NON-TUBERCULOUS MYCOBACTERIA (NTM) AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL, 2010-2017

Dr Lamla Nqwata

Student number: 521775
Student qualification: MBChB (Unitra), FCP(SA), Cert. Pulmonology (SA)

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine).

Supervisor: Prof Charles Feldman MB BCh, DSc, PhD, FRCP, FCP (SA)
: Dr Marianne Black MBChB, FCPath micro (SA), MMED (micro)

JOHANNESBURG, 2023

DECLARATION

I, Dr Lamla Nqwata, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine, at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other University.

23 _ November _ 2023

DEDICATION

I dedicate this work to my grandmother (Mrs MP Nqwata) for her resilience, her love and will forever be indebted to her for letting me stand on her shoulders. My siblings and the entire family are my motivation and my strength behind all my achievements.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and the utmost appreciation to the people that held my hand and made it possible to be able to finish this work; To my supervisors, Professor Charles Feldman, I could never thank you enough, may you live forever!! To Dr M. Black, thank you for being forever calm and amazing. My appreciation to Dr J Pasipanodya and Dr A. R. Ouedraogo for assistance with the data analysis and for your contributions in this work.

To my teachers and mentors, Dr Z Bayat, Dr M Tsitsi, Dr N Mohammed, Dr I Kalla, Dr E Shaddock and Dr FA Skosana. You are all the epitome of: “we lift each other as we rise”. You all let me learn, make as many mistakes as I could but still gave me confidence to stand my ground and believed in me when doubts got the better of me.

To my friends, Dr M Leodegard, Dr P Banda, Dr U Nqebelele, Dr J Venturas, Dr Kagiso Motse, you have been forever lovely, kind and mostly showed me true humanity especially on the days when I needed it the most.

An abstract submitted from the research report has been accepted at the World NTM/bronchiectasis (WBC) conference for presentation both as a poster and an oral presentation. The congress is to be held between July 18 and 20 in New York. The abstract was awarded a “World Bronchiectasis 2023 Rising Star Researcher” accolade.

ABSTRACT

Rationale: Diseases due to non-tuberculous mycobacteria (NTM) are difficult to diagnose and are not reportable in South Africa (SA), resulting in the disease burden and trends being under-appreciated.

Objectives: To characterize NTM disease occurrence and trends in Johannesburg and to estimate end-of-treatment outcomes.

Methods: A retrospective review of all clinical isolates that were positive for NTMs between 1 January 2010 and 30 June 2017 and the corresponding medical records of the patients at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) were analysed. A data collection form was designed, and data was collected based on information listed on the form. This was analysed using Graphpad Instat (Graphpad Inc, 3.1 version, San Diego, California, US) and STATA version 11, College Station, Texas, software. In descriptive analyses, two-tailed Fisher's exact tests were used to compare categorical variables, while Kruskal-Wallis tests and Student's T-test were used to compare continuous variables, as needed. Kaplan-Meier curves and log-rank tests were used to compare time-to-death, while Cox regression analyses were used in multivariate analyses of the same.

Results

A total of 123 patients with positive NTM isolates were enrolled in this study. In this cohort, positive NTM isolates were found mostly in males (71; 57,7%), with a median age of 39 [Interquartile range 31.5-49.5] years. *Mycobacteria avium* complex (MAC) was the most common, isolated in 90 (75%) cases. Human immunodeficiency virus (HIV) infection, found in 96(80%) patients, and prior pulmonary tuberculosis (TB), found in 38(30%), were the common comorbidities. Overall, 27(22%) were successfully treated and 28(23%) died. In multivariate Cox regression analysis the adjusted hazard rates were 2.79 (95%CI 1.20 – 6.50) in those with low CD4 cell counts and 4.01 (95%CI 1.17 – 13.77) in those with unknown HIV test results. Receipt of antimicrobials did not significantly improve survival.

Conclusion

Non-tuberculous mycobacteria (NTM) appear to be common in our setting and is associated with poor outcomes.

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ABBREVIATIONS

ATS	American Thoracic Society
ARVs	Anti-retrovirals
BMI	Body mass index
BTS	British Thoracic Society
CD4	Cluster of differentiation 4
CF	Cystic fibrosis
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
COPD	Chronic obstructive pulmonary disease
CXR	Chest radiograph
DISA	Data Intensive Systems and Applications
FS	Free State
GORD	Gastro-oesophageal reflux disease
HIV	Human immunodeficiency virus
HRCT	High Resolution Computed Tomograph
HRECM	Human Research Ethics Committee Medical
INH	Isoniazid
IDSA	Infectious Diseases Society of America
KZN	Kwa-Zulu Natal
MAC	*Mycobacterium avium* complex
M. intra	*Mycobacterium intracellulare*
M. kansasii	*Mycobacterium kansasii*
MOTT	*Mycobacteria* other than tuberculosis
MTB	*Mycobacterium tuberculosis*
NCFBr	Non-cystic fibrosis bronchiectasis

NHLS	National Health Laboratory Services
RA	Rheumatoid arthritis
SA	South Africa
US	United States of America
WBC	World Bronchiectasis conference

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Non-tuberculous mycobacteria (NTM), also referred to as “atypical mycobacteria”, are mycobacteria other than *M. tuberculosis* complex and *M. leprae*, with more than 150 species (1). The organisms are found in the environment and water systems, but without evidence of patient-to-patient transmission (2) except in a cystic fibrosis (CF) cohort where patient-to-patient transmission was documented in 11 of 31 cases (3). Other factors that predispose to the disease include immunosuppression, namely human immunodeficiency virus (HIV) infection and immune-suppressive medications, respiratory diseases including chronic obstructive airway disease, bronchiectasis and transplant patients (4,5,6). Other predisposing factors include rheumatoid arthritis (RA), gastro-oesophageal reflux disease (GORD), Lady Windermere syndrome and environmental soil exposure (7,8,9,10). The presentation vary depending on number of factors, i.e system involved, disease severity and the isolated species. NTM commonly affect the lungs, and other systems including lymph nodes, gastrointestinal tract, skin, soft tissue, bones, and bone marrow and disseminated disease. The American Thoracic Society (ATS) and Infectious Disease Society of America (IDSA), British Thoracic Society (BTS) and the recent 2020 Clinical Practice of NTM Pulmonary Treatment Guideline gives clarity to approaching management of NTM infections (11,12,13). However, the challenges in identifying optimal treatment regimens, duration and true worldwide incidence still remains. The reasons for this are NTM infections are not reported as a public health matter, isolates being colonisers and failure of cases fulfilling guideline criteria for diagnosis. These have led to uncertainties and a paucity of data locally, hence rationale for this study.

1.2 Literature review

1.2.1 Incidence of NTM

Reports from a national survey in the United States of America (US), estimated a prevalence of 4201 cases of NTM infection at a rate of 1.78 cases per 100,000 population (14). A report of US elderly patients, over a 10-year period found that there had been an annual increase of 8.2% per year (15). Studies from northern Australia during the 8-year period reported an average yearly incidence of 3.9 cases per 100,000 persons (16), and an increase of 8.4% per year was reported from Ontario (17).

With the increase in the global trends, this contrasts our experience as there is still a lack of NTM published reports in the South African (SA) setting. Corbett *et al*., from the sputum samples of miners in the Free State (FS) found 32 (27%) cases met the ATS/IDSA definition for pulmonary NTM disease (18). Among these, they isolated varying NTM species, including *M. kansasii (23)*, *M. scrofulaceum (7)*, and *M. avium (1)* and *M. abscessus (1).* In the study by van Halsema *et al.*, in gold-mine workers, (98%) with positive NTM isolates were of male gender with a median age of 44 years. MAC (38 individuals*), M. gordonae* (60) and *M. kansasii* (50) were the

common NTM species isolated. The individuals with MAC were more symptomatic, with a higher HIV rate in those that were tested 57/74 for HIV status (77%) (19). In KwaZulu Natal (KZN), Sookan and Coovadia, documented that among 200 specimens with suspected NTM isolates, 133 (65%) were confirmed to be NTM positive and MAC species was the most common isolate, in 76 (57.2%) of the cases (20). A review of NTM pulmonary samples in the sub-Saharan region reported a prevalence of 7.5% (21). Similar to van Halsema *et al.* findings*,* males were the most affected, and the patients were of younger age; with a median age of 39 years [IQR: 31.5-49.5] . MAC (28%) was the most isolated species with higher rate of HIV coinfection (40.5%) and a prior history of PTB (32.4%) (21).

1.2.2 Risk factors for NTM infection

Despite the increase in NTM trends, a positive isolate does not always equate to clinically significant disease. There are, however, a number of factors that increase the risk of patients developing the disease. These include HIV, chronic underlying lung diseases including chronic obstructive pulmonary disease (COPD), bronchiectasis, cystic fibrosis and previous pulmonary TB. Sonnenberg *et al*., among South African miners reported the independent risk factors to be a prior TB history, period of having worked underground and silicosis (22). In those without an existing respiratory disease, the risk factors include gastro-oesophageal reflux disease (GORD), chronic corticosteroid use and transplant patients. HIV-seropositive with advanced immunosuppression tend to manifest NTM disease with a disseminated presentation. This usually occurs in patients with cluster differentiation 4 (CD4) cell counts of less than 50 to 100 per µL, and the common NTM species to be isolated being MAC (23, 24). Pettipher and colleagues, in patients with CD4 counts of less than 100 cells/µL, MAC was reported to have a 10%-point prevalence in South African Black patients (25).

However, in a study on one SA gold-mine, although less common, M. kansasii disease, among the HIV positive was described to occur at earlier stages of HIV infection with high CD4 counts of 480 cells/µL. Radiologically, new hilar adenopathy was the most common finding with less cavitation (26). Among the HIV-seronegative patients, underlying lung diseases is a predisposing factor of NTM infections. In non-cystic fibrosis bronchiectasis (NCFBr) patients, a meta-analysis by Chu and colleagues among 1492 patients, the prevalence of NTM was 9.3% (27). Among the NCFBr patients, there were other additional factors that further increased likelihood of NTM infections, and these included female gender, older age, lower body mass index (BMI) (mean <23kg/m^2) and childhood infections. MAC was the most common isolate across the NCBr studies (28-31).

COPD is a common form of respiratory disease associated with pulmonary NTM infection. A Taiwan COPD study, found MAC as the common species to be isolated, both in multiple and single isolate groups. Among the multiple isolates, MAC was found in (36.2%) (32). The multiple NTM isolates in COPD have been reported to worsen exacerbations and are also associated with a decline in forced expiratory volume in one second.

