HEALTHCARE WORKERS AWARENESS ON DIAGNOSIS AND MANAGEMENT OF NON-TUBERCULOUS MYCOBACTERIAL PULMONARY DISEASE

TOGOLANI GODFREY MAYA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Non-tuberculous mycobacteria (NTM) are also referred to as mycobacteria other than tuberculosis (MOTT). These bacteria are ubiquitous in the environment and their distribution varies depending on environmental microbial distribution, climate and weather condition. Transmission occurs by aerosols for example during bathing and steaming. Over time the numbers of species and burden have been increasing. This includes saprophytes and emergence of species that cause diseases to human and animals. In general *Mycobacterium avium* complex (MAC) has been the most common isolated group of NTM. These bacteria have commonly been isolated from among patients presumed to have pulmonary Tuberculosis

Non-tuberculous mycobacteria cause a variety of clinical conditions from localized infection to disseminated disease. Commonly they have been isolated from patients with atypical pulmonary TB. This condition is also known as NTM Pulmonary Disease (NTM-PD) and it is clinically similar to TB. NTM-PD cases have remained undiagnosed or misdiagnosed for TB. This poses a high risk of unsuccessful treatment outcomes and long hospitalization. Management of NTM requires Healthcare Workers (HCW) with expertise in the field because despite of long courses of treatment required, treatment regimen also varies depending on the infecting species. Treatment of *Mycobacteria avium* Complex (MAC) and *Mycobacteria abscessus* Complex (MABC) mostly relies on the use of macrolides and aminoglycosides.

Problem of NTM-PD is not yet well addressed in Tanzania. Success in detection and management of cases is largely limited by the level of awareness among HCWs and drug

susceptibility of circulating species. The current study assessed knowledge gap, identify circulating NTM species as a single or co-infections with TB and their drug susceptibility. This study adopted a cross-sectional design. NTM-PD awareness was carried out on HCWs from Health facilities (HFs) in the four administrative Regions that make the Northern zone of Tanzania; Tanga, Arusha, Manyara and Kilimanjaro. Four cadres of HCWs from TB clinics were conveniently interviewed using standardized questionnaire. These included; clinicians, nurses, laboratory personnel and pharmacists. A list of HCWs for each cadre in each HF provided the sampling frame for the study. In a situation where more than one HCWs per cadre met inclusion criteria for survey in a particular HF, simple random selection was applied.

Analysis of culture positive isolates was carried out at the Central TB Reference Laboratory. These included sample selection, storage, DNA extraction, speciation, drug susceptibility testing (DST). Isolates that were positive for para-nitrobenzoic acid (PNB) and all isolates from Kibong'oto zonal laboratory received between November, 2019 and August, 2020 were screened for NTMs. GenoLyse® protocol was used to extract DNA that were stored at – 20^0C till analysis. DNA amplification was done on the GTQ Cycler 96. Mycobacterium species were identified using GenoType® Mycobacterium CM/AS and GenoType® NTM-DR protocol. GenoType® NTM-DR was used to determine mutations that lead to drug resistance.

An average score for awareness on Pulmonary NTM was 24.1% (n=120; 95% CI: 10.5-37.7 STD: = 11.72), for which the highest was 61% and the lowest was 3%. Only 5 (4%) of all participants had a fair level of awareness (scored 50 to 74%) while all the remaining had poor level of awareness. A strong relationship was observed between history of attending training in which NTM was a topic of study and high level of

awareness ($p<0.05$). There was no statistical significant difference among the two genders. Mean awareness score of clinicians, laboratory staff, pharmacy staff and nurses were at 26% (STD: 13), 26% (STD: 11), 22% (STD: 14) and 21% (STD: 10), respectively. Level of education did not have statistically significant impact on the level of awareness in this study. Of the four regions; Kilimanjaro had the highest average awareness score of 26% (STD: 12.9) followed by Tanga 25% (STD: 11.7) and Manyara 24% (STD: 11.7) while Arusha had the lowest mean awareness score of 18% (STD: 7.5).

A total of 188 mycobacteria isolates were GenoType® d for NTM species. Of these, 179 were positive for mycobacteria and the remaining were negative. Among the positive, 24 (13.4%) were NTM while others were MTBC. Fifteen (62.5%) of the NTM could be GenoType® d to species level. Predominant NTM groups were six (25%) MAC and four (16.7%) were MABC. Four (16.7%) isolates were *M. intracellulare*, 3 (12.5%) *M. abscessus* sub. *abscessus*, 2 (8.3%) *M. avium* sub. *avium* and 2 (8.3%) were in the *M. fortuitum group*. Other species included; 1(4.2%) *M. bollettii*), 1 (4.2%) *M. kansasii*, 1(4.2%) *M. simiae* and 1 (4.2%) *M. szulgai*.

GenoType® NTM-DR was performed on MAC and MABC (10 (67%) isolates) of the identified species. Mutations were detected in three isolates of MABC on the *erm (41)* gene. Two of them are *M. abscessus sub. abscessus* and one *M. abscessus sub. bollettii* both with negative band C and positive T band making the isolates resistant to the macrolides. On the other hand no mutation was detected in the *rrl* and *rrs* genes hence all MAC were susceptible to both macrolides and aminoglycosides.

This study makes it the first attempt to determine the level of awareness among HCW in TB clinics on NTM-PD. It provides baseline information on the level of awareness.

It is also the first study in Tanzania to investigate the drug susceptibility profiles of NTM to the recommended drugs. To address the challenges this study uncovers a low HCW awareness on the disease and existence of strains that are resistant to macrolides. Observations predict a low suspicious index (case detection) and treatment failures. In addition to increased burden especially of MAC and MABC, inability to treat with routine TB drugs and under-reporting of cases in Tanzania with no regulated cases management guidelines all these urge TB programs to take action as this has a direct impact on management of TB.

The level of awareness on NTM pulmonary disease was generally poor among health care workers at the TB clinics in Northern Tanzania despite of the existence of significant number of cases. Mycobacteria avium complex species have been found the most prevalent NTMs among tested mycobacterial isolates. Finally this study uncovered existence of macrolides resistant mutations among MABC strains.

The TB programs should consider building awareness of its team HCWs so can be able to identify cases. As this study reveals an influence of previous participant training to awareness of NTM-PD, we recommend TB programs include a topic on NTM as a module in their current TB training packages.

In management of TB, it is important for clinicians to rule out NTM (specifically MAC and MABC) for all patients presenting with TB symptoms. Clinicians should anticipate macrolides resistances especially among MABC infected individuals.

DECLARATION

I, Togolani, Godfrey Maya, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Togolani Godfrey Maya Date

(MSc. Candidate)

The above declaration is confirmed by;

_____ _____

Prof. Erick Vitus Gabriel Komba Date

(Supervisor)

_____ _____

Prof. Rudovick Reuben Kazwala Date

(Supervisor)

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LIST OF ABBREVIATIONS

AAS	Africa Academy of Science
AIDS	Acquired Immunodeficiency Syndrome
AS	Additional Species
ASPIRE	Africa Scientific Partnership for Research Excellence
ATCC	American Type Culture Collection
ATS	American Thoracic Society
CF	Cystic Fibrosis
CM	Common Mycobacteria
COPD	Chronic Obstructive Pulmonary Disease
CTRL	Central Tuberculosis Reference Laboratory
DDH	Designated District Hospital
DELTAS	Developing Excellence in Leadership, Training and Science in Africa
DH	District Hospital
DNA	Deoxyribonucleic acid
DR	Drug Resistance
DST	Drug susceptibility testing
HC	Health Centre
HCW	Healthcare Workers
HF	Health Facilities
MABC	Mycobacteria Abscessus Complex
MAC	Mycobacterium Avium Complex
MDR	Multi-Drug Resistance
MOTT	Mycobacteria Other Than Tuberculosis

MTBC	Mycobacteria Tuberculosis Complex
MUT	Mutation
NTLP	National Tuberculosis and Leprosy Program
NTM	Non-tuberculous Mycobacteria
NTM-PD	Non-tuberculous Mycobacteria Pulmonary Disease
OPD	Outpatient Department
PNB	Para-nitrobenzoic Acid
R	Resistant
RNA	Ribonucleic Acid
RRH	Regional Regional Hospital
S	Susceptible
STD:	Standard deviation
TB	Tuberculosis
WHO	World Health Organization
WT	Wild Type

LIST OF PAPERS (ATTACHED)

Paper One: Non-tuberculous mycobacterial pulmonary disease: frontline healthcare workers' awareness survey in Northern Tanzania.

Paper Two: Drug susceptibility profiles of non-tuberculous mycobacterial species circulating among patients diagnosed with pulmonary tuberculosis in Tanzania.

CHAPTER ONE

Paper One

Non-tuberculous mycobacterial pulmonary disease: awareness survey of frontline healthcare workers in Northern Tanzania

Togolani Godfrey Maya[1], Erick Vitus Gabriel Komba [1], Rudovick Reuben Kazwala [1]

Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture

P.O. Box 3021, Morogoro, Tanzania

Corresponding author email: tmaya.tz@gmail.com

1.1 Abstract

Objective: This study was conducted to determine level of awareness on non-tuberculous mycobacterial pulmonary disease among front desk Healthcare workers in Northern Tanzania. It was also aimed to determine factors that influence the level of awareness.

Design: This was a cross-sectional descriptive survey carried out among front desk HCWs in the four administrative regions that make Northern Tanzania. A standardized questionnaire was administered to consented participants from four clusters; clinicians,

laboratory technicians, nurses and pharmacists working in TB clinics from Regional and District Health Facilities.

Setting: Non-tuberculous mycobacteria form a group of mycobacteria that cause neither tuberculosis nor leprosy (*M. leprae*). More than 200 species are known to exist of which only around 30 have been associated with human diseases. Pathogenic NTM infect various body parts including the lungs. Signs of NTM pulmonary disease resembles those of TB thus patients are easily confused for pulmonary TB.

Study Participants: A total of 120 participants were interviewed of whom coincidentally males and females were in equal numbers. Of them 38 (32%) were clinicians, 33 (27%) were laboratory personnel, 35 (29%) nurses and 14 (12%) pharmacists.

Outcome measures: Awareness score was used to measure the level of awareness among healthcare workers. Each participant was asked a set of questions, scored and the total score for each participant was determined. Average score for all participants was estimated including the 95% confidence interval.

Results: Average awareness score was 24.2% ± 11.7 (95% CI: 10.5 to 37.7). History of training, experience in TB care, level of HF, age group, region of origin were found to have strong relationship with level of awareness of participants. Although there were differences in levels of awareness between gender, cadre and level of education, the differences were not statistically significant.

