW was added to the first tube (master mix).The last 2 micro-centrifuge tubes 32 and 33 were label positive and negative control.All the micro-centrifuge tubes were closed and taken to DNA addition room[7].

3.12.3 Addition of DNA to Master Mix

(This procedure was carried out inside BSC)

Master mix aliquots and DNA inside their racks were placed in the Class ii BSC.

To each remaining tube of master mix (from tube 2 onwards) was added 0.5 micro liter of the respective DNA and was mixed by pipetting up and down.5 micro liter of both positive and negative controls were added to tubes32 and 33.The tubes were closed and taken to amplification room[7].

3.12.4 Amplification in Thermocycler

The 0.2 micro-centrifuge tubes in PCR tray was placed inside the Thermocycler and the switch botton was pressed to commenced the PCR reactionwhich involved the heating at various cycles in order to **Denature, Anneal and Elongate** the DNA of the specimen.

(See Appendix xiii) At the end of the cycle, the amplified DNA was removed from the Thermocycler and was taken to the detection room for hybridization[7].

3.12.5 Hybridization

TwinCubator® was switched on to pre-warm it to 45^0C, also pre-warm HYB and STR to 37^0C inside the water bath.Tray wells were label with sample number.

20 microliter of denatured solution (blue) was dispensed in a corner of each of the wells to be used.To each well, 20microliter of amplified sample was added. The reagent and amplified sample were mixed by pipetting up and down.It was incubated at room temperature for 5 minutes. Strips were brought out with the aid of tweezers, and marked sample number with special pen underneath the colored marker.1ml of pre-warmed Hybridization buffer was carefully added to each well.Each strip was placed in a corner away from the reagents.The tray was gently shook until the solution has a homogenous color.The tray was placed in shaking Twincubator, and incubated for 30 minutes at 45minutes (step 1). Hybridization buffer was completely aspirated.1ml of Stringent wash solution was added to each well, and incubated for 15 minutes at 45^0Con shaking TwinCubator (step 2).Complete removal of stringent wash solution followed.(drained on a paper towel, by turning tray upside down.Each strip was washed once with 1ml rinse solution for 1 minutes on shaking TwinCubator (step 3). 0.9ml of diluted conjugate was added to each strip, and incubated for 30 minutes on shaking TwinCubator. (step 4). Theconjugate solution was removed and washed the strips twice for 1 minute with 1ml rinse

solution on TwinCubator (step 5 & 6). 1ml of distilled water was added to rinse the strip for 1 minute (step 7).20.0.9ml of diluted substrate was added to each strip, and incubated protecting from light without shaking (step 8).

The reaction was stopped by briefly rinsing with 1ml distilled water (step 9).The strips were removed from tray using tweezers, dried between paper towels and taped onto worksheet.

The chart of reaction zones that accompanies the kit was used to interpret result [7].

3.13 Molecular Identification of Isolates

3.13.1 Bacteria DNA Extraction

DNA was extracted using the protocol stated by (1). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer[8].

3.13.2 Bacteria DNA PCR Analysis

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaqcolourless reaction, 3 µl of 25mM $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins. And chill at 4oC.GEL (2, 3)[8].

3.13.3 Integrity

The integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated

by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel[8].

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile distilled[8].

Purification of Amplified Product.water and kept in -20oC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified productand quantified using ananodrop of model 2000 from thermo scientific[8].

3.13.4 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Bio-systems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis[8].

13.14 Data Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) 23.0 Version. The results were presentedin frequency tables, diagrams and charts.

3.15 Ethical Approval

Ethical permission was sought from the Ethical Committee, Oyo state Hospital Management Board and from UI/UCH Research Ethics Committee.

Endnotes

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Chapter Four

Results

4.1 Result of Findings

This is the analysis of collected data on the prevalence of pulmonasry mycobacterial infections and their risk Factors among inmates of Agodi Correctional Home, Ibadan, Oyo state Nigeria. Data was analyzed using Statistical Package for Social Sciences (SPSS) 23.0 Version.

Table 4.1 below (Socio-demographic Characteristics) show that all the participants 200(100.0%) were males. Majority (39.5%) were in the age group of 25-34 years. Only 26.0% had cough for two or more weeks; and 4.0% had coughing up bloodstained sputum (haemoptysis). Few (3.0%) had fevers for two or more weeks; and 7.5% have noticeable weight loss. Also, 6.5% have excessive sweating at night for two or more weeks. Concerning the participant's level of education, over half (67.5%) had secondary education as their highest educational qualifications, followed by primary education (22.0%) and higher education (10.5%) respectively. About 4.5% were HIV positive.

7.5% have noticeable weight loss and 6.5% of inmates that have excessive sweating at night for two or more weeks were observed. Speaking about the number of inmate in a cell, 4 categories of < 10, 10-20, 21-30, and >10 were housed in a cell, but the 3rd category carried the highest number of 98% which is a strong indication of overcrowding. Those smoking tobacco account for 23%, 4% of the inmate were malnourished and non 0% have diabetes. Only 0.5% have hypertension and none (0%) consumed alcohol during previous weeks. No inmate (0%) took immunosupresive or cytotoxic drugs but only 1% took illicit drugs during previous weeks. Lastly, 3% of the inmate each took BCG vaccination and was also treated for tuberculosis.

Table 4.1 Socio-demographic Characteristics

Participants' (n=200)	Frequency	Percent (%)
Sex		
Male	200	100.0
Female	0	0.0
Age (years)		
<25	54	27.0
25-34	79	39.5
35-44	32	16.0
45-54	24	12.0
55-64	8	4.0
≥65	3	1.5
Age group (years)		
≤34	133	66.5
≥35	67	33.5
Do you have cough for two or more weeks?		
Yes	52	26.0
No	148	74.0
Do you have coughing up bloodstained sputum (haemoptysis)?		
Yes	8	4.0
No	192	96.0
Do you have fevers for two or more weeks?		
Yes	6	3.0
No	194	97.0
Do you have noticeable weight loss?		
Yes	15	7.5
No	185	92.5

Do you have excessive sweating at night for two or more weeks?		
Yes	13	6.5
No	187	93.5
Level of Education		
Primary education	44	22.0
Secondary education	135	67.5
Higher education	21	10.5
Level of Education		
Lower education (Primary & secondary)	179	89.5
Higher education	21	10.5
HIV status		
Positive	9	4.5
Negative	191	95.5
Number of inmates in a cell		
<10	1	0.5
10-20	1	0.5
21-30	197	98.5
>30	1	0.5
Tobacco use currently or any time during previous 5 years:		
Yes	46	23.0
No	154	77.0
Are you malnourished?		
Yes	8	4.0
No	192	96.0
Do you have diabetes?		

Yes	0	0.0
No	200	100.0
Do you have hypertension?		
Yes	1	0.5
No	199	99.5
Heavy alcohol consumption during previous week:		
Yes	0	0.0
No	200	100.0
Are you taking an Immunosuppressive or Cytotoxic drugs?		
Yes	0	0.0
No	200	100.0
Any illicit drug use within previous week:		
Yes	1	0.5
No	199	99.5
BCG Vaccination:		
Yes	6	3.0
No	194	97.0
Previously treated for TB:		
Yes	6	3.0
No	194	97.0
Total	**200**	**100.0**

Table 4.2 show the result of laboratory investigation that was done on the sputum sample of the inmates. Out of 200 inmates that participated 21(10.5%) were positive for direct ZN stain. The result of Gene Xpert was 39(19.5%) when it was generally categorized into either positive or negative but when it was fully categorised, the positive inmate were 20 (10%), The inmate sputum sample that were indeterminate were repeated. Culture result was 42 (21%) when contamination rate was taken into cognizance, the isolates obtained was analysed using a rapid molecular method (SD Bioline) to characterize them. 30 isolates were MTBC (Mycotuberculous Complex) out of this 20 (10%) were *Mycobacerium tuberculosis*, 10 (5%) were *Mycobacerium bovis* and 12 (6%) were NTM.

LPA (Line Probe Assay) for DST with both first and second line anti TB drugs was set up. The TUB band on the third portion of the strip was positive for all the 30 isolates confirming the identity of the isolates. 9 (30%) were Rif sensitive, and 21 (70%) were Rif resistant, 17 (56.7%) were INH sensitive and 13 (43.3%) were INH resistant, Flouroquinolones was 29 (96.7%) sensitive and 1 (3.3%) resistant. Kanamycin, Amikacin and low level kanamycin were completely sensitive without any resistance.

I proceeded to setting up another type of DST using the proportion method which is the goal standard, the results gotten were as follows: Rif showed 7(23.3%) were sensitive and 23(76.7%) resistant, INH with 13(43.3%) sensitive and 17(56.7%) were resistant. EMB gave 22 (73.3%) sensitive and 8 (26.7%) resistant. FLQ gave 28 (93.3%) sensitive and 2 (6.7%) resistant. KN, AMK and LKN were altogether sensitive 30 (100%). All the above results were in accordance with the NTBLCP expected values and also with Nyasulu 2017.

Comparing the DST of LPA and that of proportion (70.0% LPA, 76.7% Prop.), it was observed that resistance was more in the proportion method this is in tandem with the international standard which stipulated that resistance is better measured with proportion method.

