COMPARISON OF LED FLUORESCENT MICROSCOPY, ZEIHL-NEELSEN AND GeneXpert ASSAY FOR DIRECT DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX IN SPUTUM IN JOS PLATEAU STATE, NIGERIA

BY

Augustina Ifunanya AGUOKOLI

(17/27/MMI001)

A THESIS SUBMITTED TO THE MICROBIOLOGY UNIT,

DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLOGY,

COLLEGE OF PURE AND APPLIED SCIENCES KWARA

STATE UNIVERSITY MALETE.

IN PARTIAL FULFILMENT OF TH E REQUIREMENTS FOR THE AWARD OF MASTERS DEGREE OF SCIENCE (M. Sc.) DEGREE IN MICROBIOLOGY.

NOVEMBER, 2019

DECLARATION

I AugustinaIfunanya AGUOKOLI hereby declare that this research work titled "comparison of led fluorescent microscopy (led-fm), Zeihl-Neelsen (zn) and GeneXpert assay (xpert) for direct detection of mycobacterium tuberculosis complex in sputum in Jos plateau state, Nigeria" was researched and carried out by me under the supervision of Prof. O. Adedayo.

_____ _____

AugustinaIfunanya AGUOKOLI Date

17/27/MMI001

DEDICATION

This research work is solely dedicated to almighty God who has make it easy from the beginning to the end and also to my husband for his overwhelming support all through the programme.

ACKNOWLEDGEMENTS

I wish to sincerely express my unreserved gratitude to God for His amazing grace and strength granted to me to complete this research work. I use this opportunity to express my profound gratitude and deep regards to my guide and supervisor, Professor O. Adedayo for his exemplary guidance, monitoring and constant encouragement throughout the course of this research. My appreciation also extends to my ever loving husband for his love and support towards my career may God continue to bless you for me in Jesus mighty name (Amen). I also take this opportunity to express a deep sense of gratitude to my mentor, boss and guardian Dr Elisha and his lovely wife Mrs Elisha for their unmeasurable support, from the beginning to the end, this day might not have come into play without them, am truly grateful. I would love to show gratitude to my other lecturers, Dr. S. Awe, Dr.Adetumbi, A.T. Ajao., Dr.(Mrs) A.E. Ajiboye and Dr.(Mrs) M.R. Adedayo.

My acknowledgement cannot come to an end without appreciating some very important people for their moral, physical and spiritual supports during this research work, they include Dr.Mrs.Bunmi Jetta Winter, Dr. Freeman, Prof .Bello, Mr Philemon, Dr. Abayomi and Mr. Adedeji. Finally, I really appreciates the entire Aguokoli family especially my most able mother, thanks mama, I love you ma.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

This study compared the effectiveness and sensitivity of three different diagnostic methods of identification of pulmonary tuberculosis. These were Led fluorescent microscopy, Zeihl-Neelsen staining technique and GeneXpert assay for detection of the bacterium in sputum specimen. A cross sectional study was conducted among 435 patient suspected to have pulmonary tuberculosis at the Bingham Teaching Hospital Jos Plateau State Nigeria. The patients sputum were collected over three months period, 196 (45%) were males while 239 (55%) were females. For all specimens' collected Z-N detected 54 (12.4%) positive cases, FM detected 46 (10.6%) positive cases while the GeneXpert detected 57(13.1%) positive cases. All patients were subjected to test for human immunodeficiency virus (HIV), only 55 (28.1%) of males were HIV positive while 94 (39.3%) females were HIV positive. Although the identification identification techniques used were found to be specific, the result showed that GeneXpert was most sensitive and accurate than the other two. Consequently health care professionals may be encouraged to use the GeneXpert more since it very fast, more realable and it can also detects the resistance of rifampicin in the sample alongside with detection of the bacterium.

CHAPTER ONE

INTRODUCTION

Tuberculosis is an infectious disease usually caused by *Mycobacterium tuberculosis*;which affects the lungs but can also affect other parts of the body. Most infections are asymptomatic, in which case it is known as latent tuberculosis (Bajramiet al., 2016). In 2016, 10.4 million people fell ill with tuberculosis, in the world and 1.7 million died from the disease including 0.4 million among people with HIV (W.H.O 2016).An estimated 250,000 children died in the world fromtuberculosis in 2016 including children with HIV associated tuberculosis.

Tuberculosis happens to be world's most infectious deadly killer with about 4500 lives lost per day and unfortunately; Nigeria is far worse hit by this global epidemic in Africa (W.H.O 2018).Nigeria currently ranked 4 in the world and 2 in Africa with the highest burden of TB/HIV and multidrug resistant tuberculosis, pulmonary tuberculosis is a disease of the respiratory system.

Nigeria is among the 14 high burden countries for TB, TB/HIV and Multi Drug Resistant TB, The country is ranked seventh among the 30 high TB burden countries and second in Africa (Aliyuet al., 2013). The problem of TB in Nigeria has been made worse by the issues of drug resistant TB and the HIV/AIDS epidemic (Skrahinaet al., 2013; Omo-Emmanuel et al., 2017). It is estimated that 407,000 people in Nigeria have TB each year. In addition there are an estimated 63,000 HIV positive people that get TB each year. An estimated 115,000 HIV negative people die from TB in Nigeria each year and an estimated 39,000 HIV positive people also die. It is difficult to appreciate what it means for 154,000 people to be dying each year from tuberculosis.

Tuberculosis (TB) is one of the leading causes of death due to an infectious agent, Ninety-five per cent of cases occur in the developing world where diagnostic and treatment facilities are rudimentary or non-existent (Butt et al., 2014). Early diagnosis is essential for TB control. However, regions with the highest burden of tuberculosis also lack the resources to institute effective control measures. Despite the recent advances in biotechnology, a rapid, accurate and inexpensive diagnosis of tuberculosis remains elusive. Existing laboratory tests either have low diagnostic accuracy or they are too sophisticated and expensive to be of any practical use in developing countries (van Ingenet al., 2012) there is an urgent need for rapid but sensitive diagnostic tests for tuberculosis which are also simple and cost-effective.

There are various methods for bacteriological diagnosis of tuberculosis; the smear examination technique which is believed to be simple, cheap, quick and practicable and effective case finding method for developing countries. As *Tuberculosis bacilli* are very slow growing organisms, culture results are available after a period of three to six weeks but microscopic examination has the advantage of the giving a result at once. The specimen most commonly examined is sputum and mucous secretion coughed up from the lungs. Microscopic examination of Ziehl-Neelsen and Led fluorescent staining specimen identifies *M. tuberculosis*in less than an hour. Ziehl-Neelsen is the most extensively used procedure for the demonstration of *Mycobacterium tuberculosis* in sputum smear also knoawn as acid-fast bacilli (AFB). Microscopy is the key tool in the diagnosis of pulmonary tuberculosis all over the world.

In India, several million smears have been performed in the DOTS programme and stained by the Ziehl-Neelsen (Z-N) method as per the Revised National Tuberculosis Control Programme (RNTCP) guidelines. However, this staining procedure is cumbersome for laboratory technicians as it involves the laborious steps of filtering Carbolfuchsin for each use, applying external heat while staining with Carbolfuchsin, decolorization with acid, and counter

staining with methylene blue. Several modifications of this Z-N method have been explored in the past to simplify the procedure, with little or no success.

Led fluorescent staining is a smear stained with fluorescent stain called auramine,The advantages of Led fluorescence staining procedure is simpler and can be examined at a lower magnification than Z-N (40x Vs 100x), It does not require heat application compared to the Ziehl-Neelsen stain. It has been estimated that Led fluorescent microscope (LFM) may take up to 75% less time than ZN. This advantage would be a tremendous benefit for overburdened laboratory system in many low resource settings. The present prospective study was under taken to compare the sensitivity of Led fluorescence staining versus Ziehl-Neelsen,fuchsinstaining and GeneXpert in diagnosing pulmonary tuberculosis. Acid-Fast bacilli (AFB) sputum smear microscopy is an established low-cost screening and diagnostic procedure for identifying patients suspected of suffering from tuberculosis (TB) worldwide. Unfortunately, the sensitivity of AFB microscopy is low, ranging from 22 to 73.2% depending on many variables. The molecular method of identification is simplified by processing method and examination time which could be several minutes shorter compared to FM than ZN. The advancement in molecular biology and nucleic acid technology, human knowledge of genetic basis of diseases and have also provided opportunity for diagnostic tests based on genetic of the infecting organism. One of those tests is the real-time PCR test such as the GeneXpert MTB/RIF, This is a DNA probe and a polymerase chain reaction based test which allows the rapid detection of M. *tuberculosis* complex, It detects theDNA from the clinical specimen in two hours, The test also detect resistant to Rifampicin by detecting the specific gene responsible for the resistance.