In the transplant recipients, NTM isolates are most commonly found in lung and heart transplants, while those with kidney and liver transplants have minute rates. The incidence of NTM infections with lung, heart, kidney and liver transplants are 4.4%, 2.4%, 0.8% and 0.04%, respectively (33-35).

1.2.3 Diagnosis of NTM infection

The NTM diagnosis depends on the system involved, which may include the lung, lymph nodes, skin, soft tissue, bursae, tendon sheaths, joints, and bones, and may also be disseminated (10). Respiratory disease is by far the most common manifestation of NTM disease. The guidelines stipulate that for a diagnosis to be made, both clinical and microbiological criteria need to be met (10,11). In addition, these criterions should be associated with typical chest radiographic patterns (cavities) or high-resolution computed tomography (HRCT) scan of multifocal bronchiectasis or nodules.

In the HIV seropositive and negative, a culture isolation of the NTM species is mandatory to diagnose disseminated disease, either from blood or other closed sites, namely bone marrow. The HIV-seropositive commonly manifests with nonspecific symptoms including pyrexia, diarrhoea, weight loss and varying degrees of cytopenias accompanied by a usually low CD4 count <50 cells/µl.

To diagnose disease in other sites, a positive NTM culture of a particular tissue involved is warranted. Lymphadenitis typically involves the peripheral nodal groups and are usually unilateral and non-tender (10). Diagnosis requires specific isolation of the pathogen from lymph node cultures. Culture from drainage material for skin and soft tissue infections and synovial biopsy for joint involvement is warranted for diagnosis.

1.2.4 Management of NTM infection

The challenges of managing NTM disease range from being able to differentiate colonisation from significant disease that needs treatment, the need for prescription of multiple antibiotics, different treatment regimens for the different species, and extended duration of the treatment, increasing risks of undesirable effects. The guidelines (10, 11), recommends a 3-drug regimen (ethambutol, macrolide and rifabutin) for MAC lung and disseminated disease for a 1-year period following culture conversion.

Wallace *et al.*, in MAC-pulmonary disease demonstrated a treatment success in 84% of the patients with the use of macrolide regimens. In addition, there was better tolerance of the drugs especially with the infrequent dosing compared to the daily approach (36). There are published data on the alternative treatment of macrolide-resistant MAC cases.

In clofazimine-containing regimen, half of the patients demonstrated culture conversion within 1-year of treatment (37). As a rescue approach, 6-months of

bedaquiline achieved culture conversion in half of the patients without any serious side effects (38). Although more data is still warranted, these results demonstrate efficacy of these drugs in cases of macrolide resistance.

In *M. kansasii* pulmonary disease, the recommended regimen includes rifampicin, ethambutol and isoniazid (INH) or a macrolide, with a minimum duration of 1-year following culture conversion. For extrapulmonary sites, including skin, soft tissue and bones, combination therapy of surgical debridement, together with a 3-drug regimen is recommended.

1.2.5 Statement of the problem and justification

Other than gold mines in South Africa, there have been relatively few studies describing the clinical and microbiological features of NTM infections in South Africa. While globally, NTM occurrence is on the rise, the data from local studies are scarce despite our significant HIV and TB burdens and their association with NTM acquisition.

CHAPTER 2: STUDY AIM, OBJECTIVES, DESIGN AND METHODOLOGY

2.1 Aim of the study

The study aims were to describe a cohort of patients, admitted to the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), from whom an NTM was cultured from clinical specimens.

2.1.1 The objectives of the study

a) Describe the demographic and clinical features of patients infected with NTM.

b) Report on the significant laboratory parameters (including HIV status, CD4 cell count, viral load, and full blood count) associated with NTM infection.

c) Describe the common microbiology of NTM infections.

d) Report on the treatment and outcome of the patients infected with NTM.

e) Compare MAC and non-MAC rates in HIV-positive and HIV-negative patients

f) Stratify the patients into survivors and non-survivors and compare the variables between both groups, including sex, HIV status, CD4 cell count and presence/absence of cytopenias or previous PTB, and whether cases were treated or not.

2.2 Methodology

2.2.1 Study design

This was a retrospective record review of all patients in whom one or more non-tuberculous mycobacterium species was isolated at CMJAH during the period between January 2010 and 30 June 2017.

2.2.2 Study site/area

CMJAH is a tertiary hospital with 1,088 beds catering for patients across Gauteng province and surrounding areas. It is estimated to have more than 4,000 personnel offering specialised services to both in and outpatients. It is based in Johannesburg, and is one of the main referral hospitals in its cluster. It also offers academic programme for the University of the Witwatersrand, Faculty of Health Sciences, ensuring training in all areas of health professions.

2.2.3 Study population

The study analysed the records of patients from whom NTM was isolated from multiple types of specimens (sputum, blood, tissue, fluid, bone marrow) at the

CMJAH between 1 January 2010 and 30 June 2017. Despite the intention and attempts to capture every single NTM positive result between the mentioned study period, the changes in the laboratory tracking results made it impossible. Most of the results from 2010 to 2013 could not be accessed due to changes in the laboratory results tracking system from Data Intensive Systems and Applications (DISA) to National Health Laboratory Services (NHLS) and a significant number of specimens (n=378) were submitted during this period.

2.2.4 Inclusion criteria

All adult patients (≥ 18 years of age) with an NTM positive specimen, from any site, at CMJAH from 1January 2010 to 30 June 2017.

2.2.5 Exclusion criteria

All patients below 18 years of age.

Patients with a positive NTM isolate reported by the NHLS but without medical records from the hospital.

2.2.6 Sample method

Records of patients admitted to CMJAH with clinical specimens that cultured an NTM were obtained from the database from the NHLS, managed by the Data Corporate Warehouse (CDW) and from the case files at the CMJAH. The two methods of culture used by the laboratory were both in liquid media and included the Mycobacteria Growth Indicator Tube (MGIT 960) system and the BACTEC 460 TB system. To identify NTM's from culture, a Genotype-Mycobacterium common mycobacterium (CM) was used for the most common clinically significant species and Genotype Mycobacterium additional species (AS) for additional less common NTM species.

2.2.7 Sample size

The initial projection was that approximately 200 patients would be included in the study. Following the search for NTM positive results reported between January 2010 and June 2017, a total of 199 cases were retrieved. Among these, 76 cases were excluded as they did not fulfil inclusion criteria for the following reasons (paediatric cases=23, patients without retrievable hospital records=53). A total of 123 cases were finally included in the analysis.

2.2.8 Data collection

A uniform data collection sheet was used to collect data for all the patients (Appendix A).

Information collected included demographic details (age, sex, race and BMI), smoking history, including pack years, presence or absence of known risk factors (structural lung diseases including COPD, childhood infections, post tuberculosis infections and others), microbiology, treatment and outcome.

The above information was recorded and then entered in Microsoft Excel 2007 relational database software by the investigator. The data set was then transferred to STATA (College Station, Texas) version 11 software for cleaning, which involved removal of duplicates and checking for missing variables, grouping and coding, determining internal consistency and then statistical analyses.

2.2.9 Statistical analysis

Data collected was analysed using Graphpad instat (Graphpad Inc, 3.1 version, San Diego, California, US) and STATA version 11 software for both descriptive and multivariate analyses. Demographic, clinical and laboratory factors, including treatment regimens, individual antimicrobial agents and therapy duration, which were recorded as continuous data, were reported as mean with corresponding standard deviations [SD] or median with interquartile range IQR], depending on whether the data was normally distributed or not. Student's T tests and the Mann-Whitney tests, were used to compare the respective distributions. Meanwhile, categorical variables were presented as raw frequencies and proportions as percentages. Fisher's exact tests were used to compare the difference in frequency between groups. For example, examination of proportions of patients who lived versus those who died at the end of follow-up among patients who were HIV-seropositive versus HIV-seronegative used cross-sectional comparison with Fisher's tests. All tests were two-sided with an alpha-level of 0.05 considered statistically significant.

On the other hand, Kaplan-Meier curves were used to examine time-to-death in patients with different NTM infections during treatment or post-treatment on follow-up. Log-rank tests were used to compared median survival from the different curves of different NTM organisms, sex and the different baseline CD4 cell counts in univariate analysis. Lastly, multivariate Cox proportional regression models with backward elimination were used to examine factors predictive of long-term survival. Specifically, the joint impact of receiving some antimicrobial agents, therapy duration and CD4 cell counts were examined in multivariate analysis. The results were presented as tables and Kaplan-Meier curves.

2.3 Ethics

The study was approved by the Human Research Ethics Committee (Medical), of the University of the Witwatersrand (180753) (Appendix B). Only serial study numbers were used on the data capture sheets of the individual patients in order to ensure confidentiality.

2.4 Funding

All necessary costs were incurred by the candidate, including costs for stationery, printing and other paperwork.

CHAPTER 3: RESULTS

3.1 Descriptive analysis of NTM isolates

The study flow chart is shown in Figure 3.1. Following the search for positive NTM isolates between January 2010 and June 2017, a total 199 cases were retrieved. Among these, 76 cases were excluded as they did not fulfil inclusion criteria for the following various reasons (paediatric cases=23, patients without retrievable hospital records=53. A total of 123 cases were included for analysis.