Conclusion: The level of awareness on NTM-PD and its consistence in symptoms with TB is poor among HCWs in TB clinics in Northern Tanzania. On job training has been found to have strongest influence to level of awareness, so NTP programs are advised to include a topic on NTM in various TB training packages for HCW.

Key words: Awareness, Non-tuberculous mycobacteria pulmonary disease, healthcare workers and Northern Tanzania.

1.2 Introduction

Non-tuberculous mycobacteria (NTM) are mycobacteria other than tuberculosis complex (MOTT). They are also known as atypical mycobacteria, environmental mycobacteria or Mycobacteria other than tuberculosis (MOTT). These bacteria are ubiquitous in the environment, can be isolated from soil and water including sanitation (chlorine treated) water (Honda *et al*., 2018). Humans acquire pulmonary NTM infections mainly through inhalation of water droplets from contaminated sources like showers. Until 2015 there

was no documented evidence of human to human transmission (Harris *et al*., 2015). The distribution of NTM varies globally depending on environmental, microbial, ecological, climatic and weather characteristics of a place (Hoefsloot and Ingen *et al.,* 2013). Mycobacteria tuberculosis complex (MTBC) cause Tuberculosis (TB) and *Mycobacterium leprae* that cause leprosy while non-tuberculous mycobacteria pulmonary infections can lead to normal lung colonization or pulmonary disease with TB-like symptoms. This condition known as NTM pulmonary disease (NTM PD) (Piersimoni, *et al.*, 2008). Diagnosis, currently relies on clinical signs, radiological features and microbilogical assessment of presumptive patients (Griffith *et al*., 2007).

Although clinical pathogenicity of NTM is not yet well established, the organisms have been associated with a variety of diseases from localized infections to disseminated diseases. People with other underlying diseases such as Acquired Immuno-deficiency Syndrome (AIDS) and chronic lung diseases such as Cystic Fibrosis (CF) are more prone to disease. These include acute or chronic respiratory diseases, lymphadenitis, sinusitis, skin and soft tissue infection (Griffith *et al*., 2007). Studies carried out in Kilimanjaro and in Arusha discovered NTM to cause septicaemia and lymphadenitis (Mfinanga *et al*., 2004, Crump *et al*., 2009). However, NTM predominantly present as chronic pulmonary disease (Tortoli *et al*., 2009). Some of NTM species have frequently been isolated from patients with TB signs. For this reason, more rigorous studies are warranted to assess pathogenesis of various mycobacteria species like avium and abseccuss complex (Duan *et al*., 2016 and Weygaerde *et al*., 2019).

The prevalence and number of NTM species cuasing disease in humans has been reported to increase over the recent decades. In US the prevalence of NTM was found high among

women and the elderly (65 years and above). Between 2008 and 2015 the prevalence of NTM increased by at least 10% in 39 states (Winthrop *et al*., 2020). Systematic review and meta-analysis of 37 studies on pulmonary NTM in the South-Saharan countries revealed prevalence of 7.5% (Okoi *et al*., 2017). This includes saprophytes and emergence of species that cause diseases in human and other animals. A study conducted in 2013 reported a total of 140 NTM species (Daley *et al.*, 2013), while Euzeby *et al.*, 2020 lists more than 200 NTM species. In Tanzania two studies carried out in Tanga indicated 9.7% (Hoza *et al*., 2016) and 8.1% (Hoza *et al*., 2016) of presumptive TB patients were infected with NTM. Despite a significantly high proportion of NTM cases among TB presumptive cases, NTM have never been considered reportable in programmatic control measures. These facts urge a careful determination of cases by competent healthcare providers. Early detection of NTM cases through culture of at least two sputa specimen taken on separate days and correct management ultimately improves treatment success among patients with the NTM-Pulmonary Disease (Haworth *et al.*, 2017).

Control of pulmonary NTM disease can be achieved partly through developing a team of Healthcare workers (HCW) that is aware and thus suspicious for patients presenting in their hospitals (Winthrop *et al.,* 2017). Low awareness among HCW affects effective control of pulmonary NTM infections as either single or co-infection with TB (Chalmers *et al*., 2018). High suspicious index on presumptive tuberculosis cases and cases under treatment helps in ruling out NTM and proper management of cases. This in turn will contribute in the reduction of TB treatment failures and deaths, and eventually contribute in attaining the 2030 milestone of reduction of incidents and deaths among Tuberculosis patients (WHO, End TB Strategy, 2015). Generation of information on level of awareness of front desk HCW is key in determining NTM diagnosis capacity.

Consequently, this study aimed to determine the levels of awareness of pulmonary NTM disease among HCWs in TB clinics from Northern Tanzania. This is important as it helps in identifying the knowledge gaps among HCW working in TB clinics on pulmonary infecting NTM. The generated information contributes to proper prevention measures, detection of cases and treatment thus reduce incident rate, treatment failures among misdiagnosed TB patients, and eventually improve the health of people. Disease control policy development process also benefits from the results that the current study has generated.

1.3 Materials and Methods

1.3.1 Study design and setting

This was a descriptive cross-sectional structured survey conducted in Health Facilities located in Northern Tanzania (Tanga, Arusha, Manyara and Kilimanjaro) from November 2019 to February 2020. This survey was designed based on WHO guideline to developing knowledge, attitude and practice surveys (WHO, 2008). From 2013 to 2018 these regions have been in the top ten list of regions that reported higher number of TB cases in the country. Of these notified cases more than 50% have been clinically diagnosed as TB after being negative for acid fast bacilli (AFB) smear microscopy and/or GeneXpert (NTLP report, 2013 to 2018).

1.3.2 Study population

Study participants were recruited randomly from among front desk HCWs from HFs in the Northern Tanzania region, consented and interviewed. Health care staffs working directly in serving patients in selected TB clinics in this region formed the study population for this survey. These included at least one front desk clinician (clinician), nurse, laboratory personnel and pharmacist.

1.3.3 Sampling methods and sample size

Front desk HCW's in TB clinics are the key personnel in control of TB and other atypical mycobacteria lung infections. A plan was made to visit a total of 33 government owned or designated Health Facilities (HF) with active TB clinics for this survey. Study participants from each clinic were divided into four clusters i.e. Nurse, laboratory personnel, Clinician and pharmacist before simple random selection was conducted to get at least one participant from each cluster. Based on the formula below to calculate sample size in estimating proportions at least 134 AFB culture positive isolates will be analysed.

$$n = \left(\frac{t \times SD}{L}\right)^2 \qquad SD = \sqrt{P \times (1 - P)}$$

Where;

n = Sample size

t = student t – value

L = Significance level

SD = Standard Deviation

t = 1.96 L = 0.05 P = 0.097

$$n \geq 134$$

1.3.4 Inclusion and exclusion criteria

Assessment of awareness on pulmonary NTM infections included all technical health care workers in major TB clinics in the four administrative regions that make the Northern Tanzania. These included physicians and nurses at the OPD/TB Clinic, laboratory personnel working in TB testing and pharmacists dispensing drugs for TB patients. HCW for other clinics in the HF and those who are not involved direct in diagnosis of pulmonary TB were not involved in this study. Of the qualified staff only those who agreed and signed the consent were interviewed.

1.3.5 Questionnaire administration and data collection

Based on the WHO guideline, face to face interviews were conducted using structured questionnaire with a total of 54 items. It was specifically developed for HCW in TB care. Questions based on information gathered from various research findings and validated by experts in Mycobacteriology. Twenty eight questions were specific for NTM while others were assessing general awareness in the field. It took 30 to 45 minutes to administer the questionnaire to each participant.

Despite of questions that assessed awareness it also captured participant socio-demographic and clinical data such as age, gender, HIV status, previous history of TB treatment and residence (district and region). Most of variables were categorical except age and awareness scores which have been numerical.

1.3.6 Data management and statistical analysis

Collected data using a questionnaire were entered into 2010 Microsoft Excel®, for which each item in the paper questionnaire was transferred to electronic format. Scoring was done for each participant based on questions which were specific for assessment of NTM awareness. Data cleaning and some basic descriptive statistics were done in the Microsoft excel. The data set was then imported to R Studio version 1.2.5033 for further analysis. This study evaluated HCW levels of awareness in terms of percentage score from a total of questions asked of which the answers were correct. According to this study all variations with p-value less than 0.1 were considered statistically significant.

1.3.7 Results interpretation

This study used among others “awareness score” as the main outcome variable. Participants were considered aware of a particular research item of the study questionnaire when they gave a correct answer. Each correct answer was scored “1” except for items that required a participant to provide more than one responses in which a score of “1” was given when a participant was able to mention only one and a score of “2” was awarded when a participant was able to mention more than one correct responses. Each participant was scored out of total score i.e. 32 and multiplied by 100 to get percentage score. Scores were ranked as poor, fair and good as indicated in Table 1.1 below (Yusof *et al*., 2014)**.** Percentage awareness of participants was also evaluated based on individual research items like ability to mention any other name for NTM and ability to mention any species.

Table 1.1: Awareness ranking

S/N	Score ranking (%)	Level of awareness
01	0 – 49	Poor/Unsatisfactory
02	50 – 74	Fair/Satisfactory/Moderate
03	75 – 100	Good/High/Excellent

1.3.8 Ethical clearance and HCWs enrolment

This study protocol was approved by the Sokoine University of Agriculture Institutional Research Review Board, Institutional Research Ethical Committee of the National Institute for Medical Research (NIMR) (NIMR/HQ/R.8a/Vol. IX/3245) and local authorities of all the four administrative Regions and 27 Districts. Participants were

approached after permission from Medical officer in-charges of the HFs. Only participants who provided consent were interviewed. Enrolment to this study was based on presence of the HCW on a day when the HF was visited. A random procedure was adopted to select two HCWs in situations where more than two met the selection criteria. Study identification numbers were used instead of patients' names to ensure that identity of each participant remains anonymous. Data obtained were kept in computer with protected access using a password; and hard copies were kept in lockable draw.

1.4 Study Results

1.4.1 Study setting analyses

A total of 29 (88%) Regional and District Hospitals were visited out of 33 available in the region. Of these HFs one (3%) was a National TB Reference Hospital, two (7%) were RRH and 26 (90%) were DH. Of the district hospitals, 13 were government owned, six were faith based HFs also called Designated District Hospitals (DDHs) and seven were HC (for districts that didn't have established district hospitals). The highest number of HFs (11, 38%) was from Tanga region, while Arusha had the lowest number (5, 17%). Four HFs could not be visited due to various reasons among them being in-accessibility during the study period which resulted from heavy rains (Ngorongoro and Bumbuli). Others were Mawenzi RHH and Mount Meru RRH, the reason being unaffordable research fees.