Table 4.2: Frequency Distribution of Tests Performed

Variables (n=200)	Frequency	Percent (%)
Gene Xpert		
MTB Positive	20	10.0
Negative	161	80.5
RR-Indeterminate	16	8.0
RR-Positive	3	1.5
Gene Xpert		
Positive	39	19.5
Negative	161	80.5
Gene Xpert (n=39)		
RR Positive	3	7.7
RR Negative	36	92.3
Gene Xpert (n=39)		
Indeterminate Positive	16	41.0
Indeterminate Negative	23	59.0
Culture		
Positive	42	21
Negative	145	72.5
Contaminated	13	6.5
Culture		
Positive	42	21
Negative	158	79
Direct Zn stain		
Positive	21	10.5
Negative	167	83.5

Other bacteria	12	6.0
Direct Zn stain		
Positive	21	10.5
Negative	179	89.5
Zn isolate		
Positive	28	14.0
Negative	172	86.0
SP Bioline		
Positive	30	100
Negative	0	0
MTB		
Positive	20	10.0
Negative	180	90.0
Mbovis		
Positive	10	5.0
Negative	190	95.0
NTM		
Positive	12	6.0
Negative	188	94.0
Line probe Assay_TUB		
Present	30	100
Absent	0	0
Line probe Assay_RIF		
Sensitive	9	30
Resistance	21	70
Line probe Assay_INH		
Sensitive	17	56.7
Resistance	13	43.3

Line probe Assay_FLQ		
Sensitive	29	96.7
Resistance	1	3.3
Line probe Assay_KN		
Sensitive	30	100
Line probe Assay_AMK		
Sensitive	30	100
Line probe Assay_LKN		
Sensitive	30	100
PROP_RIF		
Sensitive	7	23.3
Resistance	23	76.7
PROP_INH		
Sensitive	13	43.3
Resistance	17	56.7
PROP_EMB		
Sensitive	22	73.3
Resistance	8	26.7
PROP_FLQ		
Sensitive	28	93.3
Resistant	2	6.7
PROP_KN		
Sensitive	30	100
PROP_AMK		
Sensitive	30	100
PROP_LKN		
Sensitive	30	100

Keys: RR: Rifampicin Resistance, **Zn**: Ziehl neelsen, **MTB**: Mycobacteria tuberculosis, **NTM** : Non-Tuberculous Mycobacteria, **TUB**: Tuberculosis Band, **RIF**: Rifampicin, **INH**: Izoniazide, **FLQ:** Floroquinolones, **KN**: Kanamycin, **AMK:** Amikacin, **LKN**; Low Level Kanamycin.

The Table 4.3 show bivariate analysis revealed that respondents' educational status (p=0.000) and HIV status (p=0.000) were statistically significantly associated with age. On the other hand, have cough for two or more weeks (p=0.733), have coughing up bloodstained sputum (haemoptysis) (p=0.721), have fevers for two or more weeks (p=0.666), have noticeable weight loss (p=0.394), have excessive sweating at night for two or more weeks (p=0.764), number of inmates in a cell (p=0.213), Tobacco use currently or any time during previous 5 years (p=0.860), being malnourished (p=1.000), being hypertension (p=0.335), illicit drug use within previous week (p=0.335), having taken BCG Vaccination (p=1.000) and previously treated for TB (p=0.666) were not significantly associated with the inmates' age.

Table4.3: Relationship between participants' age and variables in the questionnaire

	Age Group (years)				
Some important variables in the questionnaire	**≤34**	**≥35**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**0.235**	**0.733**
Yes	36(27.1%)	16(23.9%)	52(26.0%)		
No	97(72.9%)	51(76.1%)	148(74.0%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**0.270**	**0.721**
Yes	6(4.5%)	2(3.0%)	8(4.0%)		
No	127(95.5%)	65(97.0%)	192(96.0%)		
Do you have fevers for two or more weeks?				**0.787**	**0.666**
Yes	5(3.8%)	1(1.5%)	6(3.0%)		
No	128(96.2%)	66(98.5%)	194(97.0%)		
Do you have noticeable weight loss?				**1.327**	**0.394**
Yes	12(9.0%)	3(4.5%)	15(7.5%)		
No	121(91.0%)	64(95.5%)	185(92.5%)		
Do you have excessive sweating at night for two or more weeks?				**0.154**	**0.764**
Yes	8(6.0%)	5(7.5%)	13(6.5%)		
No	125(94.0%)	62(92.5%)	187(93.5%)		
Level of Education				**19.674**	**0.000***
Primary education	37(27.8%)	7(10.4%)	44(22.0%)		
Secondary education	90(67.7%)	45(67.2%)	135(67.5%)		

Higher education	6(4.5%)	15(22.4%)	21(10.5%)		
Level of Education				**15.152**	**0.000***
Lower education (Primary & secondary)	127(95.5%)	52(77.6%)	179(89.5%)		
Higher education	6(4.5%)	15(22.4%)	21(10.5%)		
HIV status				**18.708**	**0.000***
Positive	0(0.0%)	9(13.4%)	9(4.5%)		
Negative	133(100.0%)	58(86.6%)	191(95.5%)		
Number of inmates in a cell				**4.496**	**0.213**
<10	0(0.0%)	1(1.5%)	1(0.5%)		
10-20	1(0.8%)	0(0.0%)	1(0.5%)		
21-30	132(99.2%)	65(97.0%)	197(98.5%)		
>30	0(0.0%)	1(1.5%)	1(0.5%)		
Tobacco use currently or any time during previous 5 years:				**0.044**	**0.860**
Yes	30(22.6%)	16(23.9%)	46(23.0%)		
No	103(77.4%)	51(76.1%)	154(77.0%)		
Are you malnourished?				**0.060**	**1.000**
Yes	5(3.8%)	3(4.5%)	8(4.0%)		
No	128(96.2%)	64(95.5%)	192(96.0%)		
Do you have hypertension?				**1.995**	**0.335**
Yes	0(0.0%)	1(1.5%)	1(0.5%)		
No	133(100.0%)	66(98.5%)	199(99.5%)		
Any illicit drug use within previous week:				**1.995**	**0.335**
Yes	0(0.0%)	1(1.5%)	1(0.5%)		

No	133(100.0%)	66(98.5%)	199(99.5%)		
BCG Vaccination:				**0.000**	**1.000**
Yes	4(3.0%)	2(3.0%)	6(3.0%)		
No	129(97.0%)	65(97.0%)	194(97.0%)		
Previously treated for TB:				**0.787**	**0.666**
Yes	5(3.8%)	1(1.5%)	6(3.0%)		
No	128(96.2%)	66(98.5%)	194(97.0%)		
Total	**133(100.0%)**	**67(100.0%)**	**200(100.0%)**		

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.4 above revealed that respondents' who have cough for two or more weeks (p=0.000), who have noticeable weight loss (p=0.005), who have excessive sweating at night for two or more weeks (p=0.008), and previously treated for TB (p=0.020) were statistically significantly associated with culture. However, respondents who have coughing up bloodstained sputum (haemoptysis) (p=0.067), who have fevers for two or more weeks (p=0.611), educational status (p=0.168), HIV status (p=0.209), number of inmates in a cell (p=0.841), Tobacco use currently or any time during previous 5 years (p=0.1.000), being malnourished (p=0.067), being hypertension (p=1.000), illicit drug use within previous week (p=0.215), having taken BCG Vaccination (p=0.344) were not significantly associated with the culture.

Table 4.4: Relationship between culture and variables in the questionnaire

Some important variables in the questionnaire	Culture			Pearson chi-square	p-value
	Positive	Negative	Total		
Do you have cough for two or more weeks?				**18.026**	**0.000***
Yes	22(51.2%)	30(19.1%)	52(26.0%)		
No	21(48.8%)	127(80.9%)	148(74.0%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**4.011**	**0.067**
Yes	4(9.3%)	4(2.5%)	8(4.0%)		
No	39(90.7%)	153(97.5%)	192(96.0%)		
Do you have fevers for two or more weeks?				**0.513**	**0.611**
Yes	2(4.7%)	4(2.5%)	6(3.0%)		
No	41(95.3%)	153(97.5%)	194(97.0%)		
Do you have noticeable weight loss?				**9.737**	**0.005***
Yes	8(18.6%)	7(4.5%)	15(7.5%)		
No	35(81.4%)	150(95.5%)	185(92.5%)		
Do you have excessive sweating at night for two or more weeks?				**8.619**	**0.008***
Yes	7(16.3%)	6(3.8%)	13(6.5%)		
No	36(83.7%)	151(96.2%)	187(93.5%)		
Level of Education				**2.557**	**0.278**
Primary education	7(16.3%)	37(23.6%)	44(22.0%)		

Secondary education	29(67.4%)	106(67.5%)	135(67.5%)		
Higher education	7(16.3%)	14(8.9%)	21(10.5%)		
Level of Education				**1.947**	**0.168**
Lower education (Primary & secondary)	36(83.7%)	143(91.1%)	179(89.5%)		
Higher education	7(16.3%)	14(8.9%)	21(10.5%)		
HIV status				**2.581**	**0.209**
Positive	0(0.0%)	9(5.7%)	9(4.5%)		
Negative	43(100.0%)	148(94.3%)	191(95.5%)		
Number of inmates in a cell				**0.834**	**0.841**
<10	0(0.0%)	1(0.6%)	1(0.5%)		
10-20	0(0.0%)	1(0.6%)	1(0.5%)		
21-30	43(100.0%)	154(98.1%)	197(98.5%)		
>30	0(0.0%)	1(0.6%)	1(0.5%)		
Tobacco use currently or any time during previous 5 years:				**0.002**	**1.000**
Yes	10(23.3%)	36(22.9%)	46(23.0%)		
No	33(76.7%)	121(77.1%)	154(77.0%)		
Are you malnourished?				**4.011**	**0.067**
Yes	4(9.3%)	4(2.5%)	8(4.0%)		
No	39(90.7%)	153(97.5%)	192(96.0%)		
Do you have hypertension?				**0.275**	**1.000**
Yes	0(0.0%)	1(0.6%)	1(0.5%)		
No	43(100.0%)	156(99.4%)	199(99.5%)		
Any illicit drug use				**3.670**	**0.215**

within previous week:					
Yes	1(2.3%)	0(0.0%)	1(0.5%)		
No	42(97.7%)	157(100.0%)	199(99.5%)		
BCG Vaccination:				**1.694**	**0.344**
Yes	0(0.0%)	6(3.8%)	6(3.0%)		
No	43(100.0%)	151(96.2%)	194(97.0%)		
Previously treated for TB:				**7.477**	**0.020***
Yes	4(9.3%)	2(1.3%)	6(3.0%)		
No	39(90.7%)	155(98.7%)	194(97.0%)		
Total	**43(100.0%)**	**157(100.0%)**	**200(100.0%)**		

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.5, 4.6 and 4.7 show the analysis of the relationship between Gene-xpert and variables in the questionnaire with three categories of the Gene-xpert frequency results obtained in tables 4.2. Interestingly, Table 4.7 was able to give a clear picture of degree of association simply because the parameters here were 2, the variables of cough for 2weeks coughing up blood, fewer for 2 weeks, noticeable with loss Excessive night sweat and level of education gee a p-values 0.000, 0.048, 0.014, 0.000, 0.001, 0.012, 0.037 respectively and were significantly associated which was in agreement with the work of ACM Lemus, DS Abebe and B. Seri