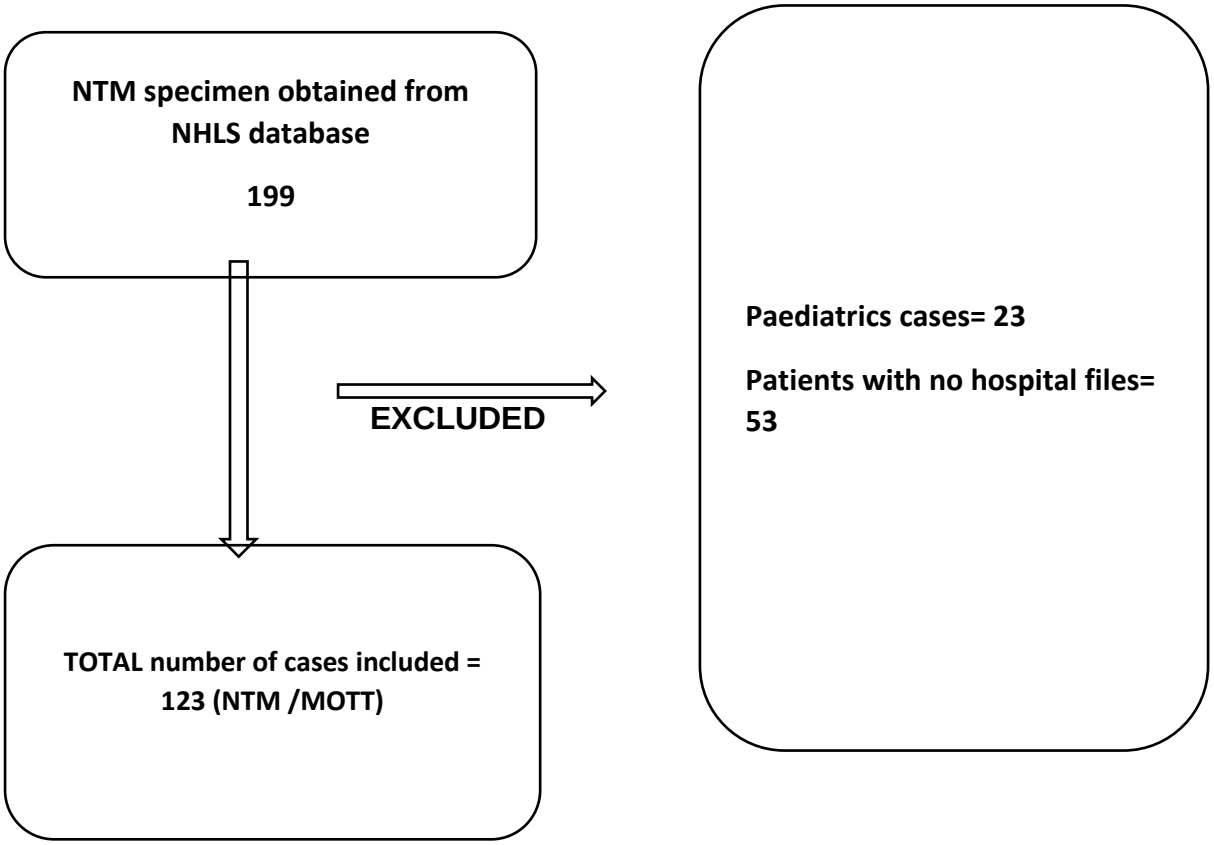

Figure 3.1 The study flowchart

3.1.1 The site of isolation of NTM species

Among the 123 patients, the majority of NTM were isolated from sputum (78 patients) using the Mycobacterial Growth Indicator Tube (MGIT) 960 system and blood specimens (40 patients) from BACTEC blood culture bottles. From the sputum cultures, 10 of the patients had more than 2 positive sputum specimens (10 [12,8%]), and 8 (20%) had more than 2 positive blood culture specimens. The least number of NTM specimens were cultured from other tissues (bone marrow aspirate and trephine, lymph node, and peritoneal fluid) (Table 3.1).

Table 3.1 Sources of NTM specimens (N=123)

Specimen source	N	%
Pulmonary		
Sputum (MGIT 960)	78	63,4
Extra-pulmonary		
BACTEC cultures	40	32,5
Lymph nodes	3	2,4
Bone marrow	1	0,8
Others (peritoneal fluid)	1	0,8
TOTAL	**123**	**100**

3.1.2 Demographic information of the cases from whom NTM were isolated

The NTM positive isolates were commonly amongst younger patients with a median age of 39 [IQR: 31.5-49.5] years, ranging between 20 to 40 years old (Figure 3.2). More males 71 (60%) were found to have positive NTM isolates compared to 52 (40%) of females (Figure 3.3). Overall, the NTM cases showed a slow but steady rise from the year 2010 reaching a peak by 2014, which was then followed by the consistent decline till year 2017 (Figure 3.4). This pattern of the NTM cases through-out the study period indicated that in males there was a steady rise, with notable increase by 2014, and a decline thereafter. The females, however had a very much slower rise to 2014 and generally plateaued for the rest of the period (Figure 3.5).

The patient's body weight (kg) was recorded, based on convenience, as there were no height measurements to calculate body mass index (BMI). A greater proportion of the patients (40%) weighed between 40 and 50kg, with just over 30% weighing between 55-70kg and a 6% of the patients weighing between 25kg and 40kg (Figure 3.6).