Rapid and accurate diagnosis of tuberculosis (TB) is indispensable to adequately manage the disease and control its transmission. The routine application of nucleic acid amplification techniques for detection of *Mycobacterium tuberculosis* results in accurate

diagnosis of tuberculosis but requires laborious processing time and dedicated biosafety conditions Example is the GeneXpert MTB/RIF assaywhich is fully automated, walk-away real-time PCR-based assay with a time to result of approximately 2hours. To detect M. *tuberculosis* and mutations associated with resistance to rifampin (RIF), the 81-bpcore region of the rpoB gene is amplified and probed with five overlapping molecular beacons. Tuberculosis is identified when 2 probes gives a positive signal within a predefined number of cycles, The rifampicin resistance detection (RIF's) mutation is detected by lack of delayed onset of fluorescence ofat least one molecular beacon ,The only disadvantages of the GeneXpertMTB/RIF are its exceeding costs, M. *tuberculosis* assaysGeneXpert MTB/RIF was endorsed by the WHO in December 2010 and is generally recognized as break-through in TB diagnosis, for investigating patients who might have tuberculosis, especially in regions where multi drug resistance tuberculosis (MDR) and HIV infection are common (CDC, 2014). The Xpert MTB/RIF Assay is a highly sensitive, specific and rapid method for diagnosing TB which has potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity (Müller et al., 2015). Its usefulness in detecting sputum smear and culture negative patients' needs further evaluation in high burden TB and HIV areas under programmatic health care settings to ascertain applicability, cost-effectiveness, robustness and local acceptance. Early diagnosis and management of TB is also critical to reduce TB transmission in communities and health care facilities.

Tuberculosis been a major cause of death and serious illness in Nigeria, this as prompt urgent need for a screening test that is sensitive, cheap, reliable ,easy to manage and does not have time limitation for clinical specimen in suspected tuberculosis cases to help eradicate the disease globally, which enhance thepresent study objective which is to compare the identificationof *Mycobacterium tuberculosis* and flexibility and sensitivity of three different methods, two of which are smear staining method, the Z-N, FM, and the molecular

method,GeneXpert assay which was also used as a standard test.It is imperative therefore, that rapid accurate diagnosis of tuberculosis must be achieved for effective management and control of disease transmission in Nigeria. Light-emitting fluorescence microscopy (LED-FM) is relatively new method that is currently advocated by the W.H.O for rapid diagnosis of tuberculosis in low income countries because it is considered to be of higher sensitivity when compared to the age long Z-N method, the FM method is relatively cheap and required limited expertise.

The World Health Organization has focused resources toward the goal of early diagnosis and treatment. Subsequently, W.H.O along with other donor organizations has combined efforts to provide financial and technical assistance to resource-limited nations such as Nigeria. Equipment and materials for both LED-FM and GeneXpert have been provided to regional hospitals in the tuberculosis endemic countries which include Nigeria. Luckily, Bingham University Teaching Hospital is one of the beneficiaries of the program. Currently, the hospital is in possession of one LED-FM Microscope and GeneXpert equipment. All the accessories and consumables were also supplied.

It is important that as scientists establish, through research, our peculiar experiences with the three diagnostic, the recommendations may provide the needed help and statistics to other centers across the country. Identification and prompt treatment of infected individuals are considered the central efforts in eradication of Tuberculosis worldwide. Tuberculosis is a curable disease, often people do not access services quickly enough, or are in other ways unable to get the help they need to stay healthy, live long and have productive lives, recently there has been an increase of drug resistant tuberculosis, including multidrug (Gerardo et al., 2012) which are extensively difficult to treat and contribute to increased mortality.

Mycobacterial culture is the gold standard means of identification, It is highly sensitive but slow way to diagnose TB due to the slow growing method of the organism (WHO 2010).To

halt the disease's spread, it is essential that TB-particularly TB that is resistant to several treatment drugs (multidrug-resistant, or MDR, TB) to be diagnosed quickly.

1.1 STATEMENT OF RESEARCH PROBLEM

The method of detecting tuberculosis are not reliable and have different rate of sensitivity. Most of the time, we have late detection of tuberculosis due to inadequate diagnostic methods and difficulty in identifying latent manifestation of tuberculosis which has contributed to the death rate and increase chance of transmission of the causative agent of tuberculosis in low income countries such as Nigeria.

1.2 JUSTIFICATION FOR THE STUDY

- The gold standard for diagnosis of tuberculosis infection is the microbiological identification of *Mycobacteria tuberculosis* in culture. However, culture of *Mycobacteria tuberculosis* demands certain amount of safety management that is general beyond the scope of most hospitals in resource limited countries such as Nigeria. Culture incubation period takes too long which is time consuming.

- This research provide useful information on prevalence of tuberculosis as a disease diagnostic method like GeneXpert and direct detection of mycobacterium and co infection in HIV patients in Nigeria, the rest of Africa and the world at large and for building a framework to protecting the spread of TB across all age group.
- The World Health Organization has recently endorsed the implementation of light-emitting diode (LED) Fluorescent microscopy and GeneXpert MTB/RIF assay for national tuberculosis programs in developing countries.

❖ 1.3GENERAL OBJECTIVE

To compare ZN and FM sputum smear methods to GeneXpert DNA-based-method in detecting Mycobacteria tuberculosis inthe sputum of suspected of Tuberculosis.

1.3.1 SPECIFIC OBJECTIVE

- ❖ To determine the sensitivity of ZN and FM sputum smear methods
- ❖ To compare the relative sensitivity of the two sputum staining methods with the GeneXpert DNA identification method.
- ❖ Determine the HIV status of all the suspected tuberculosis patients.

1.3.2 RESEARCH HYPOTHESES

There is significant difference in the use of ofZiehl-Neelsen, led florescence microscope techniques andGeneXpert for direct detection of Mycobacterium tuberculosis.

CHAPTER TWO

LITERATURE REVIEW

2.1 MYCOBACTERIUM

Mycobacterium is a genus of Actinobacteria,given its own family,the Mycobacteriaceae.Over 190 species are recognized in this genus.This genus includes pathogens known to cause serious diseases in mammals,including tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*) in humans. The Greek prefix myco-means fungus alluding to the way mycobacteria have been observed to grow in a mold-like fashion on the surface of a culture media. It is acid fast and cannot be stained by Gram stain procedure. (King et al., 2017)

Scientific classifications are Domain: Bacteria, Phylum: Actinobacteria, Class: Actinobacteria, Order: Actinomycetales, Family: Mycobacteriaceae, Genus: Mycobacterium, Species: M. tuberculosis (Ratogiet al., 2012). M. tuberculosis is part of a complex that has at least 9 members: M. tuberculosis *sensustricto*, M. *africanum*, M. *canetti,* M. *bovis*, M. *caprae*, M. *microti,*M.*pinnipedii*, M. *mungi*, and M. *orygis*.(Friedrich et al., 1898), it requires oxygen to grow, does not produce spores, and is nonmotile. M. *tuberculosis* divides every 15 – 20 hours, this is extremely slow compared with other bacteria, which tend to have division times measured in minutes (Koshet al., 1882). Mycobacteria are immobile, slow-growing rod-shaped, gram-positive bacteria with high genomic G+C content (61-71%). Due to their special staining

characteristics under the microscope, which is mediated by mycolic acidic in the cell wall, they are called acid-fast. It is also the reason for the hardiness of mycobacteria (Adam et al., 1926)

Mycobacteria can be classified into several major groups M. tuberculosis complex, which includes M. tuberculosis, M. *bovis*, M. *africanum*, and M. *microti*, M. *leprae*. The nontuberculous mycobacteria (NTM) includes all other mycobacteria, which can cause some pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, or disseminated disease.

Tuberculosis infection usually arise from patients, who suffer from active infectious pulmonary tuberculosis, the risk of infection is increased by poor hygiene conditions and in a densely populated areas. As the pathogens infect cells of the immune system, called macrophages, infants and immunocompromised persons are at higher risk, in most cases the immune system succeeds in fighting the bacteria or in encapsulating them, mycobacteria can then persist in the body for several years as latent tuberculosis without causing any symptoms (Dutta et al., 1899).

The group of nontuberculous mycobacteria (NTM), formerly called atypical or ubiquitous mycobacteria, contains over 150 species. NTM can be found ubiquitously in nature and show a broad diversity regarding where they can be found and how they adapted to certain environmental conditions. They can be detected in soil, ground and drinking water as well as in food like pasteurized milk or cheese, in general, NTM are less pathogenic. Nevertheless, they can cause illness in humans, especially in immunocompromised persons or those who suffer from previous pulmonary diseases (Mitnick et al., 2016). Mycobacteria differ so strongly from other bacteria in their cell wall architecture and metabolism that they require specific diagnostic tests, i.e. stains, culture media, identification methods.

Tuberculosis in cattle and humans is also caused by *Mycobacterium bovis*, while *Mycobacterium africanum* is a rare cause of human tuberculosis in central Africa. These pathogenic species exhibit some phenotypic differences; they are genetically very similar and are therefore often classed as the '*Mycobacterium tuberculosis* complex'. Other pathogenic mycobacteria include the *Mycobacterium avium* complex (MAC) and the non-tuberculous mycobacteria (NTM) atypical mycobacteria, or environmental mycobacteria).They are opportunistic pathogens, particularly in immunosuppressed individuals (Gcebe et al., 2017).

