Anti-tuberculosis drug resistance in Sub-Saharan Africa: The case of Uganda
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Anti-tuberculosis Drug Resistance In Sub-Saharan Africa
The Case of Uganda

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ANTI-TUBERCULOSIS DRUG RESISTANCE IN SUB-SAHARAN AFRICA: THE CASE OF UGANDA

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ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

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prof. dr. D.C. van den Boom

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Chapter 1

General introduction
General introduction

Anti-Tuberculosis Drug Resistance

Tuberculosis (TB) and HIV infection remain major public health problems in sub-Saharan Africa (SSA). TB is so far the second commonest cause of death among the infectious diseases and the commonest cause of morbidity and mortality among people living with HIV (PLHIV) in the world (1). Although HIV has existed for just a couple of decades, TB dates as far back as biblical times documented in the books of Deuteronomy and Leviticus in the old testament (2). It has devastated human kind throughout its documented history and expected to have resulted in more death than any other microbial organism(3). This pathogen, discovered by Robert Koch in 1882 is reported to have epidemiologically originated from East Africa approximately three million years ago (4)(5). Anti-TB drug resistance (DR) became apparent soon after the introduction of effective anti-tuberculosis agents (6)(7) and multidrug resistance (MDR) \textit{defined as in vitro resistance to both isoniazid and rifampicin, the two most efficacious anti-TB drugs}, is reported to increase at a rate of 2% annually. The perceived threat of DR TB, presently classified as a global pandemic, is substantial, challenging a number of TB control programs (8).
Globally an estimated 450,000 (300,000-600,000) MDR-TB cases occur annually. A total of 54,887; 61,907; and 83,715 MDR-TB cases were notified in 2010, 2011 and 2012 respectively, way below the estimated annual burden (9). Globally primary resistance to any of the TB drugs is estimated to occur at a prevalence in the range of 2% to 40%, highest against isoniazid estimated at 7.3% and lowest against ethambutol at 1.0%, while the prevalence of primary MDR-TB is estimated to be 1.4%. Although data on resistance to second-line drugs (SLDs) is still scarce in most of WHO regions, an estimated 9.6% of all the MDR-TB cases are also resistant to an injectable second-line drug and a fluoroquinolone. This combination has been coined extensively drug resistant TB (XDR-TB) after realizing severe consequences of this resistance pattern to the efficaciousness of SLD MDR-TB treatment. Prevalence of any resistance among the previously treated patients ranges between 5.3%-100% and MDR-TB rates in this patient category are estimated at 13% globally (10). It is important to note that DR among previously treated cases may not be a useful proxy of truly acquired resistance as it contains a combination of three types of resistance including (i) patients who have acquired resistance during TB treatment; (ii) patients who have been primarily infected with a resistant strain and subsequently failed therapy; and (iii) patients who have been re-infected with resistant forms (11). Global data on MDR-TB are difficult to interpret due to strong variation in drug resistance prevalence between countries and geographical regions. According to the WHO 4th global report on DR-TB, where DST results on 90,726 patients were reported from 83 countries, over 70% of all the cases were notified in only 15 countries dominated by countries of the former Soviet Union (FSU), including Azerbaijan, Moldova, Ukraine, Russian Federation, Uzbekistan, Estonia, Latvia, Lithuania, Armenia and Georgia. In addition two provinces of China contributed to this burden. In each of these countries about 6% primary MDR-TB prevalence was reported while an average of 11.7% MDR-TB rates were estimated among the previously treated patients with some countries in FSU reporting rates as high as 60%. Though the number of surveys is still small as a result of insufficient laboratory infrastructure in SSA, data from countries in this region shows levels of drug resistance as being lower than in most WHO regions with an approximated 60,000 prevalent cases annually (11), largely attributed to late introduction of rifampicin and to the unavailability of TB medicines at open market. On the other hand high levels of MDR-TB elsewhere in the world have been suggested to result from irregular supply of anti-TB drugs, use
of non-standardized TB regimens, nosocomial TB infections, migration and outbreaks in congregate settings like prisons (12)(13).

Although several biological mechanisms have been reported to result into resistance to anti-TB drugs (14), random chromosomal alterations and selection of mycobacterial mutants resistant to the most potent drugs is so far the most commonly documented, although this process occurs relatively slowly in a population of Mycobacterium tuberculosis (MTB) (15). Development of resistance in TB begins with monoresistance, and subsequent resistance to additional drugs may occur, hence resistance to more than one anti-TB drugs is the cumulative result of sequential mutations (16). Further, literature has shown that anti-TB drugs impose selective pressure in which resistant mutants gradually outnumber susceptible bacilli and emerge as the dominant strains (17). This probably explains why lack of patient adherence to treatment is a major risk factor for development of drug resistance and may predispose to high rates of MDR (10). TB treatment is complex, takes a long time and patients tend to feel better before the recommended period of therapy which may lead patients to non-adherence and therefore development of DR - TB (18). Other complex biological mechanisms of MTB leading to DR have been documented such as the physical barrier of the bacterial cell wall, efflux pumps and genetic traits that speed up clearance of drugs from the circulation (20). In the 1980s, Barnes listed possible epidemiological factors responsible for development of DR such as history of previous treatment, the patients' country of origin and duration of residence especially in the developed world (21). Treatment of latent TB infection is another practice of potential TB monotherapy in an event that patients with active disease are not correctly identified, predisposing to high rates of isoniazid resistance (22)(23) and contributing to an increased risk of MDR-TB in the population. Most importantly previous exposure to anti-TB drugs is so far the strongest risk factor documented over decades for development of DR in both individual surveys (24)(25)(26) and meta-analytic studies (27)(28). The consistency of this finding therefore clearly demonstrates how easily MTB can develop resistance when exposed to the current anti-TB drugs.
Drug Resistant Tuberculosis and HIV infection in SSA.

TB/HIV co-infection rates are highest in SSA. In 2010, of the 13% TB cases co-infected with HIV globally, SSA contributed 82% (29). Despite the high rates of TB/HIV co-infection with 13% of all HIV deaths globally attributed to TB (30), independent association between MDR-TB and HIV infection in SSA remains controversial. Some studies reported a positive association (31)(32)(33)(34)(35). However most of the population-based surveys have reported no association between MDR-TB and HIV infection (36)(37)(38)(39)(40)(41). Several biological mechanisms have been documented in support of a possible association, including the possibility of reduced fitness of the drug-resistant strains (42) with less capacity to cause disease in immune competent hosts which might lead to manifestation of such strains predominantly in immune compromised patients. Secondly, TB among HIV-infected patients is commonly due to recent infection where higher proportions of new infections may be drug resistant from the increasing pool of drug resistant MTB strains among active TB cases in the general population than before. Thirdly, there may be shared risk factors for HIV and MDR-TB such as injection drug use and hospitalization responsible for the reported nosocomial MDR-TB outbreaks. In addition, treatment of immune compromised TB patients is more likely to fail since such patients are more prone to carrying multiple genetically diverse MTB strains including strains that have been associated with higher potential to develop resistance. In addition gastrointestinal pathologies are common among TB/HIV co-infected patients which may result into reduced bioavailability of anti-TB drugs to achieve minimum required concentrations in the circulation for effective antibacterial action, thus predisposing to drug resistance (43). However, despite all the mechanisms postulated in support of this possible relationship, independent association of MDR-TB and HIV infection in SSA is yet to be confirmed (28).

Molecular aspects of anti-TB drug resistance.

Molecular epidemiological studies are important for understanding important aspects of TB control, including the ability to develop resistance to anti-TB drugs, transmissibility, immunogenicity and potential to cause disease outbreaks (44)(45). Molecular typing has been applied to study transmission within and between communities, to identify factors associated
with acquisition of infection and development of disease (46)(47)(48)(49)(50)(51), to investigate possible cases of nosocomial transmission (52) and to facilitate tracking of spread of both drug sensitive and drug resistant tuberculosis (53). Such techniques have also played a major role in differentiating between re-infection (i.e. with a new strain) and recurrence (i.e. with the same strain) of TB (54). In such studies several molecular methodologies have been used including spacer oligonucleotide typing (spoligotyping), regional difference (RD) analysis, mycobacterial insertion repetitive units (MIRU) analysis, and insertion sequence 6110 (IS6110) restriction fragment length polymorphism (RFLP), among others (55)(56). Single nucleotide polymorphisms (SNP) represent reliable markers for lineage classification of *Mycobacterium tuberculosis complex* and have proved highly sensitive and more specific than some other techniques such as spoligotyping (57). For Uganda, earlier molecular analytical studies identified the T2 genotype as an important MTB strain in this setting and more modern techniques (SNP) have been applied to further sub-classify this genotype into the Uganda I and Uganda II MTB strains (58)(59). Through such studies, the Uganda II subtype has been reported as the predominant genotype in most localities in Uganda, which has facilitated more molecular epidemiological studies of the distribution and associations of this genotype with other patient characteristics.

Genotypes of MTB have been studied in relation to drug resistance in earlier studies. The Beijing genotype in particular has been associated with outbreaks of multidrug-resistant tuberculosis in Europe and Cuba (60)(61)(62)(63) and, in the early 1990s, the W strain (a variant of the Beijing genotype), caused large outbreaks of multidrug-resistant tuberculosis in the United States. It is reported to be emerging in parts of Asia (53)(65) and still associated with drug resistance (66), confirmed by other reports which show high anti-TB DR rates where the this genotype is prevalent (67). However literature about other genotype in relation to DR patterns across the world is still limited. The Beijing genotype is documented as unique with regard to its mutation characteristics (68) that confer upon it higher ability than other strains to allow particular critical mutations in resistance genes, which enables them to acquire resistance to drugs used in a standard treatment regimens.
Diagnosis of drug resistant tuberculosis in Uganda.

Culture, MTB identification and drug susceptibility testing (DST) is recommended by the National TB/Leprosy Program as the mainstay for detection of MDR-TB cases in Uganda (Uganda national Guidelines for Programmatic management of Drug resistant tuberculosis The National TB/Leprosy program January 2011). However from 2011 through 2013, use of Xpert MTB/RIF assay has been improved across the country and diagnosis of rifampicin resistance as a proxy to MDR has been strengthened. A laboratory network exists in the country where sputum samples from patients with higher MDR-TB risk such as treatment failures, relapse cases and treatment defaulters are collected and delivered to the national TB reference laboratory (NTRL) for culture and DST and results returned to the requesting health facility through the same channel for appropriate patient care and support. Rifampicin resistant samples from laboratories with Xpert MTB/RIF are also processed at NTRL for a full DST profile. Although conventional DST by the Löwenstein-Jensen (LJ) method is characterized a by longer turnaround time (6-10 weeks) than most modern methods, it is so far the commonly used and recommended by the NTLP in Uganda.

Treatment of MDR-TB in Uganda.

Treatment of MDR-TB in Uganda follows a mixed model. Some patients are managed through an ambulatory approach in which a patient diagnosed with MDR-TB is treated in the community by a multidisciplinary team of both health care workers and community-based treatment supporters. Under this approach patients are initiated on treatment and attached to health care facilities close to their residences where directly observed treatment (DOT) for the second-line drugs (SLDs) is provided by a trained health worker on a daily basis. Each patient is reviewed by a team of experts at an established MDR-TB treatment centre at regular intervals and as/whenever need arises. A small proportion of patients are hospitalized due to such reasons as severe illness, those staying far from follow-up health facilities and those requiring monitoring of adverse events of the drugs hence the mixed model approach. International guidelines are applied with a combination of SLDs that have shown in vitro effectiveness for TB treatment (69). A standardized treatment regimen composed of five drugs that include six months of kanamycin
(KM), levofloxacin (Lfx), ethionamide (Eto), cycloserine (Cs) and pyrazinamide (Z), and 18 months of Lfx, Eto, Cs and Z is commonly used (6Km+Lfx+Eto+Z/18Lfx+Eto+Cs+Z).

Alternative standardized regimens include (6Km+Lfx+Eto+PAS/18Lfx+Eto+Cs+para-amino salicylic acid (PAS)) and (6Km+Mfx+Eto+PAS/18Mfx+Eto+Cs+PAS). Both injectable and oral treatment are directly supervised by a health worker at the follow-up health unit. Although shorter regimens have been found equally effective in some settings (70)(71), MDR-TB treatment in Uganda currently lasts a minimum of 18 months after culture conversion and treatment is standardized for all the patients, although individualized treatment has been documented to achieve relapse-free cure and higher success rates (72). Patient response to treatment is mainly monitored by culture conversion (73) but molecular and radiological methods (74)(75) as well as host makers have been reported in some studies (76) as mechanisms by which response to MDR-TB treatment can be monitored.

Challenges Associated with Diagnosis and Treatment of Drug Resistant Tuberculosis.

In Uganda and SSA at large, laboratory infrastructure has been documented as inadequate to conduct DST for routine DR surveillance, which has incapacitated most countries to generate data on rates of DR (77). Sputum microscopy, the commonest method for TB diagnosis in resource-poor settings such as SSA does not detect drug resistance, and culture and DST (phenotypic methods) are very costly and less sustainable if implemented at a wide scale. These assays are in addition not only performed in specialized laboratories but also have long turnaround times delaying timely decision-making for patient care. Although molecular methods with a shorter turnaround time are becoming available, they are expensive, and their diagnostic accuracy (specificity and sensitivity) is still debated in a number of studies when compared to conventional DST (78)(79)(80)(81). Secondly, prolonged hospitalization of patients for MDR-TB treatment where applicable does not only predispose vulnerable patients to nosocomial MDR-TB infections (82), it is too costly for the health system and poses social economic challenges for the patient. We have already provided information regarding the long duration of MDR-TB treatment in Uganda, with very expensive, less efficacious and more toxic drugs which affects treatment adherence (83). Despite the complexity of the applied regimens, SLDs have not been simplified to fixed drug combinations to ease their intake. MDR-TB treatment is further
complicated by high HIV co-infection rates and the use of antiretroviral drugs (ARVs) and SLDs, which may have shared toxicities further complicating patient care. Out of an estimated half a million MDR-TB incident cases annually worldwide, only 7% are diagnosed, of which only 1% are started on treatment from programs that use quality assured drugs (40). Diagnosis of primary resistance is commonly delayed partly as a result of the current policy. Due to limitations in laboratory capacity for DST, MDR-TB investigations for newly diagnosed TB patients only begin after failure of FLDs.

Study Site and Surveys

Uganda

Uganda is a land-locked country approximately 800 km from the Indian Ocean situated between latitude 4° 12 N and 1° 29’ S and longitudes 29° 34’ and 35° 0’ E. It stretches northwards from Lake Victoria to about 900 m above sea level and measures 241,038 sq km (197,100 sq km water and 43,938 sq km land. It lies in east of Democratic Republic of Congo, west of Kenya, North of the Republic of Tanzania and Rwanda and, to the south of the Republic of South Sudan. The population of Uganda is estimated to be 34,758,809 people. Uganda is a high HIV burden country with estimated prevalence of 7.3% in the 15-49 year age group.

Kampala City

Kampala, the capital city of Uganda, is host to the biggest TB burden of TB in this country. With an estimated 5% of the national population, Kampala’s health facilities report to the NTLP 18 to 20% of all TB cases in Uganda. About 7,500 to 8,500 TB cases are reported from Kampala annually, of which 3,500 to 4,000 are new sputum-smear positive (infectious) cases (Kampala district unpublished data). The treatment success rate (TSR) among diagnosed TB patients in Kampala is currently estimated at 77%, below the global target of 90% percent by 2015 (85). The cure rate is improving, currently estimated at about 56% and the ‘loss to follow up’ rate is still about 12%. The TB/HIV co-infection rate is about 50%. TB and HIV services are largely integrated at the health facility level, with over 90 percent of diagnosed TB patients testing for HIV since 2012.
Drug Resistance Surveys

Studies reported in this thesis are based on two anti-TB drug resistance surveys in Uganda at national and sub-national level. The sub-national survey provided sputum samples and data from the 22 clinics of the capital city (Kampala) and in the national survey 44 health facilities participated. (See map below).

Our survey in Kampala was done as part of the INTERACT program and supported financially by the European Union, and the Netherlands Organization for Scientific Research. It was in part...
meant to pilot survey tools and logistics (such as sputum-sample transportation from the enrollment sites to the NTRL), determine the laboratory capacity for culture and drug susceptibility testing (DST) and establish a link with a supranational laboratory for quality assurance. The Kampala DRS, for which data collection was concluded in December 2008, thus paved the way for the national survey that begun a year later (December 2009) with similar objectives.

The national survey was supported by the United States Centers for Disease Control and Prevention (CDC), Division of Global HIV/AIDS. At the time of the survey, there was limited population based data available on DR-TB in Uganda except for some regional data collected as part of the first global drug resistance surveillance reported over the preceding 10 years. Therefore data on DR-TB in Uganda was outdated or covered limited geographical settings. To enable implementation of programmatic management of DR-TB (PMDT), updated data was required to establish the burden of MDR-TB to guide strategic planning for its control in the country.

Although the two surveys employed different sampling methodologies, both provided a random sample of the population of smear-positive TB patients (i.e. in Kampala and Uganda, respectively). Both surveys complied with WHO guidelines for drug resistance surveillance(86) and included TB patients passively presenting to health care facilities with suggestive TB symptoms for care. Diagnosis was based on the national TB diagnostic algorithm and patients were only enrolled for the study after fulfilling the inclusion criteria. All newly diagnosed and previously treated TB patients received treatment according to the national treatment guidelines and all medications were supplied as fixed dose combinations by the NTLP free of charge to all the diagnosed TB patients.

Objectives and rationale for this thesis

The major scientific objective of the studies presented in this thesis was to establish the prevalence of anti-TB DR in SSA with focus on Uganda, and the possible risk factors, such as patient demographic characteristics, HIV infection, and the MTB genotype, in a setting with high burden of TB and HIV.
Using sputum samples collected and data generated in both drug resistance surveys we carried out five original anti-TB DR-related studies. We studied variations of M/DR-TB in SSA and associated risk factors on a regional scale through a systematic review in which we generated pooled DR data in this region with exception of the republic of South Africa assumed to have unique drivers for this epidemic that differed from those in other SSA countries. In addition to quantifying the burden of DR in SSA, we aimed to establish its potential drivers based on what has been found in both high and low MDR-TB burden settings elsewhere in the world. Since SSA is more hit by HIV than other WHO regions our findings were meant to add on the existing pool of knowledge on DR-TB in high HIV-burden settings and possible associations such as previous exposure to anti-TB drugs, HIV infection, social demographic factors and MTB genotype.

Chapter 2 reports on anti-TB drug resistance in Kampala. This study aimed to measure rates of DR-TB and associated factors including HIV infection in an urban African setting, potentially providing a different picture from nationwide estimates that include both urban and rural settings. The relatively small scale of this study allowed more in-depth collection of risk factor data than the logistically more complex nationwide survey.

Chapter 3 reports on anti-TB drug resistance in Uganda. In this study we aimed to assess the prevalence of DR among new and previously treated sputum smear-positive TB patients and associated risk factors at a nationwide scale, along with its association with HIV infection and demographic factors.

Chapter 4 provides a comparative analysis of HIV infection rates among sputum smear-positive TB patients in the DRS versus routine surveillance. Analysis of the Kampala survey data that included HIV testing results suggested a substantially lower TB/HIV co-infection rate than expected based on routine NTLP data. In order to corroborate this finding and understand possible causes of this inconsistency we collected individual patient-based data from the routine NTLP records of patients diagnosed in the same clinics over the same period and compared the HIV prevalence with that observed in the survey stratified by possible drivers of discrepancy.
Chapter 5 assesses MTB genotype variation in Kampala in relation to drug resistance and HIV infection. Using spoligotyping we aimed to establish whether in this random sample of TB patients from Kampala particular MTB strain families were associated with demographic characteristics, anti-TB DR and HIV infection.

Chapter 6 reports on a similar molecular epidemiology study done at a nationwide scale, but focusing on the Uganda II MTB genotype, a subtype of the T2 genotype that accounts for up to 85% of T2 strains circulating in Uganda. The T2 genotype, identified in earlier molecular epidemiological studies as the predominant MTB genotype in Uganda, was of particular interest as in our study in Kampala (Chapter 5) we found it to be negatively associated with anti-TB drug resistance when compared to other genotypes. This is potentially relevant for understanding the role of genotype in the epidemiology of TB DR in Uganda and possibly elsewhere in east Africa. But requires confirmation in a geographically more diverse dataset. The study in Chapter 6 therefore aimed to test the hypothesis of negative association between the Uganda II MTB genotype in a different study population that also allowed us to adjust for possible underlying regional variation across the country.

Chapter 7 provides a systematic review and meta-analysis of anti-TB drug resistance in sub-Saharan Africa. Despite the high burden of drug sensitive TB and HIV/TB co-infection rates, about half of the countries in SSA had results on anti-TB drug resistance prevalence by 2005 where low levels of DR have been consistently reported. This study aimed to establish variation of DR-TB) in SSA countries and determinants of TB DR in this region excluding the Republic of South Africa.

References


Chapter 2

Rates of anti-Tuberculosis Drug Resistance in Kampala-Uganda are Low and Not Associated with HIV Infection.

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Rates of Anti-Tuberculosis Drug Resistance in Kampala-Uganda Are Low and Not Associated with HIV Infection

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ABSTRACT

Background: Drug resistance among tuberculosis patients in sub-Saharan Africa is increasing, possibly due to association with HIV infection. We studied drug resistance and HIV infection in a representative sample of 533 smear-positive tuberculosis patients diagnosed in Kampala, Uganda.

Methods/Principal Findings: Among 473 new patients, multidrug resistance was found in 5 (1.1%, 95% CI 0.3–2.5) and resistance to any drug in 57 (12.1%, 9.3–15.3). Among 60 previously treated patients this was 7 (11.7%, 4.8–22.6) and 17 (28.3%; 17.5–41.4), respectively. Of 517 patients with HIV results, 165 (31.9%, 27.9–36.1) tested positive. Neither multidrug (adjusted odds ratio (ORadj) 0.7; 95% CI 0.19–2.6) nor any resistance (ORadj 0.7; 0.43–1.3) was associated with HIV status. Primary resistance to any drug was more common among patients who had worked in health care (ORadj 3.5; 1.0–12.0).

Conclusion/Significance: Anti-tuberculosis drug resistance rates in Kampala are low and not associated with HIV infection, but may be associated with exposure during health care.

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INTRODUCTION

An estimated 9.3 million incident tuberculosis (TB) cases and 1.4 million deaths occurred in 2007, making TB a major cause of morbidity and mortality in the world. Of major concern to TB control is the resistance to first-line anti-tuberculosis drugs. In particular with multi-drug resistance (MDR), i.e. resistance to isoniazid and rifampicin, cure rates for first-line anti-TB drug treatment are significantly reduced [1,2].

WHO estimates that 490,000 MDR-TB cases emerge every year, representing 5.3% of all TB cases globally, resulting in 110,000 deaths [3]. Data on anti-TB drug resistance for sub-Saharan Africa are often limited to hospital settings and representative data based on quality-assured susceptibility testing are scarce [2]. These data tend to show low MDR rates, but concerns have been recently fuelled by an epidemic in Southern Africa of MDR-TB, compounded by resistance to major second-line drugs (extensively drug-resistant/XDR-TB), emerging against a background of high HIV prevalence and expanding antiretroviral treatment [2,4].

HIV has a strong impact on TB incidence rates and HIV infection has been associated with MDR-TB through outbreaks in institutional settings [5]. Most studies however date from before the large-scale roll-out of antiretroviral treatment (ART). Since ART strongly reduces mortality in co-infected patients, it may have a paradoxical effect of increased transmission of MDR-TB through prolonged survival of infectious MDR-TB patients [6]. Results of the Uganda HIV sero-behavioral survey (2005) showed an HIV prevalence of 8.5% for Kampala district and ART has been widely rolled out in the district since 2004 (Kampala City Council, unpublished data).

In the first anti-TB drug resistance survey in Uganda (1996–1997), which used a similar sampling scheme as ours but covered another area of the country, the prevalence of resistance to any drugs was 19.8% and of MDR-TB 0.5% [7]. In a hospital-based study conducted two years later in Kampala, MDR prevalence was the same (0.5%), but resistance to rifampicin was 1.4%. More recently a hospital based study in Kampala found 12.7% MDR among previously treated patients [8]. Therefore data on anti-TB drug resistance in Kampala was either outdated or hospital based and no risk factors for anti-TB drug resistance were known.

To be able to estimate the current prevalence of anti-TB drug resistance and risk factors associated with it in Kampala district, we carried out a cross-sectional survey among new and previously treated TB patients. We in particular wanted to establish whether drug resistance
prevalence among new cases had increased and/or become associated with HIV status over the past 10 years during which anti-retroviral treatment has been rolled out, increasing from 44,000 in 2004 to 193,000 in June 2009 (Uganda AIDS Control Programme unpublished data).

METHODS

Ethical approval was obtained from the Research and Ethics Committees of Makerere University College of Health Sciences, Kampala. Written informed consent was obtained from all the patients who participated in the study.

Study design

We conducted this survey between 18 August and 19 December 2008 in all health care facilities in Kampala that reported TB cases to the National TB/Leprosy Programme (NTLP). This period was determined by the sample size of 483 new patients, based on the requirement to measure in this group an MDR prevalence of 1.4% with an upper boundary of the 95% confidence interval of 3.0%. Since all health care facilities were included in the sample, we assumed a design effect of 1. For logistical reasons we grouped the facilities into three, based on the number of sputum smear-positive patients expected in each group. Patient enrollment followed a rotational fashion from one group of health facilities to another so that each group participated for the same period in the study, thus keeping a self-weighted sample. All sputum smear-positive patients who were registered for treatment during the enrollment period aged 18 years or above and consented to participate were enrolled.

Data collection

Each participant who consented to participate was requested to provide two sputum samples (an early morning and spot) and a blood sample for HIV testing. HIV testing was done within 24 hours of collection at a quality-assured laboratory, independently of the routine HIV counseling and testing procedures.

Information about demographic characteristics, HIV status prior to enrollment, use of anti-retroviral treatment and history of TB treatment was collected through a structured interview. We also obtained data about risk factors for anti-TB drug resistance and HIV, including prison and health care exposure, injection drug use and involvement in commercial sexual activities.
We defined a patient as previously treated if this patient had a history of having taken first-line anti-tuberculosis drugs for one month or more and as new if otherwise. Patient treatment history was ascertained at the health facility using a standardized questionnaire recommended by WHO [9].

We carried out re-interviews on 110 (20%) participants randomly selected from the enrolled patients within 6 weeks of the original interview to check for the quality of ascertainment of treatment history. Re-interviews were conducted by staff who were independent of the clinic where data was collected and blinded to the original interview result; none showed any discrepancy in treatment history classification to the original interviews.

**Laboratory methods**

Sputum culture. For each included patient the sputum specimen with the highest bacillary count was decontaminated and inoculated onto two slopes: one glycerol and the one pyruvate Lowenstein-Jensen (L-J) medium incubated at 35–37°C and examined weekly for growth up to 8 weeks. Cultures showing no growth at 8 weeks were reported as negative. M. tuberculosis identification was based on presumptive appearance of colonies on culture and later confirmed by IS6110 based PCR. In addition, when performing drug susceptibility testing, paraminobenzoic Acid (PNB) 500 mg/ml was used to differentiate non-tuberculous mycobacteria from M. tuberculosis.

Drug susceptibility testing (DST). All M. tuberculosis isolates were tested at the National TB Reference Laboratory for resistance to isoniazid, rifampicin, ethambutol and streptomycin by the L-J proportion method using as concentrations 0.2 mg/ml for isoniazid, 4 mg/ml for rifampicin, 40 mg/ml for ethambutol, and 2.0 mg/ml for streptomycin. MDR was defined as resistance of an isolate to at least isoniazid and rifampicin. Second line DST was done on all MDR isolates using LJ proportional method using critical concentrations 2.0 mg/ml for ofloxacin and 30.0 mg/ml for kanamycin [10].

Drug susceptibility proficiency testing was performed at the Supranational Reference Laboratory (SRL) in Borstel (Germany) on all the identified MDR isolates, on 15 isolates randomly selected
from the remaining isolates of previously treated patients and on all rifampicin-monoresistant isolates.

