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**PREVALENCE AND FACTORS ASSOCIATED WITH ISONIAZID
MONO-RESISTANCE AND MULTI-DRUG RESISTANCE AMONG NEW
TUBERCULOSIS PATIENTS IN KAMPALA.**

BY

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**A dissertation submitted in partial fulfillment of the requirements for the award of a
Masters degree in Clinical Epidemiology and Biostatistics of Makerere University**

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Declaration

I, Karenye Kimani David, declare that the work submitted in this dissertation is my own compilation and has not been submitted for another degree in this or any other university or institution of higher learning. All work is original unless otherwise acknowledged.

Karenye Kimani David (Investigator)

Sign..... Date.....

This dissertation has been submitted for examination with approval of my supervisors

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Signature

Date

2. Dr. Ezekiel Mupere, MB.ChB, MMED, MSc, PhD

Signature.....

Date.....

Dedication

To my Dad, Mum & Grandma

Acknowledgement

First I am forever grateful to the Almighty God for the immense blessing that I have come this far in my studies and from the conception of the idea to the completion of this work.

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Abbreviations and Acronyms

aOR	Adjusted Odds Ratio
AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
BMI	Body Mass Index
CBTBC	Community Based Tuberculosis Care
COPD	Chronic Obstructive Pulmonary Disease
CWRU- MU	Case Western Reserve University-Makerere University
CXR	Chest X-Ray
DST	Drug Susceptibility Testing
GPS	Global Positioning Satellite
HAART	Highly Active Anti-Retroviral Therapy
HBC's	High Burden Countries
HIV	Human Immunodeficiency Virus
IQR	Inter Quartile Range
INH	Isoniazid
KCHS	Kawempe Community Health Study
LJ	Lowenstein-Jensen
LTBI	Latent Tuberculosis Infection
LPAs	Line Probe Assays
MDR-TB	Multi Drug Resistance Tuberculosis
MoH	Ministry of Health
MTB	Mycobacterium Tuberculosis
NLTP	National Tuberculosis & Leprosy Programme
OR	Odds Ratio
WHO	World Health Organization

Operational Definitions

A **new case of TB** is a patient with initial episode TB and has never had treatment for TB or who has taken anti-TB drugs for less than one month (4weeks) [1-3].

Drug Susceptibility Testing (DST) is the testing of a strain of *Mycobacterium tuberculosis* for its resistance to one or more anti-TB drug[4].

Mono-resistance is defined as resistance to only one of the first line anti-TB; First line antibiotics are Isoniazid, Rifampicin, Ethambutol, Pyrazinamide and Streptomycin[2, 5].

Multi-drug resistance (MDR) is resistance to at least Isoniazid and Rifampicin [3, 5-6].

Abstract

Background

The cornerstone of TB management is at least a 6-month course of a combination of first line drugs. The increasing frequency of anti-TB drug resistance has been a major setback. It is necessary that we prevent further development of the resistance strains and optimize the available first line drugs or TB will become once again incurable. Because Isoniazid continues to have a prominent role in treatment regimens for TB disease and for LTBI, it is essential to understand what factors may be contributing to its resistance so that clinicians can make informed treatment decisions when evaluating these patients.

Objectives

To determine the prevalence and factors associated with INH mono-resistance and Multi-drug resistance among the new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District.

Methods

This study involved two study designs; a baseline cross-sectional and a case-control study nested in an established KCHS cohort from the CWRU-MU Research Collaboration. New TB cases who had attended the clinic between January 2002 and December 2012 were considered. Cases had at least INH mono-resistance and controls had INH susceptible TB, in a ratio of 1 to 3. Factors associated with INH resistance at a level of $p < 0.2$ were considered for multivariate analysis.

Results

A total of 962 patients were considered for the prevalence study. INH mono-resistance prevalence was 5.2% (95% CI 3.8 – 6.6) and MDR 0.9 % (95% CI 0.3 – 1.5). Among the 204 case-control patients, the Bantu with aOR of 0.37 (95% CI 0.13- 1.01) and the employed 2.37

(95% CI 1.05- 5.38) were associated with INH mono-resistance. HIV status of an individual was not associated with INH mono-resistance, aOR 1.03 (95% CI 0.44 – 2.42).

Conclusion

The prevalences of INH resistance and MDR TB are relatively low and have not changed significantly over time in Kampala. Neither INH resistance nor MDR TB was associated with HIV infection. Only the employed and Bantu tribe were associated with INH mono-resistance. Molecular epidemiological studies to assess the genetic disparities among the Ugandan tribes need to be carried in order to identify the different genetic markers.

CHAPTER 1: INTRODUCTION

Background

Tuberculosis (TB) accounts for 8.4 million cases worldwide and is one of the leading infectious causes of death today[7]. A World Health Organization (WHO) 2011 comprehensive report involving 198 countries accounting for 99% of TB cases indicated that globally, the absolute number of incident TB cases per year has been falling since 2006. This is in line with the Millennium Development Goal No. 6 “To combat HIV/AIDS, Malaria and other diseases” which targets a reduction in TB incidence by the year 2015[8].

There are 22 TB high burden countries (HBCs) with Africa being represented by 9 countries. Uganda is ranked 16th out of the 22 HBCs in the world[8-9]. It has an Annual Risk of TB Infection estimated at 3 percent with incidence of TB at 330 and 136 per 100, 000 population for all TB cases and for sputum smear-positive (SS+) pulmonary TB respectively. The country in 2008 recorded a death rate of 5.3 percent among sputum smear-positive pulmonary TB patients[1].

The cornerstone of tuberculosis management is at least a 6-month course of combination of first line drugs: Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin. Rifampicin and Isoniazid both form an integral part of the initiation and continuation phase of anti-tubercular treatment regimens in all defined categories of patients – categories I – IV[1, 10]. Compliance with the treatment regimen is challenging because of the long duration of treatment and the associated adverse drug reactions and; yet it is crucial for curing the disease and prevention of drug resistance[1, 5].

Drug resistance has been a major setback in fighting this infectious disease. The resistance varies from one drug to more than one. The Mono-resistance refers to resistance to only one of the first line anti-TB, while Multi-drug resistance (MDR) is resistance to at least Isoniazid and Rifampicin[5-6]. Another rare type of resistance is XDR-TB which is a form of MDR-TB resistant to a fluoroquinolone and at least one injectable second-line drug; Kanamycin, Amikacin or Capreomycin[11]. Approximately 425,000 MDR-TB cases occur annually worldwide, representing nearly 5% of the world's annual TB burden[5]. Recent WHO estimates indicate that nearly 60,000 (14% of the global burden), MDR-TB cases occur annually in the sub-Saharan region. Patients with MDR-TB require the use of “second line” drugs and a much longer treatment period compared with drug-susceptible TB[1, 5].

A national survey conducted in Madagascar between 2005 -2006 estimated the MDR-TB to occur in new and previously treated TB cases at 2% and 7% respectively. Similarly INH drug was most often mono-resistant at 3.6% (33/926) of new cases and 3.4% (3/87) of previously treated cases[12]. The first drug-resistance study in Uganda was conducted in 1996-1997 as part of a global drug-resistance surveillance programme, but was carried out in only part of Uganda. From the survey, patients who had received no more than 1 month of anti-TB therapy in the past participated, the prevalence of resistance to any drug was 19.8%; to Isoniazid (INH) 6.7%; Ethambutol (E) 6.1%; Rifampicin(R) 0.8%; and to Streptomycin (S) 13.4%. The prevalence of MDR-TB was found to be 0.5% [1]. A Kampala survey by *Lukoye et al 2008*, reported among the new cases, MDR was found in 5 (1.1%) and any drug resistance in 57 (12.1 %). Resistance among new cases was most frequent to Streptomycin 8.7% and Isoniazid 5.7% [13].

The frequency and nature of Anti-TB resistance have been the matter of concern to the global community. In East African and more so Uganda no recent studies have been published on this subject matter. Thus, because Isoniazid continues to have a prominent role in treatment regimens for TB disease and for Latent TB infection (LTBI), it is essential to understand what factors may be contributing to its resistance so that clinicians can make informed treatment decisions when evaluating these patients.

The aim of this study was to determine the prevalence and to assess factors associated with Isoniazid mono-resistance and Multi-drug resistance among new Tuberculosis patients in Kampala District, Uganda.

Problem Statement

In sub-Saharan Africa countries with a high burden of TB and HIV infection, MDR-TB is estimated to occur in respectively 2% and 7% of new and previously treated TB cases[11]. Uganda is one of the world's 22 high burden countries for TB. Despite having a national treatment programme for drug-sensitive TB, there has been an emergence of drug-resistant strains of the disease, which are presenting a new and urgent threat to people's health. By the year 2008, treatment success rates in Uganda were lower at 70 % compared to the WHO goal targets of 85% [9].

The drug-resistance TB is on the rise and controversies do exist on factors that may be associated with its development. Prior exposure to anti-TB drugs may lead to development of resistant strains[13]. Similarly inadequate anti-TB treatment is an important factor that can contribute to the development of drug-resistant TB strains. The factors causing inadequate anti-TB treatment can be grouped into health provider factors (inadequate training and drug stock outs), drug factors (high pill burden and long duration of treatment) and patient factors (poor adherence, malabsorption and adverse drug effects)[1].