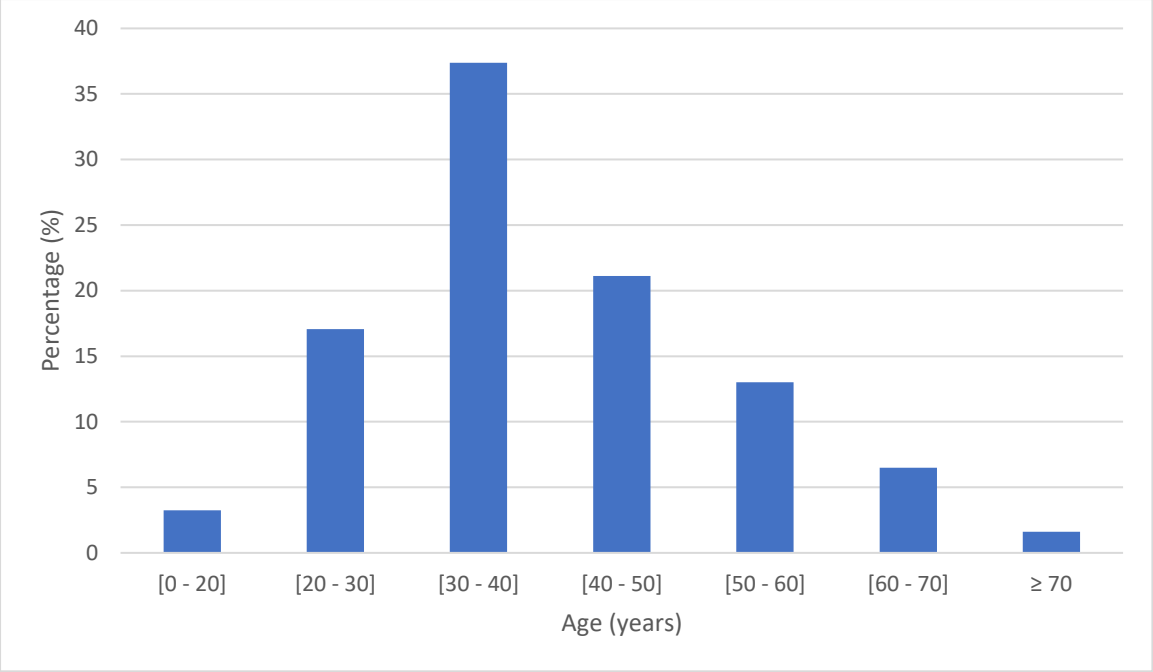

Figure 3.2 Age distribution of the study participants

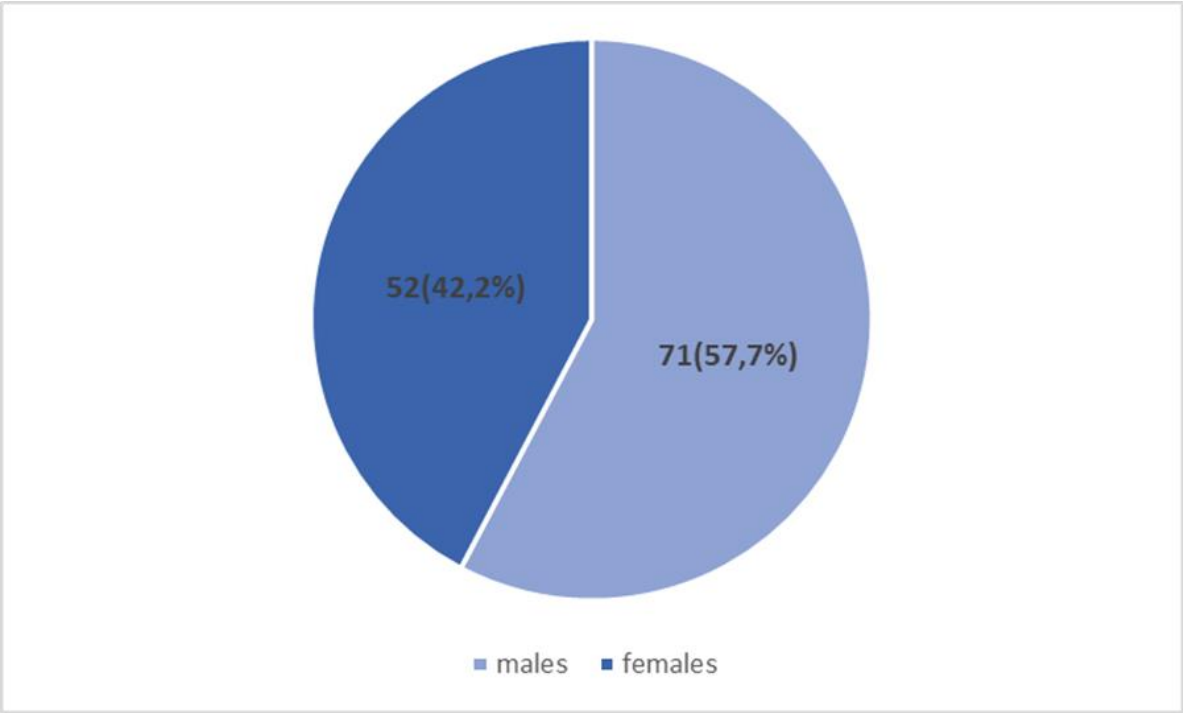

Figure 3.3 NTM cases categorised by gender

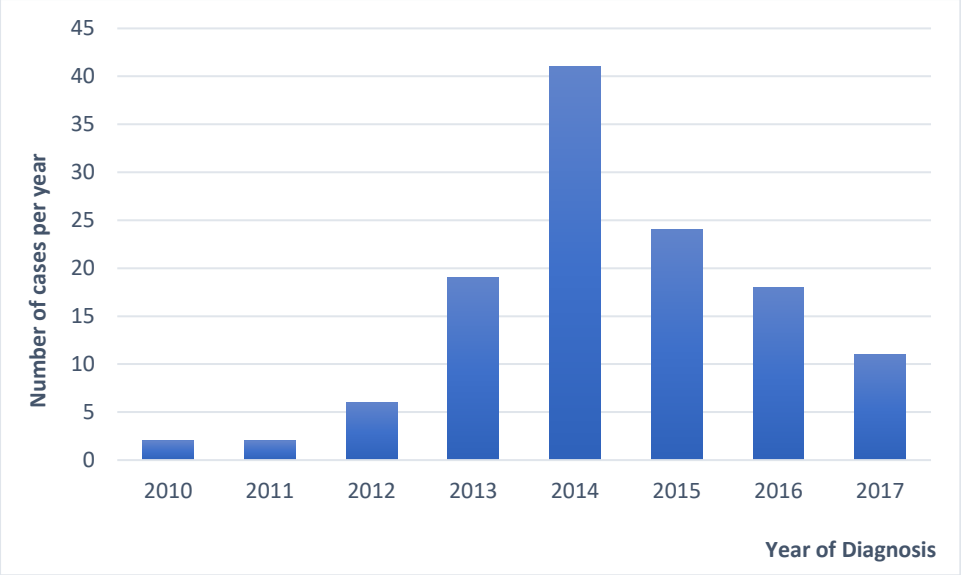

Figure 3.4 NTM cases categorised by year of diagnosis

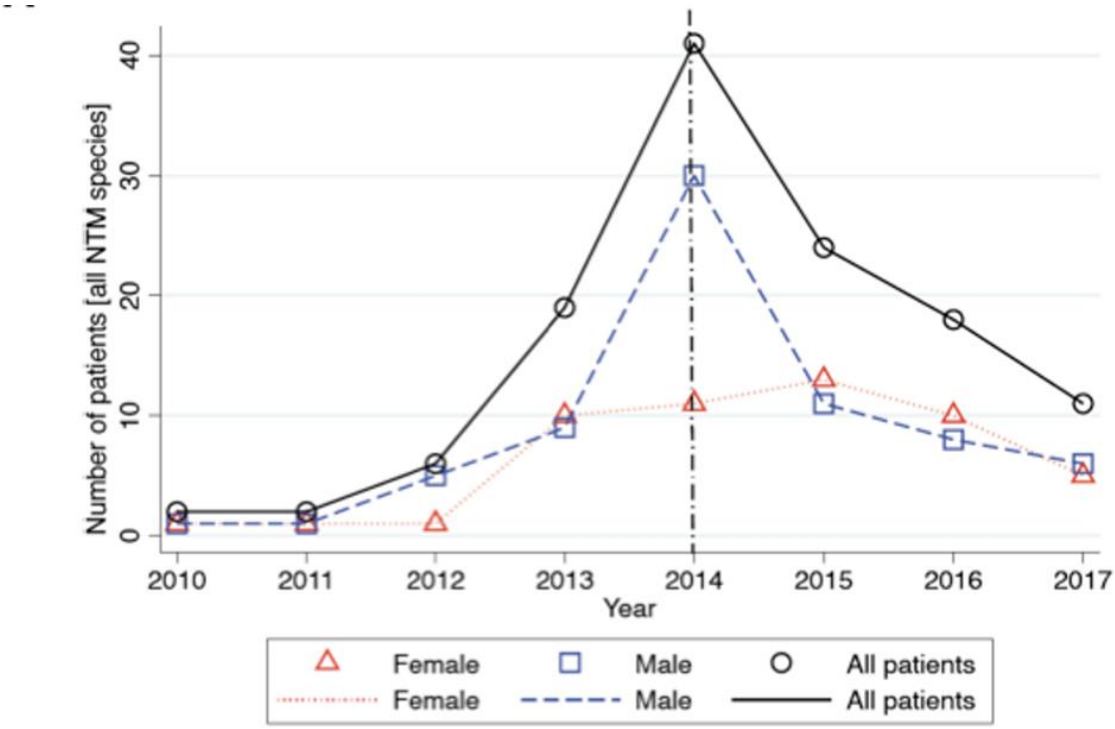

Figure 3.5 NTM cases stratified by year of diagnosis and gender

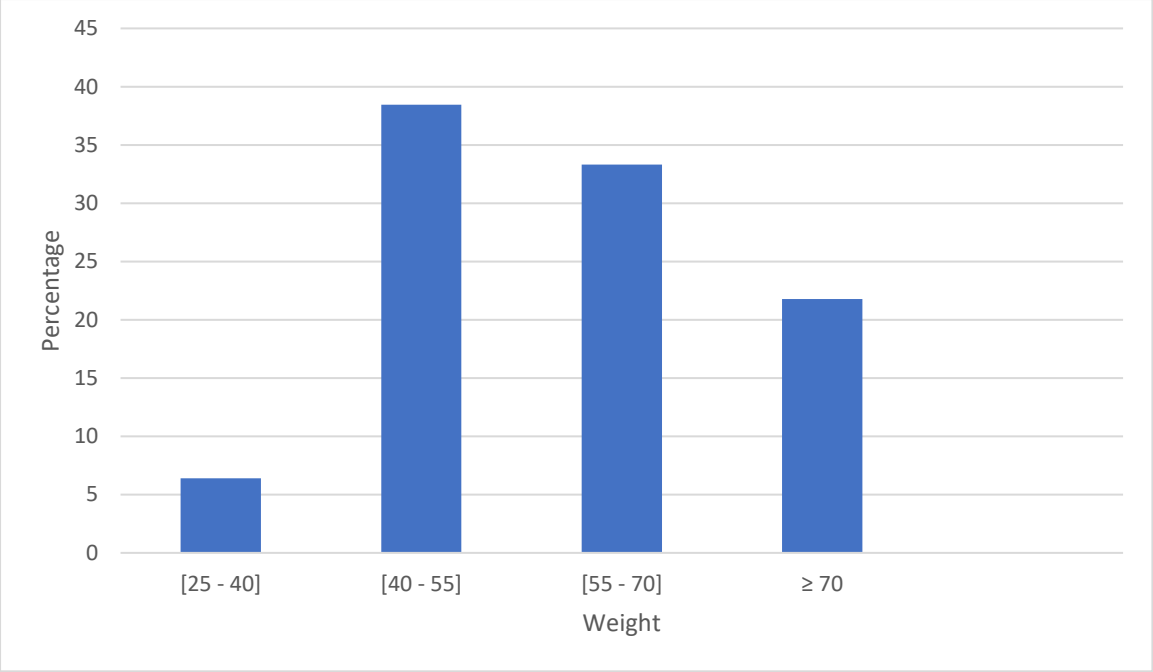

Figure 3.6 Weight (Kg) of the NTM positive patients

3.1.3 Clinical features, comorbid illnesses and imaging findings

The two most common comorbid illnesses in this cohort, as depicted in Table 3.2 and Figure 3.7, were HIV infection in 96 (78%) and a prior history of pulmonary tuberculosis (PTB) in 38 (30,8%)).

Figure 3.7, shows the rise of NTM cases related to the HIV status with a larger increase in the HIV-positive cases, peaking in 2014, while NTM cases among the HIV-negative remained relatively flat overall, and at a lower rate. Among the HIV-infected patients, 53 (55,2%) had a CD4 cell count of less than 100 cells/µL, and 11 (11,5%) had a CD4 cell count between 100-200 cells/µL (Table 3.3).

Out of the total cohort, 39 (31,7%) of the patients had cytopenias, most notably in patients with lower CD4 cell counts of <100 cells/µL, and varying from anaemia, to bi-cytopenia and pancytopenia.

Descriptions of the chest radiographs were retrieved from 29 (23,6%) of the patient case notes and the information was recorded as per the attending doctor's description with no access to the original chest radiographic films available to the investigator. The imaging findings, as indicated in Table 3.4, included cavitation in 10 (34,4%), bronchiectasis in 6 (20,6%), infiltrates in 5 (17,2%), consolidation in 4 (13,7%) and hilar lymphadenopathy in 2 (6,9%). The patients with a prior history of PTB, were most likely to have cavitation, bronchiectasis, and hilar lymphadenopathy on their imaging.

Table 3.2 Distribution of the NTM-positive patients based on HIV and PTB status

HIV status	n (%)	Previous PTB	n (%)
Negative	21 (17,0)	Negative	49 (39,8)
Positive	96 (78,0)	Positive	38 (30,8)
Unknown	6 (4,8%)	Unknown	36 (29,2)
Total	123 (100)	Total	123 (100)

HIV- human immune-deficiency virus PTB – pulmonary tuberculosis NTM - non tuberculous mycobacteria

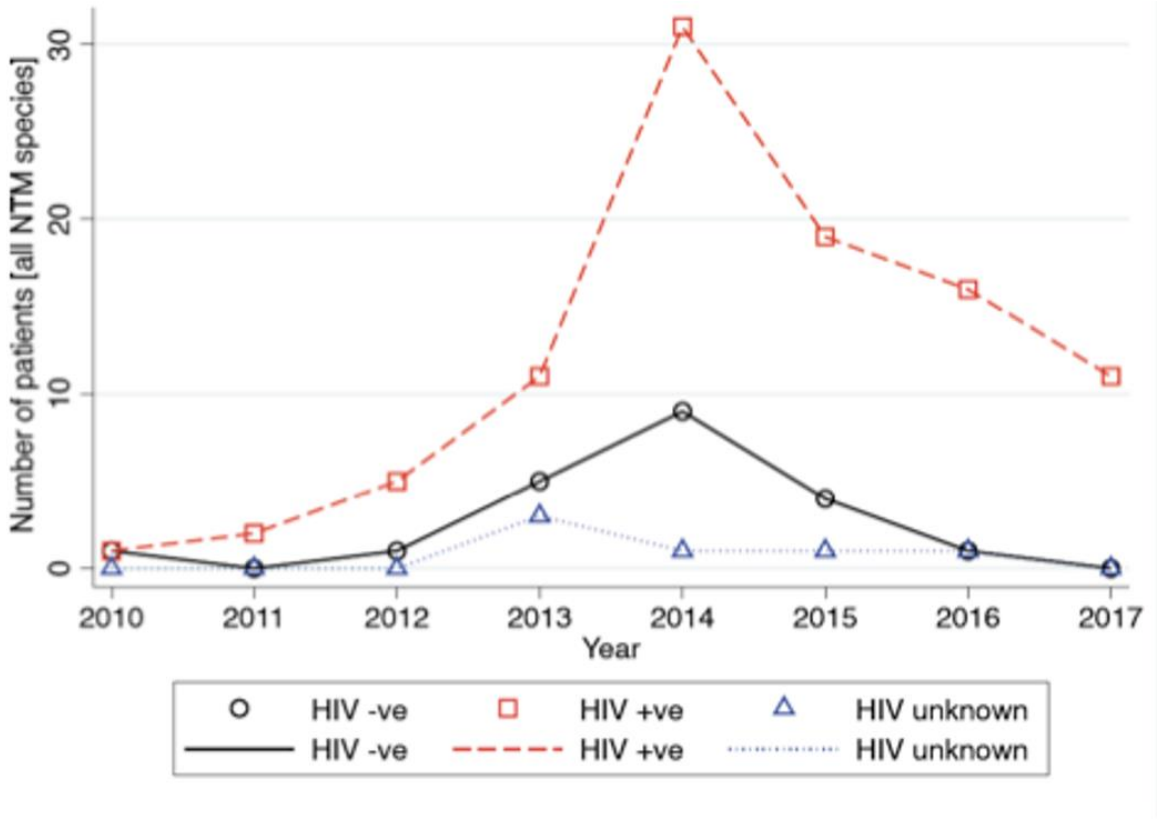

Figure 3.7 Distribution of NTM cases by HIV status

TABLE 3.3 Distribution of the NTM-positive patients based on CD4 cell counts

CD4 count (cells/μL)	Frequency N	Percent %
<100	53	58,9
100 - 200	11	12,2
200 - 300	10	11,1
300 - 400	6	6,7
400 - 500	6	6,7
500 - 600	2	2,2
600 - 800	1	1,1
≥ 800	1	1,1
TOTAL	90	100