**HIV Testing.** HIV antibody testing was done in parallel using Abbot Determine (Abbott Laboratories Abbott Park IL, USA) and double-well run Vironostika HIV Uni-form II Ag/Ab (BioMerieux Boxtel, Netherlands). The Generic Biorad HIV-1/ HIV-2 plus O-ELISA kit (Biorad Laboratories, Redmond WA, USA) was used as the tie-breaker. All tests were performed in accordance with the manufacturers’ instructions.

**Data management.** Data were double entered into Epidata 3.1 (www.epidata.dk). Discrepancies were checked against the raw data. Analyses were done in STATA v10 (Stata Corp. College Station TX, USA). For comparison of categorical variables we used the $X^2$ test or the 2-sided Fishers’ exact test as appropriate. Uni and multivariable analyses were done by logistic regression. Contribution of the variables to the model was tested using the likelihood ratio $X^2$ test. All significance testing was done at 5% confidence level.

**RESULTS**

During the rotation periods, 633 sputum smear-positive TB patients were registered with the NTLP of whom 557 (87.9%) were enrolled after submission of 2 sputum samples. Most of the non enrollments were due to declining participation and late start of enrollment due to administrative problems in some of the participating units. These did not significantly differ with regard to demographic characteristics like age, sex or history of previous TB treatment. None of the cultures grew non-tuberculous mycobacteria. Table 1 shows characteristics of the patients and proportions with complete data.

Of the study participants 327 (58.7%) were male, the modal age group was 25–34 years (216 participants, 38.8%), 50 (8.9%) were 45 years and above. There were 495 (88.9%) new and 62 (11.1%) previously treated patients. Three hundred and seventeen (59.5%) participants had been tested for HIV in the past; 91 (28.7%) were known to be HIV-positive. Of these, 34 (37.4%) were on anti-retroviral treatment; only one participant had used INH prophylaxis before (table 1).
Out of the 557 smear-positive specimens received at NTRL, those from 12 (2.2%) participants were contaminated and 12 (2.2%) were culture-negative. Therefore DST results were available for 533 (95.6%) patients of whom 473 (88.7%) were new and 60 (11.3%) previously treated (table 2). Of the latter the outcome of previous treatment had been cure for 20, treatment completion with no smear results for 16, failure for 7 and default for 17.

Among the new cases, MDR (percentage, 95% confidence interval (CI)) was found in 5 (1.1; 0.3–2.5) and any drug resistance in 57 (12.1, 9.3–15.3). Resistance among new cases was most frequent to streptomycin (8.7%) and isoniazid (5.7%).
Table 1. Characteristics of new and previously treated TB patients diagnosed in Kampala; August-December 2008.

<table>
<thead>
<tr>
<th>PATIENT Characteristic</th>
<th>Enrolled n = 557 (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>327 (58.7)</td>
</tr>
<tr>
<td>female</td>
<td>230 (41.3)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>18-24</td>
<td>183 (32.8)</td>
</tr>
<tr>
<td>25-34</td>
<td>216 (38.8)</td>
</tr>
<tr>
<td>35-44</td>
<td>108 (19.4)</td>
</tr>
<tr>
<td>45-54</td>
<td>37 (6.6)</td>
</tr>
<tr>
<td>&gt;= 55</td>
<td>13 (2.3)</td>
</tr>
<tr>
<td>Highest level of Education</td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>36/557 (6.5)</td>
</tr>
<tr>
<td>Primary</td>
<td>228/557 (40.9)</td>
</tr>
<tr>
<td>Secondary</td>
<td>228/557 (40.9)</td>
</tr>
<tr>
<td>Higher learning</td>
<td>37/557 (6.6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>216/557 (38.8)</td>
</tr>
<tr>
<td>Married</td>
<td>210/557 (37.7)</td>
</tr>
<tr>
<td>Separated</td>
<td>82/557 (14.7)</td>
</tr>
<tr>
<td>Widowed</td>
<td>23/557 (4.1)</td>
</tr>
<tr>
<td>Cohabiting</td>
<td>21/557 (3.8)</td>
</tr>
<tr>
<td>None of the above</td>
<td>5 (0.9)</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
</tr>
<tr>
<td>Public Servant</td>
<td>34 (6.1)</td>
</tr>
<tr>
<td>Self employed</td>
<td>430 (77.2)</td>
</tr>
<tr>
<td>Peasant</td>
<td>41 (7.3)</td>
</tr>
<tr>
<td>Student</td>
<td>52 (9.3)</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
</tr>
<tr>
<td>Kampala</td>
<td>373 (67.0)</td>
</tr>
<tr>
<td>Outside Kampala</td>
<td>184 (33.0)</td>
</tr>
<tr>
<td>HIV infection previously diagnosed positive</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>96 (16.6)</td>
</tr>
<tr>
<td>ART* use at enrolment</td>
<td>Yes 34 (6.8)</td>
</tr>
<tr>
<td>Previous history of TB treatment</td>
<td>Yes 62 (11.1)</td>
</tr>
<tr>
<td>No</td>
<td>495 (88.9)</td>
</tr>
</tbody>
</table>

**Column percentages.

HIV = Human Immunodeficiency Virus
ART = Anti Retroviral Therapy

Among the 60 previously treated cases, 7 (11.7, 4.8–22.6) had MDR and 17 (28.3, 17.4–41.4) had any resistance. Of the 7 MDR cases among this category, 3 (42.8%) were resistant to all four drugs, 2 (28.6%) were additionally resistant to streptomycin and 2 (28.6%) were resistant to isoniazid and rifampicin only.

Among new and previously treated cases combined, 13.9% (95% CI 11.0–17.1) had any resistance, 2.3% (95% CI 1.2–3.9) had MDR, 9.3% (95% CI 7.0–12.2) had mono-resistance and 3.4% (95% CI 2.0–5.3) had poly-resistance. Specific resistance patterns are shown in table 2.
Of the 30 samples submitted for external quality assessment, susceptibility results were concordant for 28 (93.3%) of the isolates. All resistant isolates were correctly detected, 2 isolates which were initially monoresistant to rifampicin turned out pansensitive at retesting.

Of the 517 patients with HIV results 165 (31.9%, 95% CI 27.9– 36.1) tested positive. No association was established among new or previously treated patients between any drug resistance and HIV status, neither before (table 3) nor after adjustment for potential confounders (table 4). Nor did we find any association between MDR and HIV status, although numbers were too small to allow meaningful multivariable analysis. We did not find significant associations between any drug resistance or MDR and any of the other demographic variables or potential risk factors for TB drug resistance or HIV infection, including anti-retroviral treatment among the HIV infected and history of hospitalization. We did however find a significantly increased risk of any resistance among new patients who had a history of having worked in health care (adjusted odds ratio 3.5 95% CI 1.0–12.2; p = 0.045). All 14 patients with a history of health care work had been tested for HIV. Of the 4 who had any drug resistance, 2 (50%) were HIV-infected, compared to 2 of 10 who had not (2-sided Fisher’s exact p-value = 0.520), while there was no increased prevalence of HIV infection among patients with a history of health care work (4 of 10, 28.6%) compared to those without (161 of 503, 32.0%, p=0.999). Two had monoresistance to streptomycin, one mono-resistance to isoniazid, and one combined resistance to streptomycin and isoniazid.

**DISCUSSION**

This study shows low prevalence of anti-TB drug resistance in Kampala district when new and previously treated patients are combined. Among previously treated patients, this is relatively high as established in other studies in the region. Seven of the twelve (58.3%) MDR cases were previously treated, yet this category contributed only 11.3% of the study participants. Although the MDR prevalence among new patients in this study was similar to that in a number of other studies carried out in the African region (1.4% in Burundi, 1.2% in Tanzania), it was lower than in Gambia (2.6%), Mozambique (3.4%) and Rwanda (3.9%) [11,12]. This suggests that transmission of MDR-TB in Kampala is a limited problem. We found no association between any resistance or MDR and HIV infection. Although the number of MDR cases was small and
thus limited the precision of our estimate, a clinically or epidemiologically relevant effect of HIV on the acquisition and/or transmission of MDR-TB would have resulted in sizable MDR prevalence among HIV-infected patients, which we did not observe.

The overall low MDR prevalence could result from community-based TB care with fewer chances of MDR-TB and HIV infected patients coming into close contact when seeking care in health facilities. In settings with high HIV prevalence, MDR outbreaks have been reported, generally resulting in increased anti-TB drug resistance prevalence among HIV infected patients [13]. Lack of association between drug resistance and HIV infection shows that opportunities for (nosocomial) transmission of drug-resistant TB may indeed be limited. In addition, supply of fixed dose combinations free of charge by the NTLP may contribute to patient adherence and prevent monotherapy during treatment. Finally the Uganda NTLP uses for adult new TB patients, who contribute about 80% of all the adult TB cases in Kampala, the eight-month standard regimen in which rifampicin is only given in the intensive phase. This regimen is likely to result in higher relapse rates than the six-month regimen where rifampicin is used throughout. However, it limits chances of improper use of rifampicin to the first two months, potentially reducing the risk of acquisition and hence transmission of MDR-TB in settings where observation of drug intake is not consistently applied throughout treatment.
<table>
<thead>
<tr>
<th>Pattern of resistance</th>
<th>Number (%)</th>
<th>95% CI</th>
<th>Number (%)</th>
<th>95% CI</th>
<th>Number (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Patients</td>
<td>473 (88.7)</td>
<td>60 (11.3)</td>
<td>533</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible to all</td>
<td>416 (87.9)</td>
<td>84.7–90.6</td>
<td>43 (81.6)</td>
<td>58.5–82.5</td>
<td>459 (86.1)</td>
<td>82.8–89</td>
</tr>
<tr>
<td>Any resistance*</td>
<td>572 (11.2)</td>
<td>9.2–15.3</td>
<td>17 (28.3)</td>
<td>17.4–41.4</td>
<td>74 (13.9)</td>
<td>11.0–17.1</td>
</tr>
<tr>
<td>Any resistance to:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP</td>
<td>7 (1.5)</td>
<td>0.5–3.0</td>
<td>8 (13.5)</td>
<td>6.9–23</td>
<td>15 (2.8)</td>
<td>0.4–11.6</td>
</tr>
<tr>
<td>INH</td>
<td>27 (5.7)</td>
<td>3.8–8.2</td>
<td>12 (20)</td>
<td>10.7–32.0</td>
<td>39 (7.3)</td>
<td>5.2–9.8</td>
</tr>
<tr>
<td>EMB</td>
<td>3 (0.64)</td>
<td>0.13–1.8</td>
<td>6 (10)</td>
<td>3.7–20.5</td>
<td>9 (1.7)</td>
<td>0.7–3.1</td>
</tr>
<tr>
<td>SM</td>
<td>41 (8.7)</td>
<td>6.3–11.5</td>
<td>9 (15)</td>
<td>7.2–26.5</td>
<td>50 (9.4)</td>
<td>7–12.2</td>
</tr>
<tr>
<td>H+R Resistance (MDR**)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH+RMP</td>
<td>1 (0.21)</td>
<td>0.054–1.7</td>
<td>1 (1.7)</td>
<td>0.04–8.9</td>
<td>2 (0.4)</td>
<td>0.04–13.5</td>
</tr>
<tr>
<td>INH+RMP+EMB</td>
<td>0 (0)</td>
<td>-</td>
<td>1 (1.7)</td>
<td>0.04–8.9</td>
<td>2 (0.4)</td>
<td>0.04–13.5</td>
</tr>
<tr>
<td>INH+RMP+EMB+SM</td>
<td>0 (0)</td>
<td>-</td>
<td>1 (1.7)</td>
<td>0.04–8.9</td>
<td>2 (0.4)</td>
<td>0.04–13.5</td>
</tr>
<tr>
<td>INH+EMB</td>
<td>1 (0.21)</td>
<td>0.54–1.7</td>
<td>4 (6.7)</td>
<td>1.8–16</td>
<td>5 (0.94)</td>
<td>0.3–2.1</td>
</tr>
<tr>
<td>INH+SM</td>
<td>12 (2.5)</td>
<td>1.3–4.3</td>
<td>4 (6.7)</td>
<td>1.8–16</td>
<td>16 (3)</td>
<td>1.7–4.8</td>
</tr>
<tr>
<td>RMP+EMB</td>
<td>1 (0.21)</td>
<td>0.54–1.7</td>
<td>4 (6.7)</td>
<td>1.8–16</td>
<td>5 (0.94)</td>
<td>0.3–2.1</td>
</tr>
<tr>
<td>RMP+SM</td>
<td>2 (0.4)</td>
<td>0.05–1.5</td>
<td>5 (8.3)</td>
<td>2.7–18</td>
<td>7 (1.3)</td>
<td>0.5–2.6</td>
</tr>
<tr>
<td>**Other resistance</td>
<td>0 (0)</td>
<td>-</td>
<td>1 (1.7)</td>
<td>0.04–8.9</td>
<td>1 (0.2)</td>
<td>0.005–10</td>
</tr>
<tr>
<td>Monoresistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMP</td>
<td>0 (0)</td>
<td>-</td>
<td>0 (0)</td>
<td>-</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>INH</td>
<td>10 (2.12)</td>
<td>1.0–3.8</td>
<td>4 (6.7)</td>
<td>1.8–16</td>
<td>14 (2.6)</td>
<td>1.4–3.3</td>
</tr>
<tr>
<td>EMB</td>
<td>1 (0.21)</td>
<td>0.054–1.7</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.19)</td>
<td>0.0004–0.010</td>
</tr>
<tr>
<td>SM</td>
<td>26 (12.9)</td>
<td>10–16</td>
<td>3 (5)</td>
<td>1–13</td>
<td>29 (4.4)</td>
<td>3.6–7.7</td>
</tr>
<tr>
<td>***Other resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMB+SM</td>
<td>1 (0.21)</td>
<td>0.054–1.7</td>
<td>4 (6.7)</td>
<td>1.8–16</td>
<td>5 (0.94)</td>
<td>0.3–2.1</td>
</tr>
</tbody>
</table>

*Any resistance: resistance to the drug with or without resistance to other drugs. RMP = rifampicin, INH = Isoniazid, SM = streptomycin, EMB = ethambutol. CI = Confidence Interval.

**MDR: Multidrug resistance, i.e. resistance to at least INH and RMP. Monoresistance: resistance to only one anti-tuberculosis drug.

***Other resistance: Resistance to a combination of other drugs not including INH or RMP.
Table 3. Univariable analysis for risk factors for any anti-TB drug resistance in Kampala.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>New patients</th>
<th>Previously treated patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N% OR; 95% CI P</td>
<td>n/N (%) OR; 95% CI P</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>29/278 (10.4) 1</td>
<td>14/37 (37.8) 1</td>
</tr>
<tr>
<td>Females</td>
<td>28/195 (14.4) 1.44 (0.83–2.51)</td>
<td>3/23 (13.0) 0.25 (0.06–0.98)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–24</td>
<td>20/166 (12.1) 1</td>
<td>3/11 (27.3) 1</td>
</tr>
<tr>
<td>25–34</td>
<td>21/185 (11.4) 0.93 (0.49–1.79)</td>
<td>5/22 (22.7) 0.78 (0.15–4.12)</td>
</tr>
<tr>
<td>35–44</td>
<td>12/84 (14.3) 1.22 (0.56–2.63)</td>
<td>6/18 (33.3) 1.33 (0.26–6.94)</td>
</tr>
<tr>
<td>45–54</td>
<td>4/27 (14.8) 1.27 (0.40–4.05)</td>
<td>2/8 (25) 0.89 (0.11–7.11)</td>
</tr>
<tr>
<td>≥55</td>
<td>0/11</td>
<td>1/1</td>
</tr>
<tr>
<td>HIV infection status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>40/308 (13.0) 1</td>
<td>14/44 (31.8) 1</td>
</tr>
<tr>
<td>Positive</td>
<td>15/149 (10.1) 0.75 (0.40–1.41)</td>
<td>3/16 (18.8) 0.49 (0.12–2.02)</td>
</tr>
<tr>
<td>Worked in Health care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>53/461 (11.5) 1</td>
<td>17/58 (29.3) 1</td>
</tr>
<tr>
<td>Yes</td>
<td>4/12 (33.3) 3.85 (1.12–13.2)</td>
<td>0/2 -</td>
</tr>
<tr>
<td>Admitted to hospital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>50/422 (11.9) 1</td>
<td>12/42 (28.6) 1</td>
</tr>
<tr>
<td>Yes</td>
<td>7/51 (13.7) 1.18 (0.51–2.77)</td>
<td>5/18 (27.8) 0.96 (0.28–3.29)</td>
</tr>
<tr>
<td>History of imprisonment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>56/435 (12.9) 1</td>
<td>14/53 (26.4) 1</td>
</tr>
<tr>
<td>Yes</td>
<td>1/38 (2.6) 0.18 (0.02–1.36)</td>
<td>3/7 (42.9) 2.09 (0.41–10.5)</td>
</tr>
</tbody>
</table>

*See table 2 above for definitions.

Lack of association with HIV infection suggests that the recently initiated large-scale introduction of ART in Kampala has not resulted in paradoxical increases in rates of MDR-TB at the population level. It may be that such effects will not occur, or that they will only occur after prolonged large-scale use of ART, for example, because it may require several TB transmission cycles before an effect on MDR-TB transmission becomes apparent. Therefore, continued combined TB-drug resistance and HIV surveillance remains a priority in settings where TB and HIV coexist.
Worth noting in our findings is the relatively high prevalence of any resistance to streptomycin (15%) among previously treated patients, because streptomycin is used during the first 2 months in the standard retreatment regimen. Although NTLP is implementing routine HAIN based rapid DST for previously treated patients, the results are yet to be used in guiding treatment decisions.

Table 4. Multivariate analysis for risk factors associated with any resistance to anti-TB drugs in Kampala.

<table>
<thead>
<tr>
<th>NEW PATIENTS</th>
<th>Any resistance</th>
<th>Number (%)</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV positive</td>
<td>Yes</td>
<td>15/149 (10.1)</td>
<td>0.7 (0.4–1.3)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>40/308 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worked in a health care setting</td>
<td>Yes</td>
<td>4/12 (33.3)</td>
<td>3.5 (1. –12)</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>53/461 (11.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Previously treated patients</th>
<th>Any resistance</th>
<th>Number (%)</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>3/16</td>
<td>0.6 (0.12–2.9)</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14/44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other variables used for adjusting included age, sex, patient category, and marital status.

Although HIV was not associated with anti-TB drug resistance, 32% of the study participants (and 26.7% of the previously TB treated participants) were HIV co-infected. However, TB/HIV co-infection among the patients notified to the NTLP is above 50% of those who are tested (NTLP, unpublished data). In the national referral hospital study mentioned earlier, the TB/HIV co-infection prevalence among previously treated patients was 49% [8], substantially higher than what we found. This may be due to a selection bias in the national surveillance data since not all notified TB patients are tested for HIV. In addition, TB patients diagnosed at the referral hospital may reflect a relatively ill selection, and therefore may have an increased probability of HIV infection. Finally, the wide roll-out of ART in Kampala to the level of primary care facilities may increase the average CD4 levels among the HIV infected, reducing the risk of progression from latent TB infection to TB disease. Further studies are required to explore whether HIV prevalence among TB patients has indeed declined in this setting and others where ART is provided at a large scale.
Figure 1. Patient flow chart during the study. DST, Drug Susceptibility testing; ZN –ve, Ziehl Neelsen negative; LJ, Lowenstein Jensen; Culture +ve, culture positive; Culture –ve, culture negative. doi:10.1371/journal.pone.0016130.g001
The association between transmitted anti-TB drug resistance and a history of health care work calls for confirmation in other studies since the numbers in this study were small: 4 patients with history of health care work showed drug resistance (to isoniazid and/to streptomycin) is cause for concern and calls for further exploration. It may reflect prolonged infectiousness of drug resistant cases due to delayed treatment response. This has been shown for resistance to isoniazid when current first line regimens are used, and may well occur in patients with streptomycin resistance when treated with the standard retreatment regimen [14]. As a result, health care workers could be more exposed to drug-resistant than to drug-susceptible TB. E.g. when working on a TB ward or in another facility where TB patients on treatment are encountered. HIV infection among these patients may also have increased their risk of (re) infection and subsequent TB disease, however we found no difference in HIV prevalence between patients who did and patients who did not have history of health care work, either with or without drug resistance. We did not find HIV infection to be associated with having drug resistant TB among these health care workers.

**Limitations**

This study had some limitations. The numbers of resistant cases, in particular MDR, were small, limiting the power to detect risk factors for drug resistance as statistically significant, including associations with HIV infection. However, if anything, we observed decreased risks of resistance among HIV-infected patients making it unlikely that we missed positive associations of substantial magnitude. While our sampling design in theory would result in consecutive sampling of all the eligible patient population, disrupted supply of anti-TB drugs during the survey, may have affected the sampling since some sites did not enroll participants into the study for the entire period. This could have led to selection bias if this problem was more frequent in clinics with relatively high resistance prevalence. For the same reason referral bias could not be ruled out, since larger health units were prioritized when anti-TB drug supplies were inadequate to cover all the TB treatment facilities. This probably did not significantly affect our results since health care facilities without drug supplies referred patients to other health units, and all TB care centers in Kampala were included in our study.

Anti-TB drug resistance prevalence in Kampala are low, but the occurrence of primary MDR indicating transmission of resistant M. tuberculosis strains is a threat to TB control in the district.
We therefore recommend that directly observed therapy for diagnosed TB cases with drug-sensitive disease be strengthened to prevent acquired drug resistance and a country-wide drug resistance survey be carried out to establish the national burden. Establishment of Programmatic Management of Drug-resistant TB in the country is urgently required. The finding of health care work as a risk factor for drug-resistant TB as well as the continuing risk of outbreaks of (M)DR-TB among the HIV-infected call for strengthening TB infection control in health care settings to prevent nosocomial transmission. Routine DST of previously treated patients should be strengthened in order to identify MDR cases before treatment is started, since these have over 10% probability of having MDR-TB. The introduction of rapid resistance testing methods should be supported and treatment policies adjusted so that treatment of previously treated TB cases can be better guided by their resistance patterns.

CONCLUSION AND RECOMMENDATIONS

Prevalence of anti-TB drug resistance in Kampala is low and not associated with HIV infection. Nonetheless continued and expanded surveillance of anti-TB drug resistance should be a priority. The association of drug resistance among new patients with a history of work in health care suggests nosocomial transmission and warrants further investigation.

ACKNOWLEDGMENTS

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Author Contributions

Conceived and designed the experiments: MLJ DL FGC. Performed the experiments: DL SK NE FEA. Analyzed the data: JKL FN MLJ. Contributed reagents/materials/analysis tools: FGC. Wrote the paper: DL FCG MLJ.
References


Chapter 3

Anti-Tuberculosis Drug Resistance among New and Previously Treated Sputum Smear-Positive Tuberculosis Patients in Uganda:
Results of the First National Survey

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Anti-Tuberculosis Drug Resistance among New and Previously Treated Sputum Smear-Positive Tuberculosis Patients in Uganda: Results of the First National Survey

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ABSTRACT

Background: Multidrug resistant and extensively drug resistant tuberculosis (TB) have become major threats to control of tuberculosis globally. The rates of anti-TB drug resistance in Uganda are not known. We conducted a national drug resistance survey to investigate the levels and patterns of resistance to first and second line anti-TB drugs among new and previously treated sputum smear-positive TB cases.

Methods: Sputum samples were collected from a nationally representative sample of new and previously treated sputum smear-positive TB patients registered at TB diagnostic centers during December 2009 to February 2011 using a weighted cluster sampling method. Culture and drug susceptibility testing was performed at the national TB reference laboratory.

Results: A total of 1537 patients (1397 new and 140 previously treated) were enrolled in the survey from 44 health facilities. HIV test result and complete drug susceptibility testing (DST) results were available for 1524 (96.8%) and 1325 (85.9%) patients, respectively. Of the 1209 isolates from new cases, resistance to any anti-TB drug was 10.3%, 5% were resistant to isoniazid, 1.9% to rifampicin, and 1.4% were multi drug resistant. Among the 116 isolates from previously treated cases, the prevalence of resistance was 25.9%, 23.3%, 12.1% and 12.1% respectively. Of the 1524 patients who had HIV testing 469 (30.7%) tested positive. There was no association between anti-TB drug resistance (including MDR) and HIV infection.

Conclusion: The prevalence of anti-TB drug resistance among new patients in Uganda is low relative to WHO estimates. The higher levels of MDR-TB (12.1%) and resistance to any drug (25.3%) among previously treated patients raises concerns about the quality of directly observed therapy (DOT) and adherence to treatment. This calls for strengthening existing TB control measures, especially DOT, routine DST among the previously treated TB patients or periodic drug resistance surveys, to prevent and monitor development and transmission of drug resistant TB.
INTRODUCTION

Tuberculosis (TB) remains one of the world’s leading causes of adult morbidity and mortality resulting in an estimated 8.8 million incident cases and 1.4 million deaths in 2010. Ninety-two percent of the cases occur in low and middle-income countries. Sub-Saharan Africa (SSA), a region with highest incidence of TB in the world hosts nine of the highest TB incidence countries globally [1]. The STOP TB strategy developed by the World Health Organization (WHO) aims to dramatically reduce the global burden of tuberculosis by 2015 by ensuring that all TB patients benefit from universal access to high-quality diagnosis and patient-centered treatment [2]. The HIV epidemic and the emergence of drug-resistant TB pose a serious challenge to achieving these ambitious goals. Treatment of multidrug resistant TB (MDR-TB) which is TB occurring in patients with strains of Mycobacterium tuberculosis resistant to at least rifampicin and isoniazid, was estimated to cost almost 30–40 times more than treatment of drug-sensitive disease in a recent study done in South Africa. In addition MDR-TB requires longer treatment with more toxic drugs, poorer treatment success rates, prolonged periods of morbidity and higher mortality as compared to drug sensitive TB [3,4].

MDR-TB is gaining global importance with an estimated 440,000 cases occurring annually, representing about 3.6% of all TB cases across the world [5,6]. Inappropriate drug regimens, non-adherence to treatment, transmission in congregate settings, substandard drug quality, and erratic drug supply are the major risk factors for development of drug resistant TB [7]. Mortality rates among MDR-TB patients have been reported to be as high as 37% and 89% among HIV-negative and HIV-positive patients respectively [8,9]. Anti-TB drug resistance surveillance using routine drug susceptibility testing (DST) for all TB patients prior to starting their TB treatment would be ideal for monitoring the performance of TB control programs. However, due to the lack of routine DST services in most high TB prevalent countries, periodic surveys of representative samples of TB patients in the country are the only available source of information on the prevalence of anti-TB drug resistance. Despite the importance of these periodic surveys, the most recent WHO reports show that only 22 of the 46 countries in the African region have conducted these anti-TB drug resistance surveys [10]. Some studies have shown an association between HIV infection with rifampicin monoresistance [11] and MDR-TB outbreaks have been
associated with HIV, although evidence showing HIV as an established independent risk factor for MDR is not yet documented [12]. The emergence of extensively drug resistant (XDR) TB, that is MDR-TB strains resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e, amikacin, kanamycin, or capreomycin) and its association with high mortality among people living with HIV has raised a new challenge for TB control [13].

Uganda with an estimated population of 33 million ranks 19th among the 22 high-TB burden countries in the world with an estimated incidence of 209/100,000 for all forms of TB [1]. About 8% of all the notified cases have had previous exposure to anti-TB drugs (relapses, defaulters or treatment failures). According to the WHO global report 2011, the cure rate was 31%, treatment completion 48%, death 8%, treatment failure 1% and treatment default 12%, among previously treated sputum smear-positive patients started on treatment. While among new patients, 28% were cured, 42% completed treatment, 5% died, 1% failed, 11% defaulted and approximately 14% were not evaluated.

