MAJOR ARTICLE

# Rate and Amplification of Drug Resistance among Previously-Treated Patients with Tuberculosis in Kampala, Uganda

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***Background.*** Drug-resistant *Mycobacterium tuberculosis* has emerged as a global threat. In resource-constrained settings, patients with a history of tuberculosis (TB) treatment may have drug-resistant disease and may experience poor outcomes. There is a need to measure the extent of and risk factors for drug resistance in such patients.

***Methods.*** From July 2003 through November 2006, we enrolled 410 previously treated patients with TB in Kampala, Uganda. We measured the prevalence of resistance to first- and second-line drugs and analyzed risk factors associated with baseline and acquired drug resistance.

***Results.*** The prevalence of multidrug-resistant TB was 12.7% (95% confidence interval [95% CI], 9.6%–16.3%). Resistance to second-line drugs was low. Factors associated with multidrug-resistant TB at enrollment included a history of treatment failure (odds ratio, 23.6; 95% CI, 7.7–72.4), multiple previous TB episodes (odds ratio, 15.6; 95% CI, 5.0–49.1), and cavities present on chest radiograph (odds ratio, 5.9; 95% CI, 1.2–29.5). Among a cohort of 250 patients, 5.2% (95% CI, 2.8%–8.7%) were infected with *M. tuberculosis* that developed additional drug resistance. Amplification of drug resistance was associated with existing drug resistance at baseline ($P < .01$) and delayed sputum culture conversion ($P < .01$).

***Conclusions.*** The burden of drug resistance in previously treated patients with TB in Uganda is sizeable, and the risk of generating additional drug resistance is significant. There is an urgent need to improve the treatment for such patients in low-income countries.

Drug-resistant *Mycobacterium tuberculosis* has emerged as a major global public health threat. Resistant strains have been identified in most countries [1]. For decades, tuberculosis (TB) control programs have focused their efforts on patients with newly diagnosed TB, who are responsible for most of the disease burden. However, compared with patients with new TB cases, patients presenting with TB who have a history of treatment experience poorer treatment outcomes and have increased mortality and higher rates of drug resistance [2]. Importantly, multidrug-resistant (MDR) TB, defined as resistance to at least rifampicin and isoniazid, occurs 5–10-fold more frequently among patients who previously received treatment for TB than among patients with new TB [1]. Recently, extensively drug-resistant TB has also emerged as a global concern [3, 4]. The growing worldwide threat of TB drug resistance has prompted the World Health Organization (WHO) to call on national TB control programs to address the problem [5].

Drug resistance develops primarily through the selection of naturally occurring mutations in the presence

Received 17 March 2008; accepted 30 June 2008; electronically published 22 September 2008.

Presented in part: 37th Union World Conference on Lung Health, Paris, France, 31 October–4 November 2006.

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**Clinical Infectious Diseases 2008; 47:1126–34**

1058-4838/2008/4709-0002$15.00
DOI: 10.1086/592252

of inadequate treatment [6]. Once initial resistance has developed, acquisition of resistance to additional drugs is more likely, and treatment with standard regimens may be suboptimal [7, 8]. Previous studies on the acquisition of drug resistance are limited by their retrospective design, a focus on new TB cases, selection bias of the study population, missing or incomplete *M. tuberculosis* strain genotyping, small sample size, or undue influence by the Beijing strain of *M. tuberculosis,* which may be associated with increased rates of drug resistance [9–13].

The WHO has classified Uganda as a country with a high burden of TB [14]. Common wisdom has been that MDR TB is infrequent in most of sub-Saharan Africa [15]. However, there currently is little information available regarding drug resistance in Uganda, and drug susceptibility testing is not routinely performed. Previous data on drug resistance among previously treated patients with TB in Uganda are limited to a survey involving only 45 patients [16]. We report here the frequency and factors associated with drug resistance among treatment-experienced patients with TB in Kampala, Uganda, and describe the acquisition of drug resistance in *M. tuberculosis* in a subset of patients during or after retreatment.

