IMPACT OF GENEXPERT MTBC/ RIF ASSAY USE ON *Mycobacterium tuberculosis* COMPLEX INCIDENCE IN PARTS OF NANDI COUNTY, KENYA USING GENEXPERT MACHINE.

A Thesis Submitted to the Department of Biological Sciences and Agriculture, School of Science and Technology.

University of Eastern Africa, Baraton.

In Partial Fulfillment of the requirement for the degree of Master of Science in Biomedical Sciences.

By:

RAYMOND KIBET KOECH.

ID: SKOERA1521

MAY, 2019

DECLARATION

DECLARATION BY THE CANDIDATE

This is my original work and to the best of my knowledge this work has not been published and/ or presented to any University for an award of a degree.

Raymond Kibet Koech Date _____

_____

DECLARATION BY THE SUPERVISORS

This thesis has been submitted for examination with our approval as University supervisors

Prof Lizzy Mwamburi Date_____

Signature: _____

Mr. Frank Kombo Date_____

Signature: _____

APPROVAL SHEET

IMPACT OF GENEXPERT MTBC/ RIF ASSAY USE ON *Mycobacterium tuberculosis* COMPLEX INCIDENCE IN PARTS OF NANDI COUNTY, KENYA USING GENEXPERT MACHINE.

Written and submitted by,

KOECH RAYMOND KIBET, in partial fulfillment of the requirement for the

Degree in Master of Science in Biological sciences-Biomedical option, is hereby

Accepted and approved by supervisors.

1. Prof. Lizzy Mwamburi. _____ **2.Mr Frank Kombo_____**

Date _____ Date _____

Accepted in Partial fulfillment for the degree in Master of Science in Biological Sciences –Biomedical option

Date: _____ Date: _____

Prof. Francis Ramesh, **Prof. Francis Ramesh, PhD.**

Department Chair School Dean

Signature _____ Signature: _____

Prof. Korso Gude PhD

Director, School of Graduate studies and Research

Signature: _____ Date: _____

ACKNOWLEDGEMENT

First and foremost my greatest gratitude goes to the almighty God for the guidance and chance He gave me to pursue this. I would like to thank Mr. Rop who heads the TB program in Nandi County, Prof Mwamburi and Mr. Kombo for supervision and support that they provided when writing this document. I also extend my heartfelt appreciation to Mr. Victor K. Sang for being a source of motivation. God bless you all.

DEDICATION

I dedicate this to my wonderful Parents, My lovely wife Nancy, The silent Mentors and the knowledge navigators. May you continue being a blessing to many more.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AFB	Acid fast bacilli
ART	Antiretroviral Therapy
CDC	Center for Disease Control
HIV	Human Immuno Virus
INH	Isoniazid
MDG	Millennium Development Goals
MDRTB	Multiple Drug Resistant Tuberculosis
MTBC	Mycobacterium tuberculosis
NTLD	National Tuberculosis, Leprosy and Lung Disease
RCT	Randomized Clinical Trial
RFP	Rifampicin
SDRTB	Single Drug Resistant Tuberculosis
SPSS	Statistical package for Social Sciences
TB	Tuberculosis
WHO	World Health Organization

ABSTRACT

Tuberculosis being an airborne infectious disease causes up to 2 million deaths yearly in the world. Rifampicin (RIF) is one of four first line antibiotics used to treat TB. It's the 4th leading cause of deaths in Kenya at 6.3% of all causes of deaths, according to the National Strategic plan for Tuberculosis, leprosy and Lung Health (2015). Kenya is ranked 15th of 22 high burdened TB countries all accounting for more than 80% of the world's TB cases hence the need for research for mitigation and eradication purposes. This research was carried out to scrutinize impact of the new diagnostic use of GeneXpert assay to analyze TB burden, and Rifampicin resistant *Mycobacterium tuberculosis* considering age, gender, type of patient and facility. A laboratory investigation was done using a molecular based test called gene expert system to simultaneously detect *M. tuberculosis* and Rifampin resistance. Data analysis was done using SPSS 20; to determine frequencies and crosstabs. Of the 90 samples analyzed it was found that there is a 17.8% rate of *Mycobacterium tuberculosis* and 5.6% are Rifampicin resistant. The most affected people were aged 61-65 at 4.4%, case detection with GeneXpert is more accurate than microscopy .

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Tuberculosis (TB) is an airborne infectious disease that causes up to 2 million deaths yearly in the world (Keshavjee, 2012) .It is leading cause of death, and reduction in the effectiveness of drugs commonly used to treat TB, due to drug resistance remains a significant concern. TB is a cause of morbidity and mortality throughout the world (Center For Disease Control and Prevention, 2015). In the majority of cases, the disease affects the lungs, but there are also not negligible numbers of cases (about 15%) with extra-pulmonary involvement in low-incidence countries. There are even higher rates in high-incidence countries. HIV-confected TB patients often develop extra-pulmonary involvement and may progress rapidly unless the infection is diagnosed and they are treated appropriately (Hillemann, 2011). It was declared a global emergency by the World Health Organization (WHO) in 1993 (Mwinga, 2002). The Millennium Development Goal of WHO, targeting to bring TB to a stop and reverse epidemic by 2015 was not achieved (Sujay Kumar Bhunia, 2015). This calls for further research to help on eradicating the TB.

Tuberculosis is caused by a mycobacterium called *Mycobacterium tuberculosis*. According to a report by WHO in 2016 on laboratory services in TB control, this bacterium grows slowly in cultures in laboratories hence difficult to study the resistance levels especially in developing countries, hence the low reports from African countries on the situation of the disease. TB can survive in the body of a person in the defense system cells posing a difficulty on treatment (Ernst, 2012).

Multidrug-resistant tuberculosis MDR-TB is defined as TB caused by strains of *Mycobacterium tuberculosis* resistant to at least isoniazid (INH) and Rifampicin (RFP), which are first line anti-tuberculosis treatment (Zhang, 2013). It afflicts up to 500 000 new patients yearly (Keshavjee, 2012). Development of the resistant TB to antibiotics is documented to have begun in the 1940s shortly after the first randomized clinical trial (RCT), starting with Streptomycin resistance and since then the resistance to many combinations of drugs developed and spread across geographic areas. Currently TB is treated with a combination of four drugs i.e. ethambutol pyrazinamide, isoniazid and Rifampicin). TB strains that are not susceptible to these drugs are said to be multi drug resistant (MDR).

Kenya is among the 22 high burden countries that together contribute 80% of the global TB cases. In 1990 TB cases was 11635 and in 2007 it was 116,000 showing a rise in prevalence. In Nairobi alone the cases have increased from 1048 in 1990 to 18906 in 2007 which represents almost 18 fold increase in the last 17 years (Ogaro, 2017). Hence the need for this research in Nandi county.

Piatek, (2013) in a study reports that sputum smear microscopy remains the most common way to diagnose pulmonary TB. Depending on the report and method used, smear microscopy can accurately detect TB in 20% to 80% (using fluorescence microscopy methods) of TB cases. Sputum smear microscopy has significant limitations because it can only be used to diagnose TB when sputum has sufficient bacillary load, and it cannot detect drug resistance. Thus, HIV-associated TB often goes undetected because people living with HIV (PLHIV), especially those with severe immunosuppression, generally have very low numbers of bacilli.

Gene expert technology is a cartridge-based nucleic acid amplification text (NAAT) for rapid tuberculosis diagnosis (Iram, 2014). It can detect resistance of RIF and is an easy to use method (Boehme, 2010). Globally control of TB is hampered by slow sensitively inaccurate methods particularly for detection of drug resistant forms of TB. This study was carried out in Nandi County, Kenya at Kapsabet county referral hospital, with the aim to address research gaps and providing information on MDRTB burden using first line antibiotic Rifampin sensitivity on smear positive sputum collected in patients with drug sensitive or multi drug-resistant pulmonary tuberculosis in order to help in scaling up programmatic management through providing accurate diagnostic methods for MTBC.

1.2 Problem statement

Advances in molecular biology and molecular basis of drug resistance have provided more information needed in developing new tools for rapid tuberculosis diagnosis (Carlos, 2006). A crucial impediment to global tuberculosis control is the lack of an accurate, rapid diagnostic method (Rachow, 2011) Studies of clinical and programmatic impact and operational research are few and needs scaling up in order to guide on implementation of new and point of care assays in high burden and poor countries. In this study GeneXpert technology kit will be used to study Incidence, sensitivity, accuracy and case detection in comparison to previous years when the kit was not in use.

1.3 Objectives

1.3.1 Broad objective

- To scrutinize incidence of MTBC and compare sensitivity accuracy and efficiency of GeneXpert assay with pre-existing AFB method in MTBC diagnosis among patients' samples collected.

1.3.2 Specific objectives

i). To describe incidence of MTBC and Rif resistant MTBC in Nandi county.

ii). To describe the characteristics of patients confirmed to be MTBC positive under study at Kapsabet County Referral Hospital TB clinic in Nandi County.

iii). To determine Rif resistance in MTBC positive patients' sputum samples at Kapsabet County Hospital TB clinic in Nandi County, using GeneXpert technology.

1.4 Hypotheses

Gene expert has no impact on MTBC and RIF resistant TB case detection and accuracy.

1.5 Justification of the study

There is need for thorough surveillance study on TB diagnostics across the country in order to present data on the situation to the WHO Global Project on Anti-tuberculosis Drug Resistance Surveillance. As of 2013, 136 countries had presented the data and Kenya was not in the list hence this study is important to in the fight of facing out TB as reported by WHO in the same year. Information on TB diagnosis provided by this study will be helpful to the country's ministry of health together with Nandi County TB outreach program at Large. This will create focus on scale up of emerging diagnostic technologies hence the delivery of treatment control measures offered at the hospital and its environs will be better. These findings aid reduction of the sparse information on the brutal outcome of TB and help focus on factors associated with TB, existence and continuous infection. The information deduced from this study is important in improving the people's understanding of the disease and the challenges it presents which are presented in form of recommendations.

1.6 Scope

This study aimed to provide information on incidence and compare sensitivity of diagnostic performances of GeneXpert test of *Mycobacterium tuberculosis* present among patients being tested for TB in Nandi county TB clinics and Rif resistance. This was done by collecting sputum from patients directed by clinicians to provide sputum for TB tests. Sputum samples are then analysed using gene expert for MTBC and Rifampicin resistance. Patients' samples were labeled and classified in terms of how the disease infected the patient as of new cases, previously treated case, Relapsed case, age and sex for clear variables to be treated statistically. This study was important in determining the best diagnostic method available in Nandi County and to scrutinize drug resistance against tuberculosis, finding out the situation of TB cases in terms of age, sex, location, and determine the extent of Rif's resistance in patients with TB attending the county hospitals as compared to existing information from 2016. The findings are important in backing up the ministry of health in using the appropriate method and presenting information required to achieve the goals of kicking out tuberculosis as theorized by WHO. Recommendations and conclusions were made on the drugs used and how best to manage drug resistant TB.

1.7 Limitations

- Electric blackouts.
- Patients providing saliva instead of sputum.
- The unwillingness of some health workers to release the sputum samples was limiting.

1.8 Definition of terms and concepts

Acquired drug resistance (ADR)-After treatment has started, drug-resistant variants may emerge although the bacterium initially isolated from the patient was sensitive to drugs.

Drug resistance rate- is the rate strains become resistant as assessed by drug susceptibility tests (e.g., the number of drug resistance strains/ the number of examined strains * 100%) including single drug resistance (SDR) rate and multiple drug resistance (MDR) rates.

MDR-TB-is defined as the resistance against ≥2 anti-MTBC drugs at least including INH and RFP.

New cases-TB patients who denied having prior anti-TB treatment or who received anti-TB treatments for 30 days or less.

Previously treated case-a newly registered episode of TB in a patient who, in response to direct questioning admits having been treated for TB for one month or more, or, in countries where adequate documentation is available, there is evidence of such history.

Primary Drug Resistance - (PDR)-PDR concerns MTBC isolated from patient which never used any anti-MTBC drug or used anti-MTBC drug for one month.

Relapsed case-A patient previously treated for TB and declared cured by a medical officer after one full course of chemotherapy, but who reports back to the health service bacteriologically positive (smear or culture).