Since 1997 Uganda has been using an eight-month regimen with two months of isoniazid, rifampicin pyrazinamide and ethambutol, followed by six months of isoniazid and ethambutol. For previously treated sputum smear-positive TB patients, the treatment regimen is two months of streptomycin, rifampicin, ethambutol, isoniazid and pyrazinamide, one month of rifampicin, ethambutol, isoniazid and pyrazinamide and 5 months of rifampicin, isoniazid and ethambutol. The mainstay of TB treatment in Uganda is community-based directly observed treatment (DOT). The National TB Leprosy Program initiated routine anti-TB drug resistance surveillance among re-treatment cases in 2008 although this has not been adequately implemented and improvement is still needed.

Limited anti-TB drug resistance surveys have been conducted so far, one in 1996–97 as part of global drug resistance surveillance that covered 3 zones. Two of the studies included new TB patients where the prevalence of MDR-TB was found to be 0.5% and 1.1% respectively [14–16]. Data on national anti-TB drug resistance rates and patterns in Uganda do not exist. The present study is the first national anti-tuberculosis drug resistance survey in Uganda conducted in accordance with the WHO-recommended methodology [17]. The objectives of this survey were to establish the prevalence of anti-TB drug resistance among new and previously treated smear
positive TB patients and to assess the risk for anti-TB drug resistance among HIV-infected TB patients in the country.

**METHODS**

**Study Design**
We obtained ethical approval from the ethical board at the Makerere University College of Health Sciences, the Uganda National Council of Science and technology, and Associate Director for Science at the United States, Centers for Disease Control & Prevention. All adult patients gave written informed consent before enrollment. Patients below 18 years assented and their consent was provided by guardians/parents.

**Sampling**
A cluster sampling method was used in which 30 clusters (primary sampling units) were selected randomly with probability proportional to the number of smear-positive TB patients registered in 2005. Within each cluster a fixed number of consecutively diagnosed smear-positive patients were enrolled so that all included patients had identical sampling probabilities (“self-weighted sampling design”) [18]. Four hundred and ninety eight public health facilities in Uganda had TB diagnostic and treatment centers in 2007. We used data reported in these facilities to determine the average number of TB patients diagnosed per facility. The sample size was based on the number of new sputum-smear positive TB cases notified through the National TB and Leprosy Control Program (NTLP) in 2007 (n = 20,559) and designed to detect an assumed rifampicin prevalence of 1.4% [15] with 1% absolute precision for a 95% confidence interval (CI). Assuming a design effect of 2, estimated losses due to contamination and negative cultures of 15%, the final sample size was 1500 new sputum smear-positive patients with each cluster required to enroll 50 patients within a year.

A cluster was defined as a health care facility that was able to meet the requirement of 50 new smear-positive TB cases in a year (according to the 2007 enrollment). Where a facility was noted to have achieved enrollment of less than 50 new cases, it was merged with others depending on proximity to each other to have a group that was able to enroll the required minimum number of
50 cases. Such a group was called a pseudo-cluster. Clusters and pseudo-clusters were then listed. Based on the cumulative total enrollment 30 clusters/pseudo-clusters were selected randomly with probability proportional to the number of smear positive TB patients in accordance with the WHO guidelines [18]. Participants were enrolled from 44 diagnostic facilities [fig. 1]. Of these 21 were clusters involving 18 facilities (one had four clusters) and 9 were pseudo clusters involving 26 facilities. TB patients who were already on anti-TB treatment at the beginning of the study were excluded and enrollment of eligible patients into the study was done alongside provision of other services involved in treatment initiation including registration of patients in the unit TB registers for care. Consecutive eligible and consenting patients were enrolled in the survey until the sample size for each cluster was met. Alongside enrollment of new cases, all sputum-smear positive previously treated TB cases identified at the selected health facilities during this period were also included in the survey. Health care workers used a detailed questionnaire to collect demographic and clinical information to accurately classify patients as new or previously treated. Prior to the start of the survey, staff from all the selected health facilities were trained on the survey procedures and data instruments and participated in the piloting of instruments. A national coordination team was established to oversee and implement the survey.

Data Collection
A standard clinical form was used to obtain data on patients’ demographic characteristics, HIV status prior to enrollment and previous history of TB treatment through a structured interview. In addition data about risk factors for exposure to resistant strains including imprisonment and those related to the patients’ social environment were collected. All TB patients at the sites including those eligible for enrollment were counseled and tested for HIV under routine conditions as required by the Uganda NTLP guidelines [19] and their results were included in the information sent on the case report form.

The national coordination team independently carried out re-interviews on patients randomly selected from the enrolled patients within 2 months of the original interview to validate their treatment history in order to allocate each patient to the correct category based on previous treatment.
Laboratory Methods

Sputum collection & transportation. Each eligible patient who consented provided two sputum samples, an early morning and spot sample, independent of the routine samples used for diagnostic purposes to minimize chances of contamination of samples collected for the survey. No decontaminants were added. Samples were refrigerated at 4°C and then transported to the National TB Reference Laboratory (NTRL) for processing via a local courier system. Sputum samples were accompanied by a sputum shipment form that contained information about the date of sputum collection, participant number, and laboratory serial number and quantified results of sputum smear examination from the local laboratory.
Figure 1. Map showing Health care facilities which participated in the National anti-TB drug resistance survey December 2009–February 2011.
Sputum culture and drug Susceptibility Testing (DST). At the NTRL, samples were decontaminated using 1.5% NaOH NALC method. One of the samples, preferably an early morning sample was processed while the other was kept as a backup. The backup sample was analyzed if the first sample turned out as either negative or contaminated. The other sample was inoculated on 2 slopes of egg based Lowenstein-Jensen (L-J) medium, incubated at a temperature of 37°C and monitored weekly for growth up to 8 weeks. A culture was only reported negative if no growth was shown after 8 weeks. For the positive cultures identification of M. tuberculosis was done based on presumptive phenotypic appearance of colonies on the medium, and confirmed using insertion sequence 6110-based PCR method as previously described [20].

Isolates were tested for resistance to rifampicin, isoniazid, ethambutol and streptomycin using the LJ proportional method, in concentrations of 40 mg/ml for rifampicin, 0.2 mg/ml for isoniazid, 2.0 mg/ml for ethambutol and 4.0 mg/ml for streptomycin and all identified MDR-TB isolates were tested for resistance to kanamycin and ofloxacin using the same method in concentrations of 30 mg/ml & 2.0 mg/ml respectively.

We sent all rifampicin resistant isolates, a random sample of isolates from retreatment patients that were susceptible to isoniazid and rifampicin (n = 20) and a random sample of isoniazid resistant isolates sensitive to rifampicin (n = 20) to the supra-national reference laboratory (Borstel–Germany) for blinded external quality assurance.

Definitions
A smear positive case in the study was defined as an individual in which at least one sputum sample was positive, for acid fast bacilli by direct Ziehl Neelsen staining. We defined a new patient as one who had not received first line anti-TB drugs for more than one month and previously treated if the patient had received first line anti-TB treatment for more than one month.

An MDR-TB patient was defined as one whose sputum isolate showed resistance to at least isoniazid and rifampicin while XDR-TB was defined as an MDR-TB patient whose isolate demonstrated resistance to kanamycin (as an injectable second line anti-TB drug) and ofloxacin (as a fluoroquinolone).
Data Management

Data were double entered in epi-info V6, and discrepancies were corrected using the raw data. Analysis was done in Stata v.10 (Stata/Corp. College Station TX USA/.) For comparison of categorical variables we used the Chi-square test or the 2-sided Fishers’ exact test where appropriate. Multivariate analysis was done using logistic regression. We did all significance testing at 5% confidence level.

The outcome was the proportion of patients with drug resistance stratified by history of previous treatment calculated as a proportion across all clusters after weighing for the exact sampling probabilities for each new individual patient for whom DST results were available. These sampling weights were calculated as (number of patients in the cluster with DST results/50). In all these calculations confidence intervals and p-values were adjusted for cluster design by first-order Taylor linearization and by second-order correlation of Rao and Scott of the Pearson $X^2$ respectively, as implemented by Stata svy commands. [21].

RESULTS

Of the 1537 patients enrolled at 44 health facilities, 1397 (90.7%) were new and 140 (9.3%) previously treated (fig. 2). Enrollment rate for the new sputum smear positive cases was 93.1% (1397/1500). Nine of the 30 clusters failed to enroll the required 50 new sputum smear positive patients due to insufficient number of patients registered during the enrollment period. A total of 1018 (66.2%) patients were male and the median age of the enrolled patients was 34.6 years. The national coordination team re-interviewed 130 (8.3%) patients to confirm their treatment history and the categorization of the patients as new or retreatment by the facilities was found to be completely accurate.

Culture Results, Table 1

Of 1537 enrolled patients, both LJ slants from 77 (5.0%) were contaminated leaving 1460 patients who had culture results that were included in analysis. Of these, 127 (8.7%) were negative while 8 isolates grew non-tuberculous mycobacteria (NTM). A total of 1325 (90.5%) patients with culture-positive isolates underwent DST including 1209 isolates from new and 116 from previously treated patients. There was no statistically significant difference with respect to
age, sex, history of previous treatment, and patient HIV status between patients who had culture-positive results and patients who had negative/contaminated cultures.

**Drug Resistance Prevalence, Table 2**

Among 1209 enrolled new patients with DST data, the prevalence of resistance to any of the drugs was 10.3% (n = 124; 95% CI; 8.6–12.1). Any resistance to isoniazid was found in 60 (5.0%; 95% CI; 3.8–6.3), and to rifampicin in 23 (1.9%; 95% CI; 1.2–2.8) of the isolates, while 17 (1.4%; 95% CI; 0.80–2.2) showed MDR-TB. Monoresistance to rifampicin was observed in 3 (0.24%) of the isolates.

Of the 116 previously treated patients, 53 (38.6%) had been cured, 58 (41.4%) completed treatment, 17 (12.1%) defaulted, 2 (1.4%) were treatment failures, while treatment outcomes of 9 (6.4%) patients were unknown. Thirty one (25.9%, 95% CI; 18.1–34.8) showed resistance to at least one drug. Any resistance to isoniazid was observed in 27 (23.3%, 95% CI; 15.3–32.0) and to rifampicin in 14 (12.1%; 95% CI, 6.7–19.4) patients. All the 14 (12.1%, 95% CI; 6.8–19.4) isolates resistant to rifampicin were MDR.

Overall the prevalence of any resistance and MDR when new and previously treated patients are combined was 11.6% (n = 154 95%; CI, 9.90–13.4) and 2.3% (n = 31; 95% CI 1.5–3.3) respectively. Of the 31 MDR-TB cases 17 (54.8; 95% CI 36.0– 72.6) were resistant to all the four first line drugs. We found monoresistance prevalence highest for streptomycin 3.7% (n = 49; 95% CI 2.7–4.8) and lowest for rifampicin 0.3% (n = 4; 95% CI, 0–0.7).

Out of the 73 samples sent to the SRL for external QA, accuracy was 97.3% (n = 71) for isoniazid, rifampicin, and streptomycin and 95.8% (n = 70) for ethambutol. All MDR-TB cases were correctly identified with exception of one isolate that turned out to be pan-susceptible on retesting.

**Factors Associated with Drug Resistance, Table 3**

Isolates from patients previously exposed to anti-TB drugs were more likely to show anti-TB drug resistance (odds ratio (OR) 9.02; 95% CI; 3.4–23.3 p,0.001). In multivariate analysis we
found that patients enrolled in urban clusters were more likely to have MDR-TB, compared to those from rural clusters (Adjusted OR 6.0; 95% CI 1.40–25.3; p = 0.02). We also found a significant association between age and drug resistance; those >35 years were more likely to have MDR-TB as compared to patients ≤35 years among new patients (OR 2.0; 95% CI; 1.0–4.30), while among the previously treated patients this association was not significant (OR = 1). No other associated factors were identified. Of the 1537 patients enrolled, 1524 (99.1%) had HIV testing of whom 469 (30.7%, 95% CI; 28.4–33.1) tested positive. Among the 1313 patients with complete HIV and DST results, no significant association was observed between HIV infection and any resistance (OR 1.2, 95% CI; 0.8–1.7 p = 0.38), isoniazid resistance (OR 1.2; 95% CI 0.76–2.1 p = 0.36) or MDR (OR 1.5; 95% CI, 0.52–2.5; p = 0.71) in a multivariate analysis.
Figure 2. Flow Chart of patient enrollment in the National Anti-TB drug resistance survey in Uganda; December 2009–February 2011.

Figure legend: **NTM = Non Tuberculous Mycobacteria.
XDR Prevalence

All the 31 MDR isolates were tested for susceptibility to kanamycin and ofloxacin to which all demonstrated complete susceptibility showing absence of XDR among the study patients.

DISCUSSION

This study is the first nationally representative anti-TB drug resistance survey in Uganda and one of the studies done in Sub Saharan Africa at a national scale. The survey showed an MDR-TB prevalence of 1.4% and 12.1% among new and previously treated sputum smear-positive TB patients respectively. Since settings with an MDR-TB prevalence of less than 3% among new patients are classified as having a low MDR-TB burden, [22] we conclude that the prevalence of MDR-TB among new smear positive patients in Uganda is low. MDR-TB among previously treated TB cases however was moderately high (12.1%). The prevalence of resistance to any of the first line anti-TB drugs, among new (8.3%) and previously treated (25.9%) patients was consistent with findings of a recent community based survey in Kampala city as shown in our previous report [16]. Other nationwide surveys in the region have observed the prevalence in the same range. The prevalence of any resistance among new and previously treated patients was 8.3% and 20% respectively in the United Republic of Tanzania [23]. A related survey done in Rwanda showed an MDR-TB rate of 3.9% when new and previously treated patients were combined as compared to the 2.3% that we report for the new and previously treated patients together in this survey [24].

While it’s difficult to directly compare outcomes from different countries, especially when surveys are conducted at different time periods, the data from this survey show that levels of MDR-TB among newly diagnosed smear positive TB patients in Uganda are relatively low. This could potentially be attributed among other things to the limited use of rifampicin only during the first 2 months (2EHRZ/6EH) for new TB cases who contribute over 90% of the disease burden, assuming a good adherence to TB therapy. This is contrary to the earlier reports that shorter duration of rifampicin may lead to increase in acquired resistance [25]. However, we also acknowledge the lower treatment success rate (70%) among new smear positive patients and the potential role of a rifampicin lacking TB regimen for this lower success rate [26]. In addition to the 17 cases identified as MDR-TB, an additional 43 and 6 new smear positive cases were found.
to have resistance to isoniazid and rifampicin respectively thus placing these patients just one step away from developing MDR-TB. If the national TB program plans to adopt 6 month TB regimen with 4 months of rifampicin during the continuation phase in the near future, directly observed therapy and adherence to therapy for all TB cases especially new TB cases has to be carefully monitored and completion ensured. The higher rates among previously treated TB patients as we see in this study have been attributed to stepwise selection of mutants due to drug resistance conferring genes [27].

Higher levels of MDR-TB (12.1%) and resistance to any drug (25.3%) among previously treated patients raises concerns about the quality of directly observed therapy and adherence to treatment. XDR-TB was not detected among the survey participants. Our study was not powered to assess the prevalence of XDR-TB among the study participants so no definitive conclusions could be made about the prevalence of XDR-TB in the country. However, XDR-TB might be a very limited problem if at all in Uganda especially given the limited use and availability of the second line drugs. Disaggregated by age, the older age group (.35 years) had higher levels of MDR-TB than the young age group (OR 2.0; 95% CI; 1.0–4.30) implying higher chances of exposure over time to drug-resistant TB in the community by the older than the young population. Patients diagnosed in urban clusters were more likely to have MDR-TB (OR = 6; 95% CI, 1.44–25.3 ) than those from rural facilities, probably as a result of referral of complicated TB cases including MDR-TB suspects from rural health units to referral centers (regional/district hospitals) commonly located in urban areas. Overcrowding in the towns and cities might also have facilitated primary MDR-TB transmission resulting in majority of the cases being in the urban clusters.

HIV prevalence was 30.7% (95% CI; 28.4–33.1) among study participants was lower than the 54% found among all TB cases through the TB surveillance system [1]. HIV was more prevalent among female participants than among males (OR 1.89; 95%; CI 1.45–2.50; p = ,0.01) and this finding was consistent with the gender wise HIV prevalence in the general population. According to the recent AIDS Indicator Survey (AIS) the HIV prevalence among females aged 15–59 was 7.6% and 5.6% among men of the same age group [28]. Like in other published studies, there was no statistically significant association between HIV infection and MDR-TB [29–32], although some studies have reported contrasting findings in which such an association has been documented [33].
Of major concern among the findings is the existence of primary resistance (rifampicin 1.9%, isoniazid 5%, streptomycin 6.3%) implying ongoing transmission of drug resistant strains in the community. This could imply weakness in infection control measures which should therefore be strengthened through dissemination of TB infection control guidelines by the NTLP. Priority should also be accorded to TB infection control training for health care workers in the TB diagnostic and treatment centers especially those which offer comprehensive TB/HIV care. Health care work was identified as a risk factor for resistance to any of the anti-TB drugs according to our earlier report [16], suggesting that nosocomial transmission of drug-resistant TB strains occurs.
Table 1. Demographic Characteristics of Patients enrolled in National anti-TB drug resistance survey in Uganda; December 2009–February 2011.

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<th>Not included for DST n (%)</th>
<th>p-value</th>
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<td>Outcome of previous treatment</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>successful</td>
<td>53 (37.9)</td>
<td>44 (37.8)</td>
<td>9 (37.5)</td>
</tr>
<tr>
<td>unsuccessful</td>
<td>8762 (62.1)</td>
<td>72 (62.1)</td>
<td>15 (62.5)</td>
</tr>
<tr>
<td>unknown</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>homeless</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (0.1)</td>
<td>2 (0.1)</td>
<td>–</td>
</tr>
<tr>
<td>No</td>
<td>2 (0.1)</td>
<td>2 (0.1)</td>
<td>–</td>
</tr>
<tr>
<td>Prisoner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>1325</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-values for the patients included and those not included in the analysis of drug resistance.
<table>
<thead>
<tr>
<th>Pattern of Resistance</th>
<th>New cases</th>
<th>Prevalence</th>
<th>95% CI</th>
<th>Previous Treated</th>
<th>Prevalence</th>
<th>95% CI</th>
<th>All cases</th>
<th>Prevalence</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patients</strong></td>
<td>N = 1209</td>
<td>1085 (89.7)</td>
<td>87.9–91.3</td>
<td>86 (74.1)</td>
<td>65.2–81.8</td>
<td>1171 (88.4)</td>
<td>86.5–90.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Any Resistance</strong></td>
<td>124 (10.3)</td>
<td>8.40–12.3</td>
<td>30 (25.9)</td>
<td>18.1–34.8</td>
<td>154 (11.6)</td>
<td>9.9–13.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMP</strong></td>
<td>23 (1.9)</td>
<td>1.2–2.8</td>
<td>14 (12.1)</td>
<td>6.80–19.4</td>
<td>37 (2.80)</td>
<td>1.9–3.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INH</strong></td>
<td>60 (5.0)</td>
<td>3.8–6.3</td>
<td>27 (23.3)</td>
<td>15.9–32.1</td>
<td>87 (6.56)</td>
<td>5.2–8.0</td>
<td></td>
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<tr>
<td><strong>EMB</strong></td>
<td>25 (2.1)</td>
<td>1.3–3.0</td>
<td>13 (11.2)</td>
<td>6.10–18.4</td>
<td>38 (2.90)</td>
<td>2.0–4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SM</strong></td>
<td>76 (6.3)</td>
<td>4.9–7.8</td>
<td>20 (17.2)</td>
<td>10.6–25.3</td>
<td>96 (7.24)</td>
<td>5.9–8.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INH+RMP Resistant (MDR)</strong></td>
<td>1 (0.025)</td>
<td>0.0–0.6</td>
<td>2 (1.72)</td>
<td>0.2–6.1</td>
<td>5 (0.38)</td>
<td>0.1–0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INH+RMP (Only)</strong></td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INH+RMP+ EMB</strong></td>
<td>6 (0.5)</td>
<td>0.0–1.0</td>
<td>3 (2.6)</td>
<td>0.5–7.3</td>
<td>9 (0.68)</td>
<td>0.3–1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All INH+RMP Resistant (MDR)</strong></td>
<td>17 (1.40)</td>
<td>0.6–2.2</td>
<td>14 (12.1)</td>
<td>6.80–19.40</td>
<td>31 (2.3)</td>
<td>1.5–3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INH+ Other Resistance</strong></td>
<td>5 (0.38)</td>
<td>0.0–0.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>INH+ EMB</strong></td>
<td>12 (0.9)</td>
<td>0.4–1.4</td>
<td>6 (5.2)</td>
<td>1.9–10.0</td>
<td>18 (1.4)</td>
<td>0.8–2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMP+other Resistance</strong></td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMP+ EMB</strong></td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMP+ SM</strong></td>
<td>2 (0.21)</td>
<td>0.1–0.6</td>
<td>0 (0)</td>
<td>–</td>
<td>2 (0.2)</td>
<td>0–0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMP+ EMB+ SM</strong></td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td>0 (0)</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mono Resistance to;</strong></td>
<td>3 (0.24)</td>
<td>0.0–0.7</td>
<td>4 (0.30)</td>
<td>0.0–0.7</td>
<td>0 (0)</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMP</strong></td>
<td>26 (2.1)</td>
<td>1.1–3.1</td>
<td>7 (6.0)</td>
<td>2.40–12.0</td>
<td>33 (2.5)</td>
<td>1.7–3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EMB</strong></td>
<td>11 (0.9)</td>
<td>0.4–1.4</td>
<td>1 (0.9)</td>
<td>0.0–4.0</td>
<td>12 (0.9)</td>
<td>0.5–1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SM</strong></td>
<td>47 (3.8)</td>
<td>2.7–5.0</td>
<td>2 (1.7)</td>
<td>0.2–6.1</td>
<td>49 (3.7)</td>
<td>2.7–4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other Resistance</strong></td>
<td>1 (0.05)</td>
<td>0.0–0.2</td>
<td>0 (0)</td>
<td>–</td>
<td>1 (0.1)</td>
<td>0–0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Any Resistance: Resistance to any of the anti TB drugs either in combination or as single drug.
**Mono Resistance; Resistance to only one anti-TB drug.

**RMP = rifampicin INH = isoniazid EMB = ethambutol SM = streptomycin.**
Limitations

The survey only represented patients diagnosed through the NTLP-supervised health facilities and does not account for drug resistance patterns among population not having access to the health system and, we did not have data about the size and characteristics of this patient population. Although the survey was conducted using the most recent WHO guidance, smear negative patients were not included in the survey. Our findings thus might not account for potentially different drug resistance pattern among smear negative TB patients. Moreover, inclusion of smear negative patient who are more likely to be HIV positive might impact the association between MDR-TB and HIV status. Nosocomial transmission in congregate settings has been proven to be one of the major risk factors for transmission of MDR-TB. We were not able to assess it during this survey.

Also, the sampling frame was based on TB case notification in 2005 in Uganda, and a number of changes in health care delivery system had taken place since, especially the establishment of new districts and new health facilities, which did not make part of the sampling frame but shared the patients with the included facilities. Incidents of untimely closure of the local courier system in some parts of the country might have led to delayed or non-delivery of the sputum samples from these clusters which could have contributed to the observed contamination and enrollment rates that varied from expected. However to avoid selection bias due to unequal participation rates, we controlled for this occurrence at the analysis level by weighting for the exact sampling probabilities for each individual patient for whom DST results were available across the clusters. We could have done multiple imputation for the missing drug resistance results, but with the amount of data that were available to predict drug resistance status for missing results, this method would most likely lead to biased results as well. The distances covered to reach the nearest diagnostic/treatment units (DTUs) in some clusters were too long to bring the early morning sample after submission of the spot sample as the study required. Some patients therefore failed to deliver the early morning sample within the required period. These numbers were however too small to affect the enrollment rates and the occurrence was too random to result into any bias that could significantly affect our results. Our conclusions about XDR-TB prevalence were based on resistance studies against kanamycin alone although cross resistance with other injectable second line anti-TB drugs has been documented.
Table 3. Analysis of factors associated with Multi drug resistance in Uganda; December 2009–February 2011.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>N (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td>Male</td>
<td>21/881 (2.4)</td>
<td>0.94 (0.4–2.0)</td>
<td>1.2 (0.5–3.30)</td>
</tr>
<tr>
<td>Female</td>
<td>10/444 (2.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age group (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>9/757 (1.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥35</td>
<td>22/568 (3.9)</td>
<td>3.3 (1.5–7.0)</td>
<td>2 (1.0–4.3)</td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>28/798 (3.5)</td>
<td>6.3 (1.9–20.9)</td>
<td>6.0 (1.44–25.3)</td>
</tr>
<tr>
<td>Rural</td>
<td>3/527 (0.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Previous history of TB treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14/116 (12.1)</td>
<td>8.6 (4.3–16.9)</td>
<td>8.6 (4.0–18.2)</td>
</tr>
<tr>
<td>No</td>
<td>17/1209 (1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HIV Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11/388 (2.8)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20/984 (2.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Variable included in the multivariate model were, age, sex, residence and previous history of TB treatment.

**Analysis limited to univariate level as inclusion at multivariate level masked the apparent association between MDR and potential risk factors.
CONCLUSION AND RECOMMENDATIONS

Anti-TB drug resistance among new smear positive TB cases was low and not associated with HIV infection in Uganda, despite the high TB-HIV co infection rates. We therefore recommend that strengthening and implementation of appropriate interventions is critical to keep MDR-TB levels low in the country or to reverse the trends. The NTLP needs to focus on improving the quality of directly observed therapy and develop interventions to support patient adherence in order to prevent development of acquired resistance. The NTLP should strengthen the existing specimen referral system and implementation of a routine surveillance system for anti-TB drug resistance to follow drug resistance trends over time and to identify outbreaks of drug resistant TB. Establishment of an effective MDR-TB control program and treatment strategy would be critical for effective clinical management of all cases of drug resistant TB. The introduction of rapid molecular diagnostic tests like Xpert MTB/RIF present a unique opportunity to diagnose MTB and identify rifampicin resistance within 2 hours [34,35]. WHO recommends the use of Xpert MTB/RIF as the first diagnostic test for persons at risk of developing MDR-TB and among people living with HIV [36] and NTLP should consider targeted roll out this technology. Efforts towards TB infection control including ensuring adequate ventilation for inpatient wards and outpatient waiting areas, provision of protective wear for patients and most importantly effective treatment of drug susceptible cases should be ensured to minimize emergence of new MDR-TB cases. We recommend further studies to establish whether MDR-TB cases are due to reactivation of latent disease or transmission of new infections and whether there exists predominance of a particular MTB strain among drug resistant patients as described elsewhere [37].

ACKNOWLEDGMENTS

We thank the staff from the National TB reference laboratory, the district health officials, staff from the TB diagnostic and treatment centers where the patients were enrolled, and highly acknowledge Professor FGI. Cobelens for the tremendous technical support provided during the survey and analysis of the data for this study. We also extend special thanks to the patients and their families for their participation.
REFERENCES


Chapter 4

Do drug resistance surveys underestimate the prevalence of HIV infection among sputum smear-positive TB patients?

Submitted for publication
Do drug resistance surveys underestimate the prevalence of HIV infection among sputum smear-positive TB patients?


1National Tuberculosis and Leprosy Program, Kampala, Uganda; Academic Medical Center and Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands. 2Makerere University College of Health Sciences, Kampala, Uganda; National Tuberculosis Reference Laboratory, Kampala, Uganda. 3Makerere University College of Health Sciences, Kampala, Uganda. 4National Tuberculosis Reference Laboratory, Kampala, Uganda. 5Academic Medical Center and Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands; KNCV Tuberculosis Foundation, The Hague, Netherlands.
ABSTRACT

Background: Surveillance of HIV infection among tuberculosis patients is important for monitoring tuberculosis/HIV co-infection. With the roll-out of provider-initiated HIV testing routine data on co-infection become increasingly available. HIV testing is also recommended as part of anti-tuberculosis drug resistance surveys (DRS) in high-burden HIV settings, and bias in either data source may limit their comparability.

Objective: To compare the prevalence of HIV infection among sputum smear-positive tuberculosis patients as estimated from a DRS with that obtained through routine surveillance.