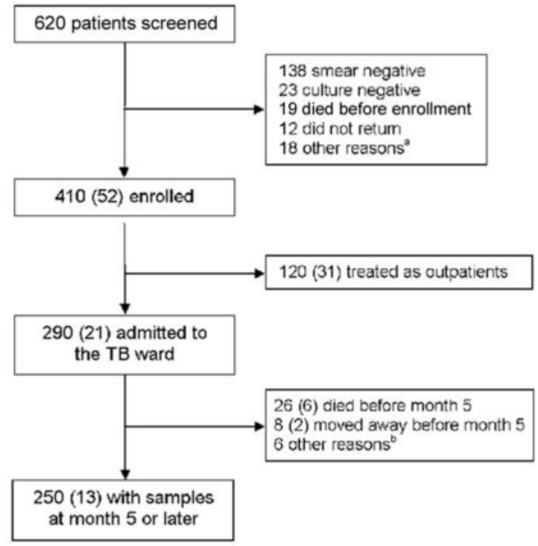


**Figure 1.** Profile of study patients. The number of patients with multidrug-resistant tuberculosis (TB) at each study time point is shown in parentheses. [a]Other reasons for nonenrollment were no samples provided (6 patients), lost or contaminated culture (3), discharge from the hospital before enrollment (3), aged <18 years (3), refusal (2), and mental illness (1). [b]Other reasons for no samples at month 5 or after were inability to produce sputum (3 patients), restart of treatment before month 5 (2), and loss to follow-up (1).

## PATIENTS AND METHODS

***Study population.*** The study examined consecutive treatment-experienced patients with TB who presented to the National Tuberculosis and Leprosy Programme (NTLP) clinic at Mulago Hospital in Kampala, Uganda, from July 2003 through November 2006 (figure 1). The NTLP clinic at Mulago Hospital serves as both the national referral center (approximately one-third of patients are referred) and the largest TB treatment clinic in Kampala, a predominantly urban area with ~25% of all TB cases that are reported to the NTLP each year. Patients were enrolled if they were aged ⩾18 years, gave written informed consent, and provided sputum specimens that had positive results by acid-fast bacilli smear microscopy and were subsequently confirmed to be positive for *M. tuberculosis* by culture. We used a standardized questionnaire to collect relevant demographic and clinical information. All patients were offered HIV testing. Patients were classified, according to the WHO subcategories [17] of previously treated patients with a recurrence of TB (relapse, treatment after failure, or treatment after default), with use of information from a previous TB-treatment card (for 59% of patients) or hospital chart (for 41% of patients). Most patients (83%) had a chest radiograph performed at the time of screening.

***Treatment and follow-up.*** Patients were treated according to NTLP treatment guidelines for recurring cases with use of the WHO-recommended regimen: 2 months of streptomycin, isoniazid, ethambutol, rifampicin, and pyrazinamide; 1 month of rifampicin, isoniazid, ethambutol, and pyrazinamide; and 5 months of rifampicin, isoniazid, and ethambutol. Hospitalization was recommended for the first 2 months of treatment. Directly observed therapy was provided during hospitalization. For patients who declined hospital admission, a 1-month supply of drugs was provided. After hospital discharge, drugs were supplied on a monthly basis, in accordance with NTLP practices. Patients were issued a treatment card on which they indicated each dose that was taken.

After hospital discharge, follow-up clinic visits were scheduled monthly during the 8-month TB treatment course, at month 9, and thereafter, quarterly for at least 2 years. Patients who missed an appointment were visited at home. At follow-up visits, we assessed treatment compliance with treatment card review, monthly pill counts, and patient self-report. Compliance was categorized into a 5-level ordinal scale (fully compliant, missed a few doses, missed approximately one-half of the doses, missed most doses, and not compliant at all). Patients who missed up to 2 months of treatment had their treatment course extended by the number of missed doses. Patients who missed ⩾2 months of treatment were started on a new treatment course.

During follow-up, 2 sputum samples were collected for acid-fast bacilli smear microscopy and culture at predefined time points (months 1, 2, 5, 8, and 12); additional samples were collected at later visits if a cough was present. Time of culture conversion was defined as the first time point when both sputum samples were negative for *M. tuberculosis* by culture.