Mycobacterium species growth differs, some grow on very simple substrates, using ammonia or amino acidsas nitrogen sources and glycerol as a carbon source in the presence of mineral salts, optimum growth temperatures vary widely according to the species and it ranges from 25 °C to over 50 °C, other species such as *M. ulcerans* and *M. paratuberculosis* produce visible growth on a solid medium only after a month or more in culture (Whittington,1999).

There has been extensive study on the growth and nutritional requirement of M. *tuberculosis* since the organism was discovered. What is known is that the organism utilizes lipids as the main source of carbon during clinical infection. However, genes that encoded for disaccharide transporter were found essential for the organism during the first week of infection suggesting there may be a switch of its main source of carbon from carbohydrate to lipids with the onset of adaptive immune response (Fritz et al., 2017). Like most other bacteria, M. *tuberculosis* requires hydrogen, oxygen and source of electrons. Phosphorus, Nitrogen and Sulphur are other required elements.

Most mycobacterium species, including most clinically relevant species, can be cultured in blood agar. However, some species grow very slowly due to extremely long reproductive cycles, M. *leprae*, may take more than 20 days to proceed through one division cycle, making

laboratory culture a slow process. In addition, the availability of genetic manipulation techniques still lags far behind that of other bacterial species. A natural division occurs between slowlysand rapidly growin g species. Mycobacteria that form colonies clearly visible to the naked eye within 7 days on subculture are termed rapid growers, while those requiring longer periods are termed slow growers.

Tuberculosis has existed throughout history, but the name has changed frequently over time. In 1720, though, the history of tuberculosis started to take shape into what is known of it today; as the physician Benjamin Marten described in his theory of Consumption (Bragazzi et al.,2017) tuberculosis may be caused by small living creatures transmitted through the air to other patients. The BCG vaccine, which was derived from M. bovis, has had limited success in preventing tuberculosis (O'Neillet al., 2018).

2.2 MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis (Gordon and Parish, 2018) It was first discovered by Robert Koch, M. *tuberculosis* has an unusual, waxy coating on its cell surface primarily due to the presence of mycolic acid, this coating makes the cells impervious to Gram staining, and as a result, M. tuberculosis can appear either Gram-negative or Gram-positive (Saleebet al., 2011). Tuberculosis is spread mainly through the air droplets. When infectious people cough, sneeze, talk, laugh or spit; droplets containing *Mycobacterium tuberculosis* are sprayed into the air. People nearby may inhale the bacteria and become infected, *Mycobacterium tuberculosis* can remain viable as airborne droplet suspended in the air for a longtime or as part of house dust for weeks, however, transmission usually occurs (Abdel et al.,2007).

A person can be infected by *Mycobacterium tuberculosis* for many years without getting sick or spreading the organism to other people, if immune system is weakened by immunosuppressive disease like HIV infection, diabetes mellitus, malignancy, chronic kidney disease, extremes of ages, and immunosuppressive agent, latent tuberculosis infection can develop into active disease. If a person with active disease is untreated, he or she will infect on the average between 10 and 15 people every year. Tuberculosis accounts for 2.5%of the global burden of disease and is the commonest cause of death in young women, killing more women than all causes of maternal mortality (Olufemiet al.,2013).

Tuberculosis can be found all around the world and other than HIV/AIDS and it is one of the most frequent infectious diseases in HIV patients. Recent estimations suggest that one third of the world's population is infected with tuberculosis (Bloom et al., 2017). According to the WHO, each year more than nine million people are newly infected with tuberculosis and about two million die from it. About 95% of all newly infected patients live in developing countries, the facts that more and more resistant mycobacteria emerge and that co-infections with HIV are frequent make it even more difficult to fight (WHO 2016a). Acid-fast stains such as Ziehl-Neelsen, or fluorescent stains such as auramine are used instead to identify M. *tuberculosis* with a microscope. The physiology of M. *tuberculosis* is highly aerobic and requires high levels of oxygen, Primarily a pathogen of the mammalian respiratory system, it infects the lungs with warning signs of cough, hemoptysis, and chest pain, shortness of breath, fever, weight loss, and drenching night sweat. The most frequently used diagnostic methods for tuberculosis are the tuberculin skin test, acid-fast stain, culture, and polymerase chain reaction (Müller et al., 2015).

2.3.1 PATHOGENESIS AND PATHOLOGY

Most infections are due to inhalation of nuclei droplet containing the organism, other modes of infections have been reported for pulmonary tuberculosis while other forms of Mycobacterium can be transmitted by other routes example, M. *bovis* from injection of contaminated milk. Skin inoculation of M. *tuberculosis* from abrasion contaminations was not uncommon particularly among pathologists; venereal transmissions were sometimes encountered (Cudahy and Shenoi, 2016).

The initial focus of pulmonary infection is normally sub pleural in the mid lung zone i.e. the lower part of the upper lobe and the upper parts of the lower middle lobes. These are the areas of greater air flows that favor bacilli deposition and propagation (Camuset al., 2002). The initial pulmonary focus is almost always single, double foci are seen in about a fourth of the cases. Upon infection, the mycobacteria are injected by alveolar macrophages which may be able to eliminate a few organisms. Most of the bacilli continue to multiply inside the macrophages resulting in the death of the macrophages. Lymphocytes and monocytes with the circulatory system are attracted to these foci (Canetti et al., 1955). Lymphocytes quickly differentiate to macrophages injecting the released organisms. This process lead to pneumonitis. Infected macrophages are carried to regional lymph nodes such as hilar, mediastinal and sometimes supraclavicular nodes, in non-immune host, hematogenous spread may occur at this point, before the development of immunity (Tuberculin reactivity). There is uninhibited bacterial growth(Cudahy and Shenoi, 2016), in general, tuberculin activities occur 3-8 weeks following infection and established the development of cellular immunity and tissue hypersensitivity, for most people, the infection process ends at this level, the only future evidence of infection for them will be a positive skin test results (Cudahy and Shenoi, 2016). Mycobacterium are intracellular organisms with the ability to grow inside phagosome and phagolysosomes by preventing fusion of bacterial containing phagosome with lysosomes when macrophages are infected, this ability is attributable to supholipids located on bacterial cell

surface. Other important virulent factor includes the lipoglycans which modulate cytokines secretion as well as macrophages effector functions" The cell wall architecture clearly controls the ability of Mycobacteria to survive inside macrophages and control immune response of the infected host. Surface products such as phenolic glycolipids and supholipids may also protect the organism from extracellular killings by removing reactive oxygen molecules and by simply activating of macrophages (Murray et al., 2005;Cudahy et al., 2016).

2.3.2 EPIDEMIOLOGY

Tuberculosis is a global disease that has affected mankind for over 5000 years and still remains a leading cause of morbidity and mortality in most part of the world to this day. A disease of antiquity that has continue to cause great suffering and economic loss (JEFFREY et al.,2017). 80% of the disease burden is located in 22 countries, 17 of which belong to the low-income countries, tuberculosis infects over 9 million people yearly leading to about 2.3 million deaths. Drug resistance to the organism as well as co-infection with the human immunodefiency virus, HIV, are two driving forces for the re-emergence of the disease worldwide (Getahunet al., 2010). In 2011, there were estimated 8.7 million cases of the disease with 13% of them co-infected with HIV,same year, 1.4 million deaths were reported worldwide of which 1 million of them were HIV negative and about 400,000 were co-infected with HIV (WHO, 2014)

2.4 MYCOBACTERIM STRUCTURE

Mycobacterium cell wall is compose of outer lipids, mycolic acid, polysaccharides (arabinogalactan), peptidoglycan, plasma membrane, lipoarabinomannan (LAM), phosphatidylinositol mannoside, which is the cell wall skeleton. Mycobacteria are aerobic, they are bacillary in form, at least in most phases that have attracted human microbiological attention to date. They are straight or slightly curved rods between 0.2 and 0.6 μm wide and between 1.0 and 10 μm long. They are generally nonmotile bacteria, except for the species *Mycobacterium marinum*, which has been shown to be motile within macrophages. They are characteristically acid-fast. Mycobacteria have an outer membrane. They possess capsules, and most do not form endospores. M. marinum and perhaps M. bovis have been shown to sporulate, however, this has been contested by further research (Woodroffeet al., 2004). The distinguishing characteristic of all Mycobacterium species is that the cell wall is thicker than in many other bacteria, being hydrophobic, waxy, and rich in mycolic acids and mycolates. The cell wall consists of the hydrophobic mycolate layer and a peptidoglycan layer held together by a polysaccharide, arabinogalactan. The cell wall makes a substantial contribution to the hardiness of this genus. The biosynthetic pathways of cell wall components are potential targets for new drugs for tuberculosis (Michael et al., 2007).