CD4 -cluster 4 differentiation

TABLE 3.4 Chest radiographic findings in 29 patients

C-X-R	Number (n)	Frequency (%)
Cavitation	10	34,4
Bronchiectasis	6	20,6
Infiltrates	5	17,2
Consolidation	4	13,7
Hilar nodes	2	6,9
Others	2	6,9

C-X-R, chest X-ray Others -ground glass, emphysema

3.1.4 NTM species isolated, and time to positivity of culture

The NTM species isolated were recorded exactly as they were reported in the laboratory results system (Table 3.5). The most common isolates reported were *M. avium* (34,1%), MAC (21,1%), *M. intracellulare* (13,8%) and MOTT (8,1%) (Table 3.5). These NTM species were common among the HIV-positive patients (Table 3.8, Figure 3.7), and in 6 of the patients with NTM species isolated, the HIV status was not known (3-*M. avium, 1*-MOTT*,* 1-*M. mucogenium,* 1- MAC). Other NTM species were isolated less frequently with cases in smaller amounts.

Table 3.5 Classification of NTM based on species isolated

Species	**Frequency** **N**	**Percent** **%**
M. avium	42	34,1
M. avium complex	26	21,1
M. intracellulare	17	13,8
MOTT	10	8,1
M. fortuitum	6	4,8
M gordonae	5	4,0
M chelonae	4	3,2
M scrofulaceum	4	3,2
M. kansasii	3	2,4
M abscessus	2	1,6
M xenopi	2	1.6
M mucogenium	1	0.8
M simiane	1	0.8

NTM species were further categorised into MAC (including those reported as M*ycobacterium avium, Mycobacterium intracellulare* and MAC) and non-MAC species (all other NTM other than MAC) (Table 3.6, Figure 3.8). The MAC group

curve showed a discernible rise compared to non-MAC cases, which maintained a relatively flatter curve throughout the study period. Furthermore, as indicated in Table 3.6 MAC cases were shown to be significantly more common in the HIV-positive patients (*M. avium* 30%, MAC 18% and *M. intracellulare* 11%) than in the HIV–negative cases (OR= 7.11 [95% CI, 2.61-17.81]; P=0.0002).

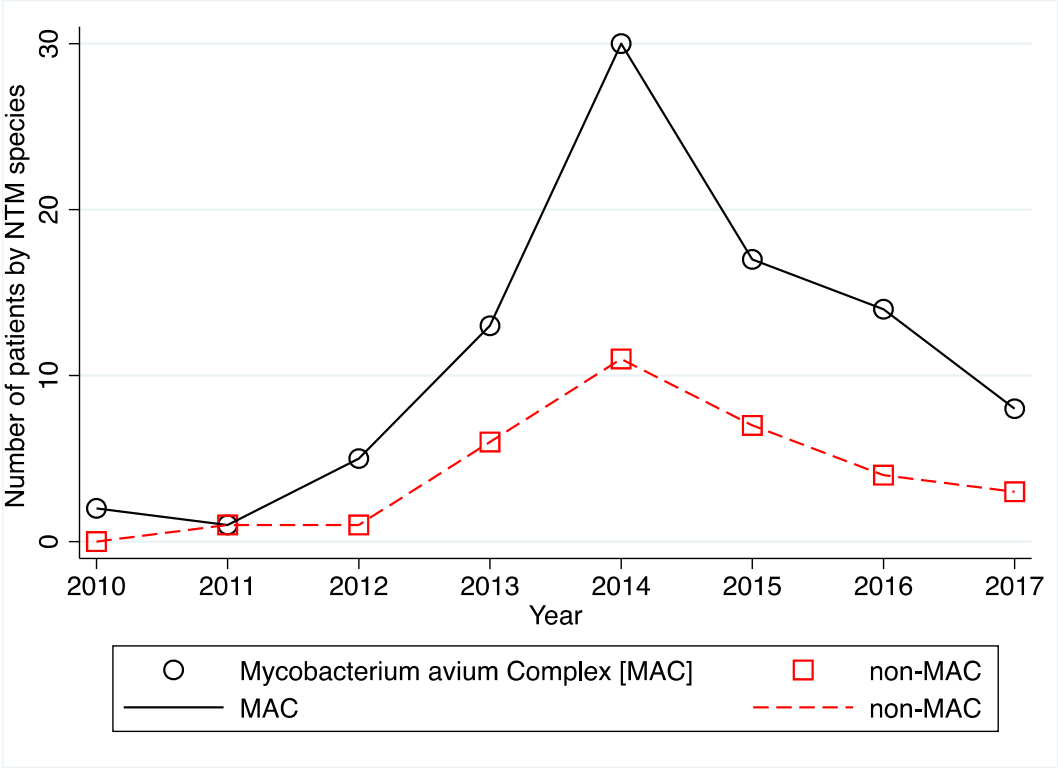

Figure 3.8 Distribution by MAC versus non-MAC

Table 3.6 MAC and non-MAC categorised by HIV status

NTM species	HIV positive, n=96(%)	HIV negative, n=21 (%)	HIV test result unknown n=6 (%)
MAC			
M. avium	37 (38,5)	2 (9,5)	1(16,6)
MAC	22 (22,9)	2 (9,5)	3(50)
M. intracellulare	14 (14,5)	3 (14,2)	-
Non-MAC			
M. fortuitum	5 (5,2)	1 (4,7)	
M. chelonae	3 (3,1)	1 (4,7)	
MOTT	3 (3,1)	6 (28,5)	1(16,6)
M. scrofulaceum	3 (3,1)	1 (4,7)	
M. kansasii	3 (3,1)	-	
M. xenopi	2 (2)	-	
M. abscessus	1 (1)	1 (4,7)	
M. gordonae	1 (1)	-	
M. simiae	1 (1)	-	1(16,6)
M. mucogenicum	-	1 (4,7)	

MAC- mycobacterium avium, mycobacterium intracellulare, mycobacterium avium complex, non-MAC all other NTM species

MAC versus non-MAC categorised per HIV status OR=7.11 [95%CI, 2.61-17.81]; P=0.0002).

The time to positivity (TTP) (Figure 3.9; Table 3.7) of the culture results vary widely with majority falling between 10 to 20 days with these being mostly *M. avium*. The TTP of sputum culture beyond 30 days was documented in only four species.

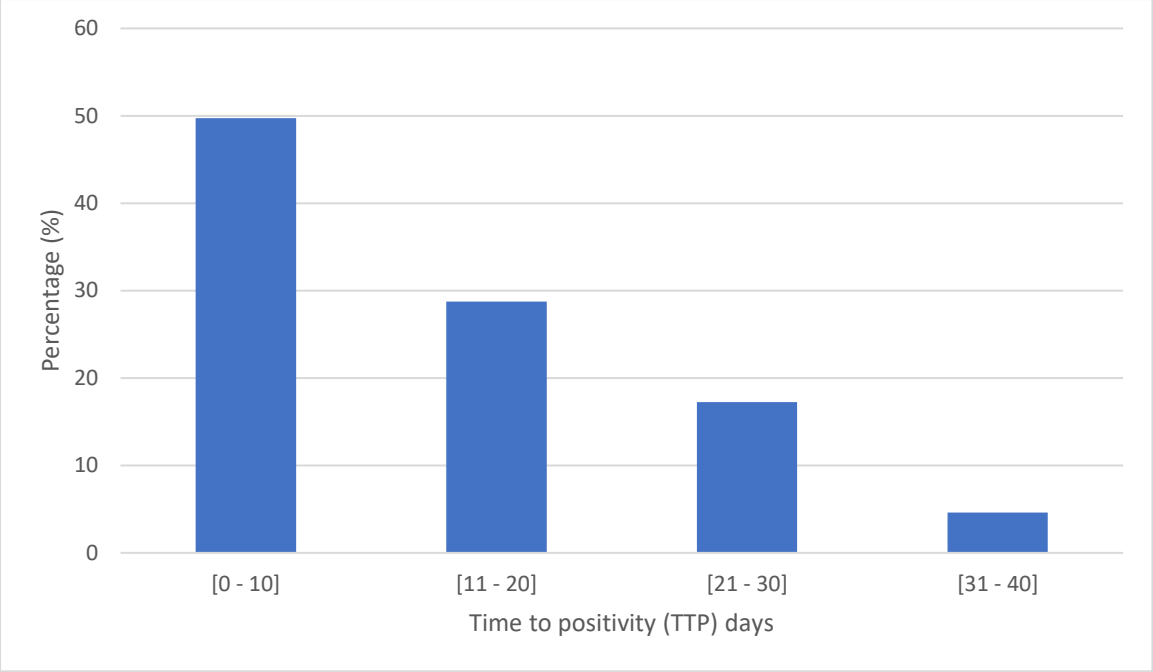

Figure 3.9 Time to positivity of mycobacterial culture

Table 3.7. Distribution of isolated species according to the time to positivity

	SPECIES							
TTP (days)	**MOTT**	**MAC**	**AVIUM**	**INTRA**	**KANSA**	**FORTUS**	**OTHERS**	**TOTAL**
[0; 10]	3	6	19	5	0	3	6	42
[10; 20]	0	4	12	5	0	0	4	25
[20; 30]	0	2	7	1	1	0	3	14
[30; 40]	0	0	0	2	0	0	2	4
Total	3	12	38	13	1	3	15	85

MOTT- mycobacteria other than tuberculous, MAC- *Mycobacterium* avium complex, INTRA- *Mycobacterium intracellulare*, KANSA- *Mycobacterium kansasii*, FORTUS- *Mycobacterium fortuitum*

TABLE 3.8 Distribution of isolated species according to weight

	SPECIES							
WEIGHT (Kg)	**MOTT**	**MAC**	**AVIUM**	**INTRA**	**KANSA**	**FORTUS**	**OTHERS**	**TOTAL**
[25 ; 40]	0	0	2	3	0	0	0	5
[40 ; 55]	1	11	9	4	1	0	3	29
[55 ; 70]	5	3	7	4	1	2	4	26
≥70	0	3	7	4	0	0	3	17
Total	6	17	25	15	2	2	10	**77**

MOTT- mycobacteria other than tuberculous, MAC- *Mycobacterium avium* complex, INTRA- *Mycobacterium intracellulare*, KANSA- *Mycobacterium kansasii*, FORTUS- *Mycobacterium fortuitum*

3.1.5 Treatment regimens

The treatment strategies varied (Table 3.9), with multiple combination regimens used. The most common regimens used were the anti-TB drugs alone, or with both a macrolide and fluoroquinolone. Out of the total cohort, 30% of the cases were not initiated on any form of therapy according to the records, and 22,7% were labelled as unknown as there was no record of whether they had received treatment or not.