**Table 1. Characteristics of treatment-experienced patients with tuberculosis (TB) at study enrollment in Kampala, Uganda, from 2003 through 2006.**

| Characteristic | Proportion (%) of patients ($n$ = 410) |
|---|---|
| Sex | |
| Male | 261/410 (64) |
| Female | 149/410 (36) |
| Reason for retreatment | |
| Relapse | 232/410 (57) |
| Treatment failure | 37/410 (9) |
| Default | 141/410 (34) |
| No. of previous TB episodes | |
| 1 | 321/410 (78) |
| 2 | 60/410 (15) |
| 3–6 | 29/410 (7) |
| HIV status[a] | |
| Infected | 197/409 (48) |
| Not infected | 212/409 (52) |
| CD4 cell count,[b] cells/$\mu$L | |
| <200 | 105/159 (66) |
| 200–349 | 25/159 (16) |
| ⩾350 | 29/159 (18) |
| Extent of lung disease on chest radiograph[c] | |
| Normal | 10/341 (3) |
| Minimal disease | 26/341 (8) |
| Moderate disease | 102/341 (30) |
| Advanced disease | 203/341 (59) |
| Cavities on chest radiograph[c] | |
| Present | 228/341 (67) |
| Absent | 113/341 (33) |

[a] One person did not consent to HIV testing.
[b] CD4 cell counts were measured for 159 (81%) of 197 HIV-infected patients.
[c] A chest radiograph was performed for 341 patients (83%).

***Drug susceptibility testing (DST).*** Sputum specimens were cultured for the presence of *M. tuberculosis* by use of either the BACTEC 460 system (through July 2006) or the BACTEC MGIT 960 system (after July 2006; Becton Dickinson), according to the manufacturers recommendations [18]. Confirmation of *M. tuberculosis* complex was determined by either the BACTEC NAP test (Becton Dickinson) or PCR for IS*6110*, as described elsewhere [19]. The number of days from inoculation of 12B media until a positive BACTEC culture result (growth index, >30) was determined for specimens obtained at baseline.

Initial *M. tuberculosis* isolates obtained at study enrollment were subjected to DST. Testing of first-line drugs (streptomycin, isoniazid, ethambutol, rifampicin, and pyrazinamide) was performed using BACTEC 460 or BACTEC MGIT 960 testing systems. As of August 2003, isolates were routinely tested for ofloxacin resistance by use of the BACTEC 460 method at a critical concentration of 2 $\mu$g/mL. Isolates that were resistant to isoniazid and rifampicin were further tested for resistance to second-line drugs. Capreomycin, kanamycin, ethionamide, and para-aminosalicylic acid were tested using the MiddleBrook 7H10 agar proportion method [20] at critical concentrations of 10, 5, 5, and 2 $\mu$g/mL, respectively. Quality control for first-line DST was performed by the US Centers for Disease Control and Prevention (CDC); ~100 isolates per year are retested at the CDC with 95%–100% concordance.

During follow-up, repeat DST was performed for patients who had a culture positive for *M. tuberculosis* ⩾5 months into the treatment course. If the DST pattern was different from that of the patient's initial isolate, IS*6110* restriction fragment–length polymorphism analysis was performed on both isolates [21]. Patients were defined as having *M. tuberculosis* that acquired drug resistance if a follow-up isolate was identical to the initial isolate but had acquired drug resistance.

***HIV testing.*** HIV infection status was determined using a rapid test algorithm, as recommended by the WHO [22]. Rare samples that could not be resolved using this algorithm were assessed using PCR. All HIV-infected patients were referred to antiretroviral treatment programs that became available during the study.

***Analysis.*** The analysis of acquisition of drug resistance included all patients who provided a sputum sample at month 5 after enrollment or later. All data were collected using predesigned questionnaires and were double-entered and managed using a Microsoft Access database system (Microsoft). Data were analyzed using Stata/SE, version 9.2 (StataCorp). Either the standard $\chi^2$ test or the $\chi^2$ test for trend was used in univariate analyses to identify factors associated with the outcomes of interest. Factors significantly associated at the 15% level were further investigated in multivariable analyses. Multiple logistic regression models were fitted, and associations were quantified by ORs with 95% CIs. Cox regression models were fitted to take into account different durations of follow-up and produced hazard ratios (HRs) with 95% CIs. The analysis period started on the date of enrollment in the study cohort and ended on the date of first acquisition of drug resistance or on the date on which the last sputum sample was provided.

***Ethical approval.*** The AIDS research subcommittee of the Uganda National Council of Science and Technology and the Institutional Review Boards at the University of Medicine and Dentistry of New Jersey and the London School of Hygiene and Tropical Medicine approved the study.