Mycobacterium is classical acid-fast organisms, Stains used in evaluation of tissue specimens or microbiological specimens include Fite's stain, Ziehl-Neelsen stain, and Kinyoun stainetc. Mycobacteria appear phenotypically most closely related to members of Nocardia, Rhodococcus, and Corynebacterium, Mycobacteria are widespread organisms, typically living in water (including water treated with chlorine) and food sources. Some, however, including the tuberculosis and the leprosy organisms, appear to be obligate parasites and are not found as free-living members of the genus. Mycobacteria can colonize their hosts without the hosts showing any adverse signs example, billions of people around the world have asymptomatic infections of *M. tuberculosis.*

Mycobacterial infections are notoriously difficult to treat. The organisms are hardy due to their cell wall, which is neither truly Gram negative nor positive. In addition, they are naturally resistant to a number of antibiotics that disrupt cell-wall biosynthesis, such as penicillin. Due to their unique cell wall, they can survive long exposure to acids, alkalis, detergents, oxidative bursts, lysis by complement, and many antibiotics. *M. tuberculosis* produces a number of surface and secreted proteins that contribute to its virulence. However, the mechanism by which these proteins contribute to virulence remains unknown.

It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall which is rich in lipids such as mycolic acid and is likely responsible for its resistance to desiccation is a key virulence factor (Desaluet al., 2013).The cell walls of mycobacteria are very thick and consist of four layers. The innermost layer is composed of peptidoglycan and the others of lipids, the presence of lipid provides the bacteria with resistance to acid and alkaline environments and renders the cells relatively impermeable to various basic dyes, which need to be combined with phenol to allow penetration fof the cell wall. The cell wall composition renders mycobacteria hydrophobic, and as a result these bacteria tend to grow in aggregates that 'float' on the surface of liquid media. (Asiko et al., 2003).Other bacteria are commonly identified with a microscope by staining them with Gram stain. The content of the cell wall of M. tuberculosis does not absorb the gram stain, instead, acid-fast stains such as Ziehl-Neelsen stain, or fluorescent stains such as auramine are used. *Mycobacterium tuberculosis* cells are curved rod-shaped and are often seen wrapped together, due to the presence of fatty acids in the cell wall that stick them together. This appearance is referred to as cording (like strands of cord that make up a rope).*M. tuberculosis* is characterized in tissue by caseating granulomas containing Langham's giant cells, which have a horseshoe pattern of nuclei (Oshiet al., 2014).

The ribosomal genes of Mycobacterium are linked into an operon in the following order S″-rrs(16s RNA)-rrl(23s RNA)-rrf(5s RNA)-3′ and each of these interjecting regions inside the operon encoded for transfer RNA. In slow growing Mycobacterium specie, the operon is present as a single copy whereas the two copies are present in rapidly growing species. The 16s r-DNA consists of two hypervariable regions refer to as regions A and B and are the specie-specific genetic signature of the organism. The specific codon is located on 138 bp portion of region A and 70 bp portion of region B. Therefore, a greater proportion of the specie-specific variability is located on region A, making region A the most desired target for Mycobacterium gene sequencing studies. Another interesting region is the ITS 16s-23s region. This region shows more variability than the 61s r-DNA gene allowing for further discrimination within specie.

2.5 CLINICAL SPECTRUM

M. *tuberculosis* can affect virtually every organ of the body but the primary presence is the lung. In most cases, spread to other organs is from the lung depending on the interaction between the host immunity and the infecting mycobacterium. The first exposure to the organism is called primary tuberculosis which is usually asymptomatic lung infection. Symptoms such as fever and cough may occur (Cudahy and Shenoi, 2016; CDC 2018). Acquired cellular immunity is responsible for the defeat of the organism during the primary infection. However, several of the organisms remain dormant inside the macrophages for several years or until there is a challenge to immunity when there is reactivation of infection called the secondary infection (Camuset al., 2002;)

From the reactivated infection, lungs, pleural and pericardial cavities may be involved; Lymph node infection remains the most common extra pulmonary manifestation of tuberculosis (Fitzgerald et al., 2015). The cervical nodes are usually involved which become swollen and

matted together and may drain, Lymph node infection is called Scrofula. Renal involvement is also common. When the kidney is involved, patients do have red and white blood cells in their urine without bacteria seen on gram staining and no growth when cultured. This is called sterile pyuria. Skeletal involvement normally affects the thoracic and lumbar spine. The intervertebral discs may be destroyed and the adjacent vertebral bodies, this is called Pott's disease. Joint involvement presents as chronic arthritis. Central nervous system may be involved leading to tuberculous meningitis with intracranial granulomas, occasionally, but common with patients with immunodepression such as HIV/AIDs. Tiny millet-seed-sized tubercles are or granulomas are disseminated all over the body (Camuset al., 2002; Ocheiet al., 2014). The kidney, liver, lungs and almost all other organs may be involved. This is called Miliary tuberculosis, and it is common in children, the elderly and immunocompromise individuals (Ocheiet al., 2017).

2.6 TB IN CHILDREN

It is estimated by the WHO that 30,000 children get TB in Nigeria each year (Kanabus et al., 2018). There are also 47,000 children that are eligible to receive preventative treatment that would help to prevent them from getting TB. However, only about 8,500 children actually receive this preventative treatment (CDC, 2014).Nigeria has however started to use the new TB treatment for children. This is the treatment that is both dispersible and flavored and so it makes it much easier for children to take (WHO, 2014). Studies have shown the important role played by treatment supporters, the support needs of patients whilst taking treatment include: monitoring and supervision of daily drug taking, motivational support to take the drugs as expected, provision of support for feeding (when there is little food) and support for provision of transportation cost to visit TB clinic when the need arises. Patients with treatment supporters who were offered these supports tended to complete their treatment regimen and were less likely to default.

There are four important parameters for the containment of TB:

(I) Early diagnosis

(II) Prevention of disease spreading

(III) Effective treatment with antituberculosis

(IV) Prevention of resistance development

2.7 TREATMENT AND CONTROL

Treatment of tuberculosis depends on the location and the extent of damage. In general, medical treatment is the rule except instances when surgical interventions are necessary. There are about 10 classes of drugs currently approved by United States Food and Drug Administration for the management of Tuberculosis depending on certain clinical circumstances in adults. For first time patients without complication, first line drugs consisting of combination of Isoniazid, Rifampicin, ethanbutol and pyrazinamide is recommended for a period of six to nine months. Injectable forms such as streptomycin is also advised (Hopewell et al., 2006). For patients who re-presented with Tuberculosis or those with evidence of resistance to any of the first three first line drugs, fluoroquinolone are added to the medication. Surgical resection of parts or entire lobe of the lung may be necessary (Ozimset al., 2016).

Mainstay of prevention is the avoidance of overcrowding and provision of well-ventilated accommodations, identification of infected individuals, prompt medication and avoidance of non-complaints such as the use of DOT centers are strongly advocated by the World Health Organization (Chhaya 2011). Prophylaxis for exposed individuals with Rifampicin has been very effective. Vaccination of children with BCG vaccines has been practiced for years. It must be mentioned however, that the occurrence of immunosuppression such as those that came to be because of HIV/AIDS and organ transplantations particularly in developed country have made Tuberculosis a re-emerging disease of worrisome proportion.

2.8 MAJOR HURDLES IN TB CONTROL AND ELIMINATION

The smear based identification method which is not expensive has limited sensitivity. Novel molecular assays such as cartridge-based PCR systems (that also test for drug resistance) have better sensitivity but still miss TB in children and/or in other organs than the lungs, and are too expensive to be used at point-of-care (Fitzgerald et al., 2015).Co-infection with HIV is associated with decreased sensitivity of sputum microscopy and specificity of chest radiography for the detection of pulmonary tuberculosis. The lack of accurate diagnostic tools may result in either 'under' or 'over-treatment' of suspects, impacting both on patient care and the efficient utilization of resources. Culture, although it offers improved sensitivity, is slow and requires specialist microbiological safety facilities that are not widely available in sub-Saharan Africa. Nucleic acid amplification methods have been shown to offer rapid diagnosis of pulmonary tuberculosis. The bacteria do not need to be viable for testing by these techniques and specimens may be heat treated prior to testing, reducing the risk of infection and facilitating transport of the specimens (Mario 2015).

Preventive treatment cannot be efficiently targeted.

Once infected, the lifetime risk of developing active TB disease is on average 10%, but increased malnutrition, smoking, diabetes and alcohol abuse (WHO;2009). Progression from latent infection to TB disease can be prevented with a 3 to 6 months course of one or two drugs ("prophylaxis"). But this present regimen of prophylaxis is too toxic, cumbersome and expensive to be used for mass prophylactic treatment. Currently available tests cannot identify those with clearly increased risk of disease progression, precluding targeted preventive treatment in a cost-effective way. Diagnostic biomarkers that identify latently infected persons at high risk of progression to disease are largely unknown (Gitauet al., 2013).

All these problems are aggravated in patients co-infected with HIV.