Table 4.5: Relationship between Gene Xpert and variables in the questionnaire

	Gene Xpert				
Some important variables in the questionnaire	**Indeterminate Positive**	**Indeterminate Negative**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**0.818**	**0.516**
Yes	10(62.5%)	11(47.8%)	21(53.8%)		
No	6(37.5%)	12(52.2%)	18(46.2%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**2.126**	**0.286**
Yes	3(18.8%)	1(4.3%)	4(10.3%)		
No	13(81.2%)	22(95.7%)	35(89.7%)		
Do you have fevers for two or more weeks?				**0.148**	**1.000**
Yes	2(12.5%)	2(8.7%)	4(10.3%)		
No	14(87.5%)	21(91.3%)	35(89.7%)		
Do you have noticeable weight loss?				**1.021**	**0.444**
Yes	5(31.2%)	4(17.4%)	9(23.1%)		
No	11(68.8%)	19(82.6%)	30(76.9%)		
Do you have excessive sweating at night for two or more weeks?				**0.335**	**0.694**
Yes	4(25.0%)	4(17.4%)	8(20.5%)		
No	12(75.0%)	19(82.6%)	31(79.5%)		

Level of Education				**0.577**	**0.749**
Primary education	2(12.5%)	2(8.7%)	4(10.3%)		
Secondary education	10(62.5%)	17(73.9%)	27(69.2%)		
Higher education	4(25.0%)	4(17.4%)	8(20.5%)		
Level of Education					
Lower education (Primary & secondary)	12(75.0%)	19(82.6%)	31(79.5%)	**0.335**	**0.694**
Higher education	4(25.0%)	4(17.4%)	8(20.5%)		
HIV status				**NOT POSSIBLE**	
Positive					
Negative					
Number of inmates in a cell				**NOT POSSIBLE**	
<10					
10-20					
21-30					
>30					
Tobacco use currently or any time during previous 5 years:				**1.158**	**0.307**
Yes	6(37.5%)	5(21.7%)	11(28.2%)		
No	10(62.5%)	18(78.3%)	28(71.8%)		
Are you malnourished?				**0.473**	**0.631**
Yes	1(6.2%)	3(13.0%)	4(10.3%)		
No	15(93.8%)	20(87.0%)	35(89.7%)		
Do you have hypertension?				**NOT POSSIBLE**	
Yes					
No					

Any illicit drug use within previous week:				**0.714**	**1.000**
Yes	0(0.0%)	1(4.3%)	1(2.6%)		
No	16(100.0%)	22(95.7%)	38(97.4%)		
BCG Vaccination:				**3.030**	**0.162**
Yes	2(12.5%)	0(0.0%)	2(5.1%)		
No	14(87.5%)	23(100.0%)	37(94.9%)		
Previously treated for TB:				**2.126**	**0.286**
Yes	3(18.8%)	1(4.3%)	4(10.3%)		
No	13(81.2%)	22(95.7%)	35(89.7%)		
Total	**16(100.0%)**	**23(100.0%)**	**39(100.0%)**		

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.6: Relationship between Gene Xpert and variables in the questionnaire

	Gene Xpert				
Some important variables in the questionnaire	**RR Positive**	**RR Negative**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**3.792**	**0.089**
Yes	0(0.0%)	21(58.3%)	21(53.8%)		
No	3(100.0%)	15(41.7%)	18(46.2%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**0.371**	**1.000**
Yes	0(0.0%)	4(11.1%)	4(10.3%)		
No	3(100.0%)	32(88.9%)	35(89.7%)		
Do you have fevers for two or more weeks?				**0.371**	**1.000**
Yes	0(0.0%)	4(11.1%)	4(10.3%)		
No	3(100.0%)	32(88.9%)	35(89.7%)		
Do you have noticeable weight loss?				**0.975**	**1.000**
Yes	0(0.0%)	9(25.0%)	9(23.1%)		
No	3(100.0%)	27(75.0%)	30(76.9%)		
Do you have excessive sweating at night for two or more weeks?				**0.839**	**1.000**
Yes	0(0.0%)	8(22.2%)	8(20.5%)		
No	3(100.0%)	28(77.8%)	31(79.5%)		
Level of Education				**0.597**	**0.742**
Primary education	0(0.0%)	4(11.1%)	4(10.3%)		
Secondary education	2(66.7%)	25(69.4%)	27(69.2%)		
Higher education	1(33.3%)	7(19.4%)	8(20.5%)		
Level of Education				**0.328**	**0.508**
Lower education (Primary & secondary)	2(66.7%)	29(80.6%)	31(79.5%)		

Higher education	1(33.3%)	7(19.4%)	8(20.5%)		
HIV status				**NOT POSSIBLE**	
Positive					
Negative					
Number of inmates in a cell				**NOT POSSIBLE**	
<10					
10-20					
21-30					
>30					
Tobacco use currently or any time during previous 5 years:				**1.277**	**0.545**
Yes	0(0.0%)	11(30.6%)	11(28.2%)		
No	3(100.0%)	25(69.4%)	28(71.8%)		
Are you malnourished?				**0.37**	**1.000**
Yes	0(0.0%)	4(11.1%)	4(10.3%)		
No	3(100.0%)	32(88.9%)	35(89.7%)		
Do you have hypertension?				**NOT POSSIBLE**	
Yes					
No					
Any illicit drug use within previous week:				**0.086**	**1.000**
Yes	0(0.0%)	1(2.8%)	1(2.6%)		
No	3(100.0%)	35(97.2%)	38(97.4%)		
BCG Vaccination:				**0.176**	**1.000**
Yes	0(0.0%)	2(5.6%)	2(5.1%)		

No	3(100.0%)	34(94.4%)	37(94.9%)		
Previously treated for TB:				**0.371**	**1.000**
Yes	0(0.0%)	4(11.1%)	4(10.3%)		
No	3(100.0%)	32(88.9%)	35(89.7%)		
Total	3(100.0%)	36(100.0%)	**39(100.0%)**		

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.7: Relationship between Gene Xpert and variables in the questionnaire

	Gene Xpert				
important variables in the questionnaire Some	**Positive**	**Negative**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**19.525**	**0.000***
Yes	21(53.8%)	31(19.3%)	52(26.0%)		
No	18(46.2%)	130(80.7%)	148(74.0%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**4.938**	**0.048***
Yes	4(10.3%)	4(2.5%)	8(4.0%)		
No	35(89.7%)	157(97.5%)	192(96.0%)		
Do you have fevers for two or more weeks?				**8.766**	**0.014***
Yes	4(10.3%)	2(1.2%)	6(3.0%)		
No	35(89.7%)	159(98.8%)	194(97.0%)		
Do you have noticeable weight loss?				**16.945**	**0.000***
Yes	9(23.1%)	6(3.7%)	15(7.5%)		
No	30(76.9%)	155(96.3%)	185(92.5%)		
Do you have excessive sweating at night for two or more weeks?				**15.653**	**0.001***
Yes	8(20.5%)	5(3.1%)	13(6.5%)		
No	31(79.5%)	156(96.9%)	187(93.5%)		
Level of Education				**7.684**	**0.012***
Primary education	4(10.3%)	40(24.8%)	44(22.0%)		
Secondary education	27(69.2%)	108(67.1%)	135(67.5%)		
Higher education	8(20.5%)	13(8.1%)	21(10.5%)		
Level of Education				**5.169**	**0.037***
Lower education (Primary & secondary)	31(79.5%)	148(91.9%)	179(89.5%)		

Higher education	8(20.5%)	13(8.1%)	21(10.5%)		
HIV status				**2.283**	**0.210**
Positive	0(0.0%)	9(5.6%)	9(4.5%)		
Negative	39(100.0%)	152(94.4%)	191(95.5%)		
Number of inmates in a cell				**0.738**	**0.864**
<10	0(0.0%)	1(0.6%)	1(0.5%)		
10-20	0(0.0%)	1(0.6%)	1(0.5%)		
21-30	39(100.0%)	158(98.1%)	197(98.5%)		
>30	0(0.0%)	1(0.6%)	1(0.5%)		
Tobacco use currently or any time during previous 5 years:				**0.741**	**0.401**
Yes	11(28.2%)	35(21.7%)	46(23.0%)		
No	28(71.8%)	126(78.3%)	154(77.0%)		
Are you malnourished?				**4.938**	**0.048***
Yes	4(10.3%)	4(2.5%)	8(4.0%)		
No	35(89.7%)	157(97.5%)	192(96.0%)		
Do you have hypertension?				**0.243**	**1.000**
Yes	0(0.0%)	1(0.6%)	1(0.5%)		
No	39(100.0%)	160(99.4%)	199(99.5%)		
Any illicit drug use within previous week:				**4.149**	**0.195**
Yes	1(2.6%)	0(0.0%)	1(0.5%)		
No	38(97.4%)	161(100.0%)	199(99.5%)		
BCG Vaccination:				**0.754**	**0.332**
Yes	2(5.1%)	4(2.5%)	6(3.0%)		
No	37(94.9%)	157(97.5%)	194(97.0%)		

Previously treated for TB:				**8.766**	**0.014***
Yes	4(10.3%)	2(1.2%)	6(3.0%)		
No	35(89.7%)	159(98.8%)	194(97.0%)		
Total	**39(100.0%)**	**161(100.0%)**	**200(100.0%)**		

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

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Table 4.8 analyse the relationship between ZN stain and variables in the questionnaire which also show significant association as above but with exception of coughing up blood.

Table 4.9 - 4.11 show the result of relationship between LPA and proportion DST result with variables in the questionnaire that was not significantly associated.

Table 4.13 - 4.14 compared relationship between Genex-pert and age the person Chi-square values gotten with P. values were as well use to calculate the sensitivity, specificity, positive predictive values and Negative predictive value which all concluded that age is not significantly associated with Gene-xpert.