CHAPTER TWO

REVIEW OF RELATED LITERATURE AND STUDIES

2.0 Introduction

The Xpert MTBC/RIF assay is a hemi-nested real-time PCR method that amplifies the 81-bp region of the RIF-resistance-determining region of the rpoB gene, positions 507–533 (Scott, 2011). The Xpert MTBC/RIF assay utilizes molecular beacon technology to detect DNA sequences amplified in a hemi-nested rt-PCR assay. Five different nucleic acid hybridization probes are used in the same multiplex reaction. Each probe is complementary to a different target sequence within the *rpoB* gene of Rifampicin-susceptible *M. tuberculosis* and is labeled with a differently colored fluorophore. Together, these overlapping probes span the entire 81 bp core region of the *rpoB* gene (Lawn, 2004). HIV-related tuberculosis is difficult to diagnose and is associated with high morbidity and mortality. Recently, the World Health Organization has endorsed the GeneXpert MTBC/RIF (Xpert) assay for the diagnosis of pulmonary tuberculosis in HIV-infected patients from developing countries, but information about the use of Xpert for the diagnosis of extra-pulmonary tuberculosis is scarce (Alvarez-Uria, 2012). The absolute number of TB cases occurring each year (9.4 million) is currently greater than at any time in history and, although the global incidence rate is estimated to have peaked and has started to decline, progress towards TB control is very slow . The case detection rate worldwide in 2009 was just 63% and the lack of rapid and accurate diagnostics remained a critical

stumbling block, which was undermining progress towards the 2015 millennium development goals for TB control (Nicol., 2011). This calls for more control measures.

2.1 Advances in Molecular Technology

Advances in molecular biology and molecular basis of drug resistance have provided more information needed in developing new tools for rapid tuberculosis diagnosis. The overall accuracy of these tests are better than the conventional methods, in clinical mycobacteriology laboratories (Carlos, 2006). Gene expert is one of the new diagnostic tools.

A lot of literature is coming up on gene expert technology. One article on diagnosis of pulmonary by Lawn published in 2013, highlights that gene expert MTBC/RIF assay use provides high diagnostic accuracy in a range of clinical settings. It also provides the information that operational implementations have been reported from South Africa. However even though there is continued momentum and optimism regarding the impact of this new fully automated molecular assays, studies of clinical and programmatic impact and operational research are few and needs scaling up in order to guide on implementation of new and point of care assays in high burden and poor countries. The author writes that this is needed to accelerate progress in TB control. Using expert MTBC/RIF Assay in TB endemic countries has elevated the numbers of TB patient's identification (Alimuddin, 2015). However, the accessibility, sustainability and scale up of emerging diagnostic technologies are lagging behind hence a lot of input is needed (Pontali, 2018).

TB is still a global emergency and kills up to 1.7 million people globally every year. Drug resistant TB has spread worldwide and most Tb patients are not being screened hence the

need for more accurate and rapid tests. Expert MTBC/RIF assays will greatly impact on this problem (O'Grady 2011).

A study done in South Africa indicates that Gene expert assay will increase the number of TB cases diagnosed by 30 – 37% and MDR-TB case diagnosis will increase by 69%- 71% and the cost of TB diagnosis will also reduce effectively (Meyer-Rath, 2011). Another study indicates that in a population of HIV-positive patients with high TB risk Xpert MTBC/RIF increased case detection by 45% (Lawn, 2011). A descriptive study done by Pervaiz, (2015) on 133 patients, 123 (92.48%) were sputum AFB smear positive and 10 (7.52%) were negative. All 133 were positive for MTBC on Gene-Xpert. In sputum smear positive patients (123), Gene-Xpert MTBC was positive in all 123 and Gene-Xpert Rifampicin resistance was positive in 113 (91.86%) and negative in 10 (8.13%). In 113 patients whose Rifampicin Resistance on Gene-Xpert was positive, 112 (99.11%) were positive for Rifampicin resistance on DST and 1(.008%)was negative. In 10 patients who were Rifampicin resistance negative on Gene-Xpert, 1 was Rifampicin resistance positive on DST and 9 were negative. In 10 cases who were sputum smear negative, Gene-Xpert MTBC was positive in all 10 and all were Rifampicin resistance positive on Gene-Xpert and DST. Gene-Xpert Specificity was 90% and sensitivity 99.18%. The authors' conclusion was that frequency of drug resistance is high in re-treatment cases of TB and using Gene-Xpert as a primary tool for early detection of Rifampicin resistance is an important clue for MDR-TB. It emphasizes the need for routine use of Gene-Xpert, Culture and DST in re-treatment cases of TB

Overview of MTBC XPERT Technology

Weyer (2013) emphasized that the GeneXpert platform, launched in 2004, simplified real-time PCR-based molecular testing by integrating and automating the key processes of sample preparation, amplification and detection. Core components of the system include the instrument, a personal computer, a barcode scanner and the software (Fig. 1), together with disease-specific, single-use, disposable cartridges containing lyophilized reagents, buffers and washes. Target detection and characterization is performed in real time using a six-color laser detection device.

An article published in the U.S.A indicates that the Xpert MTBC/RIF cartridge for the simultaneous detection of TB and Rifampicin resistance was developed within 4 years, following a unique collaboration between academia and industry, brokered by FIND and financially supported by the US National Institutes of Health (Bethesda, MD, USA) and the Bill and Melinda Gates Foundation (Seattle, WA, USA). This collaboration serves as a blueprint for TB diagnostics development, consisting of clear end-user product specifications, adequate research funding, collaboration among academic partners on the core components of the technology, pooling of research resources, controlled clinical validation trials, large-scale field evaluations under well-designed operational research protocols, and a flexible response by industry engaging early on in negotiations on cost and preferential pricing.

2.2 Financial Issues

An article written by Lawn in 2011 found that global TB control efforts have been severely hampered by the lack of diagnostic tests that are accurate, simple to use and can be applied at the point of clinical care. This has been further compounded by the widespread inability

to test for drug resistance Gglobally, 1.8 million Xpert MTBC/RIF assays per year would be needed to test patients at high risk of MDR-TB (Pantoja, 2013) and For people living with HIV with TB signs and symptoms, 3.8 million Xpert MTBC/RIF tests per year would be needed to test for TB. If all individuals presenting at health facilities with signs and symptoms of TB were tested for TB using Xpert MTBC/RIF, a best estimate of 26 million tests per year would be needed.

2.3 Unresolved Issues

A world health report tittled WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTBC/RIF Ultra compared to Xpert MTBC/RIF states that the development of the Xpert® MTBC/RIF assay (Cepheid, Sunnyvale, USA) was a major step forward for improving the diagnosis of tuberculosis (TB) and Rifampicin resistance detection globally. However, Xpert MTBC/RIF sensitivity is imperfect, particularly in smearnegative and HIV-associated TB and some limitations also remain in its determination of Rifampicin resistance. The Xpert® MTBC/RIF Ultra assay (Ultra) has been developed by Cepheid as the next-generation assay to overcome these limitations, and uses the same GeneXpert® platform as Xpert MTBC/RIF

According to Nicol, (2011), while much has rapidly been learned about this assay, further research is needed in the following four main categories and a conclusion:

1. **Pediatric populations**: Only a single study in pediatric population has been published and further data are needed in different settings and age groups.
2. **Use for routine screening high-risk populations:** Studies to date have almost exclusively examined diagnostic accuracy in symptomatic TB suspects. Only one study has examined the utility of the assay as a screening test and that was done

among HIV-infected patients preparing to start antiretroviral therapy Further screening studies in high-risk populations are needed as well studies to define how Xpert best fits into screening algorithms.

3. **Extra pulmonary TB:** Data accrued thus far on extra pulmonary TB are few and have largely been opportunistic laboratory-based studies using routine samples obtained from a variety of anatomic sites. Systematic studies of various forms of extra pulmonary TB are now needed, including investigation of requirements for optimum sample preparation.
4. **Use for monitoring response to TB treatment:** The utility of the assay for monitoring response to TB treatment (drug-sensitive and drug-resistant disease) or for detecting the emergence of drug-resistant disease is not known. The assay requires the presence of intact organisms in the sample, as these are deposited on the cartridge filter during the initial automated steps of the assay. It is not known how long intact but nonviable mycobacteria persist during TB treatment, but detection of these might cause false-positive results.
5. **Further research & development for the Rifampicin resistance read-out:** As discussed above, reports of confirmed false-positive Rifampicin resistance have emerged from recent studies and further research and development is urgently needed to develop ‘fixes’ for this and then to validate these in the field. Interim solutions also need to be identified to know how to optimally use the current form of the assay.

2.4 Light Emitting Diode Fluorescence Microscopy

A study done in India by Thapa et al. (2015), reported that The World Health Organization (WHO) recommended phasing-in Light Emitting Diode Fluorescent Microscopy (LED-FM) as an alternative to conventional Ziehl-Neelsen microscopy (ZN) for diagnosis of smear-positive pulmonary tuberculosis. Thapa et al. (2015) showed that on 2868 TB patients examined by LED-FM during follow-up, 213 (7.4 %) were smear-positive and of 2740 examined by ZN, 136 (5.0 %) were smear-positive-an additional yield of 77 follow-up smear-positives. The disaggregated results of first and second specimensThe additional yield of second specimen (negative on first specimen and positive only on second specimen) was 12.5 % with ZN microscopy whereas it was 21.6 % with LED-FM ($p = 0.3$). The increase in yield by LED-FM could be due to (1) increased absorbability of mycolic acid and damaged bacilli to auramine than fuschin, (2) the LED-FM stained background of slide is brighter, making it easier for laboratory technicians to focus and examine slides with fewer bacilli and (3) increased area of the slide examined. Since these findings come from a setting of quality-assured smear microscopy under routine programmatic settings, they are most likely to be accurate and reflect operational realities and importantly during the period of study external quality assurance did not yield any major errors.

2.5 Global Use and Impact of Gene Expert

The 2010 *Lancet* tuberculosis series provided a comprehensive overview of global control efforts and challenges. In this update we review recent progress. With improved control

efforts, the world and most regions are on track to achieve the Millennium Development Goal of decreasing tuberculosis incidence by 2015, and the Stop TB Partnership target of halving 1990 mortality rates by 2015; the exception is Africa (Raviglione., 2012). This could be due to poor early detection which is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect as stated by Boehme (2010).

Globally, case detection and treatment access are poor for Rifampicin-resistant tuberculosis (RR-TB). The Xpert MTBC/RIF test has the potential to increase detection and reduce time to treatment (TTT). However, these benefits are dependent on health system capacity to provide treatment. (Helen, 2015) . A prospective cross-sectional analytical study was conducted in National Anti-Tuberculosis Center (NATA) center-Biratnagar and Primary Healthcare Center (PHC) - Manglabare, Morang District, of eastern Nepal from January 2014 to August 2014 whereby Laboratory investigation was done by conventional AFB staining followed by Xpert assay and the conlusion was that that implementation of GeneXpert MTBC/RIF assay is a helpful tool for early and rapid detection of tuberculosis with greater sensitivity and specificity over traditional AFB staining techniques (Shrestha, 2018). On another study done in nine countries on case notification done by Creswell (2014) whereby all projects used MTBC/RIF testing for people with suspected TB, as opposed to testing for drug resistance among already diagnosed patients. The projects placed 65 machines (196 modules) in a variety of facilities and employed numerous case-finding strategies and testing algorithms. The projects consumed 47,973 MTBC/RIF tests. Of valid tests, 7,195 (16.8%) were positive for MTBC. A total of 982 Rifampicin resistant results were found (13.6% of positive tests). Of all tests

conducted, 10.6% failed. The need for continuous power supply was noted by all projects and most used locally procured solutions. There was considerable heterogeneity in how results were reported and recorded, reflecting the lack of standardized guidance in some countries. The findings of this study begin to fill the gaps among guidelines, research findings, and real-world implementation of MTBC/RIF testing. Testing with Xpert MTBC/RIF detected a large number of people with TB that routine services failed to detect. The study demonstrates the versatility and impact of the technology, but also outlines various surmountable barriers to implementation. The study is not representative of all early implementer experiences with MTBC/RIF testing but rather provides an overview of the shared issues as well as the many different approaches to programmatic MTBC/RIF implementation.