The treatment of the resistant TB forms comes at a higher cost on average, 100- to 300-fold higher than that associated with the drug-susceptible form and more drug related toxicities. Furthermore, patients with MDR-TB have lower cure rates and higher mortality than do patients with drug-susceptible TB[5].

HIV is still prevalent in Uganda, and has been associated with high TB burden globally[8] There are still no conclusive studies regarding HIV association with anti-TB drug resistance especially

among new cases of TB. In Uganda *Lukoye et al* found no association between anti- TB drug resistance with HIV infection since the MDR cases were few and this limited the precision of their estimate[13]. However, at least three African studies and one from the United States reported data indicating that HIV infection may be associated with anti-TB drug resistance[14-16].

Although the national survey on drug-resistance patterns has been done, the findings are yet to be disseminated. There exist substantial knowledge gaps on anti-TB drugs resistance that ought to be addressed. It was therefore important that its magnitude and risk factors are identified for better control the global MDR-TB epidemic.

Study Justification

In 2001 the MoH through National Tuberculosis and Leprosy Program formally adopted the community-based TB Care (CBTBC) strategy to address the TB services all over the country. By the year 2008, treatment success rates were lower at 70 % compared to the WHO goal targets of 85% [9].The development of *Mycobacterium tuberculosis* Complex drug resistance strains could be linked to the low treatment success rate in the country. The steady rise in MDR-TB, is threatening to make TB an incurable disease once again. It is important to limit the spread of MDR-TB and more recently, extensively drug-resistant TB (XDR-TB).

The current treatment guideline only identifies drug resistant suspect basing on: chronic cases (still sputum smear-positive after completing supervised retreatment regimen), contact with known drug resistant TB, relapses, treatment after failures, treatment after defaults and history of frequent interruption of drug treatment. However, the diagnosis of drug resistance is made microbiologically. The resistance can be tested by molecular line probe assay (LPAs), GeneXpert or using culture and sensitivity testing in the laboratory[17].

Uganda is a resource limited country and whose majority of sputum positive patients are not checked for drug susceptibility test (DST). There is inadequate TB laboratory infrastructure. It is therefore imperative that TB control measures are strengthened to minimize the emergence of drug resistance through rapid diagnosis, rapid identification of drug resistance, supervised treatment, and maintenance of comprehensive surveillance[11].The current treatment policy lacks procedures for identification of new drug resistance TB patient.

This study aimed to provide an insight on the prevalence and the possible risk factors associated with INH mono-resistance and MDR among new TB patients. The study findings will empower clinicians to improve on diagnosis and identification of new TB drug resistance suspects in the resource limited health facilities before initiating TB treatment. Thereafter the clinician will be able to initiate the appropriate TB regimen, isolate and counsel the patients on ways to reduce the transmission of the resistant form.

Conceptual Framework

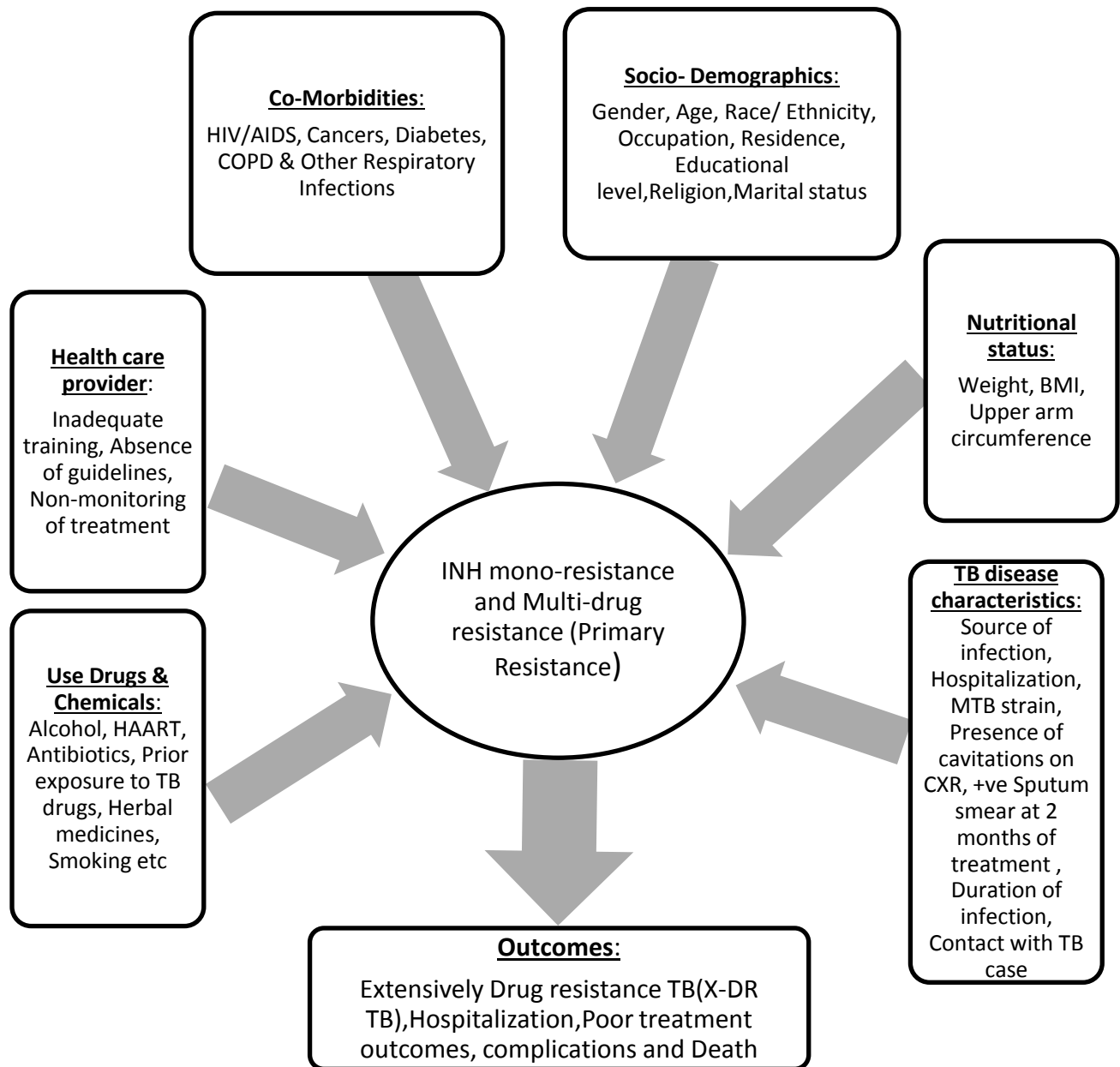


Figure 1: Conceptual framework showing the possible predictors associated with INH mono-resistance and Multi-drug resistance (Primary Resistance).

Scope of the study

Figure: 1 shows the various factors predictive of INH mono-resistance and Multi-drug resistance (Primary Resistance) as well as the consequences. The factors assessed in this study included: patients' demographics and nutritional status, clinical co-morbidities, TB disease characteristics and drug related factors that are predictive of INH mono-resistance and Multi-drug resistance (Primary Resistance). The health provider factors and consequences i.e. poor treatment response, increase in extended drug resistant strains, increased morbidity and mortality were however not assessed.

Research Questions

1. What is the prevalence of INH mono-resistance among the new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District?
2. What is the prevalence of Multi-drug resistance among the new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District?
3. What are the factors associated with INH mono-resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District?
4. What are the factors associated with Multi-drug resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District?

General Objective

To determine the prevalence and factors associated with INH mono-resistance and Multi-drug resistance among the new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District.

Specific Objectives

Primary Objectives

1. To determine the prevalence of INH mono-resistance among the new tuberculosis clients attending CWRU-MU Research collaboration clinic between 2002 -2012 in Kampala District.
2. To determine the factors associated with INH mono-resistance among the new tuberculosis clients attending CWRU-MU Research collaboration clinic between 2002 - 2012 in Kampala District.

Secondary Objectives

1. To determine the prevalence of Multi-drug resistance among the new tuberculosis clients attending CWRU-MU Research collaboration clinic between 2002 -2012 in Kampala District.
2. To determine the factors associated with Multi-drug resistance among the new tuberculosis clients attending CWRU-MU Research collaboration clinic between 2002 - 2012 in Kampala District.

Hypotheses

Primary Hypotheses

Null

- There is no association between HIV infection and Isoniazid mono- resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District.

Alternative

- There is an association between HIV infection and Isoniazid mono- resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala district.

Secondary Hypotheses

Null

- There is no association between HIV infection and Multi-drug resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District.

Alternative

- There is an association between HIV infection and Multi-drug resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala district.

CHAPTER 2: LITERATURE REVIEW

Definitions

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB). The disease is spread in the air when people who are sick with pulmonary TB expel bacteria, for example by coughing. In general, a relatively small proportion of people infected with *Mycobacterium tuberculosis* will go on to develop TB disease; however, the probability of developing TB is much higher among people infected with the HIV. TB is also more common among men than women, and affects mostly adults in the economically productive age groups; around two-thirds of cases are estimated to occur among people aged 15–59 years[8].