Design: Sputum smear-positive patients included in a DRS in Kampala, Uganda, were tested for HIV antibodies, and routine records for smear-positive patients registered for treatment during the same period in the same clinics from which the survey population was sampled were checked for HIV status.

Results: HIV prevalence in the survey was 165/517 (31.9%, 95% CI 27.9-36.1%), but 844/1608 (52.6%, 95% CI 50.1-55.0) according to the routine records (p<0.001). The HIV prevalence measured in the survey was consistently lower independent of age, sex, treatment history and residence.

Conclusion: HIV prevalence data from anti-tuberculosis drug resistance surveys may underestimate the true prevalence as a result of design and inclusion criteria.
INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality among people living with HIV\(^1\). Studies have shown HIV as a cause of major changes in the clinical presentation of TB from a slowly progressing disease with reasonable prognosis to one with high mortality\(^2\). The World Health Organization recommends provider-initiated HIV counseling and testing (PITC) among presumptive TB cases, and among patients diagnosed with drug-sensitive or drug-resistant TB\(^3-4\) to comprehensively provide the required care and support to these patients.

In addition, inclusion of HIV testing in anti-tuberculosis drug resistance surveys (DRS) has been recommended as a source of valuable information for monitoring HIV prevalence among smear-positive TB patients and assessing associations between HIV prevalence and drug resistance\(^5\).

Uganda is among the 22 TB high burden countries in the world. Approximately 7500-8500 TB cases of all forms are notified annually from the capital city Kampala. The TB/HIV co-infection rate is estimated at 57% (Kampala district unpublished data), while the HIV prevalence for the 15-49 year age group is 9.3% in the general population\(^6\).

When in 2009 we completed a DRS among smear-positive TB patients in this city\(^6\) we observed an HIV prevalence that was considerably lower than we had expected based on routine reporting data. A nationwide DRS with a different design that was completed in 2011 showed a similar discordance\(^6\), suggesting a systematic bias in either data source rather than an artifact of our study design.

In this report, we compare TB/HIV co-infection rates among participants of an anti-TB DRS in Kampala\(^6\) with rates observed in routine TB/HIV surveillance among sputum smear-positive TB patients diagnosed in Kampala during the period that the survey was conducted. The aim was to establish the consistency of TB/HIV co-infection rates reported among this category of TB patients generated by the different surveillance systems and establish the extent to which TB/HIV co-infection rates in this group can be generalized to the population.
Study Population and Methods

From August to December 2008 we enrolled new and previously treated sputum smear-positive TB patients in a DRS in Kampala. This period was determined by the required sample size and the enrollment plan of the DRS. Eligible patients were all consecutively diagnosed during a fixed period at all health facilities in Kampala reporting to the National Tuberculosis and Leprosy Control Programme (NTLP) (simple random sampling). For logistical reasons the facilities were divided into 3 groups based on the expected number of sputum samples in each group, and sputum samples were collected from each of the 3 groups in a rotational pattern. Each group participated for the same period of time to ensure a self-weighted sample. Based on our sampling method, all patients in Kampala had equal probability of participation in the study. Two sputum samples (spot and early morning) and a blood sample for HIV testing were collected from each patient who consented to participate. We defined a sputum smear-positive TB patient as one with a positive Ziehl-Neelsen stained smear examination on either sputum sample. We did HIV testing in addition to and independent of the routine testing at the sites of patient enrollment. Information on patient demographic characteristics including HIV status prior to enrollment was collected using a structured interview.

In addition we retrospectively collected data on routine HIV testing and the number of smears done for each new and previously treated sputum smear-positive pulmonary TB patient registered in the health unit TB register during the period of the survey. All health care facilities in Kampala including those which participated in the survey collected this data on a quarterly basis as a requirement by NTLP for TB/HIV surveillance and disease monitoring. Since the routine data were collected from the same clinics and during the same period of the DRS, the survey population was a random sample of the patients in the routine records. Because the survey data had been anonymized however we did not link patient HIV data on an individual basis.

Serum HIV testing for the DRS was done at the Medical Molecular Laboratory, Makerere University College of Health Sciences, using Abbot Determine (Abbott Laboratories Abbott Park IL, USA) and double well run Vironostika HIV Uni-form II Ag/Ab (BioMerieux, Boxtel, Netherlands) in parallel. The Generic Biorad HIV-1/HIV-2 plus O-ELISA kit (Biorad Laboratories, Redmond WA, USA) was used as the tiebreaker. We followed the manufacturer’s
guidelines while performing all the tests. Results were not reported back to the health facilities since patients were also offered routine HIV testing.

Routine rapid HIV testing was done at the health care facilities according to the Uganda Ministry of Health algorithm. Two rapid HIV-1 tests, Unigold recombinant HIV (Trinity Biotech, Wicklow, Ireland) and Determine HIV-1 and 2 (Abbot, Tokyo, Japan), were performed on anticoagulated blood. Samples were first tested by Abbot Determine and only reported if they turned out negative. Unigold was used as the confirmatory test while a third rapid test, (Stat-Pak; ChemBio, Medford NY, USA) was used where results to the screening and confirmatory test turned out discordant.

We received ethical approval from the High Degree Research and Ethics Committee, Makerere University School of Public Health, (HDREC-MUSPH), the Faculty of Medicine Ethical Review Board, Kampala, Uganda and Academic Medical Centre Amsterdam, The Netherlands.

RESULTS

During the period 18th August – 21st December 2008, 557 of 633 (87.9%) registered sputum smear-positive patients were enrolled in the Kampala anti-tuberculosis drug resistance survey and received HIV counseling, including 327 (58.7%) males and 230 (41.3%) females. The modal age group was 25-34 years with 216 (38.8%) of the participants. Sixty seven percent were residents of Kampala district and 62 (11.1%) had received previous TB treatment. During the same period, 1901 sputum smear-positive patients aged 18 years and above were registered for routine care in the health unit TB registers in the city. The modal age group of the patients was 25-34 years, similar to that for the survey participants, with 750 (39.5%) of the total smear-positive TB patients registered. Seventy one percent of the patients were from Kampala, and 175 (9.2%) had received previous TB treatment (Table 1).

We received HIV testing results for 517 (92.8%) of survey participants, and for 1608 (84.6%) of sputum smear-positive patients enrolled for routine care. Among survey participants, 165 (31.9%, 95% confidence interval (CI) 27.9-36.1) were HIV seropositive. The HIV prevalence in this group was 40.3% (95% CI 33.6-47.2; n=85) among female and 26.1% (95% CI 21.3-31.4;
n=80) among male patients. Of the patients under routine care, overall 846 (52.6%; 95% CI 50.1-55.0) tested positive for HIV: 416 (62.6% 95% CI 58.8-66.3) female and 430 (45.6%; 95% CI 42.3-45.7) male patients. The prevalence of HIV was consistently higher in routine surveillance than in the DRS, both overall and within various strata for age, sex, residence (Kampala versus outside Kampala) and TB treatment history. These differences were significant (p<0.001), and except for the youngest and oldest age groups remained significant even after assuming that all patients with no HIV result in the routine system were HIV seronegative (Table 2).

DISCUSSION

Here we report a mismatch of HIV prevalence rates among sputum smear-positive TB patients in the same population generated by two different surveillance systems. Given the similarity in location, patient category, DRS participants being a subset of the total patients enrolled for care, and the similarity in HIV testing rates between the surveillance systems, one would expect minimal variability of HIV prevalence between the two groups regardless of differences in the surveillance system. The observed difference in co-infection rates was substantial: 32% and 53% in the DRS and in the routine surveillance, respectively. This poses potential problems, as comprehensive patient care and support in a setting with high TB/HIV co-infection rates require accurate HIV testing.  

The low rates of HIV infection among survey participants compared to rates under routine surveillance was confirmed by later findings of a nationwide DRS in Uganda in which we observed lower rates of TB/HIV co-infection among study participants (30.1%) in the same range as findings in Kampala city. Therefore, consistency in these findings suggests that this occurrence probably does not merely reflect specific design characteristics of the Kampala survey. 

The difference in observed TB/HIV co-infection rates may represent underestimation in the DRS in at least two ways. First, at enrolment for the DRS, sputum smear-positive TB patients who already had confirmed positive HIV serostatus might have declined participation during the consenting process. Consenting is not a requirement under routine care. Of all registered patients eligible for the survey, 12% were not enrolled, and these patients may have been more often
HIV-positive than those who were. That this may have happened is suggested by the observation that 13.6% (or 30.1% of all tested HIV positive) of patients in routine surveillance were on antiretroviral treatment, whereas this was only 6.1% for the patients in the DRS (20.6% of all tested positive).

Second, it may be that, compared to patients in the DRS, patients in routine surveillance more often had their diagnosis based on a single positive smear, for example because the physician decided that treatment was warranted on clinical grounds. Since the sensitivity of smear examination for pulmonary TB is lower among HIV-positive than among HIV-negative patients, the probability of inclusion for HIV-positive patients may have been lower in the DRS than in the routine surveillance. We were unable to verify this from the routine data, because the treatment register data contained no details on the numbers of pretreatment smears that had been examined.

Alternatively, TB/HIV co-infection rates in the routine surveillance may have been overestimated. Selection bias for HIV testing may have occurred if TB patients more likely to have HIV/AIDS were offered HIV testing, so that the patients not tested had below average probability of HIV infection. At the time of the study, provider initiated HIV testing and counseling (PITC) had not yet been initiated and patients had to voluntarily accept HIV testing under the policy that was applicable at that time. In the DRS over 90% of the participants were tested compared to 85% in the routine surveillance. However, even when assuming that all patients not tested in the routine surveillance were HIV-negative, co-infection rates were still considerably lower in the DRS.

The observed mismatch could finally reflect false-positive HIV testing results or erratic register entries at the health care facilities. Indeed, under routine care conditions quality control mechanisms might not be adequately applied. However this may not account for our finding since in the national DRS that was subsequently conducted, HIV testing was included in the study design with testing done in TB diagnostic and treatment centers under routine conditions, and TB/HIV co-infection rates among survey participants remained lower than in routine care just as we find in the present study. Therefore, although we cannot exclude that some overestimation occurred in the routine surveillance, it is likely that the difference in observed TB/HIV co-infection rates reflects underestimation in the DRS.
This bias will be important to be aware of when interpreting HIV prevalence data among TB patients. For example, for countries where provider-initiated testing among TB patients was recently scaled up, trends in HIV prevalence may for earlier years be based on DRS data and thereby potentially show a spurious increase over time. Similarly, in countries with weak health systems HIV prevalence among TB patients may altogether be derived from DRS, potentially leading to marked underestimation.

CONCLUSION

Rates of HIV infection among sputum smear-positive TB patients under drug resistance survey settings are lower than rates reported in routine TB/HIV surveillance as we show in this report. This is most likely caused by selection bias of study participants during implementation of the survey but could also result from selective HIV testing under a routine care setting. Although inclusion of HIV testing in drug resistance surveys is important for monitoring outbreaks of drug-resistant TB in settings of high TB and HIV burden and for assessing associations of drug resistance patterns with HIV infection, efforts should be made to maximize participation of eligible patients and thereby reduce selection bias. In addition, HIV infection prevalence among smear-positive TB patients in drug resistance surveys in which HIV testing is included in the study design must be interpreted with caution and only generalized to sputum smear-positive TB-patients at population level after controlling for the potential non-participation of TB/HIV co-infected patients.

ACKNOWLEDGEMENTS

We acknowledge the staff at Medical Molecular Laboratory of Makerere University College of Health Sciences who processed the sample and the health care workers at the TB diagnostic and treatment centers in Kampala who provided the HIV data among TB patients under routine care.

Conflict of interest. None declared
Financial support. This work was done as part of the INTERACT program and supported financially by the European Union (SANTE/2006/105-316) and the Netherlands Organization for Scientific Research (NACCAP W 07.05.2010).

REFERENCES

Table 1. Characteristics of sputum smear-positive patients from routine surveillance data and drug resistance survey compared.

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Routine surveillance</th>
<th>Survey data</th>
</tr>
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<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Sex</td>
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<tr>
<td>Males</td>
<td>1137 (59.8)</td>
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<tr>
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</tr>
<tr>
<td>18-24</td>
<td>489 (25.7)</td>
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<td>25-34</td>
<td>750 (39.5)</td>
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<td>79 (4.2)</td>
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<td>1353 (71.2)</td>
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<td>ART Use (N=846)</td>
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<td>1726 (90.8)</td>
<td>495 (88.9)</td>
</tr>
<tr>
<td>Total</td>
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<td>557 (100)</td>
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Table 2. HIV prevalence among sputum smear-positive tuberculosis patients in the drug resistance survey and routine surveillance in Kampala compared.

<table>
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<tr>
<th>Routine TB/HIV Surveillance Characteristic</th>
<th>Tested for HIV (%)</th>
<th>HIV positive out of all patients tested (%)</th>
<th>Drug Resistance Survey Tested for HIV (%)</th>
<th>HIV positive out of all patients (%)</th>
<th>*P value</th>
<th>**P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td></td>
<td></td>
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<tr>
<td>Males</td>
<td>941/1137 (82.7)</td>
<td>430/941 (45.6)</td>
<td>430/1137 (37.8)</td>
<td>306/315 (97.1)</td>
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<td>&lt;0.01</td>
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<td>Females</td>
<td>664/764 (86.9)</td>
<td>416/664 (62.2)</td>
<td>416/764 (54.7)</td>
<td>211/218 (96.7)</td>
<td>0.44</td>
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</tr>
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<td>Age Group</td>
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<td></td>
<td></td>
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<tr>
<td>18-24</td>
<td>414/489 (84.7)</td>
<td>136/414 (32.9)</td>
<td>136/489 (27.8)</td>
<td>171/177 (96.7)</td>
<td>0.88</td>
<td>0.20</td>
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<tr>
<td>25-34</td>
<td>642/750 (85.6)</td>
<td>359/642 (55.9)</td>
<td>359/750 (48.1)</td>
<td>199/207 (96.1)</td>
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<td>35-44</td>
<td>391/456 (85.8)</td>
<td>260/391 (66.5)</td>
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<td>64/106 (60.3)</td>
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<td>&gt;55</td>
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<td>26/79 (32.9)</td>
<td>8/8 (100)</td>
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<td>0.65</td>
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<td>Residence</td>
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<td>Kampala</td>
<td>1156/1353 (85.4)</td>
<td>604/1356 (45.0)</td>
<td>604/1353 (44.0)</td>
<td>344/357 (96.4)</td>
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<td>&lt;0.01</td>
</tr>
<tr>
<td>Other</td>
<td>452/548 (82.5)</td>
<td>241/452 (53.3)</td>
<td>241/548 (44.9)</td>
<td>173/176 (98.3)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Previous history of TB treatment</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>139/175 (79.4)</td>
<td>85/139 (61.2)</td>
<td>85/175 (48.6)</td>
<td>61/61 (100)</td>
<td>0.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>No</td>
<td>1469/1726 (85.1)</td>
<td>760/1469 (51.7)</td>
<td>760/1726 (44.3)</td>
<td>456/472 (96.6)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Total</td>
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<td>846/1669 (52.7)</td>
<td>846/1900 (44.5)</td>
<td>517/533 (96.9)</td>
<td>165/517 (31.9)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* p= p-value for the difference between the HIV prevalence among all patients in routine surveillance and HIV prevalence in the drug resistance survey. **p= p-value for the difference between the HIV prevalence among patients tested for HIV in routine surveillance and HIV prevalence in the drug resistance survey.
Chapter 5

The T2 Mycobacterium tuberculosis Genotype, Predominant in Kampala, Uganda, Shows Negative Correlation with Antituberculosis Drug Resistance

The T2 *Mycobacterium tuberculosis* Genotype, Predominant in Kampala, Uganda, Shows Negative Correlation with Antituberculosis Drug Resistance

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ABSTRACT

Surveillance of the circulating *Mycobacterium tuberculosis* complex (MTC) strains in a given locality is important for understanding tuberculosis (TB) epidemiology. We performed molecular epidemiological studies on sputum smear-positive isolates that were collected for anti-TB drug resistance surveillance to establish the variability of MTC lineages with anti-TB drug resistance and HIV infection. Spoligotyping was performed to determine MTC phylogenetic lineages. We compared patients’ MTC lineages with drug susceptibility testing (DST) patterns and HIV serostatus. Out of the 533 isolates, 497 (93.2%) had complete DST, PCR, and spoligotyping results while 484 (90.1%) participants had results for HIV testing. Overall, the frequency of any resistance was 75/497 (15.1%), highest among the LAM (34.4%; 95% confidence interval [CI], 18.5 to 53.2) and lowest among the T2 (11.5%; 95% CI, 7.6 to 16.3) family members. By multivariate analysis, LAM (adjusted odds ratio [ORadj], 5.0; 95% CI, 2.0 to 11.9; \( P < 0.001 \)) and CAS (ORadj, 2.9; 95% CI, 1.4 to 6.3; \( P = 0.006 \)) families were more likely to show any resistance than was T2. All other MTC lineages combined were more likely to be resistant to any of the anti-TB drugs than were the T2 strains (ORadj, 1.7; 95% CI, 1.0 to 2.9; \( P = 0.040 \)). There were no significant associations between multidrug resistance and MTC lineages, but numbers of multidrug-resistant TB strains were small. No association was established between MTC lineages and HIV status. In conclusion, the T2 MTC lineage negatively correlates with anti-TB drug resistance, which might partly explain the reported low levels of anti-TB drug resistance in Kampala, Uganda. Patients’ HIV status plays no role with respect to the MTC lineage distribution.
INTRODUCTION

Tuberculosis (TB) is one of the oldest infectious diseases in the world and one of the most successful pathogens in the history of mankind (1). Surveillance of circulating *Mycobacterium tuberculosis* complex (MTC) strains in a given locality is important for understanding TB epidemiology, including transmission, identification of outbreak-prone strains, and resistance to anti-TB drugs in a defined population of human hosts (2–4).

Studies done elsewhere have shown associations between specific genotypes and anti-TB drug resistance (5, 6), and some genotypes might be associated with HIV infection if they are less immunogenic or virulent than others in immunocompetent hosts (7). Although factors related to the quality of TB control programs and socioeconomic conditions have been cited to predict anti-TB drug resistance (8), to a large extent intrinsic factors influencing its emergence and spread remain obscure (9). Molecular tests have therefore been used to generate data on the frequency of drug-resistant strains to better understand the impact of drug resistance on the global spread of TB, hence contributing to identification of MTC strain families/lineages associated with anti-TB drug resistance (10, 11). Most studies have focused on the Beijing genotype, which has shown associations with drug resistance in several settings (10).

Uganda is among the 22 high-TB-burden countries that host 80% of all global TB cases (12). Anti-TB drug resistance levels in Uganda are low, with any resistance to anti-TB drugs estimated at 10.3% among new cases and 25.9% among the previously treated, while multidrug-resistant TB (MDR-TB) prevalence is 1.4% and 12.1%, respectively (13). About 53% of all TB patients in Uganda are coinfected with HIV (12), while at population level the HIV prevalence rate is 7.3% in the 15- to 49-year age group (14).

Kampala, the capital city of Uganda, measuring 197 km$^2$, has a projected population of about 1.8 million people. The district is administratively divided into 5 municipalities: Central, Kawempe, Lubaga, Makindye, and Nakawa. Respiratory diseases, including TB, are among the five top causes of morbidity in this locality (15). An estimated 18 to 20% of all TB cases reported to the National Tuberculosis and Leprosy Program (NTLP) come from this city, making Kampala the highest-TB-burdened district in Uganda (NTLP unpublished data). Anti-TB drug resistance and TB-HIV coinfection rates are similar to those for the entire country (16).
TB molecular analytical studies published from Uganda so far have included relatively small numbers of patients from limited administrative entities and/or a few clinics in which particular strains may predominate through localized transmission (17–19), while none have covered larger geographical areas through representative sampling. Our aim was to examine anti-TB drug resistance patterns of different MTC lineages circulating in the entire city of Kampala and to establish their relationship with HIV infection and other patient demographic characteristics in this city where the burden of TB, HIV, and TB-HIV coinfection is high.

MATERIALS AND METHODS

Study design. This study included patients who were enrolled in the anti-tuberculosis drug resistance survey that was conducted from August to December 2008 in all of the five divisions comprising the Kampala district (16). In brief, sputum samples were collected from all health care facilities in Kampala that reported TB cases to the NTLP. Eligible for inclusion were all new or previously treated sputum smear-positive TB patients aged 18 years registered for treatment during the study period at these clinics. The study period was determined by the sample size (of 536 patients) required for the anti-TB drug resistance survey, arrived at after considering an expected prevalence of rifampin resistance of 1.4%, with a desired upper boundary of the 95% confidence interval (CI) of 3.0%. This sample size also accounted for approximated 10% losses due to negative cultures or contamination. We assumed a design effect of 1 since all the facilities were included over a fixed period.

Data collection. Each participant provided 2 sputum samples (an early morning and a spot sample) and a blood sample for HIV testing. HIV testing was done within 24 h of collection at a central laboratory independently of the routine HIV counseling and testing procedures at health facilities. We used a structured interview to collect information on demographic characteristics, including known risk factors for drug-resistant TB, such as history of previous TB treatment, imprisonment, and health care exposure.

PCR analysis which targets regions of difference and spoligotyping on all Ziehl-Neelsen (ZN)-positive cultures isolated from 533 TB cases enrolled in the survey were done to determine species and phylogenetic lineage/clade of MTC strains.
Extraction of genomic DNA, MTC identification, and spoligotyping. Cultures were harvested using Tris-HCl–EDTA buffer and heated for 2 h at 90°C. Pure genomic DNA was extracted using the cetyltrimethyl-ammonium bromide (CTAB)-phenol-chloroform method according to a standardized protocol as earlier described (20). Isolates/cultures were confirmed as MTC by IS6110-PCR, as described previously (21), and further identification to species level was done using a PCR protocol based on the presence or absence of the region of difference (RD) RV2073C (RD9), TBD1, RV3120 (RD12), and RV1510 (RD4) in the MTC genome (20) and *Mycobacterium* genus-specific 16S rRNA. Standard spoligotyping was performed using a commercial kit (Isogen Bioscience BV, The Netherlands) according to the manufacturer’s instructions (22).

HIV and drug susceptibility testing. HIV testing was done in parallel using Abbott Determine (Abbott Laboratories, Abbott Park, IL, USA) and double-well run Vironostika HIV Uni-form II Ag/Ab (bioMérieux, Boxtel, The Netherlands) tests. The generic Bio-Rad HIV-1/HIV-2 Plus O-ELISA kit (Bio-Rad Laboratories, Redmond, WA, USA) was used as the tiebreaker. All tests were performed in accordance with the manufacturers’ instructions. We tested all the MTC isolates at the National TB Reference Laboratory (NTRL) for resistance to isoniazid, rifampin, ethambutol, and streptomycin using the Löwenstein-Jensen proportion method, in concentrations of 0.2 g/ml for isoniazid, 4.0 g/ml for rifam-pin, 40 g/ml for ethambutol, 2.0 g/ml for streptomycin, and 2.0 g/ml for ofloxacin and 30 g/ml for kanamycin for second-line drug susceptibility testing (DST). Multidrug resistance (MDR) was defined as resistance of an isolate to at least isoniazid and rifampin.

Data analysis. Spoligotypes were analyzed as character types with the un-weighted pair group method using arithmetic averages (UPGMA) and Jaccard index by the BioNumerics software, version 5.1 (Applied Maths, Kortrijk, Belgium). These spoligotypes were in addition compared in binary code format with the international spoligotyping database of the Pasteur Institute of Guadeloupe (http://www.Pasteur-Guadeloupe.fr.8081/SITVITDemo/) to assign phylogenetic lineages. Isolates which were orphaned, those with contiguous deletion of spacers 33 to 36 and spacer 40 or 43 or both spacer 40 and spacer 43 missing, and those undesignated with the same signature were grouped as T2-Uganda using visual rules (23).
Data on patient demographics, HIV infection, and DST results were double entered into Epidata 3.1 (www.epidata.dk). Data quality was ensured through validation of the two files and by checking the discrepancies against the raw data. Analyses were done in STATA v10 (Stata Corp., College Station, TX, USA). We used the chi-squared test or the 2-sided Fisher exact test as appropriate to compare categorical variables and logistic regression for multivariable analyses. All testing was done at the 5% significance level.

**Ethical considerations.** This study obtained ethical approval from the research and ethics committee of the Makerere University College of Health Sciences and the Uganda National Council for Science and Technology (UNCST) as part of the Kampala Anti-TB Drug Resistance Survey. We obtained informed consent from all study participants to use samples and stored isolates for future research.

**RESULTS**

**Inclusion of isolates.** A total of 633 sputum-smear-positive TB patients were registered for care during the period of enrollment, 557 of whom (87.9%) submitted two sputum samples for the study. Patients who participated did not differ significantly with respect to age, sex, or history of previous TB treatment from those who did not participate. None of the cultures grew nontuberculous mycobacteria.

Of the study participants, 58.7% were male, the mean age was 30.1 years, and the modal age group was 25 to 34 years (38.8%) with only 8.9% patients aged 45 years and above. New and previously treated patients constituted 88.9% and 11.1% of the study participants, respectively. Out of the 557 smear-positive specimens received, 12 (2.2%) were negative and 12 (2.2%) were contaminated on culture. Of the 533 remaining isolates that were analyzed, 497 (93.2%) records had complete demographic, DST, and spoligotyping data. Three hundred one (61%) of these were from male participants. With the exception of sex, isolates with complete data did not differ significantly from those excluded from analysis at this level. New and previously treated patients contributed 442 (91%) and 55 (9.0%), respectively, of the total isolates analyzed (Table 1).
Spoligotyping. One hundred seventy different spoligotypes were identified among the 497 MTC isolates. Three hundred ninety (78.5%) isolates belonged to 61 previously known spoligotypes, while 107 (21.5%) belonged to spoligotypes not previously reported in the SITVIT database. Twelve distinct lineages were identified. In order of predominance, these included T2-Uganda (28.9%), T2 (17.1%), CAS (10.2%), LAM (6.8%), and T1 (7.3%). Haarlem, S, MANU, T2T3, T3Eth, undesignated, and EAI contributed less than 2% each to the total number of isolates identified. Only two isolates were identified as Beijing (Table 2). Of the predominant lineages, SIT135 (T2-Uganda), SIT52 (T2), SIT26 (CAS1_Delhi), ST128 (T2), SIT53 (T1), SIT125 (T2), SIT21 (CAS1_Kili), SIT420 (T2), and SIT4 (LAM3/S convergent) individually contributed a significant portion (Fig. 1).