1.4.2 Participants' demographic characteristics

In this study a total of 120 participants were interviewed with an average age of 40 years (range: 24-60). Most of the participants (34%) were between the age of 30 and 41 years old. While more participants with lower age were males, more participants with higher age were females. Figure 1.1 indicates variation in numbers of respondents among

various demographic characteristics. The number of participants with regard to their age groups is indicated in Figure 1.2. With respect to gender, coincidentally male and female participants were in equal numbers. Most female participants had lower education level when compared to males. Table 1.2 summarises numbers of participants per type of Health facility visited. This study found that for most of the cadres the number of males was higher except for nurses of which the number of females was by far higher than that of males (Figure 1.3). Figures 04 and 05 display the distribution of different cadres of participants based on gender and region.

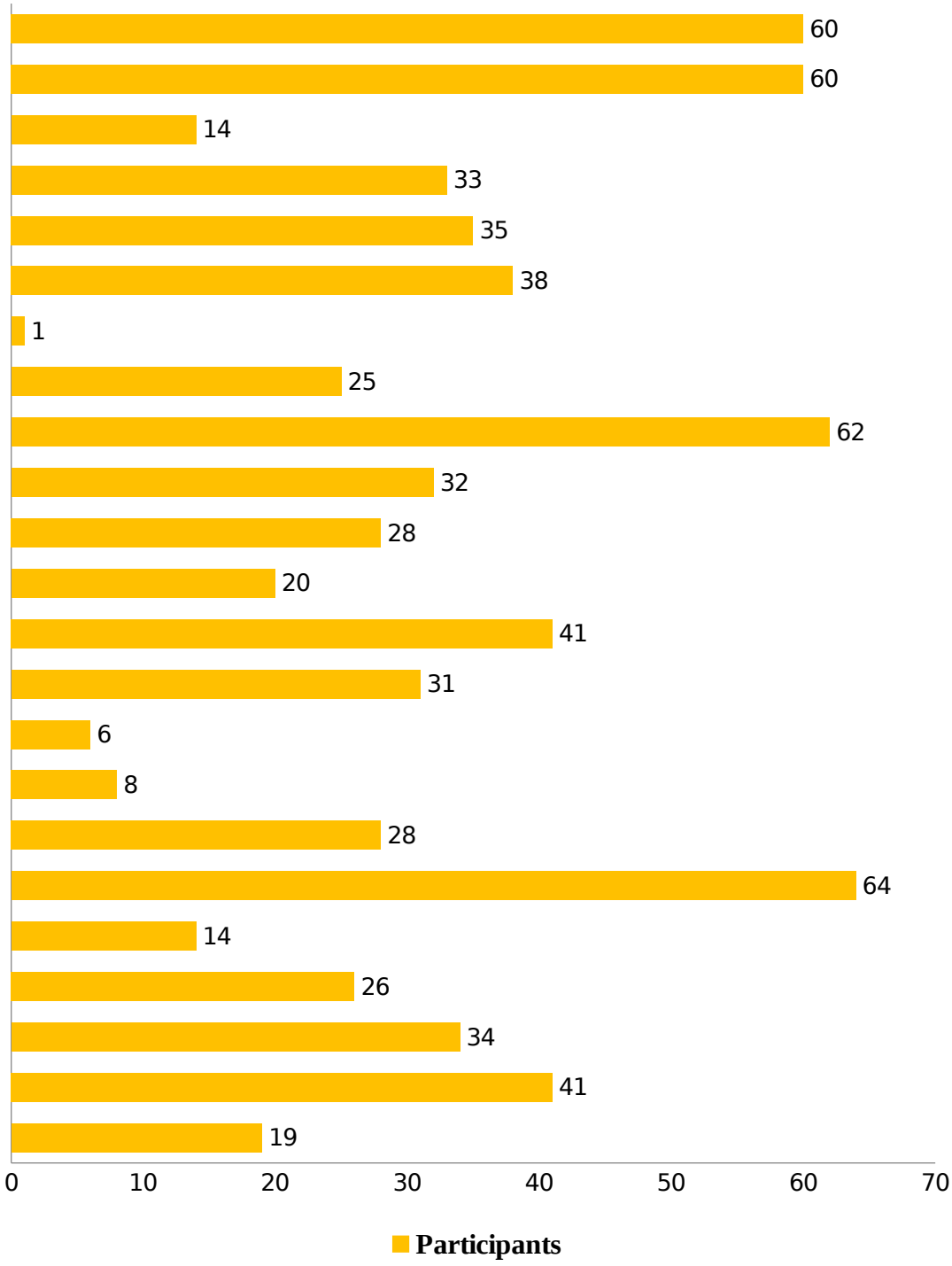

Figure 1.1: Demographic characteristics of participants

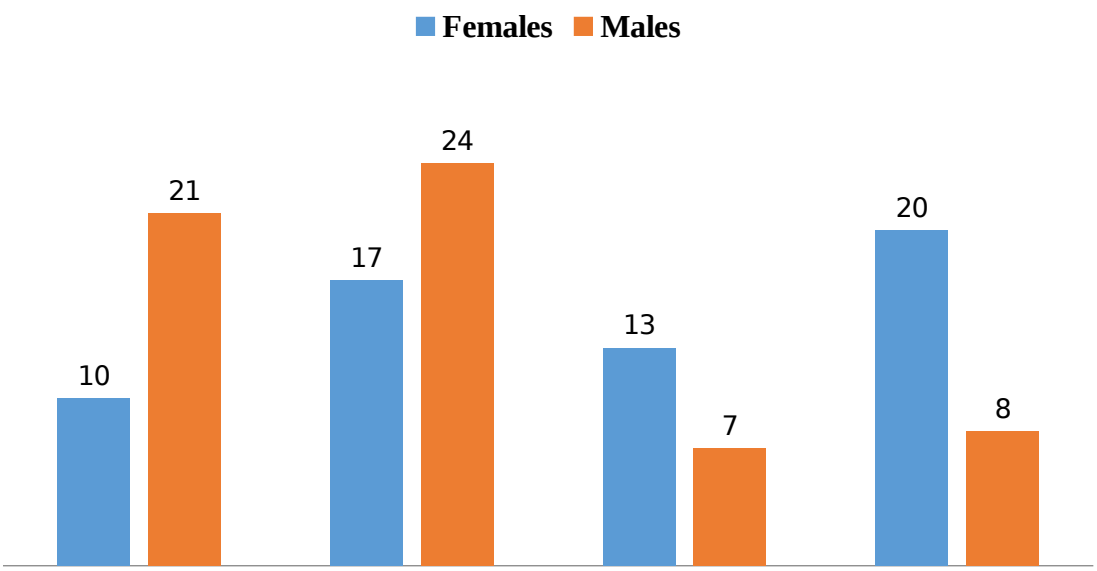

Figure 1.2: The distribution of participants by age and sex

Table 1.2: Number of study participants for each type of health facility

Type of Health Facility	Female	Male	Total (%)
NTRH	3	1	4 (3)
RRH	3	6	9 (8)
DH	27	28	55 (46)
DDH	16	13	29 (24)
HC	11	12	23 (19)
Total	**60**	**60**	**120**