Table 3.9 Treatment regimens used in the NTM patients

NTM treatment	**n**	**%**
Anti-TB treatment (Rifafour)	16	13,0
Anti-TB+macrolide	15	12,1
Anti-TB+macrolide +fluoroquinolone	19	15,4
Multi-TB-drug (MDR)	5	4,0
Macrolide only	2	1,6
Fluroquinolone only	1	0,8
Not treated	37	30,0
Unknown	28	22,7

3.1.6 NTM patient outcomes

The outcomes of many (>1/3) of the cases were documented as ‘unknown’ as this data was not available (Table 3.10). Overall, 28 (22,7%) of the cases were noted to have died from the disease, and 27 (21,9%) were recorded as having been successfully treated.

Table 3.10 Outcomes of NTM patients

Died	28 (22,7%)
Successfully treated	27 (21,9%)
Defaulted	20 (16,2%)
Transferred to other hospitals	2 (1,6%)
Unknown	46 (37,3%)

3.1.7 Characteristics of the NTM patients that died

Cross-sectional comparison of the patients' demographic characteristics, laboratory, and treatment factors against observed known and unknown outcomes were made with full results shown in Table 3.11. In the cohort, 28 (22,7%) of the patients were known to have died, while outcomes were unknown in 46 (37,3%) patients. Next, we performed separate nested case-control comparison of the 28 patients who died to the 27 patients known to be alive (Table 3.12). Of the 28 deceased, 24 (85,7%) were HIV-positive, while 4 (14,2%) were HIV-negative. Overall, 50% of the patients had a CD4 count of less than 50 cells/µL, with a prior PTB history in 39,1%, cytopenias in 60% and MAC (*M*. *avium* and *M. intracellulare)* as the most common NTM species (Table 3.12).

Table 3.11 Comparison of patients' demographic, clinical and treatment factors in patients with known and unknown outcomes after follow-up

Variable		**Outcome**			**P-value**
		Died n=28 (22,7%)	Lived n=27 (21,9%)	Unknown n=46 (37,3%)	
Sex	Male	15 (53,5)	16 (59,2)	27 (58,6)	0.888
	Female	13 (46,4)	11 (40,7)	19 (41,3)	
Age	Median (IQR)	40 (32 – 49)	38 (31 – 41)	39 (31 – 51)	0.493
NTM species	MAC	23 (82,1)	18(66,6)	32 (69,5)	0.267
	Other/MOTT	4 (14,2)	6 (22,2)	10 (21,7)	
	M. kansasii	0	0	3 (6,5)	
	M. fortuitum	1 (3,5)	3 (11,1)	1 (2,1)	
Initial bacterial load	Median (IQR)	1.94 (1.52 – 2.43)	1.39 (1.25 – 2.01)	1.63 (1.35 – 2.39)	0.150
Previous TB	None	11 (39,2)	14(51,8)	15 (32,6)	0.065
	Yes	12 (42,8)	10 (37,0)	13 (28,2)	
	N/A	5 (17,8)	3 (11,1)	18 (39,1)	
Concurrent TB	No	15 (53,5)	6 (22,2)	31 (67,3)	0.156
	Yes	12 (42,8)	9 (33,3)	15 (32,6)	
HIV-baseline	Negative	4 (14,2)	4 (14,8)	10 (21,7)	0.297
	Positive	24 (85,7)	23 (85,1)	33 (71,7)	
	N/A	_	_	3 (6,5)	
Viral loads	Median (IQR)	27 (7 – 63)	125 (64 – 291)	79 (16 – 288)	0.052
CD4 cells counts	Median (IQR)	27 (7 – 63)	125 (64 – 291)	79 (16 – 288)	***0.002***
CD4 cell counts	<50	17 (60,7)	4(14,8)		

	51-200	7 (25,0)	8(29,6)		
	201-350	1(3,5)	7(25,9)		
	351 -500	_	3 (11,1)		
	>500	_	_		
Treatment Strategy	No antibiotic	11(39,2)	3(11,1)		
	Macrolide-based	6(21,4)	18(66,6)		
	Anti-TB drugs only	8(28,5)	4(14,8)		
	Anti-TB+macrolide	6 (21,4)	16(59,2)		
Therapy duration	Median (IQR)	1 (1 - 6)	21 (18 – 24)	10 (7 – 24)	***0.010***

Table 3.12 Nested case-control comparison of cases who died versus patients who lived

Variables		**Died; n=28 (%)**	**Lived; n=27 (%)**	**P-value***
Gender	Male	15 (53,5)	16 (59,2)	0.67
	Female	13 (46,4)	11 (40,7)	
HIV status	Positive	24 (85,7)	23 (85,1)	1.00
	Negative	4 (14,2)	4 (14,8)	
CD4 cell count[##]	<50	17 (60,7)	4 (14,8)	***<0.001***
	50-350	8 (28,5)	14 (51,8)	
	>350	0	4 (14,8)	
	N/A**	3 (10,7)	5 (18,5)	
Previous TB	Yes	12 (42,8)	10 (37)	0.599
	No	11 (39,2)	14 (51,8)	
	N/A	5 (17,8)	3 (11,1)	
Cytopenias	Yes	16 (57,1)	7 (25,9)	***0.031***
	No	2 (7,1)	8 (39,6)	
	N/A	10 (35,7)	10 (37)	
NTM	*M. avium*	12 (42,8)	9 (33,3%)	0.48
	M. intrac	7 (25,0)	4 (14,8)	
	MAC	4 (14,2)	5 (18,5)	
	Others	5 (17,8)	9 (33,3)	
Chemotherapy^^	Treated	6 (21,4)	18 (66,6)	***<0.001***
	Not Treated	22 (78,5)	7 (25,9)	
	N/A**	0	2 (7,4)	

N/A data not available; * Fisher's Exact (2-tail) test; ** excluded from contingency table test; Bold and italics denote statistically significant p-values, <0.05.

3.1.8 Factors associated with time-to-death

Finally, longitudinal analysis was performed to determine patient's demographic, clinical and laboratory factors, including examination of whether therapy duration and the different NTM species, were associated with death during treatment. The median follow-up was 0.5 (IQR 0.5-4.5) years. Mortality rates were 2.90 times significantly higher between the years 2015-2017 at 0.32 (95%CI, 0.20 – 0.54) person-years as compared to 0.11 (95%CI, 0.06 – 0.18) person-years for the years 2010-2014. However, as shown in the Kaplan-Meier curves in Figure 3.10 and Figure 3.11, majority of death of these deaths occurred within a year of bacterial isolation suggesting that these deaths were during treatments. The log rank test revealed that survival did not significantly differ by the different NTM species identified in the cohort, p=0.566 (Figure 3.10).

In both unadjusted and adjusted models, mortality rates were significantly higher in patients with unknown HIV status and those with low CD4 counts at almost similar rates, which might suggest that those with unknown HIV test results probably had HIV infections (Table 3.13 and Figure 3.11). The adjusted hazard rates were 2.79 (95%CI 1.20 – 6.50) in those with low CD4 cell counts and 4.01 (95%CI 1.17 – 13.77) in those with unknown HIV test results. Interestingly, receiving antimicrobial chemotherapy did not significantly improve survival.

Table 3.13 Unadjusted and multivariate-adjusted mortality rates, in person-years, stratified by demographic, clinical, mycobacterial and treatment factors

		Unadjusted mortality rate (person-year)		**Multivariate adjusted hazard rate**	
Variable	**Level**	**95%CI**	**P-value**	**95%CI**	**P-value**
Sex	Male	0.15 (0.09 – 0.25)	0.584		
	Female	0.19 (0.11 – 0.33)			
Year of diagnosis	2010-2014	0.11 (0.06 – 0.18)	***0.006***	referent	
	2015-2017	0.32 (0.20 – 0.54)		1.76 (0.79 – 2.88)	0.164
NTM species	MAC	0.19 (0.13 – 0.29)	0.596		
	Other MOTT	0.09 (0.03 – 0.24)			
	M kansasii	0.66 (0.09 – 4.72)			
	M fortuitum	0			
Concurrent Mtb	None	0.20 (0.12 – 0.33)	0.106		
	Yes	0.21 (0.12 – 0.36)			
	N/A	0			
Previous TB	None	0.13 (0.07 – 0.23)	0.567		
	Yes	0.25 (0.15 – 0.44)			
	N/A	0.13 (0.05 – 0.33)			
Cavitary TB	None	0.31 (0.14 – 0.70)	0.585		
	Yes	0.16 (0.05 – 0.49)			
	N/A	0.15 (0.09 – 0.23)			
HIV test Result (baseline)	Negative	0.09 (0.03 – 0.28)	0.500		
	Positive	0.18 (0.12 – 0.28)			
	N/A	0.20 (0.03 – 1.42)			
CD4 counts	≤50 cells/mm^3	0.35 (0.21 – 0.58)	***0.004***	2.79 (1.20 – 6.50)	***0.017***
	>50 cells/mm^3	0.08 (0.04 – 0.15)		referent	
	N/A	0.35 (0.13 – 0.93)		4.01 (1.17 – 13.77)	***0.028***

