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

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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 [OR^{adj}], 5.0; 95% CI, 2.0 to 11.9; $P < 0.001$) and CAS (OR^{adj}, 2.9; 95% CI, 1.4.0 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 (OR^{adj}, 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.

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

Received 10 November 2013 **Returned for modification** 16 December 2013
Accepted 3 April 2014
Published ahead of print 28 April 2014
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doi:10.1128/AAC.02338-13

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 antituberculosis 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 cetyltrimethylammonium bromide (CTAB)-phenol chloroform method according to a standardized protocol as earlier described (20). Isolates/cultures were confirmed as MTC by IS*6110*-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 μg/ml for isoniazid, 4.0 μg/ml for rifampin, 40 μg/ml for ethambutol, 2.0 μg/ml for streptomycin, and 2.0 μg/ml for ofloxacin and 30 μg/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 unweighted 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/SITVIT_ONLINE/) 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 χ^2 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, T3_Eth, 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 [OR^{adj}], 3.1;

TABLE 1 Demographic characteristics of patients enrolled in the molecular analysis of *M. tuberculosis* isolates from smear-positive tuberculosis patients in Kampala

Patient characteristic	No. included (%), $n = 497$	No. not included (%), $n = 60$	P value[c]
Sex			
Male	302 (60.7)	29 (48.3)	0.06
Female	195 (39.3)	31 (51.7)	
Age (yr)			
18–24	164 (33.0)	20 (33.3)	0.12
25–34	188 (37.8)	28 (46.7)	
35–44	102 (20.5)	5 (8.3)	
>44	43 (8.7)	7 (11.7)	
From Kampala[a]			
Yes	327 (65.8)	43 (71.7)	0.36
No	170 (34.2)	17 (28.3)	
HIV result at (re)testing			
Positive	160 (32.2)	16 (26.7)	0.45
Negative	324 (65.2)	41 (68.3)	
Missing	13 (2.6)	3 (5.0)	
Previous history of TB treatment			
Yes	55 (11.1)	7 (11.7)	0.89
No	442 (88.9)	53 (88.3)	
Prisoner/has been in prison before			
Yes	47 (9.5)	5 (8.3)	0.78
No	450 (90.5)	55 (91.7)	
Health care exposure[b]			
Yes	85 (17.1)	11 (18.3)	0.82
No	412 (82.9)	49 (81.7)	

[a] Refers to patients who reported Kampala as their district of residence.
[b] Refers to exposure to health care setting as a health care worker.
[c] P value for difference between included and nonincluded patients.

TABLE 2 *M. tuberculosis* strain lineages identified among 497 isolates in Kampala

Strain lineage	No. (%)
T2	85 (17.1)
T2-Uganda	142 (28.6)
T1	36 (7.3)
CAS	17 (3.4)
CAS1_Delhi	17 (3.4)
CAS1_Kili	15 (3.0)
LAM11_ZWE	12 (2.4)
LAM3/S convergent	11 (2.2)
S	9 (1.8)
LAM9	8 (1.6)
H3	7 (1.4)
T2T3	4 (0.8)
T2_Eth	4 (0.8)
Undesignated	5 (1.0)
CAS2	1 (0.2)
EAI5	4 (0.4)
Beijing	2 (0.4)
LAM4	2 (0.4)
H4	2 (0.4)
MANU1	2 (0.4)
MANU2	3 (0.6)
LAM6	1 (0.2)
EA1ND	1 (0.2)
Unknown	107 (21.5)

95% confidence interval [95% CI], 1.4 to 6.7; $P = 0.004$). The T1 (OR^{adj}, 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.