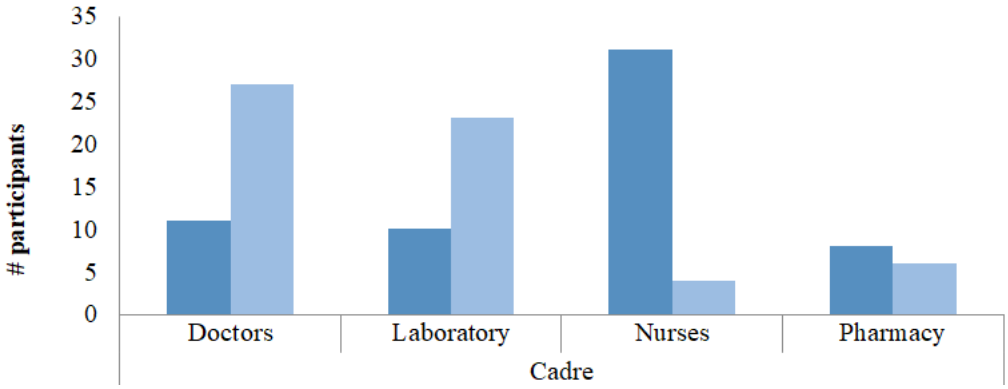

Figure 1.3: Gender variation for each cadre

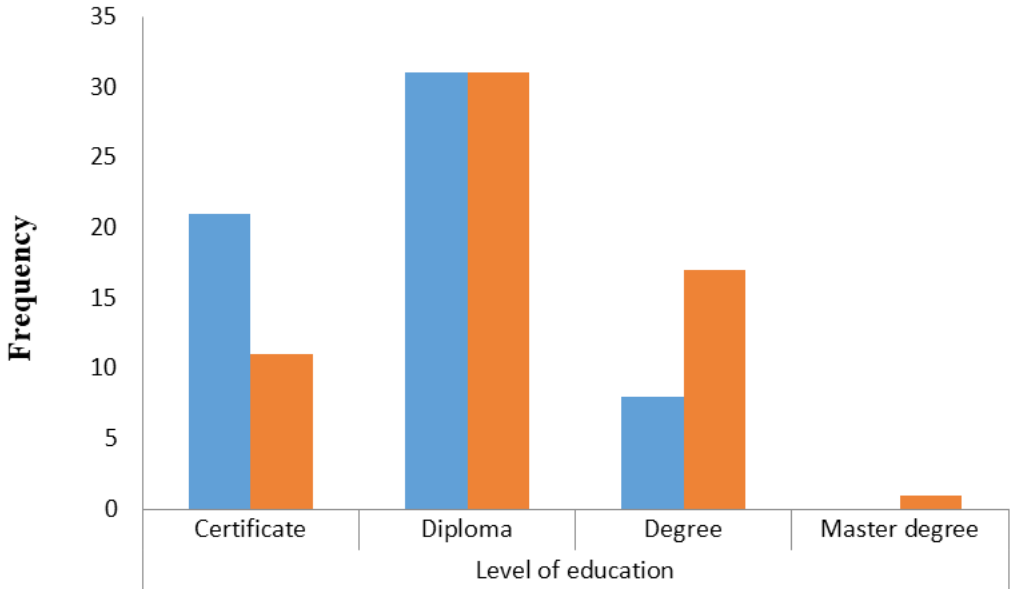

Figure 1.4: The distribution of education levels of study participants by gender

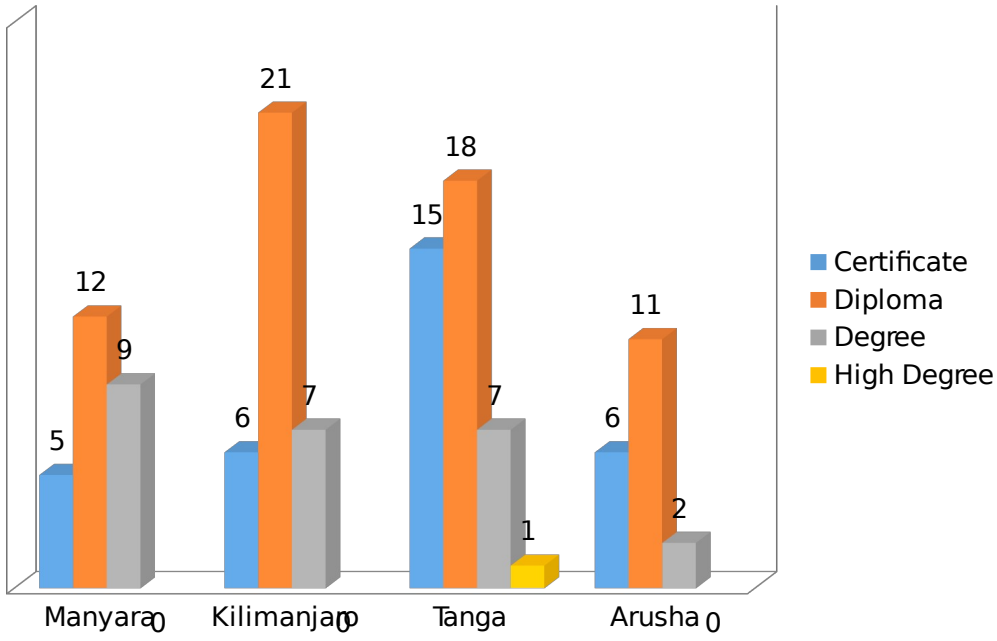

Figure 1.5: The distribution of the different cadres in TB clinics in different regions of Northern Tanzania

1.4.3 Pulmonary NTM disease awareness score results

An average score for awareness on Pulmonary NTM was 24.1% (95% CI: 10.5-37.7, STD: = 11.7), for which the highest was 61% and the lowest was 3%. Only 5 (4%) of all participants had a moderate level of awareness (scored 50 to 74%) on pulmonary NTM while all the remaining had poor level of awareness. Table 1.3 summarizes mean awareness scores, standard deviation and significance of any variations between the outcome variable and explanatory variables. Female participants had an average awareness score of 22% (STD: 10) while mean awareness score among males was 26% (STD: 13) as displayed in Table 1.3. Similarly this table displays participants mean scores with respect to their age groups. From this table mean awareness scores for clinicians, laboratory staff, pharmacy staff and nurses were at 26 (STD:13), 26 (STD: 11), 22 (STD: 14) and 21 (STD: 10), respectively.

Additionally this table indicates results of mean score between -different levels of education. Of the four regions; Kilimanjaro had highest average awareness score of 26% (STD: 13) followed by Tanga 25% (STD: 11.7) and Manyara 24% (STD: 11.7) while Arusha had the lowest mean awareness score of 18% (STD: 7.5) see Figure 1.6.

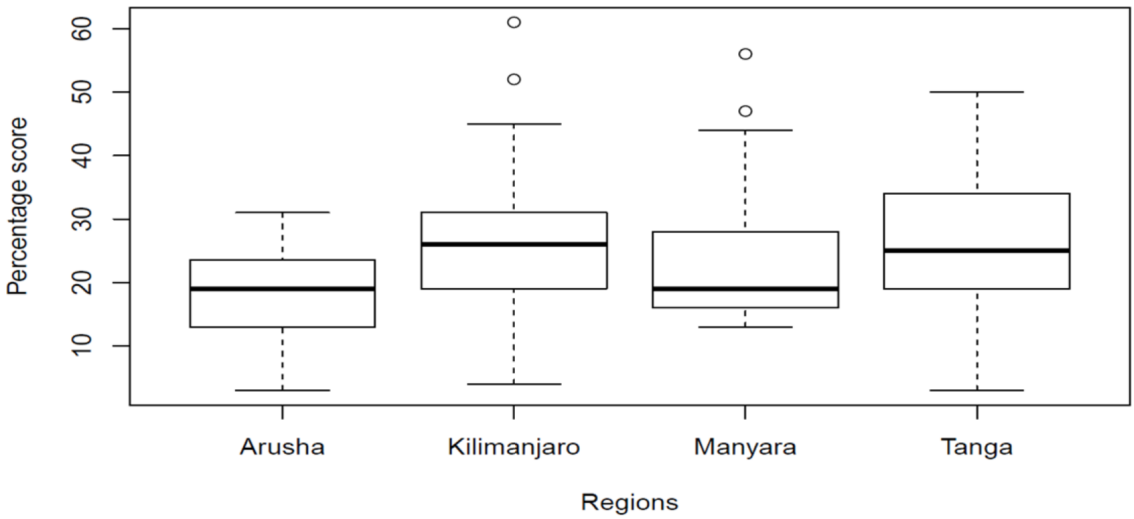

Figure 1.6: Boxplot of mean pulmonary NTM awareness scores per Region

Table 1.3: NTM awareness score among study participants in relation to different attributes

Explanatory variables	Variability	Mean Awareness Score (%)	STD: Deviation (%)	p-value
Gender	Men	26	10	0.3225
	Women	22	13	
Age group (years)	21-30	26	12	2.2e-16
	31-40	26	12	
	41-50	23	11	
	51-60	21	11	
Experience in Health Care	Less than 1	13	NA	0.97
	1-5	28	13	
	6-10	22	10	
	11-15	18	11	
	Greater than 15	23	10	

Experience in TB care (years)	Less than 1	20	8	5.419e-05
	1-5	26	13	
	6-10	24	11	
	11-15	18	9	
	Greater than 15	22	8	
Education Level	Certificate	20	9	0.8463
	Diploma	24	12	
	Degree	29	13	
	Higher degree	28	NA	
Region	Tanga	25	12	0.03982
	Arusha	18	8	
	Manyara	24	12	
	Kilimanjaro	26	13	
Cadre	Pharmacist	22	14	
	Nurse	21	10	0.7313
	Clinician	26	11	

	Laboratory personnel	26	13	
NTM training	Yes	44	7	3.727e-11
	No	24	11	
Type of HF	NRH	46	15	0.0002151
	RRH	33	15	
	DH	22	10	
	HC	23	10	

On the other hand of the 120 participants only three (2.5%) had attended a training where NTM was a topic for discussion. Of those who were asked whether it is true or false that all mycobacteria species can cause TB it was found that 79 (66%) were aware that it is not all mycobacteria species can cause TB. It was found in this study that only 48 (40%) participants had ever heard the term non-tuberculous or mycobacteria other than tuberculosis in the whole period of their career. Although only 19 (16%) of participants could correctly define NTM, 77 (64%) believed that pulmonary non-tuberculous infection is clinically similar to TB. Thirty three (28%) of all the interviewed participants were aware that not all NTM can cause diseases to human. Only 12 (10%) and 12 (12%) of the

interviewed participants could mention any other name and species of NTM. Table 04 shows responses to other questions that were asked during this survey.

Table 1.4: Participants responses on non-tuberculous mycobacterial survey questions

Category	Item	Responses	Counts	%
Diagnosis of pulmonary NTM	Pulmonary NTM and TB are clinically different	Yes	38	33
		No	77	67
	NTM presents as i) Pulmonary ii) Extra – pulmonary iii) Both	Pulmonary	22	19
		Extra – Pulmonary	28	24
		Both	67	57
	Ability to identify risk factors for NTM infection	Able	72	60
		Unable	48	40
	Microscope cannot distinguish between MTBC and NTM	True	48	40
		False	33	28
		Not Sure	39	32
	GeneXpert can detect both NTM and MTBC	True	51	43
		False	32	27
		Not Sure	37	50
	Which of these is a gold standard test for NTM	Culture	49	41
		GeneXpert	29	24
		Microscopy	7	7
		Not Sure	34	28

	DNA test is the definitive for NTM	True	44	37
		False	12	10
		Not Sure	64	53
	NTM found in soil and water	True	18	15
		False	22	18
		Not Sure	80	67
	Mention Pulmonary NTM Risk factor	Able to mention any	15	13
		Can't mention any	105	87
Transmission of NTM	Pulmonary NTM is acquired through inhalation	Yes	84	70
		No	36	30
	Can be transmitted from one person to another	True	52	53
		False	19	19
		Not Sure	27	28
	All pulmonary infecting NTM species can be treated with TB drugs	True	39	33
		False	29	24
Treatment of NTM		Not Sure	52	43
	All NTM species can be	True	15	13

	treated with same drug	False	46	38
	regimen	Not sure	59	49
	Ability to mention any	Mention at least one	13	12
	drug recommended for NTM	Couldn't mention any	98	88
		3	3	3
	How long (months) extended treatment takes after NTM	6	27	22
	case converts to culture	12	1	1
	negative	24	1	1
		Not sure	85	71

1.5 Discussion

Since pulmonary NTM infections present with signs similar to TB, competence among HCW in TB clinics will improve management of cases of this emerging public health threat. Findings of this study supports Makondo *et al.* (2014) recommandation on building of awareness among HCWs of the disease especially in TB endemic countries. This is important because the management of pulmonary NTM differs significantly from TB. Otherwise, it takes high suspicious index among HCWs for diagnosis of patients, treating and infection prevention.

The most important finding in this study was the poor awareness about pulmonary NTM infections among HCWs in TB clinics (Table 1.3). Only 4% had been found to have moderate awareness on pulmonary NTM infections, being a low proportion compared to that found in a similar study on TB awareness in Uganda in which authors reported 62% of the HCWs being (Buregyeya *et al*., 2016). The current study has found a significant association between awareness and the following; experience in TB care, history of training were NTM were discussed, age group, type of HF and administrative region (Table 1.3). Although there was a variation in mean scores between gender, cadre, level of education and experience in health care services provision, this study did not find a significant association of these attributes with the level of awareness of NTM.

The current study revealed that, history of on job training in which NTM was a topic of discussion had a strong relationship with high level of awareness of NTM. This finding is worth noting and thus formalization of NTM module in the current TB training packages for HCWs is highly recommended. This will make practitioners think of NTM as a differential diagnosis in situations of TB treatment failures instead of believing that every treatment failure is a result of *Mycobacterium tuberculosis* complex drug resistance. It will also promote inclusion of NTM in molecular tested TB negative patients who present with symptoms of atypical TB.

Participants in the age groups of 21 to 30 and 31 to 40 had the highest score compared to those in higher age groups (Table 1.3). This correlation coincided with the finding that participants with lesser experience on the job i.e. 1 to 5 years and 6 to 10 years (26.4% STD: 13), 24.0% (STD: 11%) had higher awareness scores respectively. This suggests that fresh HCWs in lower age groups and experience had advantage due to recent graduation from college where NTM is part in most of curricula. We could not find

significant difference in level of awareness between different cadres despite of high mean score among clinicians than nurses and pharmacists.