A new case of TB is a patient with initial episode of TB who has never had treatment for TB or who has taken anti-TB drugs for less than one month (4weeks) [1-3].

A definite case of TB is identified from a clinical specimen, either by culture or by a newer method such as molecular line probe assay. In countries with poor laboratory infrastructure to routinely identify *Mycobacterium tuberculosis*, a pulmonary case with one or more initial sputum specimens positive for acid-fast bacilli (AFB) is also considered to be a “definite” case, provided that there is functional external quality assurance with blind rechecking[2].

Drug Susceptibility Testing (DST) is the testing of a strain of *M. tuberculosis* for its resistance to one or more anti-TB drugs[4].

Mono-resistance has been defined as resistance to only one of the first line anti-TB drugs, while Multi-drug resistance (MDR) is resistance to at least Isoniazid and Rifampicin [3, 5-6]. The first line anti-TB drugs are; Isoniazid, Rifampicin, Ethambutol, Pyrazinamide and Streptomycin.

Prevalence of Anti-TB Drugs Resistance

In 2010, there were an estimated 8.5–9.2 million cases and 1.2–1.5 million deaths (including deaths from TB among HIV-positive people). Stop TB Partnership aims that, by 2015 TB prevalence and death rates are reduced by 50%, compared to 1990 levels and by 2050 the global incidence of active TB cases to be <1 case per 1 million population per year[8]. This can only be achieved through effective National Tuberculosis Programmes (NTPs). The MDG's targets can be achieved in all WHO regions with the exception of the African Region[8]. This can be partially explained by the development of drug resistance Mycobacterium strains.

Approximately 425,000 MDR-TB cases occur annually worldwide, representing nearly 5% of the world's annual TB burden. HIV infection epidemic has caused explosive increases in TB incidence and may be contributing to increases in MDR-TB prevalence[5]. In sub-Saharan Africa countries with a high burden of TB and HIV infection, MDR-TB is estimated to occur in respectively 2% and 7% of new and previously treated TB cases. The burden of XDR-TB remains largely unknown due to a lack of laboratory infrastructure for reliable drug susceptibility testing[11].

The first partial drug-resistance study patterns in Uganda were conducted in 1996/97 as part of a global drug-resistance surveillance programme. Among the 374 evaluable isolates from the new cases of TB patients, the prevalence of resistance to any drug was 19.8%; to Isoniazid 6.7%; to

Ethambutol 6.1%; to Rifampicin 0.8%; and to Streptomycin 13.4%. The prevalence of MDR-TB was found to be 0.5%[1]. Similarly Mulago Hospital study in 2000 on 215 previously untreated patients revealed a similar MDR prevalence of 0.9%, but resistance to Rifampicin was found in 1.4% of the patients[1].

A national TB resistance survey conducted for the first time in Madagascar between 2005 and 2007, using a cluster sampling representative of the general population of the country, involved 1275 smear-positive TB patients recruited at 34 sites, 926 new patients and 87 previously treated. Resistance among new cases was 6.5% and among previously treated cases it was 11.5%. Monoresistance among new cases was 5.8% and mainly to INH (3.7%). Nevertheless, the rate remained relatively low compared to levels in Africa overall 8.3% (95% CI 6.8–9.9).

Multi-resistance to INH and RMP was 0.2% (95% CI 0 – 0.5) and 3.4% (95% CI 0 – 7.2) among new and previously treated cases respectively. The rates of resistance among new and previously treated cases were relatively low in Madagascar compared to Africa in general 11.4% (95% CI 6.4– 16.5)[12].

Detection of Resistance

Culture and drug susceptibility tests for all cases of tuberculosis are considered the gold standard for diagnosis and surveillance of drug resistance. However, such tests are not feasible in most settings[3]. In most parts of the world, less than 5% of TB patients are tested for drug susceptibility. The diagnosis of MDR-TB requires that people with TB are tested for susceptibility to first-line anti-TB drugs. The WHO reports that, The Global Plan includes targets that by 2015 all new cases of TB considered at high risk of MDR-TB should be tested for drug

susceptibility (estimated at about 20% of all new cases) and that 100% of retreatment cases should be tested.

Conventional methods used to diagnose resistance TB rely on culturing of specimens followed by DST. The results take weeks and not all laboratories with capacity to perform DST for first line drugs have the capability to perform DST for second-line drugs[11]. The landscape of TB diagnostics is rapidly evolving; this has resulted in the endorsement of a new rapid test kits. These kits have different operation techniques as prescribed by the manufacturer hence varying sensitivity and specificity. LPAs use multiplex polymerase chain reaction (PCR) amplification and reverse hybridization to identify *M. tuberculosis* complex and mutations to genes associated with Rifampicin and Isoniazid resistance[17].

Culture on Lowenstein-Jensen (LJ) medium and the proportion method for DST are the most frequently used laboratory methods. Field demonstration studies found that Xpert MTB/RIF can detect Rifampicin resistance with 95.1% sensitivity and exclude resistance with 98.4% specificity [8].The nitrate reductase assay (NRA) is based on the ability of *M. tuberculosis* to reduce nitrate to nitrite when grown on LJ medium containing potassium nitrate and the test drug. Addition of the detection reagent causes a colour change (pink purple), indicating mycobacterial growth and thus resistance to the drug.

In a study to evaluate seven rapid tests kits using well characterized isolates for detection of MDR-TB in a Ugandan setting; nitrate reductase assay (NRA),Mycobacterium Growth Indicator

Tube 960 (MGIT 960) and Genotype MTBDR*plus* gave excellent detection of MDR TB, with significantly shorter time to results compared to conventional testing[11].

Factors Predictive of Resistance

The highest rates of HIV coinfection in TB patients are in the African Region, where 44% of TB patients with an HIV test result in 2010 were HIV-positive (range among high TB/HIV burden countries, 8%–82%), followed by the Region of the Americas at 17%[8]. A systematic review on association between MDR-TB and HIV reported that; For persons infected with *M. tuberculosis*, HIV infection is the strongest risk factor for the development of active TB either drug-susceptible or drug-resistant TB. In addition to increasing the TB burden in general, HIV infection may also be contributing to increases in MDR-TB prevalence among patients with TB and has been associated with many MDR-TB outbreaks, as well as with acquired Rifamycin resistance [4-5, 8].

Studies from; Tanzania, Botswana, South Africa, Malawi, India, Vietnam , and Russia, as well as a multi-country study of determinants of drug-resistant TB , have not demonstrated an excess association between HIV infection and anti-TB drug resistance. Their methods varied widely, the sample sizes were small, and their results were confounded by previous TB treatment and hospitalization. However, one of the studies from Mozambique, demonstrated an association between HIV infection and resistance to INH and Streptomycin, while Ethiopian study demonstrated an association between anti-TB drug resistance and HIV infection among patients never previously treated for TB[5].

From a United Kingdom (UK) study, it showed that the proportion of INH resistant TB was higher in men than in women, although the difference was not significant. However, men were significantly more likely to have MDR tuberculosis. Ethnic origin exhibited significant difference with highest proportion of INH and MDR reported in isolates from people of black African origin at 10.1% and 2.0% respectively, with 7.2% and 1.4% in those originating from the Indian subcontinent, and among 4.1% and 1.4% in those of white ethnic origin.[6].

Although several factors can contribute to the development of drug-resistant TB strains, inadequate anti-TB treatment is probably the most important. Situations of inadequate anti-TB treatment include: inadequate drug regimen, inadequate duration of treatment and drugs not taken regularly by the patient[1].

A study on anti TB drug resistance conducted between 1993- 1999 in the UK involving 7603 of the drug resistance cases, reported 1396 patients to have had a previous episode of tuberculosis. This group exhibited a significantly higher proportion of INH resistance (15.5%) and MDR (9.4%) than either those patients who had never had TB 5.7% and 0.8% respectively[6].

A case control study compared the level of alcohol use among MDR-TB patients against three control groups: 1) non-MDR-TB patients, 2) HIV infected patients without a history of TB, and 3) the general population. Alcohol use and abuse was measured with the Alcohol Use Disorders Identification Test 10 (AUDIT) questionnaire. Among patients with TB, alcohol abuse was found to be a risk factor for the development of MDR-TB[18].

A bivariate logistic regression analysis carried out to assess the possible association between INH mono-resistance and clinically significant risk factors (previous history of TB, previous treatment with a quinolone and injectable agent other than Streptomycin, and the presence of cavities in the lungs), found no association with INH mono-resistance; this might be due to the small number of patients analyzed. However a previous report from the USA has reported its association with a previous history of TB; while previous treatment with a quinolone and injectable agent other than streptomycin (OR 3.889, 95% CI 1.828 – 8.272) demonstrated a likely association with MDR-TB[3, 10].

Smear-negative pulmonary tuberculosis patients have been thought to be less contagious and therefore without the same public health impact as smear-positive cases. In the Mexico region, study findings suggest that smear-negative patients can be an important factor for the dissemination of resistant *M tuberculosis*[19].

The specific genotype family of *M. tuberculosis* has been linked to anti-TB resistance. A recent meta-analysis of 129,000 patients from 49 studies in 35 countries identified the Beijing genotype family of *M. tuberculosis* as more virulent and has been implicated in many MDR-TB outbreaks in the USA, and other specific geographic settings[5]. The wild isolates of *M tuberculosis* that have never been exposed to anti-TB drugs almost never show any resistance[3, 10].