Table 4.8: Relationship between Direct Zn stain and variables in the questionnaire

	Direct Zn stain				
Some important variables in the questionnaire	**Positive**	**Negative**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**8.487**	**0.007***
Yes	11(52.4%)	41(22.9%)	52(26.0%)		
No	10(47.6%)	138(77.1%)	148(74.0%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**1.864**	**0.200**
Yes	2(9.5%)	6(3.4%)	8(4.0%)		
No	19(90.5%)	173(96.6%)	192(96.0%)		
Do you have fevers for two or more weeks?				**10.270**	**0.016***
Yes	3(14.3%)	3(1.7%)	6(3.0%)		
No	18(85.7%)	176(98.3%)	194(97.0%)		
Do you have noticeable weight loss?				**8.997**	**0.012***
Yes	5(23.8%)	10(5.6%)	15(7.5%)		
No	16(76.2%)	169(94.4%)	185(92.5%)		
Do you have excessive sweating at night for two or more weeks?				**18.808**	**0.001***
Yes	6(28.6%)	7(3.9%)	13(6.5%)		
No	15(71.4%)	172(96.1%)	187(93.5%)		
Level of Education				**14.929**	**0.001***
Primary education	1(4.8%)	43(24.0%)	44(22.0%)		
Secondary education	13(61.9%)	122(68.2%)	135(67.5%)		
Higher education	7(33.3%)	14(7.8%)	21(10.5%)		
Level of Education				**13.017**	**0.002***
Lower education (Primary & secondary)	14(66.7%)	165(92.2%)	179(89.5%)		

Higher education	7(33.3%)	14(7.8%)	21(10.5%)		
HIV status				**1.106**	**0.602**
Positive	0(0.0%)	9(5.0%)	9(4.5%)		
Negative	21(100.0%)	170(95.0%)	191(95.5%)		
Number of inmates in a cell				**0.357**	**0.949**
<10	0(0.0%)	1(0.6%)	1(0.5%)		
10-20	0(0.0%)	1(0.6%)	1(0.5%)		
21-30	21(100.0%)	176(98.3%)	197(98.5%)		
>30	0(0.0%)	1(0.6%)	1(0.5%)		
Tobacco use currently or any time during previous 5 years:				**0.009**	**1.000**
Yes	5(23.8%)	41(22.9%)	46(23.0%)		
No	16(76.2%)	138(77.1%)	154(77.0%)		
Are you malnourished?				**1.864**	**0.200**
Yes	2(9.5%)	6(3.4%)	8(4.0%)		
No	19(90.5%)	173(96.6%)	192(96.0%)		
Do you have hypertension?				**0.118**	**1.000**
Yes	0(0.0%)	1(0.6%)	1(0.5%)		
No	21(100.0%)	178(99.4%)	199(99.5%)		
Any illicit drug use within previous week:				**8.567**	**0.105**
Yes	1(4.8%)	0(0.0%)	1(0.5%)		
No	20(95.2%)	179(100.0%)	199(99.5%)		
BCG Vaccination:				**3.432**	**0.122**
Yes	2(9.5%)	4(2.2%)	6(3.0%)		
No	19(90.5%)	175(97.8%)	194(97.0%)		
Previously treated for TB:				**3.432**	**0.122**
Yes	2(9.5%)	4(2.2%)	6(3.0%)		
No	19(90.5%)	175(97.8%)	194(97.0%)		

Total	**21(100.0%)**	**179(100.0%)**	**200(100.0%)**

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.9: Relationship between LPA-RIF and variables in the Questionnaire

	LPA-RIF				
Some important variables in the questionnaire	**Sensitive**	**Resistance**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**0.524**	**0.691**

Yes	6(66.7%)	11(52.4%)	17(56.7%)		
No	3(33.3%)	10(47.6%)	13(43.3%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**0.018**	**1.000**
Yes	1(11.1%)	2(9.5%)	3(10.0%)		
No	8(88.9%)	19(90.5%)	27(90.0%)		
Do you have fevers for two or more weeks?				**0.408**	**0.517**
Yes	1(11.1%)	1(4.8%)	2(6.7%)		
No	8(88.9%)	20(95.2%)	28(93.3%)		
Do you have noticeable weight loss?				**0.130**	**1.000**
Yes	2(22.2%)	6(28.6%)	8(26.7%)		
No	7(77.8%)	15(71.4%)	22(73.3%)		
Do you have excessive sweating at night for two or more weeks?				**0.040**	**1.000**
Yes	2(22.2%)	4(19.0%)	6(20.0%)		
No	7(77.8%)	17(81.0%)	24(80.0%)		
Level of Education				**0.332**	**0.847**
Primary education	1(11.1%)	2(9.5%)	3(10.0%)		
Secondary education	6(66.7%)	16(76.2%)	22(73.3%)		
Higher education	2(22.2%)	3(14.3%)	5(16.7%)		
Level of Education				**0.286**	**0.622**
Lower education (Primary & secondary)	7(77.8%)	18(85.7%)	25(83.3%)		
Higher education	2(22.2%)	3(14.3%)	5(16.7%)		
HIV status				**NOT POSSIBLE**	

Positive					
Negative					
Number of inmates in a cell				**NOT POSSIBLE**	
<10					
10-20					
21-30					
>30					
Tobacco use currently or any time during previous 5 years:				**0.068**	**1.000**
Yes	3(33.3%)	6(28.6%)	9(30.0%)		
No	6(66.7%)	15(71.4%)	21(70.0%)		
Are you malnourished?				**1.978**	**0.287**
Yes	0(0.0%)	4(19.0%)	4(13.3%)		
No	9(100.0%)	17(81.0%)	86.7%		
Do you have hypertension?				**NOT POSSIBLE**	
Yes					
No					
Any illicit drug use within previous week:				**0.443**	**1.000**
Yes	0(0.0%)	1(4.8%)	1(3.3%)		
No	9(100.0%)	20(95.2%)	29(96.7%)		
BCG Vaccination:				**NOT POSSIBLE**	
Yes					
No					
Previously treated for TB:				**0.055**	**1.000**

Yes	1(11.1%)	3(14.3%)	4(13.3%)
No	8(88.9%)	18(85.7%)	26(86.7%)
Total	**30(100.0%)**	**1(100.0%)**	**31(100.0%)**

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.10: Relationship between LPA_INH and variables in the Questionnaire

	LPA-INH				
Some important variables in the questionnaire	**Sensitive**	**Resistance**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**1.033**	**0.460**
Yes	11(64.7%)	6(46.2%)	17(56.7%)		
No	6(35.3%)	7(53.8%)	13(43.3%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**0.136**	**1.000**
Yes	2(11.8%)	1(7.7%)	3(10.0%)		
No	15(88.2%)	12(92.3%)	27(90.0%)		
Do you have fevers for two or more weeks?				**1.639**	**0.492**
Yes	2(11.8%)	0(0.0%)	2(6.7%)		
No	15(88.2%)	13(100.0%)	28(93.3%)		
Do you have noticeable weight loss?				**0.197**	**0.698**
Yes	4(23.5%)	4(30.8%)	8(26.7%)		
No	13(76.5%)	9(69.2%)	22(73.3%)		
Do you have excessive sweating at night for two or more weeks?				**0.305**	**0.672**
Yes	4(23.5%)	2(15.4%)	6(20.0%)		
No	13(76.5%)	11(84.6%)	24(80.0%)		
Level of Education				**0.185**	**0.912**
Primary education	2(11.8%)	1(7.7%)	3(10.0%)		
Secondary education	12(70.6%)	10(76.9%)	22(73.3%)		
Higher education	3(17.6%)	2(15.4%)	5(16.7%)		

Level of Education				**0.027**	**1.000**
Lower education (Primary & secondary)	14(82.4%)	11(84.6%)	25(83.3%)		
Higher education	3(17.6%)	2(15.4%)	5(16.7%)		
HIV status				**NOT POSSIBLE**	
Positive					
Negative					
21-30					
>30					
Tobacco use currently or any time during previous 5 years:				**0.006**	**1.000**
Yes	5(29.4%)	4(30.8%)	9(30.0%)		
No	12(70.6%)	9(69.2%)	21(70.0%)		
Are you malnourished?	<10			**1.885**	**0.290**
Yes	10-20	3(23.1%)	4(13.3%)		
No	16(94.1%)	10(76.9%)	26(86.7%)		
Do you have hypertension?				**NOT POSSIBLE**	
Yes					
No					
Any illicit drug use within previous week:				**1.353**	**0.433**
Yes	0(0.0%)	1(7.7%)	1(3.3%)		
No	17(100.0%)	12(92.3%)	29(96.7%)		
Previously treated for TB:				**0.632**	**0.613**

Yes	3(17.6%)	1(7.7%)	4(13.3%)
No	14(82.4%)	12(92.3%)	26(86.7%)
Total	**17(100.0%)**	**13(100.0%)**	**30(100.0%)**

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.11: Relationship between LPA_FLQ and variables in the questionnaire

	LPA-FLQ				
Some important variables in the questionnaire	**Sensitive**	**Resistance**	**Total**	**Pearson chi-square**	**p-value**
Do you have cough for two or more weeks?				**1.353**	**0.433**
Yes	17(58.6%)	0(0.0%)	17(56.7%)		
No	12(41.4%)	1(100.0%)	13(43.3%)		
Do you have coughing up bloodstained sputum (haemoptysis)?				**0.115**	**1.000**
Yes	3(10.3%)	0(0.0%)	3(10.0%)		
No	26(89.7%)	1(100.0%)	27(90.0%)		
Do you have fevers for two or more weeks?				**0.074**	**1.000**
Yes	2(6.9%)	0(0.0%)	2(6.7%)		
No	27(93.1%)	1(100.0%)	28(93.3%)		
Do you have noticeable weight loss?				**2.845**	**0.267**
Yes	7(24.1%)	1(100.0%)	8(26.7%)		
No	22(75.9%)	0(0.0%)	22(73.3%)		
Do you have excessive sweating at night for two or more weeks?				**0.259**	**1.000**
Yes	6(20.7%)	0(0.0%)	6(20.0%)		
No	23(79.3%)	1(100.0%)	24(80.0%)		
Level of Education				**0.376**	**0.829**
Primary education	3(10.3%)	0(0.0%)	3(10.0%)		
Secondary education	21(72.4%)	1(100.0%)	22(73.3%)		
Higher education	5(17.2%)	0(0.0%)	5(16.7%)		
Level of Education				**0.207**	**1.000**
Lower education (Primary & secondary)	24(82.8%)	1(100.0%)	25(83.3%)		