Variability of MTC lineages with patient characteristics. Analysis of the variability of strain lineages with key patient characteristics showed previously treated TB patients as being more likely to harbor the CAS lineage than new patients (23.6% versus 8.7%; p=0.001) (Table 3). Taking T2 as the reference group, there was a statistically significant association between the CAS family and history of TB treatment, which remained significant after adjusting for the HIV status (adjusted odds ratio [ORadj], 3.1; 95% confidence interval [95% CI], 1.4 to 6.7; p=0.004). The T1 (ORadj, 5.0; 95% CI, 1.7 to 19.6; p=0.005) family was more likely to occur in the age group 35 years than was the T2 family after adjusting for HIV infection status and history of TB treatment. No association was established between MTC lineage and other characteristics studied, including sex, previous exposure to a health care setting, and patient’s residence.
<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>No. Included (%)</th>
<th>No. included (%)</th>
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<td>n = 60</td>
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<tr>
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<td>302 (60.7)</td>
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<td>Female</td>
<td>195 (39.3)</td>
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<td>18-24</td>
<td>164 (33.0)</td>
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<td>188 (37.8)</td>
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<td>&gt;44</td>
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<td>117 (34.2)</td>
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<td>HIV results at (re)testing</td>
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<td>47 (9.5)</td>
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<td>55 (91.7)</td>
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<td>83 (16.8)</td>
<td>11 (18.3)</td>
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<tr>
<td>No</td>
<td>412 (82.9)</td>
<td>49 (81.7)</td>
<td></td>
</tr>
</tbody>
</table>

a= refers to patients who reported Kampala as their district of residence
b=refers to exposure to a health care setting as a health care worker
p-value for the difference between included and non included patients
Table 2: M. tuberculosis strain lineages identified among 497 isolates in Kampala

<table>
<thead>
<tr>
<th>Strain lineage</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>T2</td>
<td>85 (17.1)</td>
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<tr>
<td>T2- Uganda</td>
<td>142 (28.6)</td>
</tr>
<tr>
<td>T1</td>
<td>36 (7.3)</td>
</tr>
<tr>
<td>CAS</td>
<td>17 (3.4)</td>
</tr>
<tr>
<td>CAS1_DELHI</td>
<td>17 (3.4)</td>
</tr>
<tr>
<td>CAS1-KILI</td>
<td>15 (3.0)</td>
</tr>
<tr>
<td>LAM11_ZWE</td>
<td>12 (2.4)</td>
</tr>
<tr>
<td>LAM3/s-convergent</td>
<td>11 (2.2)</td>
</tr>
<tr>
<td>S</td>
<td>9 (1.8)</td>
</tr>
<tr>
<td>LAM9</td>
<td>8 (1.6)</td>
</tr>
<tr>
<td>H3</td>
<td>7 (1.4)</td>
</tr>
<tr>
<td>T2-T3</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>T2_ETH</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>Undesignated</td>
<td>5 (1.0)</td>
</tr>
<tr>
<td>CAS2</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>EAI5</td>
<td>4 (0.8)</td>
</tr>
<tr>
<td>Beijing</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>LAM4</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>H4</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>MANU1</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>MANU2</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>LAM6</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>EA1ND</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>107 (21.5)</td>
</tr>
</tbody>
</table>

Resistance patterns of tuberculosis lineages for first-line anti-TB drugs. Of the 497 isolates with complete drug susceptibility profiles, 75 (15.1%) showed resistance to at least one first-line anti-TB drug, with 52 resistant to streptomycin (10.5%). Fifteen (3.0%) and 40 (8.1%) isolates had any resistance to rifampin and isoniazid, respectively, while 12 (2.5%) were MDR. Eight (1.6%) had any resistance to ethambutol. As Table 4 shows, resistance to any first-line drug was most frequent among the LAM (34.4%; 95% CI, 18.5 to 53.1) and the CAS (31.0%; 95% CI, 18.2 to 45.4) lineages. The LAM family demonstrated the highest proportion of isolates resistant to isoniazid (25.0%; 95% CI, 11.4 to 43.4) followed by the T1 lineage (19.4%; 95% CI, 8.1 to 36.0). The T1 family, with the highest proportion of isolates resistant to rifampin (11.1%; 95% CI, 3.1 to 26.0), also accounted for the highest proportion of MDR isolates, since all T1 rifampin-resistant isolates were resistant to isoniazid as well. Other lineages combined, including the undesignated, Beijing, EAI, Haarlem, MANU, S, T2T3, and T2_Eth (each with less than 2% of the total number of isolates), showed no resistance to rifampin and were largely sensitive to...
isoniazid but substantially contributed to streptomycin resistance (13.9%; 95% CI, 5.2 to 27.9).

All *M. tuberculosis* lineages demonstrated minimal resistance to ethambutol compared to other drugs, with the exception of the CAS family, which exhibited this resistance pattern in 8.2% of the isolates.

Although isolates with spoligotypes unknown to the SITVIT database accounted for 21.5% of our sample, their contribution to overall resistance was low compared to those of other lineages. Among the distinctly classified lineages that contributed at least 10% to the total sample, the T2 family was the most predominant but demonstrated the lowest frequency of resistance to any of the anti-TB drugs studied (11.5%; 95% CI, 7.6 to 16.7). Multivariable analysis of the MTC lineages with anti-TB drug resistance showed that LAM (OR adj, 5.0; 95% CI, 2.0 to 11.9; *P* 0.001) and CAS (OR adj, 2.9; 95% CI, 1.4 to 6.3; *P* 0.006) families were more likely to be resistant to any of the anti-TB drugs than was the T2 family, whereas an association with the T1 family remained short of significance (OR 2.4; 95% CI, 1.0 to 6.2; *P* 0.070) (Table 5). All other MTC lineages combined were more likely to be resistant to any of the anti-TB drugs than were the T2 strains (OR adj, 1.7; 95% CI, 1.0 to 2.9; *P* 0.04). A multivariate model in which T2-Uganda was treated as a separate lineage showed no significant difference from T2 in prevalence of any resistance (OR adj, 1.2; 95% CI, 0.5 to 2.7; *P* 0.733).

**HIV infection.** Of the 497 participants with complete demographic, drug susceptibility, and spoligotyping data, 484 (97.4%) had HIV testing results, 160 of which (33.1%) were HIV positive and 324 of which (66.9%) were negative. Univariable analysis of the MTC lineages with HIV serological test results showed no significant association between HIV infection and *M. tuberculosis* lineage (Table 6). HIV infection was more frequent among patients older than 35 years than in the younger age group (*p* = 0.040).
DISCUSSION

In this representative sample of smear-positive TB patients in Kampala, we found significant variation of MTC lineages with anti-TB drug resistance and age but not with HIV infection. Although 21.5% of the spoligotypes were not previously identified and the T2 lineage contributed only 45.7% of the identified isolates, we document a clear negative correlation between the T2 lineage and resistance to any of the anti-TB drugs. The T2 strains in this study belonged to SIT420, -135, -128, -125, and -52, which were previously documented to be the predominant cause of TB in Lubaga municipality and some parts of rural Uganda (17–19, 24). Compared to other lineages that were distinctly identified and significantly contributed to our sample, specifically the LAM, CAS, and T1 families, T2 isolates were significantly less often anti-TB drug resistant. Patients infected with MTC of the LAM, T1, and CAS families had 5.0-, 2.4-, and 2.9-fold-higher likelihoods of harboring drug-resistant *M. tuberculosis*, respectively, although the difference between the T2 and T1 family with regard to anti-TB drug resistance was just short of significant (p=0.070) by multivariable analysis. It can be speculated from our findings that the observed negative correlation of the predominant lineage (T2) circulating in this population might be partly responsible for the low levels of anti-TB drug resistance in Kampala as detailed in our previous report (16). Predominance of the T2 lineage has been reported from elsewhere in Uganda, where drug resistance rates were similarly low (18, 19). Whether T2 is inherently less likely to become drug resistant or whether this is merely a reflection of environmental differences (for example, with regard to the quality of TB control measures)
remains to be investigated. However, the T1, CAS, and LAM families that were more frequently resistant to any first-line drug are widely distributed in Africa and hence more likely to be imported with preexisting drug resistance and then to spread in the local population. Compared to settings where rates of anti-TB drug resistance are high, the predominant strain has been associated with high rates of drug resistance. In addition, the Beijing genotype, documented to acquire drug resistance more easily or to be more easily transmitted when drug resistant (25), was almost absent in our sample.
Table 3. Variation of *M. tuberculosis* lineage in Kampala with key patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>T2-Uganda</th>
<th>T2- (%)</th>
<th>CAS</th>
<th>LAM</th>
<th>T1</th>
<th>Unknown</th>
<th>Others a</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>86 (28.5)</td>
<td>51 (16.9)</td>
<td>31 (9.9)</td>
<td>30 (6.6)</td>
<td>6.0</td>
<td>70 (23.2)</td>
<td>26 (8.6)</td>
<td>302</td>
</tr>
<tr>
<td>Female</td>
<td>56 (28.7)</td>
<td>24 (13.4)</td>
<td>19 (9.7)</td>
<td>12 (6.2)</td>
<td>15 (7.8)</td>
<td>37 (19.1)</td>
<td>20 (10.3)</td>
<td>105</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-34</td>
<td>102 (28.9)</td>
<td>52 (14.8)</td>
<td>30 (8.5)</td>
<td>27 (7.7)</td>
<td>33 (9.4)</td>
<td>75 (21.3)</td>
<td>33 (9.4)</td>
<td>352</td>
</tr>
<tr>
<td>&gt;=35</td>
<td>40 (27.6)</td>
<td>33 (22.8)</td>
<td>19 (13.1)</td>
<td>5 (3.5)</td>
<td>3 (2.1)</td>
<td>32 (22.1)</td>
<td>13 (9.0)</td>
<td>145</td>
</tr>
<tr>
<td><strong>Health care exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (24.7)</td>
<td>16 (18.8)</td>
<td>9 (10.6)</td>
<td>6 (7.1)</td>
<td>6 (7.1)</td>
<td>16 (18.8)</td>
<td>10 (11.7)</td>
<td>85</td>
</tr>
<tr>
<td>No</td>
<td>121 (29.4)</td>
<td>69 (16.8)</td>
<td>40 (9.7)</td>
<td>26 (6.3)</td>
<td>26 (6.3)</td>
<td>91 (22.1)</td>
<td>33 (8.7)</td>
<td>412</td>
</tr>
<tr>
<td><strong>Previously treated for TB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (16.4)</td>
<td>14 (25.5)</td>
<td>13 (23.6)</td>
<td>1 (1.8)</td>
<td>1 (1.8)</td>
<td>19 (35.2)</td>
<td>4 (7.3)</td>
<td>55</td>
</tr>
<tr>
<td>No</td>
<td>133 (30.1)</td>
<td>71 (16.1)</td>
<td>36 (8.1)</td>
<td>33 (7.0)</td>
<td>31 (7.0)</td>
<td>98 (22.2)</td>
<td>42 (9.5)</td>
<td>442</td>
</tr>
<tr>
<td><strong>Patient residence reported as Kampala</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>94 (28.7)</td>
<td>57 (17.4)</td>
<td>32 (9.5)</td>
<td>22 (6.7)</td>
<td>22 (6.7)</td>
<td>68 (20.8)</td>
<td>32 (9.8)</td>
<td>327</td>
</tr>
<tr>
<td>No</td>
<td>48 (28.8)</td>
<td>28 (16.5)</td>
<td>19 (10.6)</td>
<td>10 (5.9)</td>
<td>10 (5.9)</td>
<td>39 (22.9)</td>
<td>14 (8.2)</td>
<td>170</td>
</tr>
<tr>
<td><strong>Municipality where TB diagnosis was made</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>29 (32.2)</td>
<td>14 (15.6)</td>
<td>9 (10.0)</td>
<td>5 (5.6)</td>
<td>5 (5.6)</td>
<td>16 (17.8)</td>
<td>11 (12.2)</td>
<td>90</td>
</tr>
<tr>
<td>Kawempe</td>
<td>72 (26.7)</td>
<td>53 (19.6)</td>
<td>20 (7.4)</td>
<td>18 (6.7)</td>
<td>18 (6.7)</td>
<td>63 (23.3)</td>
<td>24 (8.9)</td>
<td>270</td>
</tr>
<tr>
<td>Luzira</td>
<td>11 (32.4)</td>
<td>4 (11.8)</td>
<td>4 (11.8)</td>
<td>3 (8.8)</td>
<td>3 (8.8)</td>
<td>9 (26.5)</td>
<td>3 (8.8)</td>
<td>34</td>
</tr>
<tr>
<td>Mokolowe</td>
<td>9 (23.7)</td>
<td>6 (15.8)</td>
<td>6 (15.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (28.9)</td>
<td>3 (7.9)</td>
<td>38</td>
</tr>
<tr>
<td>Nakawa</td>
<td>18 (29.5)</td>
<td>8 (13.1)</td>
<td>8 (13.1)</td>
<td>6 (9.8)</td>
<td>6 (9.8)</td>
<td>8 (13.1)</td>
<td>5 (8.2)</td>
<td>61</td>
</tr>
</tbody>
</table>

**Others**: includes sub lineages with less than 2% total contribution to the number of isolates analysed like, the undesignated, Beijing, EAI, Haarlem, MANU, S, T2T3, T2-Eth those unknown to the SpolDB4 database.

a = p value = 0.005 for comparison with age < 35 years for either lineage

b = p value = 0.001 for comparison with new patients for either lineage.
Table 4. Resistance patterns of M. tuberculosis isolates in Kampala, by lineage

<table>
<thead>
<tr>
<th>Strain Lineage</th>
<th>number of isolates per lineage (%)</th>
<th>Rifampicin</th>
<th>Isoniazid</th>
<th>Ethambutol</th>
<th>Streptomycin</th>
<th>MDR</th>
<th>Any resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2-Uganda</td>
<td>142 (28.6)</td>
<td>2 (1.4)</td>
<td>4 (2.8)</td>
<td>0 (0)</td>
<td>11 (7.7)</td>
<td>2 (1.4)</td>
<td>14 (9.9)</td>
</tr>
<tr>
<td>T2</td>
<td>85 (17.1)</td>
<td>3 (3.7)</td>
<td>9 (10.6)</td>
<td>1 (1.1)</td>
<td>8 (9.4)</td>
<td>3 (3.5)</td>
<td>12 (14.1)</td>
</tr>
<tr>
<td>CAS</td>
<td>49 (9.9)</td>
<td>3 (6.1)</td>
<td>9 (18.4)</td>
<td>4 (8.2)</td>
<td>8 (16.3)</td>
<td>2 (4.1)</td>
<td>15 (30.6)</td>
</tr>
<tr>
<td>LAM</td>
<td>32 (6.4)</td>
<td>2 (6.3)</td>
<td>8 (25.0)</td>
<td>1 (3.1)</td>
<td>8 (25.0)</td>
<td>1 (3.1)</td>
<td>11 (34.4)</td>
</tr>
<tr>
<td>T1</td>
<td>36 (7.2)</td>
<td>4 (11.1)</td>
<td>7 (19.4)</td>
<td>0 (0)</td>
<td>2 (5.6)</td>
<td>4 (11.1)</td>
<td>8 (22.2)</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>107 (21.5)</td>
<td>1 (0.9)</td>
<td>1 (0.9)</td>
<td>2 (1.8)</td>
<td>9 (8.4)</td>
<td>0 (0)</td>
<td>9 (8.4)</td>
</tr>
<tr>
<td>OTHERS</td>
<td>46 (9.3)</td>
<td>0 (0)</td>
<td>2 (4.4)</td>
<td>0 (0)</td>
<td>6 (13.0)</td>
<td>0 (0.0)</td>
<td>6 (13.0)</td>
</tr>
<tr>
<td>Total</td>
<td>497</td>
<td>15 (3.1)</td>
<td>40 (8.1)</td>
<td>8 (1.6)</td>
<td>52 (10.6)</td>
<td>12 (2.4)</td>
<td>75 (15.2)</td>
</tr>
</tbody>
</table>

*a* = refers to a group of isolates whose genotypes are unknown to the SITVIT database

*b* includes the undesigated, Beijing, EAI Haarlem MANU, S, T2T3 and T3-Eth.
Table 5: Association of *M. tuberculosis* strain lineage with resistance to any of the anti-TB drugs in Kampala.

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Total number (N) of isolates</th>
<th>No. (%) of isolates resistant to any anti-TB drug</th>
<th>Crude Odds ratio (95% CI)</th>
<th>Adjusted odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2/T2U ganda</td>
<td>227</td>
<td>26 (11.5)</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAS</td>
<td>49</td>
<td>15 (30.6)</td>
<td>3.4 (1.6-7.1)</td>
<td>2.9 (1.4-6.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>LAM</td>
<td>32</td>
<td>11 (34.4)</td>
<td>4.1 (1.7-9.3)</td>
<td>5.0 (2.1-11.9)</td>
<td>0.00</td>
</tr>
<tr>
<td>T1</td>
<td>36</td>
<td>8 (22.2)</td>
<td>2.2 (0.9-5.5)</td>
<td>2.4 (1.0-6.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>UNKNO WN</td>
<td>107</td>
<td>9 (8.4)</td>
<td>0.7 (0.3-1.6)</td>
<td>0.7 (0.3-1.6)</td>
<td>0.45</td>
</tr>
<tr>
<td>OTHER S†</td>
<td>46</td>
<td>6 (13.0)</td>
<td>1.2 (0.4-3.0)</td>
<td>1.1 (0.4-3.0)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Other variable included for adjusting were age, sex, patient category, history of imprisonment and health care work.

Based on multivariable logistic regression model, including age, sex, treatment history, history of imprisonment, and history of health care work.

†Includes Haarlem, EAI, MANU, S, undesignated, T2T3, T3_Eth, and Beijing.
Only 2.4% of the isolates analyzed were MDR, diversely distributed among the lineages (Table 3), making meaningful multi-variable analysis impossible. However, our results showed that T1 contributed the highest proportion to the MDR-TB isolates (9.1%), all of which belonged to the previously treated patient category (Table 4). Since previous exposure to anti-TB drugs is documented as the strongest risk factor for development of MDR-TB (26), a more in-depth molecular analysis of the T1 lineage in relation to previous exposure to anti-TB drugs in Kampala is needed.

The probability that TB infection has been acquired long ago increases with the age of the patient (27). Therefore, higher proportions of T1 among the 35-year age group, compared to T2, might point to more recent and active transmission of T1 than of T2 family strains. Since the T1 family has been associated with drug resistance, this might also indicate active transmission of drug-resistant TB in the community, albeit at a lower rate. Contrary to studies showing associations between identified MTC families and HIV infection (28), we did not find any association of this nature in our study. Given the high TB-HIV coinfection rates in this locality, it is possible that all MTC lineages in our sample had the potential to cause active tuberculosis to more or less the same extent regardless of the patients’ HIV status. It might also imply that the pathogenicity or virulence of different MTB lineages in this locality does not differ significantly between HIV-positive and HIV-negative patients.

Noteworthy was the occurrence in our sample of T3_Eth strains (approximately 1%), not previously reported in related studies so far done in this district, which might be attributed to the currently increased cross-border movements between Uganda and the Horn of Africa, where this group of strains predominates (29). In a related study done in Kenya, this strain contributed almost 4% of the samples included in the analysis (30).
Table 6. Univariable analysis of the association between HIV infection and *M. tuberculosis* lineages in Kampala.

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Total number (N) of isolates</th>
<th>HIV Positive n(%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Univariable analysis OR(95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2/T2 Uganda</td>
<td>220</td>
<td>75 (34.1)</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td>CAS</td>
<td>49</td>
<td>17 (34.9)</td>
<td>1.0 (0.5-2.0)</td>
<td>0.94</td>
</tr>
<tr>
<td>LAM</td>
<td>31</td>
<td>7 (22.6)</td>
<td>0.6 (0.3-1.4)</td>
<td>0.21</td>
</tr>
<tr>
<td>T1</td>
<td>36</td>
<td>13 (36.1)</td>
<td>0.9 (0.5-2.3)</td>
<td>0.81</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>107</td>
<td>34 (33.0)</td>
<td>1.0 (0.6-1.6)</td>
<td>0.85</td>
</tr>
<tr>
<td>OTHERS</td>
<td>46</td>
<td>14 (31.0)</td>
<td>0.8 (0.4-1.7)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

<sup>a</sup>Column percentages for contribution of each MTB lineage to the HIV positive participants in the study.

**Limitations.** Our study had limitations. The direct repeat region which spoligotyping targets is prone to convergent evolution, which leads to ambiguous classification of MTC strains in phylogenetic lineages. Similarly, the SITVIT database that we used to assign spoligotypes to phylogenetic lineages has a small representative collection compared to all the circulating strains and therefore left a significant proportion of isolates not assigned. This made it difficult to fully describe the phylogenetic structure in this city. In addition, we studied only sputum smear-positive isolates in adults aged 18 years and above. This might have biased our results with regard to the circulating MTC strains in Kampala if this differs significantly between smear-positive and smear-negative patients or between those below and those above 18 years. However, since sputum smear-positive patients are more likely to transmit the infection, our findings are most likely the true reflection of the circulating MTC strains in Kampala. Since we analyzed only sputum samples, this phylogenetic structure may not be generalized to extrapulmonary disease should there be a biological preference for some lineages to cause active TB outside the lung tissue.
CONCLUSION

Our findings show the T2 MTC lineage, predominant in Kampala, as being negatively associated with anti-TB drug resistance compared to other identified lineages that significantly contribute to the TB burden in this locality. Despite high TB-HIV coinfection rates in Kampala, no association was established between HIV infection and MTC lineage. These findings are important to the control of drug-sensitive and drug-resistant TB in Kampala in view of the high HIV infection rate among TB patients, the high burden of TB, and the indication of recent transmission of possibly more “drug resistance-prone” lineages. They should alert the NTLP of the need to sustain, if not intensify, drug resistance surveillance. Since anti-TB drug resistance rates nationally have been reported as low, we recommend a similar study at a national scale to establish the contribution of MTC strain lineage to these low drug resistance rates in Uganda.

ACKNOWLEDGMENTS

We thank the staff at the Medical Molecular Laboratory and the National TB Reference Laboratory for processing the sputum samples where these results were generated.

AUTHOR CONTRIBUTIONS


REFERENCES


Chapter 6

The T2/Mycobacterium tuberculosis Uganda II Genotype and resistance to first-line anti-tuberculosis drugs. Results of a country-wide molecular epidemiological study in Uganda.

Submitted for publication
The T2/Mycobacterium tuberculosis Uganda II Genotype and resistance to first-line anti-tuberculosis drugs. Results of a country-wide molecular epidemiological study in Uganda.

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ABSTRACT

Background: The global increase in the burden of multidrug-resistant tuberculosis (MDR-TB) underscores an urgent need for data on factors involved in generation and spread of TB drug resistance. We performed molecular analyses on a representative sample of *Mycobacterium tuberculosis* (MTB) isolates. Basing on findings of the molecular epidemiological study in Kampala, we hypothesized that the predominant MTB strain lineage in Uganda is negatively associated with anti-TB drug resistance and we set out to test this hypothesis.

Methods: We extracted DNA from mycobacterial isolates collected from smear-positive TB patients in the national TB drug resistance survey and carried out IS6110-PCR. To identify MTB lineages/sub lineages RT-PCR SNP was performed using specific primers and hybridization probes and the ‘melting curve’ analysis was done to distinguish the T2/Uganda II genotype from other MTB families. The primary outcome was the distribution of the T2/Uganda II genotype and its associations with anti-TB drug resistance and HIV infection.

Results: Out of the 1537 patients enrolled, MTB isolates for 1001 patients were available for DNA genotyping, of which 973 (97%) had conclusive RT-PCR results. Of these 422 (43.4%) were of the T2/Uganda II genotype, mostly distributed in the south west zone (55.0%; OR=4.6 for comparison with other zones; 95% CI, 2.83-7.57; p< 0.001) but occurred in each of the other seven geographic zones at varying levels. Compared to the T2/Uganda II genotype, other genotypes as a group were more likely to be resistant to any anti-TB drug (OR adj =2.9; 95% CI, 1.63-5.06; p=0.001) or MDR (OR adj=4.9; 95% CI, 1.15-20.60; p=0.032), even after adjusting for geographic zone, patient category, sex, residence and HIV status. It was commonest in the 25-34 year age group 159/330 (48.2%). No association was observed between T2/Uganda II genotype and HIV infection.

Conclusion: The T2/Uganda II genotype is a major cause of morbidity due to TB in all NTLP zones in Uganda. It is less likely to be resistant to anti-TB drugs than other MTB strain lineages.
INTRODUCTION

Tuberculosis (TB) remains one of the major causes of morbidity and mortality, estimated to affect a third of the population worldwide. Through the past two to three decades TB incidence and mortality have markedly increased as a result of HIV(1), and an estimated half million multidrug resistant (MDR) TB cases exist across the world. The association between MDR-TB and HIV infection in sub-Saharan Africa (SSA) remains a controversial subject with no association documented in most settings(2)(3)(4)(5), although some studies continue to report an association between these two disease epidemics at individual level (6)(7)(8)(9). The increasing global burden of MDR-TB underscores the urgent need for pathogen and host data on factors involved in generation and spread of drug resistant strains, so as to facilitate focussed interventions by the national programs for its control (10), especially in low resource and high burden TB, HIV and TB/HIV co-infection settings such as SSA.

Uganda is still counted among the 22 high TB burden countries worldwide with an estimated 293 per 100,000 incident cases of all TB forms in 2010. It has a high HIV prevalence, currently estimated at 7.3% among the 15-49 years age group in the general population (AIS 2011), with a TB/HIV co-infection rate of 54% in the 2010 TB cohort(11). Results from the national drug resistance survey show the prevalence of MDR-TB to be low; 2.3% among sputum smear-positive new and previously treated TB patients combined (5).

Molecular epidemiological studies are useful for understanding key aspects of TB control including transmission, potential to cause disease outbreaks, and resistance to anti-TB drugs (12)(13)(14)(15), among others. Molecular techniques commonly used in these studies (16)(17) include spacer oligonucleotide typing (spoligotyping), region of difference (RD) analysis, mycobacteria insertion repetitive units (MIRU) analysis, insertion sequence 6110 (IS6110) restriction fragment length polymorphism (RFLP) (14-15) and single nucleotide polymorphisms (SNP). Among these, SNPs represent reliable markers for lineage classification of *Mycobacterium tuberculosis complex* (18)(19).

The Uganda II genotype is a subtype of the T2 Uganda genotype. Using the highly sensitive and specific SNP technique, the T2 Uganda genotype is further subdivided into the Uganda I and
Uganda II sub-types which contribute 15% and 85% respectively to this genotype(20). Earlier studies have identified the T2 family in general as an important MTB genotype in specific localities in Uganda (21)(22) and shown a negative association with drug resistance in a limited geographic setting (23). This finding is potentially relevant for understanding the role of genotype diversity in the epidemiology of TB drug resistance in Uganda and elsewhere in East Africa, but calls for confirmation in a geographically more diverse dataset, among others to establish whether unrelated regional differences in genotype distribution could confound this association. Our hypothesis in this study therefore was that the T2/Uganda II MTB subtype is the major cause of morbidity due to tuberculosis in Uganda and is negatively associated with anti-TB drug resistance, and we aimed at testing this hypothesis in a larger and geographically representative data set.

Methods

Sampling and sample size estimation
The study included new and previously treated sputum smear-positive TB patients enrolled in a national anti-TB drug resistance survey from all the NTLP zones excluding the Central Zone (see fig 1). This was a cross-sectional survey that included 44 TB diagnostic and treatment units across the nine TB control zones (DTUs) in the country (fig 1). A cluster sampling method was used in which 30 clusters (primary sampling units) were selected randomly with probability proportional to the number of smear-positive TB patients registered in 2007. Within each cluster a fixed number of consecutively diagnosed smear-positive patients were enrolled so that all included patients had identical sampling probabilities. Each cluster was required to enrol 50 new sputum smear-positive TB patients (for a target sample size of 1500 patients) within one year, plus all the previously treated patients diagnosed during this period.

Laboratory methods

Sputum collection: Each eligible patient who consented provided two sputum samples, an early morning and a spot sample, independent of the routine samples used for diagnostic purposes to
minimize chances of contamination. Samples were refrigerated at 4°C and then transported to the National TB Reference Laboratory (NTRL) for processing via a local courier system.

**Sputum culture and drug susceptibility testing:** At the NTRL, samples were decontaminated using the 1.5% NaOH NALC method and processed. The sample was inoculated on two slopes of Löwenstein-Jensen (L-J) medium, incubated at a temperature of 37°C and monitored weekly for growth up to 8 weeks. A culture was only reported negative if no growth was shown after 8 weeks. For the positive cultures identification of MTB was done based on presumptive phenotypic appearance of colonies on the medium, and confirmed using insertion sequence 6110-based PCR method as previously described(24).

Isolates were tested for resistance to rifampicin, isoniazid ethambutol and streptomycin using the L-J proportional method, in concentrations of 40 μg/ml for rifampicin, 0.2 μg/ml for isoniazid, 2.0 μg/ml for ethambutol and 4.0 μg/ml for streptomycin, and all identified MDR-TB isolates were tested for resistance to kanamycin and ofloxacin in concentrations of 30μg/ml and 2.0 μg/ml, respectively, using the method as previously described (25). All samples resistant to rifampicin, a random sample of 20 isolates from previously treated patients susceptible to isoniazid and rifampicin, and a random sample of isoniazid resistant isolates that were susceptible to isoniazid were retested by the supranational reference laboratory in Borstel (Germany) for external quality assurance.