A significant number of HCWs 70% believes that NTM are acquired mainly through inhalation which is right but only 19% understands that there is not enough evidence of this disease being transmitted from one person to another (Falkinham *et al*., 2011). Despite evidence of NTM being acquired directly from the environment, limited findings support transmission of *M. abscessus* between patients with Cystic Fibrosis (Bryant *et al*., 2013). It is a fact that there is currently no evidence of person to person transmission of the disease but people acquire this disease mostly from water aerosols during bath, steaming and swimming. (Falkinham *et al.*, 2011). These bacteria are ubiquitous in water and soil, but only 18 (15%) of participants were aware of this (Table 1.4). Very few participants were able to mention any risk factor for pulmonary NTM disease but a significant number could spot a risk factor from a list of factors as multiple choice questions. A good understanding seems to have been influenced by their experience that most of lung infectious diseases result from similar risk factors.

Awareness on drugs and regimen recommended for treatment is crucial in control of pulmonary NTM infections. This study found poor level of HCWs awareness on drugs and/or regimen for treatment of pulmonary NTM (Table 1.4). Of the interviewed HCWs (n=120), only 13 (12%) could mention at least one drug recommended for treatment of pulmonary NTM. Although MAC and MABC cannot respond to TB drugs, it is evident that some NTM species like *Mycobactreria kansasii* respond to rifampicin, Isoniazid and Ethambutol except Pyrazinamide, most participants are not aware that not all NTM species cause pulmonary infections can be treated with TB drugs (British Thoracic Society., 1994, Ryu, *et al*., 2016, Kim *et al*., 2019). While MAC responds to macrolides

in addition to rifampicin and Ethambutol, MABC responds to macrolides in addition to aminoglycosides and other antibiotics (Wu *et al*., 2019).

1.6 Study Limitations

The questionnaire used in this study was developed based on multi-centre and systematic review studies results, researcher knowledge, experience and consultation with experts in control of mycobacteria infections and experts in mycobacteria research, of whom some were supervising this work. It needs more validation steps like piloting and comparison with other researchers' questionnaires on the same or similar topic. Many more multi-centre pulmonary NTM awareness studies need to be conducted to validate information generated here. This study only identified gaps existing on awareness which impedes successful control of NTM pulmonary disease. It does not determine, but recommends the possible solution to the problem. Moreover references made in this study were not validated for ATC criteria that distinguish between pulmonary NTM colonization and causation of pulmonary disease. More studies on how pulmonary NTM infections play a role in causing disease or impair management of other chronic lung diseases such as TB need to be done.

1.7 Conclusions and Recommendations

The level of awareness on NTM pulmonary disease is generally unsatisfactory among health care workers in TB clinics in the four administrative regions in Northern Tanzania. This urges for a need of inclusion of the NTM component in the TB training package for Health care workers. Since there is a strong relationship between training and awareness; this will improve awareness, increase NTM suspicious index among HCW in TB Clinics and in turn will reduce incidences of misdiagnoses for TB.

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CHAPTER TWO

Paper Two

Drug susceptibility profiles of non-tuberculous mycobacteria species circulating among patients diagnosed with pulmonary tuberculosis in Tanzania

Togolani Godfrey Maya[1], Erick Vitus Gabriel Komba[1], Rudovick Rauben Kazwala[1]

Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture

P.O. Box 3021, Morogoro, Tanzania

Corresponding author email: tmaya.tz@gmail.com

2.1 Abstract

Background: Number of clinical cases resulting from non-tuberculous mycobacteria infection has been observed to increase over the recent decades throughout the world. Pulmonary NTM infectious lead to TB-like disease called NTM pulmonary disease (NTM-PD). Due to variation in treatment options among NTM species it is necessary to

identify species and be aware of drug susceptibility profiles to be able to choose appropriate regimen for the disease.

Methods: A total of 188 culture positive isolates from patients diagnosed for TB at the Central Tuberculosis Reference Laboratory were screened for NTM. All NTM were further speciated using GenoType® Mycobacterium - Common Mycobacterium and Additional species (GenoType® CM/AS) kit. *Mycobacteria avium* complex (MAC) and *Mycobacteria abscessus* complex (MABC) which could not be identified to species with the test were subjected to GenoType® Mycobacteria NTM-DR for further speciation. Furthermore identified MAC and MABC were genotyped to determine drug susceptibility profile of each isolate to macrolide and aminoglycosides using GenoType® Mycobacteria NTM-DR assay.

Results: Of all samples that tested positive to mycobacteria, 24 (13%) where positive for NTM. Fifteen isolates could be identified to species level of which prevalent species was *M. avium* sub. *intracellulare* 4 (27%). A total of 10 isolates were MAC and MABC being (6 and 4 respectively), which were subjected to GenoType® Mycobacteria NTM-DR for determination of macrolide and aminoglycoside susceptibility. Three of the four MABC had mutation at the T28 position of the *erm (41).* All MAC were susceptible to both drugs.

Conclusion: In this study MAC species were the most frequent isolated species followed by MABS species. Being the leading cause of NTM-PD and due to their natural resistance to routine TB drugs existence of these bacteria can lead to treatment failures for patients misdiagnosed for TB. While all of MAC and MABC isolated species identified in this study were susceptible to aminoglycosides three MABC were resistant to the macrolides due to mutation at position 28 of the *erm (41)* gene. For these reasons HCWs need to be aware of possibility MAC and MABC pulmoanry infection from among TB presumed patients and also be able to identify species with their drug susceptibility pattern before treatment initiation for better outcomes.

Keywords: Non-tuberculous mycobacterial pulmonary disease, species, drug susceptibility profiles, Tanzania

2.1 Background

Non-tuberculous mycobacteria pulmonary disease (NTM-PD) results from lung infection of atypical mycobacteria. These bacteria have also been known as Mycobacteria other than tuberculosis (MOTT) or environmental mycobacteria. On the other hand Mycobacteria tuberculosis complex species. (MTBC) cause Tuberculosis while *Mycobacterium leprae* cause leprosy. NTM-PD presents with similar features to TB and has frequently been referred to as atypical TB (Koh *et al*., 2006, Kendall *et al*., 2011). They are also known as atypical mycobacteria or environmental mycobacteria. These bacteria are ubiquitous in the environment therefore can be isolated from soil and

water including sanitation water. Home showers, water tanks and wet soil have been the major reservoirs of NTM and transmission occurs by aerosol. *M. marimum* for example causes swimming pool granulomatous disease. The number of NTM species has been observed to increase over the recent decades due to lifestyle and climatic changes. This involves saprophytes and emergence of species that cause diseases in human and other animals. A study in 2013 reported a total of 140 NTM species (Daley *et al.*, 2013) in 2019 there are more than 200 species reported (Jean P. Euzéby, 2020) [www.bacterio.cict.fr/m/mycobacterium.html] 27th June 2020). While some NTM species cause disease especially in people with other underlying diseases, most of them are saprophytic (Jennifer *et al.*, 2015).

More than 30 NTM spp. have been found to have clinical implications on human health (Griffith *et al*., 2007). These can further be categorized into two groups; slow growing and rapid growing (Forbes *et al*., 2018). They cause a variety of clinical conditions in human including acute or chronic respiratory diseases, lymphadenitis, sinusitis, skin and soft tissue infection (Griffith *et al*., 2007). Chronic diseases like Chronic Obstructive Pulmonary Disease (COPD), Cystic Fibrosis (CF) and HIV have been associated with increased prevalence of NTM-PD (Honda *et al*., 2015). Respiratory infections can occur as a single infection or co-infected with mycobacteria tuberculosis complex (MTBC) (Kim *et al*., 2017; Mertaniasih *et al.*, 2017). The cases have frequently been mis-categorized for TB (Evans *et al*., 1996). In Tanzania a case that was misdiagnosed as MDR-TB patients after relapse and failure of first line TB drugs regimen was later discovered to be a result of *M. yongonense* infection, one of the novel NTM (Mnyambwa

et al., 2018). Studies carried out in Kilimanjaro and Arusha discovered NTM causing septicemia and lymphadenitis (Mfinanga *et al*., 2004; Crump *et al.,* 2009).

There is currently no clear evidence of human to human transmission of NTM-PD documented (Harris *et al*., 2015). A multicenter NTM prevalence study among people with CF indicated no strong enough strain similarity to prove human to human transmission (Olivier *et al*., 2003). Distribution of NTM varies globally depending on environmental microbial, climate and whether of a place. In general MAC are among the most isolated spp. of NTM; more specifically *M. avium, M. intracellular* and *M. kansasii (Mueller et al., 2008)*. Few multicenter studies have been carried out on distribution and burden of NTM in Africa especially in Southern Saharan Africa. A systematic review and meta-analysis of 37 articles on NTM in the Southern Saharan region revealed a prevalence of 7.5% of pulmonary NTM with MAC prevailing by 28% and in 19 of the studies (Okoi *et al.,* 2017). A study similar to the current which was done on culture positive samples from a National TB prevalence survey revealed NTM proportion of 54.3% with *M. fortuitum* being the most isolated species (Addo *et al.,* 2017).

The proportion of cases of NTM is significantly high among TB presumptive cases in Tanzania. Two studies that were carried out in Tanga indicated 9.7% and 8.1% of presumptive TB patients had NTM. *M. interjectum* isolates were found the leading NTM in the catchment (16.7%) followed by *M. intracelallare* 11.1%. The prevalence was high among patients with other underlying diseases like AIDS and Cystic Fibrosis (CF) (Hoza

et al., 2016, McShane *et al.*, 2015). Non-tuberculous mycobacteria diversity has also been investigated from human-animal interaction in the Serengeti ecosystem where 36.7% were *M. intracellulare (Katale et al., 2014).*

Success in management of NTM is largely limited by lack of knowledge on species circulating and their drug susceptibility profiles. This is because treatment regimen is species dependent and most of the clinically relevant NTM species are naturally resistant to conventional TB drugs (Brown *et al*., 2012; Haworth *et al*., 2017). While some of clinically relevant NTM species can respond to conventional TB drugs such as *M. kansasii*, the rest are naturally resistant to these drugs (Wang *et al*., 2014). This poses a high risk of unsuccessful treatment outcomes and long hospitalization of NTM cases misdiagnosed as TB.

The current study was focusing on identifying species and their drug susceptibility profiles of non-tuberculous mycobacteria commonly isolated at the Central Tuberculosis Reference Laboratory (CTRL) in Tanzania**.** Knowledge generated contributes to awareness of prevalent species and key drugs that should be included in the treatment regimen. The TB control policy development process can also benefit from the results that the current study has generated such as when NTM needs to be included in the TB trainin modules and control strategies.