Ardizzoni et al, (2015) describe the performance of Xpert and key lessons learned during two years of implementation under routine conditions in 33 projects located in 18 countries supported by Médecins Sans Frontières across varied geographic, epidemiological and clinical settings. The implementation of Xpert in diverse clinical settings was feasible and led to a significant increase in bacteriologically-confirmed pulmonary TB both for Xpert as first test, and as an add-on test. The choice of the best strategy took into account the epidemiological setting, including prevalence of NTM, and the test cost which may have represented a limitation in resource-constrained settings. However, the estimation of the cost took into account that the major investment is often represented by the logistical support required for installation of the system, while the higher test cost compared to microscopy was offset by the higher sensitivity and specificity compared to microscopy. This study conluded that the system was far from a “plug and play” device. High numbers

of inconclusive results in the same study by Ardizzoni et al, (2015) represented an extra expense. Significant infrastructure requirements, training, technical support and experience were indispensable to decrease errors and achieve good routine results, and time was needed for programmes to become more effective in applying sample collection strategies. To further decentralize diagnosis of drug-sensitive and drug-resistant TB strains, more robust, simpler technologies which are well-adapted to low-resource settings are still needed. In addition, as the GeneXpert system is now relatively widespread in many high-burden countries, development of a cartridge incorporating resistance detection for other drugs could boost the fight against drug-resistant tuberculosis.

2.6 Regional Use and Impact of GeneXpert

Taddese, (2018) carried out a study in Ethiopia to compare the diagnostic performances of auramine O-stained sputum smear examined by LED-FM and GeneXpert test. The study outlined that the sensitivity of GeneXpert was better than LED-FM for the diagnosis of PTB. Hence, it should be implemented as a primary diagnostic test in areas where overlapping synergy of TB and HIV/AIDS is high. The implementation of LED-FM should be supplemented by GeneXpert in areas where the prevalence of HIV/AIDS is high. For sero-negative presumptive TB patients LED-FM should be implemented as diagnostic test of PTB in Addis Ababa, Ethiopia.

Dorman (2018) conducted a prospective, multicenter, diagnostic accuracy study, recruited adults with pulmonary tuberculosis symptoms presenting at primary health-care centers and hospitals in eight countries (South Africa, Uganda, Kenya, India, China, Georgia, Belarus, and Brazil). In total of 2368 participants were recruited for sputum sampling. Another 248 participants were excluded from the analysis, and 1753 participants were

distributed to the case detection group (n=1439) and the multidrug-resistance risk group (n=314). Sensitivities of Xpert Ultra and Xpert were 63% and 46%, respectively, for the 137 participants with smear-negative and culture-positive sputum (difference of 17%, 95% CI 10 to 24); 90% and 77%, respectively, for the 115 HIV-positive participants with culture-positive sputum (13%, 6·4 to 21); and 88% and 83%, respectively, across all 462 participants with culture-positive sputum (5·4%, 3·3 to 8·0). Specificities of Xpert Ultra and Xpert for case detection were 96% and 98% (−2·7%, −3·9 to −1·7) overall, and 93% and 98% for patients with a history of tuberculosis. Xpert Ultra and Xpert performed similarly in detecting Rifampicin resistance. From this study for tuberculosis case detection, sensitivity of Xpert Ultra was superior to that of Xpert in patients with paucibacillary disease and in patients with HIV. However, this increase in sensitivity came at the expense of a decrease in specificity.

2.7 GeneXpert Use and Impact in Kenya

Muia et al, (2017) in one study found that to combat the challenges of TB epidemic in Kenya there has to be massive scale up of both treatment and diagnostic facilities. The challenges encountered in many centers is failure to accurately detect *Mycobacterium tuberculosis*. This study aimed at evaluating the performance of GeneXpert MTBC/RIF assay in detection of pulmonary TB and drug resistant testing. Out of the 400 samples analysed 37.5% were smear positive of which 60% ($p<0.05$) were male. For the culture and GeneXpert assays the positive samples were 33% and 32.25% respectively. Smear microscopy had the highest number of false positives (28%) and false negatives (9.6%). For bacilli identification the sensitivity, specificity, positive predictive value and negative predictive values for smear microscopy were 81.8%, 84.3%, 72% and 90.4% while for

GeneXpert they were 97.7%, 100%, 100% and 98.9% respectively. This implies that GeneXpert was a better method. GeneXpert MTBC/RIF assay offers high potential for rapid diagnosis of TB and drug susceptibility testing in an austere Kenyan environment.

To describe the epidemiology of childhood tuberculosis Onyango et al, (2018) conducted a retrospective analysis of the Kenyan national TB program data for patients enrolled from 2013 through 2015. A total of 23,753 children aged less than 15 years were included in the analysis. Survival analysis was performed with censorship at 9 months and mortality was the main outcome. Onyango et al, (2018) found that Childhood TB accounted for 9% (n = 24 216) of all patients with TB; 98% of the notified children (n = 23 753) were included in the analysis. TB/HIV co-infection was 28% (n = 6112). Most TB cases (71%; n = 16 969) were detected through self-referral. Treatment was successful in 90% (n = 19 088) and 4% (n = 1058) died. Independent risk factors for mortality included being HIV infected but not on antiretroviral therapy (adjusted hazard ratio [aHR], 4.84; 95% CI, 3.59-6.51), being HIV infected and on antiretroviral therapy (aHR, 3.69; 95% CI, 3.14-4.35), children aged less than 5 years (aHR, 1.25; 95% CI, 1.08-1.44), and being diagnosed with smear negative pulmonary disease (aHR, 1.68; 95% CI, 1.27-2.24).this study conluded that Most childhood TB cases in Kenya were detected through passive case finding. TB/HIV co-infection is high among children on treatment for TB, and HIV is associated with an increased risk of death. There is a need to intensify active case finding among children. TB prevention interventions among HIV-infected.

Children, early diagnosis of HIV, and early antiretroviral therapy initiation among children on TB treatment should be strengthened.

In Kenya, access to early infant diagnosis and viral load monitoring services for HIV patients on ART is significantly hampered by sample transportation challenges and long turnaround times. Near patient care testing technologies have the potential to obviate such constraints. The Cepheid GeneXpert was launched in 2010 as a TB assay and in 2014 as a potential point of care HIV viral load assay. Whereas it is widely used for TB in Kenya, its utility for HIV testing has not been evaluated. To integrate HIV diagnosis into the existing GeneXpert platforms for TB Diagnosis, there is need to scale up the infrastructure and to change the way work is done (Bwana, 2019).

A study done in Western Kenya by Cavanaugh et al., (2016) outlines that Sputum smear microscopy is widely used for diagnosis, despite limited sensitivity in PLHIV. The study investigates evidence needed to determine the optimal diagnostic approach for patients. From 778 enrolled patients, they identified 88 (11.3%) laboratory-confirmed TB cases. Of the 74 cases who submitted 2 specimens for microscopy and Xpert testing, ZN microscopy identified 25 (33.6%); Xpert identified those plus an additional 18 (incremental yield = 24.4%). Xpert testing of spot specimens identified 48 (57.0%) of 84 cases; whereas Xpert testing of morning specimens identified 50 (66.0%) of 76 cases. Two Xpert tests detected 22/24 (92.0%) TB cases with CD4 counts <100 cells/μL and 30/45 (67.0%) of cases with CD4 counts ≥100 cells/μl. It was concluded that In PLHIV, Xpert substantially increased diagnostic yield compared to smear microscopy and had the highest yield when used to test morning specimens and specimens from PLHIV with CD4 count <100 cells/μL. TB programs unable to replace smear microscopy with Xpert for all symptomatic PLHIV should consider targeted replacement and using morning specimens Cavanaugh et al., (2016).

2.8 First Line Antibiotics used to treat MTBC Patients

2.8.1 Ethambutol

Ethambutol is a substituted ethylene-diamine that was first introduced in the treatment of TB in 1962 (Hahn, 2012) and is part of the current first-line regimen to treat the disease. Ethambutol is bacteriostatic against multiplying bacilli interfering with the biosynthesis of arabinogalactan in the cell wall. In *M. tuberculosis*, the genes *embCAB*, organized as an operon, code for arabinosyl transferase, which is involved in the synthesis of arabinogalactan, producing the accumulation of the intermediate D-arabinofuranosyl-P-decaprenol (Palomino, 2009).

The recognized mechanism of resistance to ethambutol has been linked to mutations in the gene *embB* with mutations at position *embB*306 as the most prevalent in most of the studies performed (Hilleann, 2009). Some studies, however, have also found mutations in *embB*306 in ethambutol susceptible isolates. Moreover, a study with a large number of *M. tuberculosis* isolates found that mutations in *embB*306 were not necessarily associated with resistance to ethambutol but with a predisposition to develop resistance to increasing number of drugs and to be transmitted. In fact, allelic exchange studies have shown that individual mutations causing certain amino acid substitutions produced ethambutol resistance, while other amino acid substitutions had little or no effect on ethambutol resistance. The same authors have more recently reported that mutations in the decaprenylphosphoryl-B-D-arabinose (DPA) biosynthetic and utilization pathway genes, Rv3806c and Rv3792, together with mutations in *embB* and *embC* accumulate, giving rise to a range of MICs of ethambutol depending on mutation type and number. These findings could have influence on the correct detection of ethambutol resistance by current molecular

methods. Mutations in *embB*306 then, cause variable degrees of ethambutol resistance and are required but are not enough to cause high-level resistance to ethambutol. There remain about 30% ethambutol resistant strains that do not present any mutation in embB stressing the need to identify other possible mechanisms of drug resistance to this drug (Hilleann, 2009).

2.8.2 Rifampin

Rifampicin is a Rifamycin derivative introduced in 1972 as an antituberculosis agent (Deepa, 2005). It is one of the most effective anti-TB antibiotics and together with isoniazid constitutes the basis of the multidrug treatment regimen for TB. Rifampicin is active against growing and non-growing (slow metabolizing) bacilli. The mode of action of Rifampicin in *M. tuberculosis* is by binding to the β-subunit of the RNA polymerase, inhibiting the elongation of messenger RNA. The majority of Rifampicin-resistant clinical isolates of *M. tuberculosis* harbor mutations in the *rpoB* gene that codes for the β-subunit of the RNA polymerase.As a result of this, conformational changes occur that decrease the affinity for the drug and results in the development of resistance (Floss, 2005).

In about 96% of *M. tuberculosis* isolates resistant to Rifampicin, there are mutations in the so-called ―hot-spot region‖ of 81-bp spanning codons 507–533 of the *rpoB* gene (Bartfai, 2001). This region is also known as the Rifampicin resistance-determining region. Mutations in codons 516, 526 and 531 are the most commonly associated mutations with Rifampicin resistance in the majority of studies. Although less frequent, some reports have also noted the occurrence of mutations outside of the hot-spot region of *rpoB.* Cross-resistance with other Rifamycin can occur. Mutations in some codons (e.g., 518 or 529) have been associated with low-level resistance to Rifampicin but still susceptible to other

Rifamycins, such as Rifabutin or Rifalazil. This is important for TB patients Recent genome sequencing studies have uncovered the acquisition of compensatory mutations in *rpoA* and *rpoC*, encoding the α and β' subunits of RNA polymerase, in Rifampicin-resistant strains with that need to receive antiretroviral therapy since Rifabutin is a less effective inducer of the cytochrome P450 CYP3A oxidative enzyme. On the other hand, monoresistance to Rifampicin is quite rare and almost all Rifampicin-resistant strains are also resistant to other drugs, especially to isoniazid. This is the reason why Rifampicin resistance is considered as a surrogate marker for MDR-TB.

Mutations in *rpoB.* These compensatory mutations would be responsible for restoring the fitness of these strains *in vivo* and have also been associated with a higher transmissibility in some settings (Sun, 2012).

2.8.3 Isoniazid

Isoniazid (INH) is the most widely used first-line antituberculosis drug (Kalo, 2015). It is the cornerstone of all effective regimens for the treatment of TB disease and latent infection (Leibert, 2010). INH is a prodrug and is activated by the catalase-peroxidase enzyme encoded by the *kat*G gene. The target of activated INH is enoyl-acyl-carrier protein reductase (*inh*A), which is required for mycolic acid biosynthesis. There are two described mechanisms that account for the majority of INH resistance. The most common method, mutations in *kat*G, is generally associated with high-level resistance to INH (Yuan, 2012). Resistance to INH can also occur by mutations in the promoter region of the *inh*A gene, which are generally associated with low-level resistance to INH and are less frequent than *kat*G mutations. DNA sequence analysis of *inh*A and *kat*G of Isolate E revealed a G>C point mutation in the *kat*G locus resulting in serine being replaced by

threonine at codon 315 (Ser315Thr); *inhA* was wild-type (i.e., no mutations were detected) (Martin, 2014).

The recommended critical concentration and for testing INH using the AP method is 0.2 μg/ml. An additional higher concentration is also recommended, 1.0 μg/ml. The equivalent concentrations for both MGIT and Versa TREK are 0.1 μg/ml and 0.4 μg/ml. It is recommended that all laboratories perform testing at the critical concentration; if the isolate is resistant, then testing at the higher recommended concentration should be performed.