Higher education	5(17.2%)	0(0.0%)	5(16.7%)		
HIV status				**NOT POSSIBLE**	
Positive					
Negative					
Number of inmates in a cell				**NOT POSSIBLE**	
<10					
10-20					
21-30					
>30					
Tobacco use currently or any time during previous 5 years:				**0.443**	**1.000**
Yes	9(31.0%)	0(0.0%)	9(30.0%)		
No	20(69.0%)	1(100.0%)	21(70.0%)		
Are you malnourished?				**6.724**	**0.133**
Yes	3(10.3%)	1(100.0%)	4(13.3%)		
No	26(89.7%)	0(0.0%)	26(86.7%)		
Do you have hypertension?				**NOT POSSIBLE**	
Yes					
No					
Any illicit drug use within previous week:				**0.036**	**1.000**
No	28(96.6%)	1(100.0%)	29(96.7%)		
Yes	1(3.4%)	0(0.0%)	1(3.3%)		
BCG Vaccination:				**NOT POSSIBLE**	
Yes					
No					
Previously treated for				**0.159**	**1.000**

TB:			
Yes	4(13.8%)	0(0.0%)	4(13.3%)
No	25(86.2%)	1(100.0%)	26(86.7%)
Total	**30(100.0%)**	**1(100.0%)**	**31(100.0%)**

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table 4.12: Relationship between Gene Xpert and age 1

	Gene Xpert								
	Indeterminate Positive	**Indeterminate Negative**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.321**	**0.740**	**56.3%**	**34.8%**	**37.5%**	**53.3%**
≤34	9(56.2%)	15(65.2%)	24(61.5%)						
≥35	7(43.8%)	8(34.8%)	15(38.5%)						
Total	**16(100%)**	**23(100%)**	**39(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is not significantly associated with Gene Xpert (p=0.321). This means that respondents' age do not influence Gene Xpert.

Note:

Sensitivity = True positives / Total positives x 100

Specificity = True negatives / Total negatives x 100

Positive predictive value (PPV) = True positives / Tested positive x 100

Negative predictive value (NPV) = True negatives / Tested negative x 100

Table 4.13: Relationship between Gene Xpert and age 2

	Gene Xpert								
	RR Positive	**RR Negative**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**1.092**	**0.547**	**33.3%**	**36.1%**	**4.2%**	**86.7%**
≤34	1(33.3%)	23(63.9%)	24(61.5%)						
≥35	2(66.7%)	13(36.1%)	15(38.5%)						
Total	**3(00%)**	**36(100%)**	**39(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is not significantly associated with Gene Xpert (p=0.547). This means that respondents' age do not influence Gene Xpert.

Note:

Sensitivity = True positives / Total positives x 100

Specificity = True negatives / Total negatives x 100

Positive predictive value (PPV) = True positives / Tested positive x 100

Negative predictive value (NPV) = True negatives / Tested negative x 100

Table 4.14: Relationship between Gene Xpert and age 3

	Gene Xpert								
	Positive	**Negative**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.535**	**0.457**	**61.5%**	**32.3%**	**18.0%**	**77.6%**
≤34	24(61.5%)	109(67.7%)	133(66.5%)						
≥35	15(38.5%)	52(32.3%)	67(33.5%)						
Total	**39(100%)**	**161(100%)**	**200(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is not significantly associated with Gene Xpert (p=0.457). This means that respondents' age do not influence Gene Xpert.

Table 4.15 compared the relationship between Culture and Age as was done above and concluded that Culture is not significantly associated with age.

Table 4.16 compared the relationship between Direct ZN stain with age and show that age is statistically significantly associated with Direst ZN Stain (P=0.026). This mean that respondent age influence Direct ZN Stain.

Table 4.17 - 4.20 compared the relationship between ZN isolate, LPA:RIF, INH, FLQ and age to give a result that age is significantly not associated with them.

Table 4.21 vividly brought out the prevalence of Mycobacterial infections 42(21%) the infection with M. Tuberculosis gave 20(10%), M. bovis 10(5%) and NTM 12(6%).

Table 4.15: Relationship between Culture and age

	Culture								
	Positive	**Negative**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.338**	**0.587**	**62.8%**	**32.5%**	**20.3%**	**76.1%**
≤34	27(62.8%)	106(67.5%)	133(66.5%)						
≥35	16(37.2%)	51(32.5%)	67(33.5%)						
Total	**43(100%)**	**157(100%)**	**200(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is not significantly associated with Culture (p=0.587). This means that respondents' age do not influence culture.

Table 4.16: Relationship between Direct Zn stain and age

	Direct Zn stain								
	Positive	**Negative**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**5.887**	**0.026***	**42.9%**	**30.7%**	**6.8%**	**82.1%**
≤34	9(42.9%)	124(69.3%)	133(66.5%)						
≥35	12(57.1%)	55(30.7%)	67(33.5%)						
Total	**21(100%)**	**179(100%)**	**200(100%)**						

*p<0.05 (i.e. Significant). **p-value is obtained from Fisher's exact test.

Table above showed that age is statistically significantly associated with Direct Zn stain (p=0.026). This means that respondents' age influences Direct Zn stain.

Table 4.17: Relationship between Zn Isolate and age

	Zn Isolate								
	Positive	**Negative**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.489**	**0.520**	**60.7%**	**32.6%**	**12.8%**	**83.6%**
≤34	17(60.7%)	116(67.4%)	133(66.5%)						
≥35	11(39.3%)	56(32.6%)	67(33.5%)						
Total	**28(100%)**	**172(100%)**	**200(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is significantly not associated with Zn Isolate (p=0.520). This means that respondents' age do not influence Zn Isolate.

Table 4.18: Relationship between LPA_RIF and age

	LPA_RIF								
	Sensitive	**Resistance**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.062**	**1.000**	**66.7%**	**38.1%**	**31.6%**	**72.7%**
≤34	6(66.7%)	13(61.9%)	19(63.3%)						
≥35	3(33.3%)	8(38.1%)	11(36.7%)						
Total	**9(100%)**	**21(100%)**	**30(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is significantly not associated with LPA_RIF (p=1.000). This means that respondents' age do not influence LPA_RIF.

Table 4.19: Relationship between LPA_INH and age

	LPA_INH								
	Sensitive	**Resistance**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.889**	**0.454**	**70.6%**	**46.2%**	**63.2%**	**54.5%**
≤34	12(70.6%)	7(53.8%)	19(63.3%)						
≥35	5(29.4%)	6(46.2%)	11(36.7%)						
Total	**17(100%)**	**13(100%)**	**30(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is significantly not associated with LPA_INH (p=0.454). This means that respondents' age do not influence LPA_INH.

Table 4.20: Relationship between LPA_FLQ and age

	LPA_FLQ								
	Sensitive	**Resistance**	**Total**	**Pearson chi-square**	**p-value**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Age group (years)				**0.599**	**1.000**	**62.1%**	**0%**	**94.7%**	**0%**
≤34	18(62.1%)	1(100.0%)	19(63.3%)						
≥35	11(37.9%)	0(0.0%)	11(36.7%)						
Total	**29(100%)**	**1(100%)**	**30(100%)**						

**p-value is obtained from Fisher's exact test.

Table above showed that age is significantly not associated with LPA_FLQ (p=1.000). This means that respondents' age do not influence LPA_FLQ.

` Table 4.21:Mycobacterial Infections

Infections Mycobacterial	N	Positive(%)	Negative(%)
Mycobacterium tuberculosis	200	20 (10.0)	180 (90.0)
Mycobacterium bovis	200	10 (5.0)	190 (95.0)
Non-Tuberculous Mycobacterium (NTM)	200	12 (6.0)	188 (94.0)
Total	**200**	**42 (21.0)**	**158 (79.0)**

Source: Author's Field Work, 2023

Table 4.22 categorized the type of resistance to Rifampicin gotten from the DST and concluded that multi drug resistant is 13(43%) and 2(7%) Pre XDR. The MDR and pre XDR are pointers to serious problem if adequate infection control is not quickly put in place.

Table 4.23 spelt out the genes coding for resistance in each drugs Rpob gene codes for Rifampicin, InhA and KatG gene codes for Izoniazide, gyrA/B gene codes for Flouroquinolones and rrs gene codes for Aminoglycoside.

Table 4.22: Anti TB Drug Resistance Pattern

Isolate	N	RIF MONO	INH MONO	MDR	POLY RES	PRE-XDR	XDR
M.tuberculosis	20	2(10%)	6(30%)	8(80%)	5(50%)	0	0
M.bovis	10	1(10%)	4(40%)	5(50%)	3(30%)	2(20%)	0
Total	30	3(10%)	10(33%)	13(43%)	8(27%)	2(7%)	0

Table 4.23:Mycobacterial Infections Vs Antibiotic Resistance Genes

Mycobacterial Infections	N	Rpob gene n(%)	KatG gene n(%)	Inh A gene n(%)	gyrA/B gene n(%)	Emb gene n(%)	rrs gene n(%)
Mycobacterium tuberculosis	30	15(50%)	5(16.7%)	2(6.7%)	Nil	Nil	Nil
Mycobacterium bovis	30	6(20%)	4(13%)	2(6.7%)	1(3.0%)	3(10%)	Nil

NB: Rpob gene codes for Rifampicin, InhA and KatG gene codes for Izoniazide, gyrA/B codes for Flouroquinolones, rrs codes for Aminoglycosides.

Table 4.24 gave the result of phenotypic characterization of Non Tuberculous Mycobacteria with five (5) tests on each of them. 10 show rapid growth within 4 days, growth at 25°C, non production of pigment, reduced Nitrate and was positive for PNB. The distinguishing test in Nitrate was not reduced in two (2) of the NTM which brought me to conclude *Mycobacterium chelonei*.