2.2 Methods

2.2.1 Study sites

Testing of TB in Tanzania is organized into different levels of laboratories from National/central, zonal, regional, district, health center and dispensary level. However this study was conducted at the Central Tuberculosis Reference Laboratory (CTRL) in Dar es Salaam. Despite of receiving culture positive isolates from the zonal laboratories for comprehensive routine surveillance of drug resistance, CTRL serves as zonal in its catchment, Eastern zone (Dar es Salaam, Pwani, Morogoro, Lindi and Mtwara). Others include; Kibong'oto Hospital Laboratory (Northern zone), Pemba Public Health Laboratory (Pemba and Unguja), Bugando Medical Centre Laboratory (Lake zone), Mbeya Referral hospital laboratory (Southern highlands) and Dodoma Regional Referral Hospital laboratory (Central zone).

In implementing universal DST coverage strategy Tanzania implement culture of sputum specimen from bacteriologically confirmed (by AFB microscopy or Xpert MTB/Rif) TB patients for comprehensive conventional DST. In 2018 a total of 27 201 TB cases were bacteriologically confirmed in (NTLP, Annual report., 2018). This is has been incorporated into the DR-TB Surveillance system. At CTRL specimen are received for various reasons including DR-TB surveillance (by Xpert MTB/Rif, Hain line probe assay or conventional DST), TB diagnosis, and for treatment monitoring. In the year 2016 and 2017 a total of 392 and 443 cultures respectively were received at CTRL from Kibong'oto alone which serves Northern zone.

2.2.2 Study design and sampling

This was a cross-sectional study in which isolates from culture positive sputum specimen preferably at diagnosis stage were conveniently selected for DNA extraction. A priority was given to those which were positive to para-amino benzoic acid (PNB) during conventional DST. The CTRL receives these isolates from the zonal laboratories. The isolates were genotyped using GenoType® Mycobacterium, Common Mycobacteria and Additional species (GenoType® CM/AS) for NTM species identification and then the species were genotyped using GenoType® Mycobacteria NTM-DR for drug susceptibility to the recommended drugs. Central Tuberculosis Reference Laboratory culture registers and the list of culture positive isolates received between November, 2019 and August, 2020 provided the sampling frame from which samples were conveniently selected. Each of selected positive culture (mycobacteria isolate) from primary culture media was transferred to a 2 ml cryotube containing 15% glycerol in Tryptic Soya Broth (TSB). These isolates where then stored at – 20^0C until used.

This study only included isolates received at CTRL for diagnosis, treatment monitoring and conventional TB DST. While most of isolates were from newly diagnosed patients before treatment initiation, few were isolates from patients under treatment monitoring (follow up). This is because NTM, especially MAC, have been frequently isolated from patients under TB treatment (Nishiuch *et al.*, 2017).

2.2.3 Data collection

2.2.3.1 Patients information (from patients' test request/report forms)

Patients demographic characteristics (age, gender, district, region, etc) and clinical information such as HIV status, diagnostic test used, initial local GeneXpert/AFB smear and culture results were collected. Laboratory test request form and TB LIS (electronic laboratory information system) provided a source of patients data.

2.2.3.2 DNA Extraction and storage

For genotyping DNA was extracted chemically using GenoLyse® (Hain Lifescience, Nehren, Germany) protocol (Hain Life Science, 2017) [https://www.hain-lifescience.de/en/products/dna-isolation/genolyse. html] 10th July 2020). In case DNA could not be GenoType® d on the same day of extraction, it was stored in a deep freezer at – 20°C. After genotyping DNA were transferred to – 80°C for long term storage.

2.2.3.3 Species identification

PCR was carried out in the GTQ Cycler 96 using the protocol in the user guide (Hain Lifescience, Nehren, Germany). Speciation was carried out using GenoType® ® CM/AS (Hain Lifescience, Nehren, Germany) protocol on TwinCubator (Hain Lifescience, Nehren, Germany). Species specific probes are mounted to the DNA strips to determine complementary strands from amplified DNA samples (Mäkinen *et al.*, 2006; Lee *et al.*, 2009). Table 2.1 below shows that MAC and MABC that could not be speciated by this technique were further speciated using GenoType® ® NTM-DR (Hain Lifescience, Nehren, Germany) protocol (Hee *et al*., 2019). DNA amplicon that could

not be identified to species level the same day of amplification were stored at – 20^0C. On the other hand isolates that GenoType® ® CM/AS assay could not GenoType® to species level are stored at – 80^0C for sequencing of 16S rRNA gene.

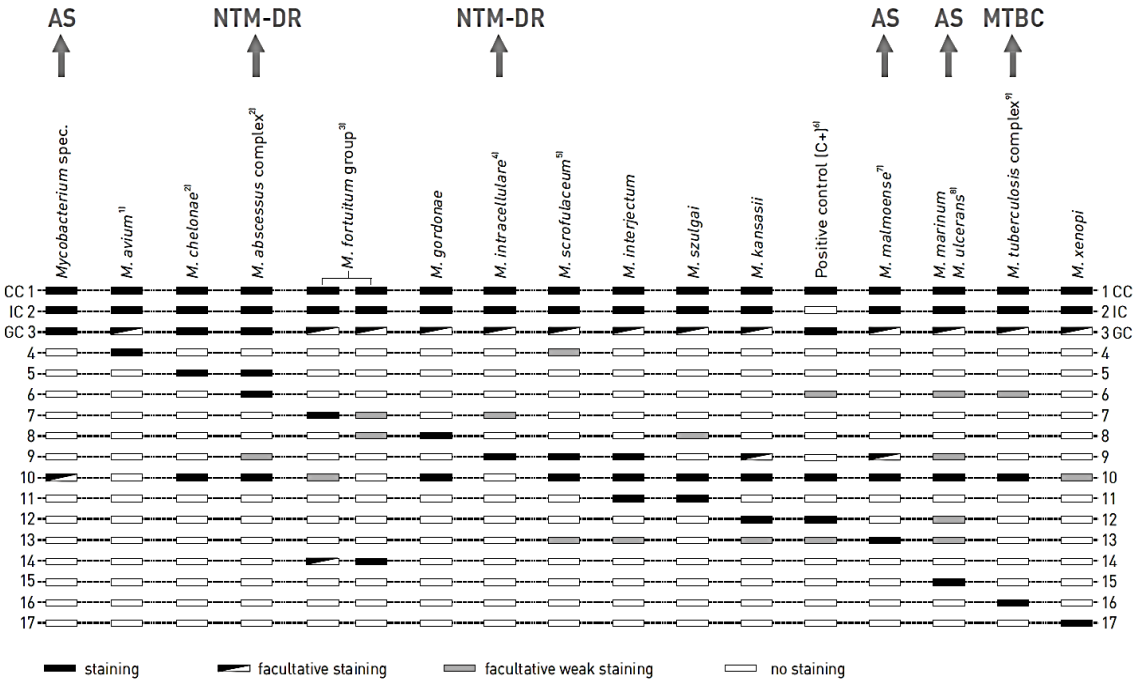

Figure 2.1: GenoType® mycobacterium CM band pattern and the corresponding spp.

2.2.3.4 NTM drug susceptibility testing

Molecular drug susceptibility profiles of *Mycobacteria avium* Complex and *M. abscessus* complex isolates were carried out using the GenoType® ® NTM-DR. This PCR technique involves amplification of the *erm (41)* (erythromycin ribosomal

methylase) gene and *rrl* gene which encodes for 23S peptidyl transferase of the large subunit (50S) of rRNA were conducted (Figure 2.2). This detects mutation that may result to development of resistance to macrolides (Kobashi *et al*., 2006 and 2012). However *erm (41)* is available only in MABS with some few spp exceptions. Moreover this also involves amplification of the *rrs* gene. Mutation(s) in the *rrs* gene which encodes for 16S rRNA results to resistance to aminoglycosides (Mougari *et al*., 2017).

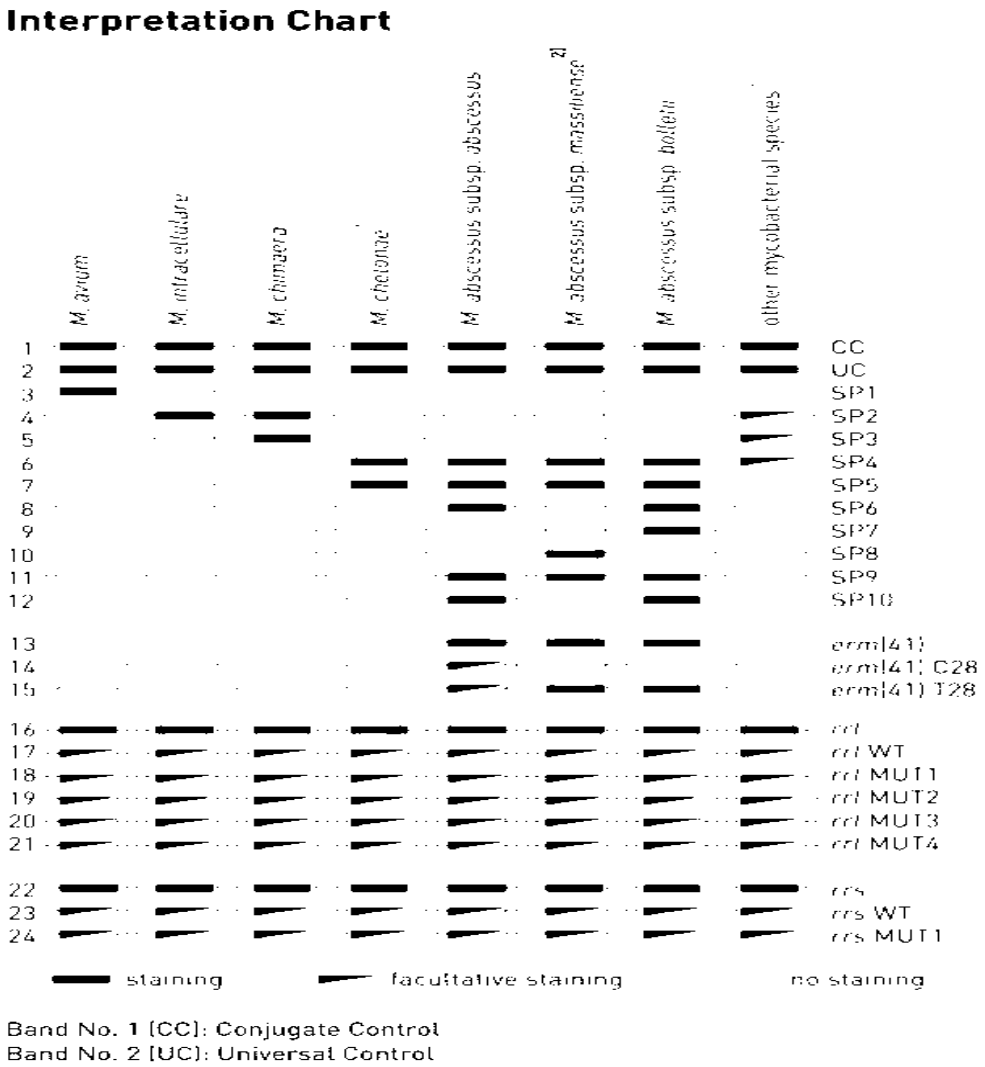

Figure 2.2: GenoType® NTM-DR for MAC and MABC species and banding pattern for Drug susceptibility to macrolides and aminoglycosides.