CHAPTER 3: METHODS

Study Design

This study involved two study designs (baseline cross-sectional and a nested case-control study). A baseline cross-sectional study was carried out on the established Kawempe Community Health Study (KCHS) cohort from the Case Western Reserve University- Makerere University Research Collaboration Kampala, to determine the prevalence of Isoniazid mono-resistance and MDR-TB among new tuberculosis clients attending CWRU-MU Research collaboration clinic between 2002-2012 in Kampala District.

A nested case-control based study was also carried using the KCHS cohort, to determine the factors associated with INH mono-resistance and Multi-drug resistance among the new tuberculosis clients attending CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District.

Kawempe Community Health Study

The KCHS commenced in the year 2002 and is still ongoing with several research questions it aims to answer. To achieve this, new TB cases and adult household contacts may be enrolled without the enrollment of their children.

The study aims to provide valuable information to the Uganda Ministry of Health that will ultimately lead to improved control of TB in the country. The study objectives are;

- To determine critical host factors associated with primary MTB infection, re-infection, reactivation, and progression of clinical disease.

- To identify and track individual strains of MTB through Ugandan households and local community.

Study Setting

This is an out-patient clinic study at Old Mulago Hospital, located adjacent to TB ward 11. The clinic is run through collaboration between the CWRU and College of Health Sciences Makerere University. The clinic receives referral patients from both government and private health institutions situated in Kampala district.

Approximately 50 patients are attended to daily in the clinic, with less than five being new patients and the rest are on scheduled clinical visits. Only patients with initial episode of pulmonary tuberculosis are screened and recruited into the ongoing study in the clinic. The provisional diagnosis of TB is established basing on chest x-ray, history, directed physical examination or sputum smear for acid-fast bacilli. If, after initial evaluation, a patient is a suitable candidate for the study, the patient is then referred to the study's medical personnel who obtains consent and completes the screening examination. After the examination, initial specimens of sputum and blood are taken as part of the standard medical management. The home health visitor then transports the patient home to record the patient's address and location coordinates of the household using a GPS device for follow up.

Population

Target population

All adults from Kampala district with initial episode of tuberculosis disease.

Accessible population

All adults from Kampala district with initial episode of tuberculosis disease who had attended CWRU- MU Research Collaboration Clinic between January 2002 and December 2012.

Study Population

All adults from Kampala district with initial episode of pulmonary TB who had attended CWRU- MU Research Collaboration Clinic between January 2002 and December 2012. The patients must fulfill the eligibility criteria.

Eligibility Criteria

Inclusion for Cross-Sectional Study

- All adults in the KCHS cohort with initial episode of pulmonary TB who had attended CWRU- MU Research Collaboration Clinic between January 2002 and December 2012.
- The participant must have had DST results.

Case

Definition

A case was a patient with initial episode of tuberculosis disease, who had INH mono-resistance, and/or Multi-drug resistance DST result between Jan. 2002 and Dec.2012. The Mono-resistance being defined as resistance to only Isoniazid (INH) a first line antibiotic. Multi-drug resistance(MDR) is defined as resistance to at least Isoniazid and Rifampicin[6] both being first line antibiotics.

Inclusion criteria

- Adults with confirmed pulmonary TB and had attended the clinic between Jan.2002 and Dec. 2012

- The drug susceptibility testing (DST) results of their sputum collected post screening indicated Isoniazid resistance
- The sputum samples should have been collected before commencement of anti-TB medication from the clinic.

Exclusion criteria

- Prior Anti-TB treatment for more than one month

Control**Definition**

A control was a patient with initial episode of tuberculosis disease, whose DST results showed no resistance to Isoniazid a first line anti-TB drug. Three controls were selected for every one case identified.

Inclusion criteria

- Adults with confirmed pulmonary TB and had attended the clinic between Jan.2002 and Dec. 2012
- The drug susceptibility testing (DST) results of their sputum collected post screening indicated NO Isoniazid resistance
- The sputum samples should have been collected before commencement of anti-TB medication from the clinic.

Exclusion:

- Prior Anti-TB treatment for more than one month.

Sampling Procedure

For the cross-sectional study, the entire database was used and participants who satisfied the eligibility criteria were included for analysis.

For the nested case control study, non-probability (convenience) sampling for the cases was used since the numbers of cases in the sampling frame were few (fifty one). The three controls were randomly enrolled per case identified from the sampling frame containing eligible controls. Simple random sampling procedures using computer generated random numbers were used to select the controls who presented around same time of two months as the case identified. In the event that the random number was repeated, then immediate next was considered.

Sample Size Determination

Primary Objectives 1

To determine sample size that is sufficient to compute the prevalence of INH mono-resistance among new TB patients, we used the Kish Leslie formula[20]. A survey conducted between 18 August and 19 December 2008 in all health care facilities in Kampala that reported TB cases to the NTLP estimated the INH resistance among new TB patients to be at 5.7%[13] .

$$N = \frac{Z_{\alpha/2}^2 p(1-p)}{d^2}$$

$Z_{\alpha/2}$ = The level of confidence at 95% = 1.96

p = The estimated prevalence of INH mono-resistance (5.7%)[13].

d = level of precision (5%)

Substituting the respective values into the Kish Leslie formula. The minimum numbers of subjects needed to determine the prevalence of INH mono-resistance were 83. However the whole database of KCHS consisting of 962 eligible patients was used, since it would give the real prevalence compared to a sample.

Primary Objective 2

To compute the minimum analytical sample size, the formula for difference in proportion of two groups was used.

- **H₀:** There is no association between HIV infection and Isoniazid mono- resistance among new tuberculosis patients attending the CWRU-MU Research collaboration clinic between 2002- 2012 in Kampala District.
- The desired power of the study is 80%, with a two sided statistical level of significance of $\alpha = 5\%$.

$$N = \frac{\left[Z_{\alpha/2} \sqrt{P(1-P) \left(\frac{1}{q_1} + \frac{1}{q_2} \right)} + Z_{\beta} \sqrt{\left(p_1(1-p_1) \left(\frac{1}{q_1} \right) \right) + \left(p_2(1-p_2) \left(\frac{1}{q_2} \right) \right)} \right]^2}{(p_1 - p_2)^2}$$

p_1 = Proportion of cases exposed to HIV;

- From a UK Antibiotic resistant TB survey between 1993-1999[6], the proportions of HIV infected persons from INH resistance isolates was 11.6% ; Similarly a recent Kampala study estimated nearly same 10.1% for the same group of cases[13]. The average percentage was used of 10.9%.

p_2 = Proportion of controls exposed to HIV;

- From a rate of anti-TB drug resistance study in Uganda, the proportion of HIV infected persons among INH susceptible isolates was estimated to be 29.3% [13].

$q_1 =$ Proportion of subjects in group 1(Cases) = 0.25

$q_2 =$ Proportion of subjects in group 2(Control) = 0.75

N = Total number of subjects,

$P = P_1 q_1 + P_2 q_2 = 0.247$

$Z_{\alpha/2} = 1.96$ at 95% confidence interval

$Z_{\beta} = 0.84$ at 80% power

From the formula above, N is 205. A total of 204 patients were studied in the ratio of cases to control 1:3, hence the minimum number of cases were 51 and controls 153.

Measurement Variables

Predictor variables

1. **Patient socio-demographics:** Gender, Age, Ethnicity, Occupation, Educational level, Marital status
2. **Patients clinical factors:** Nutritional status, History of HIV/AIDS, Cancers, Diabetes & COPD
3. **Drug and chemicals related factors:** use of Alcohol, HAART, Antibiotics, INH prophylaxis, Herbal medicines, Cigarettes
4. **TB disease characteristics:** Source of infection, MTB strain, Presence of cavitations on CXR, Sputum smear (+ve or -ve), Duration of infection, Contact to TB patients

Outcome

The primary outcomes were INH mono-resistance and Multidrug-resistant (MDR) to anti-TB drugs. Mono-resistance has been defined as resistance to only one of the first line anti-TB in this case being Isoniazid, while Multi-drug resistance (MDR) is resistance to at least Isoniazid and Rifampicin [3, 5-6].

Data Collection

Pretested structured data extraction forms were used to obtain information from the patient's medical charts and electronic database by trained research assistants and data manager respectively.

Data Management

Electronic data was extracted using Microsoft Access 2007 for the selected participants. When there was electronic data incompleteness, the structured extraction forms were used. They were cross-checked by the principal investigator to ensure correctness and completeness. The filled forms were transported in boxes to data entry room and kept in lockable cabinets accessible to only authorized personnel. The data was then entered into the Microsoft Access 2007.

Data was cleaned, frozen and kept safely backed up in the CWRU-MU Research collaboration server. A duplicate copy of the frozen data was exported to STATA V11 (Stata Corp. College Station TX, USA) for subsequent analysis.

Data Analysis

The prevalence of INH mono-resistance and MDR were determined using a cross-sectional study design. The prevalences were expressed as proportion of anti-TB drug resistance (INH mono-resistance or MDR) against the total number in the sample.