HIV infection is the strongest risk factor for progression from latent TB infection to TB disease, increasing this risk by 10 to 100-fold depending on the level of immune impairment, Patients with TB-HIV co-infection, especially those with severe immune deficiency, are more difficult to diagnose and have high mortality if left untreated. Antiretroviral treatment reduces the risk of TB disease and improves survival, but not completely. Preventive treatment with isoniazid provides additional protection against TB disease but has limited effect beyond the timespan of the preventive treatment course, and limited application because of feasibility constraints. In addition, MDR/XDR-TB outbreaks among HIV-infected individuals occur in settings where nosocomial TB transmission is poorly controlled, and prophylactic treatment for MDR TB is not available (Fitzgerald et al., 2015).

Finally, effective control of TB is hampered by poor health system responsiveness. TB is the archetypal disease of poverty, hitting hardest those with the poorest access to quality health care. TB patients and their families often face catastrophic expenditures for diagnosis and treatment, especially with MDR/XDR-TB. Public clinics and laboratories, in most countries responsible for managing TB, are often poorly staffed, poorly equipped and underfunded, while private care providers tend to delay diagnosis, provide substandard treatment and not notify TB cases. These problems are aggravated in tuberculosis, HIV co-infection, which requires integrated management, TB infections would be a possible target for intervention but little is known about how these could be identified at an early stage (Comas et al., 2013).

Tuberculosis diagnosed late result in poor treatment outcomes and continued transmission to others. Moreover, with delayed diagnosis, long-term effects of TB hamper and impair health post-TB treatment. The chronic nature of the disease, its insidious onset and non-typical signs and symptoms lead to poor clinical suspicion with patients, physicians and other health care

workers. Until recently diagnosis in resource-poor settings was based on sputum smear microscopy (Alvarez-Uriaet al., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The collection center for the study was at Bingham University Teaching Hospital (Formerly ECWA Evangel Hospital) located in Jos Plateau State. The hospital was established in 1959 and currently has approximately 160 beds. It serves medical needs of the Jos metropolis and the adjourning villages and towns. Jos city is in the North centralof Nigeria. The city is located on a Plateau at an elevation 1,217 Meters above sea level. Jos is the

administrative capital of Plateau State with a population of 900,000 and covering an area of 30,913 square kilometers based on (NPC, 2006).

3.2 DATA AND SPECIMEN COLLECTION

3.2.1 SAMPLE SIZE DETERMINATION

Convenient sampling technique was employed to determine the sample size based on the average national TB prevalence in general population portion formula was applied to obtain the actual sample size which is 303 and the actual sample size and the total sample collected for the current study is 435 TB patients, However two samples were collected from each presumptive TB patient for GeneXpert, ZN technique and LFM stain

N= (Z ∝/2)2 $x \frac{(1-p)x(p)}{d2}$

Where N= minimum sample size

Z /2=1.96 at 95% confidence interval (CI)

P= National TB prevalence

D= Margin of error 0.05 at 95%

3.2.2 SAMPLE COLLECTION AND PROCESSING

The sample size collected were 435 and two consecutive early morning sputum are obtained from each subjected in line with World Health Organization (WHO) recommendation, which is two sputum specimen were collected, one of which was early morning sputum and the other was collected from the spot after referral to the laboratory by the physician. Subjects who had earlier been instructed on methods of expectoration would be required to collect the sputum into a sputum container provided after consultation. Sputum was brought to the hospital as soon as collected in the morning or at the spot and delivered to a specified section of the laboratory.

Inclusion criteria and exclusion criteria

All neonates' patients were excluded and HIV patients were included

Ethical consideration

For ethical consideration, the research proposal was sent to the Ethical Committee of the Hospital for approval and approval was obtained.

GeneXpert

The GeneXpert is a real time PCR for identifying tuberculosis and it also simultaneously determine rifampicin resistant in the clinical sample.

3.3 GeneXpertPROCEDURE

The sputum sample for testing was2 volume of sample reagent added to 1 volume of the sample (ratio 1:2) in a tightly close the lid covered bottle. Then shake vigorously for 20 times in a forth and back movement, it then left on the bench work for 5 minutes and allow to incubate at room temperature, after the incubation the sample is shake vigorously again 20

times, incubation takes place again at room temperature for yet another 10 minutes. Using a sterile transfer pipette, transferred 2 ml of the sample into the open port of the Xpert MTB/RIF cartridge ensuring that bubbles and aerosol were not created during the process of transfer by slowly dispensing into the cartridge.Test was run on the automated machine attached to the computer, on the system “CREATE TEST” window and then scan the cartridge bar code, on the Xpert MTB/RIF cartridge using the barcode reader. After two hours the test is fully run, the module door and opens for removal of the cartridge and the Xpert system window views results.

3.4 HIV SCREENING TEST USING DETERMINE KIT

All samples were collected from patients’ and screen for human immunodeficiency virus (HIV) before the diagnosis of tuberculosis. UsingDetermine Kit was used to identify the virus in the blood sample. This was done by collecting blood samples from the referred suspected tuberculosis patients, the blood sample was then spined with centrifuge for the separation of plasma and serum. The plasma was used by using a rubber pipette to take 0.5mil and drop on the sample well. The kit takes about 5 minutes to read result.

3.5PROCEDURE OFLED FLORESCENCE MICROSCOPY METHODS (AURAMINE-RHODAMINE STAINING FOR AFB)

Thin smear of the sputum sample was done and heat fix by passing the slide through the flame of the Bunsen burner at 65-75 C, making sure no overheating of the slides. Then slides smears were stained with Auramine O- Rhodamine B solution and for 15 minutes, which was then rinsed off with chlorine free water until no color appears in the effluent.The slides was then decolorized with decolorizing agent for 3 minutes using acid alcohol which de-stain the smear was then washed off with distilled water. Addition of potassium permanganate as

counter stain to the smear slide for 2 minutes and viewed with microscope immediately using 100x oil immersion objective to observe the morphology of fluorescing organism.

3.6 ZIEHL-NEELSEN SAMPLES STAINING PROCEDURE

A Wooden applicator sticks (disposable) was used to make sputum smears of 2 cm by 1 cm on slide, which were allowed tolet to air dry, then slides were heat fixed with flame from Bunsen burner. The slides was flooded with strong 3% Carbol fuchsine and heated under to steam, avoiding boilingand ensuring that the stain did not dry up for 5 minutes. The slides were wash in a running tap water, 3% acid alcohol was added to decolorize the primary stain for 3 minutes ensuring that smears were not over decolorized, counter stain was used to stain the smear with 1% methylene blue for 1 minute, and then washed off with running tap water.

CHAPTER FOUR

RESULTS

Results were based on comparative study of different types of diagnostic methods of identifying tuberculosisusing Ziehl-Neelsen (ZN), Led florescence microscopy (LFM) and GeneXperttechniques.The results were grouped into different age brackets due to the large numbers of samples gotten was also categorize into gender.

4.1 Diagnosis of tuberculosis using Ziehl-Neelsen technique in relation to gender and age

Table 4.1presented positive cases with Ziehl-Neelsen (ZN) technique in relation to gender and age, the results were grouped into different age brackets and the samples subjected for Ziehl-Neelsen technique were 435 and the results were grouped into gender, the males were 196 (45%),females were 239 (54.9%).

Table 4.1: Diagnosis of tuberculosis using Ziehl-Neelsen techniques in relation to gender and age

Age Group (year)	Number of male Tested	Number of positive cases	Number of female Tested	Number of positive cases
0-15	57	5 (8.8%)	43	1 (2.3%)
16-20	11	1 (9.1%)	31	1 (3.2%)
21-25	17	0 (0.0%)	8	2 (25%)
26-49	91	11 (12.1%)	124	10 (8.1%)
50 above	20	5 (25%)	33	2 (6.1%)
Total	196	22(11.2%)	239	16 (6.7%)

The result of Table 4.1, the total number of sample taken were all subjected to Ziehl-Neelsen technique,the recorded result as shown female tested were 239 (54.9%) the recorded positive cases was 22 (11.2%) while the male patients on the other hand was 196 (45%) and the positive cases was 16 (6.7%).

4.2 Diagnosis of tuberculosis using Led florescence microscopy (LFM) technique in relation to gender and age

Detection of M. tuberculosis by LED fluorescent microscopy is presented in Table 2 and the data was grouped into gender and different age bracket.

4.3 Diagnosis of tuberculosis using GeneXpertmtb/rif in relation to gender and age

Positive cases with MTB/RIF GeneXpert analysis in relation to gender is presented in Table 3,Out of 435 patients tested, the percentage of males and females tested positive in the entire population was 7.5 and 5.5, In this analysis age group 0-15 and 50 and above years.

Table 4.2: Diagnosis of tuberculosis using led florescent microscopy in relation to gender and age.

Age Group (year)	Number of male Tested	Number of positive	Number of female Tested	Number of positive
0-15	57	3 (5.3%)	43	1 (2.3%)
16-20	11	1 (9.1%)	31	1 (3.2%)
21-25	17	0 (0.0)	8	2 (25%)
26-49	91	15 (16.5%)	124	13 (10.5%)
50 above	20	7 (35%)	33	3 (9.1%)
Total	196	26 (13.3)	239	20 (8.4%)

The result of Table 4.2, total number of sample taken were 435 and they were grouped into different gender, the males tested were 196(45%) while the number of females tested were 239(54.9%). The positive result for Led Florescent Microscope are 26 (13.3 %) and 20(10.2) for males and females patients respectively.

