PREVALENCE, PREDICTORS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON-TUBERCULOUS MYCOBACTERIUM AMONG PATIENTS WITH SMEAR POSITIVE RESULTS IN DODOMA

FATUMA KUCHIMBA AZIZ

MASTER OF MEDICINE IN MICROBIOLOGY AND IMMUNOLOGY

THE UNIVERSITY OF DODOMA

DECEMBER, 2021

PREVALENCE, PREDICTORS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF NON-TUBERCULOUS MYCOBACTERIUM AMONG PATIENTS WITH SMEAR POSITIVE RESULTS IN DODOMA

BY

FATUMA KUCHIMBA AZIZ

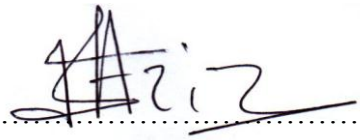
A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE IN MICROBIOLOGY AND IMMUNOLOGY

THE UNIVERSITY OF DODOMA

DECEMBER, 2021

DECLARATION AND COPYRIGHT

I, **Fatuma Kuchimba Aziz** declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award or research project.

Signature:  ……..

CERTIFICATION

The undersigned certifies that he has read and hereby recommends for acceptance by the University of Dodoma a dissertation entitled "**prevalence, predictors and anti-microbial susceptibility pattern of non-tuberculous mycobacterium among patients with smear positive results in Dodoma"** in fulfillment of the requirements for the degree of master of medicine in microbiology and immunology of the University of Dodoma.

Dr. Mkhoi L. Mkhoi

Signature……  …… Date 13/01/2022

SUPERVISOR

ACKNOWLEDGMENT

I would like to present my special thanks to Dr Mkhoi L. Mkhoi, my research supervisor for his tireless work. I thank him for guidance assistance, constructive criticism, patience, and for giving out his time which made the successful completion of this study. I would also like to give my sincere gratitude to Dr Abdallah Baja, he was my mentor, my inspiration and a brother to me. I thank him for his academic assistance and guidance.

I also like to give my thanks to Dr Charles Massambu the head of department of Microbiology and Parasitology and all other staffs of the department of Microbiology and Parasitology at the University of Dodoma. I'm happy and gladly honored to be supervised and guided by these blessed people. May our Almighty God continue to bless them.

A special thanks also goes to hospital management, laboratory managers as well the laboratory workers at DCMC hospital, Dodoma regional referral hospital, Isanga dispensary, Makole health center and Mirembe hospital for their support in data collection. They played a very important part in this research.

I would also like to take this opportunity to thank the Regional Health Management Team for their support which enabled me to do this study in their respective hospitals and health centers found in Dodoma city.

DEDICATION

This study is dedicated to my beloved late Grandmother Zuhura Khalil Ibrahim for laying down the foundation of my education which made me who I am today, and for her daily encouraging words that to never quit despite challenges which i may encounter in life.

ABSTRACT

Background: Non tuberculous mycobacterium are increasingly being recognized as human pathogens, causing morbidity and mortality to the society. Both non tuberculous mycobacterium and *Mycobacterium tuberculosis* are acid fast bacilli. Patients with non tuberculous mycobacterium pulmonary infection may present with clinical features similar to pulmonary tuberculosis infection. Because both non tuberculous and mycobacterium tuberculosis are acid fast bacilli, they cannot be distinguished by smear microscope, a routine diagnostic method in resource limited settings like Tanzania. As a result patients with non tuberculous mycobacterium may be prescribed anti-tuberculosis drugs.

Objective: The study aimed to determine the prevalence, predictors and antimicrobial susceptibility pattern of non tuberculous mycobacterium among patients with smear positive results.

Method: Between October 2020 to March 2021 this cross-sectional analytical study design consented and recruited 140 patients with positive sputum smear microscopy results for acid fast bacilli. Patients provided sputum sample for Mycobacterial culture on solid Lowenstein Jensen media. The GenoType CM/AS (Hain Life science, Nehren, Germany) kit detected species from cultures isolates. Antimicrobial susceptibility testing was performed using proportion method on Lowenstein Jensen media. Normally distributed continuous data were summarized as mean with standard deviation. While Fisher's exact test compared proportion, binary logistic regression model estimated the odds for detecting non tuberculous mycobacterium. A p value of <0.05 was considered significance

Results: Out of 140 patients with acid fast bacilli sputum smear positive, 6(4.29%) had non tubercuulous mycobacterium infection and were identified as *Mycobacterium avium* (n=4) and Mycobacterium kansasii (n = 2). All 6 non tuberculous mycobacterium species were resistant to isoniazid, rifampcin and ethambutol but were sensitive to kanamycin, amikacin and ofloxacin. In adjusted binary regression model, patients with history of previous pulmonary tuberculosis infection were 7.76 times likely to have non tuberculous mycobacterium compared to those who had no exposure to *Mycobacterium tuberculosis* infection ($p = 0.038$).

Conclusion: Non tuberculous mycobacterium is prevalent among patients with sputum smear positive for acid fast bacilli suggesting continual screening. Previous history of pulmonary tuberculosis infection was an independent predictor of non tuberculous mycobacterium infection. All non tuberculous mycobacterium were susceptible to aminoglycosides (kanamycin and amikacin) and fluoroquinolones such as ofloxacin.

Key words: Non-tuberculous mycobacterium, drug susceptibility pattern, acid fast bacilli.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATION

AFB	Acid Fast Bacilli
AS	Additional species
AST	Antimicrobial susceptibility test
ATS	American thoracic society
CLSI	Clinical laboratory standard institute
CM	Common mycobacteria
HIV	Human Immunodeficiency Virus
Lj	Lowenstein Jensen
MTB	Mycobacterium Tuberculosis
MTBC	Mycobacterium Tuberculosis Complex
NTM	Non tuberculous mycobacterium
TB	Tuberculosis

OPERATIONAL DEFINITION

ACID FAST BACILLI: These are type of bacteria which show the characteristic of acid fastness which is a physical characteristic property that gives a bacterium the ability to resist decolourization

CULTURE: A laboratory method of multiplying microbial organism under controlled laboratory conditions.

BIOSAFETY LEVEL 3: This involves handling indigenous or exotic agents that may cause serious or potentially lethal diseases through inhalation and/or pose a serious threat to the environment.

BIOSAFETY LEVEL 2: Biosafety level two cover work with agents associated with human disease, in other words, pathogenic or infectious organisms posing a moderate hazard

CHAPTER ONE
INTRODUCTION

1.0 Background

Non tuberculous Mycobacteria (NTM) belongs to the genus *Mycobacterium*. They are atypical Mycobacteria often reffered as Mycobacterial other than *Mycobacterium tuberculosis* and *M.leprae* (Griffith et al., 2007).This group contains many species which comprises of approximately 170 species which are divided into slow and fast growers. The most common pathogenic types of NTM to humans includes *M.avium* complex, *M.kansasii* and *M.abscessus* (Forbes, 2017; Haworth et al., 2017; Ratnatunga et al., 2020).

NTM are numerously found in the environment, predominantly in fresh water, salt water, biofilms and soil(Thomson et al., 2013). Humans can be infected when exposed to these environments (Falkinham, 2015). Inhalation, ingestion and dermal contact being the main route of infection (Jeon, 2019).

NTM may infect any organ and may be symptomatic or asymptomatic. The most common presentation is NTM lung disease (Griffith et al., 2007). This NTM lung disease is divided into two forms, the fibrocavitary form which is characterized by formation of cavities and nodular bronchiectatic form which is characterized by damaging and scarring of lung tissue(Griffith et al., 2007; Ryu et al., 2016). Patient with NTM lung disease may present at the clinic with similar symptoms to those of pulmonary tuberculosis including cough, hemoptysis, fatigue, malaise, weight loss etc (Kim et al., 2014).

Non - tuberculous mycobacterium lung disease is an emerging disease which affects both immunocompromised and immunocompetent individuals, and the incidence rate of this infection keeps on increasing(Haworth et al., 2017; Koh et al., 2013).

Old age, immune deficient state including HIV and AIDS, cigarette smoking, use of tap water, previous history of tuberculosis infection, structural lung diseases which may either be genetic or acquired are some of the conditions which may make an individual to be susceptible to NTM lung infection ,(Field et al., 1994; Ratnatunga et al., 2020; Zhang et al., 2019)

Diagnosis of non tuberculous mycobacterium disease involves the use of clinical feature and laboratory investigations such as culture, molecular and chemical diagnostic technique which are the standard investigations (Griffith et al., 2007; Ryu et al., 2016). Fortunately the American thoracic society proposed the minimum evaluation criteria for patients being investigated for NTM lung disease and they includes the chest radiographical abnormalities such as fibrosis and cavity, detection of three or more positive sputum specimen for acid fast bacilli in the absence of pulmonary tuberculosis (Griffith et al., 2007). However use of sputum smear for microscopy is less sensitive and it cannot discriminate NTM from *Mycobacterium tuberculosis* causative agent of TB and consequently it challenges clinical decision at TB clinic (Detjen et al., 2015; Griffith et al., 2007; Kilale et al., 2016)

Sputum culture remain the gold standard method for the diagnosis of NTM however other methods such as matrix assisted laser desorption time of light (MALDI-TOF) and molecular technique are used for the specie identification of non tuberculous mycobacterium (Ingen, 2013; Mediavilla-gradolph et al., 2015) Molecular investigation techniques involve the use of highly technological machines and equipment which are very expensive (Hoza et al., 2016; Okoi et al., 2017).

Treatment of non tuberculous mycobacterium is individualized in a manner that species differ in the regime of treatment (Griffith et al., 2007). Despite of presence of treatment regimens for each species of non-tuberculous mycobacterium, antimicrobial susceptibility tests is usually recommended because of the emergency of resistant strain of non-tuberculous mycobacterium(Griffith et al., 2007). The most common treatment for *Mycobcterium avium* lung disease includes clarithromycin or azithromycin, ethambutol and rifmpin however the severity of the disease determines the amount of dose and frequency of drug intake, the recommended treatment regime of *M.kansasi* according to American thoracic society includes isoniazid, rifampin and ethambutol daily for 12 months (Griffith et al., 2007).

Worldwide there is a increase in the prevalence of non-tuberculous mycobacterium infection as well as the mortality and morbidity rate (Marras et al., 2017; Ratnatunga et al., 2020; Kevin L Winthrop et al., 2020). The knowledge of prevalence of non-tuberculous mycobacterium in Africa and most sub Saharan countries is still low

because of the diagnostic challenges (Okoi et al., 2017). However there are several studies which have been done in Africa and have shown that there is increase of non tuberculous mycobacterium cases (Agizew et al., 2017; Chanda-Kapata et al., 2015). The study done in Botswana among HIV pulmonary suspected case reported non tuberculous mycobacterium prevalence of 56% which is the largest prevalence ever reported in Africa (Agizew et al., 2017).

The overall prevalence of non-tuberculous mycobacterium in resource constrained African countries like Tanzania is not yet known. However there are some few studies which have been done and show the prevalence in different parts of the country. Example in northern Tanzania it was 9.7% (Hoza et al., 2016), in the study done by Mnyambwa et al in different selected hospital in Tanzania the prevalence of NTM was 3.19% (Mnyambwa et al., 2017). Study done in Mawenzi and KCMC hospital showed the 9% prevalence of NTM (Crump et al., 2009). The scarce data on the prevalence of NTM in these settings may be attributed to limited diagnostic options. Therefore this study aimed to determine the magnitude of the NTM lung disease, species of non-tuberculous mycobacterium and the type of antibiotics susceptible to those NTM species found.

1.1 Problem statement

Worldwide there is an increase in prevalence, morbidity and mortality rate caused by non tuberculous mycobacterium (Diel et al., 2017; Donohue & Wymer, 2013; Marras et al., 2017; Ratnatunga et al., 2020; Kevin L Winthrop et al., 2020). Evidence is shown in the comparative study for non-tuberculous mycobacterium done in USA whereby it shows an increase in mortality rate of 10% due to no- tuberculous mycobacterium infection (Mirsaeidi et al., 2014). In canada the mortality rate of 26.6% for non-tuberculous mycobacterium pulmonary infection and 39.69 % for NTM pulmonary disease was reported (Marras et al., 2017). Study done in Botswana among HIV patients with suspected pulmonary TB infection cases reported 56% prevalence of non-tuberculous mycobacterium which was the largest prevalence ever reported in Africa (Agizew et al., 2017).

The knowledge of prevalence and number of death due to non tuberculous mycobacterium in Africa and most sub Saharan countries is still low because of the

diagnostic challenges (Okoi et al., 2017). Smear microscope investigation is mostly widely used in many developing countries especially in peripheral laboratories which do not have access to high technological machines for the diagnosis of *Mycobacteria* infection (Detjen et al., 2015)

This situation has potentially lead to missing cases of NTM pulmonary disease and mistreatment of patients with NTM infection by prescribing anti-tuberculosis drugs while they don't have *Mycobacterium tuberculosis* infection thus exposing the patients to toxic side effects of anti-tuberculosis drugs unnecessarily .Current reporting of *Mycobacterium tuberculosis* infection to public health authorities may be overestimating the burden of *Mycobacterium tuberculosis* lung diseases since in most settings only direct microscopic examination for acid fast bacilli is done..

It is important to distinguish between non-tuberculous mycobacterium and *Mycobacterium tuberculosis* infection because therapeutic regimens differ from one another. And because of evolution of multidrug strains of Mycobacterium antimicrobial susceptibility test is a very crucial matter. In limited resource settings NTM may be misdiagnosed and treated as multidrug resistance tuberculosis (Mnyambwa et al., 2018; Shahraki et al., 2015).

1.2 Study objectives

1.2.1 Broad objective

To determine the prevalence, predictors and antimicrobial susceptibility pattern of non-tuberculous mycobacterium among patients with AFB sputum smear positive results in Dodoma.

1.2.2 Specific objective

i. To determine the prevalence of non - tuberculous mycobacterium among patients with AFB sputum smear positive results in Dodoma.

ii. To determine predictors of non - tuberculous mycobacterium infection among sputum culture positive patients in Dodoma

iii. To determine antimicrobial susceptibility pattern of non-tuberculous mycobacterium infection in patients with AFB sputum smear positive results in Dodoma.