2.8.4 Pyrazinamide

Pyrazinamide was introduced into TB treatment in 1952 (Silva, 2011). It is an important first line drug that is part of short course tuberculosis chemotherapy (Mendez, 2009). It has the characteristic of inhibiting semi-dormant bacilli residing in acidic environments such as found in the TB lesions. Pyrazinamide is also a pro-drug that needs to be converted to its active form, pyrazinoic acid, by the enzyme pyrazinamidase/ nicotinamidase coded by the *pncA* gene (Mendez, 2009). The proposed mechanism of action of pyrazinamide involves conversion of pyrazinamide to pyrazinoic acid, which disrupts the bacterial membrane energetic inhibiting membrane transport (Silva, 2011).Pyrazinamide would enter the bacterial cell by passive diffusion and after conversion to pyrazinoic acid it is excreted by a weak efflux pump. (Fernandez, 2014). Under acid conditions, the protonated pyrazinoic acid would be reabsorbed into the cell and accumulated inside, due to an inefficient efflux pump, resulting in cellular damage. One study has also found that pyrazinoic acid and its n-propyl ester can inhibit the fatty acid synthase type I in replicating *M. tuberculosis* bacilli (Cheng, 2000).

A recent study, however, has challenged the previous model by proposing that pyrazinoic acid inhibits trans-translation, a process of ribosome-sparing in *M. tuberculosis* (Shi, 2011). The study was performed in pyrazinamide-resistant strains lacking mutations in pncA but that had mutations in RpsA Antibiotics identifying the ribosomal protein 1 (RpsA) as the proposed target. Over expression of RpsA conferred increased resistance to pyrazinamide and pyrazinoic acid was confirmed to be bound to RpsA . While a very intriguing hypothesis as a target for pyrazinamide, the failure to perform allelic transfers in this study makes it difficult to conclude that in fact mutations in RpsA are the target of pyrazinamide (Zhang & Yew, 2009).

Mutations in the gene pncA remain as the most common finding in pyrazinamide resistant strains. These mutations, however, are scattered throughout the gene but most occur in a 561-bp region in the open reading frame or in an 82-bp region of its putative promoter. Some few studies have reported the occurrence of pyrazinamide resistant strains without any mutation in pncA stating that the resistance could be due to mutations in another not yet identified regulatory gene. Based on the current evidence, the contribution of mutations in RpsA to pyrazinamide resistance remains limited (Silva, 2011).

2.8.5 Streptomycin

Originally isolated from the soil microorganism *Streptomyces griseus*, **s**treptomycin was the first antibiotic to be successfully used against TB (Palomino, 2014). Unfortunately, as soon as it was prescribed, resistance to it emerged, a result of being administered as monotherapy. Streptomycin is an aminocyclitol glycoside active against actively growing bacilli and its mode of action is by inhibiting the initiation of the translation in the protein synthesis. More specifically, streptomycin acts at the level of the

30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA coded by the genes *rpsL* and *rrs*, respectively (Nishimura, 2007). In the last years, it has also been reported that mutations in *gidB*, a gene encoding a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA, confers low-level resistance to streptomycin (Perdigao, 2014).

CHAPTER THREE

METHODOLOGY

3.1 Research design

This was a descriptive study with the aim to isolate *M. tuberculosis* complex and carry out gene expert tests to determine Rifampicin resistance in comparison to previous results of microscopy. Quantitative data was collected using laboratory investigations.

3.2 Population and Sampling Technique

3.2.1 Study population

The study population was sputum samples collected from patients suspected to be having TB of both sexes and across all ages in selected TB clinics in nandi county. The samples were analysed at Kapsabet county referral hospital.

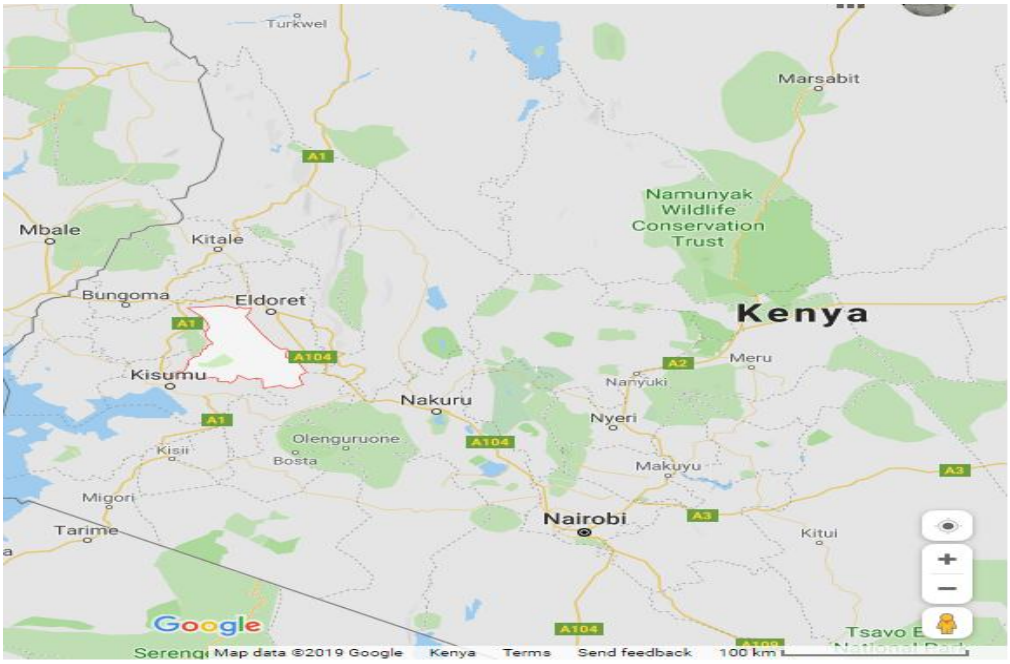
Turkwel
Marsabit
Namunyak
Wildlife
Conservation
Trust
Mbale
Kitale
Bungoma
Eldoret
Kenya
Kisumu
Nakuru
Meru
Nanyuki
Nyeri
Olenguruone
Bosta
Kisii
Makuyu
Migori
Tarime
Nairobi
Kitui
Google
Map data ©2019 Google
Kenya
Terms
Send feedback
100 km

Figure 1: *Showing Nandi county position in Kenya (google.com/maps/place/Kapsabet+District+Hospital).*

Figure 2: *Showing Kapsabet District Hospital where study was conducted (*https://www.google.com/maps/place/Nandi+County)

3.2.2 Sampling

Sample size determination

Cochran's formula (1977) will be used to determine the sample size.

$n = Z^2 * P (1 – P)/d^2$

n = required sample size

Z = confidence level at 95 %(1.96)

P = estimated prevalence of 6.2% (Ndung'u, 2011) (Group, 2011)%)

d = margin of error at 5%

$n = 1.96^2 * 0.062 (1 – 0.062)/0.05^2$

n=90 samples

3.2.3 Sampling technique

A total of 90 sputum samples were collected randomly over two months then used in the experimental design study. The sputum collections and investigations followed supranational reference laboratory procedures in accordance to the WHO guidelines on Sputum collection.

3.3 Research Instruments

The information generated from this study compared existing data of Nandi County from NLLTB, January to April 2016 when Fluorescence AFB Microscopy was in use for a better and clear understanding of its output. The data was collected at Nandi County health clinics identified randomly and analyzed using the laboratory at County Referral Hospital Kapsabet. It was done under strict supervision of the Hospital TB Lab technologist and other technical staff from February 2017 to April 2017 for a period of 12 weeks, five days a week (Monday to Friday). All the samples Negative and positive were recorded within the period and investigated. Using SPSS VERSION 21 descriptive statistics, frequencies and cross tab analysis was done to produce this information.

Materials/System requirement:

Figure 3 : GeneXpert Cartridge

1. **GeneXpert System**
    - Equipped with GX2.1 software/computer/printer/barcode wand-reader and operator manual (Cepheid Inc., Sunnyvale, and USA).
    - It is available in a one, two, four, or 16-module configuration
2. **GeneXpert Cartridge:**
    - Single-use disposable Xpert MTBC/RIF cartridges
    - Sample extraction, amplification and detection are all carried out within this self-contained cartridge.
3. **Class II biological safety cabinet (BSC)**
4. Sample reagent (provided in Xpert MTBC/RIF kit), 8ml volume pack per each cartridge. The sample reagent solution is clear, but may range from colorless to golden yellow.

5. Permanent marker pens.
6. Sterile (individually packed) disposable transfer pipettes– with single mark for minimum volume of sample transfer to cartridge (provided in Xpert MTBC/RIF kit).
7. Sterile screw-capped specimen collection containers/cups.
8. Discard containers for pipettes and sputum containers.

3.4 Data Gathering Procedures

GeneXpert assay

Basic Procedure:

Sputum sample was collected from the patient with suspected TB. The sputum was mixed with the reagent that was provided with the assay, and a cartridge containing this mixture was placed in the GeneXpert machine. All processing from this point on was fully automated. The system integrated and automated sample processing, nucleic acid amplification, and detection of the target sequences which is rpoB gene. GeneXpertMTB/RIF assay test simultaneously detected DNA of *Mycobacterium tuberculosis* complex and resistance to Rifampin (RIF) in less than 2 hours. The primers in the XpertMTB/RIF assay amplified a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with Rifampicin resistance.

Interpretation of GeneXpert results:

Results of GeneXpert was interpreted along with clinical, radiographic, and other laboratory findings. The Xpert MTBC/RIF assay in this study did not replace the need for smear with microscopy for acid-fast bacilli, culture for mycobacteria, and growth-based drug susceptibility testing, in addition to genotyping for early discovery of outbreaks.

Results from the Xpert MTBC/RIF assay indicated whether or not MTBCC was detected in the sample. In some instances, the result was "invalid, no results or erroneous" whereby the test should have been repeated. For purpose of the study this was not necessary.

Where MTBC was detected, the results stated whether resistance to RIF was;

Detected: Mycobacteria have a high probability of resistance to RIF; this was confirmed by additional testing. If RIF's resistance was confirmed, rapid molecular testing for drug resistance to both first-line and second-line drugs was performed so that an effective treatment regimen can be selected.

- **Not detected**: Mycobacteria are probably susceptible to RIF; All tests that were positive for MTBC should have growth-based susceptibility testing to first-line TB drugs hence the medical staff were informed for further procedures.
- **Indeterminate**: The test could not accurately determine if the bacteria are resistant to RIF.

Regardless of the Xpert MTBC/RIF result, Center of Disease Control (CDC) strongly recommends a comprehensive testing algorithm that includes AFB smear and mycobacterial culture as well as drug susceptibility testing (DST) and genotyping.

Limitation of Nucleic Acid Amplification (GeneXpert) System

It was noted that a negative NAA test result did not exclude the possibility of a positive culture also A positive NAA test result did not differentiate between the species of MTBC or determine the presence of other Mycobacteria species. Isolates were required for all

positive MTBC patients in order to perform culture-based drug susceptibility testing and genotyping.

3.4.1 Specimen Sources

A pulmonary specimen was used that is sputum, which is a primary specimen.

3.4.2 Specimen Collection

The sputum was collected early in the morning to ensure highest yield of AFB. It was collected in sterile, leak proof containers then sealed with tape before refrigerating the specimen to reduce overgrowth of contaminating bacteria during transit to lab. At least three consecutive specimens were collected at 8-24 hr intervals. The volume collected was in range of 5-10 ml. During collection, infection control precautions were observed. A patient who could not produce sputum by coughing underwent sputum induction, bronchoscopy, or gastric aspiration. All patients suspected of TB disease had their sputum cultured. Lastly, the specimens were collected to TB lab at Kapsabet county referral hospital laboratory. Within 24 hrs. Patients' names were labeled on test request forms and the specimen container.

3.5 Statistical treatment and Analysis

The information collected was compared to existing data at the facility from January to April 2016 when Fluorescence AFB Microscopy was in use for a better and clear understanding of its output. The data was collected at Nandi County health clinics identified randomly and analyzed using the laboratory at County Referral Hospital Kapsabet. It was done under strict supervision of the Hospital TB Lab technologist and

other technical staff from February 2017 to April 2017 for a period of 12 weeks, five days a week (Monday to Friday). All the samples Negative and positive were recorded within the period and investigated. Using SPSS VERSION 21 descriptive statistics, frequencies and cross tab analysis was done to produce this information. Quantitative data was analyzed using SPSS version 20. Descriptive statistics were used for presenting the resistance of TB to Rif. This consists of frequencies; percentages, and means.