Table 4.3: Diagnosis of tuberculosis using GeneXpertmtb/rif in relation to gender and age

Age Group (year)	Number of male Tested	Number of positive cases	Number of female Tested	Number of positive cases
0-15	57	6 (10.5%)	43	1 (2.3%)
16-20	11	3 (27.3%)	31	1 (3.2%)
21-25	17	0 (0.0)	8	3 (37.5%)
26-49	91	20 (21.9%)	124	16 (12.9%)
50 above	20	6 (30%)	33	3 (9.1%)
Total	196	33 (16.8%)	239	24 (10.0%)

The result presented the positive cases to the GeneXperttechnique,thetotal number of males tested were 196 while the number of females tested were 239 and the positive cases for GeneXpert were, 33 (16.8 %) and female positive cases were 24(12.2%).

4.4 The data in Table 4Incidence of tuberculosis using Ziehl-Neelsen technique compared to Led florescence microscopy technique and GeneXpertmtb/rif in relation to different age group

The data in Table 4.4 indicates patients who tested positive to tuberculosis using Ziehl-Neelsen technique, LFM positive cases and tested positive to MTB/RIF GeneXpert technique. The analysis also shows age dissemination in relation to positive cases for MTB/RIF.

Incidence of tuberculosis using Ziehl-Neelsen compared to LFM positive and GeneXpertmtb/rif

4.5 Incidence of HIV cases in relation to gender and age .

The table 4.5 presents the result to positive cases of human immune-deficiency virus (HIV) in relation to tuberculosis, the result were grouped into different gender and age groups.

Table 4.4 Incidence of tuberculosis using Ziehl-Neelsen technique compared to Led florescence microscopy technique and GeneXpertmtb/rifin relation to different age group

Age Group	PATIENTS TESTED	ZIEHL-NEELSEN	LED FLOURESENT MICROSCOPY	GeneXpert assay
0-15	100	6 (6%)	4 (4%)	7 (7%)
16-20	42	2(4.8%)	2 (4.8%)	4 (9.5)
21-25	25	2 (8%)	2(8%)	3 (12%)
26-49	215	21 (9.8%)	28 (13.0%)	36 (16.7%)
50 above	53	7 (13.2%)	10(18.9%)	9 (16.9%)
Total	435	54 (12.4%)	46 (10.6%)	57 (13.1%)

Table 4:4 presents positive cases with Ziehl-Neelsen (ZN) technique to Led florescence microscopy (LFM) technique GeneXpertmtb/rif in relation to different age group at Bingham Teaching Hospital, out of 435 patients tested, the percentage of patients tested were grouped into age differences of 0-15years, 100 numbers of samples within this age group has sensitivity cases to ZN 6 samples in all LFM 4 samples while GeneXpert 7 samples, for the age group 16-20 total number 42 samples, ZN 2, LFM 2, GeneXpert 4, 21-25 years, total test is 25, Z-N 2, LFM 2, GeneXpert 3, age group 26-49 years total number of test done was 215,Z-N 21, LFM 28, GeneXpert, 50years above 53 Z-N 7, LFM 10, GeneXpert 9 average aggregate number

435,Z-N 54, LFM 46 and GeneXpert 57.Comparative analysis of Ziehl-Neelsen, Led florescent microscopy and Genexpert techniques for diagnosis of tuberculosis.

Table 5:Incidence of HIV cases in relation to tuberculosis.

Age Group (year)	Number of male Tested	Number of positive cases	Number of female Tested	Number of positive cases
0 – 15	57	20 (13.4%)	43	17 (11.4%)
16 – 20	11	2 (1.3%)	31	8 (5.3%)
21 – 25	17	3 (2.0%)	8	4 (2.6%)
26 – 49	91	23 (15.4%)	124	54 (36.2%)
50 above	20	7 (4.6%)	33	11 (7.3%)
Total	196	55 (12.6%)	239	94 (21.6%)

Table 4.5: Incidence of HIV cases in relation to tuberculosis

Presents the result of the positive cases of patient tested positive to HIV relayed to tuberculosis cases, the result of male tested were 12.6%% while the female tested positive showed 21.6%,which is alarming statistically to the health sector and the data to positive HIV patient of female drastically is far much than the male positive cases .

CHAPTER FIVE

5.0 DISCUSSION

In this research, threedifferent diagnostic techniques were assessed for *Mycobacterium tuberculosis* among suspected pulmonary tuberculosis patients attending Bingham University Teaching Hospital in Jos. The GeneXpert MTB/RIF assay outperformed LED microscopy and the ZN staining. The study has no limitations, culture. However, both LED fluorescent microscopy and GeneXpert MTB/RIF have shown specificities above 95% in this study, so the possibility of bias due to false positive results is very small.The results of GeneXpert MTB/RIF with sputum specimens were more modest than previously reported (AlemuGadissaet al., 2017).

The occurrence of *M. tuberculosis* with Ziehl-Neelsen, Led florescent microscopy and GeneXpert MTB/RIF technique in selected patients living with HIV/TB attending Bingham University Teaching Hospital in Jos state of Nigeria was found to be considerable high.The results of this study indicate that the implementation of the GeneXpert MTB/RIF assay could dramatically improve the rapid diagnosis of extra pulmonary tuberculosis in HIVinfected patients, especially in cases with suspicion of tuberculosis meningitis. In settings where GeneXpert cannot be afforded, LED fluorescent microscopy may be used for the diagnosis of pulmonary tuberculosis with acceptable results compared to the GeneXpert MTB/RIF assay if several sputum specimens can be collected in different days.This is maybe due to inadequate health education of TB and HIV infections in the state. This observation is supported by work of Ozimset al. (2016). The study result shows prevalence rate of HIV among male was lower than female. It was observed that more than 70 percent of patients with HIV positive was also TB positive. This observation correlates with the report of Pinyopornpanishet al. (2015). There was general increase in the prevalence of tuberculosis as the age progresses with the age group

between 26 and 49 recording the highest proportional distribution. The possible enlightenment for the highest proportion delivery recorded for 26-49 age groups could be attributed to the weakening of the immune system and others. This is allied to the work of Causseet al. (2011). Even in the absence of HIV, this is the age group in general population where reactivation of latent tuberculosis takes place as reported earlier by Bajramiet al. (2016). It has been projected that the diagnosis of active TB with a sputum based assay with similar sensitivity rate has the potential to save half a million lives per year (Ocheiet al 2016; Oforet al., 2016; Olusola-Falaeet al., 2016). But in this work the findings disagree and the improvement of smear microscopy services remains necessary to increase patients' access to treatment. This is in agreement with that reported by Danish et al. (2018) which stated that test appeared to be as sensitive as culture for the detection of tuberculosis in smear positive, smear negative and extra pulmonary tuberculosis.

The alteration in the figure diagnosed by GeneXpert and LFMis a source of public health alarm to embracing this current practice without consideration of the money. Surplus of the first line drug, capital, adverse side effect such as audio-toxicity and death are avoided for LFM, GeneXpert MTB detected and RIF's resistant cases directly (Omo-Emmanuel et al., 2017). The chain of transmission of those that would have been infected with tuberculosis by the undiagnosed RIF's resistant cases with ZN technique can be promoted by the early and prompt detection of such by GeneXpert technique or Led florescent microscopy. These findings are supported by work of (Ozimset al., (2016). The general commonness of tuberculosis among HIV patients in this research population was high and coinfection with tuberculosis is possible. This can be recognized to the immunocompromised state of the patient which causes the patient to unattached defense mechanism against invading pathogens (Ocher et al., 2017)

The current conventional techniques, i.e. GeneXpert MTB/RIF and LED-FM system not only require the Biosafety Level III and trained personnel (Gelalchaet al., 2017). This is a proven technology in TB diagnosis. The GeneXpert MTB/RIF assay is independent of the user's skills and routine staff with minimal training can use the test. The efficacy of GeneXpertMTB/RIF was proved to be much higher than conventional LED-FM and comparable to that of culture techniques Ziehl-Neelsen (Steingartet al., 2006; Gelalchaet al., 2017).

GeneXpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis*over existing AFB smear microscopy, Led florescent microscope and other molecular methodologies in an HIV- and TB-endemic region. These is because the prevalence of *M. tuberculosis*complex on people living with HIV and TB examined, reveals that some patients were negative to acid alcohol fast bacilli test. Few were found positive by Led florescent microscopy techniques compared to those tested or found positive by MTB/RIF GeneXpert test. This could be due to the ability of MTB/RIF GeneXpert to detect very low numbers of bacilli compared to acid alcohol fast bacilli which may require higher number of bacilli. MTB/RIF is a cartridge-based, automated diagnostic test that can identify *Mycobacterium tuberculosis*(MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification technique (NAAT). This is similar to that report by Gelalchaet al. (2017)&Ocheiet al. (2017).