In the Case-Control study design; the baseline characteristics were summarized in the forms of proportions and. At the univariate level, patient characteristics between the two groups (cases

and controls) were compared using the Chi-square(X^2) or Fisher's exact test for categorical variables.

Factors statistically significant at 5% and those having an association with INH resistance at a level of $p < 0.2$ in the univariate analysis were included in the multivariate logistic regression model. Similarly, factors known from literature were included in multivariate analysis even though they did not meet the former criteria. We performed multivariate analysis using stepwise backward removal method at 5% level of significance.

The logistic model was evaluated for possible interaction by forming interaction terms between the significant variables and the major predictor (HIV status). The Chunk test was used to identify the significant interaction terms. Confounding was assessed individually for dropped predictor variables, and was considered present if the change on the main predictor's effect of measure (adjusted OR) was more than 10%. The identified confounders were included in the final logistic model. Hosmer- Lemeshow goodness of fit was used to assess the final multivariate logistic model. All the analyses were performed using commercially available statistical software STATA V11 (Stata Corp. College Station TX, USA).

Quality Control

A total of 20 structured data extraction forms were pretested by the principal investigator and necessary amendments made to improve their validity and reliability. The research assistants and data managers were oriented before data collection. Regular cross checking and inspection of the collected data was done to ensure the accuracy, consistency and uniformity. Data cleaning was done to further minimize errors.

Ethical Considerations

Approval to carry out the research was sought from Clinical Epidemiology Unit, School of Medicine Research and Ethics Committee, Case Western Reserve University-Makerere University Research and Ethics Committee.

Informed consent waiver was granted by both research and ethics committees as most of the eligible participants could not be contacted. To ensure patient protection and confidentiality, any possible patient identifiers were eliminated by use of serial numbers.

CHAPTER 4: RESULTS

Study subjects

The study consisted of patients who had been enrolled in KCHS at CWRU-MU clinic between January 2002 and December 2012. A total of 2,859 patients were enrolled but only 963 were eligible for the study inclusion. Household contacts (n= 1773) were excluded for lack of both initial episode of TB and baseline DST results.

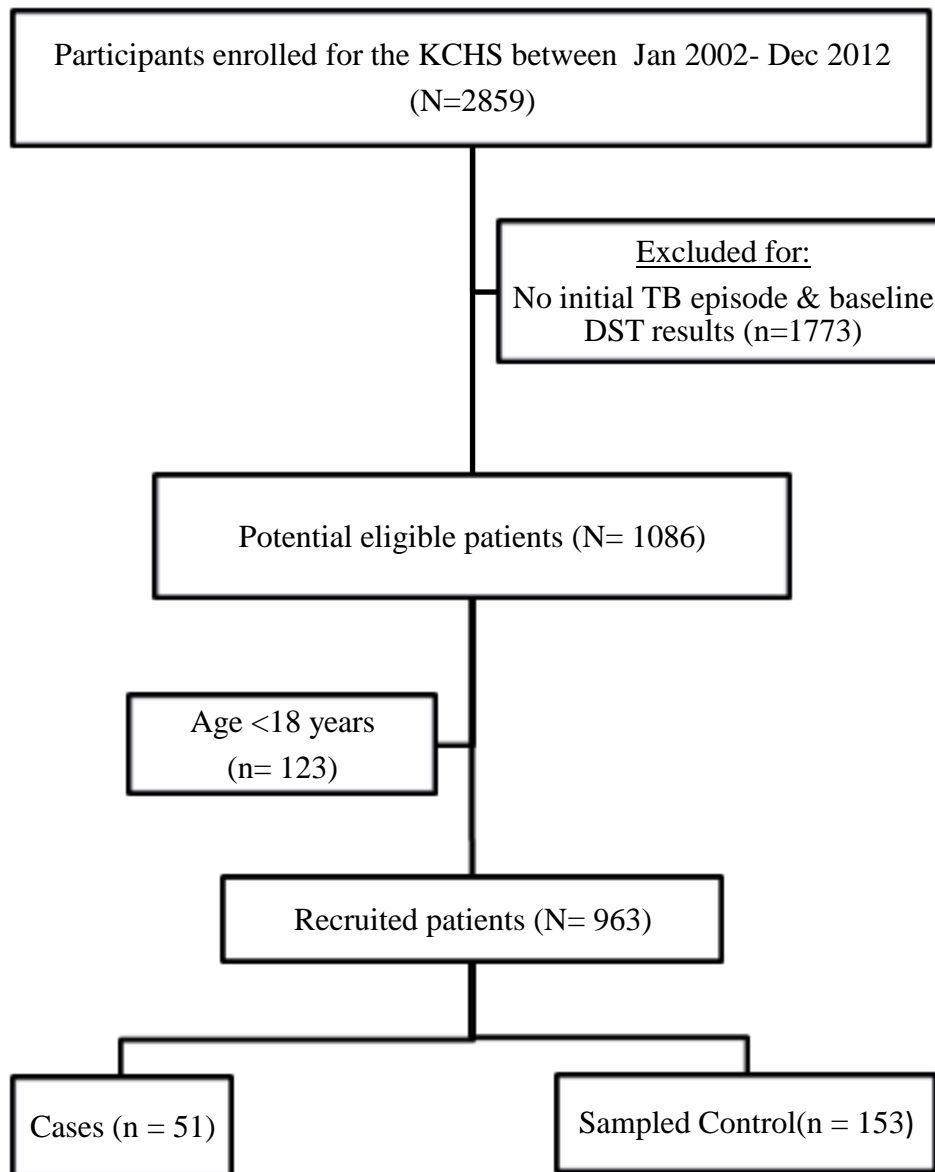


Figure 2: Patient flow chart during the study

Prevalence Study Results

A total of 962 patients were considered for the cross-sectional study. The median age was 28years (IQR 12), and there were 524 (54.5%) male participants. The socio-demographic characteristics of the participants are summarized in Table1.

Table 1: Socio-demographic characteristics of new TB patients in Kampala

Characteristics	N=962	%
Sex		
Female	438	45.5
Male	524	54.5
Age (median) years		
≤35 (25)	749	77.9
36 and above (42)	213	22.1
Marital status		
Never married	293	30.5
Married	493	51.2
Divorced/Widowed	176	18.3
Education level		
None	55	5.8
Primary	393	41.3
Secondary	440	46.3
Tertiary	63	6.6
Religion		
Catholic	275	28.5
Anglican	269	28.0
Muslim	208	21.6
SDA	18	1.9
Others *	192	20.0
Occupation		
Unemployed	340	35.3
Employed	593	61.6
Unknown	29	3.0
Tribe		
Non- bantu	76	7.9
Bantu	869	90.3
Foreigners	17	1.8

* *Pentecostal, Lutherans & Jehovah witnesses*

The prevalence of INH mono-resistance was found to be 5.2% (95% CI 3.8 – 6.6). The proportions of INH mono-resistance were 4.1% (95% CI 2.2 - 6.0) and 6.1% (95% CI 4.1 - 8.2)

for female and male respectively. The participants' age was dichotomized into the young adults (35 years and below) and 36 years and above, with median ages of 25 and 42 years respectively. The proportion of INH mono- resistance in the two age groups were 5.1% (95% CI 3.5 – 6.6) and 5.6% (95% CI 2.5 – 8.7) respectively (figure 3).

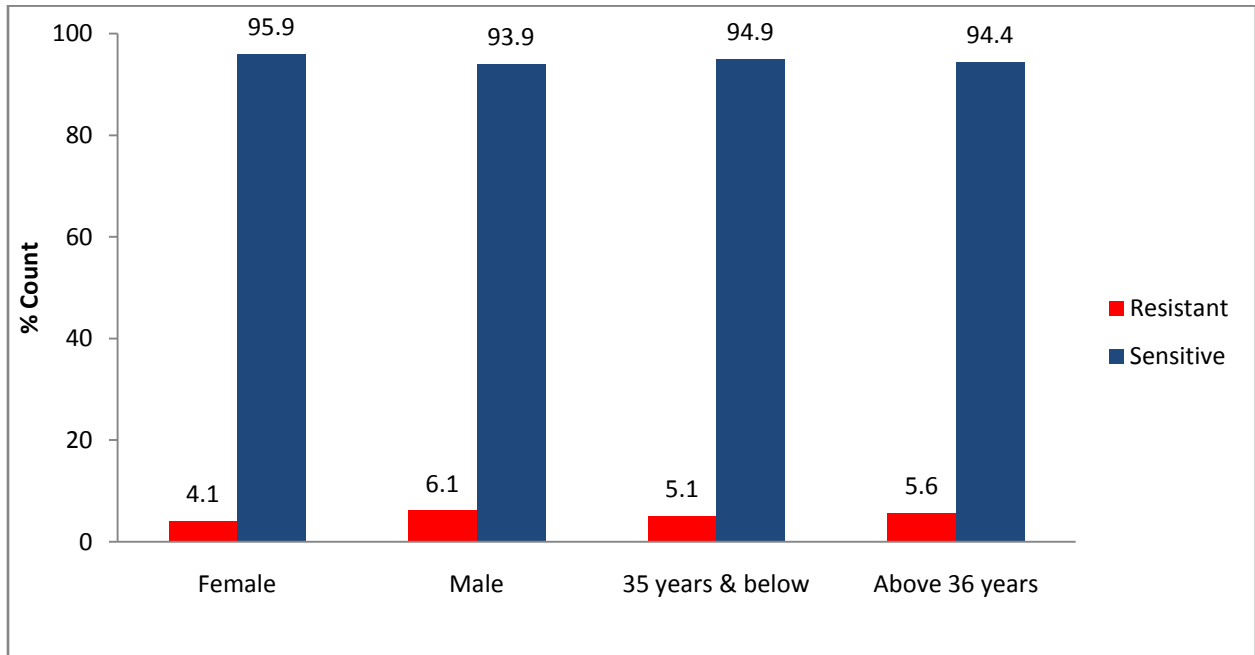


Figure 3: Proportions of INH mono-resistance across the population's gender and age with initial episode of TB in Kampala

The proportions of the INH mono-resistance for the participants' tribe/ ethnicity and occupation are represented in the figure 4 below. The INH mono-resistance across the different ethnicities; Non-bantu and Bantu were 9.2% (95% CI 2.7- 15.8) and 5.0% (95% CI 3.5- 6.4) respectively whereas none of the foreigners exhibited it. Similarly the participants' prevalence for the unemployed was 4.1% (95% CI 2.0- 6.2) and employed 6.1% (95% CI 4.1- 8.0). For those whose occupation was unknown, no INH mono-resistance was observed.