3.6 Ethical consideration

This research was presented to the UEAB institution Review Ethics Committee (IREC) for ethical approval and permission for this study to proceed. This request passed through the Deputy Vice Chancellor Students Affairs and services in order to protect the institution and provide an avenue to handle any legal matters that might arise during the study. Additionally, National Council for Science and Technology approval will be sought through the office of graduate studies.

A request was also sent to Kapsabet county referral hospital laboratory management through the medical superintendent, seeking permission to collect specimen from the institution.

The patients' names were not revealed against the samples to be analyzed. Instead, each sample was assigned a unique number (code) that ensured that their identity was not disclosed to other parties. This ensured confidentiality through the research process. Furthermore, collected data and information was kept under lock and key to ensure non-disclosure of patients' information. Since there was no contact between the researcher and the patients, no informed consent was obtained. However, utmost full professionalism was observed throughout the study.

CHAPTER FOUR

PRESENTATION OF FINDINGS, ANALYSIS AND INTERPRETATION

4.0 Introduction

This chapter presented analysis and interpretation of the findings from information generated from the gene expert method. This data was helpful in investigating and recommending the best method of diagnosis for *Mycobacterium tuberculosis* as the county moves forward in providing safe, fast, accurate and reliable healthcare to the community and adjacent communities

4.1 Objective I: To Determine incidence rate of MTBC in Nandi County

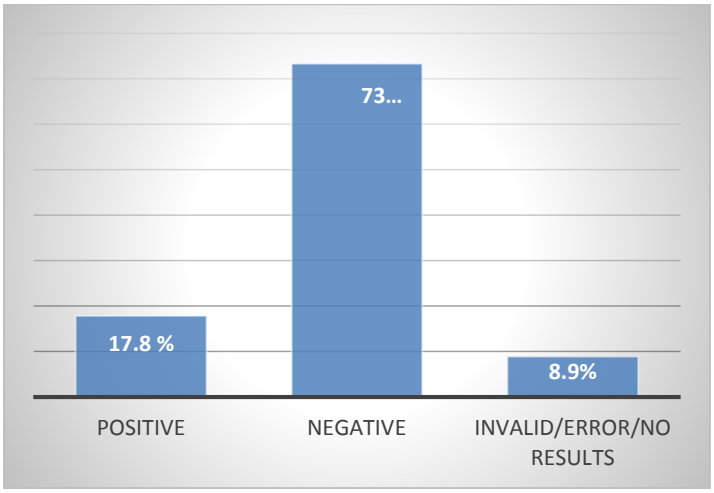

Figure 4: ***Results on incidence of MTBC in Nandi County***

Figure 4 presents the general information on the samples analyzed. Figure 4 shows results on incidents of MTBC in Nandi County. The incident cases of MTBC in Nandi County during the months of January, February and March. The diagram indicates 17.8% (16) of the samples tested were MTBC positive implicating that there was a rise in case detection as compared to results of the same months that the samples were analyzed in the previous year from NLLTB which was 9.5%. There was a notable increase in case detection

since the rate of MTBC in South Saharan was reported in 2016 as 13.7%. while in High income North America the incidence rate was as low as 0.4 (Hmwe., 2018). A rate of 8.9% (8) of the samples tabulated was grouped as samples that did not generate results due to human machine errors. Human machine error occurs when the GeneXpert cartridge doesn't work or the samples were not mounted appropriately, at times it's because of Electric blackouts during sample processing.

4.2 Objective II: To Describe Characteristics of Patients Confirmed to be MTBC Positive under Study at Kapsabet County Referral Hospital Tb Clinic in Nandi County.

Cross tabs analysis was done to investigate MTBC status within group of gender, Age, facility and type of patient as determining factors. Figures 5 below are summary of results generated. The figures below aimed at testing the null hypotheses stating that Gene expert has no impact on MTBC and RIF's resistant TB case detection and accuracy. GeneXpert technology provides a better option for TB diagnostic due to its accuracy and efficacy and also provide information on prevalence of MTBC considering the mentioned factors.

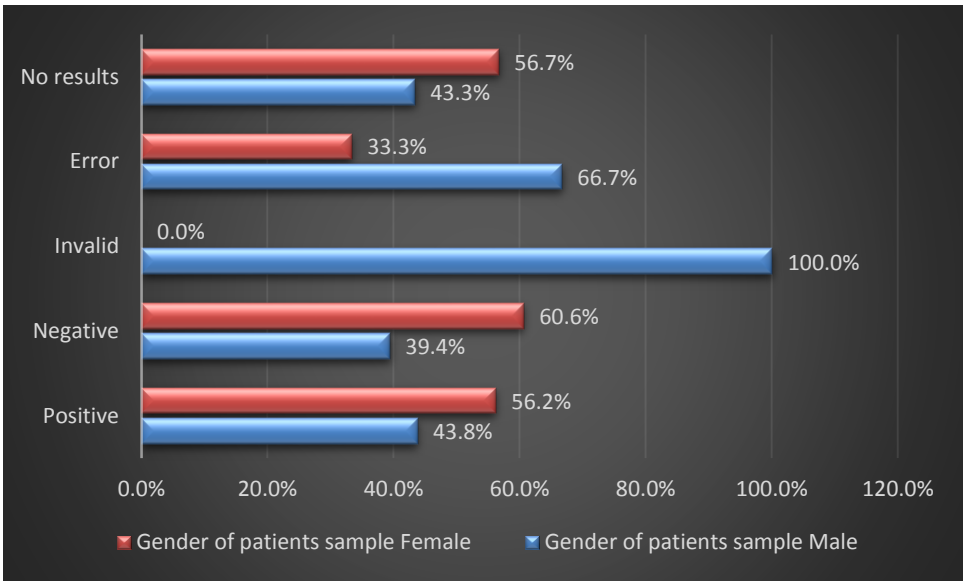

Figure 5: ***Showing Proportions of sputum samples within MTBC status against Gender***

Figure 5 shows the heights of MTBC considering the gender factor. Results from Figure 5 are important in looking at the efficiency, accuracy and prevalence after comparison with the results from previous year and date in time, i.e. same months of the previous year. Female cases were at 56.2% (9) against 43.8% (7) male samples. This is contradictory with NLTP-P database quarterly report of 2016 for Nandi County for the Month of January to March 2016. There were 53%(37) Male MTBC positive samples against 46%(32) female samples and in agreement to a study done in Bangladesh that concluded that the gender difference observed in routine tuberculosis diagnosis is real, and is not due to lesser accessibility of women to the health services (Salim, 2004). The same results were found in Ethiopia by Khan (2013). The reasons for males being more or less affected with TB than females are not well known.

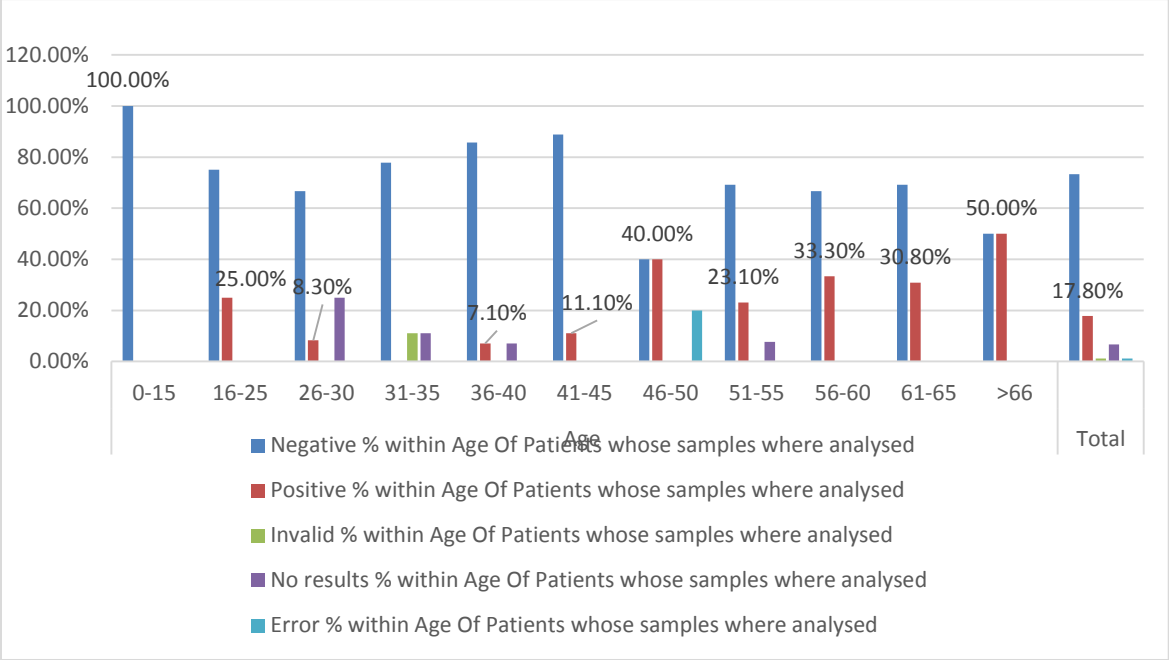

Figure 6 : ***Results on MTBC Status within Age.***

Figure 6 indicates the results of MTBC positive samples considering Age as a factor. The mean for age as a factor was 41. In the results individuals below the age of 15 generated 100% (2) results that were negative, followed closely by results of age groups that recorded over 75% negative results i.e. People aged 31 to 45 (32) and those aged 16-25 at 25% (2). At old age beyond 66 years cases of MTBC positive results was high at 50% (1). The figure also indicated high rates of MTBC among people aged between 16-25, 46-50, 56-60, and 61-65. It's important to note the age groups of 46-50 at 40% (2) to 40% (2) for positive and negative results, and that of people aged beyond 66 years of age whereby negative and positive results don't have a wide margin at 50% (1) to 50 (1) %. This results is in agreement with NTLD-P quarterly report 2016 whereby of reported MTBC cases,

people older than 15 years had a rate of 94.2% (65) and patient samples from age 15 and below was 5.8% (4) with total tests done being 394.

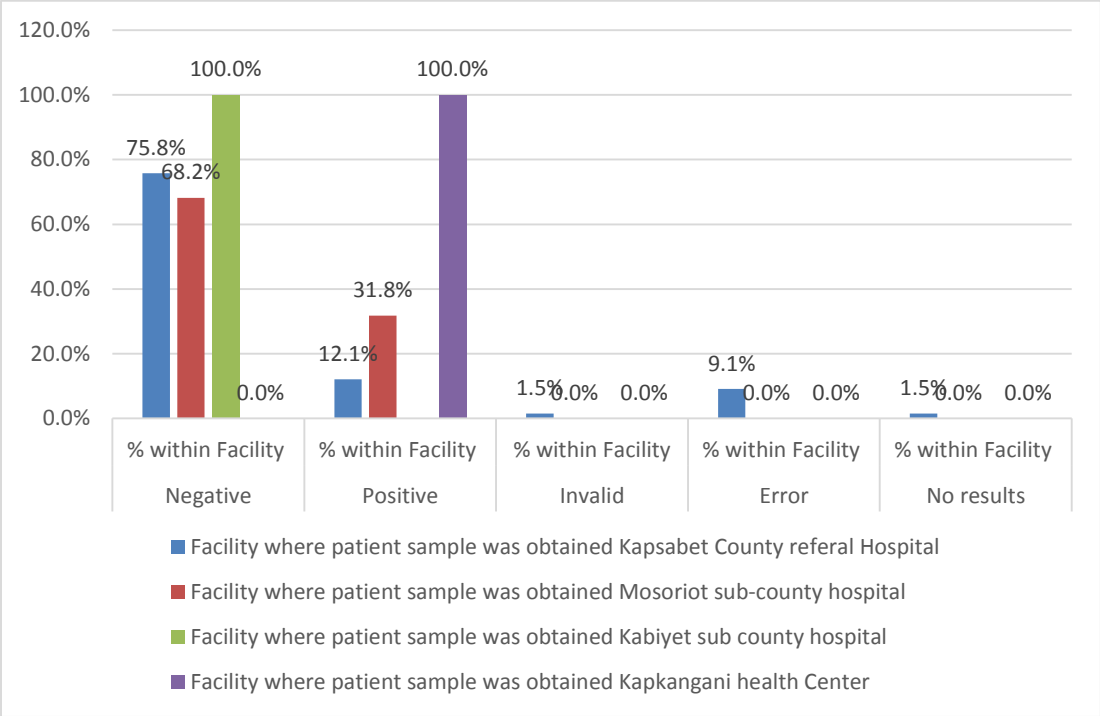

Figure 7: ***Results on MTBC status within facilities***

Figure 7 is indicative of results showing MTBC status of patient sputum samples against facilities where the samples were collected. It's clear that there were more MTBC negative cases at Kabiyet sub-county hospital at 100% this fact has been exaggerated due to the sample size from the Hospital. It's followed by Kapsabet County referral Hospital at 75.8% then Mosoriot sub-county hospital at 68.2% and lastly Kapkangani health center. It is also clear from the results that Kapkangani health center had a high rate of MTBC positive results at 100 %(1) followed by Mosoriot sub county hospital 31.8 %(7) and lastly Kapsabet County referral hospital at 12.1% (8). Kapkangani health center did not yield any MTBC positive results. This results cannot be compared to those tabulated by

NTBLLDP since in 2016 the GeneXpert only existed in one facility which had the highest rate recorded as 9.5% within the facility.