The positive cases of Human Immune-Deficiency virus and the sensitivity test of sputum samples in relation to gender and age group in this study showed that out of 435 patients tested, the percentage of males and females tested was 45.10 and 54.90% respectively.

In this study, we found that some TB patients wereco-infectedwithHIVduringthestudy period, and the positive cases of Human Immune-Deficiency virus among male and females was 12.6 and 21.6 % respectively.

Positive cases with MTB/RIF GeneXpert analysis in relation to gender is presented in table 4.Out of 435 patients tested, the percentage of males and females tested positive in the entire population was 7.5 and 5.5. In this analysis age group 0-15 and 50 and above years' males had recorded the same value of positive case of MTB and were higher than females, generally female's patients tested positive by MTB/RIF GeneXpert technique was low compared to males in this study.

5.1 Conclusion

This research work has shown that molecular diagnosis (GeneXpert)in diagnosis of tuberculosis was most sensitive and reliable than the two staining techniques used. Although according to the research results it has proved that the L ed florescent microscopy staining method was more sensitive than the Ziehl-Neelsen method. The incorporation of molecular technique such as GeneXpert for identification of *Mycobacterium tuberculosis* has showed that itis dependably accurate and saves time, it is the most adequate method of diagnosis due to it higher sensitivity, detection of drug resistance (Rifampicin) ,it does not involve sporadic steps, it quality control is grantee, there is control of infection by the usage of the buffer solution which decontaminate the infectious agents in the sputum samples, it is easy to operate. It convenient due to the fact that it a walk-away PCR and it is time limit is approximately 2 hours. The GeneXpert was more effective when compared with other identification techniques used in this study.

Tuberculosis amongst people living with human immuno-deficiency virus of patients who attended Bingham University Teaching Hospital Jos cannot be over emphasized on this research.

5.2 Recommendation

Based on this research the following are recommended;

- More research should be focused on quickest, cheapest, simplest means of identification of tuberculosis for easy detection of this diseases thereby reducing transmission rate and mortality with cases related to tuberculosis.
- It was recorded that HIV/TB in the region of research was a little high, myrecommendation is for scientists to put extra attention to diagnosis, treatments and awareness measures should also be implemented.
- Incorporation of molecular techniques such as Genexpert techniques in the final identification means of *M. tuberculosis*complex should be encouraged by all facilities,
- It is recommended that GeneXpertmethod be introduced to all centers for proper diagnosis of the tuberculosis to save life.
- The florescent microscope for LFM should be encouraged more than the regular compound microscope for Z-N in the laboratory because it view is more reliable for morphology of *Mycobacterium tuberculosis*.

REFERENCE

Aliyu G., El-Kanlem S., Abimaku A., Esati N., and Obasanya S. (2013), Mycobacteria etiology of pulmonary tuberculosis and association with HIV infection and multi-drug resistance in Northern Nigeria.Tuberculosis and research and treatment 1-9.

Alvarez-Uria C., Azcona J.M., Middle M., Nalk P.K., and Reddy S. (2012),Rapid diagnosis of pulmonary and extra-pulmonary tuberculosis in HIV infected patients; comparison of LED fluorescent microscopy and the GeneXpert assay in district hospitals in India, vol., ID 932862, 1-4

Adam J.,Caulfied.,Nancy L.,Wenganack (2016) Journal for clinical tuberculosis and other mycobacterial disease.Volume 4,pages

AsikoOngaya.,Mattew B.,Huante.,Paul Mwangi.,PhlipKeise.,Evans Amukoye.,Janice Endsley.(2013).Mycobacterium tuberculosis specific CD8+ cell recall in convalescing TB subjects with HIV co-infection tuberculosis,volume 93:pp s60-s65

Anwar S. K., Sajid, A., Muhammad, T.K, Sajjad, A., Yasir, K., Abduljabbar, Muhammad, I., and Wasim, S. (2018). Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan.Brazilian journal of microbiology /49/: 909–913

Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L.(2016) Comparison of genexpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo. J Infect DevCtries. 10/4/:418-22.

Barberis,BragazziNL,GalluzzoF.,Martini M.(2017).The history of tuberculosis from the first historical records to the isolation of Koshbacillus.J.prev med Hyg.58(1):E9-E-12

Brynildsrud OB, Pepperell CS, Suffys P, Grandjean L, Monteserin J, Debech N, Bohlin J, Alfsnes K, Pettersson JO, Kirkeleite I, Fandinho F, da Silva MA, Perdigao J, Portugal I, Viveiros M, Clark T, Caws M, Dunstan S, Thai PV, Lopez B, Ritacco V, Kitchen A, Brown TS, van Soolingen D, O'Neill MB, Holt KE, Feil EJ, Mathema B, Balloux F, Eldholm V (2018).[Mycobacterium tuberculosis lineage 4 shaped by colonial migration and local adaptation] Science Advances. 4 (10):5869.

Barry R Bloom,RifatAtun,Tedcohen.,Christopher Dye.,HamishFraser.,Gabriela B Gomez.,Gwen Knight.,MegenMurry.,Edward Nardell.,Eric Ruben.,Josua Solomon. (2017).Major infectious diseases,3rd edition Washington(DC).

Butt, T., Ahmad, R. N. Kazmi, S. Y. Mahmood, A. (2014). Rapid diagnosis of pulmonary tuberculosis by mycobacteriophage assay. INT J TUBERC LUNG DIS 8(7):899–902

Camas J.C., Pryor M.J., Medique C., and Cole S.T. (2002).Re-annotation of the genome sequence of Mycobacterium tuberculosis H337RV. Tuberculosis, 148, 2967-2973

Camus JC.,Pryor MJ.,MedigueC.,Cole ST.(2002).Re-annotation of the genome sequence of mycobacterium tuberculosis.148(pt 10):2967-73

Canetti G. (1955). The tubercle bacillus in pulmonary lesion of man: histobacteriologyand its bearing on the therapy of pulmonary tuberculosis-Spinster publishing company volume 43,issue 3,pages 301-321

Cadmus SI.,Gordon SV.,Hewinson.,Smith NH.(2002).Exploring the use of molecular epidemiology to track bovine tuberculosis in Nigeria:Veterinary microbiology-Elesevier

Camus JC, Pryor MJ, Médigue C, Cole ST (2002). "Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv". Microbiology. 148 (Pt 10): 2967–73.

Causse M, Ruiz P, Gutiérrez-Aroca J.B, Casal M. (2011).Comparison of two molecular methods for rapid diagnosis ofextrapulmonarytuberculosis.JClin Microbiol.; 49:3065–3067.24.

Center for Disease Control (2014). Drug-resistant TB".

Center for Disease Control.(2018)."How TB Spreads". Retrieved 14 March.

Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, Parkhill J, Malla B, Berg S, Thwaites G, Yeboah-Manu D, Bothamley G, Mei J, Wei L, Bentley S, Harris SR, Niemann S, Diel R, Aseffa A, Gao Q, Young D, Gagneux S (2013). "Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans". Nature Genetics. 45 (10): 1176–82

CD Mitrick.,RAWhite.,CA.Rodriguez.,JBayona.,MC Becerra (2016) European Respiratory Journal 48(4)1160-1170.

Cudahy, P. and Shenoi, S.V. (2016)."Diagnostics for pulmonary tuberculosis".Postgraduate Medical Journal. 92 (1086): 187–93.

Chaya mittal and SC Gupta (2011)Noncomplice to DOTS,how it can be decreased :Indian J community med 36(1):27-30

Danish, Z., Anjum, F., Farhat, K. and Munazah, M. (2018). Evaluation of smear microscopy and geneXpert for the rapid diagnosis of pulmonary and extrapulmonary tuberculosis in a

tertiary care hospital in North India: a descriptive prospective study. International Journal of Research in Medical Sciences 6(5):1756-1760

DianielW.Fitzgerald,DavidW.Haas.,(2015)"Mandell,Douglass and Bennetts principles and practice of infectious dieases"eighth edition

Dutta NK.,Karakousis PC.(2014) Latent tuberculosis infection: myths, models and molecular mechanisms.microbiology and molecular biology 78(3)343-371

Desalu O.O., Adeoti A.O., Fadeyi A., Salami A.K., Fawibe A.E., and Oyedepo O.O. (2013) Awareness of the warning signs, risk factors and treatment for tuberculosis among urban Nigerians. Tuberculosis research and treatment, volume ID 369717, 1-5

Friedrich N., Xavier A.,Kayigire and Sven, O (2013). Culture was confirmed by Ziehl-Neelsen staining and microscopy,51(6):1894

Fritz Titegemeyer.,Johannes Amon.,StephenParche.,Maximillian Schlicht.,DietmarHillmann.,Nadine Rehm and Michael Niederweis. (2017). A ecomomic view of sugar transport in mycobacterium smegmatis and mycobacterium tuberculosis.PMID 1755:7815

Fitzgerald, D.W., Sterline, T.R. and Haas, D.W. (2015)."251-Mycobacterium tuberculosis".In Bennett JE, Dolin, R., Blaser, M. J., Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Elsevier Saunders. p. 2787. ISBN 978-1-4557-4801-3

Gerardo, A., Jose, M., Azcona, M., Praveen, K., Naik.,Srinivasulu, R. and Raghuprakash, R. (2012). Rapid Diagnosis of Pulmonary and ExtrapulmonaryTuberculosis in HIV-Infected Patients. Comparison of LED FluorescentMicroscopy and the GeneXpert MTB/RIF Assay in a District Hospital in IndiaHindawi Publishing Corporation Tuberculosis Research and Treatment8 (4): 134-401

GitauMburu and D Arcy Richardson (2013) Supporting community-based tuberculosis and HIV,integration. First edition:ISBN:978-0-9572888-5-0

Getahun H, Gunneberg C, Granich R, Nunn P (2010). HIV infection-associated tuberculosis: The epidemiology and the response. Clin Infect Dis 50: 201-207.