2.2.3.5 Quality control

For each investigation known positive control strains *M. fortuitum* (ATCC® 6841™) and *M. kansasii* (ATCC® 12478™) were included. On other hand DNA free water (moelcular grade) was used as negative control for quality assurance.

2.2.4 Data management, confidentiality and statistical analysis

Patients' descriptive data and genotyping analytical data were collected and cleaned in Microsoft Excel® 2010 and then imported to R software for analysis. These include independent variables such as age, gender, NTM species, HIV status, patients category, and reason for testing (diagnosis or treatment monitoring) (Table 2.4). Dependent (response) variables include; NTM species and drug susceptibility (i.e susceptible or resistant).

2.2.5 Ethical clearance

This study protocol was approved by the Sokoine University of Agriculture Institutional Research Review Board, Institutional Research Ethical Committee of the National Institute for Medical Research (NIMR) (NIMR/HQ/R.8a/Vol. IX/3245). Study Identification numbers, hospital registration numbers and laboratory serial numbers was used instead of patients' names to ensure that identity of each participant remains

anonymous. Data obtained was kept in computer having its access protected using a password and hard copies was kept in lockable draw.

2.3 Results

2.3.1 Patients' demographic characteristics

Specimen analyzed in this study came from 188 patients diagnosed with pulmonary TB of whom 45 (24%) were females. Overall mean age was 43 (STD: = 15) years with females being 38 and males 48. Most (30%) of study subjects had age starting from 35 to 44 years. The mean age of patients with NTM was 47 (STD:=15). Figure 2.3 indicates study subjects distribution by age groups. Number of patients who had a known HIV status was 144 of whom 34 (24%) were positive for the virus. While of 110 who were HIV negative 11 (10%) had NTM, 34 patients with HIV 10 (29%) had NTM infection. On the other and of the patients with NTM (n= 24), 21 had the records available for HIV status of whom 9 (42%) were positive. With regard to treatment history, a total of 176 patients records were available of which 113 (64%) were new TB patients while 63 (36%) had previous treated for the disease.

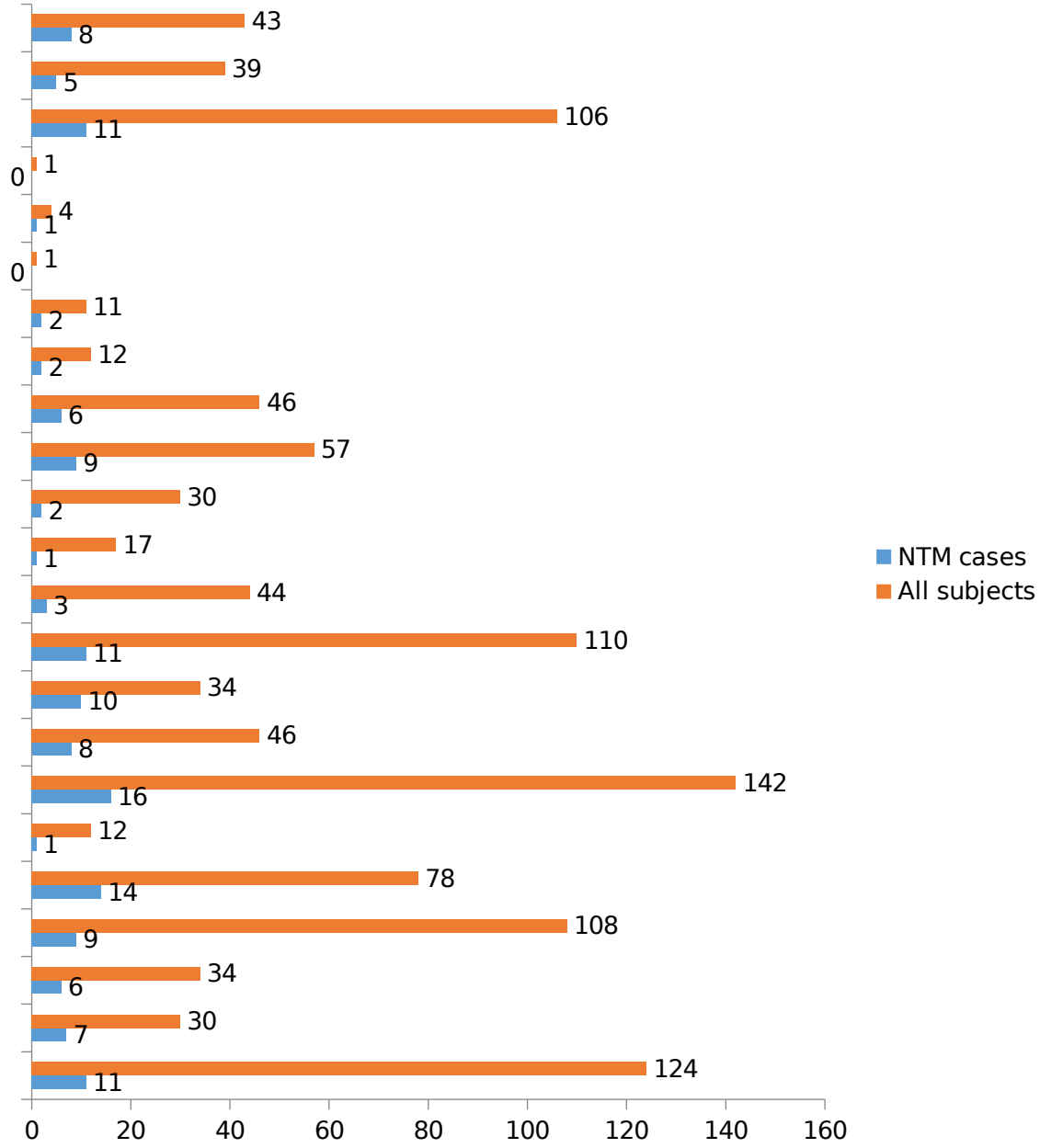

Figure 2.3: Variation of number of non-tuberculous mycobacteria between various independent variables

2.3.2 Non-tuberculous mycobacteria species and drug susceptibility

Isolates analyzed in this study resulted from culture positive sputum from patients diagnosed with pulmonary TB. Of the 188 isolates tested, 179 (95%) were positive for the genus mycobacteria while the remaining were negative. Among the positive samples 24 (13%) were NTM while the rest were MTBC. GenoType® Mycobacterium CM/AS and GenoType® Mycobacterium NTM-DR could identify 15 (63%) NTMs to their species levels. Result interpretation chart for GenoType® Mycobacterium CM and banding pattern for each positive result are shown in Figure 2.3 and Table 2.1. Deoxyribonucleic acids that could not be genotyped to species level are available for speciation by sequencing of the 16S rRNA gene. This study revealed *Mycobacterium Avium* complex (MAC) as the most prevalent group. There were 6 (25%) species belonging to this group (4 *M. intracellulare and 2 M. avium)*. On the other hand there were 4 (17%) *Mycobacterium abscessus* complex (MABC) (3 *M. abscessus sub. abscessus and 1 M bollettii*) and 2 (8%) Fortuitum group. Other species include; 1 (4%) *M. kansasii*, 1 (4%) *M. simiae* and 1 (4%) *M. szulgai*.

All MAC and MABC (10 isolates) were subjected to GenoType® NTM-DR for susceptibility testing to macrolides (azithromycin and capriomycin) and aminoglycosides (kanamycin, amikacin and gentamycin). While MABC resistance to macrolides results from mutation conferred in either or both of *rrl* and *erm (41)* genes,

MAC resistance to this drug results from mutation in the *rrl* gene only. Of the 10 NTMs tested for drug susceptibility, three were found to have mutation (T28 point mutation) in the *erm (41)* of which Cytosine is replaced by Thymine at position 28 of the gene. However there was no macrolide resistance mutation detected in the *rrl* gene. Likewise no mutation was detected for aminoglycosides resistance (in the *rrs* gene) for both MABC and MAC (Table 2.2).

Table 2.1: Banding pattern and presumed species

Band pattern CM	**Frequency**	**Band pattern AS**	**Band pattern NTM-DR**	**Presummed species**
1,2	9	Not Applicable	Not Applicable	Negative for Mycobacteria
1,2,3,10,16	155	Not Applicable	Not Applicable	MTBC
1,2,3,5,6,10	3	Not Applicable	SP4, SP5, SP6, SP9, SP10	*M. abscessus sub.abscessus*
1,2,3,5,6,10	1	Not Applicable	SP5, SP6, SP7, SP9, SP10	*M. abscessus sub.bolletti*
1,2,3,9	4	Not Applicable	SP2	*M. avium sub. intracelluare*
1,2,3,7,14	2	Not Applicable	Not Applicable	*M. fortuitum* group
1,2,3,10,11	1	1,2,3,4,6	Not Applicable	*M. simiae*
1,2,3,6,10,11	1	1,2,3	Not Applicable	*M. szulgai*

1,2,3,10	4	1,2,3	Not Applicable	Indeterminate*
1,2,3,10	3	1,2,3,12	Not Applicable	Indeterminate*
1,2,3,5,6,10,16	1	1,2,3,12	Not Applicable	(Mixed MTBC and NTM)
1,2,3	1	1,2,3	Not Applicable	Indeterminate*
1,2,3,4	2	Not Applicable	SP1	*M. avium*
1,2,3,10,12	1	Not Applicable	Not Applicable	*M. kansasii*

* Need targeted sequencing of 16S rRNA gene to identify species

Table 2.2: Summary wild type bands and associated mutations in the GenoType® Mycobacterium NTM-DR assay

	Gene								
MAC/MABC Species	***erm (41)***		***rrl***		***rrs***		**Macrolides**	**Aminoglycosides**	**Frequency**
	C28	**T28**	**WT**	**MUT**	**WT**	**MUT**			
Intracellulaire	NA	NA	+	-	+	-	S	S	4

Abscessus	-	+	+	-	+	-	R	S	2
Abscessus	+	-	+	-	+	-	S	S	1
Bollettii	-	+	+	-	+	-	R	S	1
Avium	NA	NA	+	-	+	-	S	S	2

WT – Wild type, MUT – Mutation, S – Susceptible, R – Resistant and

NA – Not applicable

2.3.3 Non-tuberculous mycobacteria statistical associations

Figure 2.4 illustrates NTM proportions among levels of some important variables investigated in this study. The prevalence of NTM was higher among people who were previously treated (category) for TB 14 (58%) compared to new patients, the difference was significant at 90% (p= 0.0656). Similarly this study supports at 90% confidence existence of association of NTM cases with age of patients with p-value = 0.07367. There was a significant relationship between gender and NTM infection where males had higher chances of being positive than females (p=0.008655). While 11(46%) tested positive for NTM among patients for specimen collected just before treatment initiation, six (25%) patients tested positive for NTM after a number of months of treatment. Pearson's Chi-square p-value of 0.004426 supports that more NTM are recovered at

diagnosis than during treatment monitoring. NTM were recovered in nine (26.5%) patient among those who were positive for HIV (n=34) representing 18% of the total number of tested individuals. This study could not find a significant relationship between type of mycobacteria infection (MTBC or NTM) and HIV status (p=0.1708).