1.3 Research questions

i. What is the prevalence of non - tuberculous mycobacterium infection among patients with AFB sputum smear positive results in Dodoma?
ii. What are the predictors of non - tuberculous mycobacterium tuberculosis among AFB sputum smear positive patients in Dodoma?
iii. What is the antimicrobial susceptibility pattern among non-tuberculous mycobacterium, patients in Dodoma?

1.4 Significance of the study

The study has created awareness on the presence of non-tuberculous mycobacterium among health workers and especially those who attend suspected cases of tuberculosis. This study has added update knowledge in the literature from Dodoma that patients with prior history of TB infection are at risk of contact NTM lung disease supporting the argument or continual screening of the population

Moreover the study generated the knowledge for clinician that NTM do not respond to first line ant TB and therefore regime containing aminoglycosides and fluoroquinolones would have good patient's outcome.

CHAPTER TWO
LITERATURE REVIEW

2.0 Prevalence of non tuberculous mycobacterium

The prevalence of NTM infection has increased worldwide in recent decades due to improved diagnostic methods and increased physician awareness (Y. M. Lee et al., 2021; Lin et al., 2018; Okoi et al., 2017; Ryu et al., 2016). However majority of the data available about non tuberculous mycobacterium and its epidemiology are from developed countries, only few are available from middle and poor countries (Okoi et al., 2017)

Epidemiology of non - tuberculous mycobacterium in most of the low-income countries is less known as there is a diagnostic challenge (Okoi et al., 2017). Culture and molecular technique are not routinely used for the diagnosis of pulmonary infection with non - tuberculous mycobacterium, and more emphasis is kept in the mycobacterium tuberculosis diagnosis (Okoi et al., 2017).

Worldwide studies about non-tuberculous mycobacterium are still continuing to be done. Between 2009 and 2014 there was a study done in Germany about NTM which showed increase in prevalence from 2.3% to 3.3% in the year 2014 (Ringshausen et al., 2016). The study done in Hyderabad NTM infection among non HIV patients showed the prevalence of 22.26% (Thangavelu et al., 2021). The study done in China among HIV infected individuals suspected to have pulmonary TB infection the prevalence of NTM was 47% (Lan et al., 2011).

In Africa there are not much data available about non tuberculous mycobacterium compared to other parts of the World because of the resource scarcity but there are some few areas in Africa which have managed to do studies on NTM. The study done in Botswana in the year 2017 showed NTM prevalence of 56% among suspected pulmonary TB cases (Agizew et al., 2017). This is the largest prevalence of NTM ever recorded among African countries (Agizew et al., 2017). The study done in Malawi on presumptive cases of multidrug resistant TB patients showed NTM prevalence of 18% between year 2015 to 2018 (Mabaso & Mughogho, 2020).

There are several studies done in Tanzania about non tuberculous mycobacterium (Hoza et al., 2016), (Crump et al., 2009), (Katale et al., 2014), (Mnyambwa et al., 2018) but still the prevalence of NTM in many parts of the country is yet unknown compared to *Mycobacterium tuberculosis* infection. The prevalence of non tuberculous mycobacterium in northern Tanzania was found to be 9.7% from the study done in 2016 by Hoza (Hoza et al., 2016). Also the study done in patents admitted at Kilimanjaro Christian medical center and Mawenzi hospital in northern Tanzania having axillary temperature of $>38^0$ C, 9% were found to have non tuberculous mycobacterium infection (Crump et al., 2009).

Prevalence variation of species of non tuberculous mycobacterium differs in regions and in different geographical condition worldwide (Hoefsloot et al., 2013; Shao et al., 2015; Spaulding et al., 2017).

2.1 Predictors for non-tuberculous mycobacterium infection.

Many researches done in several parts of the world have shown different predictors for acquiring non-tuberculous infection. Literatures shows that old age, immune deficient state including HIV and AIDS, cigarette smoking, use of tap water, previous history TB infection, structural lung diseases which may either be genetic or acquired are termed as predictors for non-tuberculous mycobacterium lung infection ,(Field et al., 1994; Ratnatunga et al., 2020; Zhang et al., 2019).

2.1.1 Age with non-tuberculous mycobacterium infection

Literatures have shown that people with from 50 years or more are more susceptible to infection by non tuberculous mycobacterium compared to other age groups. The result of the study done in south Korea on the epidemiology of non tuberculous mycobacterium have shown that people with 70 years and above were more at risk of acquiring non tuberculous mycobacterium (H. Lee et al., 2019) .In the study done between 2008 to 2013 on NTM within five states of USA, old age starting from 50 years and above to be one of the factors which increases the susceptibility to acquire NTM infection (Donohue & Wymer, 2013). Similar study which was done in Hawaii starting from the year 2005 up to 2013 also have shown that those with age of 75 years and more were more susceptible to non tuberculous mycobacterium infection (Adjemian et al., 2017).

Impaired immunity in people with old age may be one of the reasons for them to acquire many different types of infection including non tuberculous mycobacterium infection. The immune system protection usually decreases as one ages. As the immune system ages the less protection against diseases occurs (Weyand & Goronzy, 2016). Impairment in immune status as a normal outcome of aging and changes which occur in the lungs as well as the whole respiratory system are the major causes of elderly people to be more prone to infection of the respiratory system (Hannah et al., 2020; Meyer, 2005).

2.1.2 Non-tuberculous mycobacterium infection with HIV

In 1982 the first case of non-tuberculous mycobacterium was detected and the organism isolated was from the species of mycobacterium avium complex (Falkinham, 2003)

People living with HIV are more prone to NTM infection and about 56% of patients were revealed to have non tuberculous mycobacterium infection among people living with HIV in the study done in Botswana (Agizew et al., 2017). This was the largest prevalence of NTM ever obtained compared to other studies done in the sub Saharan countries. HIV infection is associated with rise in number of NTM isolation (Borland et al., 2019)

There are many studies which have been done worldwide which shows that HIV infection paves ways to non-tuberculous mycobacterium infection (Borand et al., 2019; Hannah et al., 2020; Lan et al., 2011). Species of non tuberculous mycobacterium which are commonly isolated in HIV infected individuals are from Mycobacterium avium complex (Bjerrum et al., 2016; Borand et al., 2019; Varley et al., 2017). Non tuberculous mycobacterium infection is associated with high mortality rate among people with HIV/AIDS and MAC species being the most common isolated group (Mirsaeidi et al., 2014).

HIV infection was shown to be the most common predictor of NTM infection in the study done in Northern Tanzania (Hoza et al., 2016).In the study done in the U.S.A to assess the NTM mortality rate the results shows that the percentage of NTM infected individuals were higher in HIV compared to other diseases e.g. TB

(Mirsaeidi et al., 2014). Also the study done in Mexico showed that HIV infection was significantly associated with non-tuberculous mycobacterium, in this study more NTM were isolated from people living with HIV infection (Lopez-luis et al., 2020).

The state of decreasing immunity caused by decline in the levels of CD4+ cell count contributes to their vulnerability to pulmonary infection with non-tuberculous mycobacterium (Hannah et al., 2020; Lopez-luis et al., 2020). Highest incidence of non tuberculous mycobacterium infection were observed in patients with CD4 >50cells/mm^3 in a study done to determine the incidence of non-tuberculous mycobacterium in HIV infected individuals done in Oregon, USA (Varley et al., 2017).

Impairment in T cell mediated immune response makes HIV infected individuals vulnerable to non-tuberculous mycobacterium respiratory system infection (Lapinel et al., 2019). Impairment in the immune system not only causes them to be directly susceptible to NTM but also causes other disease conditions which increase the risk of acquiring non-tuberculous mycobacterium infection such as structural lung diseases such pulmonary tuberculosis.

However the use of antiretroviral drugs has caused improvement in immune status among patients who are HIV positive and this has remarkably decreased the rate of non-tuberculous mycobacterium infection (Kobayashi et al., 2016). The ART's have helped in reducing viral load as well as increase in CD4 cell count.

2.1.3 Other immune deficiency disease and factors for non-tuberculous mycobacterium

Apart from HIV/AIDS, hematological malignancies, hairy leukemia and other immunodeficiency state, hematopoietic stem cell transplantation and solid organ transplantation immunosuppressive drugs including anti-tumor necrosis factor (anti-TNF) have been associated with NTM disease (Axson et al., 2019).

Use of certain drug predisposes or put a patient at risk of NTM infection and steroids is one of the groups of these drugs. Effect on the use of steroid was revealed by the case-control study done in Denmark where by patients who were in long time use of steroids were seen to be at the risk of acquiring non tuberculous mycobacterium

infection because steroids have the character of suppressing the immunity (Andréjak et al., 2013). In the study done by Winthrop it has been seen that the use of anti-TNF leads to increased chances for non tuberculous mycobacterium infection in cases of rheumatoid arthritis because it is associated with decrease in immunity (K L Winthrop et al., 2013). Tumor necrosis factor alpha (TNF-α), is a pro-inflammatory cytokine that is required for formation and maintenance of granulomas that effectively inhibit bacterial growth.

Patients suffering from acquired Anti-IFNγ auto antibodies and GATA2 deficiency are susceptible to non tuberculous mycobacterium. Study done in Bangkok Thailand to find the risk factors for NTM revealed that majority of men and women with anti IFN- γ autoantibody were the one with a great extent of suffering from non tuberculous mycobacterium infection (Phoompoung et al., 2017) (Browne et al., 2012). IFN- γ is necessary for the control of intracellular bacteria such as NTM.

2.1.4 Non-tuberculous mycobacterium in water system

Many research studies have indicated water as a main source of transmission of non tuberculous mycobacterium (Loret & Dumoutier, 2019a), (Falkinham, 2011), (Edirisinghe et al., 2014). Water pools, hot tub, house or hospital water systems are few examples of sources of infection. Concentration of non tuberculous mycobacterium is higher in surface water than in ground water (Loret & Dumoutier, 2019b). In the study done in Japan many respiratory active non tuberculous mycobacterium were isolated in households water system (Ichijo et al., 2014)

Non tuberculous are resistant to local water disinfectants used for water treatment due to their hydrophobic nature. Hydrophobic characteristic and ability to grow at low level of organic carbon are the factors which enable them to attach to surface and multiply in biofilms respectively (Loret & Dumoutier, 2019b).

2.1.5 History of previous TB infection with non tuberculous mycobacterium

History of pulmonary tuberculosis infection has been seen to be associated with the risk of developing non tuberculous mycobacterium lung infection. This has been shown in several studies including the one done in Taiwan whereby people who had

a history of previous TB infection and other structural lung diseases were seen to be more affected by non tuberculous mycobacterium infection (Huang et al., 2017).

TB infection after healing have a tendency of causing scars, destruction of lung parenchyma as well as airway flow which causes impairment of the normal immune system of the respiratory system (Amaral et al., 2015). With abnormal lung structure and impairment in the immune system it becomes easy to get non-tuberculous mycobacterium infection to individuals who had a previous history of TB infection.

2.1.6 Structural lung diseases with non tuberculous mycobacterium

With abnormal lung structure and impairment in the immune system it becomes easy to get non tuberculous mycobacterium infection (Griffith et al., 2007). Cavity formation leads to destruction of the lungs parenchyma, which results in respiratory failure. The study which was done in United Kingdom on the risk factor for NTM lung disease shows that cavity formation in bronchiectasis was one of the predictor for NTM infection (Axson et al., 2019). Study done in Hawaii also have shown similar results were destruction of lung parenchyma and nodules formation to be one of the predictors for NTM infection (Adjemian et al., 2017).

In the study done in Israel between 2002 and 2011 have shown that there is high prevalence of NTM infection in people with cystic fibrosis (Bar-on et al., 2015). In cystic fibrosis there is impairment in airway clearing system because of mucus plugging thus it becomes easy for infection to flourish.

2.2 Laboratory diagnosis of non tuberculous mycobacterium

Non tuberculous mycobacterium belongs to the genus *Mycobacteria* which comprises of acid fast bacilli characterized by resistance to decolourization by acid alcohol during staining and when viewed under microscope they all appear the same (Griffith et al., 2007; Jawetz et al., 2010; Pennington et al., 2021). Diagnosis confirmation of non-tuberculous mycobacterium disease involves the use clinical features and laboratory investigations such as culture, molecular and chemical diagnostic techniques which are the standard investigations (Griffith et al., 2007; Ryu et al., 2016)

The most practiced criteria for diagnosis of patient suspected to have non tuberculous mycobacterium are the one developed by American thoracic society (ATS) (Griffith et al., 2007). The minimum evaluation criteria described by the American thoracic society for patient suspected to have non tuberculous mycobacterium lung disease are chest x ray, two or more sputum specimen for acid fast bacilli analysis including specie identification and exclusion of other disorders such as pulmonary tuberculosis (Griffith et al., 2007)

Microbiological diagnosis involves performing culture where by for pulmonary infection the guideline provided by ATS requires at least two sputum specimen collected at two different occasion or single lower respiratory specimen and in non pulmonary infection source of culture is determined by the site which is affected (Ingen, 2015). In the diagnosis, non tuberculous mycobacterium should be identified to specie level as treatment differ between species thus after culture of the specimen identification be done (Pennington et al., 2021).

Matrix assisted laser desorption time of light (MALDI-TOF) and molecular technique are used for the specie identification of non tuberculous mycobacterium (Ingen, 2013; Mediavilla-gradolph et al., 2015). MALDI-TOF identification is based on measuring the fingerprint in protein extraction of an organism while molecular methods based on identification of respective genes (Mediavilla-gradolph et al., 2015). The most common molecular methods which are used are line probe assay and gene sequencing which uses hsp65, rpoB gene and 16s-23s for discrimination of species (Ingen, 2013).

2.3 Treatment and antimicrobial susceptibility of non-tuberculous mycobacterium

Treatment of non tuberculous mycobacterium involves the use of antibiotics for a long time, however the presence of NTM does not mark the initiation of therapy against NTM species, potential risks and benefits of therapy for individual patients must be considered (Ryu et al., 2016).

Treatment of non tuberculous mycobacterium is not generalized as compared to tuberculosis infection, treatment of non tuberculous mycobacterium is individualized

in a manner that species differ in the regime of treatment (Griffith et al., 2007). Macrolide based regime show much efficacy in the treatment of nodular/ bronchiectatic non tuberculous lung disease (Griffith et al., 2007; Killingley et al., 2014).