## RESULTS

***Description of cohort.*** From July 2003 through November 2006, we screened 620 individuals and enrolled 410 patients (66%). The most common reason for exclusion (161 [77%] of 210 patients) was a negative acid-fast bacilli smear or culture

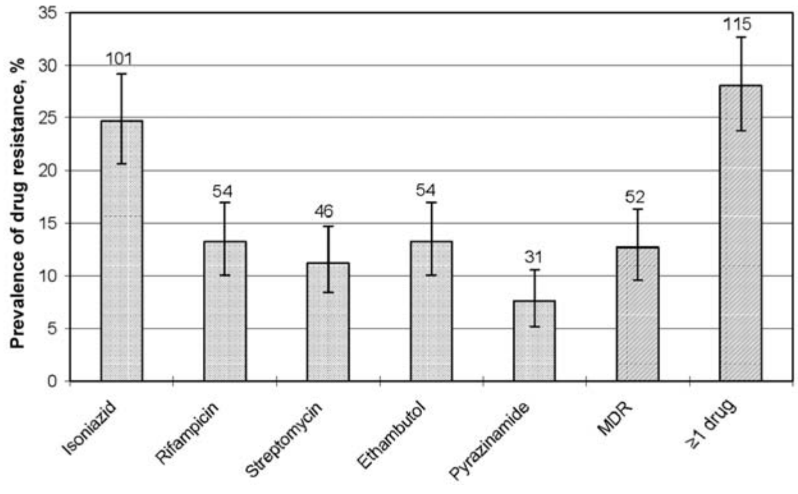


**Figure 2.** Prevalence of resistance to first-line tuberculosis (TB) drugs among 409 previously treated patients with TB in Kampala, Uganda, from 2003 through 2006 (95% CIs are indicated). The number of patients with TB resistant to each drug or drug combination are indicated. MDR, multidrug resistant.

result (figure 1). The majority of patients (258; 63%) lived in Kampala, and 125 (30%) of the patients were referred to the clinic; most of these patients (86; 69%) were referred for TB treatment. Study patients were predominantly young (median age, 33 years) and male (261 patients; 64%). The most common reason for retreatment (232 patients; 57%) was relapse (table 1). Of the 341 patients for whom a chest radiograph was performed, most demonstrated advanced (203; 59%) or moderate (102; 30%) disease on chest radiograph, and 228 (67%) had cavitary disease. Almost one-half (197 patients; 48%) were infected with HIV. Of the 159 HIV-infected patients who had a CD4 cell count measured, 105 (66%) had a CD4 cell count <200 cells/$\mu$L. Eighty-nine patients (22%) had experienced $\geqslant$2 episodes of TB prior to enrollment and had received therapy for each.

***Frequency of drug resistance at enrollment.*** DST results were available for 409 (99.8%) of the 410 patients enrolled. Isolates from 115 patients (28.1%; 95% CI, 23.8%–32.7%) were resistant to at least 1 first-line drug (figure 2). The prevalence of isoniazid monoresistance was 24.7% (95% CI, 20.6%–29.2%). Fifty-four (13.2%) of the 409 isolates were resistant to rifampicin; 52 of these isolates were also resistant to isoniazid, which resulted in a rate of MDR TB of 12.7% (95% CI, 9.6%–16.3%). Four (1.3%; 95% CI, 0.4%–3.4%) of 297 isolates tested were resistant to ofloxacin, including 1 non-MDR TB isolate and 3 MDR TB isolates.

Forty-seven (90%) of the 52 MDR isolates demonstrated resistance to other first-line drugs; 14 (27%), 19 (36%), and 14 (27%) isolates were resistant to a total of 3, 4, and 5 first-line drugs, respectively. Overall, 43 MDR isolates (83%) were resistant to ethambutol, 26 (50%) were resistant to pyrazinamide, and 25 (48%) were resistant to streptomycin. Second-line DST results were available for 51 MDR isolates (98%); we detected resistance to ethionamide (8 isolates; 16%), ofloxacin (3 isolates; 5.9%), and kanamycin (1 isolate; 2.0%). None of the isolates was resistant to capreomycin or para-aminosalicylic acid. The 1 isolate that demonstrated resistance to kanamycin was also resistant to ofloxacin, representing the first known case of extensively drug-resistant TB in Uganda.