Table 4.24:Phenotypic Characterization Of Non Tuberculous Mycobaceria

TEST TYPE NTM NAME	RATE OF GROWTH	GROWTH TEMPERATURE	PIGMENT PRODUCTION	NITRATE REDUCTION	GROWTH ON 4-PNB ACID
M.fortuitum	Rapid (4days)	25 degree C	Non	10 Positive	Positive
M.chelonei	Rapid (4days)	25 degree C	Non	0 Negative	2 Positive

Table 4.25 show the NCBI blast of the sequence identify of the isolates edited. The percentage identity of 7 of *Mycobacterium tuberculosis* is 100% while that of 3 *Mycobacterium bovis* are lesser, 99.86%, 99.73% and 99.93%. Ascension numbers of each of the mycobacterial isolate were written on the table.

Table 4.26 gave the genetic distance analysis showing genetic relatedness between the isolated Mycobacteria and reference sequence generated from the NCBI base.

Figure 4.1 clearly show the position of the standard band compared with that of any newly isolated mycobacteria. The Agarose show the positive amplification of ITS regions.

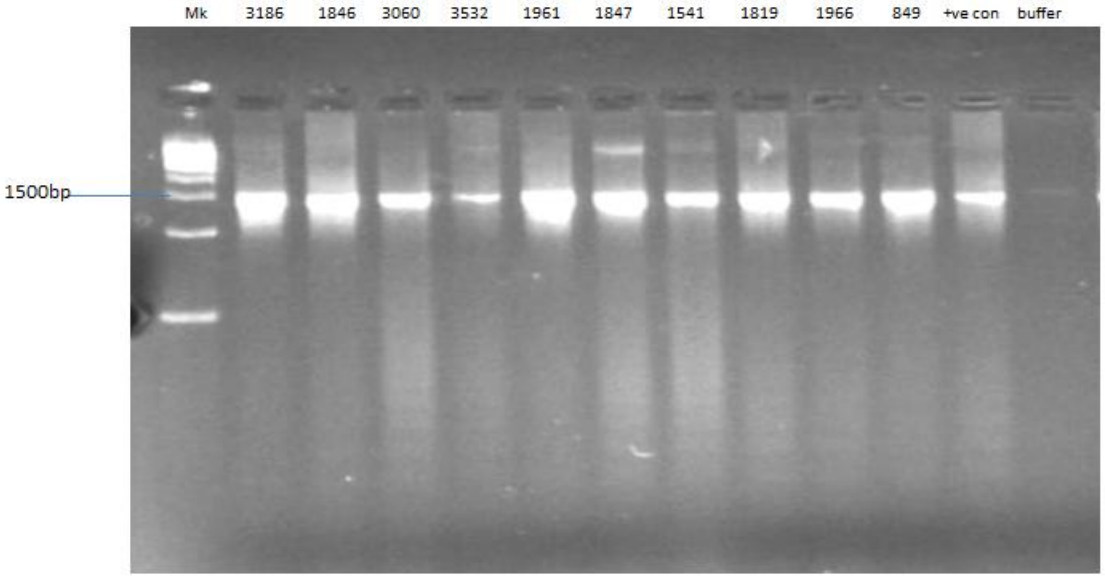

Figure 4.1: Agarose Gel showing the positive amplification of the ITS regions amplified from the selected mycobacterial samples

Source: Author's Field Work, 2023

Table 4.25: NCBI Blast showing the sequence identity of the isolates edited sequences

samplID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
3186	Mycobacterium tuberculosis	2706	2706	99%	0	100.00%	OR452345
1846	Mycobacterium tuberculosis	2706	2706	100%	0	100.00%	OR452346
3060	Mycobacterium tuberculosis	2706	2706	99%	0	100.00%	OR452347
3532	Mycobacterium tuberculosis	2706	2706	99%	0	100.00%	OR452348
1961	Mycobacterium tuberculosis	2706	2706	100%	0	100.00%	OR452349
1847	Mycobacterium tuberculosis	2706	2706	99%	0	100.00%	OR452350
1541	Mycobacterium tuberculosis	2706	2706	99%	0	100.00%	OR452351
1819	Mycobacterium bovis	2689	2689	100%	0	99.86%	OR452352
1966	Mycobacterium bovis	2678	2678	100%	0	99.73%	OR452353
849	Mycobacterium bovis	2695	2695	100%	0	99.93%	OR452354

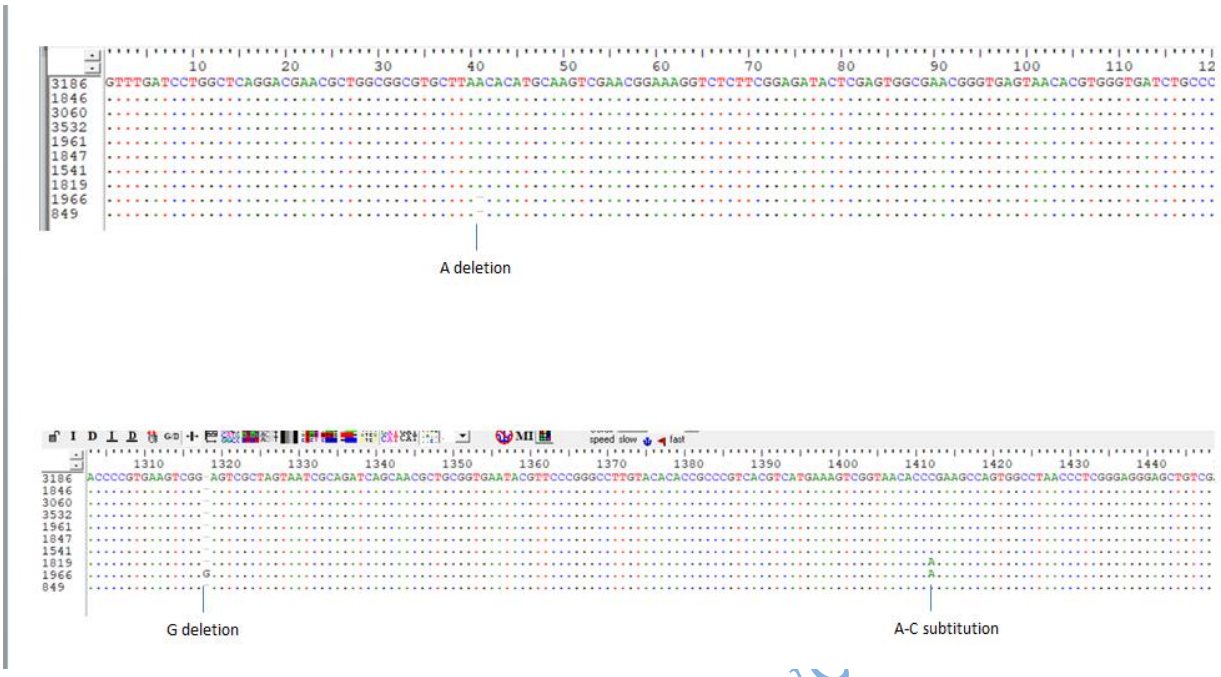

Figure 4.2: Sequence alignment revealing locations of nucleotide changes along the isolates edited sequences.

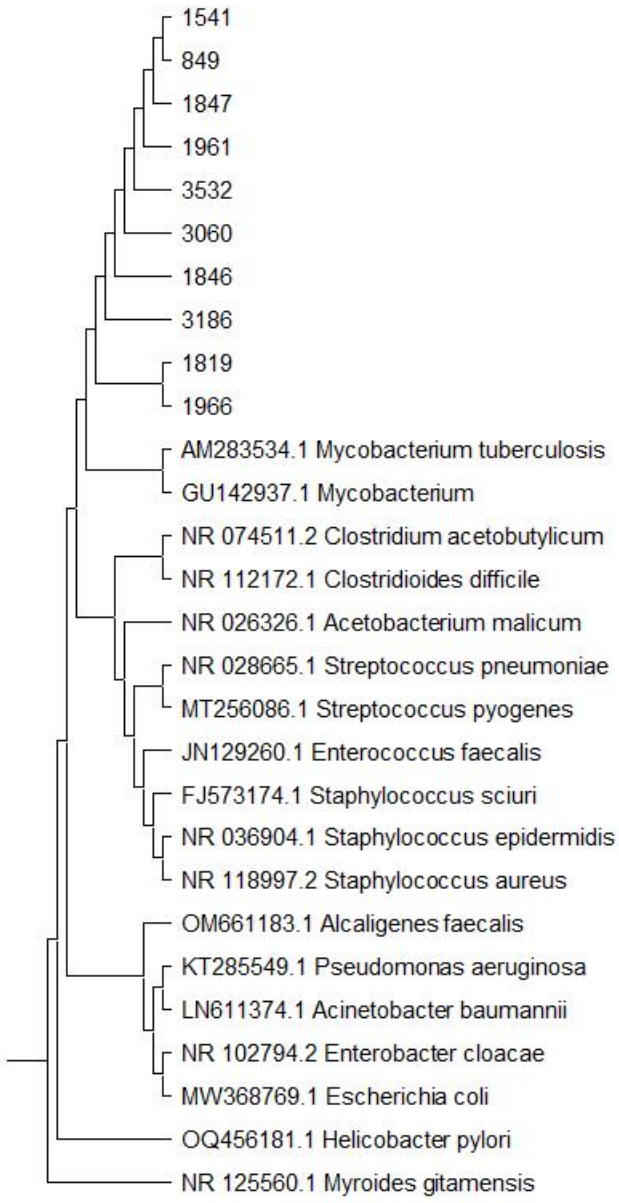

Figure 4.3: Phylogenetic analysis showing the genetic relatedness between the isolated mycobacteria and reference sequence generated from the NCBI data base

Table 4.26: Genetic distance analysis showing the genetic relatedness between the isolated mycobacteria and reference sequence generated from the NCBI data base.