The results from the study done by Li et al on fast and slow growers non tuberculous mycobacterium, Streptomycin, Amikacin, Fluoroqunolones and Tetracycline were found to be the most sensitive drugs (Li et al., 2013). The results of antimicrobial susceptibility in the study done in China about rapid growing Mycobacterium is shows that they were sensitive to Amikacin, Linezolid, cefmatazole, , macrolides and carbapenems and they were highly resistant to first line anti TB (Pang et al., 2015).. Another study done in China among TB suspected patients the NTM isolates showed different pattern towards antibiotics were by they were resistant to Rifmpcin, levofloxacin, ofloxacin, ethambutol, ofloxcin and amikacin (Zhou et al., 2021). Susceptibility of drug toward bacteria differs in different geographical location. Susceptibility pattern may differ from one part to another

2.4 Conceptual frame work

In this study the conceptual frame work shows the relationship of non-tuberculous mycobacterium with different independent variables. Age, gender HIV/AIDS, diabetes, history of previous tuberculosis infection, cigarette smoking, use of tap water, use of steroids or other immunosuppressive drugs. These independent variables put an individual at a risk to acquire non-tuberculous mycobacterium infection.

INDEPENDENT VARIABLES **DEPENDENT VARIABLE**

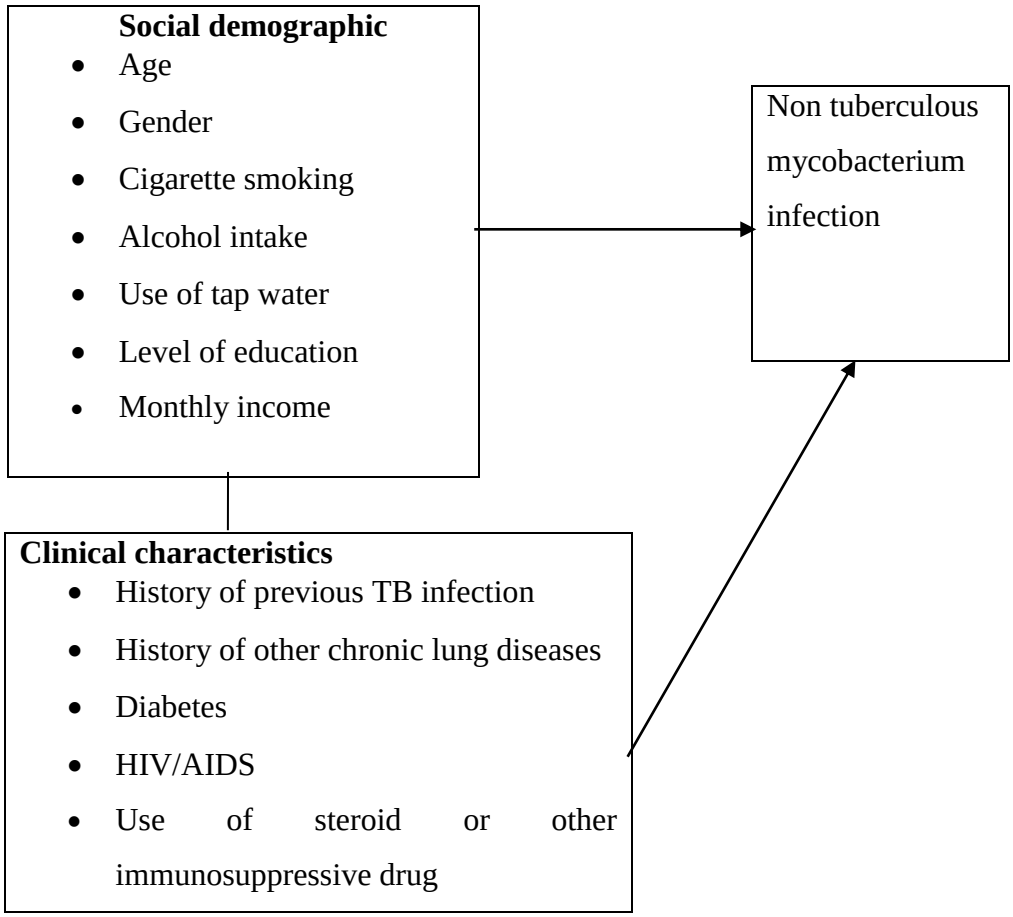


Figure 1: Conceptual frame work

CHAPTER THREE
METHODOLOGY

3.0 Study design

This was the cross-sectional analytical study conducted from October 2020 to March 2021to assess the prevalence, predictors and antimicrobial susceptibility pattern of non-tuberculous mycobacterium in patients with acid fast bacilli sputum smear positive in Dodoma.

3.1 Study location

The study was conducted at Dodoma regional referral hospital, Isanga dispensary, Makole health center, St. Gemma hospital, DCMC and Mirembe hospital found in Dodoma city which is found in Dodoma region. Dodoma is a capital city of Tanzania found in the central zone bounded by Iringa, Singida, Morogoro and Manyara region. These hospitals serve patients from Dodoma region and all its districts (Bahi, Dodoma urban, Mpwapwa, Kondoa, Chemba and Kongwa. Dodoma region has a total number of 2,083,588 people according to census of 2012. The residents of Dodoma region are Gogo and Rangi. The Dodoma Region is a capital city of Tanzania which is just about 480 km from the coast. The region covers an area of 41,311 square kilometers (15,950 sq m). There is marked increasing of reports suspected TB patients according to local data found at Dodoma Region from Makole main central of Reporting data concerning tuberculosis (DHIS2). In national tuberculosis report of 2018 Dodoma region is among the seven regions in the country which contributes about 50% of tuberculosis cases in the country.

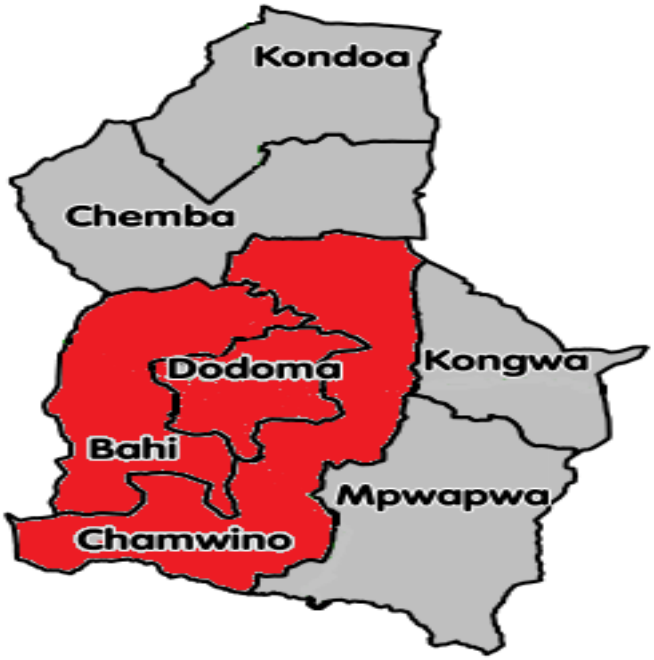


Figure 2: Map of Dodoma region

3.2 Study population

The study population included adult patients with acid fast bacilli (AFB) sputum smear positive who have not yet started anti tuberculosis drugs attending Dodoma regional hospital, Isanga dispensary, Makole health center, St. Gemma hospital, Dodoma Christian medical center hospital and Mirembe hospital.

3.2.1 Inclusion criteria

a) Adult patients from 18 years and above who are acid fast bacilli sputum smear positive in Dodoma.
b) Accepting to sign an informed consent and providing sputum sample.

3.2.2 Exclusion criteria

All patients who are acid fast bacilli sputum smear positive and have started anti-tuberculosis drugs in Dodoma.

3.3 Sampling procedure

This study used a non-probability sampling technique. Purposively sampling technique was used to obtain a sample population for the study. Also Dodoma regional hospital, Isanga dispensary, Makole health center, St. Gemma hospital, DCMC and Mirembe hospital were purposively selected to collect sample population

for the study. These hospitals have designated clinics which offer Tuberculosis services to patients suspected of having tuberculosis infection. Sputum AFB smear positive patients were purposively selected from laboratory in the selected hospitals. Recruitment of study participants was done from each hospital. Interview and smear microscopy was done in each hospital, culture was done at Dodoma regional referral hospital because it is the only hospital in Dodoma which is capable of performing Mycobacteria culture. Non tuberculous mycobacterium specie identification test was done at central tuberculosis reference laboratory (CTRL).

3.3.1 Sample size estimation

Sample size was calculated by Leslie and Kish formula. Prevalence of non - tuberculous mycobacterium used was 9.7%, adapted from the study done in northern Tanzania (Hoza et al., 2016).

$$n = \frac{Z^2 p (1- p)}{e^2}$$

n = minimum sample size.
e =sampling error
P= prevalence
Z = value of the standard normal distribution corresponding to a
Significance level of a (1.96 for 95% confidence level).
p = 9.7%
Z=1.96
e=0.05

$$n= \frac{(1.96) (1.96) x0.097 (1-0.097)}{0.05x0.05}$$

n= 134

The minimum sample size = 134 patients

Table 1: Number of participant in each hospital (N=140)

Name of the study center	Number of participant enrolled
Dodoma regional referral hospital	70
Dodoma Christian medical center	5
St. Gemma hospital	25
Isanga dispensary	5
Mirembe hospital	15
Makole hospital	20

3.4 Data collection

Data were collected by the principal investigator and research assistant per each hospital who were trained to assist in assessing and collection of laboratory investigations.

3.4.1 Data collection method

Data collection involved the use of questionnaire to patients who are AFB sputum smear positive, laboratory investigation and chest radiography investigation.

3.4.2 Study procedure

Study populations were selected from laboratory in each designated hospital involved in the study. Microbiology laboratory in TB section was receiving sputum samples of presumptive TB cases for diagnosis. Apart from other investigations smear microscope was also done as a routine investigation and as a tool of identifying AFB smear positive patients. Patents whose sputum was found to be AFB smear positive and met criteria were asked for consent by the research assistant and aim of the study was explained to the patient, those who consented were interviewed by using the prepared structured questionnaire. Demographic and clinical information were asked such as age, level of education, source of domestic water, alcohol intake, cigarette smoking, history of diabetes, history of previous TB infection, presence of cough, fever, loss of weight etc.

All AFB smear positive sputum samples were transported to Dodoma regional referral hospital for culture. After culture the isolates were sent central tuberculosis reference laboratory for specie identification.

3.4.3 Laboratory procedure

All samples which are acid fast bacilli (AFB) sputum smear positive were cultured into Lowenstein Jensen media. Colony growths were tested by using Genotype CM/AS kit by using LPA (hains lifescience, Germany). Sample collection and smear microscope investigation was done in each hospital involved in the study. Sputum culture was done in Dodoma regional referral hospital which has central zone tuberculosis laboratory and it is the only hospital in Dodoma capable of doing Mycobacteria culture. Genotype CM/AS investigation was performed at central tuberculosis reference laboratory (CTRL) in Dar es salaam.

3.4.4 Sample collection and processing

I. Smear microscopy

Sputum collection and transportation process were done by following the guideline established by clinical laboratory standard institute(CLSI) guideline of 2018 and guideline from Textbook of diagnostic microbiology 5th edition (Mahon R et al., 2015).Two sputum specimens were collected from wide mouth sterile container. The first specimen collected on the spot when the suspected patient presents at the hospital. The patient was given another sputum container and instructed to collect an early-morning specimen on the next day sputum smears were stained using auramin stain and viewed under fluorescent microscopes. This is usually done as a routine activity in the laboratory. Patients who were found to be AFB smear positive from the laboratory results were asked for consent to be enrolled in the study.

Sputum sample received in microbiology laboratory were first documented in the registry book followed by doing smear microscope examination. Smear preparation was done inside level 2 safety cabinet. The purulent part of the sputum was kept on a clean glass slide with the help of applicator stick. The sputum was spread on the slide to make an oval shape with 2-3 cm in length and 1-2 cm wide then it was allowed to dry by air. After drying the smear was fixed by using heat and let to cool before staining.

Staining was done by using auramine stain. First the auramine was flooded onto the slide containing the smear and allowed to stain for 15 minutes then the slide was rinsed by using water. After rinsing, 0.5% acid alcohol was flooded into the slide and

allowed to decolorize for three minutes then it was rinsed with water. Potassium permanganate was added into slide and let to stain for 2 minutes. After 2minutes it was rinsed with water and the slide was allowed to air dry. After drying the slide was viewed in fluorescent microscope at 200x , 250x and 400x magnification lens for diagnosis.

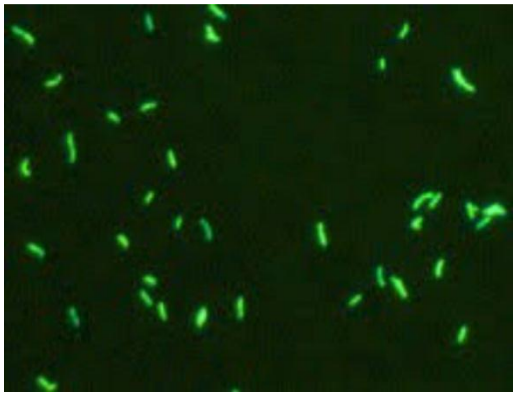


Figure 3: AFB positive view on fluorescent microscope

Figure 4: Fluorescent microscope

II. Culture and species identification

I. Specimen decontamination

The process of sputum culture was done by following the clinical laboratory standard institute (CLSI) guideline of 2018, handbook on tuberculosis laboratory diagnostic method in the European union (ECDC, 2016) and Dodoma regional referral hospital laboratory standard operating procedures.

At first the sputum specimen for culture were registered in specific registry book for *Mycobacteria* culture and then samples were kept in a level two biosafety cabinet for processing. Inside the safety cabinet, at least 2mls but not more than 5mls of sputum from special sputum collecting container were transferred to a sterile centrifuge tube.