***Characteristics of patients with MDR TB at enrollment.*** Patients who presented with MDR TB at enrollment (table 2) were more likely to have a history of treatment failure ($P<.001$) and to have had $\geqslant$2 episodes of TB ($P<.001$). On average, individuals with MDR TB were younger and demonstrated more cavitary disease on chest radiograph. Infection with HIV was not associated with MDR TB. In a logistic regression model, MDR TB at enrollment was strongly associated with a previous history of treatment failure (OR, 23.6; 95% CI, 7.7–72.4), the presence of cavities on chest radiograph (OR, 5.9; 95% CI, 1.2–29.5), and multiple previous episodes of TB (table 2).

***Acquisition of drug resistance.*** Two hundred ninety patients (including 21 of 52 patients with MDR TB) were admitted to the ward and followed up prospectively. Of these 290 patients, 250 (86%, including 13 with MDR isolates) provided sputum specimens at month 5 or later, contributing a total of 384 person-years of follow-up (figure 1). Most patients reported full compliance with treatment (84%) or reported having only missed a few doses (6%). Most patients with non-MDR TB (81%) experienced conversion of sputum culture by month 5 of treatment.

Of the 250 patients, 32 (13%) had a culture positive for *M. tuberculosis* at month 5 or later, and a second round of DST was performed; 13 (5.2%; 95% CI, 2.8%–8.7%) of these patients had isolates that showed newly-acquired resistance to at

**Table 2. Crude and adjusted ORs for characteristics associated with multidrug-resistant tuberculosis (MDR TB) at enrollment, for treatment-experienced patients with TB in Kampala, Uganda.**

| | Proportion (%) of patients | | | | |
|---|---|---|---|---|---|
| Characteristic | Non-MDR TB ($n$ = 357) | MDR TB ($n$ = 52) | Crude OR (95% CI) | Adjusted OR[a] (95% CI) | $P$ |
| Age, years | | | | | |
| 18–29 | 138/357 (39) | 29/52 (56) | 1.5 (0.7–3.2) | 2.5 (0.8–8.3) | .12 |
| 30–39 | 139/357 (39) | 12/52 (23) | 0.6 (0.3–1.5) | 0.5 (0.1–1.9) | .29 |
| ⩾40 | 80/357 (22) | 11/52 (21) | Ref | Ref | |
| Sex | | | | | |
| Male | 233/357 (65) | 27/52 (52) | Ref | Ref | |
| Female | 124/357 (35) | 25/52 (48) | 1.7 (1.0–3.1) | 1.7 (0.7–3.9) | .24 |
| Reason for retreatment | | | | | |
| Relapse | 215/357 (60) | 17/52 (33) | Ref | Ref | |
| Treatment failure | 8/357 (2) | 29/52 (56) | 45.8 (13.5–155.3) | 23.6 (7.7–72.4) | <.001 |
| Default | 134/357 (38) | 6/52 (11) | 0.6 (0.2–1.5) | 0.5 (0.2–1.5) | .20 |
| No. of previous TB episodes | | | | | |
| 1 | 303/357 (85) | 17/52 (33) | Ref | Ref | |
| 2 | 41/357 (11) | 19/52 (36) | 8.3 (3.8–17.9) | 3.4 (1.2–9.5) | .02 |
| ⩾3 | 13/357 (4) | 16/52 (31) | 21.9 (8.1–59.5) | 15.6 (5.0–49.1) | <.001 |
| BCG scar | | | | | |
| Present | 172/337 (51) | 27/50 (54) | 1.1 (0.6–2.0) | … | |
| Absent | 165/337 (49) | 23/50 (46) | Ref | … | |
| Known contact with TB | | | | | |
| Yes | 85/315 (27) | 16/49 (33) | 1.3 (0.7–2.5) | … | |
| No | 230/315 (73) | 33/49 (67) | Ref | … | |
| HIV status | | | | | |
| Infected | 173/356 (49) | 24/52 (46) | 0.9 (0.5–1.6) | … | |
| Not infected | 183/356 (51) | 28/52 (54) | Ref | … | |
| CD4 cell count,[b] cells/$\mu$L | | | | | |
| <200 | 96/144 (67) | 9/15 (60) | Ref | … | |
| 200–349 | 23/144 (16) | 2/15 (13) | 0.9 (0.2–4.6) | … | |
| ⩾350 | 25/144 (17) | 4/15 (27) | 1.7 (0.5–6.0) | … | |
| Extent of lung disease on chest radiograph[c] | | | | | |
| Advanced | 183/314 (58) | 20/27 (74) | 2.0 (0.8–5.0) | … | |
| Not advanced | 131/314 (42) | 7/27 (26) | Ref | … | |
| Cavities on chest radiograph[c] | | | | | |
| Present | 204/314 (65) | 24/27 (89) | 4.3 (1.3–14.8) | 5.9 (1.2–29.5) | .03 |
| Absent | 110/314 (35) | 3/27 (11) | Ref | Ref | |

**NOTE.** BCG, bacille Calmette-Guérin; Ref, reference.

[a] Adjusted for the characteristics for which adjusted ORs are shown.