Gitau, M, D'Arcy R (2013). Community TB-HIV integration guide AMLSN (2014): Learning update on tuberculosis. Molecular Diagnosis of Tuberculosis 12: 69-74.

GcebeNomakorinte., Michel A., Hlokwe Tiny M (2017) Non-tuberculosis mycobacteria in South Africa Wildlife:Neglected pathogens and potential impediments for Bovine Tuberculosis Diagnosis:Front cell infect microbial 10.3389.00015.

Gordon, S.V and Parish, T. (2018)."Microbe Profile: Mycobacterium tuberculosis: Humanity's deadly microbial foe".Microbiology. 164 (4): 437–439.

Hopewell P, Pai M, Maher D, Uplekar M (2006) International standards for tuberculosis care. Lancet Infec Dis 6: 710–725.

Jeff Schoery.,GeorgeB.,Craig Jr.(2019).Research demonstrates how immunotherapy may be effective for fighting tuberculosis.574-631-3933

King HC.,Khara-Butler,Janes.,P,Oakley.,BB,Erenso.,G,aseffa.,A,Knight., R,Wellington EM., Courtenary O., (2017) Environmental reservoirs of pathogenic mycobacteria across the Ethiopian biogeographically landscape 12(3):e0173811.

Kanabus and Anabel (2018) Information about tuberculosis:GHE.www.lb facts.org

Müller, R., Roberts, C. A., Brown, T. A. (2015). "Complications in the study of ancient tuberculosis: non-specificity of IS6110 PCRs". Science and Technology of Archaeological Research. 1 (1): 342-367

Mario Raviglione,Giorgiasulis (2015) Tuberculosis burden,challenges and strategy for control and elimination.8(2):6520.6570

Mohammed Abdel-Aziz Elizayat.,MohammedFaragDabour.(2017).Prevalence of undiagnosed asymptomatic bacteriuria and associated risk factors during pregnancy:BMJ7:13-98

Micheal B Reed,SebastienGagneux,KathrynDeriemer.,Peter M.,Clifton E Barry.(2007).The lineage of mycobacterium tuberculosis over produces triglycerides and the DosR dormancy reguloncondistitutivelyupregulated:Journal of bacteriology 189(7)2583-2589.

Murray PR, Rosenthal KS, Pfaller MA (2005). Medical Microbiology. Elsevier Mosby:e book ISBN:9780323064606

NPC (2006) Report of Nigeria population commission on the 2006 census population and development review,vol.33:pp 210

Ochei, K.C, Obeagu, E.I, Odo, M, Ugobo, E.E. (2016) Laboratory diagnosis of Mycobacterium tuberculosis in resource constraint setting: Direct sputum smear microscopy is comparable with automated mycobacterium growth indicator tube (BACTEC MGIT 960). Int J CurrMicrobiol App Sci. 5: 563-569.

Ochei, K.C., Adagbonyin, E., Obeagu, E.I and Aminu, B. (2017) Comparative Analysis of Ziehl-Neelsen and Genexpert Techniques for the Diagnosis of Tuberculosis in Human Immuno-Deficiency Virus Positive Patients in Benin City.Annals of Clinical and Laboratory Research5 (4): 208.

Ofor, I.B, Obeagu EI, Ochei KC, Odo M (2016) Evaluation of lipids and protein profiles in tuberculosis (Tb) patients on antituberculosis therapy in general hospital Umuguma, Owerri. Int J Curr Res Chem Pharm Sci 3: 20-28

Olufemi O.,Desalu.,Adekunle O.,Adeoti.,AbayomiFadeyi.,AlaskijaJA.,SalamiAdemola and Olarewaju O Oyedepo.,(2013).Awearness of the warning signs,risk factors and treatment for tuberculosis among urban Nigerians. Vol 2:ID-369717,Page 5

Olusola-Falae, B., Obeagu, E.I., Odo, M., Ochei, K.C, Solanke E (2016) Impact of community based tuberculosis care interventions on TB Case detection in Nigeria – What works andwhat does not? Int J AdvMultidiscip Res 3: 30

Omo-Emmanuel UK, Onuzulike N, Ochei KC, Achu ET, Obeagu (2017) HIV and TB co-infection among patients who used directly observed treatment short-course centres in Yenagoa, Nigeria. IOSR J Phar Bio Sci 4: 70-75.

O'Neill, Mary B.; Shockey, Abigail C.; Zarley, Alex; Aylward, William; Eldholm, Vegard; Kitchen, Andrew; Pepperell, Caitlin S. (2018). "Lineage specific histories of Mycobacterium tuberculosis dispersal in Africa and Eurasia".2101-61

Oshi D.C., Oshi S.N., Alobu I., and Ukwaja N. (2014).Profile, outcome and determination of unsuccessful tuberculosis treatment outcomes among HIV-infected tuberculosis patients in a Nigerian State.Tuberculosis research and treatment vol. ID 202983, 1-8.

Ozims SJ, Agu GC, Amah H, Izuchukwu IF, Nwanjo HU. (2016) Influence of socio-economic factors on treatment of tuberculosis patients in Owerri municipal. Int J AdvMultidiscip Res 3: 7-20.

Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, et al. (2015). Comparison of Xpert MTB/RIF assay and the conventional sputum microscopy in detecting mycobacterium tuberculosis in Northern Thailand.Tuberculosis Res Treat.2015; 571782.

Saleeb, P. G., Drake, S.K., Murray, P.R. and Zelazny, A.M. (2011). "Identification of mycobacteria in solid-culture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry". Journal of Clinical Microbiology. 49 (5): 1790–4.

Skrahina A, Hurevich H, Zalutskaya A, Sahalchyk E, Astrauko A, Hoffner S, Rusovich V, Dadu A, de Colombani P, Dara M, van Gemert W, Zignol M (2013). "Multidrug-resistant tuberculosis in Belarus: the size of the problem and associated risk factors". Bulletin of the World Health Organization. 91 (1): 36–45.

Steingart KR, Henry M, Ng V, et al. (2006). Fluorescence versus conventional sputum smear microscopy for tuberculosis: asystematic review. Lancet Infect Dis.;6(9):570–581.8.

vanIngen, J., Rahim, Z., Mulder, A., Boeree, M.J., Simeone, R., Brosch, R. and van Soolingen, D. (2012). "Characterization of Mycobacterium orygis as M. tuberculosis complex subspecies".Emerging Infectious Diseases. 18 (4): 653–5.

WHO (2010).Global tuberculosis control.World health Organization (WHO) report, Geneva, Switzerland.p. 118.

Wipperman, M.F., Sampson, NS, and Thomas, S.T. (2014). "Pathogen roid rage: cholesterol utilization by Mycobacterium tuberculosis". Critical Reviews in Biochemistry and Molecular Biology. 49 (4): 269–93.

Whittington RJ., Marsh I.,MC AllisterS,TurnerMJ,MarshallDJ.,Fraser CA. (1999)"Evaluation of modified Bactec 12B radiometric medium and solid media for culture of mycobacterium avium sub spp".J Clincalmicriobiology 37(4)1077-1083.

Wodarska M., Johnson J.C., Garty J.L., and Tang B. A microbiological revolution meets an ancient disease: improving the management of tuberculosis with genomics, 2015; 28(2) 521-545

World Health Organization (2009) Treatment of tuberculosis: Guidelines for national programmes, (4th edn). World Health Organization. Switzerland.

World Health Organization (2006) Global tuberculosis control: World health organization report, Geneva.

WoodroffeR.,Donnelly CA.,CallyHam.,Seth Jackson.,Kavna Chapman (2016).Badgers prefer cattle pasture but avoid cattle:implications for bovine tuberculosis control: control 19(10)12012-1208

World Health Organization (2008) Global tuberculosis-epidemiology, strategy, financing: World health organization report, UN declaration, WHO/HTM/TB/2018.23.

World Health Oranization (2006)WHO treatment guidelines for drug resistant tuberculosis:2016 update.

World Health Oranization (2014) Drug resistant tuberculosis surveillance and response: supplement to global tuberculosis report 2014.WHO/HQ/TB/2014.