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 T3_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 SIT135 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%)

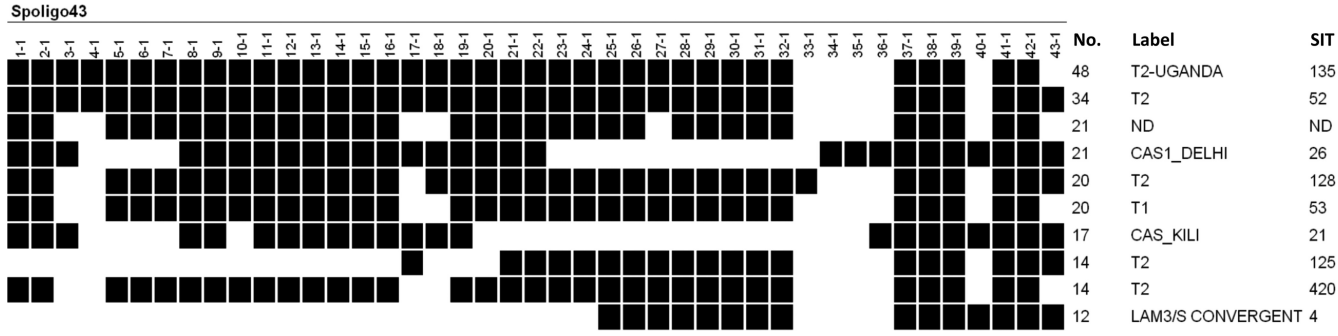

FIG 1 Spoligotype patterns of the 10 major clusters of *M. tuberculosis* strain lineages circulating in Kampala. A dark square indicates the presence of a particular spacer, and a clear square indicates its absence. SIT, international shared spoligotype; ND, not existing in the database.

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 Rubaga 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 an-

TABLE 3 Variation of *M. tuberculosis* lineage in Kampala with key patient characteristics

Characteristic	No. (%) by lineage:							
	T2-Uganda	T2	CAS	LAM	T1	Unknown	Other[a]	Total
Sex								
Male	86 (28.5)	51 (16.9)	31 (9.9)	30 (6.6)	19 (6.3)	70 (23.2)	26 (8.6)	302
Female	56 (28.7)	34 (17.4)	19 (9.7)	12 (6.2)	17 (8.7)	37 (19.0)	20 (10.3)	195
Age (yr)								
18–34	102 (28.9)	52 (14.8)	30 (8.5)	27 (7.7)	33 (9.4)	75 (21.3)	33 (9.4)	352
≥35	40 (27.6)	33 (22.8)	19 (13.1)	5 (3.5)[b]	3 (2.1)[b]	32 (22.1)	13 (9.0)	145
Health care exposure								
Yes	21 (24.7)	16 (18.8)	9 (10.6)	6 (7.1)	7 (8.2)	16 (18.8)	10 (11.7)	85
No	121 (29.4)	69 (16.8)	40 (9.7)	26 (6.3)	29 (7.0)	91 (22.1)	36 (8.7)	412
Previously treated for TB								
Yes	9 (16.4)	14 (25.5)	13 (23.6)[c]	1 (1.8)	5 (9.1)	9 (16.4)	4 (7.3)	55
No	133 (30.1)	71 (16.1)	36 (8.1)	31 (7.0)	31 (7.0)	98 (22.2)	42 (9.5)	442
Patient residence								
Kampala	94 (28.7)	57 (17.4)	31 (9.5)	22 (6.7)	23 (7.0)	68 (20.8)	32 (9.8)	327
Elsewhere	48 (28.8)	28 (16.5)	18 (10.6)	10 (5.9)	13 (7.7)	39 (22.9)	14 (8.2)	170
Municipality where TB diagnosis was made								
Central	29 (32.2)	14 (15.6)	9 (10.0)	5 (5.6)	6 (6.7)	16 (17.8)	11 (12.2)	90
Kawempe	72 (26.7)	53 (19.6)	20 (7.4)	18 (6.7)	20 (7.4)	63 (23.3)	24 (8.9)	270
Lubaga	11 (32.4)	4 (11.8)	3 (12.1)	3 (8.8)	1 (2.9)	9 (26.5)	3 (8.8)	34
Makindye	9 (23.7)	6 (15.8)	8 (21.1)	0 (0)	1 (2.6)	11 (28.9)	3 (7.9)	38
Nakawa	18 (29.5)	8 (13.1)	9 (14.8)	6 (9.8)	6 (9.8)	8 (13.1)	5 (8.2)	61

[a] "Other" includes sublineages with less than 2% total contribution to the number of isolates analyzed, including undesignated, Beijing, EAI, Haarlem, MANU, S, T2T3, and T2_Eth, and spoligotypes unknown to the SITVIT database.
[b] $P = 0.005$ for comparison with age of <35 years for either lineage.
[c] $P < 0.001$ for comparison with new patients for either lineage.

TABLE 4 Resistance patterns of *M. tuberculosis* isolates in Kampala, by lineage

Strain lineage	No. (%) of isolates						
		Resistant to anti-TB drug:					
	Per lineage	Rifampin	Isoniazid	Ethambutol	Streptomycin	Multidrug resistance	Any drug
T2-Uganda	142 (28.6)	2 (1.4)	4 (2.8)	0	11 (7.7)	2 (1.4)	14 (9.9)
T2	85 (17.1)	3 (3.7)	9 (10.6)	1 (1.1)	8 (9.4)	3 (3.5)	12 (14.1)
CAS	49 (9.9)	3 (6.1)	9 (18.4)	4 (8.2)	8 (16.3)	2 (4.1)	15 (30.6)
LAM	32 (6.4)	2 (6.25)	8 (25.0)	1 (3.1)	8 (25.0)	1 (3.1)	11 (34.4)
T1	36 (7.2)	4 (11.1)	7 (19.4)	0	2 (5.6)	4 (11.1)	8 (22.2)
Unknown[a]	107 (21.5)	1 (0.9)	1 (0.9)	2 (1.8)	9 (8.4)	0	9 (8.4)
Other[b]	46 (9.3)	0	2 (4.4)	0	6 (13.0)	0	6 (13.0)
Total	497	15 (3.1)	40 (8.1)	8 (1.6)	52 (10.6)	12 (2.4)	75 (15.1)

[a] Refers to a group of isolates whose genotype is unknown to the SITVIT database.
[b] Including undesignated, Beijing, EAI, Haarlem, MANU, S, T2T3, and T3_Eth.

ti-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. In 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.

Only 2.4% of the isolates analyzed were MDR, diversely distributed among the lineages (Table 3), making meaningful multivariable 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

TABLE 5 Association of *M. tuberculosis* strain lineage with resistance to any of the anti-TB drugs in Kampala

Lineage	Total no. of isolates	No. (%) of isolates resistant to any anti-TB drug	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)[a]	*P* value
T2/T2-Uganda	227	26 (11.5)	Reference	Reference	
CAS	49	15 (30.6)	3.4 (1.6–7.1)	2.9 (1.4–6.3)	0.006
LAM	32	11 (34.4)	4.1 (1.7–9.3)	5.0 (2.1–11.9)	<0.001
T1	36	8 (22.2)	2.2 (0.9–5.3)	2.4 (1.0–6.2)	0.070
Unknown	107	9 (8.41)	0.7 (0.3–1.6)	0.7 (0.3–1.6)	0.450
Other[b]	46	6 (13.0)	1.2 (0.4–3.0)	1.1 (0.4–3.0)	0.820

[a] Based on multivariable logistic regression model, including age, sex, treatment history, history of imprisonment, and history of health care work.
[b] Includes Haarlem, EAI, MANU, S, undesignated, T2T3, T3_Eth, and Beijing.

TABLE 6 Univariable analysis of the association between HIV infection and *M. tuberculosis* lineages in Kampala

Lineage	Total no. of isolates ($n = 484$)	No. (%) HIV positive	Odds ratio (95% CI)	*P* value
T2/T2-Uganda	220	75 (34.1)	Reference	
CAS	49	17 (34.9)	1.0 (0.5–2.0)	0.94
LAM	31	7 (22.6)	0.6 (0.3–1.4)	0.21
T1	36	13 (36.1)	0.9 (0.5–2.3)	0.81
Unknown	103	34 (33.0)	1.0 (0.6–1.6)	0.85
Other	45	14 (31)	0.8 (0.4–1.7)	0.70

(29). In a related study done in Kenya, this strain contributed almost 4% of the samples included in the analysis (30).

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.

M.L.J., D.L., and F.G.J.C. conceived and designed the experiments. F.A.K., B.B.A., D.P.K., and M.O. performed the experiments. D.L., F.G.J.C., F.A.K., and M.L.J. analyzed the data. M.L.J., F.A.K., and F.G.J.C. contributed reagents/materials/analysis tools. D.L., F.G.J.C., and F.A.K. wrote the paper.

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 07.05.20100).

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