By using a sterile transfer pipette equal amount NaOH solution was added in a centrifuge tube containing the sputum sample. The tube contained the mixture was then vortexed for approximately 1minute then left to incubate for 15 minutes so the decontamination can occur. After 15 minutes distilled water was added to 50ml mark in order to neutralize the NaOH. The mixture was then centrifuged at 3000xg at 4-12^0 C. After centrifugation samples were sent to safety cabinet again were supernatant was removed and remained with sediments for inoculation on Lowenstein Jensen (LJ) media

II. Specimen inoculation into the Lowenstein Jensen media.

First culture bottles containing the media were labeled by using specific identification codes of patients. Excess water was removed from the media by using sterile transfer pipette. After removing the supernatant of the centrifuged specimen, 0.2ml of the specimen sediments was inoculated on to the slanting surface of the Lowenstein Jensen media by using sterile Pasteur pipette. The inoculum was spread evenly over the entire surface of the media. Bottle cap was loosely tightened and incubated in a slanting position. After one week when the inoculum is fully absorbed it was tightly closed. Specimen was incubated at 37^0 C (±1^0 C).

After 48hours culture were checked for absorption of liquid inoculated and to detect early contaminants. Culture were then examined and recorded for growth after every week for a period of 8 weeks. Cultures bottles with positive growths were then subjected to species identification test by PCR based on Genotype CM/AS assay (hains life science, Germany) at central tuberculosis reference laboratory in Dar es Salaam. Those with negative growth after 8weeks were recorded as negative and discarded.

Figure 5: Mycobacterium culture isolates on Lj media

III. Genotype CM/AS assay for NTM specie identification

All the steps in this laboratory procedure followed manufacture instruction of Genotype CM/AS assay (hains lifescience, Germany). The procedure involved three steps which are DNA extraction from cultured material, multiplex amplification with biotynylated primers and reverse hybridization

i DNA extraction

The procedure was done in biosafety level 3. By using 1µl sterile loop a small amount of growth was transferred to 1.5ml screw top eppendorf tube containing 100µl of molecular grade water. For negative control 100µl of molecular grade water was added to 1.5ml centrifuge tube. 100µl of chloroform was added and vortexed. Specimen was then placed in covered water bath at 80^0 C for 30 minutes. After this procedure *Mycobacterium* species were considered safe and other procedure followed were done outside biosafety level 3. After being kept in water bath the specimen was kept in microcentrifuge tube at -20^0 C for 10 minutes. After 10 minutes the sample was taken out of the freezer.

ii PCR amplification

This procedure was done in a clean room without DNA and PCR amplification products. The procedure first involved making of PCR reaction mix which involved mixing of 35µl of prime/nucleotide/dye mix (PNM), 5µl PCR buffer, 2µl of magnesium chloride, 2.8µl of molecular water, 0.2µl of Hot-start Taq.

45µl of amplification mix was kept in 0.2ml strip thin wall tube.

iii **Amplification**

This procedure was conducted in DNA specimen preparation room.5µl of the DNA extract was pipette into appropriate tube containing 45µl of PCR master mix and mixed. The 0.2 ml strip thin wall tubes with their individual cap being sealed were kept into the diagnostic thermocycler and amplification cycle was started. Cycles with varying temperature was as followed:

15 min- 95^0 C — Denaturation

30 second- 95^0 C

2 minutes- 58^0 C step was repeated 10 times — Annealing

40 seconds- 53^0 C

40 seconds-70^0 C step was repeated 20 times

8 minutes- 70^0 C — Extension

End - 4^0 C

This reaction took approximately 1hour and 50 minutes

iv Hybridization of PCR amplicons

Twincubator was switched on and hybridization solution and stringed wash solution (STR) are pre warmed to 37-45^o before use. Conjugate concentrate (CON-C) and substrate concentrate (SUB-C) was warmed to room temperature. CON-C and SUB-C were diluted into a ratio of 1:100 with the respective buffer which are CON-D and SUB-D and mixed well. For each strip, 10µl concentrate was added to 1ml of the respective buffer. 20µl of the denatured solution (DEN-blue) was added to each well

used and then 20µl of the amplified sample was added to the solution and mixed well then it was incubated at room temperature for five minutes.

Strips to be used for identification were labeled according to sample patient identity. 1ml of the pre-warmed hybridization buffer was added to each well and then the tray was shaked until the solution had homogenous color and strip were kept in each well then the tray was kept in twincubator and incubated for 30 minutes at 45^0C. After 30 minutes hybridization buffer was aspirated from the wells. 1ml of stringed wash solution (STR-red) was added to each strip and incubated for 15 minutes at 45^0C in twincubator. After 15 minutes the stringent wash solution was removed. Each strip was then washed with 1ml of rinse solution (RIN) for 1 minute in twincubtor then 1ml of dilute conjugate was added to each strip and incubated for 30 minutes in twincubtor.

After that, the solution was removed and each strip was washed twice for 1 minute with 1ml of rinse solution and once for 1 minute with 1ml of distilled water in twincubator. Then water was removed after the last wash. After water removal 1ml of diluted substrate was added to each strip and incubated without shaking for 10 minutes. The reaction was stopped by rinsing with 1ml of distilled water. Water was then removed and strips were removed from the tray and dried. After drying they were interpreted according to the bands of lines appeared on the strips.

Figure 6: Twincubator

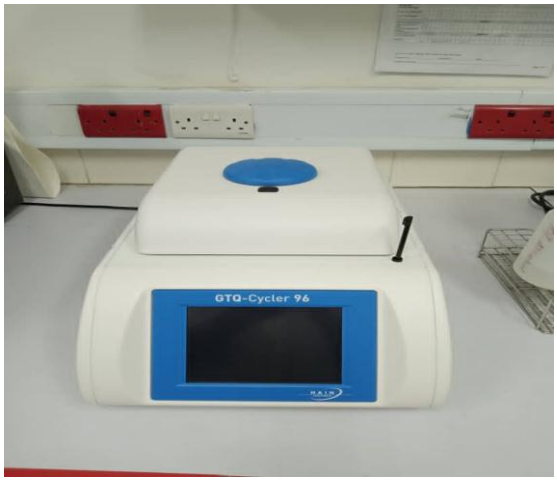


Figure 7: Thermocycler

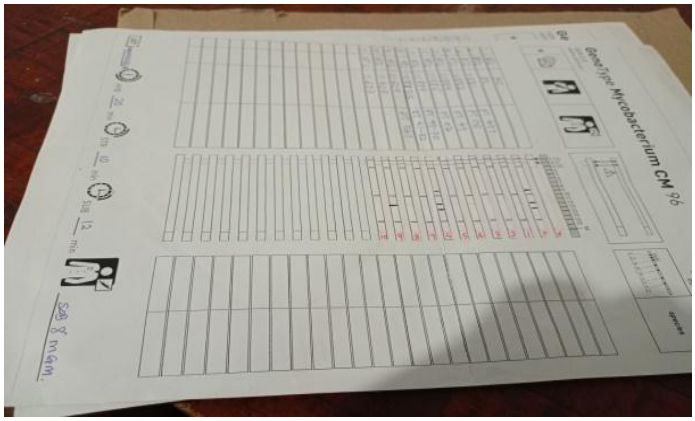


Figure 8: Genotype CM/AS kit test strips

IV. Antimicrobial susceptibility test

Drug sensitivity test was done by using proportion method as per standard operating procedure established by central tuberculosis reference laboratory and per the clinical and laboratory standards institute guideline (CLSI, 2018).First line anti TB drugs (Rifampcin, Isoniazid, and Ethambutol), amikacin, ofloxacin and kanamycin drugs were tested to all NTM positive isolates after detection from Genotype CM/AS kit by line probe assay.

Drug concentrations used were as follows: rifampcin 40µg/ml, isoniazid 0.2µg/ml, ethambutol 2µg/ml, ofloxacin 2µg/ml, amikacin30µg/ml, kanamycin 30µg/ml.

i Procedure for antimicrobial susceptibility test

The steps involved preparation of inoculums followed by inoculation of the specimen into the media. A portion of growth was taken and transferred to glass tube containing 5-6mls of sterile normal saline. 3mm beads were used to homogenize the suspension and then it was vortexed for 2-3 minutes and left to settle for approximately 30 minutes to allow large clumps of bacteria to settle.

2-4ml of supernatant was transferred to a sterile tube. Turbidity of the suspension was adjusted and compared by using McFarland number 1 standard. Then a serial of 10-fold dilution of the standard suspension was made by diluting sequentially 1ml of culture suspension in tubes containing 9ml of sterile distilled water. Dilution 10^{-2} (for control 1) and 10^{-4} (for control 2) were inoculated as control growth that is there media did not contain any antimicrobial agent. Media containing antimicrobial agent were inoculated with only 10^{-2} dilution. Volume of the inoculum used was 0.1ml and it was placed on to the middle two third of the slant. After finishing the tubes were incubated at 37^{0} Cfor 4-6 weeks.

ii Interpretation of the antimicrobial susceptibility results

Resistance was expressed as the number of colonies on drug containing media in comparison with the growth on drug free medium inoculated with 10^{-2} dilution (plain LJ) and the media inoculated with the 10^{-2} dilution containing antimicrobial agent at critical concentration of 0.2µg/ml for isoniazid, 40µg/ml for rifampcin, 2µg/ml for ethambutol, 2µg/ml for ofloxacin, 30µg.ml for amikacin, 30µg/ml for kanamycin. The results were interpreted by using 1% proportion method. If the ratio of the number of colonies on the medium containing antimicrobial to the number of colonies on the control media is less than 1% then the strain was reported susceptible and if more than 1% was reported as resistance.

V. HIV testing

HIV testing was done to all patients who were AFB sputum smear positive. And the procedure for testing followed the national algorithm for testing HIV. Before any procedure patients were asked for consent. Pre-counseling done. Then 20µl of whole blood was taken from the patients by following the aseptic technique and kept in a well of test strip SD bioline which was used for HIV screening. Four drops of buffer

were also kept in a well. The readings were interpreted after 10- 20 minutes. All tests which became positive were subjected to unigold test for confirmation of HIV infection. For unigold test 20µl of blood was kept in unigold test strip and 1 drop of buffer was added. After 10 minutes the results were interpreted.

Figure 9: SD biolin for HIV testing

3.5 Definition of Variables

3.5.1 Dependent variable

The dependent variable for this study is non - tuberculous mycobacterium infection

3.5.2 Independent variables

Age, sex, HIV infection, history of diabetes, previous TB history or any chronic lung disease, history of smoking cigarette, use of immunosuppressant drugs (steroids use of tap, occupation, level of education, alcohol intake.

3.6 Measurement of variables

3.6.1 Measurement of non-tuberculous mycobacterium

Non-tuberculous mycobacterium diagnosis and species identification was done by using Genotype CM/AS kit (hains life science, Germany) from sputum culture of patients who are AFB sputum smear positive patients.

3.6.2 Measurement of social demographic variable

Age, gender, residence, source of domestic water, level of education, occupation, was measured by using structured questionnaire.

3.6.3 Measurement of health status

SD bioline for HIV was used for HIV testing and unigold test to confirm positive HIV, previous history of TB disease, history of smoking, current use of steroid or any other immunosuppressive drugs were asked in the structured questionnaire.

3.6.4 Validity and Reliability of Data

The research assistants (intern doctor and laboratory scientist) were trained on how to fill in the questionnaires. There were frequent meetings to discuss and resolve challenges arising from the questionnaire and recorded data. Sputum culture was done by the principle investigator, AFB staining and microscope investigation where done by laboratory scientist and principle investigator, HIV testing was done intern doctor who is a research assistant. Specie identification test and anti-microbial susceptibility test was done by laboratory scientist and principle investigator. Chest x-ray films were interpreted by a certified radiologist.

3.6.5 Laboratory quality control and accuracy

All the equipment used were checked for quality and zero error. Laboratory standard operating procedure, clinical laboratory standard institute guidelines of 2018 was followed. Every day before performing any laboratory test the reagents and machines were checked for accuracy by testing with the positive and negative controls .Laboratory safety rules and principles were observed.

3.7 Data management and statistical analysis

3.7.1 Data management and quality assurance

Detailed quality assurance procedure was used to keep the quality of data. Training was given to data collector. The collected data was checked for completeness at the end of each day of data collection. Standard operating procedures were completely followed.

3.7.2 Statistical analysis

The data analysis was performed using SPSS-IBM version 25. Normally distributed continuous data were summarized as mean with standard deviation. While Fisher's exact test compared proportion, binary logistic regression model estimated the odds for detecting non-tuberculous mycobacterium. A p value of <0.05 was considered significance.

3.8 Ethical consideration

Patients involved in the study were given a clear and enough explanation on what is the intention of the research. Written informed consent was given to all individuals

eligible to participate in the research. Respect for the patient and confidentiality was observed, there was application of codes to questionnaire that hide identity of a patient. Patients were asked to participate in the study in their free willing and no use of force was applied to make them be a part of the study thus their autonomy was respected and protected. Refusal of patients to participate in this study didn't interfere their right to get medical treatment. The protocol for this study was reviewed and approved by the University of Dodoma research ethics committee with approval letter having a reference number MA, 84/261/02.