Chemo-therapy	None	0.17 (0.10 – 0.32)	0.542	--ref	
	Some	0.16 (0.10 – 0.26)		0.93 (0.42 – 2.04)	0.853
Chemo-therapy	Macrolide	0.08 (0.03 – 0.21)	0.123		
	TB/MDR	0.28 (0.15 – 0.55)			
	TB+Macrolide	0.17 (0.06 – 0.44)			

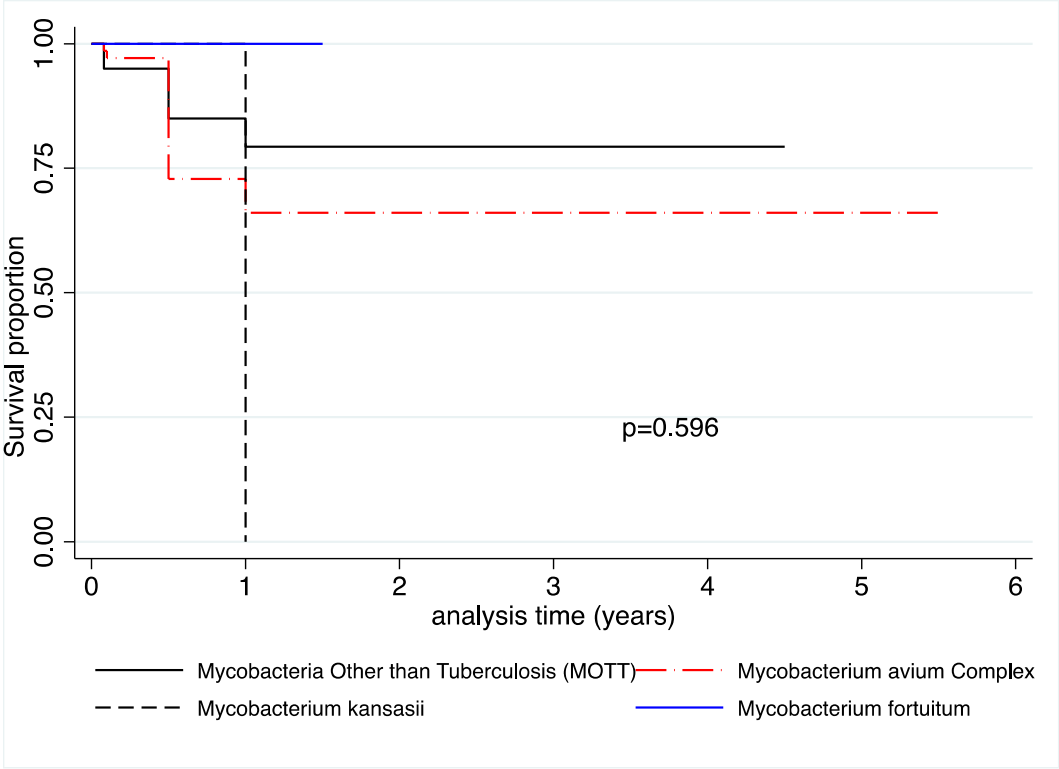

Figure 3.10 Unadjusted Kaplan-Meier curves of the different Non-tuberculous Mycobacteria (NTM) species isolated from patients and time-to-death for the respective patients.

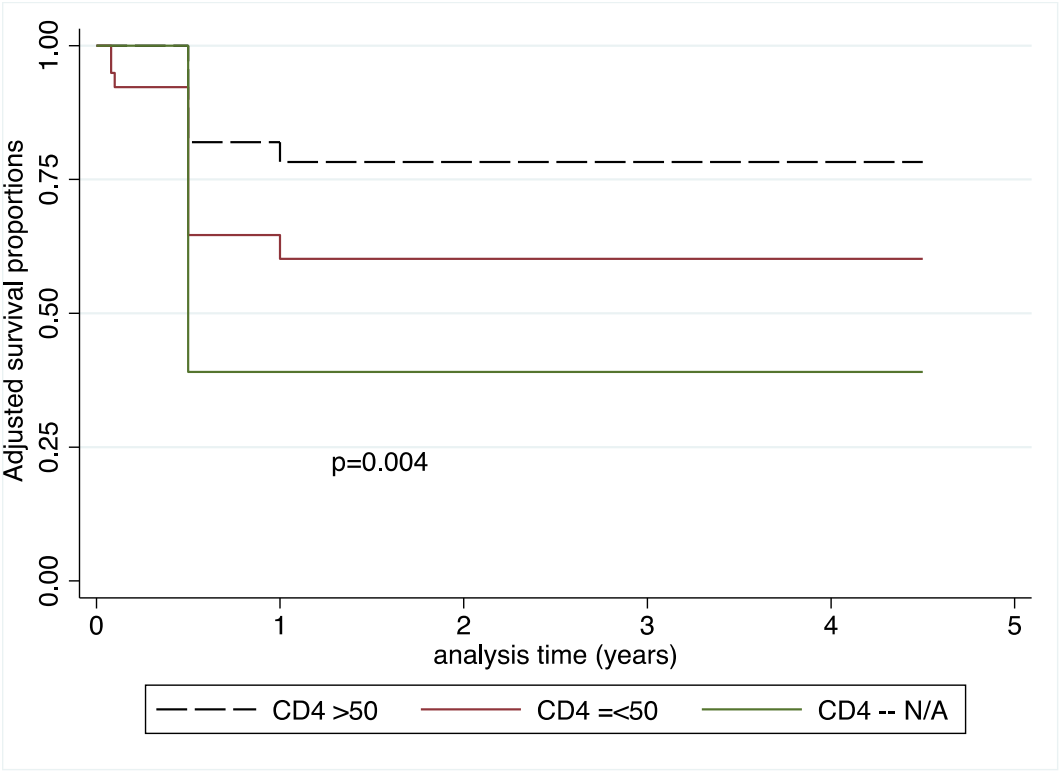

Figure 3.11 Adjusted Kaplan-Meier curves comparing time-to-death for patients with varying CD4 counts at baseline in multivariate Cox regression models that adjusted for diagnosis year and receipt of chemotherapy.

CHAPTER 4. DISCUSSION, POTENTIAL LIMITATIONS AND STRENGTHS OF THE STUDY, CONCLUSIONS AND FUTURE RECOMMENDATIONS

4.1 Discussion

Clinical data on NTM are scarce, with only a few local reports despite the high burden of NTM risk factors in our setting. In the last decade, there has been a rise in the number of reports of NTM globally, postulated to be from advanced diagnostic methods, greater awareness and knowledge, as well as an aging population with more comorbidities (12, 13, 15).

In this cohort, a significant increase in the NTM isolates was found between the years 2012-2014, followed by a steady decline after 2014 onwards. These findings are not surprising since globally ARV initiation has been shown to reduce the risk of opportunistic infections. In a US-based study by Kaplan *et al*., a 39.9% decline of MAC incidence per year was seen during 1996-1998, which coincided with the increase use of highly active antiretroviral therapy (HAART) in the US (39). This is similar to US study by Buchacz *et al*., which showed decline in MAC incidence from 26.9% (1994-1997), to 6.2% (1998-2002) and then 2.5% (2002-2007), highlighting the decline of NTM with increasing ARV use (40). In the current cohort, the high NTM trends in 2014 are possibly explained by a number of other factors. Johnson, in his study undertaken in South Africa investigating ARV enrolment rates, noted that by 2004, only a total of 47,500 patients were on ARVs (men 17 700, women 25 600 and children 4200) highlighting the delays in the ARV initiation rates (41). The delays in ARV initiation in the sub-Saharan setting have been reported elsewhere in the literature with the reasons attributed to this varying from inability to access health-care centres, poverty, medication side-effects and behavioural myths, especially among males, with men that seek health-care being seen as ‘weak’ (42). Also, in the rollout of ART in South Africa, this was initially only in patients with a CD4 cell count of <200/µl, followed in subsequent years with the inclusion of those with higher CD4 cell counts, followed ultimately by all patients at HIV diagnosis. Another possible reason for the peak on NTM in the year 2014, may be the low CD4 count of most patients at the time of ARV initiation, as this had been shown to still be associated with an increase in the risk of opportunistic infections even after ART has been initiated (43).

In this study, NTM positive isolates were noted to be more frequent among males of a younger age group (median age of 39 years). This is in contrast with the global reports which showed more females, of an older age group (>65 years), being affected more commonly. These differences in demographics (gender and age) might be explained by the high burden of HIV (and possibly TB), which is more common in the younger age group, making this population at a higher risk of acquiring NTM infection. A US-based study of NTM lung disease showed a higher prevalence in women (127 cases versus 93 cases in men) (15). Similarly, a population-based study of NTM prevalence in five states in the US from 2008-2013, showed higher cases among the elderly, between 50-80 years of age (44). This is duplicated in many other parts of the world including a recent study from China (77.8% females over 50 years of age) and reports from Korea (61.1% females; mean

age 55.8 years) (45-47). In addition, there is a higher risk in postmenopausal women that are lean, with no underlying lung disease, referred to as Lady Windermere syndrome (48). There appears to be only one USA study, by Lapinel *et al.*, which reported similar results to that of the current study cohort, showing more younger males (median age 44 years) with isolation of NTM.

The high HIV burden in the younger age group is documented in the current study, with 96 (78%) of the cases being found to be HIV-positive, associated with low CD4 cell counts of less than 200/µL, and 40 (41,6%) out of the 96 patients with CD4 cell counts of less than 50/µL. These results resonate with the reports of NTM in sub-Saharan Africa by Okoi *et al*., which describes NTM commonly found in the 33-44 years age group, also with the same findings of high HIV and TB burden (21). Similar results were documented in the study by Lapinel and colleagues, with all their participants being HIV-positive, and with lower median CD4 cell counts of 64 cells/µL (49).

Amongst the HIV-positive patients, the most frequent NTM isolates were MAC in 22 (22,9%) and *M. avium* in 37 (38,5%) as depicted in Table 3.6 This is in contrast with the lower rates in the HIV-negative group with MAC isolated in 2 (9,5%) and *M. avium* in 2 (9,5%). Similar results have been shown in other studies. In a Japanese study of 24 HIV-positive patients, *M. avium* was isolated in 20 (83%) of the patients and *M. intracellulare* in 3 (13%) of the patients with lower CD4 cell counts (50). Furthermore, Lapinel *et al*., found that MAC was the most frequent species isolated 34 (17.1%) in a group of 196 HIV-infected patients (48). Similar results were reported in the USA by Miguez-Burbano *et al.*, with the prevalence of NTM being 265 (11%) in HIV-patients admitted with lower respiratory tract infection (51). MAC was isolated in 45 (71%) patients in that cohort; in which 93% had a CD4 cell count of less than 200 cells/µL and 73% of the subjects had a CD4 cell count of less than 50 cells/µL (50). The current study patients with MAC isolates were more likely to have cytopenias, together with lower weight measurements less than 60kg, with the lowest being 38kg. In other reports, NTM infections (pulmonary and disseminated) were associated with smaller body habitus, as has been described in the Lady Windermere syndrome, and in NTM patients with bronchiectasis (52). Low body weight in our cohort could be explained, at least partly, by advanced HIV infection (HIV wasting syndrome).