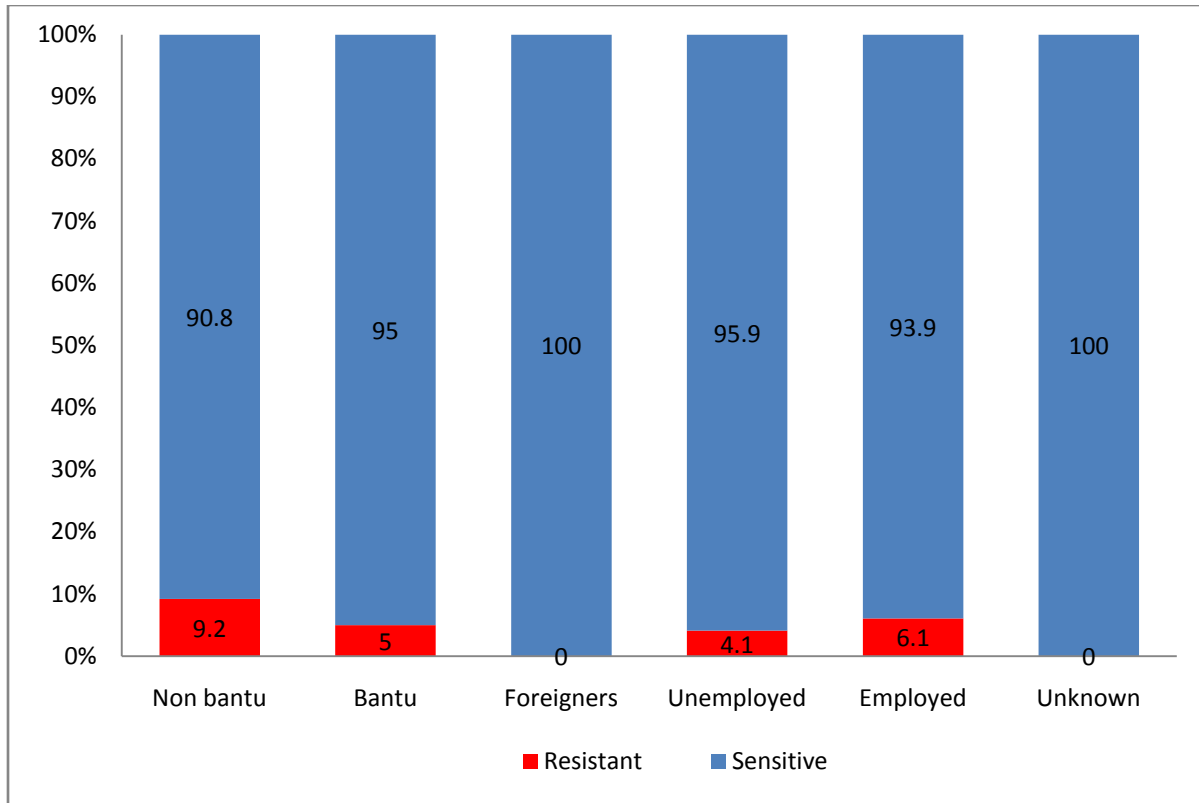


Figure 4: Proportions of INH mono-resistance across the population's tribe and occupation with initial episode of TB in Kampala

Multi Drug resistance (MDR) as described by resistance to at least Isoniazid and Rifampicin, prevalence was found to be 0.9% (95% CI 0.3 – 1.5).

Analytical Study Results

A total of 204 patients were included in this retrospective study. There were 51 cases and 153 controls, in the ratio of 1:3. The females were 91(44.6%) and males 113 (55.4%), and most of the patients were young adults (35 years and below) 161 (78.9%) (Table 2).

Table 2: Socio- Demographic characteristics of patients with Isoniazid Mono-resistant and Drug Susceptible TB in Kampala

Characteristic	No. (%)		p- value
	Isoniazid Mono-resistant TB (n= 51)	Drug Susceptible TB (n=153)	
Sex			
Female	18 (35.3)	73 (47.7)	0.122
Male	33 (64.7)	80 (52.3)	
Age, years			
≤35	39 (76.5)	122 (79.7)	0.620
≥36	12 (23.5)	31 (20.3)	
Marital status			
Single	26 (51.0)	74 (48.4)	0.746
Married	25 (49.0)	79 (51.6)	
Education level			
Primary	28 (54.9)	73 (47.7)	0.374
Secondary & Above	23 (45.1)	80 (52.3)	
Religion†			
Catholic	12 (23.5)	47 (30.7)	0.603
Anglican	14 (27.5)	36 (23.5)	
Others	25 (49.0)	70 (45.8)	
Occupation			
Unemployed	11(21.6)	53 (34.6)	0.081
Employed	40 (78.4)	100 (65.4)	
Tribe / Ethnicity†			
Non bantu	8 (15.7)	10 (6.5)	0.046*
Bantu	43 (84.3)	143 (93.5)	

† Evaluated by Fisher's Exact test

* Significant at 5% level

Socio-Demographic characteristics

From the Table 2 above, the highest proportions of Isoniazid mono-resistant patients 39 (76.5%) and drug-susceptible TB controls 122 (79.7%) occurred in the young adults group. The level of education was nearly equal between the two education levels: primary group 101(49.5%) and

secondary and above group 103 (50.5%). There more employed cases 40 (78.4%) than the unemployed 11 (21.6%). The Non-bantu ethnic group were fewer 18 (8.8%) than the Bantu 186 (91.2%) and exhibited a significant difference ($p= 0.046$). These people represented the various tribes in Uganda.

Clinical characteristics

From the patient's self reporting during enrollment into the KCHS, most were not suffering from other co-morbidities. Cancer and Diabetes were not reported in 200 (98.0%) participants. A total of 63 (30.9%) participants had chronic obstructive pulmonary disease (COPD), while the other never knew if they had the disease.

In Table 3, HIV status was based on confirmed laboratory testing. Only 11 (21.6%) of the cases were HIV positive. Notably, there was no significant association between INH mono-resistance and positive HIV status. The use of alcohol and history of cigarette smoking among the cases were at 11 (21.6%) and 10 (19.6%) respectively. Only 25 (12.3%) of all participants had ever used both alcohol and cigarettes.

From Table 3 below. The nutritional status was gauged by BMI, 21 (41.2%) INH mono-resistant individuals were below the 18.50 kg/m^2 the lower mark of the normal range. Acid fast bacilli (AFB) tests were done on the three sputum obtained from each patient during enrolment, 46 (90.2%) who had a positive smear were resistant to Isoniazid. Cavities in the lung parenchyma were reported as seen on the CXR by qualified medical personnel. 97 (63.4%) had cavities while 27 (52.9%) had cavities and exhibited INH resistant.