	3186	1846	3060	3532	1961	1847	1541	1819	1966	849
3186										
1846	0.0000									
3060	0.0000	0.0000								
3532	0.0000	0.0000	0.0000							
1961	0.0000	0.0000	0.0000	0.0000						
1847	0.0000	0.0000	0.0000	0.0000	0.0000					
1541	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000				
1819	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007			
1966	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0000		
849	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0007	0.0007	

GTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC
GGAAAGGTCTCTTCGGAGATACTCGAGTGGCGAACGGGTGAGTAACACGTGGGTGA
TCTGCCCTGCACTTCGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATAGGACCA
CGGGATGCATGTCTTGTGGTGGAAAGCGCTTTAGCGGTGTGGGATGAGCCCGCGGC
CTATCAGCTTGTTGGTGGGGTGACGGCCTACCAAGGCGACGACGGGTAGCCGGCCT
GAGAGGGTGTCCGGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGC
AGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGGG
GGATGACGGCCTTCGGGTTGTAAACCTCTTTCACCATCGACGAAGGTCCGGGTTCTC
TCGGATTGACGGTAGGTGGAGAAGAAGCACCGGCCAACTACGTGCCAGCAGCCGCG
GTAATACGTAGGGTGCGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGG
TGGTTTGTCGCGTTGTTCGTGAAATCTCACGGCTTAACTGTGAGCGTGCGGGCGATA
CGGGCAGACTAGAGTACTGCAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATG
CGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCAGTAACTGAC
GCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGGTGGGTACTAGGTGTGGGTTTCCTTCCTTGGGATCCGTGCCGTAGCTAA
CGCATTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGA
ACCTTACCTGGGTTTGACATGCACAGGACGCGTCTAGAGATAGGCGTTCCCTTGTGG
CCTGTGTGCAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTGTCTCATGTTGCCAGCACGTAATGGTGGGGACTC
GTGAGAGACTGCCGGGGTCAACTCGGAAGGTGGGGATGACGTCAAGTCATCATGCC
CCTTATGTCCAGGGCTTCACACATGCTACAATGGCCGGTACAAAGGGCTGCGATGCC
GCGAGGTTAAGCGAATCCTTAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTC
GACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAA
GCCAGTGGCCTAACCCTCGGGAGGGAGCTGTCGAAGGTGGGATCGGCGATTCT

Figure 4.4: 3186 *Mycobacterium tuberculosis*

4.2 Discussion of Findings

The prevalence of Mycobacterial infection couple with its associated risk factors among inmates in any prison of developing countries is of outmost concern and it is constituting a great deal of hindrance in its eradication. Although, Prisons are increasingly becoming breeding grounds from which infection is transmitted to the general population and accounts for up to 25% of a country's TB burden WHO[1]. From this study,the overall prevalence of Mycobacterial infections in Agodi prison was found to be 21%,andthis is slightly higher than the general population.The high prevalence of Mycobacterial infections in custodial centers, show case the importance of policy formulation and its implementation followed by systematic Tuberculosis screening program, this high prevalence could have huge effect on the TB control, for the reason of spread from prisoners and discharged inmates to staffs and visitors, B. Moges [2]. The bivariate analysis revealed that respondents' educational status (p=0.000) and HIV status (p=0.000) were statistically significant when related with age, this finding is in agreement with studies of P.Nyasulu & D.S Abebe,[3].The youths are the strength of tomorrow of any nation,but it is sad to note from the above result that, greater percentage of them were dying with tuberculosis in custodial centers. Only 13(6.5%)of the culture got contaminated and this in agreement with FMOH'scontamination rate[4].When the relationship between culture and variables in the questionnaire were calculated, it was discovered that respondents' who have cough for two or more weeks (p=0.000), who have noticeable weight loss (p=0.005), who have excessive sweating at night for two or more weeks(p=0.008), and previously treated for TB (p=0.020) were statistically significantly associated.As stated above, cough for more than two weeks is significantly associated with TB, this finding is in tandem with the studiesof A.C.M Lemus,D.S Abebe,& B.Seri,[5,3B,5B].Also, Weight loss and Malnourishment has much to do with TB in that

they were statistically significantly associated with p-values of 0.005 and 0.048 respectively.This is in agreement with the findings of S.Banus,S.X,Jittimanee,& B.Moges, [6,6B,2]. Commenting on the number of inmate in a cell which is the same as overcrowdinalthough very glaring as noted during one of my visit to the centerthat I peeped and later confirmed from the frequency of inmates of21-30 in a cell. There seem to be no significant association, which surprisingly tally with the study of P.Nyasulu[3]. But, in fact, the progression of TB bacilli involves both exogenous and endogenous risk factors, with the former playing a key role and paramount is the distance of the individual to a productive TB patient with the bacillary load in the sputum, hence my inquisitiveness[9]. In all, Tobacco use currently or any time during previous five years, Hypertension, Illicit drug use within past weeks and BCG vaccination revealed no significant association with TB whatsoever.This study under lines cogent strengthand weaknesses.For purpose of elucidation, this study may be the first to document the high burden of TB in Agodi Custodial Center Ibadan.Very much attention seemed not focused on the welfare of inmates in most regions of the world,B.M.Prasad &B.Thapz[7]. Nigeria in terms of policy formulations is very good but, in implementing it to letter, she becomes deficient. This study findings clearly stated the importance of periodic and timely screening of TB (which is the practice in Agodi prison, that arouse my curiosity toformulate hypothesis to experiment) and timely application of its treatment to control the transmission of the infectionM. Dara & C. D. Acosta, [8]. Proper screening of TB symptoms is advocated for, in all newly admitted inmates.

Table 4.21in the result chapter clearly indicated and spelt out the prevalence of Mycobacterial infections, out of 42(21%) of the 200 inmates that were screened duringthe study, 20 (10%) were Mycobacterium tuberculosis disease, 10 (5%) came up to be Mycobacterium bovis and 12 (6%) were Non Tuberculous Mycobacterium (NTM).In Table 4.1, when the DST (drug susceptibility

testing) was analyzed using both proportion method and LPA (Line Probe Assay)method, it was discovered that out of 30 isolates ran, 21 (70%) were LPA-RIF, while 23 (76.6%) were Proportion RIF, this simply showed that more resistance was possible with the proportion method. Also, 2 (6.7%) with proportion method showed resistance to Floroquinolones, but only 1 (3.3%) with LPA showed resistance to Floroquinolones,this simply depicts that Pre XDR TB higher with Proportion method than LPA.

Table 4.23, goes further to bring out the resistant genes in Mycobacterium tuberculosis and Mycobacterium bovis. Overall, rpob gene dominated, which is an evidence of MDR (Multi drug resistance).The characterization of Non Tuberculous Mycobaceria was done to know the possible species and in all, 10 of the NTM were Mycobacterium fortuitum and 2 of the NTM were Mycobacteria chelonie.

Chapter Five

Conclusion and Recommendation

5.1 Summary of Findings

Siiiiiiiiiiiiiiiiiiiiiiiii

5.2 Conclusion

A second look at the significant associated risk factors of Tuberculosis and other Mycobacterial infections in this studythat was conducted in Agodi Custodial Center, give some indications of where targeted interventions must be focused. For instance, in respondent with cough up to two or more weeks, there must be proper and adequate establishment of infection control program. Like-wise in respondent with noticeable weight loss, a qualified Nutritionist must be involved in the preparation of the inmates meal/food, the policy of punishing prisoners with non-nutritious and sub-standard food must stop. In respondent with excessive sweating, the infrastructure of prisons mustchange to the modern construction of well ventilated cells as it is in developed continents of United State of America and United Kingdom. Furthermore, in respondent with HIV and previously treated TB the intervention must be inform of timely conducting of initial screening tests on all newly admitted inmates. Lastly, in inmate's respondent with Primary and Secondary educational status, there must be constant public enlightenment on TB and career talk, not only in prisons, but as well in schools.

Prisons therefore areideal environments for Tuberculosis infection control, Mycobacterial infections and other infections control. In planning and implementing TB control, prison health servicestherefore, must take advantage of the special features of the prison environment.Having

prisoners all in one place facilitated identification of prisoners with Mycobacterial infections, promotion of adherence treatment, and accurate recording and reporting. Some inmates had little access to health care in the community. For these people, a prison with effective health care services could provide an opportunity for access to health care, including TB.Hence, the opportunity for effective TB control in prisons is an opportunity to contribute to effective TB control in the general population.

5.3 Recommendations

Implementation of infection prevention program in prisons at various level of environmental, administrative and personal aspect is highly recommended, for instance, isolation of infected inmates and protection of medical staff.

Full implementation of DOTS and incorporating the elements of the stop TB strategy in prisons will serve as the entry point for improved health services.(The last target of which is by 2050,eliminate TB as a public health problem.

Agodi Custodial Center must assume financial responsibility for the training of their personnel.

There must be keeping of adequate record on their consumption and submission of timely indent for quarterly supply with consumption report.

They must support supervisory and mentoring visits by the State Tuberculosis and Leprosy Control Officer whenever necessary.

5.4 Contribution to Knowledge

The findings of the study will help to develop prevention and control strategies for Mycobacterial infections and its associated factors prisons. This intervention will go a long way

in eradicating TB and other mycobacterial infections by the year 2030 as proposed by the World Health Organization (WHO).

5.5 Suggested Area for Further Research

Next Generation Sequencing (NGS) is needed to elucidate the drug resistance mutations and strains genotypes among the Mycobacterium species isolated from the inmates.

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Appendices

Appendix I

Preparation of Slants from Commercial Lowenstein Jensen Medium

1. Weigh and dissolve 37.4g of commercial LJ medium powder in 600ml of distilled water, add 12ml of glycerol (depending on LJ manufacturer).
2. Mix thoroughly.
3. Heat with frequent agitation just until the medium boils.
4. Autoclave at 121°c for 15 minutes.
4. Cool to approximately 50°c. Keep warm so that it does not set.

Fresh hens egg not more than 6 days old are cleaned by scrubbing thoroughly with a hand brush in warm water and a plain alkaline soap. Let the eggs soak for 30 minutes in the soap solution. Rinse eggs thoroughly in running water and soak in 70% ethanol for 15 minutes. Before handling the clean dry eggs scrub the hands and wash them. Crack the eggs with a sterile knife into a sterile 800mls flask. Add sterile beads, cover and mix vigorously, then sieve it with a 4 layered cotton gauze into another sterile beaker.

1. Add 1liter of filtered eggs into the warm solution in step 5, mix and aliquot in 7.0ml amounts in sterile universal containers or 15mls Falcon tubes.
2. On a preheated inspissator at 85°c, set the universal containers with the medium on a wooden rack.
3. Leave for 45-60 minutes to coagulate.