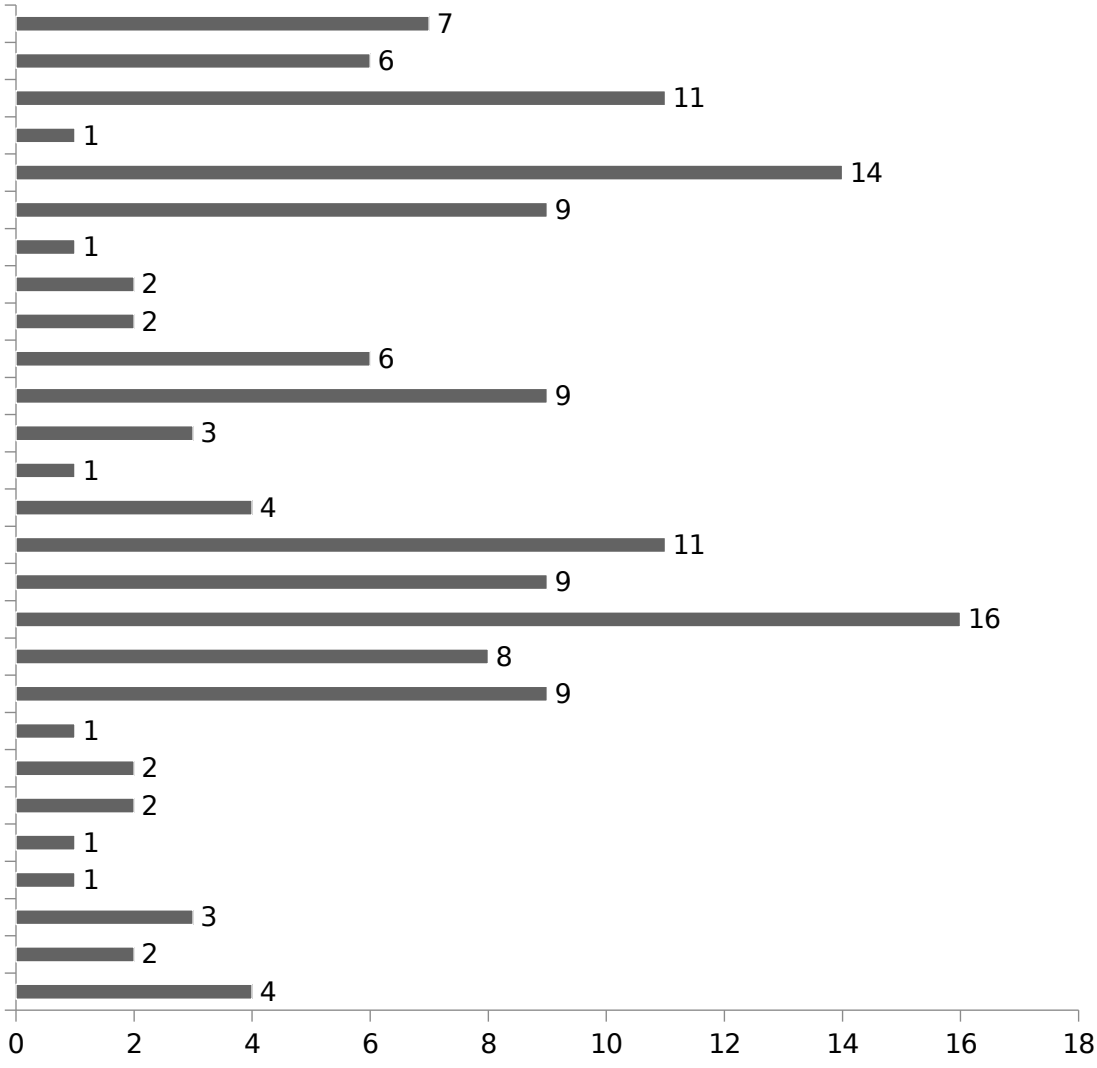

Figure 2.4: Number of Non-tuberculous mycobacteria in relation to other variables

2.4 Discussion

Increasing in numbers and burden of NTM among presumptive TB cases raises attention for National TB program (NTP) to consider screening for NTMs from among TB presumptive cases especially for patients at risk is of paramount importance (Makondo *et al.*, 2014; Hoza *et al.,* 2016). Furthermore, knowledge on prevailing species and their drug susceptibility pattern is critical for correct diagnosis and selection of appropriate drug regimen (Griffith *et al.,* 2007).

The current study aimed at determining NTM species circulating among patients diagnosed for TB and their drug susceptibility profiles. Drug susceptibility profiles were specifically performed to MAC and MABS on the recommended drugs. Several incidences of NTM have been observed among patients on TB treatment in Tanzania (Mnyambwa *et al*., 2016; Mbelele *et al*., 2019). In this particular study the interest was into characterizing NTM oftenly recovered at CTRL for species and molecular drug susceptibility to the recommended drugs.

In this study *Mycobacteria intracellulare* (MAC) was the most prevalent species, a similar observation to previous studies (Griffith *et al*., 2007; Okoil *et al.,* 2017; Daley *et al*., 2020). These were followed by MABS species of which both are well documented in causation of NTM-PD. Moreover recovered *M. kansasii* has commonly been associated with pulmonary disease. Use of rapid bioline tests to screening out of NTM among mycobacteria culture

positive and exclusion of NTM in laboratory reporting in Tanzania blinds existence of these pathogenic NTM among the patients. For this it is advised to reporting culture positive NTM among patients with chronic or acute pulmonary pulmonary disease. This study is the first in Tanzania to recover *Mycobacterium abscessus* sub. *bolletii and M. szulgae* isolates in Tanzania (Crump *et al*., 2009; Hoza *et al*., 2016; Katale *et al.*, 2016; Mnyambwa *et al*., 2017). Greater number of species identified (eight different species) indicates the vastness of NTM species circulating among patients treated for TB in Tanzania. A national NTM prevalence survey is warranted to enable estimation of the burden and treatment options in Tanzania.

Similar to González *et al*. (2017) NTM are more frequently isolated from males when compared to females. In this study the number of males with NTM was twice that of females. Chi-square supports the existence of a statistically significant difference between the two genders contrary to previous reports (Honda *et al.*, 2014; Park *et al.*, 2019). This is similar to Tuberculosis were more male contract the disease than females with reason being higher number of daily close and casual contacts in men (Dodd *et al*., 2015). NTM distribution by age groups is also similar to the distribution of TB where more patients are from the age of 35 to 44 years. We observed a proportional increase with age where patients with higher age have higher chances of getting NTM-PD (Shteinberg *et al.*, 2018).

Moreover this study supports the fact that patients previously treated for TB (relapse, loss to follow-up, treatment failure and MDR contact) have higher chances of being positive for NTM infection than new cases (Zhang *et al*., 2019). More epidemiological studies are required to investigate the factors that favor NTM colonization and infection in this group of patients. While most of the recovered NTM were from samples collected at the time of TB diagnosis, some were identified from samples of patients after several months of TB drug administration. On the other hand the current study could not support the hypothesis that people with HIV have higher chances of NTM infection in comparison to HIV free, a similar observation to the one made earlier (McCarthy *et al.,* 2012). This might have resulted from differences in the sample size and sampling methods employed.

Erythromycin resistance methylase gene (*erm (41)*) exists in MABS species like *M. abscessus* sub. *abscessus* and *M. abscessus* sub. *bollettii* (Daley *et al.,* 2020). Other species in this group like *M. massilience* lack the target base at position 28 which results from large deletion of a 397 base-pair sequence in which this target region is included. Such species have a nonfunctional *erm (41)* gene thus do not express resistance to macrolides. Mutation at nucleotide position 28 of this gene where Cytosine is replaced by Thymine results to phenotypic drug resistance of these two species to macrolides (Wallace *et al*., 1996 and Bastian *et al*., 2011). Two of the three identified *M.abscessus* sub. *abscessus* and one *M. bollettii* were detected with this mutation indicating prevailing resistances among MABS population. However these strains were all susceptible to the aminoglycosides. Management of MABS that are resistant to macrolide can follow other recommended guidelines (Haworth *et al*., 2017). On the other hand no mutation were detected in both *rrl* and *rrs* genes in this study. This indicates susceptibility of the

remaining identified MABS and MAC to both drugs. A study carried out in Ghana on National prevalence survey samples also revealed susceptibility of all isolated MAC and MABS to aminoglycosides (Addo *et al*., 2017).

2.5 Study Limitations

While most of the isolates characterized in this study came from patients within their first month of TB treatment few were from patients after a number of months of treatment. There is no evidence of these isolates to meet diagnostic criteria for non-tuberculous mycobacteria causation of lung disease as described in the ATS/IDSA Statement (Griffith *et al*., 2007) and the British Thoracic Society Guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD) (Haworth *et al*., 2017). Moreover the genotyping techniques employed in this study could not identify all isolated NTM to species level; nine isolated were left un-speciated. Aminoglycosides and macrolides are the recommended important components treatment of NTM-PD however many other drugs can be combined in developing an efficient regimen.

2.6 Conclusion

This study has recovered a total of eight different NTM species circulating among patients diagnosed with pulmonary tuberculosis whose sputum specimen were submitted for routine culture and drug susceptibility testing. Mycobacteria avium complex being the prevalent group of species (6) has been followed up by MABS (4). Both of these require medical attention when diagnosed as they have been associated with pulmonary disease. On the other hand while all MAC and MABS have been found susceptible to

aminoglycosides, three MABC have been found with a mutation that causes resistance to macrolides.

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COMPETING INTEREST

The authors declare that they have no competing interests in this work.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. However findings can be shared to clinicians upon request only if doing so will enhance patient management.

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