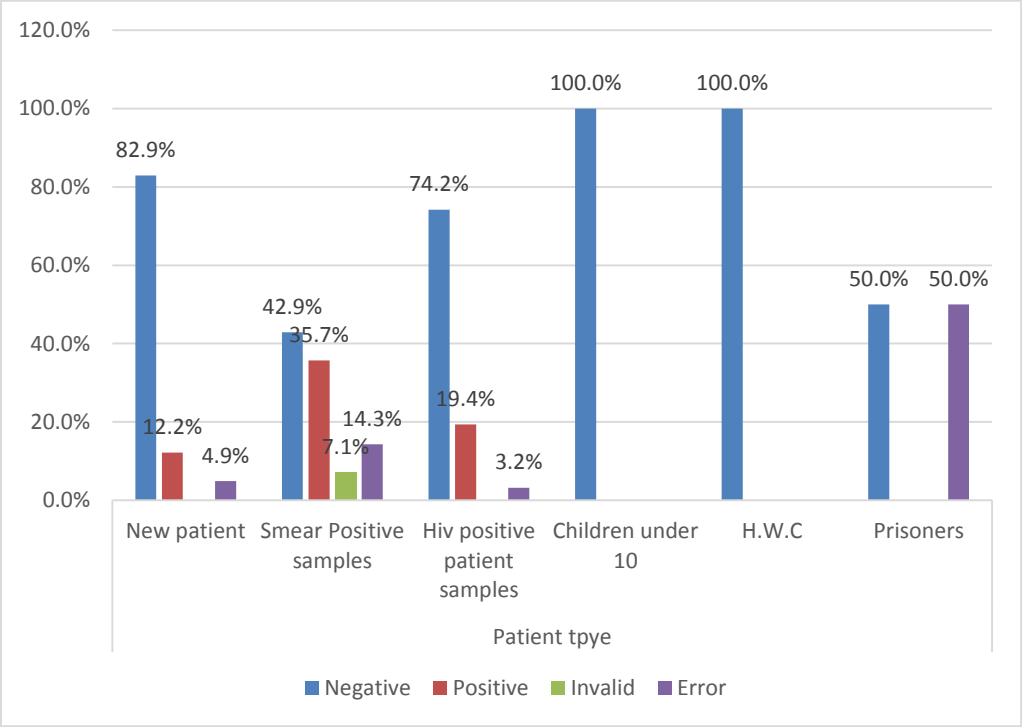

Figure 8: ***Results on MTBC Rif status within patient types***

Figure 8, shows results of MTBC status of sputum samples based on patient type as classified in this study. The figure highlights that percentages of within patient type with patients classified as H.W.C and children under the age 10 having the highest rate of MTBC negative results at 100% (1) for each, followed closely by new patient at 82.9% (34) , then HIV positive patients at 74.2% (23). Patient samples classified as smear positive at two months had the highest cases of MTBC positive results generated at 35.7% (5) followed by HIV positive patient samples at 19.4% (6) which is a low rate compared to NTLLD-P quarterly report of 2016 which indicated 96.1% (98) rate of MTBC occurrence. This study

also indicated that new patient rate at 12.2% (5). Results from prisoners' samples indicated a 50% (1) to 50 %(1) for error and MTBC negative.

4.3 Objective III: MTBC Rif resistance in patients' sputum samples at Kapsabet County Hospital TB clinic in Nandi County

Generally, 5.6% were Rif resistant while 85.5% were negative as shown in table 1.

MTBC RIF Status	Frequency	Percent	Valid Percent	Cumulative Percent	
Valid	Positive	5	5.6	5.6	5.6
	Negative	77	85.6	85.6	91.1
	Invalid	1	1.1	1.1	92.2
	Error	6	6.7	6.7	98.9
	No results	1	1.1	1.1	100.0
	Total	90	100.0	100.0	

Table 1: ***Result on prevalence of MTBC Rif resistance***

The results indicated in Table 1 indicates the incidents of Rif resistant MTBC in Nandi County as per the data analyzed randomly over the 3 months that they were collected. This results provides the answer to the hypothesis stating that Case detection of Rif resistant MTBC has improved and also provides information on the incidents of MTBC Rif resistance. The current study having found Rif resistance of 5.6%, it was slightly lower than 23.9% as in accordance to a research done in informal settlements in Nairobi Kenya by Kerubo in 2016, another study indicated a 9.9% which was noted by Kaur in 2016, in South Africa during 2007–2009 by Coovadia (8.8%) and in Iran (7.4%) by Velayati (2014). NTLDP 2016 quarterly REPORT 2016 indicates of 79 MTBC positive cases reported from January upto March there was only one case of Rif resistance occurring. The Rif resistance

from this study was however higher than a previous study in India (4.69%) by Lahiri (2015). Probable reasons for this would be differences in sample sizes.

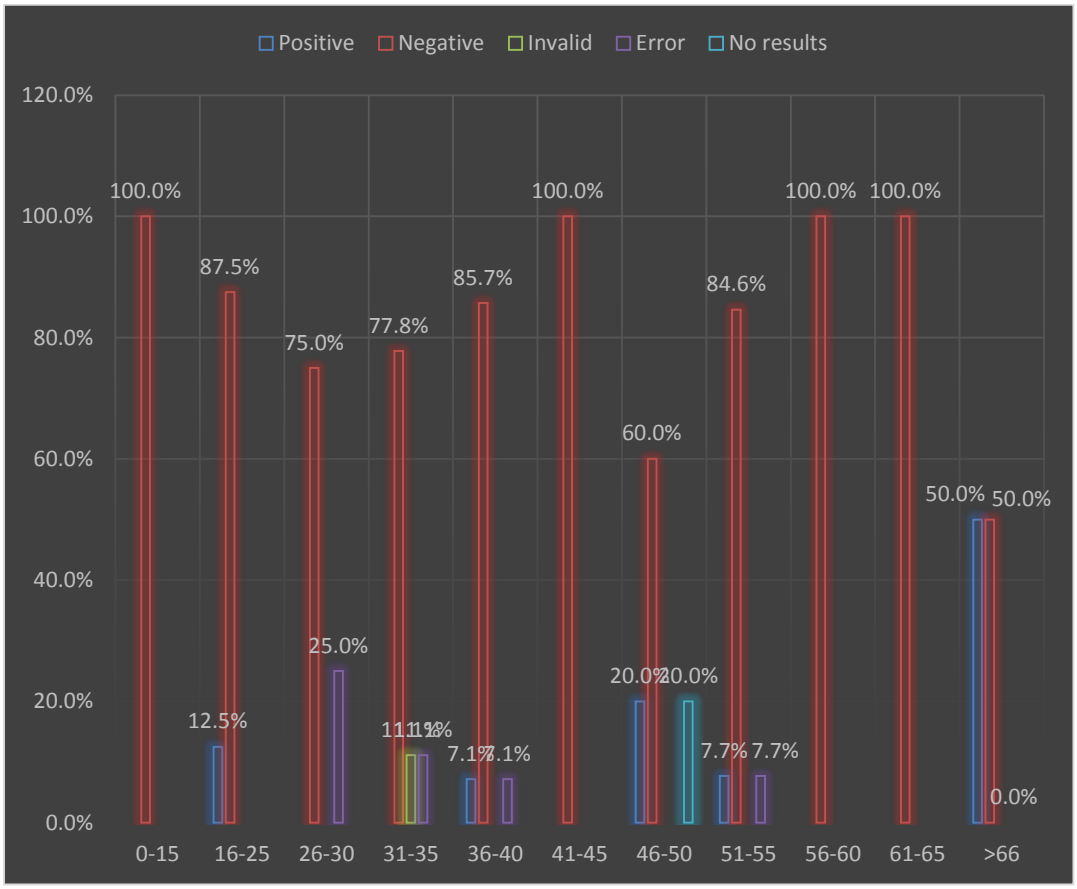

Figure 9: ***Bar graph Results showing MTBC Rif resistance within Age groups***

The results above indicate that the highest number of MTBC Rif distance was from age bracket of 66 years and above at 50% followed by people aged 16-25 at 12.5% then people aged 51-55 at 7.7% and lastly the age group of 7.1%. Its important notice that for people aged beyond 66 the rate of MTBC positive occurrence and MTBC negative occurrence is at 50%, 50% respectively.

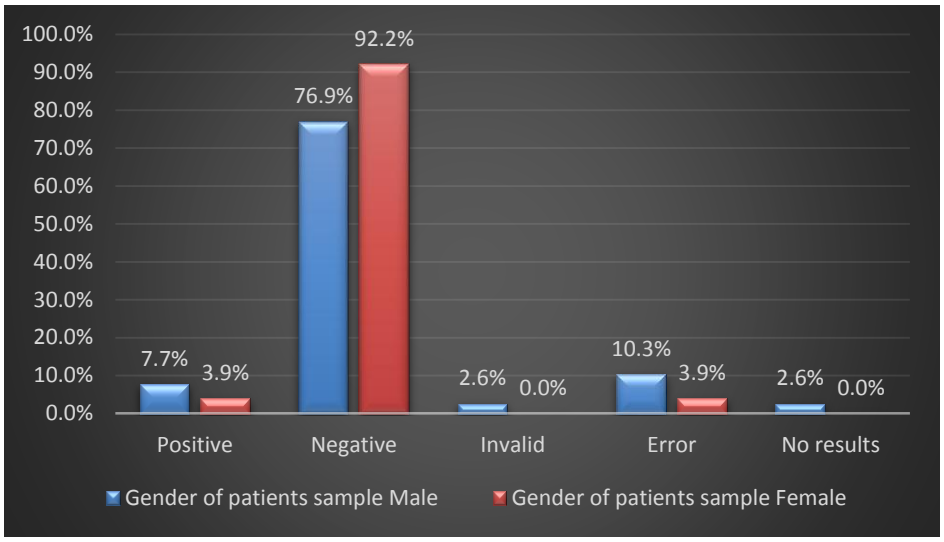

Figure 10: ***Figure showing MTBC Rif Status within Gender***

Figure 10, shows results of MTBC Rif resistance among patient sputum samples that were analyzed. MTBC Rif resistant cases were higher in Males at 7.7% against Females at 3.9%.

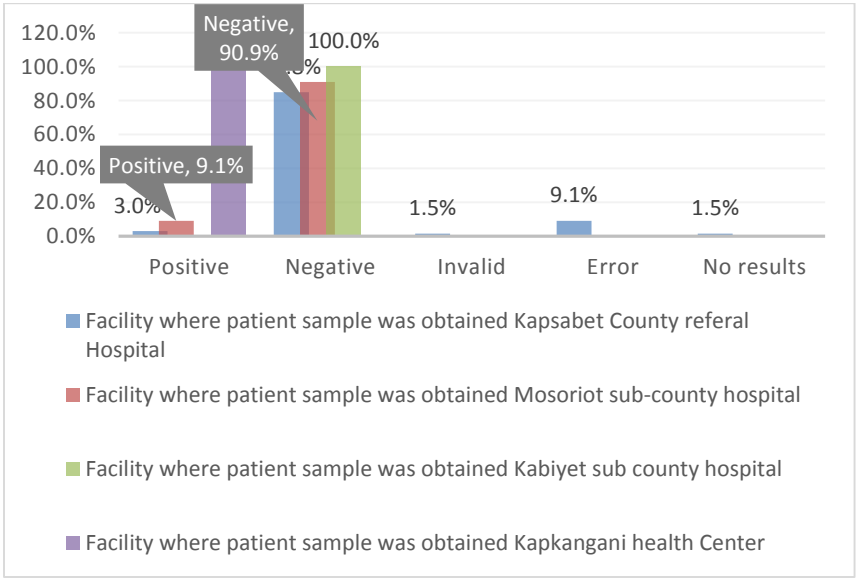

Figure 11: Figure 11: ***Bar Chart showing MTBC Rif resistance within facilities***

MTBC Rif resistance is high at Kapkangani health center 100 %(1) followed by MTBC Mosoriot sub-county hospital.