**Preparation of crude genomic DNA from Mycobacterium tuberculosis for use as template in PCRs:** A total of 1039 isolates were stored in replicates at the (NTRL) at –80°C. To extract DNA, the selected isolates were thawed overnight at – 20°C and later at room temperature for 12 hours. The vials were centrifuged at 15,000g for 30 min and the pellet was washed twice with 500 ul of Qiagen PCR water. The final pellet was re-suspended in 100 μl of Qiagen PCR-water, heated at 95°C for 30 minutes to kill and lyse the bacilli and later sonicated for 15 min at room temperature. The extracted crude genomic DNA in the supernatant was recovered by centrifugation at 15,000g for 30 min; the latter was used immediately in the real time PCR (RT-PCR) assay or stored at -20°C for future use.
Genotyping *Mycobacterium tuberculosis*: A SNP (Rv0040c-0619n) identifying the T2/Uganda II (MTB) with its accompanying designed primers and probes as previously described (18) was used in a real-time PCR ([RT]PCR) SNP assay. The (RT) PCR analysis involved 2 steps. The first was amplification (40 cycles of 95°C for 10 sec, 57°C for 10 sec and 72°C for 10 sec of the target region(s)) to generate amplicons for the melting curve analysis. The results of melting curves were analyzed using LightCycler® software version 1.5 to assign an isolate to a particular lineage depending on the melting temperature at which the hybridization probes dissociates from the amplicons. In all the assays we used MTB Uganda (MTB L4-U) genomic DNA from our laboratory, H37Rv genomic DNA (MTB lineage 4) and Central Asian strain (lineage 3) genomic DNA as control DNA.

**HIV testing:** HIV testing at all the 44 health care facilities followed the national algorithm using Unigold® (Trinity Biotech, Wiclock, Ireland) and Determine® (Abbot, Tokyo, Japan) as the screening and confirmatory tests respectively. A third kit, Stat-Pak® (Chethambutolio Medford NY, USA) was used when a mismatch between the confirmatory and the screening HIV test results on the same sample were reported. All participants were offered this test algorithm in accordance with the Uganda Ministry of Health guidelines.

**Data management and analysis**
Data on demographics, microscopy, culture and DST results was double entered in epi-info V6 (2000 CDC Atlanta GA USA), as part of the anti-TB drug resistance study, while results on molecular studies was entered in MS Excel. Analysis was done in Stata v10 (STATA Corp. College Station TX USA.) The Chi-square or the 2-sided Fisher’s exact tests were used where appropriate for comparison of categorical variables. Univariate and multivariate analysis was done using logistic regression to identify variables associated with the T2/Uganda II MTB genotype. Contribution of the variables to the model was done using the likelihood ratio Chi square test. All significance testing was at the 95% confidence level.

Our primary outcome was the proportion of MTB patients with the T2/Uganda II genotype calculated as a proportion across all clusters after weighing for the exact sampling probabilities for each new individual patient for whom DST results were available. Associations with MDR, HIV infection, patient age, previous history of TB treatment and urban or rural residence were
also studied. In all these calculations, confidence intervals and p-values were adjusted for cluster design by first order Taylor linearization and by second order correlation of Rao and Scott of the Pearson $X^2$ respectively as implemented by Stata svy commands(26).

Ethical and scientific approval of the study was granted by Makerere University College of Health Sciences, Faculty of Medicine review board and the Uganda National Council of Science and technology. All adult participants gave written informed consent before participation. The consent process included storage and use of the collected sputum samples for further studies. We obtained assent from participants below the age of 18 years.

RESULTS

Out of the 1537 patients included in the survey, 127 were culture negative, 77 samples were contaminated, eight samples grew non-tuberculous mycobacteria, and 1325 samples grew MTB based on IS6110-PCR analysis. Of these 1325 isolates, 324 did not grow on subculture (done for molecular analysis), leaving 1001 available for genotyping. Twenty-eight of these were excluded from further analysis due to inconclusive genotyping results. Thus, results on MTB genotype were available for single isolates of 973 patients. Of these isolates, 630 (64.8%) belonged to male participants. Mean age of participants was 34.7 years, 25-34 years being the modal age group (n=330; 34.3%). Out of the eight zones that participated in this study, south west zone contributed the highest number of isolates (n=313; 32.0%). Of the 973 patients, 297 (30.5%) tested positive for HIV, 217 (22.3%) of whom had already tested HIV positive before enrolment. Previously treated TB patients contributed 78 (8.1%) of the isolates. Generally there was no difference with regard to demographic characteristics (with exception of the zone where the patient was enrolled) of the patients included in the survey between those whose isolates were included and those whose were not included in the molecular typing (table 1).

Of the 973 MTB isolates analysed with valid results, 422 (43.4%) were of the T2/Uganda II genotype. Among the eight NTLP zones that participated in this survey, south west zone had the highest proportion of this genotype, 172/313 (55.0%; 95% CI, 49.2-60.5), while south east had the lowest 7/35 (19.4%; 95% CI 8.1-36.0). We found the T2/Uganda II genotype less distributed in the east and south east zones as compared to the south west zone ($p<0.001$) (table 2). The 25-
34 year age group had the highest proportion of the T2/Uganda II genotype (48.2%) although there was no statistical significance of the differences in distribution of this genotype among the different age groups. We found no association between the T2/Uganda II genotype distribution with previous history of TB treatment and rural/urban residence.

On multivariable analysis, isolates belonging to other genotypes other than the T2/Uganda II genotype were more likely to have resistance to any of the first-line drugs (OR adj 2.9; 95% CI, 1.63-5.06; p=0.001) and more likely to be MDR (OR adj 4.9; 95% CI, 1.15-20.60; p=0.032) than the T2/Uganda II genotype, even though MDR isolates were few (n=23). Included in our model for adjustment were the NTLP zone where the patient was enrolled, patient category (new or previously treated), age group, and participant residence (rural/urban) (table 3). In NTLP zones where the T2/Uganda II genotype was less distributed, participants had an increased risk of up almost twice (OR adj 1.8; 95% CI, 1.25-2.62; p=0.001) of having drug resistance as compared to south west zone where this genotype contributed a larger proportion to the sample (table 4). No association between HIV infection and the T2/Uganda II genotype (OR 1.09; 95% CI, 0.73-1.63; p=0.648) was established at univariable analysis.

DISCUSSION

In this cross-sectional molecular analysis of *M. tuberculosis* strains circulating in Uganda, we clearly demonstrate the T2/Uganda II genotype as the major cause of morbidity due to TB in the country although at different levels of predominance in the eight NTLP (i.e., geographic) zones. Its frequency was highest in the south west and lowest in the south east zone (see fig 1). Our study showed that this genotype is less likely to develop resistance to any of the anti-TB drugs compared to all the other genotypes taken as a single group, but no association with HIV infection was demonstrated.

We found the T2/Uganda II genotype about 2.9 times less likely to be resistant to any of the first-line anti-TB drugs and almost 5 times less likely to be MDR as compared to the non T2/Uganda II MTB genotypes. It confirms our observation from a similar survey done in Kampala where the T2 Uganda genotype strains were less likely to be drug resistant compared to other, geographically more diverse genotypes such LAM, CAS and T1(23). That the T2/Uganda II
genotype is negatively associated with M/DR-TB is further supported by the higher levels of M/DR-TB in zones where this genotype is less prevalent such as the east zone. Here the prevalence of drug resistance was up to almost 2 times higher than in the south west zone where the majority of the isolates belonged to the T2/Uganda II genotype after adjusting for patients’ HIV status, treatment category, rural or urban residence and sex (table 4). The negative association reported in our earlier analysis (23) between the T2 Uganda genotype and any drug resistance is thus confirmed in this study, and also exists for MDR (stronger than even the association with any resistance), possibly due to larger numbers of MDR-TB patients to establish a statistically significant difference.

Contrary to our findings, in some high burden TB settings the predominant MTB genotype has been associated with M/DR. The Beijing genotype in particular has been documented to cause outbreaks of MDR-TB where this strain is the major cause of TB morbidity(27). It remains a subject for further analysis whether the T2/Uganda II genotype is inherently less likely to become drug-resistant (i.e. has a lower potential for acquiring drug resistance), or that drug-resistant T2/Uganda II genotype strains are less easily transmitted than other drug-resistant strains. The latter could occur if for example (multi)drug-resistant T2/Uganda II strains cause clinical infections with lower bacterial load, or show higher cure rates with first-line treatment, than do (multi)drug-resistant strains of other genotypes. This could reflect differences in adaptation to the Ugandan host population between the more indigenous T2/Uganda II genotype strains and more recently imported genotype strains such as LAM, CAS and T1. In addition, one could speculate that predominance of the T2/Uganda II genotype plays a causative role with regard to reported low levels anti-TB drug resistance in Uganda(5), and that similar genotypic differences in association with drug resistance exist elsewhere in sub-Saharan Africa where low levels of (M)DR-TB have been reported.

Despite a high TB/HIV co-infection rate in our sample (30.2%) we found no association between HIV infection and T2/Uganda II strains similar to findings of related studies elsewhere in SSA(28). This finding suggests that the predominance of the T2/Uganda II strains is unlikely to be due to changes in immunological pressure on the circulating MTB population as a result of the HIV epidemic.
Since we observed significant geographical variations with respect to the distribution of the T2/Uganda II genotype, it can be speculated that the north east and south east zones where this genotype is less distributed might have a diversity of MTB strains that are imported through cross-border movement with neighbouring countries such as Kenya, Tanzania and beyond. Indeed the eastern border of Uganda acts as the entry/exit (Busia and Malaba) for goods and people from Mombasa port and the northern part of Kenya and, the Kilimanjaro region in Tanzania, while Kampala being the capital city is the major business centre and a pathway for people entering and exiting Uganda from all over the world. Human traffic at these entry/exit points might enhance transmission of communicable diseases, including TB, with higher probability of strain diversity than in the south west zone. The other possibility could be that the T2/Uganda II genotype might be genetically more adapted to indigenous human populations of the south west than the east and south east zones. Both hypotheses can only be confirmed through further research.

Limitations.

Our study had limitations. We did not fully characterise the non-T2/Uganda II genotypes into specific lineages that have been previously documented as significantly contributing to the TB burden in this setting. Therefore we could not fully conclude which particular strain drives drug resistance in this locality and any associated factors such as transmissibility and potential to develop drug resistance as documented elsewhere. However our aim was to find out the role of the predominant genotype in the observed low levels of anti-TB drug resistance in this locality and our earlier report (14), though limited in terms of geographical coverage, fully characterised the less predominant MTB lineages in relation M/DR-TB and HIV infection. We however strongly recommend a more in depth analysis to characterize the ‘non-T2/ Uganda II genotype’ group and its distribution throughout Uganda. Secondly, we employed a typing method (identification of genotype-specific SNPs) that was different from the method that was used for the Kampala survey (spoligotyping) in which the negative association of the T2/Uganda II genotype with drug resistance was initially observed. However, earlier studies have shown that the T2 and the Uganda II genotype strongly overlap (20). Thirdly, only sputum smear-positive TB patients were included in the study since this was an extension of a drug resistance survey. This sampling frame could limit generalizability of our results to smear negatives should there be
a difference in the distribution of the T2/Uganda II genotype strains. Since differences in bacterial load and resulting transmission fitness of drug resistant strains may in part explain the observed association, it would be interesting to extend the current study with sputum smear-negative patients.

CONCLUSION
This study shows the T2/Uganda II genotype to be the predominant MTB genotype in Uganda and confirms earlier findings that this genotype is less likely to be drug resistant compared to other genotypes combined. This may in part explain the low levels of drug resistance found in Uganda. Similar negative associations between the predominant indigenous MTB strains and drug resistance may exist elsewhere in SSA(29).

ACKNOWLEDGEMENT
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REFERENCES


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Table 2: Analysis of risk factors associated with T2/MTB Uganda II genotype among sputum smear-positive TB patients in Uganda.

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<th>Proportion N/N (%)</th>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>South East</td>
<td>7/36 (19.40)</td>
<td>0.20 (0.14-0.28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>North East</td>
<td>26/59 (44.1)</td>
<td>0.64 (0.35-1.17)</td>
<td>0.146</td>
</tr>
<tr>
<td>North</td>
<td>10/28 (35.7)</td>
<td>0.45 (0.31-0.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>North West</td>
<td>20/66 (30.3)</td>
<td>0.32 (0.18-0.55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Western</td>
<td>53/104 (51.0)</td>
<td>0.86 (0.43-1.72)</td>
<td>0.667</td>
</tr>
<tr>
<td>Previously treated for TB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31/78 (39.7)</td>
<td>0.85 (0.58-1.25)</td>
<td>0.403</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>130/442 (29.4)</td>
<td>1.09 (0.73-1.63)</td>
<td>0.648</td>
</tr>
<tr>
<td>Diagnosis made in an urban setting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>267/585 (45.6)</td>
<td>0.80 (0.49-1.32)</td>
<td>0.385</td>
</tr>
</tbody>
</table>

** Odds ratios and confidence intervals adjusted for cluster design.
Table 3: Multivariate analysis of T2/Uganda II genotype and anti-TB drug resistance among sputum smear-positive TB patients in Uganda.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Any resistance</th>
<th>MDR</th>
<th>Resistance Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion</td>
<td>**OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Uganda II</td>
<td>26/422 (6.2)</td>
<td>Ref.</td>
<td>3/422 (0.7)</td>
</tr>
<tr>
<td>Other</td>
<td>91/549 (16.6)</td>
<td>2.9</td>
<td>1.63-5.06</td>
</tr>
</tbody>
</table>

Others included in the model were NTLP zone, patient category (new or previously treated), HIV status, age group, and residence (rural/urban)

** Odds ratios adjusted for cluster design
Table 4: Multivariable analysis of ‘any resistance’ and ‘NTLP zone’ in relation to the proportion of the T2/Uganda II genotype among the sputum-smear positive TB patients in Uganda.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Total number of isolates N=973</th>
<th>Proportion of T2 isolates n (%)</th>
<th>Univariate analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>**OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>South West</td>
<td>313</td>
<td>172 (55.0)</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Kampala</td>
<td>278</td>
<td>112 (40.3)</td>
<td>1.9 (1.34-2.79)</td>
<td>0.004</td>
</tr>
<tr>
<td>Eastern</td>
<td>89</td>
<td>22 (23.6)</td>
<td>1.9 (0.76-4.79)</td>
<td>1.57</td>
</tr>
<tr>
<td>South East</td>
<td>36</td>
<td>7 (19.4)</td>
<td>1.7 (1.26-2.35)</td>
<td>0.001</td>
</tr>
<tr>
<td>North East</td>
<td>59</td>
<td>28 (44.1)</td>
<td>1.9 (1.27-2.73)</td>
<td>0.030</td>
</tr>
<tr>
<td>North</td>
<td>28</td>
<td>10 (35.7)</td>
<td>0.8 (0.58-1.08)</td>
<td>0.137</td>
</tr>
<tr>
<td>North West</td>
<td>66</td>
<td>20 (30.3)</td>
<td>0.5 (0.27-1.10)</td>
<td>0.295</td>
</tr>
<tr>
<td>Western</td>
<td>104</td>
<td>53 (51.0)</td>
<td>1.5 (0.22-1.61)</td>
<td>0.134</td>
</tr>
</tbody>
</table>

** Odds ratios confidence intervals and p-values adjusted for cluster design

*= P value for the difference in resistance between the south western zone and each of the other zones included in the study.

Other variables included in the model are patient category, sex, residence (rural/urban),
HIV status,
Ref= reference category.
Fig. 1 Map of Uganda showing health facilities which participated in the study and the NTLP zones.
Chapter 7

Variation and Risk Factors of Drug Resistant Tuberculosis in Sub-Saharan Africa: A Systematic Review and Meta-analysis.

Submitted for publication
Variation and Risk Factors of Drug Resistant Tuberculosis in Sub-Saharan Africa: A Systematic Review and Meta-analysis.

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ABSTRACT
Background: Prevalence of multidrug resistant tuberculosis (MDR-TB) in sub-Saharan Africa (SSA) is reportedly low compared to other regions. These estimates are based on data reported to the World Health Organization (WHO) on drug resistance surveys, which may suffer from a reporting bias. We set out to evaluate the variation in prevalence of drug resistant tuberculosis (DR-TB) and its determinants among SSA countries among new and previously treated TB patients.

Purpose: To perform a systematic review and meta-analysis of the prevalence of DR-TB and associated risk factors in SSA.

Data Sources: We searched PubMed, EMBASE, Cochrane and bibliographies of DR-TB studies.

Study selection: Surveys at national or sub-national level, with reported DR-TB prevalence (or sufficient data to calculate a prevalence) to isoniazid, rifampicin, ethambutol, and streptomycin conducted in SSA excluding the Republic of South Africa, published between 2003 and 2013 with no language restriction.

Data extraction: Two authors searched and reviewed the studies for eligibility and extracted the data in pre-defined forms.

Data synthesis: We tabulated the data extracted and presented forest plots of all prevalence estimates by resistance outcome. Summary estimates were calculated using random effects models, when appropriate. Associations between any DR-TB and MDR-TB and potential risk factors were examined through subgroup analyses stratified by new and previously treated patients.

Results: A total of 726 studies were identified, of which 27 articles fulfilled the inclusion criteria. Studies reported DST results for a total of 13,465 new and 1,776 previously treated TB patients. Pooled estimate of any DR-TB prevalence among the new cases was 12.6% (95% CI 10.6-15.0) while of MDR-TB this was 1.5% (95% CI 1.0-2.3). Among previously treated patients, any DR-TB and MDR-TB prevalence were 27.2% (95% CI 21.4-33.8) and 10.3% (95% CI 5.8-17.4%), respectively. DR-TB (any and MDR-TB) did not vary significantly with respect to sub-region, HIV prevalence, sampling design, or study coverage.

Conclusion: The reported prevalence of DR-TB in SSA is low compared to WHO estimates. MDR-TB in this region does not seem to be driven by the high HIV prevalence rates.
BACKGROUND

Globally, the World Health Organization (WHO) reports an estimated prevalence of 1.6% (range 0.6-3.9) and 11.7% (range 4.9-20.9) among notified TB cases for primary and acquired multidrug resistant tuberculosis (MDR-TB), respectively, with significant country and regional variation(1). Despite the high burden of TB in sub-Saharan Africa (SSA) fuelled by HIV(2), drug resistance surveillance has not been widely done, with only 22 of the 46 countries reporting drug resistance data by 2005. These studies have been designed to establish a nationwide MDR-TB prevalence only, and most of them had small sample sizes to assess variations between subpopulations or identify potential risk factors of the prevalence of drug resistance(3). Yet, the use of inferior TB drug regimens, high HIV infection rates, and a wide roll-out of ART may predispose countries in this region to high levels of DR-TB(4). In particular, previous exposure to anti-TB treatment is a well-established risk factor for DR-TB(5). By 2010, a number of TB programs in SSA were still using the eight-months regimen (2EHRZ/6EH) that has been associated with lower cure rates and higher rates of relapse than the currently recommended six-months regimen in which rifampicin is given throughout treatment (2EHRZ/4RH)(4).

Conversely, duration of rifampicin treatment beyond four months has been associated with increased risk of acquiring drug resistance(6). Therefore there have been concerns that, in SSA, six months of directly observed therapy are often unfeasible, and rifampicin throughout would increase the incidence of MDR-TB, in particular in the context of high HIV and pre-existing isoniazid resistance(7). While some drug resistance studies have shown an association between HIV and DR-TB/MDR, data showing HIV as an independent risk factor for MDR-TB in individuals have been limited to particular settings(8). Nevertheless, high mortality among HIV/MDR or XDR-TB (extensively drug-resistant tuberculosis) co-infected patients(9) as well as the association between rifampicin mono-resistance and HIV infection are a major concern for TB control programs in SSA(10). Understanding the role of these and other potential ‘drivers’ of DR-TB in SSA is therefore important to guide intervention policies and future drug resistance monitoring in the region. We did a systematic review and meta-analysis of published and unpublished studies to establish the variation of DR-TB in SSA countries and its determinants.
METHODS

Data sources

We searched PubMed, EMBASE, and Cochrane for original publications from 2003 to 2013 without language limitations. Search terms used were anti-TB drug resistance, drug resistant tuberculosis, M/DR/XDR-TB, and (isoniazid or rifampicin or ethambutol or streptomycin or ofloxacin or fluoroquinolone or kanamycin or amikacin) resistance for each country in SAA, excluding the Republic of South Africa (RSA). Each term was searched separately with a text string ending with the specific name of the country. We excluded RSA because drivers of DR-TB in this country are likely to be different and prevalence has been reported to be substantially higher than the rest of SSA countries (12). We additionally searched bibliographies of other reviews and citations of the original articles identified. Reviewers obtained unpublished DR-TB studies through personal communication with experts and authors of papers identified.

Study selection

We reviewed surveys carried out both at national or sub-national level reporting M/DR-TB prevalence or sufficient data to calculate a prevalence of resistance to isoniazid (INH), rifampicin (RMP), ethambutol (EMB), streptomycin (SM), and/or MDR (INH and RMP). Conference proceedings, chapters of books, and correspondences were excluded. Studies were considered of sufficient quality for inclusion if participants were classified as new or previously treated based on the WHO definition, the study covered a large geographical area (district, region, or entire country), and recommended laboratory procedures for culture and DST were followed(11). Studies conducted in a single health unit e.g. a referral hospital or a TB center, or those where fewer than 50 participants had DST were excluded to minimize bias of including non-representative samples of the population. Where cluster sampling was used, adjustment for the cluster design was a requirement for inclusion for review. Two authors conducted the electronic searches independently; the last search was conducted in June 2014. Selection of articles was done by both reviewers independently. Disagreements on articles to be included were resolved by consensus among the two authors.

Data extraction

We extracted data using pre-defined forms on: country of the study; sampling method; description of the facilities where the study was done; total number of patients enrolled in the study as per treatment category; number of patients with DST results; number of patients with a positive result for resistance to INH, RMP, EMB, SM, or MDR; and HIV prevalence among the
participants (if available). HIV prevalence at national level for each country of interest was collected from the UNAIDS report 2013 (http://www.who.int/hiv/pub/me/ua_indicator_guide/en/).

Two authors extracted data independently and any discrepancies in the data extracted were resolved through discussions.

Data synthesis and analysis

According to WHO, primary resistance is defined as resistance to one or more anti-tuberculosis drugs in patients that have never been treated for TB. Acquired resistance, on the other hand, is defined as resistance in patients diagnosed with pan-sensitive TB who have started TB treatment and subsequently acquire resistance to one or more of the drugs used during the treatment. To generate data stratified for the initial and acquired resistance, we calculated the pooled resistance prevalence along with the 95% confidence interval through meta-analysis using random effects models for MDR-TB and any DR-TB to the first line drugs (INH, RMP, EMB and SM). We assessed the heterogeneity among reported prevalence using the I2 statistic.

To explore the variation observed in the prevalence estimates, we did a subgroup analysis by stratifying studies by predefined variables. In particular, we categorized variables as follows: 1) by sub-region (Eastern sub-region included Burundi, Ethiopia, Kenya, Rwanda, Somalia, Uganda, and Tanzania; West Africa sub-region included Benin, Burkina Faso, Cameroon, Equatorial Guinea, Gambia, Ghana, Ivory Coast, and Nigeria; Southern sub-region: Botswana, Zambia, Mozambique, Madagascar, Swaziland, and Zambia; and Central Africa sub-region: Central African Republic and Chad); 2) HIV prevalence at a national level (countries with a prevalence of less than 5% compared to those with a prevalence of more than 5% in the general population); 3) type of survey (national or sub-national); 4) sampling method (random sampling or cluster sampling); 5) sample size (studies of less than 100 patients or more than 100 patients); and HIV prevalence among study participants (less than 15% compared to more than 15%).

We defined primary resistance as resistance in a patient who had not received anti-TB drugs for more than one month and acquired resistance as otherwise. However the we acknowledge limitations of this definition for acquired resistance as it does not put into consideration possibilities of re infection with resistant forms and initial infection with resistant strains contributing to treatment failure, since capacity to ascertain resistance patterns prior to treatment initiation is rarely available under routine settings.
RESULTS

Qualitative assessment

We identified 725 citations through electronic data searches and one completed study with unpublished data. Out of these, 47 articles were selected for full text review, of which 20 articles were excluded for various reasons (figure 1). Characteristics of the 27 articles included are summarized in table 1. Of these 27 studies, 19 (70%) reported DR-TB data on both new and previously treated patients. Seven studies reported primary resistance only, while one study assessed acquired DR-TB. Sixteen (59%) studies reported HIV testing, and HIV prevalence estimates at country level were available for more than 90% of the studies. Thirteen (48.1%) studies in total reported data at national level. Compared to other regions, the eastern region contributed the highest number of articles, five of which were from national surveys.

Levels of DR-TB

DR-TB data was reported for a total of 15,462 sputum smear-positive TB patients in the 27 included articles published from 2003 to 2013. Of the 15,462 patients, 13,645 (88.4%) and 1,776 (11.6%) were new and previously treated patients, respectively. All reported estimates for any resistance and MDR among new and previously treated patients are presented separately by study in figure 2. In figure 3, we then present pooled estimates for all resistance patterns, including MDR among new and previously treated patients. Prevalence of any DR-TB and of MDR-TB were higher among patients who had been previously treated for TB (figures 2 and 3).

Overall, the pooled prevalence of any primary and acquired DR-TB was 12.6% (95% CI 10.6-15.0%) and 27.2% (95% CI 21.4-33.8), respectively, while for primary and acquired MDR this was 1.5% (95% CI 1.0-2.3) and 10.3% (95% CI 6.1-17), respectively. Summary estimates for any primary and acquired DR-TB were highest for INH [7.8% (95% CI 6.5-9.4) and 23.5% (95% CI 15.9-33.2)] and lowest for ethambutol [1.9% (95% CI 1.3-2.8) and 8.6% (95% CI 4.9-14.8)] (figure 3).

Variation of DR-TB with key study characteristics. In figures 4 and 5, we present the subgroup analyses for the prevalence of any DR-TB and MDR-TB by study characteristics. Overall, we observed larger variations in the pooled estimates by subgroup with respect to any DR-TB, compared to MDR estimates.

Regional variations of DR-TB.

Prevalence of any primary DR-TB varied from 10.4% (95% CI 8.2-13.1, n=6) in the Southern region to 17.0% (12.4-23.0, n=2) in the Central region. Any acquired DR-TB was highest in East...
Africa with levels of 29.2% (95% CI 21.4-38.6, n=8) and lowest in the Southern African countries, 24.0% (95% CI 13.0-40.0, n=6). Primary MDR was the lowest in Central Africa at 1.2% (95% CI 0.3-5.5, n=2) and highest in Western Africa, 2.3% (95% CI 1.0-4.8, n=3), while acquired MDR was highest in Southern region, 11.7% (95% CI 5.0-25.0, n=6) and lowest in Eastern region, 9.6% (95% CI 4.7-18.4, n=9). We did not observe significant variations in pooled estimates of any DR-TB or MDR in the sub-regions as shown by the overlap in the 95% CIs of our estimates (figures 4 and 5).

Drug resistance and HIV prevalence rates

HIV prevalence at population level. Analysis of any primary DR-TB in relation to HIV infection rates showed somewhat higher resistance rates of 13.9% (95% CI 10.5-18.2, n=12) in countries where HIV prevalence was lower than 5%, compared to countries where the prevalence was equal to or higher than 5% [11.2% (95% CI 8.7-14.2, n=12)], while acquired DR-TB was almost the same among settings with the different HIV prevalence rates (26.1%, n=8 vs 25.4%, n=9). Primary MDR in settings with less than 5% HIV prevalence was 1.9% (95% CI 1.1-3.2 n=9) as compared to 1.5% (95% CI 0.8-2.8 n=12) in settings where the HIV prevalence equal to or higher than 5%. Acquired MDR in countries with lower than 5% HIV prevalence was 8.3% (95% CI 3.4-18.8, n=11) compared to 11.0 (95% CI 5.8-19.9, n=9) in countries with HIV prevalence of equal to or higher than 5%. However, differences were small with largely overlapping 95% confidence intervals.

TB/HIV co-infection in the survey. Where HIV testing was done as part of the survey, we observe a lower prevalence of primary DR-TB in studies where HIV was lower than 15% among the study participants [8.0% (95% CI 5.1-12.4, n=1)] as compared to 14.6% (95% CI 11.6-18.3, n=14) in studies where HIV prevalence among participants was equal to or higher than 15%. Analysis of acquired DR-TB in relation to these HIV co-infection rates shows a reverse picture to what we observe in primary DR-TB. Higher rates of any acquired DR-TB were observed in studies with lower than 15% TB/HIV co-infection among study participants, 41.7% (95% CI 18.5-69.2, n=11), as compared to 29.4% (95% CI 21.4-38.8, n=8) where TB/HIV co-infection rates were equal to or higher than 15%. Primary MDR in studies where TB/HIV co-infection rates were lower than 15% was 2.2% (95% CI 0.9-5.3%, n=1) and 1.5% (95% CI 0.8-2.8% n=12) in studies with equal to or higher than 15% TB/HIV co-infection. Acquired MDR where TB/HIV co-infection was lower than 15% among the participants was 25% (95% CI= 8.3-55.2, n=1) and 10.8% (95% CI 4.5-24.1, n=10) where equal to or higher than 15% of the participants were HIV co-infected, although this larger difference was again not significant.
Variation of drug resistance with geographical coverage

Generally, articles reporting national surveys estimated somewhat lower rates of any primary DR-TB 11.3% (95% CI= 9.0-14.3, n=13) as compared to sub-national reports 14.2% (95% CI 10.6-18.6, n=13). Any acquired DR-TB was similar in the national (26.0%; 95% CI 18.1-35.9, n=11) and sub-national surveys (28%; 95% CI- 23.1-33.5, n=7). Primary MDR estimates were the same in both national and sub-national studies at 1.6% (95% CI 0.9- 2.8, n=11) and 1.6% (95% CI 1.0-2.5, n=12) respectively, as were acquired MDR rates: 10.5% (95% CI 4.7-21.7, n=13) versus 11.0% (95% CI 5.8-19.9, n=8), respectively.

Variation of drug resistance with sample size

Studies with sample sizes less than 100 participants reported significantly higher rates of any primary DR-TB, 22.4% (95% CI 10.8-40.0, n=2) compared to studies where 100 or more participants were recruited, 12.1% (95% CI 10.1-14.4, n=24). Levels of acquired DR-TB were almost the same in both categories of sample size, 26.9% (95% CI 20.0-35.0 n=13) vs 27.8% (95% CI 17.5-42.1, n=5). For either category of study size, primary MDR levels followed similar trends, significantly higher 6.7% (95% CI 2.5-16, n=1) in studies with less than 100 participants as compared to 1.4% (95% CI 1.0-2.1, n=22) in studies with larger sample sizes. Although slightly higher, levels of acquired MDR in studies with less than 100 participants, 11.8% (6.4%-20.8%, n=14), this difference was not statistically significant as compared to studies with 100 participants or more, 8.5% (95% CI 3.1%-21.3%, n=7)

Finally, in figure 6, we explored graphically the possibility of a publication bias. We did not observe an indication of such a bias.

DISCUSSION

While data on DR-TB from SSA is limited(12), more information on rates and factors associated with of DR-TB in this region is emerging as more countries conduct surveys at national and sub-national level(13). In this report we reviewed rates and variation of DR-TB in SSA, including MDR. We analyzed data from 27 surveys in 20 of the 46 countries. As documented elsewhere, levels of any DR-TB and MDR are lower in SSA than reported globally (3). In particular, our results show MDR prevalence estimates as almost half as compared to the global average reported by WHO for both new (1.5 % vs 3.6%) and previously treated TB patients (10.3% vs 20.2%)(2). These consistent low levels despite high rates of HIV and incidents of political instability in some countries have been attributed, among other factors, to late introduction of
_levels of MDR-TB in a country have been documented as a reflection of the performance of the national TB program. Specifically, countries where standardized regimens are available and properly implemented, where quality drugs are regularly supplied, and where systems are in place to ensure patients’ adherence are less likely to report high rates of DR-TB. This can be supported by the high rates of MDR-TB from the Horn of Africa included in our review, which could have resulted from poor functioning of the TB program due to political turmoil, as observed elsewhere in the world(15)(16). Therefore, regional variations in MDR-TB rates might be considered a proxy measure for functionality of national TB programs, which calls for focused interventions to mitigate acquisition of (M) DR during first-line treatment. However transmission-related factors such as late diagnosis, nosocomial spread and delay in initiation of second line treatment might play an equally important role in the observed DR levels in this region. Also, the role of Mycobacterium tuberculosis (MTB) strains in transmissibility and its potential to develop DR in this region should not be ignored. As observed in some settings, particular MTB strains predominant in specific localities have been associated with varying rates of MDR-TB(17). Hence, more molecular studies are required to examine and explain possible associations of the predominant MTB strains with the observed prevalence of DR-TB in SSA. Higher rates of resistance to isoniazid and streptomycin than other drugs observed in our analysis have been documented earlier and have been attributed to the long history of isoniazid and streptomycin use in management of TB and to the stepwise acquisition of DR by MTB to these two drugs(18).

Lower levels of MDR (1.5%) in settings with higher HIV prevalence at population level, also observed where HIV testing was included in the study design, could result from less participation rates of MDR-TB/HIV co-infected patients in surveys due to either severe illness or higher risk of death(8). Where collection of individual HIV data was included in the study design, we found higher rates of acquired MDR (25%) in studies where HIV prevalence was lower, possibly due to the same explanation and the possibility of suspected high MDR-TB rates in the population.

We observe levels of any rifampicin resistance close to the reported prevalence of MDR-TB in our review. This finding is of significant relevance in the current global and regional efforts to accurately diagnose MDR-TB with the scale-up of molecular technology like GeneXpert MTB/RIF, to provide quick results of RMP resistance as a proxy to MDR. In fact, in many SSA countries, access to culture and DST facilities is limited and molecular technologies might ease access to MDR-TB diagnosis and reduce the time spent between diagnosis and patient care and
support. The high levels of isoniazid and streptomycin resistance we found in our review, also documented elsewhere, pose challenges of possible increase in treatment failure and relapse rates with the current first line drugs (20) and should alert TB programs of the need to monitor the correct use of rifampicin in drug-sensitive patients to avoid adding rifampicin resistance likely to lead to high MDR rates.

Finally, we observe higher rates of MDR in smaller studies as compared to larger ones as expected. Such studies are usually done to explore possibilities of high MDR-TB rates in a given setting hence prone to biases. Similarly, DR-TB rates in sub-national studies are higher than in the national surveys since in most cases sample sizes in such studies tend to be smaller, non-representative of the population, and sometimes do not apply standardized methodologies, although we aimed to exclude such studies from our analysis.

Limitations

Our review had some limitations. Of 44 countries in SSA that were considered for this study, only 20 countries had done studies that fulfilled our inclusion criteria of which studies from five countries were not on a national scale. Many of the DR-TB surveys identified during our searches were excluded because they took place at a single health facility or had not stratified patients as new or previously treated.

Although the association between HIV infection and DR-TB is still controversial and deserves further exploration, ten of the 27 studies included did not include HIV testing. It was, therefore, difficult to study this relationship fully and draw meaningful conclusions. Similarly, we did not review data on national ART coverage due to challenges associated with accessing accurate data to examine a possible relationship between ART roll-out and levels of MDR-TB. However, since current levels of MDR-TB are low, including this analysis would not significantly change our findings. Finally, results on second line DST were not reported for the majority of studies. This could be a reflection that most countries in SSA had not initiated MDR-TB treatment at the time of the study and the possibilities of finding XDR-TB were limited, although this analysis would be important especially in SSA settings where some fluoroquinolones (a cornerstone of second-line drug regimens) are widely used for treatment of other bacterial infections.

We excluded the republic of South Africa on the basis of high levels of MDR-TB and XDR rates in comparison to other countries of SSA(2)(9), possibly fuelled by high nosocomial transmission...
rates in the context of very high rates of TB/HIV co-infection reported in this country. We assumed that including such studies could potentially skew our results towards higher DR-TB or MDR estimates than the correct estimates.

CONCLUSION
Our analysis showed low levels of MDR-TB in sub-Saharan Africa compared to WHO estimates, with higher resistance to INH and SM as reported elsewhere in the world. There are no major variations in MDR-TB burden by sub-region and evidence of association between MDR-TB and HIV infection rates did not show statistical significance. We attribute these low levels to the limited existence of anti-TB drugs outside the national programs, late introduction of RMP in SSA, and wide use of fixed drug combinations. Since these factors may apply to other settings where rates of MDR-TB are higher, more studies are required to explore other possible explanations for the low levels of MDR in SSA such as the role of predominant MTB strains in generation and transmission of DR-TB in this region.

ACKNOWLEDGEMENT
Authors thank Dr. Nathan Kapata (National TB Program of Zambia) for sharing results from a national survey that was not yet published at the time of this analysis and authors of included studies whose full text were not accessible through electronic searches.

Conflict of interest. None declared.
Figure 1. Summary of literature search and study selection

Records identified through database searching  
(n =726)  
Additional records identified through other sources  
(n = 1)

Records after duplicates removed  
(n =537)  
Titles and abstracts screened  
(n =537)  
Records excluded  
(n = 490)

Full-text articles assessed for eligibility (n =47)

Studies included (n = 27)  
Studies included in quantitative synthesis (meta-analysis) (n =27)

Full-text articles excluded, with reasons (n = 20):  
Only one hospital participated, n=8  
Full text not available, n=5  
New and previously treated patients not presented separately, n=4  
Sample size too small, n=1  
Other reasons, n=2
Table 1. Characteristics of the studies included.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study year</th>
<th>Country</th>
<th>Study description</th>
<th>Patient category</th>
<th>Sample size (included in DST)</th>
<th>HIV prevalence in the study (%)</th>
<th>Country HIV prevalence (%)</th>
<th>DST method</th>
<th>Type of resistance tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdelhadi O. et al (23)</td>
<td>2009-2010</td>
<td>Chad</td>
<td>Sub-national survey. Number of TB facilities not provided.</td>
<td>New patients</td>
<td>135</td>
<td>25</td>
<td>3</td>
<td>LJ</td>
<td>INH, RMP, SM, EMB</td>
</tr>
<tr>
<td>Yimer S.A. et al (24)</td>
<td>2008</td>
<td>Ethiopia</td>
<td>Sub-national survey. Number of TB facilities in Amhara not provided.</td>
<td>New patients</td>
<td>112</td>
<td>26.9</td>
<td>1.9</td>
<td>MGIT</td>
<td>INH, RMP, SM, EMB</td>
</tr>
<tr>
<td>Irenious S. et al (31)</td>
<td>2011</td>
<td>Somalia</td>
<td>National survey.</td>
<td>New and PT patients</td>
<td>946</td>
<td>N/A</td>
<td>N/A</td>
<td>Hain</td>
<td>INH, RMP only</td>
</tr>
<tr>
<td>Chonde TM et al (32)</td>
<td>2006-2007</td>
<td>Tanzania</td>
<td>National survey.</td>
<td>New and PT patients</td>
<td>1,167</td>
<td>N/A</td>
<td>5.8</td>
<td>LJ</td>
<td>INH, RMP, SM, EMB</td>
</tr>
<tr>
<td>Study/Year</td>
<td>Country</td>
<td>Survey Type</td>
<td>Number of TB Facilities</td>
<td>New and PT Patients</td>
<td>Treatment Regimen</td>
<td>Notes</td>
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<tr>
<td>Tessema B. et al (33)</td>
<td>2009 Ethiopia</td>
<td>Sub-national survey, Five TB health facilities in north west Ethiopia</td>
<td>N/A</td>
<td>260 25.4 1.7</td>
<td>INH, RMP, SM, EMB, OFX, AM, MFX, Amino Salicylic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Chanda M. et al (34)</td>
<td>2006 Zambia</td>
<td>Sub-national survey, Six TB health facilities in Ndola district</td>
<td>N/A</td>
<td>361 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
<td></td>
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<tr>
<td>Nunes E.A. et al (35)</td>
<td>2002-2003 Mozambique</td>
<td>Sub-national survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>111 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
<td></td>
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<tr>
<td>Nelson L.J. et al (36)</td>
<td>2002 Botswana</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>2,425 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Ramarokoto H. et al (37)</td>
<td>2005-2007 Madagascar</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>1,275 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
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<tr>
<td>Siemo-Gado P. et al (38)</td>
<td>2007-2008 Swaziland</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>1,200 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Sanchez-Padilla E. et al (39)</td>
<td>1999 Gambia</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>633 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Antolabi D. et al (40)</td>
<td>2002-2004 Benin</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>470 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Tado G. et al (1,41)</td>
<td>2004 Equatorial Guinea</td>
<td>Sub-national survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>236 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>N’guesesan K. et al (42)</td>
<td>2005 Ivory Coast</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>320 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sangare L. et al (43)</td>
<td>2010 Burkina Faso</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>416 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Ellis Awusu-Dabo et al (44)</td>
<td>2001-2004 Ghana</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>216 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Jurgen Noesk et al (45)</td>
<td>2012 Cameroon</td>
<td>Sub-national, Twenty-nine TB health facilities in Litoral region, 29 PT patients</td>
<td>N/A</td>
<td>233 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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<tr>
<td>Mbulo G.M.K. et al (46)</td>
<td>2008 Zambia</td>
<td>National survey, Number of TB health facilities not provided.</td>
<td>N/A</td>
<td>883 25.4 1.7</td>
<td>INH, RMP, SM, EMB</td>
<td></td>
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</tbody>
</table>
Abbreviations: DST, drug susceptibility testing; INH, isoniazid; RMP, rifampicin; EMB, ethambutol; SM, streptomycin; Km, kanamycin; GFX, gatifloxacin; CPM, capreomycin; OFX, ofloxacin; AM, amikacin; N/A, not available; LJ, Löwenstein Jensen; MGIT, mycobacteria growth indicator tube; PT, previously treated patients.
Figure 2. Forest plot of prevalence of any resistance and MDR among new and previously treated patients.
Figure 3. Pooled estimates for all resistance patterns among new and previously treated patients. Abbreviations: n, number of studies; ES, estimate; INH, isoniazid; RMP, rifampicin; EMB, ethambutol; SM, streptomycin.
Figure 4. Subgroup analysis: prevalence of any drug resistance. 
Abbreviations: n, number of studies; ES, estimate.
Figure 5. Subgroup analysis: prevalence of MDR.
Abbreviations: n, number of studies; ES, estimate.
Figure 6. Funnel plot exploring publication bias.
REFERENCES


Chapter 8

General Discussion

Summary

Samenvatting

Acknowledgements

About the author
General Discussion

The major aim of this thesis was to add to the understanding of anti-tuberculosis (TB) drug resistance (DR) including multidrug resistant TB (MDR-TB) in sub-Saharan Africa (SSA), with focus on Uganda. It documented levels of anti-TB DR and its association with key socio-demographic determinants and HIV infection. It attempted to explore explanations for the established low levels of HIV infection among sputum smear-positive TB patients enrolled in the drug resistance surveys (DRS’s) as compared to similar patients under routine surveillance. Molecular analysis on the same sample of the DRS isolates enabled exploration of the distribution of the predominant M. tuberculosis (MTB) lineages and associations of those lineages with patient demographic characteristics, HIV infection and anti-TB DR. Through a systematic review and meta-analysis, the thesis eventually provided a summary of variation of anti-TB drug resistance across different regions of SSA. Findings from the surveys, molecular analyses and the systematic review add to available knowledge on the epidemiology of anti-TB DR in SSA to guide interventions by the TB programs towards MDR-TB control.

General Findings

Prevalence and Risk factors of Anti-TB Drug Resistance.

Chapter 2 and 3 reported levels of anti-TB drug resistance among new and previously treated sputum smear-positive TB patients in Uganda at sub-national and national level, and its association with HIV infection and other risk factors. The surveys employed similar methods but to some extent allowed us to assess different aspects. The survey done in Kampala (Chapter 2), more limited in geographic scope and sample size, offered more opportunity for detailed interviews about risk factors, whereas the national survey (Chapter 3) provided higher precision of e.g. MDR estimates and allowed studying regional variation in drug resistance prevalence.

We found levels of primary MDR (<1.5%) in both studies to be in line with the lower end of global estimates (3.6% on average)(1). Settings where MDR-TB levels are <3% among new patients are regarded as low MDR-TB burden. Earlier studies in this setting showed levels of
combined MDR-TB, i.e. among both new and previously treated patients (0.5%) that were 0.4 fold less than in our study (2.3%). Although this may reflect random variation it suggests that overall anti-TB DR in Uganda has slightly increased over the last decade (2). Results from our surveys were consistent with findings from similar studies in the East African region with exception of a study in Rwanda that showed slightly higher primary MDR rates than what we found (3)(4)(5). Although HIV has been documented to be associated with MDR-TB in some settings (6)(7) we did not find such an association, consistent with most reports from studies done elsewhere in this region (8)(9). Studies done in the early 1990s in New York associated HIV infection with MDR-TB (10) and in 2006, Gandhi et al reported an outbreak of extensively drug-resistant TB (XDR-TB) in the high HIV prevalence setting of KwaZulu Natal in which 98% of co-infected patients died within 16 days (11). Given the high TB/HIV co-infection rates in Uganda and in SSA in general, one would be concerned about high MDR-TB rates assuming a higher probability of MDR-TB and HIV patients coming into regular close contact, for instance during the course of seeking care in health facilities leading to high MDR-TB HIV co-infection rates, but this is not what we observed. One explanation may be that in Uganda TB treatment is done largely in the community, thus limiting possibilities of such close interactions and nosocomial transmission of MDR-TB. The low rates of DR observed in our studies could also be attributed to the use of fixed-dose combinations of anti-TB drugs which limits functional monotherapy that may potentially result from use of loose formulations (12). In addition, by the time of the study, NTLP used the eight months regimen in which rifampicin is only used for intensive phase treatment of adult new TB patients that contribute over 80% of the TB burden. This may prevent misuse of rifampicin in a setting where daily observation of a patient (DOT) throughout treatment is still a challenge, and thereby prevent DR amplification. Although this regimen has been documented to result in more relapses and results in lower treatment success rates than the 'rifampicin throughout' regimen (13), it might play a key role in keeping MDR-TB levels low, especially in countries at risk of rifampicin misuse during prolonged use. Further, in a setting with high rates of TB/HIV co-infection such as Uganda, TB and HIV therapy is concomitantly administered which is documented to result in significant drug interactions. Pharmacokinetic studies have shown that rifamycins including rifampicin are potent inducers of the Cytochrome P-450 pathway leading to subtherapeutic levels of some anti-retrovirals. Rifampicin has specifically been documented to reduce the bioavailability of ARVs through
complex biological mechanisms involving adenosine triphosphate (ATP) binding cassette transporter P-glycoprotein. On the other hand, rifabutin concentrations are affected by the antiretrovirals that induce or inhibit CYP enzymes (14) that may result into sub-therapeutic levels of this key component of the anti-TB regimen in circulation, predisposing to development of rifampicin resistance. However co-administration of anti-TB drugs and ARVs especially in the intensive phase of treatment at program level in Uganda is a recent development and its role in the current rates of MDR might be minimal if any. We recommend further research in this area since such conditions leading to these low levels anti-TB DR in Uganda might as well prevail in settings in the world with relatively higher MDR rates.

The association between primary DR and health care work is one major finding in this survey consistent with observations from Estonia (15), a high MDR-TB prevalence setting. It is interesting to note that even in this low MDR-TB prevalence setting this association still exists. It might reflect prolonged infectiousness of TB patients, especially those with pre-existing drug resistance due to poor treatment response, exposing health care workers more to drug-resistant than to drug-susceptible TB. It might also be a proxy for the insufficient TB infection control measures at TB diagnostic centers that predispose health care workers to frequent infections during the course of patient care.

In addition to the 17 new cases resistant to both isoniazid and rifampicin in the national survey (Chapter 3), we found 43 cases with any resistance to isoniazid and six with any resistance to rifampicin. Although isoniazid resistance rates tend to be high relative to those for other drugs and not necessarily matched by high MDR-TB prevalence in all settings (e.g. in some Asian countries such as Vietnam(16), any resistance to rifampicin should alert the program of potential MDR since in most settings up to 99% of rifampicin resistant isolates have been found to be also resistant to isoniazid (17). At the time of this study, the NTLP was transitioning from the use of the eight months to the six months regimen with rifampicin use throughout treatment. It is therefore prudent that effective interventions by the NTLP are implemented to ensure direct observation of first-line TB treatment to protect rifampicin from misuse and thereby from stepwise acquisition of resistance.

As already documented in similar surveys, previous history of TB treatment was associated with high rates of DR including MDR. Although our studies were not powered to assess XDR to
reliably conclude about its prevalence, none of the identified MDR-TB cases turned out to be XDR-TB. This probably reflected limited use of second line drugs in Uganda, since PMDT had not been rolled out at the time of the study and second-line treatment in public hospitals or the private sector, probably major drivers of XDR-TB in China and India respectively (18)(19), is rare. We can reliably conclude that XDR-TB in Uganda is a very limited problem if it existed at all since levels of MDR-TB are also low. We found more MDR-TB cases among participants >35 years old. This epidemiological picture can be best explained by increasing cumulative exposure to MDR-TB over time with age. This finding is nonetheless paradoxical. Given the high TB-HIV co-infection rate (30.7% in the study population) one would expect most cases of active TB including MDR-TB to result from recent infections hence more MDR among the young and more active population, which we did not observe. Future studies assessing trends in age- and HIV-specific patterns of drug resistance will point out whether this expectation is realized in the longer term.

**Consistency of HIV surveillance data**

We found a lower HIV prevalence in our survey participants compared to the sputum smear-positive TB patients reported under the continuous TB/HIV surveillance system by the NTLP; 31% vs 53% for Kampala (Chapter 2), and 31% vs 54% nationwide (Chapter 3). Our direct comparison of rates of HIV infection among participants included in the drug resistance surveys with HIV prevalence among TB patients in the routine NTLP TB/HIV co-infection surveillance confirmed this discrepancy (Chapter 4). In this analysis we included patients of the same category enrolled for care in the same clinics and period as for the Kampala drug resistance survey. Therefore study participants were a subset of all patients enrolled for care and we expected minimal if any mismatch between the HIV infection rates from the two surveillance systems. The discordance between two surveillance systems however persisted even after stratification for age, sex, treatment history and residence.

This mismatch could be explained by lower participation rates of the patients with a known HIV status in the survey as compared to routine surveillance, since the survey required consent, which is not mandatory in routine surveillance. Secondly patients in routine surveillance often had their TB diagnosis based on a single positive sputum smear as observed in the unit TB registers. Since the sensitivity of smear examination for pulmonary TB is lower among HIV-positive than among
HIV-negative patients (20), the probability of inclusion for HIV-positive patients might have been lower in the drug resistance survey, although we could not verify this from the unit TB registers which contained no details on the numbers of pretreatment smears examined. Overestimation of HIV prevalence under routine surveillance at testing, registration errors and/or errors at data collection might have contributed to this discrepancy. This area needs further exploration since our study can only provide a few inconclusive possibilities. Whatever the cause of the mismatch, implications of this finding are significant for TB/HIV control. Although these results are valid for the survey setting, generalizability of TB/HIV co-infection rates obtained from DRS may not be applicable to the population of TB patients at large. At most, these rates should be used to study possible outbreaks of MDR-TB, for instance in hospitals, prisons and other congregate settings. Secondly, validation of data collected under routine surveillance may be important to check for possible overestimation of TB/HIV co-infection rates under routine surveillance.

The role of MTB genotype

As we report in chapters 2 and 3, levels of MDR-TB in Uganda have been consistently low since the first sub-national survey done over 10 years earlier, despite the operational challenges of TB control in this country. Although not documented, conditions like uncertainties surrounding DOT, irregular supply of TB medicines and limited availability of ‘over the counter’ TB drugs in the private sector are not uncommon in Uganda. This coupled with delayed implementation of PMDT in this high HIV burden setting would provide a fertile ground to breed higher rates of M/DR than what is currently established in our surveys.

Chapters 5 and 6 therefore explored the association of *M. tuberculosis complex* lineages with anti-TB DR and HIV infection in Uganda to establish the role played by the molecular characteristics of MTB in these low MDR-TB rates. In our molecular analyses we found significant variations of MTB lineages with anti-TB DR and established a clear negative correlation between the predominant genotype and any resistance to first-line anti-TB drugs, compared to other MTB lineages identified. Early molecular epidemiological reports from Uganda (21)(22) had documented the T2 genotype as the predominating strains in this locality, contributing 45% of the circulating MTB strains. Later studies employing different molecular methods showed the importance of the Uganda II MTB family, a sub-group of the T2 genotype
contributing about 85% of the T2 strains, as a cause of TB in Uganda (23)(24). We confirmed the predominance of the T2/Uganda II MTB genotype as the causative agent of smear-positive TB in the country.

Genotype associations with drug resistance had not been studied in detail previously due to limited sample sizes or MDR-TB isolates being too few to study associations with sufficient statistical power. Our study in Kampala, using spoligotyping to characterize genotypes (Chapter 5), showed patients infected with LAM, T1 and CAS MTB families as 5.0, 2.9 and 2.4-times more likely to be drug resistant than those infected with the T2 strains. In the molecular epidemiological analysis done at a national scale based on single nucleotide polymorphism (SNP) typing (Chapter 6) we found the Uganda II MTB family (the predominant strain) 0.4-fold less likely to be resistant to any of the first line drugs and 0.2-times less likely to be MDR as compared to the ‘non Uganda II’ genotypes. When stratified by the NTLP zone where the samples were collected, rates of any DR were 3-times higher in the eastern zone where the Uganda II MTB family only contributed 23% of the total isolates as compared to the south western zone where the majority of the isolates (55%) belonged to the Uganda II MTB family. We speculate from our findings that this inverse association could partly be responsible for these observed low levels of MDR-TB in this setting (25). However it remains to be established whether this finding is due to environmental factors (shared risk factors for having DR-TB and for being infected with a particular genotype) or to this genotype being inherently less likely to become drug resistant (26), which is further discussed below.

Where we characterized all the isolates according to their respective strain lineages (Chapter 5), we also noted that the genotypes that were often drug resistant in our sample (CAS, LAM and T1) are more widespread across Africa and elsewhere. This suggests that these strains were more likely to be imported with pre-existing drug resistance before spreading in the local population. Although the Beijing genotype has been documented as widespread across the world and associated with TB drug resistance (27)(28)(29)(30) we did not encounter it in this sample, implying that its existence in our study population is very limited (31). Settings where MDR-TB is documented to be on the increase have often seen a rapid change in their MTB population structure towards the predominance of the Beijing genotype (32). Contrary to our findings,
studies from high burden TB settings elsewhere found that the predominating phylogenetic lineages were often also associated with M/DR-TB (33).

Bifani and others reported particular lineages of MTB as possibly well adapted to particular human populations and maladapted to others (34). In a study done in San Francisco, patients infected with the Euro-American strain were 3-times more likely to generate a secondary case of TB over an 11 year period compared patients infected with any other strain. Such variations in adaptation have been documented to occur elsewhere too (35). The predominance of the Uganda II MTB family in our study suggests increased adaptation of this strain to the Ugandan population, e.g. through higher potential than other genotypes for transmission or for progression from latent infection to disease. More insight into such adaptive mechanisms should be gained through in-depth TB molecular epidemiological studies focusing on Uganda II MTB family in this locality. For reasons of study efficiency these would preferably be done in settings where TB transmission is expected to be high, such as prisons and other congregate settings, as has been done in some countries (36). Such studies will be potentially be important in SSA since genotype-associated risks for acquisition and transmission of MDR-TB may affect effectiveness of long term TB control (37).

The inverse association between the T2/Uganda II MTB family and DR (Chapter 5) could be explained by this genotype, compared to other genotypes, being less likely to acquire DR during patient treatment (for instance due to lower mutation rates in resistance genes), having lower failure rates on first-line treatment when drug resistant, or being less transmissible when drug resistant otherwise. However this inverse correlation between the predominant strain and DR could be confounded by environmental factors that affect both the risk of DR and the predominance of particular genotypes, for instance geographic variations in treatment adherence that coincide with geographic variations in genotype distribution. Due to their broader geographical scope and larger sample size, the molecular epidemiology data from the national survey (Chapter 6) allowed us stratify the association between the T2/Uganda II genotype and drug resistance by geographic zone and some other possibly shared risk factors including age, sex, urban or rural residence, and TB treatment history. The analyses showed that no such confounding existed. Although there may be yet unidentified confounders, these results suggest that the inverse genotype association with DR is inherent (biological) rather than environmental.
Some studies have associated HIV infection with MTB families (in particular the Beijing strain) (38) but we did not observe such association in our study. Genotype associations with HIV infection may be environmental (e.g. shared risk factors through nosocomial transmission) but could also exist if certain strains are more likely to cause TB in immunocompromised compared to immunocompetent individuals(39). That we did not find such associations probably reflects minimal if any difference between the circulating MTB lineages with regard to immunogenicity or virulence.

Based on our findings and the available literature, it is plausible that the T2/Uganda II MTB family has selective advantage over other MTB strains in Uganda independent of HIV infection while it is inherently less likely to be drug resistant. We speculate that similar population structures of MTB lineages, with a predominant genotype that is less likely to be resistant, might exist elsewhere in SSA, thus contributing to the low levels of DR observed in this region.

Findings elsewhere in SSA

Chapter 7 is a systematic review and meta-analysis examining regional variations of anti-TB DR in SSA (with exception of the Republic of South Africa) in relation to HIV infection rates. We report low levels of drug resistance in this region from 27 anti-tuberculosis DR surveys reporting drug susceptibility testing (DST) data for 13,645 new and 1,776 previously treated patients. Concisely, our results show MDR prevalence estimates as almost half as compared to those reported globally by WHO for both the new (1.5 % vs 3.6%) and previously treated TB patients (10.3% vs 20.2%)(1). These levels of MDR established through this analysis compare very well with what we found in the DR surveys done in Uganda (1.4% among new and 12.1% among previously treated patients, Chapters 2 and 3). These low levels of DR-TB can probably be largely attributed to the functionality of the TB programs in this region. Indeed, studies have shown that where TB programs are functional resistance levels have been low as compared to countries with dysfunctional TB programs(40), characterized by the use of non-standardized regimes, poor access to TB medicines, uncontrolled use of anti-TB drugs on the open market and insufficient knowledge among the health care workers. This finding is consistent with the observations in countries like Somalia where, after 2 decades of civil war, levels of MDR-TB are among the highest in SSA, and in some regions such as Eastern Europe where the functionality of the TB programs was compromised due to deteriorating health care delivery systems as a
consequence of the breakdown of the Soviet Union public health structures (41). Literature has however shown that the functionality of the TB control program may not necessarily match the levels of DR and this relationship is rather complex (42). Conversely, wide use of fixed-dose combination drugs in this region might significantly contribute to the low prevalence of resistance observed, as it prevents development of resistance due to monotherapy, a recognized risk factor for acquired DR (12). Similarly, most TB programs in SSA are donor-funded and obtain their TB drugs from quality-assured suppliers which limits possibilities of using counterfeit drugs with low bioavailability and therefore less protection against resistance for the companion drug regimen.

From what we documented earlier in this thesis the regional variation in DR TB rates may also reflect variations in the predominant MTB lineages in the different countries. The role of the predominantly circulating MTB genotype in a given locality in DR rates need to be studied widely in SSA to further add on the existing knowledge of the known risk factors for DR in this region, and potentially to predict future MDR-TB levels. However, the relationship may be bidirectional. While predominance of lineages with low potential for DR may partially explain low resistance levels in the region, these lineages may remain predominant due to conditions not being conducive for spread of drug resistant strains (43). Changes in such conditions might thus induce changes in MTB population structure. As observed in some settings, particular MTB strains have been associated with differential rates of MDR-TB (44)(45). The Beijing genotype, shown to be associated with MDR in various settings, is widespread across the world and wide exploration of its distribution in SSA is important to predict the possibilities for higher MDR-TB rates in the future in such countries where this genotype is found in significant proportions or where the population structure of TB tends towards an increase in occurrence of Beijing strain infections (32).

The high TB/HIV co-infection rates coupled with use of chemotherapy has led to concerns about possible association between HIV and MDR-TB, and led to this subject being explored widely (8)(9)(46)(47)(48)(49). Drug malabsorption leading to acquired rifampicin resistance and the increased risk of exposure of HIV-infected patients to MDR-TB in health care settings are some of the mechanisms through which possibility of this association was generated (50).
Furthermore, in the WHO Africa region, which hosts the highest burden of HIV, ART coverage had increased to about 7.5 million patients by the end of 2012 (UNAIDS 2013). This may potentially complicate co-management of TB and HIV leading to higher rates of MDR which we did not observe in the analysis.

In our meta-analysis, levels of any rifampicin resistance were in the same range as the reported prevalence of MDR-TB. This finding is particularly relevant for the current global and regional efforts to timely diagnose MDR-TB through the scale-up of molecular technology to provide quick rifampicin resistance results. The use of Xpert MTB/RIF to diagnose rifampicin resistance as a proxy to MDR-TB can reliably be expanded for timely diagnosis and initiation of second-line treatment for MDR-TB patients. In fact, access to culture and DST facilities in many sub-Saharan countries is limited due to high costs, long turnaround time, and laboratory biosafety challenges, hence the use of molecular techniques in diagnosis of MDR-TB is rapidly expanding. Finally, due to the long history of isoniazid and streptomycin use in the treatment of TB and the microbiological characteristics of the drugs, resistance to isoniazid and streptomycin is the most frequent bacillary resistance among new patients in most settings, consistent with what has been documented in earlier studies (51).

**Targets for intervention/Implications for policy**

Our studies revealed potential opportunities and feasibility of MDR-TB control in Uganda and elsewhere in sub-Saharan Africa. The low levels of anti-TB drug resistance including MDR, the absence of a positive association with HIV infection and the negative association of the predominant MTB strain with anti-TB DR are opportunities that need to be harnessed by the national TB programs to ensure effective control of MDR-TB in this region. However, important challenges with TB infection control at health facility level emerged through one of the studies where relatively high levels of anti-TB drug resistance were found among health care workers.

The first drug resistance survey in Uganda, establishing existence of MDR-TB in this country, was done over 15 years ago (2). Programmatic management of MDR-TB (PMDT), i.e. second-line treatment following WHO guidelines under NTLP auspices, was rolled out more than 10 years later although some patients received second-line treatment under research settings. Given
the low levels of MDR-TB that existed even before roll out of PMDT in Uganda (initiated in 2012), it can be speculated that such levels will not become higher if the existing cases are effectively treated and therefore, transmission interrupted. One of the key priorities of the NTLP in MDR-TB management should be to identify most, if not all of the existing MDR-TB cases and timely initiate them on treatment. Globally, of the estimated 450,000 MDR-TB cases expected, only 94,000 (20.9%) and 77,000 patients (17.1%) were started on treatment in 2010 and 2011 respectively(1), implying that the majority of MDR-TB cases are not detected and that not all those detected are timely started on treatment leading to prolonged transmission. Therefore, countries where a tuberculosis specimen referral system (i.e., routine collection of sputum specimens from previously treated TB patients for culture and drug susceptibility testing at reference laboratories) exists should ensure its effectiveness for timely detection of MDR-TB, just as implemented by the national TB reference laboratory (NTRL) in Uganda. Support to expansion of the WHO recommended molecular techniques like the Xpert MTB/RIF assay for timely diagnosis of rifampicin resistance is also a very important strategy in the fight against MDR-TB in this region. Establishment of a referral laboratory at regional level (e.g., in regional hospitals) with capacity to diagnose rifampicin resistance will go a long way to facilitate this process.

Existence of primary MDR alerts TB programs to transmission of MDR-TB in the community, which can be attributed to late initiation of effective treatment and should be addressed through timely diagnosis. Current efforts and resources to establish more MDR-TB centers in Uganda should be redirected towards strengthening those already in existence and to support MDR-TB diagnosis, since such centers might be underutilized in the near future despite the enormous resources put to establish and run them. Secondary, to ensure sustained efforts for MDR-TB control, the national governments in SSA need to commit adequate resources in the annual budgetary allocations or strengthen international collaboration in order to minimize lapses experienced in supplies required for MDR-TB diagnosis and treatment. Thirdly, during PMDT implementation, national programs need to strengthen directly observed therapy (DOT) for patients with drug susceptible TB to minimize 'loss to follow up' and generation of more MDR-TB cases through irregular intake of TB drugs. Although some studies have challenged contribution of DOT in prevention of microbiological failure as compared to self-administered treatment (52), this intervention is more important today than ever before since many countries
in SSA are transitioning from the 8 months to the 6 months regimen with rifampicin throughout. Absence of XDR-TB in Uganda likely reflects limited use of second-line drugs so far, either by the TB program or in the private sector. For countries where PMDT has been rolled out, national programs ought to be more vigilant and strengthen second-line DST for purposes of XDR-TB surveillance and management of identified cases as they occur.

Community-based TB care should be strengthened as the mainstay for management of resistant and susceptible TB. Uganda and SSA in general are a high HIV burden region. Chronic management of M/DR-TB in health care settings under hospital admission would not only impose undue costs to the health care system but also increase chances of transmission of (M)DR-TB to already immune compromised hosts through frequent and close interactions of MDR-TB and HIV patients, whose treatment outcomes may not be predicted with sufficient certainty (53).

The NTLP in Uganda is currently rolling out isoniazid preventive therapy (IPT) at national level and data on IPT use was only available for 12/46 SSA countries by 2008, the widest coverage reported so far (54). It has been argued, and made plausible through modeling, that large-scale use of a drug for prophylaxis will in the end compromise its use for treatment while isoniazid resistance is a risk for failure and relapse with first line drugs (55). From our surveys, 5-10% of the isolates were already resistant to isoniazid (Chapters 2 and 3) and thus at risk of becoming MDR. National programs should therefore ensure logistical and technical capacity for effective implementation of IPT with focus on excluding active TB before its initiation in order to prevent ‘a dilemma of short term gains (prevention of active tuberculosis among the vulnerable groups) against long term losses (exacerbated isoniazid resistance)’, and a consequently increased risk of MDR.

The association between TB-DR and health care work should be further studied to quantify its magnitude at national level. TB infection control is one of the WHO recommendations that all national programs should embrace, and regular assessment of DR levels among health care workers with TB might be one of the proxy measures for the effectiveness of TB infection control implementation in health facilities. This finding also emphasizes the need to strengthen HIV testing among health care workers before deployment to the (MDR-) TB wards where they run a higher risk of contracting MDR-TB. On the other hand, this finding may reflect a delay in
diagnosing drug resistant TB, frequent visits of the affected patient to the health facility and therefore regular interactions with health workers before initiation of appropriate treatment. Although this is not amenable to simple interventions, it is worth noting and deserves serious consideration.

Results from our molecular epidemiological studies suggested that M/DR-TB was more likely to be imported than locally generated, and elsewhere in the developed world (56) studies have shown drug resistant TB as commoner among immigrants than among the indigenous population. However, this picture might not be generalizable to other populations with low MDR-TB rates especially in settings where such levels are low such as SSA. Our study showed a negative association of the predominant genotype with anti-TB DR and, since settings where TB is highly prevalent are likely to have strains adapted to that population over a long period of time, (57), levels of drug-resistant TB in Uganda may not increase beyond the current rates. Whether the predominance of the well-adapted genotype prevents a large increase will depend on whether the association is indeed biological or the conditions determine that this adaptation persists which might determine future MDR-TB rates in this setting

Strength of the studies.

Our studies had several strengths. Both the Kampala and the national survey were based on WHO guidelines for surveillance of drug resistance tuberculosis (58). Resistance results were stratified by new and previously treated patients and presented accordingly. We included HIV testing in the study design to enable analysis of possible associations between HIV infection and drug resistance patterns a subject that had been heavily debated in this region. They were designed to measure anti-TB drug resistance at population level to minimize bias in selection of study participants and we analyzed samples large enough to allow for enough power to study associations between anti-TB drug resistance and included key risk factors. Since our surveys were laboratory-based, the national TB reference laboratory where the samples were processed had built enough capacity to process large numbers of samples with high degree of reliability as per the concordance with external quality assurance results from the supranational laboratory engaged for this activity. Molecular analyses were done at the medical molecular laboratories
that also provided high quality results to allow analysis of possible associations of MTB strain lineages with anti-TB DR.

Limitations of the studies

Our studies also had limitations. The number of MDR-TB cases was small, limiting the power to study associations between MDR-TB and some key risk factors for instance between MDR-TB and HIV infection, MTB strain lineage and social demographic characteristics of the patient, although associations with any resistance could be assessed. We only included TB diagnostic and treatment units directly supervised by NTLP, well aware that some patients are diagnosed through private health units that might have not been reporting directly. Following the guidelines for surveillance of anti-TB DR we did not study sputum smear-negative patients, limiting the generalizability of our findings to this category of TB patients should there be variation in DR patterns with bacillary load and therefore smear status (i.e. between the sputum smear-positives and smear-negatives). Spoligotyping, used in the molecular analytical studies in Chapter 5, targets repeat regions that are prone to convergent evolution leading to ambiguity in classification of the MTB strains. Similarly the SITVIT database that we used to assign spoligotypes to phylogenetic lineages has a limited representative collection compared to the circulating strains, which left about 20% of the isolates unclassified.

Our comparative study of the HIV prevalence levels among the participants in the drug resistance surveys and those under routine surveillance (Chapter 4) was limited by incomplete records in the unit TB registers especially the number of pretreatment sputum smears done per sputum smear-positive TB patient registered for care, which in our view was important for this comparison.

Of the 44 countries in SSA that were considered for the systematic review (Chapter 7), only 20 countries had done studies that fulfilled our inclusion criteria with only 15 countries reporting DR data at national level. Only 10/27 studies included HIV testing in the survey to enable studying possible associations between MDR-TB and HIV infection. Also, it was not possible to analyze ART coverage in relation to DR rates in the countries included although, given the low MDR-TB rates we found, this analysis would not significantly change our findings.
CONCLUSION

We studied anti-TB drug resistance and associated risk factors in Uganda and reviewed variations of drug resistance in SSA. Our results show low levels of anti-TB DR including MDR-TB as compared to global estimates. Through these studies we established a negative association between anti-TB DR and the predominant MTB lineage that circulates in the Ugandan population as compared to other strains that are more likely to be imported through cross-border movement. This finding might partly explain the observed low levels of drug resistance in this locality. We also found health care work as a risk for resistance to any of the anti-TB drugs. Despite a high burden of HIV and TB/HIV co-infection, we found no association between having any drug resistance (including MDR) and HIV infection. Finally, we noted that TB/HIV co-infection rates among sputum smear-positive patients reported through drug resistance surveys are significantly lower than HIV prevalence rates among the same category of patients reported under routine NTLIP, TB/HIV surveillance. From these findings, we draw our conclusion that control of MDR-TB in Uganda and possibly elsewhere in SSA is feasible if efforts are sustained to treat prevalent cases and the emergence of new MDR-TB cases is halted.

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Summary

This thesis reports findings of six studies including two tuberculosis (TB) drug resistance surveys, a comparative study of HIV infection rates among patients enrolled in the survey and those under routine TB/HIV surveillance, two TB molecular epidemiological analyses and a systematic review and meta-analysis of drug-resistant TB in sub-Saharan Africa.

Chapter 1 is the general introduction to anti-tuberculosis drug resistance in the world, summarizing aspects of the history of tuberculosis, the global burden, associated risk factors with focus on HIV infection, and diagnosis and treatment of multidrug resistant TB (MDR-TB). In addition it describes the surveys through which the data were obtained and provides justification for each of the studies undertaken.

Chapter 2 is a sub-national survey of anti-TB drug resistance in Kampala City, Uganda, assessing the levels and patterns of resistance to anti-TB drugs in relation to patient demographic characteristics, HIV infection status and previous history of TB treatment. We found low levels of any resistance and of MDR-TB, and no association between HIV infection and resistance to anti-TB drugs including MDR. Any resistance to first-line drugs was associated with exposure to a health care setting as a health care worker.

Chapter 3 reports results of the first national anti-TB drug resistance survey in Uganda. Anti-TB drug resistance rates were in the same range as the findings of our sub-national survey in Kampala. TB/HIV co-infection rates among study participants were lower than the TB/HIV co-infection rates reported under routine surveillance. No resistance pattern was associated with HIV infection.

Chapter 4 is a comparative study of HIV infection rates among the sputum smear-positive patients enrolled in the Kampala drug resistance survey with TB/HIV co-infection rates reported under routine TB/HIV surveillance by the National TB and Leprosy Program (NTLP). This study showed consistently lower levels of HIV infection among survey participants compared to TB/HIV co-infection rates reported to the NTLP after controlling for key patient characteristics. Possible reasons for this mismatch could be selection bias during the survey, TB diagnosis under routine surveillance being based on a single sputum smear patients for routine care more likely to
be HIV infected hence clinically diagnosed than survey participants or erratic HIV testing/recording under routine care. These findings need further exploration.

Chapter 5 is a molecular epidemiological study aimed at exploring the association of the *Mycobacterium tuberculosis* complex (MTC) strain lineages with anti-TB drug resistance patterns and HIV infection in Kampala. Results from this study show the predominant MTC lineage in Kampala (the T2 genotype) as being less likely to be resistant to any of the first-line anti-TB drugs compared to other identified lineages. We found no association between HIV and MTC strain lineages. Strain lineages found in this study that are more widely distributed across the world were more likely to be resistant to any of the drugs and possibly imported with pre-existing drug resistance and spread locally.

Chapter 6 is a TB molecular epidemiological study in Uganda done at a national scale. This report explores the distribution of the T2/Uganda II genotype, the predominant subtype of the T2 genotype. Our results show that the T2/Uganda II genotype is the predominant genotype causing TB in Uganda, be it with substantial geographical variation. Compared to all other MTC strain lineages grouped together, the T2/Uganda II genotype was less likely to be resistant to any anti-TB drug or MDR, even after adjusting for geographic variation, thereby confirming our findings in Chapter 5. The study in addition shows that areas where the T2/Uganda II genotype is less distributed are more likely to host more drug-resistant TB cases than regions where this strain is predominant.

Chapter 7 reports findings of a systematic review and meta-analysis of anti-TB drug resistance in sub-Saharan Africa excluding the republic of South Africa. Results show that pooled estimates of anti-TB drug resistance including MDR-TB in this region are almost half of the global WHO estimates. Any resistance and MDR-TB is not associated with HIV, even though TB/HIV co-infection rates are high. Sub-group analyses of the studies done in eastern, central, southern and western Africa show regional variations of drug resistance rates that are limited in size and not statistically significant.

Chapter 8 provides the general discussion of the findings from all studies and policy implications of these findings. Overall, levels of anti-TB drug resistance are low in Uganda and elsewhere in sub-Saharan Africa, the republic of South Africa excluded. High HIV infection rates in this
region do not seem to be a driver for anti-TB drug resistance. The predominant strain in Uganda is less likely to be drug resistant than other MTC lineages that are more widely distributed across the globe, and MTC genetic characteristic might therefore play an important role with respect to the observed low levels anti-TB drug resistance in the sub-Saharan Africa region.
Samenvatting

Dit proefschrift omvat zes onderzoeken, waaronder twee surveys naar geneesmiddelenresistentie bij tuberculose (TB resistentie), een vergelijkende studie naar de HIV infectieprevalentie onder patiënten zoals waargenomen in een van die surveys versus de HIV infectieprevalentie gerapporteerd in het routine surveillancesysteem, twee moleculair epidemiologische analyses en een systematische review en meta-analyse van TB resistentie in Afrika ten zuiden van de Sahara.

Hoofdstuk 1 is een algemene inleiding tot het probleem van TB resistentie wereldwijd, en geeft een overzicht van de geschiedenis van tuberculose, de wereldwijde ziektebelasting, risicofactoren voor TB met nadruk op TB/HIV co-infectie, en de diagnose en behandeling van multiresistente TB. Tevens beschrijft het de surveys die de basis vormden voor de gegevensverzameling voor dit proefschrift en een verantwoording voor elk van de opgenomen onderzoeken.

Hoofdstuk 2 beschrijft een op subnationaal niveau uitgevoerde survey naar TB resistentie, te weten in de Oegandese hoofdstad Kampala. Het onderzoek richtte zich op prevalentie en patronen van TB resistentie in relatie tot demografische kenmerken van de patiënten, hun HIV infectiestatus en hun voorgeschiedenis met betrekking tot TB behandeling. Wij stelden lage prevalenties vast van resistentie, zowel tegen enigerlei TB geneesmiddel als multiresistentie, en vonden geen associatie tussen HIV infectie en TB resistentie, inclusief multiresistentie.

Hoofdstuk 3 geeft de resultaten weer van de eerste landelijke TB resistentiesurvey in Oeganda. De prevalentie van TB resistentie was in dezelfde grootteorde als die in de survey in Kampala. De prevalentie van HIV infectie onder de TB patiënten in de survey (TB/HIV co-infectie) was lager dan gerapporteerd in het routine surveillance systeem. Er was geen verband tussen enigerlei resistentiepatroon en HIV infectie.

Hoofdstuk 4 betreft een vergelijking tussen de HIV infectieprevalentie onder de sputumpositieve patiënten die waren geïncludeerd in de TB resistentiesurvey in Kampala en die welke waren gerapporteerd in het routine TB/HIV surveillancesysteem van het Nationale TB en Lepra Programma. Dit onderzoek liet consequent lagere HIV infectieprevalenties zien onder TB patiënten in de survey, ook na correctie voor verschillen in de meest belangrijke patiëntkenmerken. Mogelijke oorzaken voor deze discrepantie zijn selectiebias in de survey, verschillen in criteria voor de diagnose “sputumpositieve TB” (met name in de
Hoofdstuk 5 beschrijft een moleculair epidemiologisch onderzoek met als doel inzicht te krijgen in de associatie tussen specifieke stammengroepen (lineages) van Mycobacterium tuberculosis complex (MTC) en TB resistentie en TB/HIV co-infectie in Kampala. De resultaten laten zien dat de dominante MTC stammengroep in Kampala, het T2 genotype, minder vaak resistentie vertoont tegen een of meer van de eerstelijns TB geneesmiddelen dan andere in deze studie aangetroffen stammengroepen. Wij vonden geen associatie tussen HIV infectie en MTC stammengroep. Van de stammengroepen aangetroffen in dit onderzoek waren die welke ook elders in de wereld veel voorkomen vaker resistent tegen TB geneesmiddelen. Het is mogelijk dat deze stammengroepen geïmporteerd zijn met reeds aanwezige resistentie en vervolgens lokaal verspreid.

Hoofdstuk 6 is eveneens een moleculair epidemiologisch onderzoek in Oeganda, maar verricht op landelijke schaal. Het exploreert de distributie van het T2/Uganda II genotype, het belangrijkste subtype van het T2 genotype. De resultaten laten zien dat het T2/Uganda II genotype de belangrijkste verwekker is van tuberculose in Oeganda, zij het wel met aanzienlijke geografische variatie. Vergeleken met alle andere MTC stammengroepen tezamen vertoonde het T2/Uganda II genotype minder vaak resistentie tegen een of meer TB geneesmiddelen, en minder vaak multiresistentie. Dit verschil bleef bestaan na correctie voor geografische variaties in de distributie van MTC stammengroepen en TB resistentie, daarmee onze bevindingen in hoofdstuk 5 bevestigend. Verder toonde dit onderzoek dat in gebieden in Oeganda waar het T2/Uganda II genotype minder voorkomt ook minder TB resistentie voorkomt.

Hoofdstuk 7 geeft de bevindingen van een systematische review en meta-analyse van TB resistentie in Afrika ten zuiden van de Sahara. Schattingen van TB resistentie op basis van samengevoegde gegevens komen ongeveer twee keer lager uit dan door de WHO geschatte gemiddelde resistentieprevalenties voor de gehele wereld. Resistentie tegen een of meer TB geneesmiddelen noch multiresistentie is geassocieerd met HIV infectie, zelfs in het geval van hoge TB/HIV co-infectieprevalenties. Subgroepanalyses van de onderzoeken uit oostelijk,
centraal, zuidelijk en westelijk Afrika laten regionale variaties zien in TB resistentie die gering van omvang zijn en niet statistisch significant.

Hoofdstuk 8 tenslotte biedt een bespreking van de bevindingen van alle onderzoeken in dit proefschrift en de daaruit voortvloeiende beleidsimplicaties. Samengevat komt TB resistentie in Oeganda en elders in Afrika ten zuiden van de Sahara, uitgezonderd de Republiek Zuid Afrika, weinig voor. De hoge prevalentie van TB/HIV co-infectie lijkt geen aanjager van TB resistentie. De predominantte MTC stamengroep in Oeganda vertoont minder vaak geneesmiddelenresistentie dan stamengroepen welke een bredere wereldwijde verspreiding kennen, en de genetische achtergrond van MTC stammen speelt daarmee mogelijk een belangrijke rol in de verklaring voor de waargenomen lage prevalentie van TB resistentie in dit deel van de wereld.
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About the Author

Deus Lukoye was born in Masaka, Uganda on August 12 1970 to Ms Teddy Nansamba and Mr. Clement William Lukoye (RIP). He attended Kyetume primary school and Nakonyenyi Senior Secondary school in Masaka before joining Makerere University in 1994 to pursue a bachelor’s degree in human medicine. He graduated in 1999, joined Mulago national referral hospital for internship in internal medicine and obstetrics and gynecology before he started working at Holy Cross Orthodox Mission Hospital Namungoona as a medical officer in 2000.

He joined Kampala City Council (currently Kampala Capital City Authority) as a medical officer, where he was assigned to supervise tuberculosis control services and oversee curative and preventive health services in Labaga division as a division medical officer. He joined Makerere University School of Public Health (MukSPH) in 2006 supported by the Infectious disease Network for treatment and Research in Africa (INTRACT) where he graduated with a master’s degree in Public Health (MPH) in 2010. On January 1 2011, he joined the Uganda Ministry Of Health (MOH) as an operations officer for the East African Public Health Laboratory Networking Project (EAPHLNP), before he was nominated to work with Management Sciences for Health (MSH) as a technical advisor for urban tuberculosis control where he serves up to the time of writing this thesis.

He enrolled for a PHD in 2011 under support of Amsterdam Institute for Global Health and Development (AIGHD) and registered at Academic Medical Centre Amsterdam (AMC) in the same year. He coordinated two drug resistance surveys one which was the first national anti-tuberculosis drug resistance survey in Uganda. His aim is to pursue a career in control of drug resistant tuberculosis in resource poor settings especially in sub-Saharan Africa.
**PHD Portfolio**

**Courses attended**

ICH-GCP Course; November 9 - 10 2006 - Kampala

ICH-GCP Course; January 8 - 10 2007 – Kampala

Promoting Evidence Based Medicine and Critical Appraisal Course October 29th - 31st 2017: Infectious Disease Institute, Kampala

Using Epidata program for data management and analysis; January 12th -16th 2009: Infectious Disease Institute, Kampala

Multivariate analysis Course (ACREM); May 25-29 2009; Makerere University John Hopkin's University Kampala

Diagnostic Accuracy course (ACREM); September 28th to October 2nd 2009; School of Public Health Kigali National University of Rwanda

Clinical Epidemiology and biostatistics Course: March 2013. Amsterdam Institute for Global Health and Development (AIGHD) Amsterdam

Seven 2-3 weeks trainings in research methods, data analysis using STATA and scientific writing; November 2011- May 2014 AIGHD Amsterdam

Five scientific workshops organized by the INTERACT program; Between November 2007 and June 2012; both in Rwanda and Uganda

Weekly 1-2 hours research forums; November 2007- June 2013: at the Infectious Disease institute and AIGHD- Amsterdam