Table 3: Clinical characteristics of patients with Isoniazid Mono-resistant and Drug Susceptible TB

Characteristic	No. (%)		p- value
	Isoniazid Mono-resistant TB (n= 51)	Drug Susceptible TB (n=153)	
Alcohol use			
No	40 (78.4)	118 (77.1)	0.847
Yes	11 (21.6)	35 (22.9)	
History of smoking			
No	41 (80.4)	126 (82.4)	0.753
Yes	10 (19.6)	27 (17.6)	
Use of HAART			
No	40 (78.4)	126 (82.3)	0.20
Yes	1 (2.0)	0 (0.0)	
Unknown	10 (19.6)	27 (17.7)	
Ever used INH prophylaxis †			
No	31 (60.8)	103 (67.3)	0.073
Yes	2 (3.9)	0 (0.0)	
Unknown	18 (35.3)	50 (32.7)	
Antibiotics use in the last 2weeks †			
No	7 (13.7)	16 (10.5)	0.762
Yes	5 (9.8)	13 (8.5)	
Unknown	39 (76.5)	124 (81.0)	
HIV status			
Negative	40 (78.4)	126 (82.3)	0.533
Positive	11 (21.6)	27 (17.7)	
BMI			
<18.50 kg/m ²	21 (41.2)	59 (38.6)	0.741
≥ 18.50 kg/m ²	30 (58.8)	94 (61.4)	
Sputum smear			
Negative	5 (9.8)	17 (11.1)	0.794
Positive	46 (90.2)	136 (88.9)	
History of contact with TB case †			
No	10 (19.6)	26 (17.0)	0.828
Yes	2 (3.9)	9 (5.9)	
Unknown	39 (76.5)	118 (77.1)	
Presence of cavities as seen on CXR			
No	24 (47.1)	56 (36.6)	0.185
Yes	27 (52.9)	97 (63.4)	

† Evaluated by Fisher's Exact test

*Significance at 5% level

Factors Associated with INH Mono-Resistance

Factors associated with Isoniazid mono-resistance with a value of $p < 0.2$ in the univariate analysis were included in the multivariate logistic regression model. These factors included: Sex ($p = 0.122$), Occupation ($p = 0.081$), Tribe/Ethnicity ($p = 0.046$), Ever used INH prophylaxis ($p = 0.073$), Presence of cavities as seen on CXR ($p = 0.185$) and Use of HAART ($p = 0.20$). The HIV status ($p = 0.533$) had to be included in the model as it was the main predictor.

In the analysis, some of the predictor variables had zero observations in one of the cells and could not have their OR computed and hence omitted. These were Use of HAART and Ever use of INH prophylaxis.

Table 4: Unadjusted Odds Ratios for characteristics of new TB patients with Isoniazid Mono-resistant and Drug Susceptible TB in Kampala

Characteristic	No. (%)		Unadjusted Odds Ratios (95% Confidence Level)
	Isoniazid Mono-resistant TB (n=51)	Drug Susceptible TB (n=153)	
Sex			
Female	18 (35.3)	73 (47.7)	1 [Reference]
Male	33 (64.7)	80 (52.3)	1.67 (0.87 – 3.22)
Occupation			
Unemployed	11(21.6)	53 (34.6)	1[Reference]
Employed	40 (78.4)	100 (65.4)	1.93 (0.91- 4.06)
Tribe/ Ethnicity			
Non bantu	8 (15.7)	10 (6.5)	1[Reference]
Bantu	43 (84.3)	143 (93.5)	0.38 (0.14 – 1.01)
Alcohol use			
No	40 (78.4)	118 (77.1)	1 [Reference]
Yes	11 (21.6)	35 (22.9)	0.93 (0.43 – 1.99)
History of smoking			
No	41 (80.4)	126 (82.4)	1 [Reference]
Yes	10 (19.6)	27 (17.6)	1.14 (0.51 – 2.55)
Use of HAART			
No	40 (78.4)	126 (82.3)	1 [Reference]
Yes	1 (2.0)	0 (0.0)	Omitted
Unknown	10 (19.6)	27 (17.7)	1.17 (0.52 – 2.62)
Ever use INH prophylaxis			
No	31 (60.8)	103 (67.3)	1 [Reference]
Yes	2 (3.9)	0 (0.0)	Omitted
Unknown	18 (35.3)	50 (32.7)	1.20 (0.61 – 2.34)
Antibiotics use in last 2weeks			
No	7 (13.7)	16 (10.5)	1[Reference]
Yes	5 (9.8)	13 (8.5)	0.88 (0.23 – 3.43)
Unknown	39 (76.5)	124 (81.0)	0.72 (0.28 -1.87)
HIV status			
No	40 (78.4)	126 (82.3)	1[Reference]
Yes	11 (21.6)	27 (17.7)	1.28 (0.58 – 2.82)
Sputum smear			
Negative	5 (9.8)	17 (11.1)	1[Reference]
Positive	46 (90.2)	136 (88.9)	1.15 (0.40 – 3.29)
Contact with TB case			
No	10 (19.6)	26 (17.0)	1[Reference]
Yes	2 (3.9)	9 (5.9)	0.58 (0.11 – 3.15)
Unknown	39 (76.5)	118 (77.1)	0.86 (0.38 – 1.94)
Presence of cavities as seen on CXR			
No	24 (47.1)	56 (36.6)	1[Reference]
Yes	27 (52.9)	97 (63.4)	0.65 (0.34 – 1.23)

Table 5: Factors associated with Isoniazid Mono-resistance among new TB patients in Kampala

Characteristic	No. (%)		Odds Ratios (95% Confidence Level)	
	Isoniazid Mono-resistant TB (n= 51)	Drug Susceptible TB(n=153)	Unadjusted	Adjusted
HIV status				
No	40 (78.4)	126 (82.3)	1[Reference]	1[Reference]
Yes	11 (21.6)	27 (17.7)	1.28 (0.58 – 2.82)	1.03 (0.44- 2.42)
Occupation				
Unemployed	11(21.6)	53 (34.6)	1[Reference]	1[Reference]
Employed	40 (78.4)	100 (65.4)	1.93 (0.91- 4.06)	2.37(1.05- 5.38)
Tribe/ Ethnicity				
Non bantu	8 (15.7)	10 (6.5)	1[Reference]	1[Reference]
Bantu	43 (84.3)	143 (93.5)	0.38 (0.14 – 1.01)	0.37 (0.13 -1.01)
Use of HAART				
No	40 (78.4)	126 (82.3)	1 [Reference]	1[Reference]
Yes	1 (2.0)	0 (0.0)	Omitted	Omitted
Unknown	10 (19.6)	27 (17.7)	1.17 (0.52 – 2.62)	1.03 (0.44-2.42)
Ever use INH prophylaxis				
No	31 (60.8)	103 (67.3)	1 [Reference]	1[Reference]
Yes	2 (3.9)	0 (0.0)	Omitted	Omitted
Unknown	18 (35.3)	50 (32.7)	1.20 (0.61 – 2.34)	1.17(0.57- 2.39)
Presence of cavities as seen on CXR				
No	24 (47.1)	56 (36.6)	1[Reference]	1[Reference]
Yes	27 (52.9)	97 (63.4)	0.65 (0.34 – 1.23)	0.72(0.36-1.45)

From Table 5 above, only two factors were found to be significantly associated with Isoniazid mono-resistance among the new TB patients. The Bantu tribe with aOR of 0.37 (95% CI 0.13- 1.01) and the employed 2.37 (95% CI 1.05- 5.38). The HIV status of an individual was not significantly associated with INH mono-resistance, aOR 1.03 (95% CI 0.44 – 2.42). The model fitness was assessed by the Hosmer- Lemeshow goodness of fit.

CHAPTER 5: DISCUSSION

Prevalence

The prevalence of INH resistance was found to be 5.2% (95% CI 3.8 – 6.6) and that of Multi Drug resistance was 0.9% (95% CI 0.3 – 1.5). This suggested that the prevalence of anti-TB drugs resistance (Isoniazid and Rifampicin) are relatively low compared to Africa and have not changed significantly since the last national survey. The Uganda national survey conducted between in 1996-1997 as part of a global anti-TB drugs resistance surveillance programme, among patients who had received no more than 1 month of anti-TB therapy, carried out in only part of Uganda, found the prevalence of INH resistance to be 6.7% [1]. Similarly, a Kampala survey by *Lukoye et al 2008*, reported resistance among new cases was most frequent to Streptomycin 8.7% and Isoniazid 5.7% and MDR found to be 1.1% [13]. A Mulago national hospital study in 2000 on previously untreated patients revealed a similar MDR prevalence of 0.9%[1].

In Africa, the rate of anti TB drugs resistance among new patients in general, is 11.4% (95% CI 6.4– 16.5). However mono-resistance to INH in Africa overall is 8.3% (95% CI 6.8– 9.9). Similarly, the rates of primary and acquired MDR-TB in African countries with a low incidence of HIV, are respectively 1.5% (95% CI 1.1– 4.3) and 12.4% (95% CI 8.9– 12.4%). Nevertheless, the rate remains relatively low compared to levels in Africa overall[12].

The patients who had attended this clinic were referrals from some of the health facilities located around Kampala district which are randomly spread out. The health facilities are either government funded health centres III and IV or private for profit facilities. The use of the whole

database provided an appropriate representative sample of the district. This generated the true burden of INH mono-resistance as well as MDR TB in the district. Although these study findings are for time duration (2002 to 2012) providing a period prevalence, they seem not to be deviant from the other studies carried out in parts of Uganda.

Factors Associated with INH Mono-Resistance

This study indicated that there was no significant difference noted with regard to sex or age and the rates of INH mono-resistance, although it showed that the proportion of INH resistant TB was higher in men than in women. This was also exhibited in a UK and Madagascar national survey [6, 12]. There were more cases who were young adults, 39 (76.5%) compared to those of older age 12 (23.5%). A Germany study identified younger age as a risk factor for INH resistance but which we did not find in this study[21]. Gender has not been associated with INH mono-resistance even in bigger national survey such as that conducted in Madagascar between 2005 and 2006. Therefore our findings are in agreement with the studies.