3.9 Dissemination of Results

The results from this study were disseminated to the University of Dodoma, hospitals which were included in the study. The data is also expected to be published in international medical journals.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Flow chart for patient enrolment

During the study period from October 2020 to march 2021, 750 patient's sputum was screened for AFB. 140 patents were eligible and enrolled in the study. 91 (65%) sputum sample were culture positive and 6 NTM were isolated were by 4 were *M.avium* and 2 were *M.kansasii*

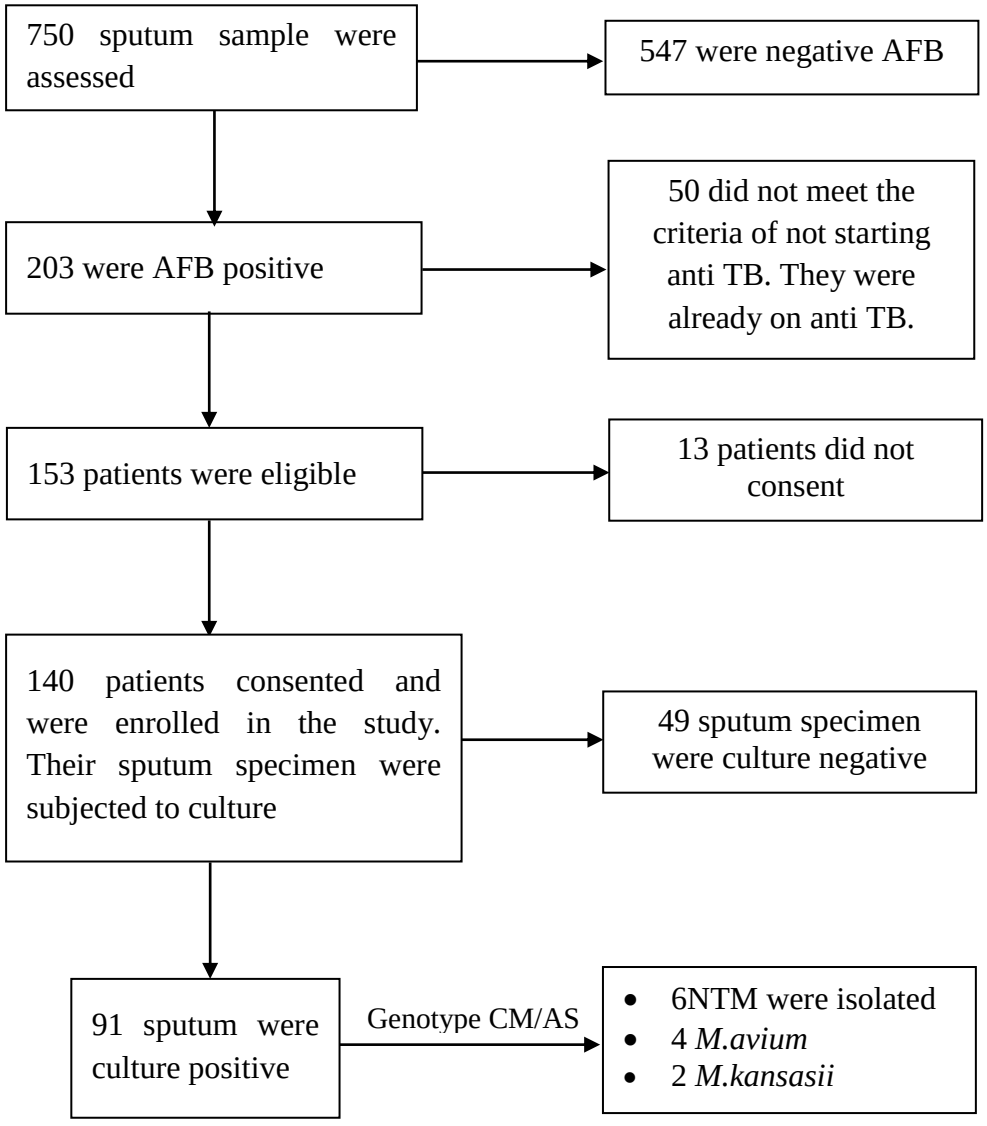


Figure 10: Flow chart of study enrolment

4.1 Social demographic characteristics of the patients who are AFB sputum smear positive in Dodoma

A total of 140 patients with positive sputum smear for acid fast bacilli were enrolled in the study from October 2020 to March 2021.The mean standard age was 42 years and SD of 14.4. Young adults were 46.4% followed by 40% of adults and elderly comprises 13.6%. Male patients accounted for 68.6% while female were 31.4%. Among the patients those who were smoking cigarette were 32.1% and non smokers were 67.9%. Tap water was the leading source of domestic water used which comprises of 61.4% followed by well water which was 33.6% and other sources of water were 5%. Alcohol users were 47.1% and non users were 52.9%. in the level of education about 19.29% were illiterate, 43.57% were those who have attained primary level of education, those with secondary education were 33.57% and tertiary education were 3.57%. Monthly income of less than 100000tsh was 32.86%, 51.43% had between 100000tsh and 500000tsh and those who had a monthly income of more than 500000tsh were15.71%. The leading social demographic characteristic with highest proportion was male gender. Many patients involved in the study were male. The Social demographic characteristics are presented in table 2 below.

Table 2: Social demographic characteristics of the patients who are AFB sputum smear positive in Dodoma (N=140)

Variable	Frequency (N)	Percentage (%)	Mean ±SD
Age groups in years			42.00± 14.42
Young adults (18-39)	65	46.4	
Adults(40-59)	56	40	
Elderly(≥60)	19	13.6	
Gender			
Male	96	68.6	
Female	44	31.4	
Smoking cigarette			
Smokers	45	32.1	
Non-smokers	95	67.9	
Source of domestic water			
Tap water	86	61.4	
Well water	47	33.6	
Other sources	7	5	
Alcohol			
Users	66	47.1	
Non-users	74	52.9	
Level of education			
Illiterate	27	19.29	
Primary education	61	43.57	
Secondary education	47	33.57	
Tertiary education	5	3.57	
Monthly income (TZS)			
<100000	46	32.86	
100000-500000	72	51.43	
>500000	22	15.71	

Clinical characteristics of patients who are AFB sputum smear positive in Dodoma

Table 3 below shows the clinical characteristics of patients who are AFB sputum smear positive. Weight loss was the commonest clinical characteristic where by 65.74% of the patients had loss of weight and those who didn't have were 34.53%. Patients who presented with fever were 53.57% and those with no fever were 46.43%. HIV positive patients were 23.6% and 76.4% were HIV negative. Patients with diabetes were7.9% and 92.1% did not have diabetes. Patients who had a history of previous tuberculosis infection were 19.3% and 80.7% didn't have the history of tuberculosis infection. Those with other chronic lung disease other than tuberculosis were7.1% and those who did not have were 92.9%.

Table 3: Clinical characteristics and radiographic findings of patients who are AFB sputum smear positive in Dodoma (N=140)

Variable	Frequency (N)	Percentage (%)
Loss of weight		
Yes	91	65.47
No	49	34.53
fever		
yes	75	53.57
No	65	46.43
HIV status		
Positive	33	23.6
Negative	107	76.4
Diabetes		
Yes	11	7.9
No	129	92.1
TB history		
Yes	27	19.3
No	113	80.7
Chronic lung disease other than TB		
Yes	10	7.1
No	130	92.9
Use of Steroids		
Users	12	8.6
Non-users	128	91.4

4.2 Prevalence of non-tuberculous mycobacterium

In sputum culture growth, 6 patients were found to have non-tuberculous mycobacterium infection which makes the prevalence of non-tuberculosis mycobacterium among patients with AFB sputum smear positive in Dodoma to be 4.29%. Species of NTM were Mycobacterium avium and *M.kansasii*. Mycobacterium avium were 4 isolates and *M.kansasi w*ere 2 isolates. Figure 11 below shows the prevalence of non-tuberculous mycobacterium in Dodoma

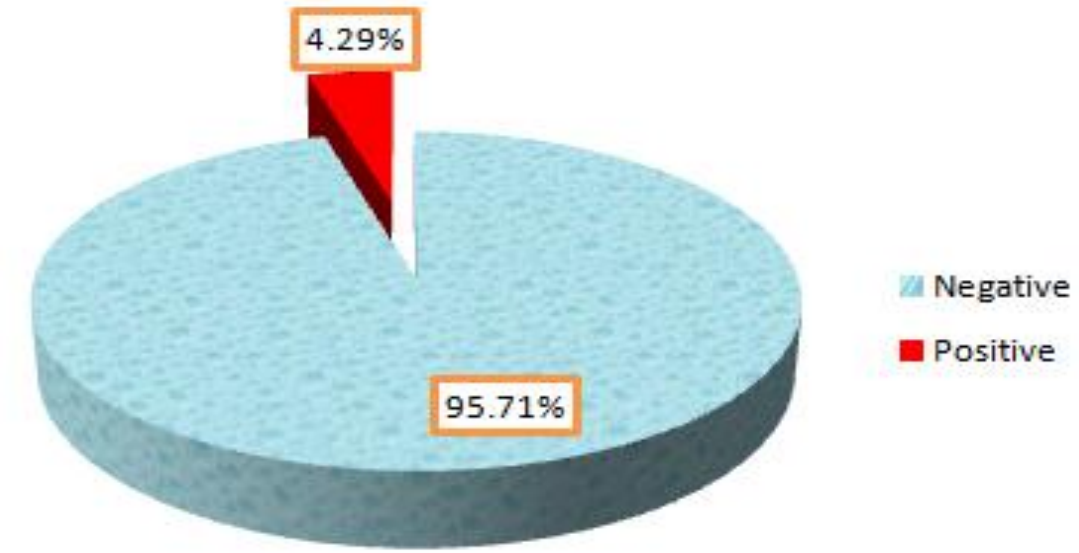


Figure 11: Prevalence of non tuberculous mycobacterium among patients with AFB sputum smear positive at Dodoma

4.3 Factors associated with non-tuberculosis mycobacterium infection

Based on fishers exact test table 3 below presents association of non-tuberculous mycobacterium with independent factor among patients with culture positive, P value less than 0.2 was considered significant here. Age is significantly associated with non tuberculous infection with P=0.0900 and those with age more than 60 years were more at risk of NTM compared to other age groups with proportion of 20% of NTM cases compared to other age groups. Source of domestic water shows association with non-tuberculous mycobacterium P=0.0341 and tap water was seen to be more associated with NTM than other sources of water with proportion of 13.95% of NTM positive cases. Monthly income shows association with non-tuberculous, P=0.0346. Those with the monthly income less than 100000 TZS with the proportion of 14.29% were seen to be more associated with NTM compared to

other groups who have an income of more than 100000 TZS. Smoking shows association with non-tuberculous mycobacterium infection with P value of 0.0340 and those who were smoking cigarette were more at risk of non tuberculous mycobacterium infection than nonsmokers with the proportion of 13.89% of positive NTM cases. Diabetic infection also shows association with NTM, P=0.0660. Patients with positive diabetes were more at risk of NTM than negative diabetes cases. History of tuberculosis infection is associated with NTM P=0.0149 and those with positive history of Tb are at more risk with a proportion of 21.05% of positive NTM cases than those with negative history.

Table 4: Factors associated with non tuberculous mycobacterium among positive sputum culture based on fisher's exact test in Dodoma (N=91)

Variable	Positive NTM N (%)	Negative NTM N (%)	Fishers' P-Value
Age groups (years)			0.0900*
Young adults (18-39)	1(2.56)	38(97.44)	
Adults (40-59)	2(5.41)	35(94.59)	
Elderly (>60)	3(20.00)	12(80.00)	
Gender			0.6648*
Male	4(6.06)	62(93.94)	
Female	2(8.00)	23(92.00)	
Source of domestic water			0.0341*
Tap water	6(13.95)	37(86.05)	
Well water	0(0.00)	32(100.00)	
Other sources	0(0.00)	16(100.00)	
Level of education			0.2627*
Illiterate	2(9.52)	19(90.48)	
Primary education	4(10.81)	33(89.19)	
Secondary education	0(0.00)	30(100.00)	
Tertiary education	0(0.00)	3(100.00)	
Monthly income (Tsh)			0.0346*
< 100000	4(14.29)	24(85.71)	
100000-500000	2(4.17)	46(95.83)	
>500000	0(0.00)	15(100.00)	
Loss weight			0.6601*
Yes	5(8.47)	54(91.53)	
No	1(3.23)	30(96.77)	
Smoking			0.0340*
Smokers	5(13.89)	31(86.11)	
Non smokers	1(1.82)	54(98.18)	
Alcohol			0.6798*
Users	2(4.65)	41(95.35)	
Non users	4(8.33)	44(91.67)	
HIV status			1.0000*
Positive	5(6.58)	71(93.42)	
Negative	1(6.67)	14(93.33)	
Diabetes			0.0660*
Yes	2(28.57)	5(71.43)	
No	4(4.76)	80(95.24)	
TB history			0.0149*
Yes	4(21.05)	15(78.95)	
No	2(2.78)	70(97.22)	

4.4 Predictors of non tuberculous mycobacterium infection

The following is the table for regression analysis to assess the predictor for non tuberculous mycobacterium infection among culture positive patients. Those independent variable which have shown association with non tuberculous mycobacterium in fisher's exact test and doesn't contain empty cell were taken for regression analysis.

Table 5 shows that in unadjusted logistic regression analysis, the odds ratio for developing NTM in patients with history of previous tuberculosis infection was 9.33(95%CI: 1.56-55.71, p=0.014). Adjusting for other factors, prior history of TB infection was an independent predictor of developing NTM lung disease, AOR = 7.76(95%CI: O.93-64.97, p=0.039)

Table 5: Binary logistic analysis for predictors of non-tuberculous mycobacterium infection in patients with sputum culture positive in Dodoma (N=91)

Independent Variable	Unadjusted logistic		Adjusted logistic	
	OR[95%CI]	p-value		p-value
Age groups (years)				
Young adults(18-39)	**Ref**		**Ref**	
Adults (40-59)	2.17[0.189-25.012]	0.534	1.57[0.105-23.385]	0.746
Elderly(>60)	9.50[0.902-100.052]	0.061	4.00[0.235-68.112]	0.337
Smoking cigarette		0.053		0.340
Smokers	8.71[0.973-77.974]		3.34[0.253-43.955]	
Non smokers	**Ref**		**Ref**	
TB history				**0.039**
Yes	9.33[1.564-55.714]	0.014	7.76[0.928-64.968]	
No	**Ref**		**Ref**	
Diabetes				
Yes	8.00[1.170-54.738]	0.034	6.39[0.556-73.516]	0.137
No	**Ref**		**Ref**	

4.5 Antimicrobial susceptibility pattern of non tuberculous mycobacterium

Table 6 below shows antimicrobial susceptibility pattern of non-tuberculous mycobacterium. *M.avium* was 100% sensitive to Kanamycin, Amikacin and Ofloxacin. *M.kansasii* were also 100% sensitive to Kanamycin, Ofloxacin and Amikacin. All species of M.avium complex and *M.kansasii* were resistant to Isoniazid, Rifampcin and Ethambutol.