After coagulation remove from inspissator and cool

Quality Control and Quality Assurance: Each tube of LJ prepared is label with the date of preparation, expiration date and lot number. This can be done by printing stickers and printing on the side of the slant.Sterility check shouldbe performed on a representative sample of LJ by incubating at 37°c for 24 hours, this must be documented.

Performance checks should be done on each lot by inoculating with control strains of MTBC and NTM. The time and quality of growth should be documented.

Appendix ii

Pyruvic Acid Medium: All the above is the same accept the substitution of Glycerol for Pyruvic Acid.

Appendix iii

Basic fuchsin Stain

1. Weigh out 10g of basic fuchsin powder.
2. Measure out 100ml of 99.9% absolute ethanol, and mix with the fuchsin.
3. Weigh out 50g of phenol crystal
4. Dissolve in 900ml distilled water and label as solution B.
5. Combine solution A and B together to get stock solution.
6. Place in a water bath at 100^0
7. Allow stain to cool.
8. Filter in 1.0l glass sterile bottle and dispense in labelled staining washing bottle.
9. Record the date of preparation, the batch number, name of person preparing the media and the expiry date on the bottles.
10. Conduct quality control on stain, if the quality control is passed, stain is ready for use.
11. Complete and sign media preparation worksheet.

Quality Control

- Test new batch of stain with positive and negative control smears.
- Controls should give the same result as the current batch of reagents used.

Appendix iv

Preparation of 3% Acid/Alcohol

1. Measure out 970ml of absolute ethanol and label as solution A.
2. Measure out 30.0ml of Hydrochloric acid label as solution B.
3. Add solution B into solution A in a 1.0 liter sterile flask.
4. Record the date of preparation the batch number, person preparing the reagent and the expiry date on the label.
5. Conduct quality control on reagent.

6. If quality control is passed, reagents are ready for use.
7. Complete and sign media preparation worksheet.

Quality Control– As above

Appendix v

Preparation of 0.1% Methylene Blue Solution

1.Weigh out 1.0g of methylene blue powder

2.Measure out 1.0l of distilled water.

3. Dissolve the methylene blue powder in the distilled water in a volumetric sterile flask.

4. Place in a water bath at 100^{o}c to dissolve stain.

5. Allow stain to cool

6. Filter in 1.0l glass sterile bottle.

7. Record the date of preparation, the batch number, person preparing the reagents and the expiry date on the label.

8. Conduct quality control together on the new and old reagents staining two smear.

9. If quality control is passed, reagent is ready for use.

10.If not, perform investigation and corrective actions.

11.Complete and sign stain preparation worksheet.

Appendix vi

Preparation Of Auramine O.Phenol Solution

1. Weigh out 0.3g of Auramine O.

2. Measure out 100ml of Phenol and warm to 40C.

3. Dissolve the Auramine O in the warm Phenol ,adding slowly and shaking vigorously for 10 minutes in a volumetric flask.

4. Filter stain and store in a dark brown bottle.

5. Allow stain to cool.

6. Record the date of preparation,the batch number person preparing the reagent and the expiry date on the label.

7. Conduct QC on the reagent.

8. If QC is passed,reagent is ready for use.

9. Complete and sign media preparation worksheet.

10. Use within 3weeks of preparation.

Appendixvii

Preparation Of 0.1% Potassium Permanganate Solution

1. Wejgh out 0.1g of potassium permanganate .
2. Measure out 100ml of distilled water.
3. Dissolve the KMNO4 in the distilled water in a volumetic flask.
4. Label with name of reagent,person preparing it and date of preparation.
5. Store in a brownbottle at room temperature and use up to 3months.

Appendix viii

Preparation of Sodium Hydroxide-Sodium Citrate

Solution A

1. Weigh out 20g of NaOH pellet.
2. Measure out 500ml of distilled water and place in a volumetric flask.
3. Add pellet to distilled water and mix to dissolve.
4. Label as Solution 1(4%NaOH).

SolutionB 2.9% Na-Citrate

1. Weigh out 5.8g of Sodium citrate.
2. Measure out 200ml distilled water and place in a volumetric flask.
3. Add salt to distilled water and mix to dissolve.

Working Solution of NaOH-Citrate

1. Combine equal volume of Solution 1 and 2 in 500ml volumetric flask.
2. Label as working solution (NaOH/Citrate) with date of preparation.
3. Autoclave at 121C for 15 minutes.

4. Refrigerate at 2-8C and use within 1 week.

Nalc-Naoh-Citrate

1. Weigh out required amount of NALC as shown in the table below.
2. Add to Sodium hydroxide-Citrate solution.
3. Use within 24hours.
4. Discard unused portions.

TOTAL VOLUME NEEDED(ML)	NaOH-NaCITRATE(ML)	NALC(g)
50	50	0.25
100	100	0.50
200	200	1.00
400	400	2.00

Quality Control and Quality Assurance

1. Each bottle of solution is labelled with the name of the reagent, date of preparation and lot number.
2. Sterility check should be performed on the solution by inoculating at 37C for 24hours and must be documented.
3. Store reagent at 2-8C.

Appendix ix

Preparation Of Phosphate Buffer

A-Stock Alkaline Buffer

1. Weigh out 4.73g of NaHPO4 and place in a 1.0Lvolumetric flask.
2. Measure out 500ml of distilled water and add to salt.
3. Swirl to dissolve.
4. Label as NaHPO4.

B-Stock Acid Buffer

1. Weigh out 4.53g of KH2PO4 and in a volumetric flask.
2. Measure out 500ml distilled water and add to in a flask.
3. Swirl to dissolve.
4. Label as stock acid buffer(KH2PO4)

C-Working Phosphate Buffer, PH 6.8

1. In a 2.0Lvolumetric flask, combine equal volume of stock alkaline and acid buffer.
2. Check Ph and adjust as necessary by adding more of one or the other of the stock buffer.
3. Autoclave at 120C for.
4. Label as working phosphate buffer solution, batch number and date of preparation.
5. Perform sterility check and store at 2-8C.
6. Discard after, if not finish after a week.

Quality Control :As in previous solution.s

Appendix x

Table For Grading of Microscopy

IUATD/WHO SCALE	BRIGHT FIELD (1000X)	FLUORESCENCE (200X)	FLUORESCENCE (400X)
	1 length =2cm 100HPF	1 length=30 field= 300HPF	1 length= 40HPF
Negative	Zero AFB/ 1length	Zero AFB/ 1length	Zero AFB/ 1length
Actual count	1-9/100 HPF	1-29 AFB /1length	1-19 AFB / 1length
1 +	10-99AFB/100HPF	30-299 /1length	20-199 AFB/1length
2 +	1-10 AFB/HPF	10-100 AFB /HPF on	5-50 AFB /HPF
3 +	>10 AFB/HPF	>100 AFB /HPF on average	>50 AFB /HPF

Appendix xi: CharacterizationOf Non Tuberculous Mycobaceria

TEST TYPE NTM NAME	RATE OF GROWTH	GROWTH TEMPERATURE	PIGMENT PRODUCTION	NITRATE REDUCTION	GROWTH ON 4-PNB ACID
M.fortuitum	Rapid (4days)	25 degree C	Non	Positive	Positive
M.chelonei	Rapid (4days)	25 degree C	Non	Negative	Positive

Appendix xii; Quantification Scale for Culture

QUANTIFICATION SCALE

Number of colonies	Quantification code
None	0
Contamination	C
<50 colonies	Actual count
50-100 colonies	1+
100-200 colonies	2+
>200,almost confluence	3+
Confluence growth	4+

Appendix xiii: Table illustrating the heating cycles used in PCR reaction

STEPS	Temperature	Time	Sample Type:Culture	Sample Type:Clinical
1	95^0C	5 minutes	1 cycle	1 cycle
2	95^0C		10 cycless	10 cycle
	58^0C			
3	95^0C		20 cycle	30 cycle
	53^0C			
	70^0C			
4	70^0C		1 Cycle	1 cycle

Bio Data

A . Personal Data:

1, Full Name:	Adegoke Olusola Andrew
2. Contact Address:	Bukola Moses Drive, Temidayo lane 1 Ajadi Ologuneru, Ibadan, Oyo State.
3. Email Address:	andybee4000@yahoo.com
4. Phone Number:	08055373564, 08134572175
5. Date of Birth:	14th December 1967
6. Place of Birth:	Ibadan
7. Nationality:	Nigerian
8. State of Origin:	Osun
9. Local Government Area:	Boluwaduro
10. Religion:	Christianity
11. Next of Kin:	Mrs Adedunni Oluwabimpe Adegoke
12. Next of Kin's Contact Address:	Bukola Moses Drive Temidayo Lane 1, Ajadi Ologuneru, Ibadan
13. Next of Kin's Phone Number:	08052432144

B. Education Background:

Educational Institution Attended with Dates and Qualification:

School Attended	Dates	Qualifications
• St Cyprian Catholic Primary School	1973-1979	Primary School Certificate
• Estate High School, Akobo, Ib.	1980- 1985	West Africa School Certificate
• School of Medical Laboratory	1987-1990	Medical Laboratory Technician Certificate
• Sch. of Med. Lab. Sci. UCH. Ib.	1992-1996	Associate M.L.S.C.Nig.
School. of Med. Lab. Sci.UCH.Ib	2019-2021	Fellow Med. Microbiology.
• Lead City University, Ibadan.	2021-2023	M.Sc in view

C. Working Experience with Date:

- Acelab Diagnostic Center, Ibadan 1991
- Gamma Lab. Limited, Ibadan 1992
- General Hospital Kebbi State 1997-1998
- O.L.A. Catholic Hospital Oluyoro 1999-2011
- University College Hospital. 2011 till date.

D. Publication

- Olugbenga Enoch Olabiyi[a b], Pius Abimbola Okiki[a c] **,** Mumuni Idowu Adarabioyo

E. Date and Signature ----------

The University Compliance Certification

This is to certify that, this Thesis written by**Olusola Andrew, ADEGOKE with Matric No. LCU/PG/002537**in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan is in full compliance with the approved University format and style.

_____ _____

Signature **Date**