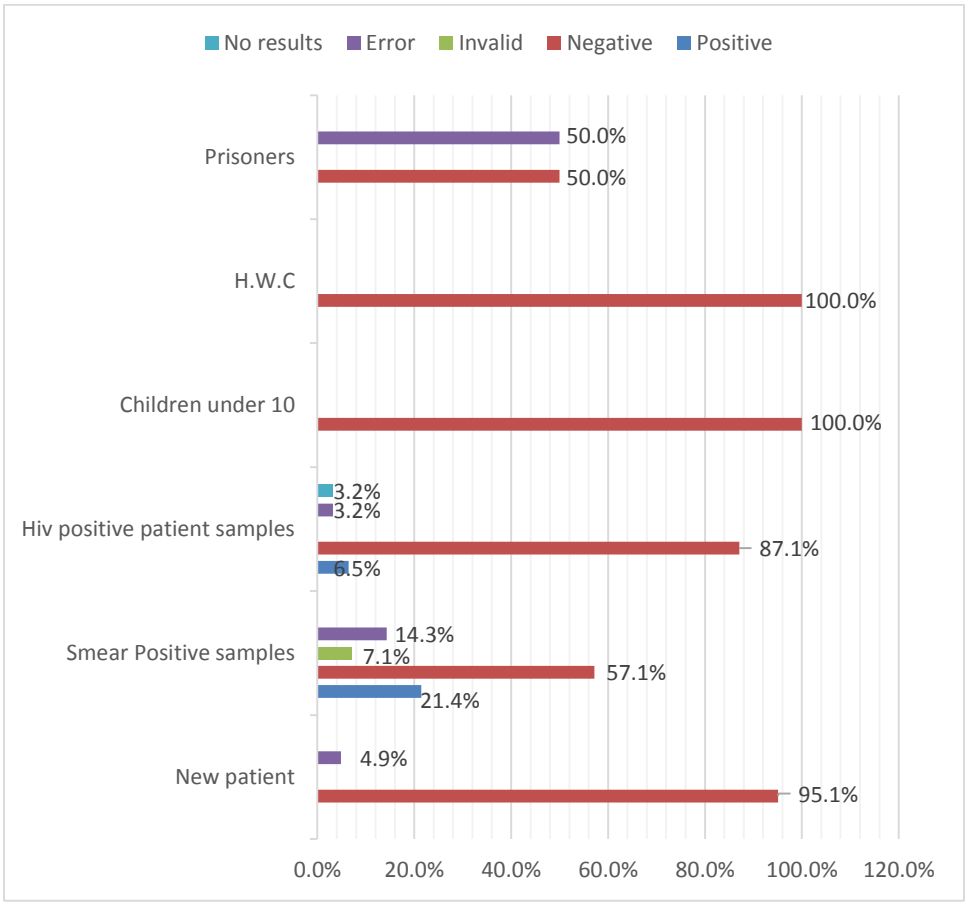

Figure 12: ***Bar graph showing MTBC Rif resistance within patient types.***

The figure above shows results Of MTBC Rif resistance status based on patient type. Smear positive samples had the highest number of MTBC Rif resistant samples at 21.4%, followed by HIV positive patient samples at 5% which is within range as compared to a study by Adrizzoni, 2015, which indicated a rate of 3-19%.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This study was conducted at Kapsabet county referral hospital TB laboratory. The study was conducted to investigate the trend of TB infection among different groups i.e. age groups, sex, facility visited, and patient types. The study was done using randomized experimental design in data collection.

Data collected and analyzed using gene expert machine at the Kapsabet County referral hospital TB laboratory was categorized and analyzed using descriptive statistics.90 samples selected randomly over a period of three months of January 2017 and March 2017, were used in this study.

Guidelines recommend routine nucleic-acid amplification testing in patients with presumed tuberculosis (TB), but these tests have not been widely adopted. Xpert could greatly reduce the frequency and impact of unnecessary empiric treatment, contact investigation, and housing, providing substantial patient and programmatic benefits if used in management decisions (Davis, 2014). Most cases reported in this study indicated that facility based TB tests is intense and significant as the trend points out that some facilities receive more patients than others because of better infrastructure. GeneXpert Machine was only present and functional at only Kapsabet county referral Hospital at the time of study. It is important for TB Program stakeholders in Nandi County to ensure TB cases are managed in all clinics if we are to achieve zero TB within the county and moving forwards.

5.2 Summary of the findings

OBJECTIVE 1: To describe incidence of MTBC and Rif resistant MTBC in Nandi county.

1. Tuberculosis infection is in existence in Nandi County based on the samples analyzed. The sampled analyzed generated positive results at 17.8% (20).

Objective 2:To describe the characteristics of patients confirmed to be MTBCC positive under study at Kapsabet County Referral Hospital TB clinic in Nandi County

1. Female cases were at 56.2% (9) against 43.8% (7) male samples.
2. The highest rate of MTBC infection after cross tabs of within age was in people aged above 66 years at 50%, followed by age 46-50 at 40%.
3. In terms of facility, patients from Kapkangani and had a 100% turn out, Kapsabet county referral hospital also had the highest number of MTBC positive results at 31.8%. Most erroneous results were from Kapsabet County referral Hospital
4. Smear positive patient type reported a high occurrence within patient type at 35.7%. Followed by HIV positive patients group at 19.4% and new patient at 12.2%.
5. Approximately 5.6 % of the MTBC positive cases were Rif resistant.
6. MTBC positive results from new patients and HIV positive patients with symptoms of TB had a 1.1% Rif's resistance.

7. Mosoriot sub county hospital had a higher case of Rif's resistance at 3.3% followed by Kapsabet sub-county hospital at 2.2%.

Objective 3: To determine Rif resistance in MTBCC positive patients' sputum samples at Kapsabet County Hospital TB clinic in Nandi County, using GeneXpert technology.

1. The current study found Rif resistance of 5.6%, it was slightly lower than 23.9% as in accordance to a research done in informal settlements in Nairobi Kenya by Kerubo in 2016, another study indicated a 9.9% which was noted by Kaur in 2016, in South Africa during 2007–2009 by Coovadia (8.8%) and in Iran (7.4%) by Velayati (2014). NTLDP 2016 quarterly REPORT 2016 indicates of 79 MTBCC positive cases reported from January upto March there was only one case of rif resistance occurring.

2. The results above indicate that the highest number of MTBCC rif distance was from age bracket of 66 years and above at 50% followed by people aged 16-25 at 12.5% then people aged 51-55 at 7.7% and lastly the age group of 7.1%.

3. . MTBCC Rif resistant cases were higher in Males at 7.7% against Females at 3.9%.

4. MTBCC Rif resistance is high at Kapkangani health center 100 %(1) followed by MTBCC Mosoriot sub-county hospital at 9.1%

5. Smear positive samples had the highest number of MTBCC rif resistant samples at 21.4%, followed by HIV positive patient samples at 5% which is within range as compared to a study by Adrizzoni, 2015, which indicated a rate of 3-19%.

5.3 Conclusions

Objective 1

- MTBC in Nandi county incidence is at 17.8% (20). prevalence calculated is 0002% hence as per the world health organization global health observatory data repository, in association with Kenya Ministry of health, the national average of tuberculosis prevalence is 223 per a population of 100000 (0.223%) and Nandi being 0.00083% (83) and Nairobi leading at 0.0049% ((490) .

Objective 2

- Female cases were at a high of 56.2% than male cases at a high of 43.8%, hence more research need to be done on age factor in TB occurrence on people in a population.
- Cross tabs analysis indicates special attention should be paid to age beyond 66 years, cases of MTBC positive results was high at 50% (1). The figure also indicated high rates of MTBC among people aged between 16-25, 46-50, 56-60, and 61-65. It's important to note the age groups of 46-50 at 40% (2) to 40% (2) for positive and negative results, and that of people aged beyond 66 years of age whereby negative and positive results don't have a wide margin at 50% (1) to 50

(1) %. Many being new patients and followed closely by HIV positive patients with symptoms of TB and smear positive samples at two months.

Objective 3

- Rif is high in male samples of age groups of relatively young adults within the age range of 16-30 followed by 36-40. 46-55 many being registered as new patients followed by HIV positive patients with symptoms of HIV and smear positives at two months. many samples were sampled at Mosoriot sub county hospital .more the rate at which Rif resistance occurred in this two months was higher than in 2016 which was 4.44 according to the National report on tuberculosis and lung diseases in Nandi county.

5.4 Recommendations

1. In order to overcome the burden of MTBC in Nandi County prevalence studies need to be done and also to increase results accuracy the timeline of such study needs to be extensive in order to improve its relevance.
2. GeneXpert machine has greatly improved case detection of within Age, Sex, Facility, type of patient. Research driven management of TB and resistance patterns should be embraced to enable evidence based decision making and solution finding now that the time taken to get results is an advantage to researchers. It is now easier to study trends of MBT in Nandi county and countrywide.
3. All sub-county hospitals need to watch out for MTBC Rifampicin resistance since most samples captured from Mosoriot Sub County had Rifampicin resistance hence easily missed. The fact that small clinics such as Kapkangani health Center has

fewer tests for MTB should not be ignored. Thorough attention to such clinics should help dig the problem to its roots.

5.5 Recommendation for further studies

- A study needs to be conducted and establish the patterns of MTBC resistance countrywide on all first line antibiotics for TB treatment. Furthermore GeneXpert MTB/RIF ASSAY use needs to be used widely upto remote areas with little or no proper healthcare.

REFERENCES

Abe, C. K. (2008). Biological and molecular characteristics of Mycobaterium tuberculosis clinical isolates with low- level resistance to isoiazid in Japan. *46*(7), 2263-2268.

Almeida (2011, July 09). Molecular basis and Mechanism of drug Resisntance in Mycobacterium tuberculosis. *Journal of Antimicrobial Chemotherapy, 66*(7), 1417-1430.

Amy, M. V. (2013). GeneXpert for TB diagnosis: planned and purposeful implementation. *Global Health*, 18-23.

Andrea , C. F. (2013). Xpert MTBC/RIF for diagnosis of tuberculosis and drug-resistant tuberculosis: a cost and affordability analysis. *European Respiratory Journal* , 708-720.

Andrzej, M. J. (2012). Tuberculosisi and HIV Co-infection. *Plos, 8*, 2.

Badri, L. W. (2015, Oct 23). Light Emitting Diode Fluorescence Microscopy increased the detection of smear-positives during follow-up of Tuberculosis patients in India: program implications. *BMC Research Notes*. Retrieved from https://doi.org/10.1186/s13104-015-1584-z

Boja,D. M. (2018). Comparison of GeneXpert against Light-Emitting Diode Fluorescent Microscopy for the Diagnosis of Pulmonary Tuberculosis in Addis Ababa, Ethiopia. *Journal of Microbial & Biochemical Technology*, 1948-5948.

Brock, I. W. (2004). Comparison of tuberculin skin test in tuberculosis contacts. *American journal of respiratory and critical care medicine,, 170*(1), 65-69.

Catharina,M. P. (2010). Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. *The New England Journal of Medicine*, 1005-1015.

CDC. (2007). *Trends of tuberculosis incidence.* New york: MMWR Morb Mortal Weekly report.

Creswell J1, C. A. (2014). Results from early programmatic implementation of Xpert MTBC/RIF testing in nine countries. *BMC Infectious Disease.* .

Curry International Tuberculosis Center and California Department of Public Health. (2011). *Drug Resistant Tuberculosis: A Survival Guide for Clinicians.* Carlifonia: Curry International Tuberculosis Center.

Deepa, P. T. (2005). *Detection and characterisation of mutations in Rifampicin resistant mycobacterium tuberculosis clinical isolates by DNA sequencing.*

Dickens,M. C. (2018). Epidemiology of Pediatric Tuberculosis in Kenya and Risk Factors for Mortality during Treatment: A National Retrospective Cohort Study. *The Journal of Pediatrics*, 115-121.

Doris,S. R. (2011). Rapid Molecular Detection of Extrapulmonary Tuberculosis by the Automated GeneXpert MTBC/RIF System. *JOURNAL OF CLINICAL MICROBIOLOGY, 49*, 1202–1205.