Table 6: Antimicrobial susceptibility pattern of patients who are AFB sputum smear positive in Dodoma urban

Non tuberculous Mycobacterium specie		**Kanamycin N (%)**	**Amikacin N (%)**	**Ofloxacin N (%)**	**Isoniazid N (%)**	**Rifampcin N (%)**	**Ethambutol N (%)**
M. avium							
	Sensitive	4(100.00)	4(100.00)	4(100.00)	0(0.00)	0(0.00)	0(0.00)
	Resistant	0(0.00)	0(0.00)	0(0.00)	4(100.00)	4(100.00)	4(100.00)
M. kansasii							
	Sensitive	2(100.00)	2(100.00)	2(100.00)	0(0.00)	0(0.00)	0(0.00)
	Resistant	0(0.00)	0(0.00)	0(0.00)	2(100.00)	2(100.00)	2(100.00)

4.6 Discussion

In this study the prevalence of non-tuberculous mycobacterium infection among patients who are AFB sputum smear positive was found to be 4.29%. History of previous tuberculosis infection was found to be significantly associated with non-tuberculous mycobacterium infection. *Mycobacterium avium* and *M.kansasi* were the NTM isolates obtained and they both have shown resistance to first line anti TB drugs and they were sensitive to Amikacin, Ofloxacin and Kanamycin.

Non - tuberculous lung disease is an emerging disease and its incidence and prevalence keeps on increasing (Y. M. Lee et al., 2021; Lin et al., 2018; Ryu et al., 2016). Worldwide the rate of awareness is rising which is mostly caused by improvement in laboratory techniques which help in the diagnosis of the NTM infection (Okoi et al., 2017).

The result of prevalence of non-tuberculous mycobacterium obtained in this study was found to be different when compared with prevalence obtained in some other studies done in other parts of the world. The prospective study in India among pulmonary and extra pulmonary TB suspected to assess the prevalence of non-tuberculous mycobacterium the prevalence obtained was 22.26% which is higher than the one obtained in this study (Thangavelu et al., 2021). The differences in the prevalence may be because of the study nature whereby in the study from India different types of specimen such as sputum, gastric aspirate, pleura fluid, ascitic fluid etc were studied for NTM while in this study only sputum was used. The isolation frequency of non-tuberculous mycobacterium may be influenced by the type of clinical material or specimen used (Maurya et al., 2015).

Also difference in prevalence is seen from other studies for example the one done in Botswana among people living with HIV which shows the prevalence of non-tuberculous among pulmonary TB cases of 56% (Agizew et al., 2017). This prevalence was high compared to the one obtained in this study. In the study done in Botswana the whole study population was HIV positive while in this study only 23.6% were HIV positive. Low immunity increases the chances of acquiring disease. The state of decreasing immunity caused by decline in the levels of CD4+ cell count contributes to their vulnerability to NTM pulmonary infection (Hannah et al., 2020;

Lopez-luis et al., 2020).The type of underlying disease influence the prevalence of non tuberculous mycobacterium to be high or low (Griffith et al., 2007; Thangavelu et al., 2021). Similar case is seen in the study done India among HIV patients showed the prevalence of 22.26% (Thangavelu et al., 2021). Also in china were the prevalence of NTM was 47% (Lan et al., 2011).

There are also other studies which have been done and shows low prevalence of non-tuberculous mycobacterium compared to the one in this study. For example in Germany the prevalence was 3.3% (Ringshausen et al., 2016). In India the prevalence was 0.6% (Thangavelu et al., 2021).Apart from this study there are also other studies which have been done in Tanzania and shows difference prevalence of no- tuberculous mycobacterium in different parts within the country. Study done at Kibong'oto to assess the proportion of non tuberculous among TB presumptive cases have shown the prevalence of 3.19% (Mnyambwa et al., 2017). Study done in northern Tanzania by Hoza et al have shown the prevalence of 9.7%. (Hoza et al., 2016).

These differences found in prevalence may also be explained by differences in geographical location and climatic conditions which have a major impact in the prevalence of non tuberculous mycobacterium. The study done by Hoefsloot et al in the European continent to determine species distribution among NTM isolated from pulmonary specimens found that geographical location variation has an impact in the occurrence of non-tuberculous mycobacterium (Hoefsloot et al., 2013). Studies have shown that living in warmer areas and area with high humidity increases the chances of acquiring non tuberculous mycobacterium, also certain soil properties likely promote the growth and persistence of NTM in the environment for example soil which have higher copper and sodium content, NTM are more frequent isolated (Adjemian et al., 2007). So with this evidence it is clearly seen why there is variation in prevalence of non tuberculous mycobacterium from this study and other studies done in other areas.

Tanzania is found among the thirty countries with high burden tuberculosis infection (World Health Organisation (WHO), 2020). The results of this study show that a history of previous pulmonary TB infection is the predictors of nontuberculosis

mycobacterium lung infection. Previous pulmonary tuberculosis infection impairs or alters the morphology of the lung parenchyma hence creating the suitable environment for multiplication of non tuberculous mycobacterium infection(Griffith et al., 2007).

History of pulmonary tuberculosis infection been seen to be associated with the risk of developing non tuberculous mycobacterium lung infection and this has been shown to correlate with the results obtained in several studies (Axson et al., 2019; Lopez-luis et al., 2020; Okoi et al., 2017). The results of the study done in Taiwan shows that people who had a history of previous TB infection and other structural lung diseases were seen to be more affected by non tuberculous mycobacterium infection compared to those who did not have the history of TB infection (Huang et al., 2017). TB infection after healing have a tendency of causing scars, destruction of lung parenchyma as well as airway flow which causes impairment of the normal immune system of the respiratory system (Amaral et al., 2015). When the immune system of the respiratory system is impaired it becomes easy to acquire NTM infection. With abnormal lung structure and impairment in the immune system it becomes easy to get non tuberculous mycobacterium infection (Griffith et al., 2007).Pulmonary tuberculosis may cause cavity formation within the lungs and destruction of the lungs parenchyma which provides the suitable environment for non tuberculous mycobacterium to multiply (Adjemian et al., 2017; Axson et al., 2019; Griffith et al., 2007).

In this study all non tuberculous isolates found were resistance to three first line anti tuberculosis drug regimen. They were resistant to Isoniazid, Rifampcin and Ethambutol but they were sensitive to Amikacin, Kanamycin and Ofloxacin. Several studies have shown different susceptibility pattern of these bacteria towards antibiotics, some of them were sensitive and others were resistant. The susceptibility results of this study differ with the one obtained in the study done in Iran whereby species of M.avium complex and *M.kansasii* acted different towards some few similar drugs like the one which were tested in this study (Heidarieh et al., 2016). Species involved in the study done in Iran were the same like which were found in this study but reaction toward these drugs differed. Difference is also seen in the study done in China where by some species of Mycobacterium avium complex and

M.kansasii obtained in the study were sensitive to rifampcin and ethambutol (Zhou et al., 2021). The results of this study correlates with the results of the study done in China whereby all species of NTM tested showed resistance to first line anti TB (Pang et al., 2015)

Strains of microorganisms response to drugs differ in different geographical location (Brown-Elliott & Woods, 2019). Bacteria strain found in one area may differ in their susceptibility toward drugs with the same type of bacteria found in another area. This may be because of resistance which may be acquired by these bacteria toward the drugs (Munita & Arias, 2016). Gene mutation contributes in variation of bacteria susceptibility towards antimicrobial.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.0 Conclusion

Non-tuberculous mycobacterium is prevalent among acid fast bacilli sputum smear positive patients and people with the history of previous tuberculosis infection are more at risk of acquiring non tuberculous mycobacterium. And isolated species of non-tuberculous mycobacterium were resistant to rifampcin, isoniazid and ethambutol.

5.1 Recommendation

There is a need of emphasizing continual screening of NTM by doing culture and molecular diagnosis of *Mycobacteria* infection in cases. Clinician should be aware of non tuberculous mycobacterium infection especially when attending suspected cases of pulmonary TB with history of previous pulmonary tuberculosis infection. Whenever a patient is diagnosed with non-tuberculous mycobacterium infection, antimicrobial susceptibly test is important to be done as bacteria have tendency of developing resistance towards drugs.

5.2 Strength of the study

The study was done in six hospitals whereby patients from several areas around Dodoma visit these hospitals so most of the captured cases represent the population.

5.3 Limitations of the study

The study has the following limitations:

- Failure to use macrolides drugs for antimicrobial susceptibility test which are the key drugs in the NTM treatment regimens. However performing susceptibility for first and second line drugs like aminoglycoside and fluoroquinolones provides options to clinicians.
- Failure to use broth microdilution method for antimicrobial susceptibility test which is the gold standard method for non-tuberculous mycobacterium. However proportion method was used and it is one of the method which can be employed for antimicrobial susceptibility test for NTM.
- The sampling method was non-probability, so the chance was not equally distributed to present the population.

REFERENCES

Adjemian, J., Frankland, T. B., Daida, Y. G., Honda, J. R., Olivier, K. N., Zelazny, A., Honda, S., & Prevots, D. R. (2017). *Epidemiology of Nontuberculous Mycobacterial Lung Disease and Tuberculosis, Hawaii, USA*. *23*(3), 439–447.

Adjemian, J., Olivier, K. N., Seitz, A. E., Iii, J. O. F., Holland, S. M., & Prevots, D. R. (2007). *Spatial Clusters of Nontuberculous Mycobacterial Lung Disease in the United States*. https://doi.org/10.1164/rccm.201205-0913OC

Agizew, T., Basotli, J., Alexander, H., Boyd, R., Letsibogo, G., Auld, A., Nyirenda, S., Tedla, Z., Mathoma, A., Mathebula, U., Pals, S., Date, A., & Finlay, A. (2017). *Higher-than-expected prevalence of non- tuberculous mycobacteria in HIV setting in Botswana : Implications for diagnostic algorithms using Xpert MTB / RIF assay*. *228*, 1–13.

Amaral, A. F. S., Coton, S., Kato, B., Tan, W. C., Studnicka, M., Janson, C., Gislason, T., Mannino, D., Bateman, E. D., Buist, S., Burney, P. G. J., Zhong, N., Liu, S., Lu, J., Ran, P., Wang, D., Zheng, J., Zhou, Y., Kocabaş, A., … Islam, M. (2015). Tuberculosis associates with both airflow obstruction and low lung function: BOLD results. *European Respiratory Journal*, *46*(4), 1104–1112. https://doi.org/10.1183/13993003.02325-2014

Andréjak, C., Nielsen, R., Thomsen, V. Ø., Duhaut, P., Sørensen, H. T., & Thomsen, R. W. (2013). *Chronic respiratory disease , inhaled corticosteroids and risk of non-tuberculous mycobacteriosis*. 256–262. https://doi.org/10.1136/thoraxjnl-2012-201772

Axson, E. L., Bual, N., Bloom, C. I., & Quint, J. K. (2019). Risk factors and secondary care utilisation in a primary care population with non-tuberculous mycobacterial disease in the UK. *European Journal of Clinical Microbiology and Infectious Diseases*, *38*(1), 117–124. https://doi.org/10.1007/s10096-018-3402-8

Bar-on, O., Mussaf, H., Mei-zahav, M., Prais, D., & Steuer, G. (2015). *Increasing nontuberculous mycobacteria infection in cystic fi brosis*. *14*, 53–62. https://doi.org/10.1016/j.jcf.2014.05.008

Bjerrum, S., Oliver-Commey, J., Kenu, E., Lartey, M., Newman, M. J., Addo, K. K., Hilleman, D., Andersen, A. B., & Johansen, I. S. (2016). Tuberculosis and non-tuberculous mycobacteria among HIV-infected individuals in Ghana. *Tropical Medicine and International Health*, *21*(9), 1181–1190. https://doi.org/10.1111/tmi.12749

Borand, L., Lauzanne, A. De, & Nguyen, L. (2019). *Isolation of Nontuberculous Mycobacteria in Southeast Asian and African Human Immunodeficiency Virus – infected Children With Suspected Tuberculosis*. *68*, 1750–1753. https://doi.org/10.1093/cid/ciy897

Brown-Elliott, B. A., & Woods, G. L. (2019). Antimycobacterial susceptibility testing of nontuberculous mycobacteria. *Journal of Clinical Microbiology*, *57*(10), 1–26. https://doi.org/10.1128/JCM.00834-19

Browne, S. K., Burbelo, P. D., Chetchotisakd, P., Suputtamongkol, Y., & Kiertiburanakul, S. (2012). Adult-Onset Immunodeficiency in Thailand and Taiwan. *T h e n e w e Ngl a Nd j o u r Na l o f m e Dic i n E*, 725–734. https://doi.org/10.1056/NEJMoa1111160

Chanda-Kapata, P., Kapata, N., Klinkenberg, E., Mulenga, L., Tembo, M., Katemangwe, P., Sunkutu, V., Mwaba, P., & Grobusch, M. P. (2015). Non-tuberculous mycobacteria (NTM) in Zambia: Prevalence, clinical, radiological and microbiological characteristics. *BMC Infectious Diseases*, *15*(1), 1–7. https://doi.org/10.1186/s12879-015-1264-6

Crump, J. A., Van Ingen, J., Morrissey, A. B., Boeree, M. J., Mavura, D. R., Swai, B., Thielman, N. M., Bartlett, J. A., Grossman, H., Maro, V. P., & Van Soolingen, D. (2009). Invasive disease caused by nontuberculous mycobacteria, Tanzania. *Emerging Infectious Diseases*, *15*(1), 53–55. https://doi.org/10.3201/eid1501.081093

Detjen, A. K., DiNardo, A. R., Leyden, J., Steingart, K. R., Menzies, D., Schiller, I., Dendukuri, N., & Mandalakas, A. M. (2015). Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: A systematic review and meta-analysis. *The Lancet Respiratory Medicine*, *3*(6), 451–461. https://doi.org/10.1016/S2213-2600(15)00095-8

Diel, R., Jacob, J., Lampenius, N., Loebinger, M., Nienhaus, A., Rabe, K. F., & Ringshausen, F. C. (2017). Burden of non-tuberculous mycobacterial pulmonary disease in Germany. *European Respiratory Journal*, 1–11. https://doi.org/10.1183/13993003.02109-2016

Donohue, M. J., & Wymer, L. (2013). *Increasing Prevalence Rate of Nontuberculous Mycobacteria*. 2008–2013. https://doi.org/10.1513/AnnalsATS.201605-353OC

ECDC. (2016). *Handbook on TB laboratory diagnostic methods in the European Union*. https://doi.org/10.2900/216384

Edirisinghe, E. A. R., Dissanayake, D. R. A., Abayasekera, C. L., & Arulkanthan, A. (2014). Occurrence of nontuberculous mycobacteria in aquatic sources of Sri Lanka. *International Journal of Mycobacteriology*, *3*(4), 242–246. https://doi.org/10.1016/j.ijmyco.2014.10.007

Falkinham, J. O. (2003). The changing pattern of nontuberculous mycobacterial disease. *Canadian Journal of Infectious Diseases*, *14*(5), 281–286. https://doi.org/10.1155/2003/323058

Falkinham, J. O. (2011). Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. *Emerging Infectious Diseases*, *17*(3), 419–424. https://doi.org/10.3201/eid1703.101510

Falkinham, J. O. (2015). Environmental sources of nontuberculous mycobacteria. *Clinics in Chest Medicine*, *36*(1), 35–41. https://doi.org/10.1016/j.ccm.2014.10.003

Field, S. K., Fisher, D., & Cowie, R. L. (1994). review Mycobacterium avium complex Pulmonary Disease in Patients Without HIV Infection *. *CHEST*, *126*(2), 566–581. https://doi.org/10.1378/chest.126.2.566

Forbes, B. A. (2017). crossm Mycobacterial Taxonomy. *Journal of Clinical Microbiology*.

