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Time to sputum culture conversion of Multi-Drug Resistant Tuberculosis in HIV positive versus HIV negative patients in Lesotho

MSc Clinical Epidemiology

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree Master of Science (MSc) in Clinical Epidemiology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ABSTRACT

Background: Multi-Drug resistant tuberculosis (MDR-TB, resistance to at least Isoniazid and rifampicin) is a disease of public health importance, accounting for an estimated 490,000 new cases globally in 2016. Historically, cure rates in MDR-TB/HIV co-infected patients were lower than in HIV negative patients, partly due to high morbidity and mortality associated with retro-viral disease.

Lesotho has one of the highest levels of tuberculosis and probably multidrug-resistant TB in the world. However, efforts to control this disease including the introduction of the World Health Organization (WHO)-recommended DOTS-Plus and recently endTB strategies for multi-drug resistant TB contributed significantly to the decline of the disease.

Aim: This study evaluated the association between HIV and time to sputum culture conversion in MDR-TB patients who were on the MDR-TB regimen and Antiretroviral treatment (if HIV positive), between January 2011 and December 2016.

Method: The study used a retrospective cohort study design of consecutive patients who were initiated on MDR-TB regimen from January 2011 to June 2016 in Lesotho. All patients were followed up until death, loss to follow-up, sputum conversion or censored at the end of December 2016, whichever came first.

Results: A total of 346 patients with confirmed MDR-TB records were included in the study. Of these, 58.02% (n=199) were male, a third of the patients were married (n= 122 [35.67%]) and about four fifths (n=277 [81.15%]) were HIV positive. The HIV positive patients achieved sputum culture clearance at a median of 54.22 (IQR 22-117) days, while the HIV negative patients achieved conversion at 60.84 (IQR 24-129) days. There was no statistically significant difference in the sputum culture conversion rates by HIV status (AHR: 1.11, CI: 0.82-1.50, p-value: 0.486). Residing in rural area (AHR: 1.60 CI: 1.20-2.14, P-value: 0.001) and good adherence (AHR: 15.84 CI: 2.21-113.60, P-value: 0.006) independently predicted higher sputum culture clearance rates.

Conclusion: HIV-status does not affect sputum culture conversion in MDR-TB patients on ART in Lesotho.

LIST OF ABBREVIATIONS

ART Anti-Retroviral Therapy

BMI Body Mass Index

CDC Centre for Disease Control

DOTS Directly Observe Therapy Strategy

DST Drug Susceptibility Testing

Hb Hemoglobin

HIV Human Immunodeficiency Virus

MDR Multi-Drug Resistant

NGO Non-Governmental Organization

PIH Partners in Health

PUD Peptic Ulcer Disease

TB Tuberculosis

TX Treatment

USAID United States Agency for International Development

WHO World Health Organization

XDR Extensively Drug Resistant

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CHAPTER ONE: INTRODUCTION

Multi-Drug resistant tuberculosis (MDR-TB, resistance to at least Isoniazid and rifampicin) is a disease of public health importance [1, 2] accounting for an estimated 490,000 new cases globally in 2016. An additional 100,000 cases of rifampicin-resistant TB cases were also eligible for MDR-TB treatment in 2016. [3] As opposed to drug-susceptible TB, MDR-TB is found to have poorer treatment outcomes due to less effective medications which may be associated with life threatening adverse events. Also, these medications have to be taken for up to a minimum of 18-24 months [4] and about 14,000 tablets [4] will be ingested together with daily injections in the first eight months of treatment. In the past, the MDR-TB epidemic was concentrated in Eastern Europe, however, evidence have shown that MDR-TB is on the increase in Sub-Sahara Africa, posing a threat to the population already overwhelmed by the HIV epidemic. [5] Historically, cure rates in MDR-TB/HIV co-infected patients were lower than in HIV negative patients [6] partly due to high morbidity and mortality associated with retro-viral disease. [7, 6] However, these MDR-TB studies [5, 6] were conducted before the availability of antiretroviral treatment (ART). The addition of antiretroviral therapy may improve survival and cure rates for MDR-TB/HIV co-infected patients. [7]

Sputum culture conversion from positive to negative is an important intermediate outcome of treatment success in MDR-TB. [8] Due to this, sputum culture conversion is now being used as an interim treatment outcome for predicting success in MDR-TB. [9] To date studies regarding culture conversion in MDR-TB/HIV patients on ART has been reported but mostly with very small sample sizes. [10, 11] A study which compared MDR-TB treatment success by HIV status, reported significant association between HIV negative patients and 2 months sputum conversion however, no significant association was reported for co-infected MDR-TB patients. [12] In contrast, Brust et al. [10] reported culture conversion rates in HIV co-infected MDR-TB patients were high with favorable treatment outcomes.

Lesotho's MDR-TB prevalence is said to be comparable to that of South Africa. [13] South Africa experienced a dramatic rise in MDR-TB cases over the past decade. MDR-TB cases increased 10 fold between 2000 and 2007. The HIV infection rate among MDR-TB cases is approximately 70%. [10] Additionally, Lesotho has the second highest prevalence of HIV (26%) in the world. It has been

noted that a lot of Basotho people work, or have worked in South African mines and, TB incidence rates in mines are high.[14] This may lead to most of Basotho people contracting TB in mines. Upon diagnosis, they are referred to Lesotho in order to initiate and complete their treatment but they usually return back to South Africa before treatment completion because of their economic situation. Due to this, they tend to migrate back and forth between their homes in Lesotho and their workplace in South Africa and hence default the treatment, which may lead to developing resistance to some or all of the TB drugs. Incomplete treatment of TB and transmission of resistant TB strains fuels MDR- TB.

To address the high MDR-TB prevalence rate and rates of HIV co-infection, Partners In Health, an NGO responsible for MDR-TB management in Lesotho, established an integrated community-based treatment program in Lesotho. All patients who had at least Rifampicin resistance in their sputum were referred to Botsabelo MDR-TB Hospital in Maseru, the only MDR-TB treatment facility in the country for initiation of treatment. Thereafter patients were seen at MDR-TB Clinics in their respective districts by the team from the MDR-TB Hospital on a monthly outpatient basis. This is a community based model which was started in 2007 in Lesotho by Partners in Health and has been adopted by many other countries. This model made use of trained treatment supporters, whose responsibility included visiting patients twice daily to administer medications and charting, recording common side effects and calling the medical team when required, following patients to monthly clinic visits to observe changes in prescriptions and giving psychosocial support.

In this study, we compared time to sputum conversion in HIV co-infected MDR-TB patients who were on ART, with time to sputum conversion in HIV negative MDR-TB patients.