Elisa,E.L. (2015, Dec 15). Implementing the Xpert® MTBC/RIF Diagnostic Test for Tuberculosis and Rifampicin Resistance: Outcomes and Lessons Learned in 18

Countries. *Plos One*. Retrieved from https://doi.org/10.1371/journal.pone.0144656

ERNEST, J. D. (2012). The immunological life cycle of tuberculosis. *Nature Reviews.*, 581-591.

Falzon, D. (2011). *WHO guidlines for the programmatic management of drug-resistant tuberculosis.* WHO.

Falzon, D.(2016). World Health Organization treatment guidlines for drug-resistance tuberculosis, 2016 update. *European Respiratory Journal,, 49*(3), 1602308.

Fernandez, J. P. (2014). Synthesis and evaluation of a pyrazinoic acid prodrug in Mycobacterium tuberculosis. *Saudi pharmaceutical journal: SPJ: the official publication of the saudi pharaceutical society,*, 376-380.

Gerardo,J. M. (2012). Rapid Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in HIV-Infected Patients. Comparison of LED Fluorescent Microscopy and the GeneXpert MTBC/RIF Assay in a District Hospital in India. *Tuberculosis Research and Treatment*, 4.

Hahn, F. E. (2012). *Mechanism of action of antibacterial agents.*

Helen S. J. (2015). Impact of Decentralized Care and the Xpert MTBC/RIF Test on Rifampicin-Resistant Tuberculosis Treatment Initiation in Khayelitsha, South Africa. *Open Forum Infectious Diseases, 2*(1).

Hilleann, D. R.-G. (2009). Visibility of Genotype MTBCDRsl assay for fluoroguinolone, amikacin capreomycin, and ethambutol resistance testing of mycobacterium

tuberculosis strains and clinical specimens. *Journal of clinical microbiology, 47*(6), 1767-1772.

Hmwe, K. E. (2018). Global, regional, and national burden of tuberculosis, results from the Global Burden of Diseases, Injuries, and Risk Factors 2016 Study. *The Lancet, 18*(12), 1329-1349.

Lucian,L. M. (2014, June 15). Impact of GeneXpert MTBC/RIF on Patients and Tuberculosis Programs in a Low-Burden Setting. A Hypothetical Trial. *ATS journals, 189*.

Jann, L. (2007). Journal of Antimicrobial Chemotherapy. *Fluoroquinolone resistance in Mycobacterium tuberculosis isolates*, 860-865.

Joseph , S. M. (2016). Comparative Yield of Different Diagnostic Tests for Tuberculosis among People Living with HIV in Western Kenya. *Plos One*. doi:10.1371

Kalo, D. K. (2015). Pattern of drug resistance of Mycobacterium tuberculosis clinical isolates to first line antituberculosis drugs in pulmonary cases. *Lung india: official organ of india chest society,, 32*(4), 339.

Karin,F. M. (2013). Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTBC/RI. *European Respiratory Journal*, 252-271.

Khutlang, R. K. (2010, September 1). Classification of Mycobacterium tuberculosis in images of ZN-stained sputum smears. *Pubmed, 14*(4), 949-957.

Lawn, S. D. (2004, January). AIDS in Africa: the impact of coinfection on the pathogenesis of HIV-1 infection. *Journal of infection, 48*(1), 1-12.

Leibert, E. (2010). New drugs and regimens for treatment of TB. *Expert review of anti-infective therapy,, 8*(7), 801-813.

Leimane, V. R. (2005). Clinical outcome of individualised treatment of multidrug-resistance tuberculosis in Latvia: a retrospective cohort study. *The Lancet, 365*(9456), 318-326.

Lienhardt, C. F. (2005, August 1). Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. *International journal of epidemiology, 34*(4), 914-923.

Lonnroth, K. (2009, June). Drivers of tuberculosisis epidemics: the role of risk factors and social determinants. *Social science & medicine,, 68*(12), 2240-2246.

Manzanillo, P. S. (2012). Mycobacterium tuberculosis activates the DNA-dependent cytosolic surveillance pathway wothin macrophages. *Cell host & microbe, 11*(5), 469-480.

Martin, J. C. (2/07/ 2014). *Drug Resistance Mechanisms in Mycobacterium tuberculosis.* Gent,Belgium: Open acces.

Martin, J. P. (2014). Drug Resistance Mechanisms in Mycobacterium Tuberculosis. *OPEN ACCESS*, 317-340.

Mendez, S. T. (2009). The antituberculosis drug pyrazinamide affects the course of cutaneous Leishmaniasis in vivo and increases activation of macrophages and dendritic cells. *Antimicrobial agents and chemotherapy,, 53*(12), 5114-5121.

Muia P. K, N. M. (2017). Performance of GeneXpert Assay in Detecting Pulmonary Tuberculosis and Rifampicin Resistance in Patients Attending Kitui County Hospital, Kenya. *Journal of Tropical Disease*.

Nicol, S. D. (2011, September). Xpert® MTBC/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and Rifampicin resistance. *Future Microbiology, 6*.

Nicol., S. D. (2011, Sept 30). Xpert® MTBC/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and Rifampicin resistance. *Future Microbiology, 6*.

Nishimura, K. H. (2007). Mutation in rsmG, encoding a 16S rRNA methyltransferase, result in low-level streptomycin resistance and antibiotic overproduction in streptomyces coelicolor A3. *Journal of bacteriology,, 189*(10), 3876-3883.

Palomino, J. C. (2009). Molecular detection, identfication and drug resistance detection in mycobacterium tuberculosis. *Pathogens and disease,, 56*(2), 102-111.

Palomino, J. C. (2014). Drug resistance mechanisms in Mycobacterium tuberculosis. *Antibiotics, 3*(3), 317-340.

Pepper, T. J. (2008). Normal chest radiography in pulmonary tuberculosis: implications for obtaining respiratory s[ecimen cultures. *The internatioal journal of tuberculosis and lung disease,, 12*(4), 397-403.

Perdigao, J. M. (2014). GidB mutation as a phylogenetic marker for Q1 cluster Mycobacterium tuberculosis isolates and intermediate-level streptomycin

resistance determination in Lisbon, Portugal. *Clinical microbiology and infection, 20*(5).

Prakash,H. K. (2018). Programmatic Impact of Implementing GeneXpert MTBC/ RIF Assay for the Detection of Mycobacterium Tuberculosis in Respiratory Specimens from Pulmonary Tuberculosis Suspected Patients in Resource Limited Laboratory Settings of Eastern Nepal. *The Open Microbiology Journal*, 9–17.

Prevention, C. f. (2008). *Transmission and Patogenesis of Tuberculosis.* Atlanta,Georgia: U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES.

Priska,J. A. (2019, March 22). Performance and usability of Cepheid GeneXpert HIV-1 qualitative and quantitative assay in Kenya. *Plos One*.

Mario,B. M. (2012). Scaling up interventions to achieve global tuberculosis control: progress and new developments. *The Lancet*, 1902-1913.

Susan,P. S. (2018). Xpert MTBC/RIF Ultra for detection of Mycobacterium tuberculosis and Rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Science Direct*, 76-84.

Roy, L. (1983, Jan 1). The Contagiosity of Tuberculosis. *Europe PMC, 3*(113), 75-79.

Salmaan,M. P. (2012). Tuberculosis, Drug Resistance and the History of Modern Medicine. *The New England Journal of Medicine*, 931-936.

Shahid, M. W. (2015). Gene-Xpert for rapid detection of MDR-TB in re-treatment cases;a comparison with standard culture and DST. *European Respiratory Journal*, 1526.

Shao-Ji, L. T. (2000, March). pncA Mutation as a Major Mechanism of Pyrazinamide Resistance in Mycobacteriumtuberculosis:Spread of a Monoresistant Strain in Quebec Canada. *ASM Journals, 44*(3), 528-532.

Shargie, E. (2007). Determinants of treatment adherence among smear-positive pulmonary tuberculosis patients in Southrn Ethiopia. *PLos medicine,, 4*(2).

Sharma, S. (2004, October). Extrapulmonary tuberculosis. *Indian Journal of Medical Research, 120*(4), 316.

Shi, W. Z. (2011). Pyrazinamide inhibits trans-translation in mycobacterium tuberculosis. *Science, 333*(6049), 1630-1632.

Simon,P. B. (2016). Epidemiology,Diagnosis and Management of Extra-Pulmonary Tuberculosis in a low prevalence country. *PLOS*, 3.

Straetemans, M. G. (2011, June 27). Assesing tuberculosis case fatality ratio a meta-analysis. *Plos One, 6*(6), 20755.

Sujay,M. S. (2015). An update on pathogenesis and managment of tuberculosis with special reference to drug resistance. *Asian Pacific Journal of Tropical Disease.*, 673-686.

Sun, G. L. (2012). Dynamic population changes in Mycobacterium tuberculosis during acquisition and fixation of drug resistance in patients. *The Journal of infectious diseases, 206*(11), 1724-1733.

Tiemersma, E. (2011). Natural History of Tuberculosis: duration and fatality of untreated pulmonary tuberculosis in HIV negative patients:A systematic review. *Plos one, 4*(6).

Van Deun, K. M. (2013). Rifampicin drug resistance test for tuberculosis: challengeing the gold standard. *Journal of linical Microbiology*, 2641-2645.

Wang, Z. (2016). The characteristics of TB Epidemic and TB/HIV Co-infection Epidemic. *PLOS one*, 10.

Wejse, C. G. (2008). TB score: Signs and symptoms fromtuberculosis patients in a low-resource setting have predictive value and may be used to asses clinical course. *Scandinavian journal of infectious disease, 40*(2), 111-120.

WHO. (2013). *Global Tuberculosis Report.* New York: WHO.

WHO. (2013). *Global tuberculosis report.* World Health Organization.

WHO. (2015). *MTD-TB.* WHO GLOBAL TB PROGRAMME.

Yuan, X. Z. (2012). Molecular characterisation of multidrug and extensive drug-resistant mycobacterium tuberculosis srains in Jiangxi. *Journal of clinical microbiology*.

Zhang, Y. (2013). *Drug Susceptibility of Mycobacterium tuberculosis from 10 Provinces in China.* Emory University .

Zhang, Y., & Yew, W. (2009, November 1). Mechanism of drug resistance in Mycobacterium tuberculosis[State oft the art Series.Drug-resistant tuberculosis. *The International Journal of Tuberculosis and Lung Disease, 13*(11), 1320-1330.

PPENDICES

Appendix 1: Ethical Clearance Letter

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH

UNIVERSITY OF EASTERN AFRICA, BARATON

P. O. Box 2500-30100, Eldoret, Kenya, East Africa

September 19, 2017

Raymond Koech
Department of Biological Science
UEAB.

Dear Raymond,

RE: ETHICS CLEARANCE FOR RESEARCH PROPOSAL (REC: UEAB/11/8/2017)

Your research proposal entitled ***"Drug Resistance in Mycobacterium tuberculosis, Isolates from Mosoriot Sub-county Hospital, Nandi County, Kenya"*** was discussed by the Research Ethics Committee (REC) of the University and your request for ethics clearance was granted approval.

This approval is for one year effective September 19, 2017 until September 19, 2018. For any extension beyond this time period, you will need to apply to this committee one month prior to expiry date. Note that you will need a clearance from the study site before you start gathering your data.

We wish you success in your research.

Sincerely yours,

Prof. Jackie K. Obey
Chairperson, Research Ethics Committee

University of Eastern Africa, Baraton
19 SEP 2017
P. O. Box 2500-30100, ELDORET
Research Ethics Committee

A SEVENTH-DAY ADVENTIST INSTITUTION OF HIGHER LEARNING
CHARTERED 1991

REPUBLIC OF KENYA

MINISTRY OF HEALTH

The Medical Superintendent
Kapsabet County Referral Hospital.
P.O Box 5 - 30300
KAPSABET.

Telegrams; MEDICAL;
Telephone; 52081, 52623
When replying please quote

Ref. **RAYMOND KOECH**

9/11/2017

RE: RAYMOND KOECH

The above named person is a M.S.C student from East Africa (Baraton) conducting research on DRUG RESISTANCE in MTB, in laboratory department which is ongoing and to complete on 30th November 2017.

He has received approval to conduct his research

Kindly accord him necessary assistance

Yours faithfuLLY

MEDICAL SUPERINTENDENT
09 NOV 2017
KAPSABET COUNTY REFERRAL HOSPITAL

G.K.SIELE
FOR MEDICAL SUPERINTENDENT
KAPSABET COUNTY REFERRAL HOSPITAL