Higher proportion of INH mono-resistant (84.3%) isolates were reported among the Bantu people compared to the non-bantu (15.7%). Being a Bantu was protective and was associated with approximately 63% (aOR 0.37) reduction on development of INH mono-resistance. The non-bantu were represented by the Acholi, Luos, Kakwa, Sabins and Nubians. Kampala district is a metropolitan city in which nearly all native ethnic communities in Uganda are represented by its inhabitants. Nationally the country of Uganda has more Bantus compared to other tribes hence the high number of the former in the study.

Studies comparing different races/ ethnicities found that black Africans were more likely to have INH resistance strains compared to other races [6, 21]. The genetic mapping studies have tried to explain these differences across the races. In this study the difference in acquisition or development of INH mono-resistance could be explained by the genetic composition differences between the Bantu and Non-bantu.

From this study, employed patients were about 2.4 times more likely to be having INH mono-resistance. The nature of the employment varied from the blue collar jobs to white collar jobs with majority in the former group. These blue collar jobs included: motor mechanics, hair dressers, carpenters and construction workers. The association could possibly be explained by the harsh working environmental conditions experienced by the majority in this group. Poor aeration or low air circulation in rooms increases the transmission of TB pathogens. Only history of working in health centre suggests nosocomial transmission of drug resistance TB[13]. People who work usually come into contact with different kinds of the populations who are either asymptomatic or symptomatic and this increases their chances of contracting all forms of the disease.

There was no significant association between INH mono-resistance and positive HIV status. The lack of the association is not entirely surprising, as multi-country studies have failed to demonstrate excessive association between HIV status and antiTB drug resistance. A Mozambique study demonstrated an association between HIV and INH resistance[5]. The small numbers of HIV positive patients 38 (18.6%), in the study could not have allowed us detect any significant association.

It was expected that previous exposure to Isoniazid would be associated with its resistance; however this was not the case. Prior exposure to anti-TB drugs has been explained as a major factor to the development of resistant TB strains[1]. Nevertheless the large amount of missing data due self reporting obscured the true association between INH mono-resistance and ever use of INH for prophylaxis in this study. A US TB survey between 1993 and 2003 failed to show the association due to missing data[21]. Similarly, the poor self reporting affected other variables namely: history of HAART use and antibiotics use in the last 2 weeks, which failed to exhibit an association with the outcome.

There was no significant association between INH mono- resistance and presence of cavities as seen on radiographs, aOR 0.73 (95% CI 0.36-1.47). A binary logistic analysis of an Indian cohort found that the presence of cavities was not associated with MDR-TB. This was not observed probably because of the small number analyzed in the cohort[10].

The use of alcohol and history of smoking were not significantly related to INH resistance. This was contrary to a Botswana case control study involving 114 MDR cases and equal controls with non-MDR pulmonary TB which found that alcohol abuse was a risk factor for the development of MDR-TB [18]. Failure to observe an association might have been explained by the few alcohol users 46 (22.5%) studied.

The results of sputum smear showed no relationship with INH resistance. Studies from Mexican region, suggested that smear negative patients could be important factor for dissemination of

resistant forms of TB[19]. History of contact with a TB was based on self report and most of the patients did not know 157 (77.0%).

Limitations

Selection bias could have resulted from the referral processes as only the health workers with knowledge of existence of CWRU-MU clinic referred patients to it. The referrals are from the government health centres III and IV as well as private clinics and hospitals. Although some hospitals such as: Mengo, Nsambya and Rubaga offer the TB services, we assumed that the patients had almost similar characteristics, since all these centres get referrals and offer the services freely. This might have minimized the bias given the fact that the referring health facilities are randomly spread out in all divisions of the Kampala district.

The natural occurring clusters (household units) of the participants were not considered in the prevalence study. This could have led to a random error. However the small numbers of the participants from the same household could not have greatly distorted the true prevalence of drug resistance.

This retrospective study used patients' medical charts and self report forms obtained during medical history taking at the enrollment into the clinic. There could have been problems of patient's recall which resulted to information bias in the study. This bias restricts the generalizability of these findings.

The numbers of resistant cases, in particular MDR, were small, limiting the power to detect risk factors for the drug resistance, including associations with HIV infection.

The development of drug resistance is based on numerous parameters, many of which might not have been assessed in this study but could be significant in a real-life scenario e.g Prior hospitalization and TB strain genotype.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

Conclusion

The prevalences of INH Mono-resistance and MDR TB are relatively low compared to Africa in general and have not changed significantly over time in Kampala since the conduction of the national global anti-TB drug resistance survey in 1996/97 and the Kampala survey in 2008.

Employed persons were approximately two and a half times more at risk of developing INH mono-resistance than those unemployed. Bantus are less likely to develop anti-TB drug resistance compared to Non-bantu.

Positive HIV status was neither associated with INH mono-resistance nor MDR TB.

Recommendations

- Molecular epidemiological studies to assess the genetic disparities among the Ugandan tribes need to be carried out in order to identify the different genetic markers.
- Further investigations using a prospective cohort study design with large sample size are needed to assess the anti-TB drug resistance relationships with use of Isoniazid for prophylaxis and use of HAART.

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APPENDICES

Appendix 1: Data Extraction Form

PREVALENCE AND FACTORS ASSOCIATED WITH ISONIAZID MONO-RESISTANCE AMONG NEW TUBERCULOSIS PATIENTS IN KAMPALA.

Name of Research Assistant.....

Participant's ID No.....

(Write in bold or tick where appropriate)

Socio-Demographic Characteristics

1. Age *(should be complete in years)* _____
2. Gender:
 1. Female 2. Male
3. Religion
 1. Catholic 2. Protestant 3. Muslim 4. Other (Specify) _____
4. Marital status
 1. Single 2. Married 3. Separated/Divorced/Widowed
5. Highest level of education attained
 1. Never gone to school 2. Primary 3. Secondary 4. Tertiary
6. Occupation
 1. House wife 2. Farmer 3. Business person 4. Salaried employee
 5. Other (specify) _____
7. Tribe
 1. Bantu 2. Not Bantu 3. Unknown

8. Division of residence

1. Kawempe 2.Makindye 3.Rubaga 4.Nakawa 5.Central

Drug-Related Factors

9. Alcohol use

0. No 1. Yes

9b. Frequency of alcohol use

1. < or equal to 1 Day in a week 2. 2-3 Days/week 3. 4-6 Days/week
4. Daily

10.Smoking

0. No 1. Yes

10b. If yes, average number of cigarettes/day? _____

11.Use of other habit forming drugs

0. No 1. Yes

11b. if yes, specify the type _____

12. Ever taken Herbal(Local) medicines

0. No 1. Yes

13.Are you on HAART

0. No 1. Yes

14. Ever been on Isoniazid(INH) prophylaxis

0. No 1. Yes

14b. if yes, for how long _____ week(s)

15. Other antibiotics used in last 2 weeks

Medical History

Ever been diagnosed with any of the following conditions?

0= No 1= Yes 9= Unknown

16.HIV _____

17.Cancer _____

18.Diabetes _____

19. Chronic Obstructive Pulmonary Disease _____

Nutritional Status

20.Weight in Kgs _____

21.Height in cm _____

TB Characteristics

22. Nature of the sputum

1. Positive 2. Negative

23.History of ever had a close contact with TB?

0. No 1.Yes 2. Unknown

23b. If yes, for how long _____ months

24. History of living with a contact with TB?

0. No 1. Yes 2. Unknown

25. Presence of cavities as seen on CXR

0. No 1. Yes

26. The strain of *Mycobacterium tuberculosis* present

Resistance to Anti-TB

Is there resistance to any of the following anti-TB?

27. Isoniazid (INH) _____

28. Rifampicin (R) _____

29. Ethambutol (E) _____

30. Pyrazinamide (P) _____

31. Streptomycin (S) _____

Appendix 2: Waiver of Consent request

2nd November 2012

The Chair,
School of Medicine Research and Ethics Committee,
Makerere University College of Health Sciences,
P.O. Box 7072 Kampala Uganda.

Thru:

The Director Clinical Epidemiology Unit,
College of Health Sciences, Makerere University

Dear Sir,

RE: Request for a waiver of consent for a study entitled “Prevalence and Factors Associated With Isoniazid Mono-Resistance and Multi-Drug Resistance among New Tuberculosis Patients in Kampala”

I am writing to request a waiver of consent for our proposed above medical records review study. This is a retrospective chart review that involves no more than minimal risk to the participants. The study will use extant records whose owners may be impossible to contact because of loss to follow-up either due to change of address or death. Moreover no medical procedures or interventions will be performed as part of this study, and no new medical conditions will be discovered that could increase economic, legal, or social risks for study participants.

In an effort to protect patient confidentiality, each subject will be assigned a unique identification number. All study related documentation will be stored under lock and key with restricted access. Access to computer records will be strictly controlled and will require simultaneous knowledge of the database structure, language, and multiple passwords. Names and other identifying information from subjects are obtained for quality assurance purposes only, and will be kept separately as an electronic file protected by multiple passwords. No individual will be identified by any study reports or publications.

The risks to subjects are minimal, and the findings are of potential benefits to society in general. The study will provide critical information on the prevalence and the possible risk factors associated with INH mono-resistance and MDR among new TB patient. This will help to improve on diagnosis and identification of new TB drug resistance suspects in the resource limited health facilities before initiating TB treatment. We appreciate your consideration of our request for a waiver.

Yours faithfully,

.....
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