Aim

To evaluate the association between HIV and time to sputum culture conversion in MDR-TB patients who were on the MDR-TB regimen and antiretroviral treatment (ART) (if HIV positive) between January 2011 and December, 2016**.**

Objectives

1 To estimate the time to sputum culture conversion in MDR-TB patients who were started on the standardized MDR-TB regimen between January 1, 2011 and June 30, 2016 in Lesotho.

2 To determine the association between HIV status and time to sputum culture conversion in MDR-TB patients who were started on MDR-TB regimen and ART (if HIV positive) between January 1, 2011 and June 30, 2016 in Lesotho.

3 To investigate clinical and demographical factors (Hemoglobin level; extent of disease; presence of cavity; Albumin level; body mass index; Age; Sex) associated with time to sputum culture conversion in MDR-TB patients who were started on MDR-TB regimen between January 1, 2011 and June 30, 2016 in Lesotho.

Null Hypothesis

Treatment outcome of MDR-TB patients on second-line tuberculosis drugs does not depend on their HIV status.

Alternative Hypothesis

Treatment outcome of MDR-TB patients on second-line tuberculosis drugs does depend on their HIV status.

Lesotho has one of the highest levels of tuberculosis and probably multidrug-resistant TB in the world. [3] However, efforts to control this disease, including the introduction of the World Health Organization (WHO)-recommended DOTS strategy for basic TB control in 1993 and the introduction of the DOTS-Plus strategy for multi-resistant TB in 1998, resulted in a considerable decline in multidrug-resistant TB among patients with newly diagnosed sputum culture-positive disease, from 14.4% in 1996 to 9.2% in 2009. The DOTS strategy is a 5-component program for TB control that consists of the following: 1) political commitment, 2) case detection through bacteriologic evaluation, 3) standardized treatment with supervision and patient support, 4) an effective drug supply system, and 5) a reporting and recording system that allows assessment of treatment. The DOTS-Plus strategy was designed to manage patients with multidrug-resistant TB using the second-line anti-TB drugs in the context of functioning DOTS program.

Additionally, tuberculosis case findings have been intensified by screening for TB in all HIV positive patients so as to put them on treatment on time and at large to prevent further infections. [15] Recently, the above strategy has been replaced by End TB strategy [16] which is not yet fully implemented in Lesotho. End TB strategy became imperative after a comprehensive review of the global tuberculosis situation between 1993 and 2015. End TB strategy has the following pillars: integrated, patient-centered care and prevention, bold policies and supportive systems, intensified research and innovation. [16] The third pillar, intensified research and innovation is still not happening as expected in a nation with very high TB incidence.

Despite this, outcomes have been encouraging in patients with multidrug-resistant TB who were treated under Lesotho's national DOTS-plus strategy, which uses regimens tailored to individual patients' anti-TB drug susceptible profiles. In 2007, the first year of full treatment availability for all patients with multidrug-resistant TB in Lesotho, 76% of patients' adherent to a full course of therapy converted early and achieved successful outcomes. [17]

Early sputum culture conversion during MDR-TB treatment is a good indicator for a favorable treatment outcome. This is supported by a study in Latvia which demonstrated that MDR-TB patients whose culture converted within the first 2 months of treatment were more likely to have pauci-bacillary disease.[10] Again, other studies have also demonstrated [18] that culture conversion earlier in the treatment period is linked to successful outcome. KJ Seung et al. reported that 96%

of patient who did not die during MDR-TB treatment in the early days of MDR-TB program in Lesotho were already sputum converted. [17]

HIV infection has been linked to an increased development of MDR-TB. Three meta-analysis and systematic review studies conducted between the years 2009 and 2015 revealed an association between HIV infection and MDR-TB. [19] [20] [21] Other studies also have similar findings.[22] [23] One possible explanation for this, can be attributed to mal-absorption of first line TB drugs in TB/HIV co-infected patients and as such leads to resistance to any of the drugs including rifampicin. However, some studies got contradictory findings that there is no association between HIV infection and MDR-TB but they all had small sample sizes. [24] [23]

Also, sputum conversion and treatment outcome is generally poor in HIV co-infected MDR-TB patients. [25] There are very few studies done on this topic globally including Sub-Sahara Africa. Furthermore, most of them also had a very small sample size. [26] [27]

Although, it is already established that HIV infection is associated with poor clinical outcomes in MDR-TB patients, [26], [28] there is limited data comparing the time to sputum culture conversion in MDR-TB patients who are HIV co-infected and on ART and those who are HIV negative.

Four studies done in Botswana [26], South Africa [10] and Lesotho [29] respectively compared sputum conversion in relation to HIV status of MDR-TB patients. Hafkins et al. [26] found no statistically significant association between HIV status and the proportion of sputum conversion in MDR-TB patients by HIV status in the Botswana study. Interestingly, the study found that HIV co-infected MDR-TB patients converted earlier (median of 78 days) than the HIV negatives (median of 95 days). In the South African study, Brust et al. found no difference in the proportion of MDR-TB patients' culture conversion in relation to their HIV status within 6 months of treatment. Again, the study also reported HIV co-infected patients converting earlier than the non-infected MDR-TB patients. In agreement, the study done in Lesotho by Satti et al. [29] reported no statistically significant association between HIV status and treatment outcome. Furthermore, the study showed that the median sputum culture conversion rate occurred within two months of treatment initiation. In all three studies discussed above, there were no significant difference in proportion of sputum conversion in MDR-TB patients in relation to their HIV status but HIV positive MDR-TB patient achieved sputum culture conversion slightly earlier. Another study done in South Africa by Mohr et al. also showed that, HIV co-infected MDR-TB patients on ART achieved culture conversion faster than HIV negative patients. [28]

However, these findings needed to be interpreted carefully due to small sample size used in all the studies. With regards to predictors of sputum culture conversion in MDR-TB, a study done in Latvia [30] found the followings ;

- HIV infection generally predisposes to poor sputum outcome in many ways. This ranges from poor drug absorption, presence of other opportunistic infections to increased mortality.
- Extra-Pulmonary site which is infection of any part of the body other than the lungs. Tuberculosis infection of extra-pulmonary sites are not only difficult to diagnose but also respond slowly and poorly to treatment due to poor drug penetration of the infectious sites, such as the Blood-Brain barrier in TB meningitis.
- The study also found low BMI was a statistically significant predictor of delayed sputum culture conversion in MDR-TB patients. Body Mass Index (BMI) is the value derived from the mass and height of an individual. It is an attempt to quantify the amount of tissue mass in an individual and categorize that person as underweight, normal weight, overweight or obese based on that value.

Other studies [31] [32] [33] reported the following as predictors to sputum conversion in MDR-TB;

- Bilateral cavitation [31] which is the presence of cavities in both lungs. Cavitation is an immune response whereby the body walls off the bacteria to reduce its spread. This thickened wall also reduces penetration of anti-tuberculosis drugs thereby prolonging sterilization.
- Adherence [32] is measurement of patients' compliance to prescription. Patients with good adherence convert faster.
- Female sex and BMI. [33] Female patients tend to seek help for their health condition earlier than men hence, are diagnosed and start treatment earlier with resultant good biological outcome. Patients with low BMI convert slower than normal weight patients.

In agreement to this, studies done in Lesotho also found that low BMI and low Hemoglobin were statistically significantly associated with delayed sputum culture conversion. [29]

CHAPTER THREE: METHODOLOGY

Study design

A retrospective cohort study of consecutive patients who were initiated on MDR-TB regimen from January, 2011 to June, 2016 in Lesotho was carried out. All patients were followed up until death, loss to follow-up, sputum conversion or censored at the end of Dec 2016, whichever came first.

Study setting

Lesotho, is a mountainous country entirely enclosed by South Africa. It is one of the least developed countries in the world and as such, diseases that have been associated with poverty are common. This study used Partners In Health data which included all the MDR-TB patients from the entire country.

Population

All bacteriologically diagnosed MDR-TB Patients, who received treatment through the MDR-TB Hospital in Maseru, were eligible. Patients initiated on treatment between January 1, 2011 and June 30, 2016 were included in the study. The data used were only for those patients who had bacteriologically proven MDR-TB, and patients who were followed up for a minimum of 6 months on treatment. As soon as multidrug-resistant TB was diagnosed, treatment with second-line therapy was started with an empirical individualized regimen considering any previous anti-TB drugs received. This was done because second-line drug susceptibility testing takes an average of 2 weeks to be processed. The initial regimen consisted of between 4 and 6 drugs, including 1 anti-TB drug that was injected. Regimens were then modified according to the results of the second-line drug susceptibility testing and included at least 5 drugs to which the patient's TB isolate was susceptible. An injected drug, usually an aminoglycoside, was included in the treatment regimen for the first 8 months of treatment. However, if the second line DST indicated resistance to second line drug(s) or confirmed XDR-TB, such patient was excluded from the study. Surgery was considered an adjuvant therapy for patients with more advanced localized disease not responding well to treatment provided they could tolerate the procedure.

Inclusion criteria

Patients were eligible if they had lived in Lesotho, had culture confirmed MDR-TB, and started on second-line Tuberculosis treatment in the period from 1 January, 2011 until 30 June, 2016.

Exclusion criteria

Patients were excluded if they had resistance to Amikacin, Kanamycin, Capreomycin or any Fluoroquinolone at the start of the treatment. Resistance to any of the second line injectables and fluoroquinolones in addition to Isoniazid and rifampicin is defined as Extensively Resistant Tuberculosis (XDR-TB) and resistance to any of the injectable or fluoroquinolone drugs is defined as Pre-XDR-TB. [34]

Study duration

The medical records of MDR-TB patients were reviewed to obtain sputum culture results information for patients, who were initiated on the MDR-TB regimen between January 2011 and June, 2016 and patients were followed up until December, 2016. Lost to follow up was defined as defaulting from care for the period of at least one month, and not reached by treatment supporters or community team.

Sample size

Based on literature, a study done in Botswana by Hafkins et al. reported that there is not much difference between HIV negative (53%) versus HIV positive (50%) MDR TB patients on ARTs in terms of culture conversion. [26] In agreement to this, Brust et al reported that about 89% of MDR-TB patients, regardless of their HIV status get culture converted within 6 months of treatment in a South African study. [10]

Alpha	Power	N	N1	N2	N Ratio	HR	S1	S2	Pr E
0.05	-	346	276	69	0.25	1	0.5	0.5	-
0.05	0.07668	346	276	69	0.25	0.9	0.5	0.5359	0.4928
0.05	0.1889	346	276	69	0.25	0.8	0.5	0.5743	0.4851
0.05	0.3735	346	276	69	0.25	0.7	0.5	0.6156	0.4769
0.05	0.5987	346	276	69	0.25	0.6	0.5	0.6598	0.4680
0.05	0.7982	346	276	69	0.25	0.5	0.5	0.7071	0.4586

Estimate power table for two-sample comparison of survival function

The sample of 276 HIV positive individuals who had a sputum conversion rate of 50%, and 69 HIV negative individuals who had a sputum conversion rate of 53%. Power varies from 8% for

HR of 1(assuming no difference in incidence of sputum conversion) to 80% for HR of 0.5(at 50% of difference) to detect statistically significant difference between HIV positive and negative on TB sputum conversion.

Measurements

This study used data from treatment charts and bacteriological laboratory reports that were entered into the Lesotho multidrug-resistant TB clinic documents. Side effect reports, expert consultation reports, and radiological reports were also used. This study collected baseline demographic characteristics and risk factor information, including the following variables: age, BMI were analyzed as continuous variables while sex, previous treatment for TB and multidrug-resistant TB, adherence to treatment (measured by treatment supporters using a daily treatment chart), alcohol use, household location and size, employment status, HIV status, and drug side effects were analyzed as categorical variables. Adherence can either be good or poor. It is indicated to be good when the patient was at least 98% compliant for the month and poor if less than 98%. The Lesotho program makes use of treatment supporters that visit the patients at their homes twice daily to administer the second line TB drugs and also check for any adverse event.

All mycobacterial cultures were performed according to international standards at the Lesotho national TB reference laboratory in Maseru by using conventional Lowenstein-Jensen solid media.[35] Drug susceptibility testing were performed on *M. tuberculosis* isolates from sputum at the National Reference Laboratory following available international standards; quality was assured by the Uganda Tuberculosis Reference Laboratory, Kampala, WHO-designated supranational reference laboratory for Lesotho. The second line Drug susceptibility testing was performed in Path care Laboratory in South Africa. Laboratory performance, measured through evaluation of control strains were provided by the supranational reference laboratory.

Patients were seen daily by clinicians while hospitalized and, monthly while receiving outpatient treatment. Sputum specimens were collected monthly for smear and culture examination and drug-susceptibility testing, and data were obtained on improvement of symptoms, treatment adherence, and side effects. All treatment was directly observed during the entire course of therapy by the treatment supporters. After resistant *M. tuberculosis* sputum culture confirmation, the injected medication was continued for at least eight months. Then, the rest treatment continued for another 12 to 18 months, depending on the severity of lung disease, history of treatment for TB, and general response to treatment. After treatment completion, patients were followed for 2 years with sputum testing done every 6 months.

Treatment outcome definitions

For this analysis, we have used the multidrug-resistant TB treatment and outcome definitions which were developed recently by an international expert consensus group. [36] Treatment default was considered to have occurred in patients who interrupted treatment for 1 or more consecutive months. Patients who began multidrug-resistant TB treatment with a positive sputum culture and had 2 negative consecutive sputum cultures taken at least 30 days apart after initiation of treatment were considered to have sputum culture conversion. Among those with documented conversion, initial time to sputum culture conversion was calculated as the time in days between the date of treatment initiation for multidrug-resistant TB and the collection date of the first of two consecutive negative sputum cultures. Proportion of sputum conversion in both groups was calculated similarly. In addition, we recognized that patients could achieve sputum culture conversion and become culture-positive again. [37] In this study, we planned to consider these patients as non-converted. During the follow-up period we did not encounter such cases amongst those who were eligible for this study.

This study used patients' clinic files as they contained monthly sputum culture test results.

Data Analysis

Data was collated and analyzed using STATA 13. [38] Categorical variables were summarized by frequencies and proportions, and continuous variables were summarized by means and standard deviation for normally distributed data while medians and interquartile range values were used for skewed distributed data. Simple unadjusted comparisons for differences between groups were assessed using Chi-square tests for categorical data and T-tests or Wilcoxon rank-sum tests for continuous data were undertaken. Low body mass index BMI, was calculated by dividing weight in kilograms by the square of height in meters. A low BMI was defined as less than 18.5 kg/m^2. [37]

To estimate time to sputum conversion, Kaplan Meier Curves were used. Log rank tests were used to compare survival curves describing sputum conversion between HIV positive and negative groups. During the analysis, time dependent co-variates and proportionality using Schoenfield residuals were assessed to test if proportional hazard assumption was violated. To investigate the association between sputum conversion and HIV status, as well as to investigate factors associated with sputum conversion, Cox Proportional Hazards models were used. Unadjusted uni-variable

and adjusted multi-variable Cox Proportional Hazards models were used. Results are reported with 95% confidence intervals and p-value of 0.05 significance level.

A pre-selection test for all potential explanatory variables was performed where each variable was tested at a time and included in the multivariate model by measure of relaxed p-value of ≤ 0.25 as the initial model except the variables which were well-known predictors from literature such as BMI, Adherence, Hb level and Cavitation. All candidate explanatory variables were included in the initial model, where one by one variable was removed based on the level of significance until the final model was reached. We maintained BMI in the final model regardless of its level of insignificance. This was so because it cofounded the result.

Ethics

The study proposal was submitted and approved by the Ethics Committees of the University of Pretoria and the Ministry of Health, Lesotho under reference number 76/2017 and reference number ID12/2017 respectively. Data for this study are routine medical data entered into patients' files. All patient names were replaced with a traceable unique study Identification numbers in the data set.

CHAPTER FOUR: RESULTS

Baseline characteristics

According to table 1, a total of 346 patients with confirmed MDR-TB records were eligible for inclusion in the study. Of these, 58.02% (n=199) were male. About a third of the patients were married (n= 122(35.67%). The median age of the study participants was 41 years (Interquartile range [IQR] 40 – 43). More than half (54.72%, n=145) of male patients were HIV positive and 45.28% (n=120) of the female patients were HIV positive. The median BMI was 18.81 (IQR: 18.38-19.24). By HIV-status, the BMI was 18.58 (IQR: 18.09-19.06) for HIV positive patients and 19.61 (IQR: 18.64-20.57) for HIV negative patients.

During the follow up period, 245 (71.64%) patients attained sputum culture clearance. Out of the HIV positive, 187 (70.83%) were converted and 58 (74.36%) were converted among HIV-negative patients. At the end of study follow up period 97 patients (28.36%) remained positive, whereby, 77 (29.17%) were HIV positive and 20 (25.64%) were negative.

Most of the patients (n=83, 24.27%) that were still culture positive died within the follow up period. This study found that the mortality is higher amongst HIV positive patients (n=64, 77%) than HIV negative patients (n=19, 23%). A good percentage of the mortality occurred within 8 weeks of treatment initiation before they could attain culture clearance. Few patients were lost to follow up (n=6, 1.75%) and failed MDR-TB treatment (n=8, 2.34%) respectively. All of the patients that failed treatment were HIV positive.

Table 1: Baseline characteristics of MDR-TB patients by HIV status

Variable Name	Frequency (n) (N=346)	HIV-positive (N= 265) (77.26%)	HIV-negative\ (N=78) (22.74%)	P-value[1]
Age in years, mean (SD)	41.35(13.44)	40.05(11.83)	45.74(17.23)	**0.001**
Height (meters)median(IQR)	1.64(1.63-1.65)	1.64(1.63-1.65)	1.62(1.60-1.64)	0.913
Body weight (Kilograms) mean(SD)	50.07(9.60)	49.75(9.67)	51.16(9.29)	0.126
BMI median(IQR)	18.81(18.38-19.24)	18.58(18.09-19.06)	19.61(18.64-20.57)	**0.025**
Hb_Level mean(SD)	10.65(2.32)	10.66(2.39)	10.64(2.05)	0.520

Table 2: Baseline characteristics of MDR-TB patients by HIV status

Variable Name	Frequency (n) (N=346)	HIV-positive (N= 265) (77.26%)	HIV-negative\ (N=78) (22.74%)	P-value[2]
Sex				**0.022**
Male	199 (58.02)	145(54.72)	54(69.23)	
Female	1444(41.98)	120(45.28)	24(30.77)	
Marital status				0.124
Single	72(21.05)	54(20.45)	18(23.08)	
Married	122(35.67)	99(37.50)	23(29.49)	
Stable relationship	38(11.11)	33(12.50)	5(6.41)	
Widow/Separated	110(32.16	78(29.55)	32(41.03)	
Employment status				0.920
Not Employed	182(53.06)	141(53.21)	41(52.56)	
Employed	161(46.94)	124(46.79)	37(47.44)	
Residential Location				0.794
Urban	112(32.65)	87(32.83)	25(32.05)	
Rural	212(61.81)	164(61.89)	48(61.54)	
Semi-urban	19(5.54)	14(5.28)	5(6.41)	
Comorbidity status				0.738
No comorbidity	75(21.93)	59(22.73)	15(19.23)	
Diabetes	10(2.92)	9(3.41)	1(1.28)	

[1] T-test was used to test for significance by p-value

[2] Chi[2] was used to test for significance by p-value

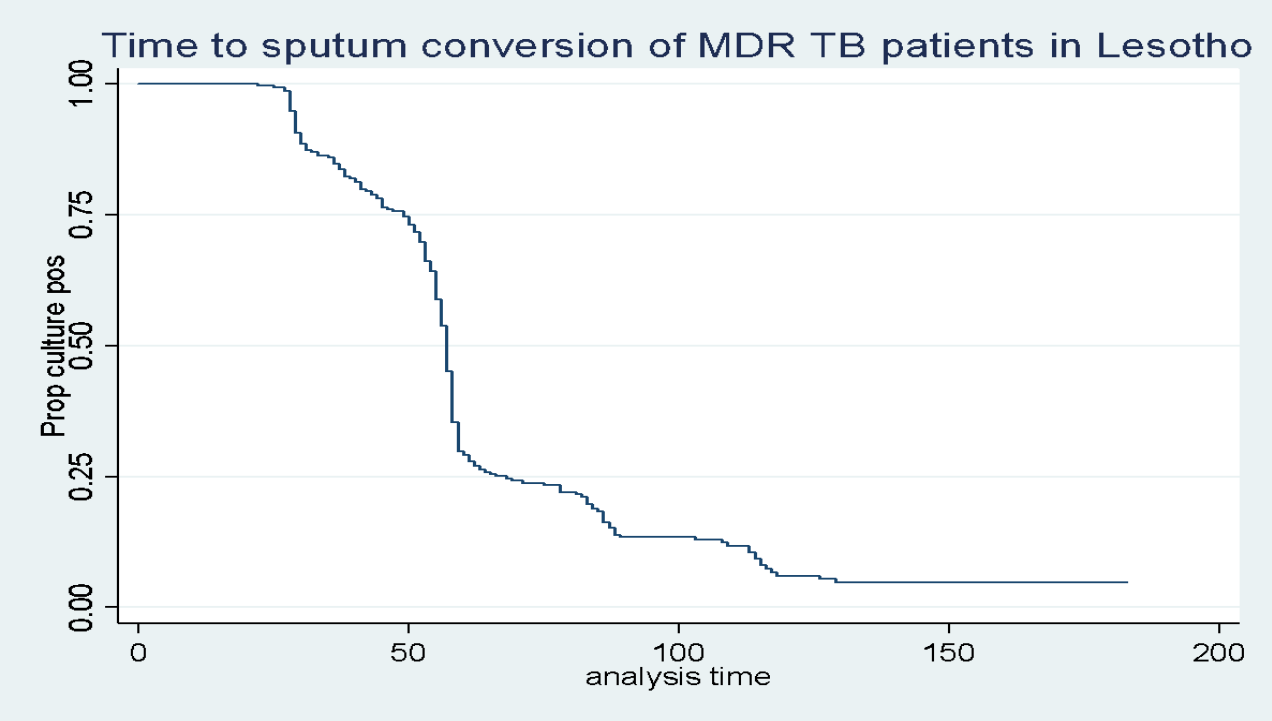
Hypertension	24(7.02)	18(6.82)	6(7.69)	
Psychosis	23(6.73)	19(7.20)	4(5.13)	
Hypothyroidism	184(53.80)	137(51.89)	47(60.26)	
Peptic Ulcer disease	26 (7.60)	21 (7.95)	5 (6.41)	
TB Treatment History				0.613
First Line	243(70.85)	187(70.57)	56(71.79)	
Second Line	14(4.08)	9(3.40)	5(6.41)	
Unknown	19(5.54)	15(5.66)	4(5.13)	
Extent of the disease				0.286
One Lung involved	119(34.69)	88(33.32)	31(39.74)	
Both Lungs involved	224(65.31)	177(66.79)	47(60.26)	
Presence of cavity				0.361
No Cavity Present	213(62.10)	168(63.40)	45(57.69)	
Cavity Present	130(37.9)	97(36.60)	33(42.31)	
Adherence to Treatment				0.208
Poor adherence[3]	17(4.97)	11(4.17)	6(7.69)	
Good Adherence	325(95.03)	253(95.83)	72(92.31)	
Sputum outcome[4]				0.544
Negative Culture	245(71.64)	187(70.83)	58(74.36)	
Positive Culture	97(28.36)	77(29.17)	20(25.64)	
Outcome of the disease				0.458
Converted	245(71.64)	187(70.83)	58(74.36)	
Died	83(24.27)	64(24.24)	19(24.36)	
Treatment failure	8(2.34)	8(3.03)	0(0.00)	

[5] A patient is said to have poor adherence when he/she have used less that 98% of the prescribed medication for the month during pill count.

[6]A patient considered to have negative culture when there is two consecutive culture results at least one apart

Time to sputum culture conversion, in HIV-positive versus HIV-negative patients

The median time to sputum conversion was 55.78 (IQR 22 – 129) days after treatment initiation (Fig 1). When stratified by HIV status, the HIV positive patients achieved sputum culture clearance earlier at a median of 54.22 (IQR 22-117) days, while HIV negative achieved sputum culture conversion at 60.84 (IQR 24-129) days (Fig 2).



Numbers at risk

Days	0	50	100	150	200
Number at Risk	346	203	26	7	0

Figure 1: Kaplan Meier curves of time to sputum conversion in MDR-TB patients in Lesotho

According to figure 1, the probability of remaining sputum culture positive among Lesotho MDR-TB patients' increases to above 50% after about 110 days of MDR-TB treatment.

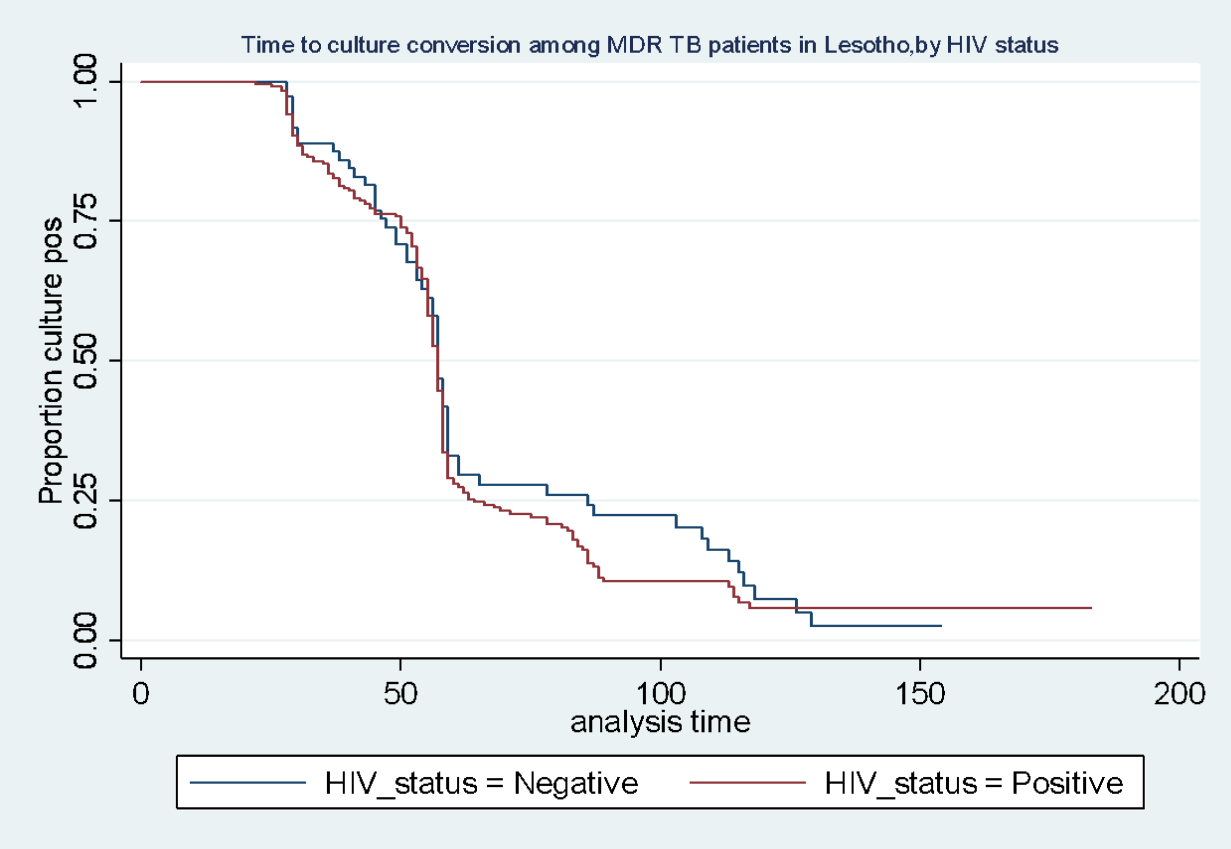


Figure 2: Kaplan Meier of time to sputum culture conversion among MDR-TB patients in Lesotho by HIV status

	HR	St. Err	Z	P-value	95% CI
HIV Status	1.13	0.17	0.8	0.43	0.84-1.52

Number at Risk

	0	50	100	150	200
HIV Neg	78	45	11	1	0
HIV Pos	264	158	15	6	0

In figure 2, the probability of achieving sputum culture conversion is relatively the same in both HIV positive and HIV negative patients until after about 55 days of MDR-TB treatment when the HIV positive patients achieve culture conversion slightly earlier than HIV negative patients.

The MDR-TB patients on treatment in Lesotho have minimal chance of sputum clearance after 120 days if they are HIV positive and up to 130 days if they are HIV negative.

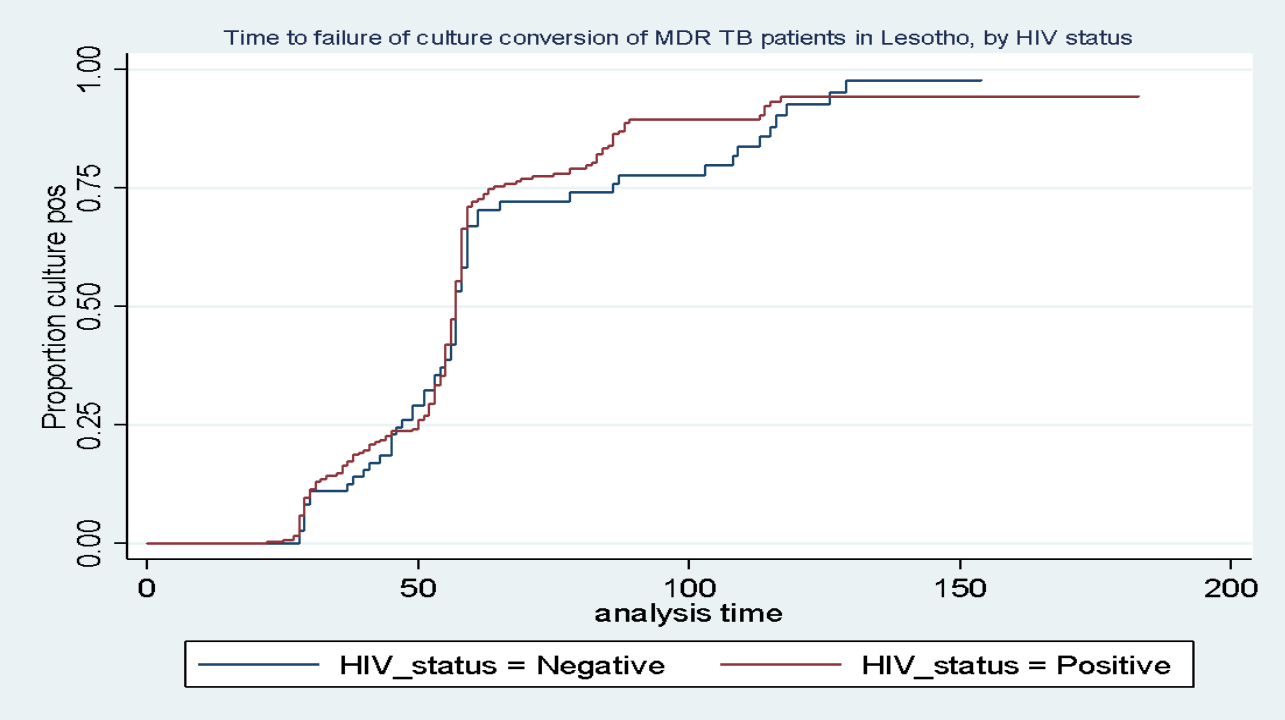


Figure 3: Time to failure of sputum conversion in MDR-TB patients in Lesotho by HIV status

Figure 3 illustrates that the longer the MDR-TB patient in Lesotho are on treatment without culture conversion, the higher the probability of remaining culture positive. The probability of not converting are fairly the same for HIV positive and HIV negative MDR-TB patients until about 75 days of treatment. Although, the probability of not converting still increases by treatment duration but it is higher amongst HIV negative MDR-TB patients.

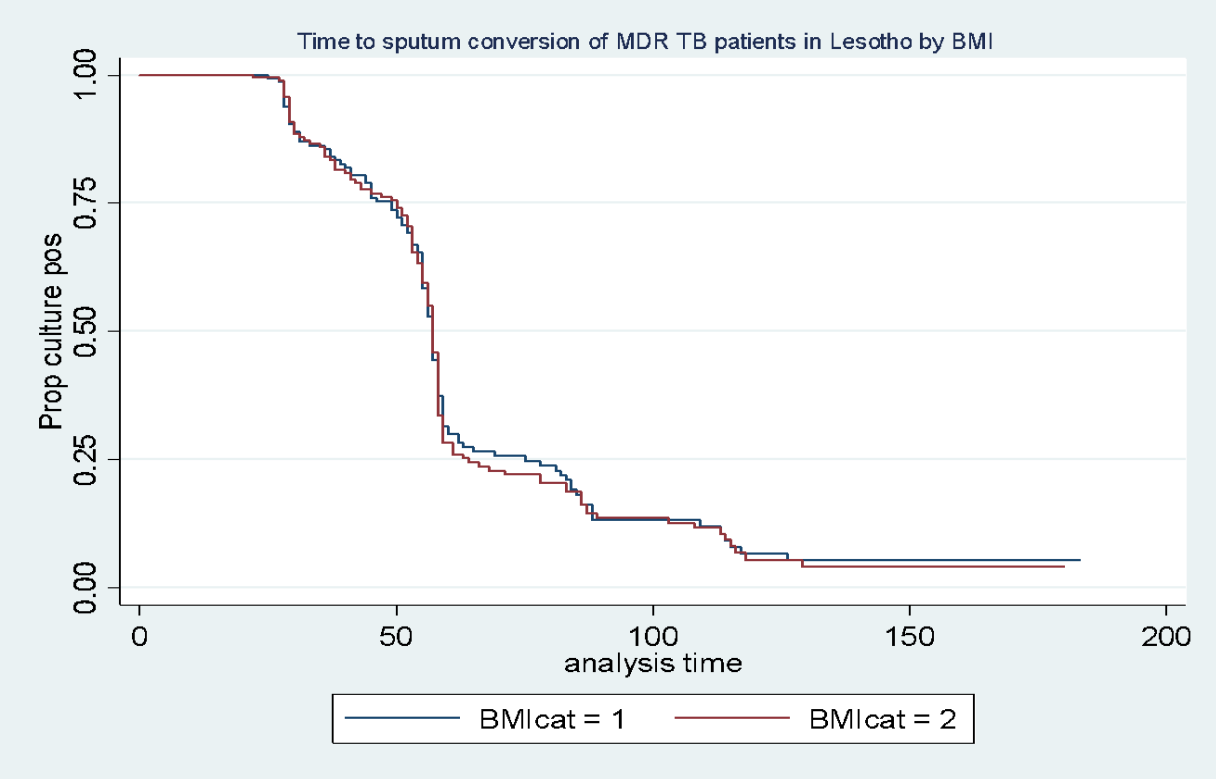


Figure 4: Kaplan Meier of Time to sputum conversion in MDR-TB patients in Lesotho by BMI

Figure 4 illustrates that there is no significant difference in the probability of achieving sputum culture conversion while on treatment in both MDR-TB patients with low or high BMI at start of treatment.

Crude and adjusted cox proportional hazard rates of sputum conversion in HIV-positive versus HIV-negative MDR TB patients

The study conducted both unadjusted uni-variable and adjusted multi-variable Cox proportional Hazards models. By using univariate analysis, age above 50 years (HR: 0.67 CI: 0.46-0.97 P-value: 0.03), presence of psychosis (HR: 1.99 CI: 1.16-3.41 P-value: 0.012), and presence of hypothyroidism (HR: 1.51 CI: 1.06-2.16 P-value: 0.024) had statistically significant associations with time to sputum culture conversion. Being HIV positive in MDR-TB patients (HR: 1.14 CI: 0.85-1.53 P-value: 0.381) increased the chance of culture conversion but this association was not statistically significant.

In multivariable analysis, residing in rural area (AHR: 1.60 CI: 1.20-2.14 P-value: 0.001) was associated with improved sputum culture conversion compared to patients who stay in urban areas

and having good adherence to MDR-TB treatment (AHR: 15.84 CI: 2.21-113.60 P-value: 0.006) independently increased culture conversion rates.

After adjusting for confounders, being HIV positive (AHR: 1.23 CI: 0.69-2.21 P-value: 0.49), also predicted higher conversion rates but this association was not statistically significant.

This study also checked for varying co-variates and interaction between factors, but there were no statistically significant factors found.

Table 3: Cox proportional hazards regression of factors associated with sputum conversion

Variable Name	**Crude Hazard Ratio (95 % CI)**	**p-value**	**Adjusted Hazard Ratio (95% CI)**	**p-value**
Disease Extent				
One lung involved	1			
Both lungs involved	1.36 (1.04 – 1.78	**0.027**	1.23(0.93-1.62)	0.145
HIV Status				
HIV Negative	1			
HIV Positive	1.14(0.85-1.53)	0.381	1.11(0.82-1.50)	0.486
Hb_Level				
<5.9mg/dl	1			
6 – 9.9 mg/dl	3.02(0.42-21.81)	0.272	4.13(0.57-29.93)	0.160
≥10mg/dl	4.26(0.60-30.44)	0.149	5.14(0.72-36.93)	0.103
BMI				
Below 18.5	1			
>18.5	0.97(0.76-1.25)	0.828	1.03 (0.80-1.33)	0.801
Residence				
Urban	1			
Rural	1.62(1.22-2.15)	**0.001**	1.60(1.20-2.14)	**0.001**
Semi-Urban	1.60(0.91-2.80)	0.101	1.64(0.93-2.90)	0.087
Adherence				
Poor adherence	1			
Good adherence	16.85(2.36-120.31	**0.006**	15.84(2.21-113.60))	**0.006**

Age				
Less than 30 years	1			
30-50 years	0.82(0.61-1.11)	0.203		
Above 50 years	0.67(0.46-0.97)	**0.032**		
Sex				
Male	1			
Female	1.04(0.81-1.34)	0.741		
Marital status				
Single	1			
Married	0.85(0.61-1.19)	0.348		
Stable relationship	0.85(0.54-1.33)	0.473		
Widow/Separated	0.78(0.55-1.10)	0.160		
Comorbidity status				
No comorbidity	1			
Diabetes	0.87(0.34-2.22)	0.774		
Hypertension	0.91(0.52-1.62)	0.756		
Psychosis	1.99(1.16-3.41)	**0.012**		
Hypothyroidism	1.51(1.06-2.16)	**0.024**		
PUD	1.28(0.73-2.21)	0.387		
TB Treatment History				
Never on TB TX	1			
First Line	1.09(0.79-1.50)	0.611		
Second Line	0.92(0.45-1.88)	0.810		
Unknown History	0.80(0.40-1.58)	0.515		

Presence of cavity in Lungs				
No Cavity present	1			
Cavity present	0.99(0.76-1.28)	0.915		
Employment				
Not Employed	1			
Employed	1.09(0.85-1.40)	0.502		

CHAPTER FIVE: DISCUSSION AND CONCLUSION

According to table 1, this study found that there were more males than females with MDR-TB. This finding is in agreement with a meta-analysis study done by Faustini et al. [39] in Europe. This could be explained by the fact that males are more in occupations that increase risk to acquire MDR-TB, such as mining. [40] Also, HIV infection was extremely common in patients with MDR-TB in this study. To concur, Faustini et al. [39], Hafkins et al. [26] and Suchindran et al. [19] reported similar findings. Like drug susceptible TB, HIV infection predisposes to developing MDR-TB due to reduced immunity as reported by Mette D et al.. [41]

This study found that most of the mortality amongst MDR-TB patients in Lesotho occurred within the first 8 weeks of treatment. This finding is similar to a study done in South Africa by Schnippel et al. where mortality was reported highest in the first 12 weeks following MDR-TB treatment initiation. This can be attributed to late presentation and diagnosis of patient as such by the time of treatment initiation the disease is already at an advanced stage with risk of poor outcome. On the other hand, since most of MDR-TB patients in Lesotho are HIV positive, they might also have other more aggressive opportunistic infections that can contribute to death.

HIV positive MDR-TB patients converted slightly earlier (54.22 days) than the negative patients (60.84 days). However, there was no statistical significant association with time to sputum conversion in MDR-TB patients in HIV-positives versus HIV-negatives using Kaplan Meier Curves. This result conforms to findings of a study done in Botswana by Hafkin et al. [26] and another done in Ethiopia by Agumas Shibabaw et al. [42] where they reported no difference in time and proportion of sputum conversion in MDR-TB patient by HIV status. Possible explanations for this finding might be as a result of pauci-bacillary infection amongst HIV patients allowing for earlier clearance. In addition, HIV negative patient have higher likelihood of developing cavities which are less penetrable to wall off pulmonary infection. Also, HIV positive patients tolerate second line treatment better and thereby have good adherence. This could be attributed to the fact that HIV infection affects the gastrointestinal system thereby leading to reduced absorption with consequent lesser adverse effect.

Low or normal BMI had no effect on failure to sputum culture conversion. Contrary to this, studies done in China and Indonesia by Shenjie Tang et al. [43] and Putri et al. [33] respectively found low BMI to be a predictor of delayed sputum culture conversion.

This study reported that living in rural area and good adherence increased the chances of culture conversion in MDR-TB in Lesotho. A possible explanation for better results for rural dwellers could be that most of the patients living in these settings are not working. Treatment supporters meet them at home and they tend to have good adherence. This findings are in conformity by the study done by Satti et al. in Lesotho. [29] In addition, there was a statistically significant association in patients who developed hypothyroidism and psychosis during treatment and sputum culture conversion. A possible explanation for this finding could be that their comorbidity is a criterion for hospitalization. Admitted patients receive specialized care and are adequately monitored, which improves adherence with favorable culture outcomes.

Study Limitation

The retrospective data that we collected had missing values in certain variables because the data was not collected at the real time. The proposed sample size could not be achieved because less than anticipated patients fulfilled the definition of Multi-Drug resistant Tuberculosis. This resulted into a very low power to detect a difference between the groups. We may have obtained different results with a larger sample size.

Conclusion

In conclusion, this study found good adherence to treatment and living in rural area as the main predictors to improved time to sputum conversion. There is no significant difference in the time and proportion to sputum conversion by HIV status in MDR-TB patients on ART in Lesotho.

Most mortality occurred in the early stage of treatment. Therefore, we recommend intensified case finding with early diagnosis for prompt treatment initiation to reduce death.

Due to the knowledge gap in this area, hence the reason of conducting this study.

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APPENDIXES

Appendix 1: Reverse Sample size calculation

A reverse calculation was done by Statistician in order to determine the power to detect a pre-determined difference given the current sample size of about 346.

The power to detect a statistically significant difference of magnitude of $p_1 - p_2$ between two groups with prevalence p_1 and p_2 respectively is given by

$$1 - \beta = \Phi\left(z - z_{1-\frac{\alpha}{2}}\right) + \Phi\left(-z - z_{1-\frac{\alpha}{2}}\right), z = \frac{p_1 - p_2}{\sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}}$$

Where:

Φ is the standard normal distribution function

α – is the Type I error

β is the Type II error, and 1 – β is power (35)

The simple power calculation in STATA is "sampsi 0.5 0.53, n(277) r(0.2)" if we assume sputum conversion rate of 50% and 53% in HIV negative and HIV positive respectively and a 4:1 in HIV negative/HIV positive in our sample. The Power here is very low ~5%

Appendix 2: Data Collection Tool

Biographic data **Date of data extraction……**	CODE ------/------/----------
1. Age in complete years	☐☐
2. **Marital status** Single ----------1 Married ----------2 stable relationship----------3 Widow/Separated……..4	☐
3. **Place of residence** Urban ----------1 Rural ----------2 Semi – urban----------3	☐
Health status **Past Medical condition**	
4. **HIV status** Negative ……0 **HIV positive……1** Unkown ……3	☐
5. **Hb Level?**	☐☐.☐ g/dL
6. **BMI**	☐☐.☐ kg/m^2
7. Extent of the disease Involved one lung……1 Involved both lungs……..2	☐
Treatment Start Date	
Date of First Negative	
Date of Second Negative Result	
Time to sputum conversion in days	☐☐☐ Days
Outcome for sputum conversion at the end of follow up: Negative……0 Positive……1	☐

Date of Last Sputum result recorded if Positive	
All missing values will have code	**99**

Appendix 3 Ethical approval certificate

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

30/03/2017

Approval Certificate
New Application

Ethics Reference No.: 76/2017

Title: Time to sputum culture conversion in Multi-Drug Resistant Tuberculosis patients that are HIV positive versus HIV negative in Lesotho

Dear Dr Odunayo Alakaye

The **New Application** as supported by documents specified in your cover letter dated 22/03/2017 for your research received on the 22/03/2017, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 29/03/2017.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year
- Please remember to use your protocol number (**76/2017**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Additional Conditions:
- Approval is conditional upon the Research Ethics Committee receiving permission from the Lesotho REC.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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