A number of the current patients 38(30,8%)) had a prior history of pulmonary tuberculosis, making HIV and TB the most commonly associated comorbid conditions and could possibly serve as risk factors for NTM in this cohort. This is similar to a recent study in China by Hu *et al.*, which looked at the prevalence of NTM pulmonary disease and found previous PTB in 64% of the patients, and HIV in 19.5% of the patients with clinical bronchiectasis (19.5%); these being the most frequent comorbid conditions in their cohort, with COPD reported in only 6.9% (45). Similar results were reported in two recent Korean studies of NTM incidence, showing TB as the most frequent comorbid illness in 10% and 33.7% of patients, respectively (46, 47).

The time to culture positivity (TTP) in the current study, varied from a minimum of 3 days to a maximum of 40 days; however, the majority had TTP that was over 20 days. This, together with the fact that there is overlap in both clinical manifestations and radiological findings of NTM and PTB, could be the reasons why most of patients in this cohort were initiated on conventional anti-TB treatment only, further resulting in poor outcomes. Furthermore, there were a significant number of specimens labelled as “contaminated” or simply as a MOTT, and these required a repeat sample submission in order to speciate the MOTT to direct appropriate therapy, hence delaying diagnosis and treatment, once again potentially impacting on outcome (data not shown).

In those cases in which the doctors’ description of the chest radiograph was available, the majority were reported as showing cavities and bronchiectasis, especially in the patients with MAC. These findings are in keeping with the current literature from China, by Hu *et al.,* who showed bronchiectasis (39.1%) and cavities (37.9%) as the major imaging features (45). In the current cohort, the imaging findings might also be explained by the high number of patients who were found to have a prior TB history, with this infection likely resulting in structural lung damage.

Managing patients with NTM is complex as specific diagnostic criteria (e.g. ATS/IDSA criteria) need to be met prior to initiating therapy. Also, the treatment outcomes have been poor in most studies, with cure rates reported to be 4% in cavitary disease and 14% in non-cavitary pulmonary disease in a US study of 91 patients (53). Several other studies echo the same results with poor outcomes, and further demonstrating factors that are associated with these outcomes, including MAC isolate, erythrocyte sedimentation rate (ESR) > 60mm/hr, elderly males > 60 years and low BMI (54, 55). In this cohort, the outcomes as depicted in Table 3.12, were that 28 (22,7%) were known to have died and 27 (21,9%) of cases were known to have lived. This mortality rate reported might be underestimated considering that 46 (37,3%) outcomes were recorded as ‘unknown’ and 20 (16,2%) as defaulted therapy; therefore, it seems reasonable for us to postulate that this number might be much higher than we have reported. In the sub-analysis of the patients that died, comparing them to the ones that lived, there were common factors that were found to be predictors of poor prognosis and these included cytopenias (p= 0.031), low CD4 cell count <50/µL (p=<0.001), whether patients were treated (p=<0.001) or not, MAC isolates, although there was a difference in numbers (23 versus 18) but was not statistically significant (p=0.48). In the study by Hu *et al*., looking at the case fatality rates among NTM cases, death was associated with older age, higher HIV viral load, and comorbidities, while other reports showed the importance of a low BMI <18kg/m² (56, 57). Unfortunately, in this cohort, we could not calculate the BMI as the height was not routinely recorded in the patients and hence conclusions could not be made based on weight alone.

These poor outcomes that are reported with NTM pulmonary disease are also accompanied by longer duration of therapies with high risk of adverse events. In the current cohort, a third of the patients were initiated on NTM therapy, and a third were not on any form of treatment. Despite having half of the MAC patients initiated on therapy, 10% still died from the disease. In a recent South Korean reports of

treatment patterns, 18.8% of patients were initiated on macrolide-based regimens, and 76.6% were not initiated on any form of therapy after at least 1-year from the time of diagnosis (47).

4.2 Potential limitations of the study

This is a single centre study and the results are not to be generalised to other centres in South Africa or to other geographical regions. The study is retrospective and hence the data were obtained from existing records and not all the information was available.

We also had to exclude a significant number of cases due to changeover in the laboratory tracking system that took place in 2013 changing from DISA to NHLS, resulting in being unable to obtain results that fell within the DISA system (378 cases).

As a consequence, the study has significantly under-estimated the number of cases from whom NTM were isolated during the study period at CMJAH.

4.3 Strengths of the study

It is one of the few reports of NTM in our setting, giving some insight into the disease burden, and based on the findings may assist in future recommendations and guide further research.

4.4 Conclusions

NTM is a common disease in the younger age group in our setting, predominantly in the male gender, with HIV and TB being the most commonly associated comorbidities. The were no significant gender differences among the patients that died (54% males vs 46% females); however, the MAC group (including *M. avium* 43%, *M. intracellurale* 25% and MAC 14% were the most common species isolated among this group. Other factors that predicted mortality included HIV-positive status (86%) with a CD4 count of less than 50/µL (50%), a previous PTB episode (39%) and cytopenias (60%). There were major delays in the NTM diagnosis with time to positivity (TTP) of 40 days being the longest delay, and hence patients being initiated only on conventional anti-TB therapy, or no treatment at all, and some defaulting therapy with devastating outcomes. It is unknown whether the patients' records that reported that the patients had defaulted was associated with patient demise or survival, but the former is most likely as they were not on any therapy. Despite not many reports of the condition in South Africa, it appears from this study that NTM is a common disease with devastating clinical outcomes. Major challenges include delays in diagnosis, poor patient treatment compliance, longer duration of therapy required, with higher risk of adverse events and also inappropriate treatment, all potentially resulting in poor outcomes.

4.5 Future recommendations

Based on the findings from this study of the patients having a high burden of NTM risk factors (TB and HIV), one would have expected a greater number of patients with NTM than what was found; however, this was not the case. Possible explanations might be lack of awareness of the potentially important role that NTM play in the South African setting, potentially overshadowed by the massive TB burden, and lack of testing for NTM using cultures, particularly in the period after roll-out of Gene Expert test. In addition, the cases that fell within the DISA system (378 cases) we had to exclude as they were no longer accessible to laboratory tracking system change. There clearly needs to be significant improvement of follow-up of culture results for suspected NTM patients, in order to document these cases, and for institution of treatment timely, monitoring compliance, adherence and adverse events. Drug sensitivity testing for macrolides may be a consideration as some of the patients in the current study, despite them being on macrolide-based regimen had poor outcomes.

These findings highlight the need for multi-centre prospective studies, specifically focussing of documenting the burden, clinical features, microbiology, treatment and outcome of NTM infections, particularly in high-risk populations. More local data will serve as a foundation for the development of guidelines for the management of infections with NTM, applicable to our population.

4.6 References

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APPENDIX A. DATA COLLECTION SHEET

Demographics

Patient number: _____

Age: _____

Gender: ☐ Male ☐ Female

Body mass index (kg/m^2): ☐ <18 ☐ 18-23 ☐ 23-25 ☐ >25

Race: ☐ Black ☐ White ☐ Coloured ☐ Indian ☐ Other

Smoking: ☐ Current ☐ Previous ☐ Never

Smoking pack years: _____

Risk Factors

HIV status: ☐ Positive ☐ Negative ☐ Unknown

CD4 count: _____Date:

Structural lung disease

☐ None

☐ COPD FEV1/FVC:____ FEV1:_____Date:

☐ NCF-Bronchiectasis

☐ Post-TB infections

☐ Childhood infections

☐ Transplant

☐ Lung

☐ Heart

☐ Kidney

☐ Liver

☐ Other: _____

NTM species

☐ *M. kansasii*
☐ MAC
☐ *M abscessus*
☐ M avium
☐ M intracellulare
☐ M gordonae
☐ M chelonae
☐ M xenopi
☐ Others _____
☐

Number of isolates
Clinically significant Y N

Time to positivity (TTP)

Culture site

☐ Lung
☐ Lymph Nodes
☐ Blood culture
☐ Skin
☐ Bone
☐ Soft tissue
☐ Other:_____

Treatment

	Duration of treatment
Macrolides	_____

Rifabutin/Rifampicin _____

Ethambutol _____

Injectables _____

Other _____

Outcome (Outcome definitions adapted from BTS guidelines 2017)

☐ Culture conversion

☐ Recurrence

☐ Refractory disease

☐ Died

☐ Lost to follow up

APPENDIX B

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Ms. N. Mzila
Office of the Clinical Director
Tell: (011): 488-4812
Email: Nolwazi.Mzila@gautemg.gov.za
20 February 2019

GP_201901_027

Dear Dr. L. Nqwata

STUDY TITLE: Non-tuberculosis mycobacteria (NTM) at Charlotte Maxeke Johannesburg Academic Hospital, 2010-2017

Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic Hospital will not anyway incur or inherit costs as result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the HOD and Unit Manager or sister in charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~**not supported**~~

pp
Dr. M.I. Mofokeng
Clinical Director
DATE: 22/02/2019

Approved/~~**not approved**~~

Ms. G. Bogoshi
Chief Executive Officer
Date: 26.02.2019

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr L Nqwata
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine - Pulmonology
Medical School
University

E-mail: drlamla@yahoo.com

CC: Supervisor: Profesor C Feldman <Charles.Feldman@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 03/12/2018

REF: *R14/49*

PROTOCOL NO: **M180753** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Non-tuberculosis mycobacteria (NTM) at Charlotte Maxeke Johannesburg Academic Hospital, 2010-2017*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr L Nqwata

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M180753

NAME: (Principal Investigator)	Dr L Nqwata
DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine - Pulmonology Medical School University
PROJECT TITLE:	Non-tuberculosis mycobacteria (NTM) at Charlotte Maxeke Johannesburg Academic Hospital, 2010-2017
DATE CONSIDERED:	27/07/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Profesor C Feldman
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/12/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore reports and re-certification will be due early in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES