CHAPTER 1
INTRODUCTION

1.1 BACKGROUND
Tuberculosis (TB) is a major public health problem affecting one third of the world’s population and killing almost two million people every year. According to the WHO in 2008 there were 8.9 – 9.9 million incident cases and 9.6 – 13.3 million prevalent cases of TB globally, with an estimated 1, 82 million deaths of which nearly 0.52 million were in HIV infected individuals. Zimbabwe is ranked 18th out of 22 countries with a high burden of TB (countries that contribute 80% of global cases) with about 90,000 cases in 2008 (about 8 cases per 1,000 population). The emergence of resistance to anti-tuberculosis drugs, and particularly of multidrug-resistant TB (MDR-TB), has become a major public health problem in a number of countries and an obstacle to effective global TB control. MDR-TB is defined as TB caused by strains of M.tuberculosis which are resistant to at least Isoniazid and Rifampicin (the most effective drugs used in treatment of TB). In 2008 nearly half a million cases of MDR-TB emerged worldwide. This probably is a result of under-investment in basic activities to control TB, poor management of the supply and quality of anti tuberculosis drugs, improper treatment of TB patients and transmission of the disease in congregate settings. Microbial factors (genetic mutations) may also result in the emergence of resistant strains. It is estimated that prevalent cases could be two or three times more than the incident cases.
The global burden of MDR-TB is not known since only 59% (114 countries) submit data on patterns and proportions of drug resistance. In Africa, only 22 out of 44 counties have conducted a drug resistance survey since 2000. It is presumed that non-reporting is attributable to challenges and cost of establishing continuous surveillance of drug resistance (culture, drug susceptibility testing [DST] for all TB cases and associated logistic and human resource costs)³.

South Africa is ranked 4th out of 27 countries with a high MDR-TB burden and it reported approximately 10 000 cases of MDR-TB and 415 cases of Extremely drug resistant TB (XDR-TB) in 2008³. The proximity of Zimbabwe to South Africa and the significant movement of populations across the SADCC borders put Zimbabwean population at a high risk of M/XDR-TB.

HIV is a powerful risk factor for all forms of TB ². A study in Harare concluded that HIV was increasingly and strongly associated with TB⁷. The proportion of TB and HIV co-infection is high, of the 9.4 million cases of incident TB reported globally in 2008, 1.2-6 million were HIV positive¹. Almost 68% of TB patients in Zimbabwe have HIV¹.⁸.

Significant associations of MDR-TB and HIV status have been shown in Ukraine (OR 1.7 95% CI 1.3-2.3)⁹. Although the study is not yet complete, preliminary results are showing a similarly positive association in Mozambique, an odds ratio of 0.7 (95% CI 0.2-2.2)³.

MDR-TB may be associated with HIV because HIV patients have a weak immune system which makes them vulnerable and they progress to MDR-TB disease easily once they are infected with an MDR-TB strain. Thus in high MDR-TB prevalence a large number of
MDR-TB patients could develop TB disease\(^1,2\). HIV patients under TB treatment may develop resistance to rifampicin due to mal-absorption of the anti-TB drugs\(^2\).

MDR-TB is difficult to treat and treatment goes up to 2 years. Less potent drugs with severe side effects are used \(^2,8\). In 2008 about 150 000 deaths occurred including those with HIV and an estimated 97 000 that were HIV negative\(^1,3\). Currently, there is insufficient data on MDR-TB treatment outcomes disaggregated by HIV status at population level worldwide. This is due to the under detection of MDR-TB\(^1,2\). However, there seems to be a high case fatality among HIV infected M/XDR-TB patients where death has been reported to occur within 36 days of diagnosis\(^10\).

Knowledge of the burden of MDR-TB is important for effective TB control and in some countries, trends over time have shown a reduction in MDRTB \(^3\). In Russia, this reduction is attributable to the efforts to diagnose and treat MDR-TB that have been put in place since 1990\(^3\).

1.2 PROBLEM STATEMENT

- It has been noticed that there is a 10% increase in the number of MDR-TB positive samples received at National Tuberculosis Reference Laboratory (NTBRL) from Mpilo OI Clinic since 2009.

- In Zimbabwe there are no clear guidelines for MDR-TB case finding, and drug susceptibility is not routinely done on samples for TB suspects that would have been found to have TB. Hence MDR-TB is not diagnosed before commencing first-line treatment. This may lead to mis-treatment as all new TB cases are initiated
on first line drugs. Thus all new TB cases that have MDR-TB go for 6-8 months on medication that is ineffective. The patients are only suspected of having MDR-TB after treatment failure. A sample is then sent to the laboratory for MDR-TB diagnosis. This takes a further 3 months after which the patient is initiated on second line medication. Effectively a new TB patient with MDR-TB is commenced on the correct regimen 9-12 months from the time they showed signs and symptoms of TB.

- Mal-absorption of anti TB drugs has been noticed in HIV positive patients who are on TB treatment and it could be a risk factor for drug resistance especially Rifampicin. Zimbabwe is a high HIV burden country and most patients are on Antiretroviral therapy (ART) this could mean that a high number of HIV patients could develop resistance to rifampicin. The current burden of TB drug resistance in these patients has not been assessed.

- Increasingly HIV patients in Zimbabwe are given Isoniazid prophylaxis, and prolonged drug use could be a risk factor for drug resistance

- Zimbabwe has undergone serious economic hardships which together with a very high burden of HIV, may have a negative impact on MDR-TB. Despite the high risk of MDR-TB in HIV positive patients, little has been done to find the burden of MDR-TB in these patients.

Therefore the purpose of this study was to:

a) Assess the burden of MDR-TB in HIV positive patients.
b) to determine the prevalence and possibly associated risk factors (age, sex occupation, recent travel to South Africa, TB contact, Isoniazid prophylaxis, health worker, duration of stay in congregate areas, hospitalization, duration on ART) of MDR-TB in HIV positive patients.

c) To assess the relationship of CD4 count and MDR-TB in these patients.

1.3 JUSTIFICATION

The last MDR-TB survey in Zimbabwe was done 16 years ago in 1995 and an MDR-TB prevalence of 3.5% among new TB cases and 6% among re-treatment cases was reported. However, the relationship was not assessed for HIV positive patients. Since then the prevalence of HIV has increased tremendously so it now becomes imperative that the prevalence of TB and MDR-TB be assessed in HIV patients.

In South Africa[S.A], TB Drug resistance is an increasingly recorded problem among HIV positive patients. Our proximity and movement of people between SA and Zimbabwe (especially that most people in the southern region often frequent SA), there is then a need to assess MDR-TB prevalence in Zimbabwe.

Knowing the association of MDR-TB in HIV positive patients and hospitalization or frequency to congregate settings is important in Zimbabwe. If found to be positively associated, it can influence policy change in prioritizing the setting up of World Health Organisation [WHO] recommended minimum infection control measures which are almost next to none in Zimbabwe OI clinics and other congregate settings.
Knowledge of Prevalence of MDR-TB can influence policy change in setting up guidelines for active MDR-TB case finding in HIV positive patients. New laboratory techniques that are fast to diagnose MDR-TB so as to reduce the time it takes before MDT-TB suspects are put on treatment can be advocated for as well.

The extent of drug resistance is not known in Africa and in most resource limited settings treatment of a patient with MDR is absent or is inadequate. Continuous surveillance through culture and DST on all TB cases is done in only 42 countries. The other 72 countries with TB program rely on surveys that are done periodically on representative samples of patients. Of these 72, only 47 have conducted a survey since 2000\textsuperscript{1,2}. Currently, there is insufficient data worldwide on MDR-TB treatment outcomes that are disaggregated by HIV status at community level. This is due to the under detection of MDR-TB\textsuperscript{2,3}. 
1.4 LITERATURE REVIEW

A number of studies in various European countries and South Africa have shown a high prevalence and associated risk factors of MDR-TB in HIV patients. Pablo et al carried out a study to determine prevalence of MDR-TB in HIV positive patients in Peru. They found an MDR-TB prevalence of 43% in HIV positive patients and this was very high compared to 3.9% in HIV negative or with unknown status. They concluded that MDR-TB is an ongoing problem in HIV infected persons receiving care in public hospitals.

In Spain, John Rullan et al., showed that being hospitalized and prolonged contact with potentially infective ward mates resulted in outbreaks of MDR-TB in HIV positive patients. In their study all the 48 patients who had MDR-TB were HIV positive and they were hospitalized. They finger printed the isolates and showed that the patients were infected by the same strain of MDR-TB species. They reached the conclusion that nosocomial transmission was the cause of the MDR-TB outbreak.

In Mumbai, India. Pal Ramprasad et al. reported a prevalence of 37% of MDR-TB in HIV positive patients. An almost similarly high prevalence of MDR-TB has been reported in Chennai, India (39.9%) and Siberia 40.3%.

Gandhi et al., carried out a cross sectional study, on Extensively drug resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area (Msinga district) of South Africa. They reported a prevalence of 39% of MDR-TB and 6%XDR-TB. Other important findings in this study were that 52 patients had XDR-TB and half of the patients with XDR-TB had never been treated for TB before thus their primary infection was caused by the XDR-TB. transmission could have been
nosocomial because two thirds had a history of recent hospitalization before the onset of XDR-TB. Two health workers died of it (There was 98% mortality with median survival time of 16 days (IQR6-37) from day of sputum collection, in the patients with XDR-TB. They concluded that MDR-TB was more prevalent than they had realized and that XDR-TB had been transmitted to HIV patients and the combined infection was associated with high mortality. They concluded that these observations warrant urgent intervention and threaten the success of the treatment program for tuberculosis and HIV.

Hassim et al 2010 on detection of a substantial rate of multidrug –resistant tuberculosis in an HIV infected population in Phidisa, South Africa by active monitoring of sputum samples. They reported a prevalence of 20.6%. They concluded that there is need for better management strategies to reduce the development of MDR-TB, Most of these patients investigated had received prior anti tuberculosis therapy that was presumably not curative.

March 2010 Michael Canter et al, determined prevalence of drug resistant TB amongst HIV patients. They found that 21% had MDR-TB. They also found that a significantly higher rate of MDR-TB was seen in patients with a history of TB therapy than those without (27% versus 12%, p = 0.05). All these studies concluded that there is a high prevalence of MDR-TB amongst HIV patients in South Africa.

In 2009, Sujit Suchindran et al., conducted a systematic review of studies assessing HIV infection as a risk factor for MDR-TB. They aimed to summarize and critically appraise studies in order to quantify the association between MDRTB and HIV infection. However, they found that most studies in North America showed an association between
HIV infection and MDR-TB but results from other regions were conflicting. They could not demonstrate an overall association between acquired MDR-TB and HIV but their results suggest that HIV infection is associated with primary MDR-TB. They concluded that there is need for future well-designed studies and surveillance in all regions of the world so as to clarify the relationship between MDR-TB and HIV.

In another study by A Hamze et al on the characterization of *Mycobacterium tuberculosis* in Lebanese patients they showed that male patients appeared more likely to be infected with or to develop MDR-TB compared to women OR 3.1(95% CI 1.2-7.39)\(^{19}\). They concluded that genotype characterization of MTB should be adapted in routine TB control activities.

Quality assured bacteriological confirmation of the presence of *Mycobacteria* is the cornerstone of TB diagnosis. Several studies have shown that the laboratory methods currently being used for detecting the presence of MDR-TB in Zimbabwe are valid.

Solid culture on Lowenstein Jensen[LJ] media is the “Gold standard” method for tuberculosis culture in the laboratory recommended by International Union Against Tuberculosis and Lung Diseases(IUATLD) and Centers for Disease Control(CDC)\(^{20,21}\).

C Rodrigues et al., did an evaluation of the liquid culture technique using a BACTEC Mycobacterium Growth Indicator Tube(MGIT) 960 against the conventional solid LJ culture technique (the gold standard) and found that using the MGIT 41% of the samples were positive for mycobacteria and using LJ only 24% were positive\(^{22}\). Similar results were obtained in Thailand by La-ong Srisuwanvil et al where the MGIT 53% of samples were positive and using the LJ media only 39% were positives\(^{23}\). Thus the MGIT is more
sensitive than the ‘Gold standard’ so it can be run in parallel with the solid LJ media. The MGIT has an added advantage of a shorter mean turnaround time of 9 days compared to 38 days for solid culture in smear positive samples.

The Ziehl Neelsen (ZN) stain is the method recommended by CDC and IUTLD for the confirmation of *Mycobacterium species* grown on culture media. Though its sensitivity is low, it is also the method of choice worldwide for Direct smear examination of sputum samples.

It is imperative to distinguish strains that are in the *Mycobacterium tuberculosis* complex from *mycobacterium* other than tuberculosis (MOTT) or BCG strains (especially in Zimbabwe and other countries where BCG vaccination is done) for clinical and therapeutic reasons. Oettinger et al showed that MPT64 antigen is absent in BCG strains, *M. bovis* and *M. leprae* and in other Mycobacterial species. The rapid immunochromatographic test kit (SD MPT64TB Ag Kit) is currently in use in Zimbabwe. Vijay GS Kumar et al evaluated this kit and found that both the sensitivity and specificity was 100%. Ismail et al compared the SD MPT 64 Ag kit to the conventional biochemical tests and the sensitivity, specificity, positive predictive value and negative predictive values were 97%, 100%, 100% and 92% respectively. These results makes it a very reliable and useful diagnostic technique.

The LJ Proportion method is the ‘Gold standard’ for TB drug susceptibility testing which has been approved by Centers for Disease Control (CDC) and International Union against Tuberculosis and Lung Diseases as the method of choice for drug susceptibility testing. It is used as a standard when evaluating other drug susceptibility methods.
1.4.1 Research Question

What is the prevalence and associated risk factors of MDR-TB in adult HIV positive patients presenting at Mpilo OI clinic?

1.4.2 Hypothesis

That in a cross sectional study HIV positive men and women 18 years and above, registered at Mpilo OI clinic, diagnosed and suspected of TB have an MDR-TB prevalence above 20% and this is significantly associated with TB contact, recent travel to South Africa exposure to high frequency (more than two times per month) in congregate settings, low CD4 count (<200) and being hospitalized.

1.4.1 Objectives

Primary objective:

To assess the burden of MDR-TB in adult HIV positive patients attending Mpilo OI clinic.

1.4.2 Secondary Objectives:

To assess the prevalence of MDR-TB in adult HIV positive patients attending Mpilo OI clinic

- To assess risk factors associated with MDR-TB in HIV positive patients.
- To assess the relationship of CD4 count and MDR-TB infection at the time of MDR-TB diagnosis
CHAPTER 2

METHODOLOGY

2.1 Methodology

2.1.1 Study Design

A health facility based cross-sectional study was conducted. Either sputum, Bone-marrow, gastric washings, Aspirates or Pus and whole blood that were collected from consenting adult ≥ 18 years HIV positive patients attending Mpiolo OI clinic from 01 March 2012 to 31 July 2012 were tested for MDR-TB and CD4 cell count respectively. Risk factors were also assessed.

Study Setting:

The study was carried out at Mpiolo OI clinic in Bulawayo, Zimbabwe. Mpiolo OI clinic is one of the clinics that administers anti-retroviral treatment (ART) and treats any opportunistic infections of HIV positive patients in Bulawayo.

Reference population: All adult ≥ 18 years HIV positive patients in Zimbabwe.

Source population: Adult HIV positive individuals who were TB suspects and TB patients and registered at Mpiolo OI clinic.

Sampling frame:

- Convenience sampling was used and all consenting adult patients attending Mpiolo OI Clinic, during the study period who fitted the inclusion criteria were recruited daily until at least 274 samples were collected.
Inclusion criteria:

- All adult (≥18) patients who were suspected to have TB (signs and symptoms e.g. fever, weight loss, night sweats, swellings at site of infection in the case of extra-pulmonary TB) and patients who were confirmed TB cases visiting the OI clinic between 01 March 2012 to 31 July 2012 were eligible. Those who consented to participate in the study were recruited.

Exclusion criteria:

- HIV patients without TB or who were not suspected of TB.

- HIV patients below the age of 18 years

**Study Sample:**

All patients who consented after meeting the inclusion criteria.

**Sample Size**

From the literature, the prevalence of MDR-TB in HIV positive patients ranges between 20-39% in South Africa. It was expected that the prevalence in Zimbabwe to be lower than that of South Africa.

Setting alpha at 5%, power at 80% and prevalence of 20%, this implied that the sample size should be at least 246.

Taking into consideration the 10% laboratory contamination of TB cultures implied that at least 274 samples should be examined to achieve 80% power at 5% significance level.
Study Factors

- Human samples [Sputum/gastric washings, bone marrow, aspirate, CSF]
- Risk factors (age, sex, occupation, recent travel to South Africa, TB contact, Isoniazid prophylaxis, duration of stay in congregate areas, hospitalization, duration on ART, CD4 count)

Outcome Factor:

The outcome factor to be measured was MDR-TB as measured by drug susceptibility testing.

2.1.2 Pilot Testing

A feasibility study was done to assess if the study was doable and also to assess any gaps in the collection of data. Consenting patients meeting the inclusion criteria were recruited and samples for MDR-TB diagnosis and CD4 count were collected. Information on demographics and key sections on risk factors was captured. Samples were sent to the laboratory and tested. Results of the whole process were reviewed and a gap analysis was done. Recommended improvements on the gaps indentified, were made on the designed questionnaire (such as removal of health worker on risk factors) and logistics for sample collection, transport and processing. The modified tools were used in the main project.

2.2. Data collection

1. Demographics and associated risk factors

A research assistant was recruited and trained to interview using the questionnaire: to allocate a unique Identifier on each patient; register patients in the TB register and to
collect sputum and whole blood for CD4 count. Each patient meeting the inclusion criteria and who consented to enter the study was interviewed using the questionnaire appendix 7. Their responses were recorded against the response (by circling). Each completed questionnaire was given a unique identifier.

2. Clinical samples (collection and transportation)

Sample collection and transport

1a). Sputum samples were spot samples collected from the patients using standard sputum collection procedure. Clean and properly labeled, wide mouthed containers were used to collect sputum. A nurse gave instructions and supervised how to collect sputum to the participant who was suspected of pulmonary TB as per standard clinic procedure.

1b) the physician collected pus, gastric lavage, aspirates, CSF or bone marrow for Extra pulmonary TB [EPTB] suspects and EPTB patients.

2. Whole Blood for CD4 was collected from the vein using syringes and put into EDTA tubes. The samples were transported to the National Tuberculosis Reference laboratory[NTBRL] as soon as possible (at most within two days from the time of collection) for quality assured bacteriological examination: Appendix 8: certificates of participation in External Quality Assurance[EQA] with a Supra-National Reference laboratory

Whole blood in EDTA tubes for CD4 count was sent to the Hematology laboratory for quality assured testing Appendix 9: participation in EQA
2.3 Laboratory Methods (Adopted From National TB Reference Laboratory
Standard Operation Procedures)

Sample processing

At the NTBRL standard laboratory procedures were followed. The samples were logged into the log book. The information on the patient sample was matched with the information on the form. If they were matching, the sample passed to be tested for Tuberculosis. The sample was then assigned a culture number which was put on both the form and the sample. The following procedures were performed on each sample: Direct Smear microscopy using the ZN technique, decontamination/digestion, inoculation on solid/liquid media, identification and drug susceptibility testing and reading and interpretation of results.

1. Protocol for Direct Smear Microscopy and the ZN Method

**Direct smear microscopy:**

Working in the Biological Safety Cabinet (BSC)

A new and clean slide was labeled with the assigned culture number, using a diamond marker. A smear was made using an applicator stick. The smears were left to air dry for at least 20 minutes.

The ZN method was used to stain the smears and the following steps were followed:

1. Slides were put on a staining rack with the smear facing up.
2. A known positive and negative control was put on each batch before staining for quality assurance.

3. Slides were heat fixed using a flame.

4. Carbolfuchsin was flooded onto the slide and heat was applied until steaming, waited for 5 minutes and heated again and waited for 5 minutes.

5. Rinsed gently with running water

6. Added Acid Alcohol decolorizer for 3 minutes

7. Rinsed gently with water

5. Added Methylene Blue for a minute.

6. Rinsed with water.

7. Left to dry.

The smears were examined using a light microscope for the presence of Acid Fast Bacilli [AFB] which take up the red color of the carbolfuchsin in the stain. For quality control: the known positive and negative controls were examined first and if they are correct, then the rest of the samples were examined.

Results were recorded on the data collection sheet. If there were no AFBs a negative result was reported. If there were AFB present, a grading chart was used to quantify the bacilli. Results were then recorded
2. Protocol for Decontamination/Digestion and Inoculation Procedure:

1. A 50ml, sterile centrifuge tube was labeled with the assigned culture number.

2. Two more tubes for the known positive and negative controls were also labeled.

3. Using a sterile Pasteur pipette, 1-2ml of sample was put into the labeled 50ml tube.

4. Sample digestion and decontamination was done using the Petroff’s sodium hydroxide method. An equal volume of 4% sodium hydroxide was added to the sample (sputum/gastric washings),

   Positive control (H37Rv) and negative control were included for quality control.

   Note: Sterile samples such as bone marrow, pleural/knee aspirates, were not decontaminated with sodium hydroxide.

5. After vortexing samples are left to digest for 15-20 minutes.

6. Phosphate buffer then was added up to the 50ml mark to neutralize the sodium hydroxide.

7. All samples including the sterile ones (bone marrow, pleural aspirate) were loaded into the refrigerated centrifuge. The aerosol containment caps were clipped onto the buckets, closed the centrifuge lid tightly, and turned on for 15 minutes and centrifuged at 3000 gravitational force.

8. during centrifugation:

   i) Liquid culture media was prepared by reconstituting the commercially made PANTA with the growth supplement. Reconstitution of the PANTA was done within the BSC.
The prepared media was labeled with the assigned culture numbers of samples and also for *known Positive and negative controls for quality control*.

ii) The prepared and quality controlled Lowenstein Jensen solid culture slopes (one with pyruvate and another one plain media) were labeled with the assigned culture numbers, *known positive and negative control slopes for quality control* were included.

9. The centrifuge was allowed to come to a coasting stop (*Note*: the brake was not applied to the centrifuge. The brake creates friction, which disturb the pellet)

10. Once the centrifuge came to a stop, 5 minutes were allowed for any aerosols within the tubes to settle (remember centrifugation does contribute to aerosol formation), the lid was then opened, carefully to remove the buckets, whilst taking care not to disturb the sediments. The buckets were placed into the BSC.

Never open the centrifuge buckets outside of the BSC!

11. The safety caps were removed from the buckets, and carefully removed the individual tubes so as not to disturb the pellets, and inspected for leakage (*Note*: if tube breakage had occurred, the broken tube was discarded into the contaminated waste within the BSC, the bucket and aerosol containment cap were decontaminated with 5% phenol, and the BSC was allowed to purge for 15 minutes, before resuming the inoculation of samples to culture

12. A funnel was placed over a discard waste container, to which 5% phenol had been added. All supernatant was decanted into the discard container, care was taken not to pour off the pellet.

13. 1-2 mL phosphate buffer was added to all tube sediments.

14. All centrifuge tubes were vortexed to mix the sediments
15. Caps of all MGIT tubes were completely loosened (but were not removed)
16. 0.8 mL of reconstituted PANTA was added to each tube, changing tips between tubes
17. A major source of contamination is the process of adding of PANTA anti-microbial suspension to MGIT tubes. Thus this was done within the BSC, and caps were removed and recapped one at a time when PANTA was added.
18. Following the addition of the PANTA to each MGIT tube, the caps of all MGIT tubes were tightened, and inverted to mix the PANTA.
19. The cap of the first MGIT tube was loosened, but was left in place
20. The MGIT tube was inoculated using a sterile disposable pipette, with 0.5 mL of well mixed inoculum (mixed gently, careful not to create bubbles, using disposable pipette). The cap on the MGIT tube was replaced but was not tightened
21. After inoculating the MGIT tube, the Lowenstein Jensen slope were also inoculated with 4 drops of well mixed inoculum from the same sterile disposable pipette, and then went straight down with the loaded pipette to inoculate the slide, making a 1X2 cm smear, great care was taken not to create any bubbles in the process. The pipette was then discarded into the waste container at this point.
22. With hands free of any pipettes, the LJ slope were manipulated so that the inoculum was spread over the entire surface area, and then placed slope in wire rack, horizontally, with the cap loosened.
23. Inoculation of the MGIT vial, LJ slope and smear were all be done using the same pipette (of course a different pipette for each sample, and as long as care was taken to prepare the smear last)
24. Remaining sediments were transferred to labeled screw cap cryotubes, and frozen

25. Smears were laid flat on the work surface of the BSC, until they had air dried. With each smear that was prepared, it was inspected closely for any bubbles, and if a smear did have bubble(s), i that smear was isolated well away from all other smears, while it was left to dry.

26. The rack of LJ slopes were placed in the 37°C incubator, in the horizontal position, with caps loosened. Following overnight incubation, caps were tightened, and tubes were placed in upright position

27. The caps of the inoculated MGIT tubes were tightened, and were inverted to mix inoculum,

28. The outside of MGIT tubes and caps was wiped with 5% phenol

29. The individual bar codes were swiped on the BACTEC 960 instrument, and loaded into the appropriate spaces, indicated by the green light

3. Protocol for Culture Observation for Growth

Observation for growth and no growth was done for both solid and liquid cultures using following steps:

a) Culture On Solid Media (LJ Slopes)

1. Lowenstein Jensen slopes were observed within 24 hours of initial incubation.

If the slants were overgrown with contamination (either media had completely liquefied or more than half the surface was contaminated with non acid fast growth), they were discarded
2. If the slants did not appear contaminated, the caps were tightened, and placed back in the incubator, in an upright position

3. They were continued to be observed on a weekly basis, through 8 weeks

4. Using a class II biological safety cabinet, any suspicious colonies of *M. tuberculosis* were smeared onto a slide in a drop of sterile saline, allowed to air dry, heat fixed, and stained with the ZN AFB stain procedure, and observed microscopically for acid fastness and morphology consistent for *M. tuberculosis* (AFB in “cords” or tight clumps)

5. LJ slopes that became overgrown (either completely liquefied or more than half the surface has overgrown with non acid fast contamination) at some point between inoculation and the end of 8 weeks, were discarded

6. LJ slopes yielding no growth after 8 weeks incubation were discarded

7. Results from ZN stained microscopic examination were so noted on the data collection tool

b) Cultures On Liquid Media:

1. While MGIT vials were incubated in the MGIT 960 instrument, they remained undisturbed, so as not to alter the oxygen gradient, until they were read out as positive

2. Once MGIT tubes had been placed in the BACTEC MGIT 960 instrument, the front panel was observed daily for a red light signal indicating a positive vial

3. Positive vials were removed from the instrument, and were observed grossly with the naked eye for particulate flakes of growth, which was consistent with *M. tuberculosis*.

4. Using a class II biological safety cabinet, one free falling drop of commercial Bovine Serum Albumin was placed onto a pre-cleaned microscope slide and then mixed with 3-4
drops of liquid from a positive MGIT vial, allowed to air dry, heat fixed, stained with the ZN AFB stain procedure, and observed microscopically for acid fastness and morphology consistent with *M. tuberculosis* (AFB in “cords” or tight clumps)

5. MGIT tubes that showed no growth at the end of 6 weeks were removed from the BACTEC MGIT 960 instrument and discarded, however before discarding a visual check of each tube was made for turbidity, for slow growers that might not have depleted the oxygen source to trigger a positive reading on the 960 instrument. For these samples, a smear was prepared to rule out AFB

6. Results from ZN stained microscopic examination are so noted on the data collection tool

7. Positively flagged MGIT vials, yielding only non acid fast bacteria, were discarded and recorded as contaminated.

8i. The results of the concentrated smear were checked, and, if positive, the frozen cryotube was retrieved, re-decontaminated, and inoculated on a fresh Lowenstein Jensen slope, MGIT vial, and concentrated smear.

8ii. If the concentrated smear was negative, over growth of culture media was reported and no attempt was made to re-decontaminate the frozen concentrate

9. Positively flagged MGIT vials, yielding a mixture of AFB and non acid fast bacteria were re-decontaminated.

10. Once a positive vial had been flagged as positive, and, by ZN appeared to be pure (not contaminated with NAF), it was sub cultured to a Blood Agar Plate for purity check. If no contamination was present then the primary MGIT tube was used for further testing (e.g., drug susceptibility testing). An LJ slant was also sub cultured at this point for further
testing and back-up. If this non-contaminated MGIT tube was over 3 days old, it was sub cultured to another MGIT tube for upcoming drug susceptibility testing.

4. Protocol for Identification from Culture:

1. All positively flagged MGIT tubes, which had been confirmed to be ZN smear positive were then identified using MPT 64 antigen detection kit (SD Bioline) which identifies MPT 64 antigen on *Mycobacterium tuberculosis species*

2. Cultures that were positive with SD Bioline were reported as *Mycobacterium tuberculosis* (MTB) or *Mycobacterium* other that tuberculosis [MOTT] was reported for cultures that were negative with SD Bioline. *The reagent strip has a positive control imbedded for quality control and it shows on every strip as a line when a sample has been added. A known positive and negative slide was added to quality control the ZN technique.*

*N.B. If it is MOTT, drug susceptibility was not done.*

5. Protocol for Drug Susceptibility Testing (DST) Using The 1% Proportional Method on Solid Culture Media

If it was MTB from the SD Bioline, first –line drug susceptibility testing (DST) was performed on solid culture, the conventional 1% proportional method was used. The drugs that were tested are Isoniazid, Ethambutol, Rifampicin and Streptomycin.

**Inoculation of Drug Susceptibility Battery:**

With Para Nitro Benzoic Acid [PNB]) and 16 drugs (4 drug, each two concentrations) Containing LJ slopes:

- LJ without drugs
- LJ with PNB
- LJ with Rifampicin (RA), 32 ug/mL, 64 ug/mL
- LJ with Isoniazid (INH), 0.2 ug/mL, 1.0 ug/mL
- LJ with Ethambutol HCl (EMB), 2.8 ug/mL, 4.0 ug/mL
- LJ with Dihydrorosptomycin sesquisulfate (SM), 8.0 ug/mL, 16 ug/mL

2. For each sample that was being tested, one sterile bijou bottle containing 6-8 sterile glass beads was labeled, with approximately 3 mL sterile distilled water.

3. Using either a sterile orange stick or inoculation loop, several colonies (appx. 5-10 mg), were picked within 1-2 weeks after appearance of growth, and were macerated into the glass tube containing beads.

4. The tube was secured tightly and vortexed at full speed for several minutes, then left to settle, undisturbed, for 5 minutes.

5. While inoculum was settling, the 1.0 McFarland was pulled from the dark (Note: McFarland Standard is kept in the dark at room temperature), about 10 mL sterile distilled water was added to a bijou bottle. The bijou bottle had the same volume of liquid and had the same dimensions as the tube containing the McFarland standard.

6. Using a sterile Pasteur Pipette, supernatant inoculum was withdrawn from the patient sample bijou bottle. This was done carefully so as not to disturb settled out heavier particles of inoculum.

7. This inoculum, was added drop wise, to a bijou bottle containing 10 mLs sterile distilled water, so as to approximate the 1.0 McFarland Standard. A third “Blank” tube that contained plain distilled water was available for comparison.

8. Necessary adjustments were done using more inoculum or sterile distilled water.

9. A white card with several horizontal black lines was used for background to help determine comparison of turbidity.
10. Necessary time was taken to properly adjust the inoculum to the 1.0 McFarland turbidity standard.
11. From this standardized suspension, two 100 fold dilutions (10^{-2} and 10^{-4}) were prepared
12. Ten slopes (8 drug containing slopes, a drug free LJ, and an LJ with PNB) were inoculated with 10 ul of the 10^{-2} dilution, a loop was used to spread the inoculum over the surface of the slope
13. Another ten slopes (8 drug containing slopes, a drug free LJ, and an LJ with PNB) were inoculated with 10 ul of the 10^{-4} dilution, and a loop was used to spread the inoculum over the surface of the slope
Culture inoculum adjusted to 1.0 McFarland

Prepare two 100X dilutions, 10^{-2}, 10^{-4}

10.0ul of 10^{-2}, 10^{-4} (drug free control only)

14. Slopes were incubated overnight at 37°C, with caps loosened,
15. The following day, the caps were tightened, and continued to re-incubate for 4 weeks, and were checked weekly.
16. Growth on slopes was read and recorded as:

Confluent growth = 3+
Innumerable discrete colonies = 2+
20-100 colonies = 1+
<20 colonies = record the number of colonies
No growth = NG
Contaminated = C

17. The expectation was that the test isolate would not grow on the LJ with PNB (typical of M. tuberculosis), and should grow on the drug free LJ
18. For any growth observed, a smear was prepared from that slope, and stained with ZN checking for typical acid fast morphology, in the absence of contamination. Growth on drug containing media was not assumed as resistance, without confirming with ZN smear

19. For isolates demonstrating resistance, the pattern was confirmed in MGIT

Quality Control:

For each batch of DTS run, *M tuberculosis* H37Rv or H37Ra isolate was included, as a drug susceptible control. Results of controls were read first and if they were acceptable, then proceeded to read the results of samples of patients.

8. Results And Interpretation

1. Culture and Sensitivity:

   Results interpretation

   • Growth was the presence of TB colonies on solid culture media and turbidity in liquid culture media.

   • Resistance was when there was growth in the presence of anti-TB drug

   • Sensitive was when there was no growth in the presence of anti-TB drugs

   Outcomes/Analysis of results

   • Samples susceptible to all drugs

   • Samples resistant to all drugs

   • Samples resistant to isoniazid and rifampicin(MDR)

   • Samples with mono resistant to isoniazid ,rifampicin, streptomycin or ethambutol

   • Samples with poly resistance –resistant to any three combinations of drugs.
2. CD4 count

Samples for CD4 count were logged using a unique identifier into the data collection sheet used in the study. A qualified laboratory scientist, who was trained to process CD4 cell counts, was hired to perform the test. After sample preparation, a calibrated and certified Facs Calibur machine was used to calculate the CD4 cell counts. The following steps are followed:

1. The sample was mixed thoroughly using an automated rocker

2. Controls with known values were loaded into the machine and the identification was typed in,

   *The results of the controls were read, and if they were within the acceptable range, patient samples were then run.*

3. Each sample was loaded into the machine one at a time and the identification was typed in

4. Waited for the machine to perform the CD4 count

5. Got the results from the printer.

6. Results for the CD4 were recorded on the data collection sheet.

   * The information on CD4 count was attached to the data collection sheet for culture and sensitivity

*Results for controls with known values were recorded into the quality control register* and patients’ results were recorded onto the data collection tools
2.4. Data Management and Statistical analysis

Data Entry:

Data that was on the data collection tools (demographics, TB culture and drug susceptibility and CD4 count) was compiled for each participant. Data was then entered into STATA version 10. Double data entry was done by two people, to cross check if the entries were correct.

Data Cleaning:

Cross checking the data to see whether all parameters were available was done to assess which samples were to be included in the final analysis.

Statistical Analysis

Baseline characteristics of the participants were presented using percentages for categorical variables such as sex and median (range) for continuous variables such as age. Risk factors for MDR TB were determined using Fisher’s exact test for categorical risk factors and students t-test for continuous risk factors such as age. Logistic regression was used to calculate odds ratio. P value of less than 0.05 was considered statistically significant.
2.5 Ethical Issues:

Permission to carry out the study was sought from Mpilo hospital authorities, Laboratory Directorate

Approval was sought from Joint Research Ethics Committee (JREC) and Medical Research Council Zimbabwe [MRCZ].

No names appeared on the questionnaire and unique identifiers were used.

The purpose of the study, the possible benefits, the confidentiality of the findings, and discomfort (during collection of whole blood) were highlighted to the patients. All patients were given a chance to enter the study of their own free will and were told that there would be no repercussions for not participating. It was highlighted to the patients that they would not be paid since they had come for their routine check-up and not solely for the study. Patients who consented to enter the study were asked to complete a consent form in English or their vernacular language.
CHAPTER 3

RESULTS

To assess the prevalence of MDR-TB and associated risk factors among adult HIV positive patients registered at Mpilo OI Clinic, 275 HIV positive patients were recruited between 01March 2012 and 31July 2012 as they came to the clinic. The majority 178(65.4%) of the participants were females and the median (range) age in years was 39(18-72). (see Table 1). One sample was collected from each one of these HIV positive patients. Sputum samples for Pulmonary TB diagnosis were collected from 205 patients and aspirates for Extra-pulmonary TB diagnosis were collected from 70 patients making a total of 275 samples. These 275 samples were all cultured for MDR-TB using both the liquid (MGIT) and Solid LJ methods. Thirty-four 34(12.4%) samples were positive for *M tuberculosis*, 19(6.9%) had Mycobacteria other than tuberculosis (MOTT), 211(76.7%) were culture negative and 11 (4.0%) were contaminated thus were excluded from analysis (see Table 2). Susceptibility to first-line drugs (Rifampicin, Isoniazid, Streptomycin and Ethambutol) was performed on all 34 *M tuberculosis* isolates of which 7 isolates had resistance to both Rifampicin and Isoniazid( MDR-TB), 24 were susceptible to all the four anti-tuberculosis agents and 3 had Isoniazid mono- resistance. Considering all adult≥18years HIV participants who visited Mpilo OI Clinic during the study period and participated in the study, 7 participants had MDR-TB and 257 did not have MDR-TB. Therefore the crude prevalence of MDR-TB was 7(2.6%) among HIV positive patients who were registered at Mpilo OI clinic and visited the clinic between 01March 2012 and 31July 2012.
Table 1: Baseline Characteristics of adult ≥18 years HIV positive patients registered at Mpilo OI Clinic and attended the clinic between 01March to 31July 2012(figures in parantheses indicate %)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>39 (18-72)</td>
</tr>
<tr>
<td>Means (SD)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>180(65.4)</td>
</tr>
<tr>
<td>Male,</td>
<td>95(34.6)</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>131(47.8)</td>
</tr>
<tr>
<td>Widowed</td>
<td>73(26.6)</td>
</tr>
<tr>
<td>Single</td>
<td>70(25.6)</td>
</tr>
<tr>
<td>Patient category n(%)</td>
<td></td>
</tr>
<tr>
<td>Suspect</td>
<td>212(81.5)</td>
</tr>
<tr>
<td>Relapse</td>
<td>51(18.5)</td>
</tr>
<tr>
<td>Recent visit to South Africa</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>59(21.5)</td>
</tr>
<tr>
<td>No</td>
<td>206(78.5)</td>
</tr>
<tr>
<td>Previous history of TB contact:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>113(41.5)</td>
</tr>
<tr>
<td>No</td>
<td>159(58.5)</td>
</tr>
<tr>
<td>On Isoniazid Prophylaxis:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6(2.2)</td>
</tr>
<tr>
<td>No</td>
<td>268(97.8)</td>
</tr>
<tr>
<td>History of Hospitalisation past 12 months</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38(13.8)</td>
</tr>
<tr>
<td>No</td>
<td>237(86.2)</td>
</tr>
<tr>
<td>Frequency to OIC past 18 months</td>
<td></td>
</tr>
<tr>
<td>≤4times:</td>
<td>14(5.1)</td>
</tr>
<tr>
<td>&gt;4 times:</td>
<td>26(94.9)</td>
</tr>
<tr>
<td>Duration on ART</td>
<td></td>
</tr>
<tr>
<td>≤6mnths</td>
<td>37(13.5)</td>
</tr>
<tr>
<td>&gt;6mnths</td>
<td>238(86.5)</td>
</tr>
<tr>
<td>CD4 count</td>
<td></td>
</tr>
<tr>
<td>≤200cells/ul</td>
<td>90(32.7)</td>
</tr>
<tr>
<td>&gt;200cell/ul</td>
<td>185(67.3)</td>
</tr>
<tr>
<td>Type of TB</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>205(74.5)</td>
</tr>
<tr>
<td>Extra Pulmonary</td>
<td>70(25.5)</td>
</tr>
</tbody>
</table>
Table 2: Outcomes for TB culture done on samples submitted from adult ≥18 years HIV positive patients registered at Mpilo OI Clinic and attended the clinic between 01 March and 31 July 2012

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative MTB</td>
<td>211 (76.7)</td>
</tr>
<tr>
<td>MTB</td>
<td>34 (12.4)</td>
</tr>
<tr>
<td>MOTT</td>
<td>19 (6.9)</td>
</tr>
<tr>
<td>Contaminated</td>
<td>11 (4.0)</td>
</tr>
<tr>
<td>Total</td>
<td>275 (100)</td>
</tr>
</tbody>
</table>

The contamination rate was 4.0%
Table 3: Characteristics of MDR-TB positive and MDR-TB negative HIV positive adults (≥18 years) registered at Mpilo OIC and attended the clinic between 01 March-31 July 2012 (figures in parentheses indicate %)

<table>
<thead>
<tr>
<th>MDRT-TB STATUS</th>
<th>Total(%)</th>
<th>MDR-TB (7.2%)</th>
<th>No MDR-TB (257.97%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean (SD)</td>
<td>39.1 (2.8)</td>
<td>41.1 (0.7)</td>
<td>0.635</td>
</tr>
<tr>
<td>Sex n (%)</td>
<td>Female</td>
<td>5 (2.9)</td>
<td>167 (97.1)</td>
<td>0.0100</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>2 (2.2)</td>
<td>89 (97.8)</td>
<td></td>
</tr>
<tr>
<td>Marital Status n(%)</td>
<td>Married</td>
<td>5 (4.1)</td>
<td>118 (95.9)</td>
<td>0.361</td>
</tr>
<tr>
<td></td>
<td>Widowed</td>
<td>2 (2.8)</td>
<td>70 (97.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>0</td>
<td>68 (100)</td>
<td></td>
</tr>
<tr>
<td>Patient category n(%)</td>
<td>Suspect, Relapse</td>
<td>6 (2.8)</td>
<td>208 (97.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>Recent visit to South Africa</td>
<td>Yes, No</td>
<td>4 (7.1), 3 (1.4)</td>
<td>32 (92.9), 205 (98.6)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Previous history of TB contact:</td>
<td>Yes</td>
<td>4 (3.6)</td>
<td>105 (96.4)</td>
<td>0.458</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3 (2.0)</td>
<td>149 (98.0)</td>
<td></td>
</tr>
<tr>
<td>On Isoniazid Prophylaxis:</td>
<td>Yes</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>6 (2.7)</td>
<td>257 (97.3)</td>
<td></td>
</tr>
<tr>
<td>History of Hospitalisation past 12 months n(%)</td>
<td>Yes</td>
<td>2 (5.4)</td>
<td>35 (94.6)</td>
<td>0.255</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5 (2.2)</td>
<td>222 (97.8)</td>
<td></td>
</tr>
<tr>
<td>Frequency to OIC past 18 months n(%)</td>
<td>≤4 times:</td>
<td>1 (7.7)</td>
<td>12 (92.3)</td>
<td>0.301</td>
</tr>
<tr>
<td></td>
<td>&gt;4 times:</td>
<td>6 (2.4)</td>
<td>245 (97.6)</td>
<td></td>
</tr>
<tr>
<td>Duration on ART</td>
<td>≤6 months</td>
<td>0</td>
<td>36 (100)</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>&gt;6 months</td>
<td>2 (2.7)</td>
<td>257 (97.4)</td>
<td></td>
</tr>
<tr>
<td>CD4 count</td>
<td>≤200 cells/ul</td>
<td>5 (5.8)</td>
<td>82 (94.2)</td>
<td>0.041*</td>
</tr>
<tr>
<td></td>
<td>&gt;200 cells/ul</td>
<td>2 (1.1)</td>
<td>175 (98.9)</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05
Age

Although not statistically significant those with MDR-TB were younger with a mean age of 39.1(+/−2.8) than those without MDR-TB with a mean age of 41.1 (+/−0.7) (see table 3 above)

CD4 Count

There was a statistically significantly difference in CD4 Count between participants with MDR-TB and those without MDR-TB (p=0.041).(see Table 3 above)

Recent visit to South Africa

There was a statistically significant association between MDR-TB Status and Recent visit to South Africa (p-value 0.039). (see Table 3 above)
Table 4

Univariate regression analysis of risk factors for MDR-TB among HIV positive adults (≥18years) registered at Mpilo OIC and attended Mpilo OI Clinic between 01March 2012 to 31July 2012

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.9 (0.91-1.05)</td>
<td>0.635</td>
</tr>
<tr>
<td>Sex</td>
<td>0.75 (0.14-3.95)</td>
<td>0.735</td>
</tr>
<tr>
<td>Patient category</td>
<td>1.4 (0.17-12.01)</td>
<td>0.751</td>
</tr>
<tr>
<td>Previous history of TB contact:</td>
<td>4.5(0.67-29.81)</td>
<td>0.165</td>
</tr>
<tr>
<td>Recent visit to S.Africa</td>
<td>5.3(1.14-24.22)</td>
<td>0.033*</td>
</tr>
<tr>
<td>Patient on Isoniazid Prophylaxis:</td>
<td>0.12(0.01-1.18)</td>
<td>0.069</td>
</tr>
<tr>
<td>History of Hospitalization past 12 months</td>
<td>0.39(0.07-2.11)</td>
<td>0.277</td>
</tr>
<tr>
<td>Frequency to OI Clinic past 18months</td>
<td>0.29(0.03-2.6)</td>
<td>0.274</td>
</tr>
<tr>
<td>CD4 count</td>
<td>0.19 (0.04-0.97)</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

*p(< 0.05)
Univariate Analysis

Visit to South Africa in the past 18 months

In univariate analysis, MDR-TB prevalence was statistically significantly higher among participants who had recently visited South Africa compared to those who had not. Those who had visited South Africa had 530% risk of MDR-TB compared to those who had not (OR 5.3, p=0.033). Recent visit to South Africa was a predictor of MDR-TB prevalence. (See table 3)

CD4 Count.

A CD4 count of >200 cells/uL showed a statistically significantly protective characteristic to MDR-TB prevalence at 95% significance level (OR 0.19, p=0.048) (see table 3 above)

NOTE: CD4 count and recent travel to South Africa were statistically significantly associated with MDR-TB prevalence, they were then considered for a multivariate analysis. However, although not statistically significant on univariate analysis the following factors were selected for multivariable analysis because of their importance and also for known biological plausibility-Age, Gender, Previous history of TB contact, History of hospitalization, Receiving Isoniazid prophylaxis and Frequency to OI Clinic.
Table 5 Multivariate regression analysis of risk factors for MDR-TB prevalence among adult ≥ 18 years HIV positive adult patients registered at Mpilo OIC, who attended the clinic between 01 March and 31 July 2012.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Multivariate OR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.01 (0.92 - 1.11)</td>
<td>0.811</td>
</tr>
<tr>
<td>Sex</td>
<td>0.72 (0.10 - 5.3)</td>
<td>0.755</td>
</tr>
<tr>
<td>Category of patient</td>
<td>1.49 (0.13 - 15.17)</td>
<td>0.733</td>
</tr>
<tr>
<td>Recent visit to S. Africa</td>
<td>4.4 (0.72 - 27.43)</td>
<td>0.11</td>
</tr>
<tr>
<td>On Isoniazid prophylaxis</td>
<td>0.13 (0.01 - 1.68)</td>
<td>0.120</td>
</tr>
<tr>
<td>Previous history of TB contact</td>
<td>0.51 (0.09 - 2.99)</td>
<td>0.459</td>
</tr>
<tr>
<td>Frequency to OIC past 18 months</td>
<td>0.08 (0.0 - 1.47)</td>
<td>0.089</td>
</tr>
<tr>
<td>CD4 T cell count</td>
<td><strong>0.14 (0.02 - 0.94)</strong></td>
<td><strong>0.043</strong></td>
</tr>
</tbody>
</table>

*p < 0.05

Multivariate Analysis

In multivariate analysis CD4 count was statistically significantly associated with MDR-TB prevalence. MDR-TB prevalence decreased significantly in the patients with a CD4 count > 200 cells/μL by 86% (OR 0.14, p = 0.043)

Age, sex, category of patient, recent visit to South Africa, frequency to OI Clinic, duration on ART, Previous history of TB contact, and receiving Isoniazid prophylaxis did
not show a statistical significant association with MDR-TB prevalence at 5% significance level in the multivariate analysis. Therefore the final model can be presented as follows:

\[
\text{MDR-TB prevalence} = 3.06 - 1.93 \text{CD4 count.}
\]

This implies that for a change in CD4 Count from \(\leq 200\) cells/\(\mu\)L to > 200 cells/\(\mu\)L when other factors are held constant, MDR-TB prevalence decreases by 193%.

Comparison of Laboratory methods

The overall contamination rate was 4.0%. The individual contamination rates of both methods was high 26(9.5%) and 32(11.7%) for LJ and MGIT respectively. The pick up rate of positive cultures was 36(13.2%) for MGIT and 23(8.4%) for LJ. The ZN stain picked 10(3.7%) positives from both extra-pulmonary and pulmonary samples. Only 5(2.6%) were picked from sputum samples (PTB cases). Of the 19 Mycobacterium other than tuberculosis (MOTT) the LJ method managed to pick only 1(5.2%) and the MGIT method picked 18(94.6%).

NB

The 34 TB Culture positive samples give a TB prevalence of 12.8% among all the adult HIV positive patients who attended Mpilo OI Clinic between 01 March and 31 July 2012.

The proportion of MDR-TB among the 34 TB Culture positive HIV positive patients who attended Mpilo OI Clinic between 01 March and 31 July was 20.6%.
Chapter 4

DISCUSSION AND CONCLUSIONS

MDR-TB Prevalence

In this study, an MDR-TB prevalence of 2.6% was found among adult HIV positive patients registered at Mpilo OIC who visited the clinic between 01 March and 31 July 2012. The null hypothesis that the prevalence would be higher than 20.6% was thus refuted.

This prevalence of 2.6% among adult HIV positive patients registered at Mpilo who visited the clinic between 01 March and 31 July was very low compared to findings from studies carried out in Europe. Prevalence of 43%, 39.9%, 40.3%, 37% and 36% was found in Peru; Chennai, India; Siberia; Mumbai, India and Italy respectively. In South Africa MDR-TB prevalence among HIV patients of 20.6%, 21.0% and 39% was documented in Phisidia, a rural area in Kwa-Zulu Natal and Msinga respectively. The prevalence is very high compared to what was found in this study because other studies documented MDR-TB prevalence among TB culture positive patients (culture negatives are not included) whereas in this study prevalence of MDR-TB was calculated among all patients who participated in this study (including culture negative results). Most of the studies documented prevalence of MDR-TB during outbreaks therefore there was a possibility of selection bias which could have resulted in the selection of participants (hospitalized patients) who were at a high MDR-TB prevalent area. The prevalence was therefore bound to be high. Whereas in this study the subjects were recruited as they came routinely to collect their medication and a possibility of not having been exposed to a high MDR-TB prevalent area.
An MDR-TB prevalence of 5.1% (95% CI 2.4–9.5) of rifampin or rifampin plus INH resistance was found among sputum culture positive patients in a study done in Durban among patients who were initiating ART. This was similar to a prevalence of 2.6% found among patients who participated in this study. Considering the fact that Durban is in South Africa and the prevalence was calculated among TB culture positives only, the prevalence of MDR-TB is expected to be much higher than in this study. There could have been selection bias in the study carried out in Durban as participants were recruited from a private hospital—hence affluent meaning that they might not have been exposed to known predictors of TB transmission such as overcrowding contrary to participants in this study (who are mainly from low social class), however further studies need to be done to ascertain potential environmental disparities. Although the sample size in the study carried out in Durban was larger compared to this study, 1035 participants versus 275, prevalence of MDR-TB was low maybe because only sputum samples for PTB were tested thus they might have missed other cases who had EPTB and grossly underestimated the prevalence.

A prevalence of 2.6% of MDRTB in HIV positive patients who visited Mpilo OI Clinic between 01 March and 31 July could have been an underestimation of the actual because patients who normally visit the OI clinic are generally well (and might not be having MDRTB), the very sick ones who could have a possibility of harboring MDR-TB sometimes send relatives to collect their medication while they remain at home or will be hospitalized. Thus potential cases of MDRTB could have been missed.

Although a lot of potential MDR-TB positive cases could have been missed, a prevalence of 2.6% among all adult HIV positive patients who came to IO clinic is very high.
compared to a prevalence of 3% found in new cases (only) and 6% found in retreatment cases (only) in a drug resistant survey carried out in Zimbabwe in 1996. However, the prevalence was not stratified by HIV status. There is need to conduct a drug resistant survey and stratify MDR-TB by HIV status to establish the actual burden in the HIV positive patients.

Risk factors

CD4 Count

It is known that individuals who are HIV positive have compromised immune systems, and they are thus more susceptible to reactivation of their latent TB infection as their CD4 T lymphocyte count falls. This was also shown in this study whereby in multivariate analysis CD4 count was statistically significantly associated with MDR-TB prevalence. It was shown that for a change in CD4 T cell Count from $\leq 200$ cells/ul to $> 200$ cells/uL when other factors are held constant, MDR-TB prevalence decreases by 193%. This was also in line (though not statistically significant) with a high frequency to OI Clinic which tended to be protective of MDR-TB prevalence (OR 0.29 (CI 0.03-2.6)) – HIV positive patients who frequent the OI clinic many times show that they are compliant to taking medication, thus are bound to maintain a high CD4 count.

Travel to South Africa

Although recent travel to South Africa did not show a statistical significance association with MDR-TB prevalence in multivariate analysis, the odds of having MDR-TB was 4.4 times more for patients who travelled to South Africa compared to those who did not in univariate analysis. It has been documented that close proximity to a high MDR-TB
prevalent area/country is a risk factor for neighboring countries. This was in line with the report by Dr Gandhi that the majority of MDR/XDR TB cases in the region, was no longer limited to Tugela Ferry but were found in other neighboring countries—Botswana, Mozambique, Lesotho, Zambia and possibly Zimbabwe. There is need to carry-out a bigger survey that includes many OI sites in Zimbabwe to establish the risk of MDR-TB posed by travelling to countries in the SADCC Region. In this study the number of MDR-TB positive patients was small.

Previous History of TB

Previous history of reported TB was not statistically significantly associated with MDR-TB in this study. This was similar to findings in other studies. As observed in Tugela Ferry, substantial proportion of patients with no prior history of TB had evidence of drug resistance. In a study carried out in Peru although participants with HIV infection had had previous history of TB, it was also not significantly associated with MDR-TB in HIV positive patients. However this is contrary to current findings in Europe where the incidence of MDR-TB is particularly high in Eastern Europe and in the eastern Mediterranean and the percentage of MDR-TB among retreatment cases is approaching or exceeding 60% in three oblasts of the Russian Federation. This difference could be attributable to the limitation of small number of MDR-TB positive patients in this study compared to a large study which was done in Russia. A similarly contrary observation was found in Phidisa, South Africa where a prevalence of MDR-TB 20.6% was reported and that most of the patients investigated had received prior anti-tuberculosis drugs that was presumably not curative. There could have been reporter or information bias which could have overestimated or underestimated the proportion of previously treated patients.
in the Phisidia study and this study respectively, since prior history of TB treatment is based on self report.

Results from a systematic review by Sujit et al suggested that HIV infection is associated with primary MDR-TB\textsuperscript{17}. It was also reported that a significant number of new TB cases are diagnosed with MDR-TB\textsuperscript{36}. In this study all the MDR-TB positive patients were suspects who had not reported prior history of TB treatment. This was also in line with findings in other studies which showed that the HIV infected and not previously treated for TB are at risk for exogenous infection with drug –resistant \textit{mycobacteria} (primary resistance)\textsuperscript{10,35,31}. However in all these studies there was no statistical significance association of MDR-TB and category of TB patient (i.e. suspect/new TB case/ Relapse /Defaulter). The lack of association could also be attributed to misclassification bias posed when the participants wrongly reported past history of TB treatment. Hence there is need for future well designed studies in all regions of the world so as to clarify the relationship between MDR-TB and HIV\textsuperscript{17}

History of Hospitalization

Although not statistically significant the lack of a prior history of hospitalization was protective (OR 0.39 CI 0.07-2.11) of having MDR-TB prevalence, compared to participants who had been hospitalized. These findings were in line with findings in a study carried out in Peru\textsuperscript{4}. This was however contrary to studies done in other countries where being hospitalized was found to be a significant predictor of an increased MDR-TB prevalence. In Spain –all patients who had MDR-TB were hospitalized, they finger printed the isolates and showed that the patients were infected by the same strain of MDR-TB
strain. Just like in South Africa, Italy and Argentina it was concluded that nosocomial transmission was the cause of MDR-TB outbreaks. Nosocomial transmission occurs in hospitals hence the study subjects had been hospitalized when the studies were done and hospitalization was bound to be significantly associated with MDR-TB prevalence. In this study there was a probable underestimation of prevalence of MDR-TB among participants who had a previous history of hospitalization. Those who were hospitalized at that particular time of the study were not included in the study because they were admitted in medical wards and did not visit the OI Clinic. The very sick ones recently discharged from hospital might also have been left out in this study if they could not visit the OI Clinic during the time of the study. This study also focused on ambulatory patients contrary to the other studies which were conducted on hospitalized patients. This selection of hospitalized patients only could have resulted in an overestimation of association of MDR-TB prevalence and hospitalization in these studies.

TB contact

Prior History of TB contact and high frequency to congregate settings were not significantly associated with MDR-TB prevalence in this study and similar findings were reported in Peru. However, the rate of exposure of susceptible persons to TB is one of the factors that drive TB transmission. Despite the prevalence of HIV and TB co-infection throughout the world there is ongoing work to determine conclusively whether there is a correlation between HIV status and transmission of TB. Failure to establish an association could have been attributable to reporter bias of previous history of TB contact and also to the fact that the number of MDR-TB patients was small. Recall bias could
have reduced the chances of establishing an association if patients did not remember whether they had been in contact with a TB patient or not.

Gender

Contrary to findings from a study done in Lebanon which showed that male patients appeared more likely to be infected with and to develop MDR-TB compared to women, there was no significant difference in gender between MDR-TB positive patients and those without MDR-TB in this study. Gender was also not found to be predictor of MDRTB prevalence in Peru. However in this study there were more females than males so it was not comparable.

Laboratory methods

The laboratory methods used fairly reduced measurement bias and maintained the internal validity of the study. The samples were cultured using both the Liquid using the BACTEC Mycobacterium Growth Indicator Tube(MGIT) 960) machine and Solid LJ methods, being run in parallel. The best result was taken as the final culture result. This means that if a result was negative with MGIT and was positive with LJ, then the final result which taken was positive or if a sample was contaminated with LJ but negative or positive with MGIT, the MGIT result was considered as the final result. Contamination was only recorded when the culture was contaminated on both methods. Hence the overall (contaminated on both LJ and MGIT) contamination rate was lower 4.0% compared to the individual contamination rates of 9.5% and 11.7% using the Solid LJ(contamination on solid culture only) and Liquid (MGIT ) (contamination on MGIT only) methods.
respectively. Therefore contamination did not affect the statistical power of this study since the sample size had been calculated catering for a 10% contamination rate.

The algorithm used of running the two methods at the same time maximized the chances of diagnosing MDR-TB. The pick up rate of positive cultures was higher 36(13.2%) on liquid culture technique (MGIT) 960) compared to 23(8.4%) the conventional solid LJ culture technique-which is the ‘Gold standard’\textsuperscript{20,21}. These results were similar to results obtained from an evaluation of the liquid culture technique (MGIT) against the conventional solid (LJ) culture technique (the Gold standard) where 41% of the samples were positive using the MGIT and 24% were positive using LJ\textsuperscript{22}. Similarly in another study 53% of the samples were positive using the MGIT and 39% were positive using the LJ media\textsuperscript{23}

The direct sputum smear microscopy using the ZN technique showed a very low sensitivity as it managed to pick only 5(2.6%) positives from sputum samples. It is known that the sensitivity of the ZN technique is low 22.3% - 43 %\textsuperscript{32,33}, and has been shown to be even lower in HIV positive patients\textsuperscript{34}. Thus the low sensitivity was expected and only confirmed that the ZN method is a poor method of choice in the diagnosis of TB/MDRTB in HIV positive patients.

**Limitations**

The major limitation of this study was that the number of MDR-TB positive cases was small.

It was a hospital based research and had bias over the selection study subjects. HIV positive patients who did not attend the OI clinic during the study period are excluded;
hospitalized patients who could go to OI clinic were also excluded. Those who volunteered to participate in the study could have been different from those who refused to participate. Hence the results cannot be generalized to all adult HIV patients.

The prevalence could have been underestimated because potential MDR-TB patients were left out when patients below 18 years were not included and all adults who did not visit the OI clinic during the study period were excluded.

There was potential survivor bias where only patients who were alive were studied.

The temporal sequence of events of associated factors and MDR-TB could not be established.

There was potential measurement bias as some participants did not produce a good quality sputum despite the number of times they repeated hence a false negative result could have been reported.

There was interviewer bias since information on risk factors was collected by a nurse who interviewed the study subjects.

Recall bias could have reduced the chances of establishing an association if patients did not remember whether they had been in contact with a TB patient or not.

**Conclusion**

A high prevalence of MDR-TB was found in this study. This means that the burden of MDR-TB is high among HIV-positive patients. Risk factors such as previous history of TB contact, previous history of TB treatment, hospitalization, frequency to congregate settings or receiving isoniazid prophylaxis were not significantly associated with this high MDR-TB prevalence. This shows that all HIV-Positive patients are at risk of contracting
MDR-TB. I then recommend that measures such as MDR-TB active case finding (through culture and DST before treatment with First line drugs) be adopted. New technologies such as PCR techniques to be advocated for since they have a very short turn around (hours to at most 2 days) time for results compared to the conventional LJ solid culture technique (up to 70 days for Culture and DST).

I also recommend that NTP and Government ensures the availability of and compliance with infection control measures. The WHO guidelines recommend a hierarchy of Infection control (which include administrative, environmental and personal respiratory protection)\(^3\) these can be the first step into the initiation of infection control since most of these do not require a lot of funds if at all. Looking at the high individual contamination rates of the Liquid and Solid culture techniques and the much reduced overall contamination rate of the combined methods, it is recommended that both methods be run in parallel so as to increase timeous case detection.

The 12.8% prevalence of TB among all the adult HIV positive patients who attended the Clinic during the study period and 20.6% MDR-TB prevalence among TB culture positive patients found in this study is very high and it warrants further investigation. A hypothesis can be generated with a large sample size to find the relative risk of developing MDR-TB given that an HIV Positive patient has been exposed to TB. The high proportion of MOTT also warrants further investigations.
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03896@upch.edu.pe Received 15 February 2008.


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Appendix 1: letter to CEO Mpiolo Hospital

30 September 2011

No 14583, Selborne Park

Ihlosi road

Bulawayo

The Chief Executive Officer

Mpiolo Hospital

Box 2096, Mzilikazi Township

Bulawayo

Dear sir/madam

RE: Request to carryout a research at Mpiolo opportunistic Infections (OI) clinic

I am currently enrolled in the MSC Clinical epidemiology program with the Institute of Continuing Health Education, University of Zimbabwe. As part of the program I am required to carry out a research.

I would like to carry out a cross sectional study on Prevalence and associated risk factors of Multi-drug resistant tuberculosis in adult HIV positive patients attending Mpiolo OI Clinic. Anonymity of patients will be guaranteed as no names or identifying features will be recorded. Please find a copy of research proposal.

Thank you

Yours sincerely

Barbara Manyame
Appendix 2: letter to Director laboratory services

30 September 2011

No 14583, Selborne Park

Ihlosi road

Bulawayo

The Director Laboratory Services

Parirenyatwa Hospital

CY430

Causeway, Harare

Dear Mr Mangwanya

RE: Request to carryout a research in the National TB Reference Laboratory and Haematology laboratories

I am currently enrolled in the MSC Clinical epidemiology program with the Institute of Continuing Health Education, University of Zimbabwe. As part of the program I am required to carry out a research.

I would like to carry out a cross sectional study on Prevalence and associated risk factors of Multi-drug resistant tuberculosis in adult HIV positive patients attending Mpilo OI Clinic. I would like to run the samples for CD4 cell count, TB culture and Drug susceptibility testing for these patients. Anonymity of patients will be guaranteed as no names or identifying features will be recorded. Please find a copy of research proposal.

Thank you

Yours sincerely

Barbara Manyame
APPLICATION TO CONDUCT HEALTH/MEDICAL RESEARCH

This form must be completed by all persons/teams intending to conduct health/medical research in Zimbabwe. Upon completion by the investigator(s) it should be submitted to the Institutional Review Board (IRB) of the institution in which/under which the research is to be conducted. Upon completion of the relevant section by the IRB, the form should be submitted to the Secretary, Medical Research Council of Zimbabwe, P O Box CY 573, Causeway, Harare.

Protocol Version Number :........1........

Details of Research Team

Name of Principal Investigator (P.I) | Barbara Manyame
Nationality of P.I | Zimbabwean
Existing Qualifications | Specialist laboratory scientist
Academic Title | Laboratory Scientist
Institution & Dept. | Clinical Epidemiology, UZ
Postal address | Box FM 637 Famona, Bulawayo
E-mail address | manyamebarbara@yahoo.com
Telephone No. | 0772 742 277
Is this research expected to lead to the award of a higher degree? (Yes/No) | Yes
University/Institution where registered | University of Zimbabwe

Co-investigators Names | Qualifications | Institution/Department
Prof Robertson | UZ Mycology Department
Prof Rusakaniko | UZ department of Community medicine

Details of Research Coordinator

Name
Postal Address
E-mail Address
Telephone Number
Mobile Number
Details of the Proposed Research

Title of proposed research: Prevalence of Multi drug resistant tuberculosis in adult (>18 years) HIV positive patients attending Mpilo Opportunistic infection clinic

Proposed starting date: 01 February 2012
Proposed ending date: 30 June 2012
Performance site(s) in Zimbabwe: Mpilo OI Clinic
Performance sites (outside Zimbabwe): N/A
Total number of study personnel: 4
Budget (state currency): $23,252.00
Name and address of Funding agency: MOHCW
Status of funding: a)Submitted for funding ☐ b)Pending ☒ c)Funded ☒

Collaborating Institutions

1st 2nd 3rd

Population: Proposed inclusion criteria
(Check all that applies)
Males: ☒
Females: 
Adolescents (12 – 17 years): 
Children (Under 12 years of age): 
Pregnant women: 
Foetuses: 
Elderly (over 65 years): 
Prisoners: 
Cognitively impaired: 
Hospital inpatients: 

Type of study (check all that applies)
Survey: ☒
Secondary data: 
Program Project: 
Clinical community trial: 
Case control: 
Longitudinal study: 
Record review: 
Course activity: 
Other (specify): ...................................................

Consent Process (Check all that applies)
Written: ☐ English: ☐x Local Language: ☐x

Proposed sample size: 274

Reading level of consent document:
Below Grade 3 ☐ Below Grade 6 ☐ Below Form 2 ☐ Below Form 4 ☒ Above O level ☐ Graduate level ☐

Determination of Risk (Check all that applies)

Does the research involve any of the following

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human exposure to ionizing radiation</td>
<td>☒</td>
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<tr>
<td>Fetal tissue or abortus</td>
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<tr>
<td>Investigational new drug</td>
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<tr>
<td>Investigational new device</td>
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<td></td>
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<tr>
<td>Observation of public behaviour</td>
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<tr>
<td>Is the information going to be recorded in such a way that participants can be identified</td>
<td></td>
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<tr>
<td>Does the research deal with sensitive aspects of the participants behaviour, sexual behaviour, alcohol use or illegal conduct such as drug use</td>
<td></td>
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<tr>
<td>Could the information recorded about the individual if it became known outside of the research, place the participants at risk of criminal prosecution or civil liability</td>
<td></td>
</tr>
<tr>
<td>Could the information recorded about the individual if it became known outside of the research, damage the participant’s financial standing, reputation and employability?</td>
<td></td>
</tr>
</tbody>
</table>

- **Do you consider the proposed research**
  - A) greater than minimal risk  
  - B) minimal risk  
  - C) no risk

  *Minimal risk is a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as part of routine physical examinations.*

- Do any of the participating investigators and or their immediate families have an equity relationship with the sponsor of the project or the manufacturer or owner of the drug or device under investigation or serve as a consultant to any of the above?  YES  NO

  *If yes, please submit a written statement of disclosure to the Chairperson of the MRCZ*
Appendix 5: Informed consent form

Title: Prevalence of Multi-drug resistant tuberculosis among adult HIV positive patients registered at Mpilo OI clinic

Principal Investigator: Barbara Manyame, Msc Clinical Epidemiology student

Phone number(s) +263-09-200737

PURPOSE

Many factors suggest that HIV positive patients have a high risk for having Multi-drug resistant tuberculosis. This study seeks to assess prevalence of Multi-drug resistant tuberculosis (MDR-TB) in patients registered at Mpilo OI Clinic and to assess possible factors associated with MDRTB. You were selected as a possible participant in this study because you have been identified as TB suspect or you are already a known TB case. Approximately 276 patients will be recruited in this study.

PROCEDURES AND DURATION

If you decide to participate, you will undergo collection of a sample for Tuberculosis investigations. Either of the following samples will be collected depending on the doctor’s request: sputum, gastric washing, Cerebro-spinal fluid, pus, bone marrow or aspirate. A volume of 3-5ml will be withdrawn. The same sample will be used for your routine testing and also for the study. All collected samples will be incinerated after testing. Information on demographics and possible risk factors is solely for the study.

RISKS AND DISCOMFORTS

There is discomfort in the withdrawing of blood for CD4 count. For patients suspected to have extra pulmonary tuberculosis, there is discomfort in the collection of either pus, Cerebrospinal fluid, Bone marrow or aspirates.

BENEFITS AND/OR COMPENSATION

You will benefit from knowing whether you have Multi-drug Resistant Tuberculosis or not. There will not be any financial benefits.

CONFIDENTIALITY

If you indicate your willingness to participate in this study by signing this document, we plan to disclose the findings to the National Tuberculosis Control program and Mpilo OI. Any information that is obtained in connection with this study that can be identified with
you will remain confidential and consent forms will be kept under lock and key in the National TB Reference laboratory for 3 years and then destroyed.

Responsible person : Barbara Manyame +263- 09-200737 cell +263 772 742 277

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you decide not to participate in this study, your decision will not affect your future relations with the Mpilo OI Clinic its personnel, and associated hospitals such as the City council Clinics and Mpilo hospital. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time without penalty.

QUESTIONS/Problems

Before you sign this form, please ask any questions on any aspect of this study that is unclear to you. You may take as much time as necessary to think it over.

AUTHORIZATION

I have read and understood this paper about the study. I understood the possible discomforts and benefits of the study. I know being in this study is voluntary and I will not lose any benefits entitled to me .I will get a copy of this consent form(initial all the previous pages of the consent form)

__________________________________________ __________
Name of Research Participant (please print) Date

__________________________________________
Signature of Witness Signature of Staff Obtaining Consent
Appendix: 6 I Fomu Lesivumelwano

Inhloko: Ukukhula kwe Multi-drug resistant tb.kwabantu abadala abagula nge hiv ababhaliswe e Mplilo OI clinic.

Umongameli wesifundo Barbara Manyame :isifundi se Msc Clinical Epidemiology

I nombolo zefoni +263-09-200737

Isizatho sesifundo

Kule zizatho ezinengi ezitshengisa ukuthi izigulane zeHIV zilakho ukubamba umkhuhlane we Multi-drug resistant TB.Lesisifundo sidinga ukwazi ukukhula kwe Multi-drug resistant TB(MDR-TB) kuzigulane ezibhaliswe eMplilo OI Clinic njalo lokudinga izizatho ezibangela i MDR-TB.Ukhethiwe njengo munye ongaphathisa kulesisifundo ngoba ungabe utholakale mhlawumbe uleTB loba utholakale ulayo iTB.Phose izigulane ezingamatshumi amabili lesikhombisa okulesithupha bazanxuswa kulesi sifundo.


INHLUNGU EZINGATHOLAKALA :Kulobuhlungwana ekuthatheni kwedwazi kudingwa inani leCD4.Kuzigulane ezicatshangelwa ukuthi zile TB yamaphaphu(extra pulmonary tuberculosis),kulobuhlungwana ekuthathweni kobomvu,lananzi atholakala emgogodhleni(cerebrospinalfluid,umkantsho kumbe ukuthatha amasampuli emzimbeni.

OZAKUTHOLA KULESISIFUNDO :Uzobakwazi ukuthi ule (Multi-drug Resistant Tuberculosis kumbe hatshi.Awusoze uthole imva ngemva kwalesisifundo.
INFIHLO YALESISIFUNDO: Ngemuva kokuvuma kwakho ukungena kulesisifundo umaususayinile leliphepha, sizahambisa esikuthole kulesisifundo ku (National Tuberculosis Control program) laseMpilo OI, Yonke imbhalo etholakale kuwe kulesisifundo izahlala iyimfihlo wonke amafomu azavalalwa eNational TB Reference Laboratory okweminyaka emithathu, ngemva kwalokho azotshabalaliswa.

UMPHATHI WALESI SIFUNDO: Barbara Manyame +263-09-200737 : 0772 742 277

UKUZIKHETHELA KWAKHO UKUNGENA KULESISIFUNDO: Ukungena kulesisifundo kuyangokuzikhethela kwakho umaukhethe ukungangeni kulesisifundo, lokho akusoz e kuhlambanise ubudlelwano bakho leMpilo OI Clinic, kunye labasebenzakhona, kunye lezibilhedlela ezizilelela leMpilo OI clinic, lezi zibilhedlela Ngamakilinika ekhansili le sibilhedlela se Mpilo. Umavumile ukungena kulesisifundo, ulelungelo lokuphuma kulesisifundo ungasaqhubekeli phambili kungela kukusola.

IMIBUZO/INKINGA: Ungakasayini leliphepha, uyakhuthazwa ukuthi ubuze imibuzo mayelana ngalesisifundo, nxa kulalokho ongakuzwisisiyo, ungathatha isikhathi osifunayo ukuthi ukukhangelise.

ISIVUMELWANO: Sengibalile ngazwisa iphepha leli mayelana ngalesisifundo, njalo ngiyazi ukuthi kungaba lobohlungwana ekuthatheni kwamasampuli, lenzuzo yalesisifundo. Ngiyazi ukuba ngizikhethele ukuba kulesisifundo njalo angisozengilahlekelwe zimfanelozami. Nzaphiwa iphepha laleli fomu (bhala amabala akuqala egama lakho lesibongo sakho emapepheni onke alelifomu)

____________________________  __________

IGAMA LAKHO  ILANGA

____________________________  __________

IGAMA LOMUMELI WAKHO  IGAMA
Appendix 7: Questionnaire

Questionnaire with demographics and key sections

OI number……………………………………

Age………. Years               DOB…./…../……(fill in day/month/year)

Sex:   Male                                          Female       (circle)

Marital Status   (circle)

KEY SECTIONS

1. Category Of Patient   (circle)

2. Previous History Of TB Contact   (circle)
   1. Stayed with a TB patient      2. Never stayed with a TB patient

3. Recent Travel To South Africa Or Any SADC Region (Circle)

Visited South Africa or any country in the SADCC Region in the past 2 years
   1. Yes                                                            2. No

If yes ,which country /countries were visited (list)

1.………………………………2.……………………………………3.………………

4. TB Prophylaxis (circle)

Are you receiving Isonizid prophylaxis ?
   1. Yes                                                            2. No

5. History Of Hospitalization (circle)

Have you been hospitalized in the past 12 months?
   1. Yes                                                            2. No
If yes, how long were you in hospital (state length of stay)

6 Frequency To Congregate Settings (circle)

How many times did you visit the OI clinic in the past 18 months?

1. Two  
2. Three times  
3. Four times  
4. More than four times

7. If on TB treatment: duration on treatment

How long have you been on TB treatment?

1. <2 months  
2. 2-6 months  
3. 6 months

8. Duration of ART (circle)

Have you been on ART for:

1. <3 months  
2. 3-6 months  
3. >6 months

9. CD4 count (to collect a blood sample)

..........................................................
Appendix 8: Certificate of Participation in External Quality Assurance Program (National TB Reference Laboratory)

Instituut voor Tropische Geneeskunde
Institut de Médecine Tropicale
Institute of Tropical Medicine
België - Belgique – Belgium

The Head of the Mycobacteriology Unit (Antwerp Supra-National TB Reference Laboratory) hereby certifies that

TB National Reference Laboratory, Bulawayo, Zimbabwe

has participated in the 2010 quality assurance round of Drug Susceptibility Testing of *Mycobacterium tuberculosis*
under the WHO / IUATLD global project for surveillance of TB drug resistance, and has successfully passed for the following drugs:
isoniazid, rifampicin, streptomycin, ethambutol

Antwerpen, 6 October 2011

For the Head, Prof. B. de Jong,
Dr. Armand Van Deun
Coordinator of the rounds

This document is accompanied by a supplement to the certificate.