Griffith, D. E., Aksamit, T., Brown-Elliott, B. A., Catanzaro, A., Daley, C., Gordin, F., Holland, S. M., Horsburgh, R., Huitt, G., Iademarco, M. F., Iseman, M., Olivier, K., Ruoss, S., Von Reyn, C. F., Wallace, R. J., & Winthrop, K. (2007). An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American Journal of Respiratory and Critical Care Medicine*, *175*(4), 367–416. https://doi.org/10.1164/rccm.200604-571ST

Hannah, C. E., Ford, B. A., Chung, J., Ince, D., & Wanat, K. A. (2020). Characteristics of nontuberculous mycobacterial infections at a midwestern tertiary hospital: A retrospective study of 365 patients. *Open Forum Infectious Diseases*, *7*(6), 1–9. https://doi.org/10.1093/OFID/OFAA173

Haworth, C. S., Banks, J., Capstick, T., Fisher, A. J., Gorsuch, T., Laurenson, I. F., Leitch, A., Loebinger, M. R., Milburn, H. J., Nightingale, M., Ormerod, P., Shingadia, D., Smith, D., Whitehead, N., Wilson, R., & Floto, R. A. (2017). British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). In *Thorax* (Vol. 72, Issue Grade D). https://doi.org/10.1136/thoraxjnl-2017-210927

Heidarieh, P., Mirsaeidi, M., Hashemzadeh, M., Feizabadi, M. M., Bostanabad, S. Z., Nobar, M. G., & Shahraki, A. H. (2016). In Vitro Antimicrobial Susceptibility of Nontuberculous Mycobacteria in Iran. *Microbial Drug Resistance*, *22*(2), 172–178. https://doi.org/10.1089/mdr.2015.0134

Hoefsloot, W., Van Ingen, J., Andrejak, C., Ängeby, K., Bauriaud, R., Bemer, P., Beylis, N., Boeree, M. J., Cacho, J., Chihota, V., Chimara, E., Churchyard, G., Cias, R., Daza, R., Daley, C. L., Dekhuijzen, P. N. R., Domingo, D., Drobniewski, F., Esteban, J., … Wagner, D. (2013). The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: An NTM-NET collaborative study. *European Respiratory Journal*, *42*(6), 1604–1613. https://doi.org/10.1183/09031936.00149212

Hoza, A. S., Mfinanga, S. G. M., Rodloff, A. C., Moser, I., & König, B. (2016). Increased isolation of nontuberculous mycobacteria among TB suspects in Northeastern, Tanzania: Public health and diagnostic implications for control programmes. *BMC Research Notes*, *9*(1), 1–9. https://doi.org/10.1186/s13104-016-1928-3

Huang, H. L., Cheng, M. H., Lu, P. L., Shu, C. C., Wang, J. Y., Wang, J. T., Chong, I. W., & Lee, L. N. (2017). Epidemiology and Predictors of NTM Pulmonary Infection in Taiwan - A Retrospective, Five-Year Multicenter Study. *Scientific Reports*, *7*(1), 1–8. https://doi.org/10.1038/s41598-017-16559-z

Ichijo, T., Izumi, Y., Nakamoto, S., Yamaguchi, N., & Nasu, M. (2014). *Distribution and Respiratory Activity of Mycobacteria in Household Water System of Healthy Volunteers in Japan*. *9*(10), 1–5. https://doi.org/10.1371/journal.pone.0110554

Ingen, J. Van. (2013). *Diagnosis of Nontuberculous Mycobacterial Infections*. *1*(212).

Ingen, J. Van. (2015). M i c ro b i o l o g i c a l Diagnosis of N o n t u b e rc u l o u s M y c o b a c t e r i a l Pulmonary Disease Nontuberculous mycobacteria Pulmonary disease Microbiology Diagnosis. *Clinics in Chest Medicine*. https://doi.org/10.1016/j.ccm.2014.11.005

Jawetz, Melnick, & Adelber's. (2010). medical microbiology. In *Principles and Practice of Pediatric Infectious Diseases* (26th ed.). https://doi.org/10.1016/B978-0-323-40181-4.00114-6

Jeon, D. (2019). Infection source and epidemiology of nontuberculous mycobacterial lung disease. *Tuberculosis and Respiratory Diseases*, *82*(2), 94–101. https://doi.org/10.4046/trd.2018.0026

Katale, B. Z., Mbugi, E. V, Botha, L., Keyyu, J. D., Kendall, S., Dockrell, H. M., Michel, A. L., Kazwala, R. R., Rweyemamu, M. M., Helden, P. Van, & Matee, M. I. (2014). *Species diversity of non-tuberculous mycobacteria isolated from humans , livestock and wildlife in the Serengeti ecosystem , Tanzania*. 1–8.

Kilale, A. M., Ngadaya, E., Muhumuza, J., Kagaruki, G. B., Lema, Y. L., Ngowi, B. J., Mfinanga, S. G., & Hinderaker, S. G. (2016). Who has mycobacterial disease? A cross sectional study in agropastoral communities in Tanzania. *PLoS ONE*, *11*(5), 1–17. https://doi.org/10.1371/journal.pone.0153711

Killingley, J., Wilson, R. W., York, D. S., & Shepherd, S. (2014). Macrolide / Azalide Th erapy for Nodular / Bronchiectatic Mycobacterium avium Complex Lung Disease. *CHEST*, *146*(2), 276–282. https://doi.org/10.1378/chest.13-2538

Kim, Y. K., Hahn, S., Uh, Y., Im, D., Lim, Y. L., Choi, H. K., & Kim, H. Y. (2014). *Comparable characteristics of tuberculous and non-tuberculous mycobacterial cavitary lung diseases*. *18*(December 2013), 725–729.

Kobayashi, T., Nishijima, T., Teruya, K., & Aoki, T. (2016). *High Mortality of Disseminated Non- Tuberculous Mycobacterial Infection in HIV- Infected Patients in the Antiretroviral Therapy Era*. 1–11. https://doi.org/10.1371/journal.pone.0151682

Koh, W. J., Chang, B., Jeong, B. H., Jeon, K., Kim, S. Y., Lee, N. Y., Ki, C. S., & Kwon, O. J. (2013). Increasing recovery of nontuberculous mycobacteria from respiratory specimens over a 10-year period in a tertiary referral hospital in South Korea. *Tuberculosis and Respiratory Diseases*, *75*(5), 199–204. https://doi.org/10.4046/trd.2013.75.5.199

Lan, R., Yang, C., Lan, L., Ou, J., Qiao, K., Liu, F., & Gao, Q. (2011). *Mycobacterium tuberculosis and non-tuberculous mycobacteria isolates from HIV-infected patients in Guangxi , China*. *15*(January), 1669–1674.

Lapinel, N. C., Jolley, S. E., Ali, J., & Welsh, D. A. (2019). Prevalence of non-tuberculous mycobacteria in HIV-infected patients admitted to hospital with pneumonia. *International Journal of Tuberculosis and Lung Disease*, *23*(4), 491–497. https://doi.org/10.5588/ijtld.18.0336

Lee, H., Myung, W., Koh, W. J., Moon, S. M., & Jhun, B. W. (2019). Epidemiology of nontuberculous mycobacterial infection, South Korea, 2007-2016. *Emerging Infectious Diseases*, *25*(3), 569–572. https://doi.org/10.3201/eid2503.181597

Lee, Y. M., Kim, M. J., & Kim, Y. J. (2021). Increasing trend of nontuberculous mycobacteria isolation in a referral clinical laboratory in South Korea. *Medicina (Lithuania)*, *57*(7). https://doi.org/10.3390/medicina57070720

Li, G., Lian, L. L., Wan, L., Zhang, J., Zhao, X., Jiang, Y., Zhao, L. L., Liu, H., & Wan, K. (2013). Antimicrobial susceptibility of standard strains of nontuberculous mycobacteria by microplate Alamar Blue assay. *PLoS ONE*, *8*(12), 4–9. https://doi.org/10.1371/journal.pone.0084065

Lin, C., Russell, C., Soll, B., Chow, D., Bamrah, S., Brostrom, R., Kim, W., Scott, J., & Bankowski, M. J. (2018). Increasing prevalence of nontuberculous mycobacteria in respiratory specimens from US-affiliated pacific Island Jurisdictions. *Emerging Infectious Diseases*, *24*(3), 485–491. https://doi.org/10.3201/eid2403.171301

Lopez-luis, B. A., Sifuentes-osornio, J., Pérez-gutiérrez, M. T., Chávez-mazari, B., Bobadilla-del-valle, M., & Ponce-de-león, A. (2020). Original article Nontuberculous mycobacterial infection in a tertiary care center in Mexico , 2001 – 2017. *Brazilian Journal of Infectious Diseases*, *24*(3), 213–220. https://doi.org/10.1016/j.bjid.2020.04.012

Loret, J. F., & Dumoutier, N. (2019a). Non-tuberculous mycobacteria in drinking water systems: A review of prevalence data and control means. *International Journal of Hygiene and Environmental Health*, *222*(4), 628–634. https://doi.org/10.1016/j.ijheh.2019.01.002

Loret, J. F., & Dumoutier, N. (2019b). Non-tuberculous mycobacteria in drinking water systems: A review of prevalence data and control means. *International Journal of Hygiene and Environmental Health*, *222*(4), 628–634. https://doi.org/10.1016/j.ijheh.2019.01.002

Mabaso, G. C., & Mughogho, T. (2020). Prevalence and Distribution of Non – Tuberculous Mycobacteria (NTM) Among MDR-TB Suspected Patients in Northern Malawi. *Research Square*, 1–13.

Mahon R, C., Lehman C, D., & Manuselis, G. (2015). *Diagnostic microbiology* (5th ed.). Elsevier Health Sciences.

Marras, T. K., Campitelli, M. A., Lu, H., Chung, H., Brode, S. K., Marchand-austin, A., Winthrop, K. L., Gershon, A. S., Kwong, J. C., & Jamieson, F. B. (2017). Pulmonary Nontuberculous mycobacteria-assocated death. *Emerging Infectious Diseases*, *23*(3).

Maurya, A. K., Nag, V. L., Kant, S., Kushwaha, R. A. S., Kumar, M., Singh, A. K., & Dhole, T. N. (2015). *Prevalence of Nontuberculous Mycobacteria among Extrapulmonary Tuberculosis Cases in Tertiary Care Centers in Northern India*. *2015*. https://doi.org/10.1155/2015/465403

Mediavilla-gradolph, M. C., Toro-peinado, I. De, Bermúdez-ruiz, M. P., García-martínez, M. D. L. Á., Ortega-torres, M., Quezel-guerraz, N. M., & Palop-borrás, B. (2015). *Use of MALDI-TOF MS for Identification of Nontuberculous Mycobacterium Species Isolated from Clinical Specimens*. *2015*.

Meyer, K. C. (2005). *Age-associated changes in lung structure and function*. *2*, 433–439. https://doi.org/10.1513/pats.200508-081JS

Mirsaeidi, M., Machado, R. F., Garcia, J. G. N., & Schraufnagel, D. E. (2014). *Nontuberculous Mycobacterial Disease Mortality in the United States , 1999 – 2010 : A Population-Based Comparative Study*. *9*(3), 1–9. https://doi.org/10.1371/journal.pone.0091879

Mnyambwa, N. P., Kim, D. J., Ngadaya, E., Chun, J., Ha, S. min, Petrucka, P., Addo, K. K., Kazwala, R. R., & Mfinanga, S. G. (2018). Genome sequence of Mycobacterium yongonense RT 955-2015 isolate from a patient misdiagnosed with multidrug-resistant tuberculosis: First clinical detection in Tanzania. *International Journal of Infectious Diseases*, *71*, 82–88. https://doi.org/10.1016/j.ijid.2018.04.796

Mnyambwa, N. P., Ngadaya, E., Kimaro, G., Kazwala, L., Petrucka, P., & Mfinanga, S. G. (2017). *Assessment of sputum smear-positive but culture-negative results among newly diagnosed pulmonary tuberculosis patients in Tanzania*. 199–205.

Munita, J. M., & Arias, C. A. (2016). *Mechanisms of Antibiotic Resistance*. *May 2017*, 1–24.

Okoi, C., Anderson, S. T. B., Antonio, M., Mulwa, S. N., Gehre, F., & Adetifa, I. M. O. (2017). Non-tuberculous Mycobacteria isolated from Pulmonary samples in sub-Saharan Africa - A Systematic Review and Meta Analyses. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/s41598-017-12175-z

Pang, H., Li, G., Zhao, X., Liu, H., Wan, K., & Yu, P. (2015). *Drug Susceptibility Testing of 31 Antimicrobial Agents on Rapidly Growing Mycobacteria Isolates from China*. *2015*.

Pennington, K. M., Vu, A., Challener, D., Rivera, C. G., Shweta, F. N. U., Zeuli, J. D., & Temesgen, Z. (2021). Journal of Clinical Tuberculosis and Other Mycobacterial Diseases Approach to the diagnosis and treatment of non-tuberculous mycobacterial disease. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, *24*, 100244. https://doi.org/10.1016/j.jctube. 2021. 100244

Phoompoung1, P., Ankasekwinai1, N., Pithukpakorn1, M., Foongladda2, S., Umrod3, P., Suktitipat4, B., Mahasirimongkol5, S., Kiertiburanakul6, S., & Suputtamongkol1, Y. (2017). *Factors associated with acquired Anti IFN- γ autontibody in patients with NTM.pdf*. https://doi.org/10.1371/journal. pone. 0176342

Ratnatunga, C. N., Lutzky, V. P., Kupz, A., Doolan, D. L., Reid, D. W., Field, M., Bell, S. C., Thomson, R. M., & Miles, J. J. (2020). The Rise of Non-Tuberculosis Mycobacterial Lung Disease. *Frontiers in Immunology*, *11*(March), 1–12. https://doi.org/10.3389/fimmu.2020.00303

Ringshausen, F. C., Wagner, D., de Roux, A., Diel, R., Hohmann, D., Hickstein, L., Welte, T., & Rademacher, J. (2016). Prevalence of nontuberculous mycobacterial pulmonary disease, Germany, 2009-2014. *Emerging Infectious Diseases*, *22*(6), 1102–1105. https://doi.org/10.3201/eid2206.151642

Ryu, Y. J., Koh, W., & Daley, C. L. (2016). *s*. *3536*, 74–84.

Shahraki, A. H., Heidarieh, P., Bostanabad, S. Z., Khosravi, A. D., Hashemzadeh, M., Khandan, S., Biranvand, M., Schraufnagel, D. E., & Mirsaeidi, M. (2015). "Multidrug-resistant tuberculosis" may be nontuberculous mycobacteria. *European Journal of Internal Medicine*, *26*(4), 279–284. https://doi.org/10. 1016/j.ejim.2015.03.001

Shao, Y., Chen, C., Song, H., Li, G., Liu, Q., Li, Y., & Zhu, L. (2015). *The Epidemiology and Geographic Distribution of Nontuberculous Mycobacteria Clinical Isolates from Sputum Samples in the Eastern Region of China*. 1–10. https://doi.org/10.1371/journal.pntd.0003623

Spaulding, A. B., Lai, Y. L., Zelazny, A. M., Olivier, K. N., Kadri, S. S., Prevots, D. R., & Adjemian, J. (2017). *Geographic Distribution of Nontuberculous Mycobacterial Species*. *14*(11), 1655–1661. https://doi.org/10.1513/AnnalsATS. 201611-860OC

Thangavelu, K., Krishnakumariamma, K., Pallam, G., Dharm Prakash, D., Chandrashekar, L., Kalaiarasan, E., Das, S., Muthuraj, M., & Joseph, N. M. (2021). Prevalence and speciation of non-tuberculous mycobacteria among pulmonary and extrapulmonary tuberculosis suspects in South India. *Journal of Infection and Public Health*, *14*(3), 320–323. https://doi.org/10.1016/j.jiph.2020.12.027

Thomson, R., Tolson, C., Carter, R., Coulter, C., & Huygens, F. (2013). *Isolation of Nontuberculous Mycobacteria (NTM) from Household Water and Shower Aerosols in Patients with Pulmonary Disease Caused by NTM*. *51*(9), 3006–3011. https://doi.org/10.1128/JCM.00899-13

Varley, C. D., Ku, J. H., Henkle, E., Schafer, S. D., & Winthrop, K. L. (2017). *Disseminated Nontuberculous Mycobacteria in HIV-Infected Patients, Oregon, USA, 2007–2012*. *23*(3), 533–535.

Weyand, C. M., & Goronzy, J. J. (2016). Aging of the immune system: Mechanisms and therapeutic targets. *Annals of the American Thoracic Society*, *13*(December), S422–S428. https://doi.org/10.1513/AnnalsATS.201602-095AW

Winthrop, K L, Baxter, R., Liu, L., Varley, C. D., Curtis, J. R., Baddley, J. W., Mcfarland, B., Austin, D., Radcliffe, L., Suhler, E. B., Choi, D., Rosenbaum, J. T., & Herrinton, L. J. (2013). *Mycobacterial diseases and antitumour necrosis factor therapy in USA*. https://doi.org/10.1136/annrheumdis-2011-200690

Winthrop, Kevin L, Marras, T. K., Adjemian, J., Zhang, H., Wang, P., & Zhang, Q. (2020). *Incidence and Prevalence of Nontuberculous Mycobacterial Lung Disease in a Large U . S . Managed Care Health Plan , 2008 – 2015*. *17*(2), 2008–2015. https://doi.org/10.1513/AnnalsATS.201804-236OC

World Health Organisation (WHO). (2020). *global tuberculosis report, 202O* (Vol. 148).

Zhang, Z. X., Cherng, B. P. Z., Sng, L. H., & Tan, Y. E. (2019). Clinical and microbiological characteristics of non-tuberculous mycobacteria diseases in Singapore with a focus on pulmonary disease, 2012-2016. *BMC Infectious Diseases*, *19*(1), 1–8. https://doi.org/10.1186/s12879-019-3909-3

Zhou, W., Zhao, H., Yuan, H., Zhang, G., Liu, Q., & Yang, J. (2021). Prevalence and antimicrobial susceptibility of non-tuberculous mycobacteria isolated from sputum samples of patients with pulmonary infections in china. *Jundishapur Journal of Microbiology*, *14*(1), 1–7. https://doi.org/10.5812/JJM.109676

APPENDICES

Appendix 1: Consent form

ASSESSMENT OF PREDICTORS OF NON - TUBERCULOUS MYCOBACTERIUM INFECTION AMONG PATIENTS WITH SPUTUM SMEAR POSITIVE RESULT IN DODOMA

PATIENT CODE:

My name is Dr Fatuma Aziz, a second year student from University of Dodoma pursuing Masters of medicine in Microbiology and immunology. As a part of a test to be qualified as a doctor with a master degree I'm supposed to do research. My research is about determining the prevalence, predictors and anti-TB drug susceptibility for non - tuberculous mycobacterium infection among AFB sputum smear positive patients at Dodoma . NTM doesn't cause Tuberculosis but pulmonary infection may present with features similar to Pulmonary TB infection. The research will involve taking sputum and asking questions through a prepared questionnaire. If you will be diagnosed to have NTM infection, antimicrobial susceptibility test will be done and results will be given to your physician.

You, as a participant, are free to accept participating in this study or not, and you will have the right to withdraw from the study anytime .Your refusal to participate will not affect the provision of health service in this hospital.

In case of any questions regarding this study, please contact 0717819824 or email address fatmaaziz1@yahoo.com

Patient part:

I understood the information above, that my participation is voluntary and I am free to withdraw, without giving any reason, while my medical care or legal rights are unaffected. Signature of patient _____ Date_____

Signature of the researcher_____

Appendix 2: Fomu ya ridhaa

NAMBA Y USAJILI:

Mimi naitwa Fatuma Kuchimba Aziz, mwanafunzi wa mwaka wa pili katIka chuo kikuu cha Dodoma nausea shahada ya uzamili ya udaktari bingwa katika mikrobaiolojia na kingamwili. Ili kutunukiwa shahada hii mojawap o ya sifa ni kufanya utafiti. Na utafiti wangu unahusu uchunguzi wa vijidudu vilivyopo katika kundi la Mycobacteria ambao wanafanana na vijduduvinavyosababisha ugonjwa wa kifua kikuu lakini wenyewe hawasababishi kifua kikuu. Maambukizi ya vijidudu hivi katika mfumo wa hewa husababisha dalili kama za kifua kikuu. Utafiti huu utahusisha uchukuaji wa makohozi kutoka kwa mgonjwa na kwenda kuyapima maaabara kama kutakuwepo na vidudu hivyo. Utafiti huu utatumia njia ya mahojiano ya ana kwa ana ili kujua hali ya afya yako pia

Kwa ujumla. Iwapo tutagundua uwepo wa vijidudu hivyo tutachunguza dawa zilizo bora na zinazoweza kuangamiza vijidudu hivyo kabisa. Tutapendekeza dawa hizo/hiyo kwa daktari wa kituo atakaye kutibia.

Ningependa pia kukuhakikishia usiri kuhusu taarifa utakazotupa, tutatumia namba badala ya jina katika kuhifadhi hizi taarifa ili kuficha utambulisho. Kushiriki kwenye utafiti huu si kwa lazima, unaweza ukaamua kutoshiriki kabla au baada ya kuanza utafiti wenyewe. Na kutoshiriki kwako hakutoathiri upataji wako wa huduma za kawaida kituoni hapa

Kuweka sahihi inamaanisha umeelewa na upo tayari kushiriki.

Kwa maswali au mawasiliano zaidi tafadhali wasiliana na mtafiti kupitia namba ya Simu ya mkononi ambayo ni 0717819824 au kwa anuani ya barua pepe ambayo ni fatmaaziz1@yahoo.com

Tafadhali, weka tiki panapohusika

1. Ndio, nimeridhia _____

2.Hapana,sijaridhia_____ Tarehe…… ……

3.Sahihiyamtafiti…….

Appendix 3: Questionnaire

A. Demographic information

1) Age: ……
2) Which Gender
    a) Male:
    b) Female:
3) Level of education
    a) Illiterate (never gone to school)
    b) Primary education:
    c) Secondary education
    d) Tertiary education
4) Residence …….. District……
5) From which Source of water are using for domestic purpose……
6) Monthly income
    a) Less than 50 thousand:
    b) Less than 100 thousand:
    c) Less than 500 thousand shillings:
    d) More than 500 thousand shillings

B. CLINICAL INFORMATION

Symptoms and signs

1) Do you have cough?
    a) Yes. If yes for how long……..
    b) No
2) Do u have fever?
    a) Yes.
    b) No
3) Loss of weight?
    a) Yes
    b) No

4) HIV status?
    a) Negative
    b) Positive
5) History of diabetes mellitus?
    a) Yes
    b) No
6) History of cigarette smoking?
    a) Yes
    b) No
7) Do you have a history of tuberculosis infection?
    a) Yes
    b) No
8) When were you diagnosed with tuberculosis disease?..... ago.
9) Are you currently on anti-tuberculosis drugs?
    a) Yes
    b) No
10) Any history chronic lung disease other than TB?
    a) No history of chronic lung disease
    b) There is history of chronic lung disease…….. Which disease……..
11) Are you currently using steroids?
    a) Yes
    b) No

Appendix 4: Ethical clearance form

THE UNIVERSITY OF DODOMA

OFFICE OF THE DEPUTY VICE CHANCELLOR-ARC

DIRECTORATE OF RESEARCH, PUBLICATIONS AND CONSULTANCY

P.O. Box 259
DODOMA, TANZANIA
TEL: +255-026-2310002 FAX: +255-026-2310012
EMAIL: dvcarc@udom.ac.tz;
Website address:www.udom.ac.tz

Ref. No. MA.84/261/02 10th September 2020

To: Fatuma Aziz
The University of Dodoma

RE: REQUEST FOR ETHICAL CLEARANCE

This is to inform you that the proposal titled *"Prevalence, Predictors and Anti-Tuberculosis Drug Susceptibility of Non-Tuberculous Mycobacterium among AFB Sputum Smear Positive Patients at Dodoma Regional Referral Hospital, Tanzania"* has been granted ethical clearance.

Furthermore, as the Principal Investigator of the study, the following conditions must be fulfilled:

- Progress report is submitted to the University of Dodoma.
- Permission to publish the results is obtained from the University of Dodoma.
- Copies of final publications are made available to the University of Dodoma.
- Sites: Dodoma Regional Referral Hospital

Approval is valid for a duration provided for under clause five (5) of the Ethical Clearance Form.

Best Regards,

Dr. Alex Mongi
For Chairperson- Institutional Research Review Committee (IRREC)

C: C: Deputy Vice Chancellor-Academic, Research and Consultancy

Appendix 5: Research permit

THE UNIVERSITY OF DODOM
OFFICE OF THE VICE CHANCELLOR
P.O. BOX 259
DODOMA, TANZANIA
TEL: +255 (0)26 2323001 FAX: +255 (0)26 2323001 EMAIL: vc@udom.ac

Ref. No. MA.84/261/02 1st September, 2020

Regional Administrative Secretary
Dodoma Region

Director
Dodoma Regional Referral Hospital
Dodoma

RE: REQUEST FOR RESEARCH CLEARANCE

The purpose of this letter is to introduce to you **Ms. Fatuma Aziz with Reg. No. HD/UDOM/00143/T.2018** who is a bonafide student of the University of Dodoma and who is at the moment required to conduct research. Our students undertake research activities as part of their study programmes.

In accordance with government circular letter Ref. No. MPEC/R/10/1 dated 4th July 1980; the Vice-Chancellor of the University is empowered to issue research clearances to staff members and students of the University on behalf of the government and the Tanzania Commission for Science and Technology (COSTECH). I am pleased to inform you that I have granted a research clearance to the student listed above.

I therefore, kindly request you to grant her any help that may help her to achieve her research objectives. Specifically, we request your permission for her to work at Dodoma Regional Referral Hospital to meet and talk to all patients who are sputum AFB smear positive attending DRRH and other relevant stakeholders in connection with her research.

The title of her research is "***Prevalence, Predictors and Anti-Tuberculosis Drug Susceptibility of Non-Tuberculous Mycobacterium Among AFB Sputum Smear Positive Patients at Dodoma Regional Refferral Hospital, Tanzania***". The period of her research is from September 2020 to February, 2021 and it will cover planned area.

Should there be any restrictions, you are kindly requested to advise us accordingly. In case you require further information, please do not hesitate to contact us through the Directorate of Research and Publication, Consultancy, and Institutional Collaboration. P.O Box 251, Dodoma. Tel. No. + (255) 262310301 Email:research@udom.ac.tz

Yours Sincerely,

VICE CHANCELLOR
THE UNIVERSITY OF DODOMA
P.O. BOX 259
DODOMA, TANZANIA

Prof. Faustine K. Bee
VICE CHANCELLOR