[b] For HIV-infected patients only.

[c] For patients for whom a chest radiograph was performed.

least 1 first-line drug (table 3). All 13 isolates had a restriction fragment–length polymorphism pattern that matched that of the baseline isolate, indicating that the infecting strain had developed resistance during or after treatment. Of the 13 patients, 6 (46%) already had MDR TB and acquired resistance to additional drugs. Of 5 (38%) who developed incident MDR TB, 2 had an initial isolate susceptible to all drugs and 3 had an initial isolate resistant to isoniazid plus 2 other drugs (table 3). Of 29 patients with non-MDR TB that was resistant to isoniazid at baseline, 3 (10%) acquired resistance to rifampicin. Of the 2 patients with rifampicin-monoresistant isolates and 9 with isolates resistant to drugs other than isoniazid or rifampicin, none acquired additional resistance. The median time to detection of acquisition of resistance was 36 weeks (interquartile range, 30–51 weeks).

For the analysis of acquired drug resistance, baseline DST results were categorized into 3 groups: resistance to both isoniazid and rifampicin (MDR), resistance to either isoniazid or rifampicin but not both, and any other resistance pattern or full susceptibility. The following baseline characteristics were

**Table 3. Characteristics of previously treated patients with tuberculosis (TB) who acquired resistance to first-line drugs during the follow-up period.**

| | Relevant characteristics | | | | | | | | Drug resistance pattern | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | HIV status | No. of previous TB episodes | AFB microscopy result[a] | Time to positive culture result, days[b] | Cavitary disease on CR | Time to culture conversion, months | Fully treatment compliant | Time to acquisition, weeks | INH | RMP | PZA | EMB | STM | MDR status |
| 1 | Pos | 1 | 3 | 6 | Yes | 2 | Yes | 51 | … | A | … | … | … | … |
| 2[c] | Neg | 3 | 4 | 3 | Yes | >5 | Yes | 38 | A | A | … | A | … | Acquired |
| 3 | Neg | 1 | 4 | 1 | Yes | >5 | Yes | 24 | A | A | … | … | A | Acquired |
| 4 | Pos | 1 | 3 | 3 | Yes | >5 | Yes | 73 | R | … | … | … | A | … |
| 5 | Pos | 2 | 4 | 3[d] | Yes | >5 | Yes | 30 | R | A | … | … | R | Acquired |
| 6 | Pos | 2 | 4 | 2 | Yes | >5 | Yes | 45 | R | R | … | A | … | Initial |
| 7 | Neg | 4 | 4 | 5 | Yes | >5 | Yes | 71 | R | R | … | A | A | Initial |
| 8 | Neg | 1 | 4 | 1 | Yes | >5 | Yes | 20 | R | R | … | A | A | Initial |
| 9 | Neg | 1 | 4 | 1 | Yes | >5 | No[e] | 21 | R | R | A | R | A | Initial |
| 10 | Neg | 3 | 4 | 3 | No | >5 | Yes | 36 | R | A | R | R | … | Acquired |
| 11 | Neg | 1 | 4 | 3 | Yes | >5 | Yes | 32 | R | A | R | A | R | Acquired |
| 12 | Neg | 1 | 4 | 1 | No | >5 | Yes | 59 | R | R | R | R | A | Initial |
| 13 | Neg | 2 | 4 | 3[d] | Yes | >5 | Yes | 31 | R | R | R | R | A | Initial |

**NOTE.** A, acquired resistance; AFB, acid-fast bacilli; CR, chest radiograph; EMB, ethambutol; INH, isoniazid; MDR, multidrug resistance; Neg, negative; Pos, positive; PZA, pyrazinamide; RMP, rifampicin; STM, streptomycin; R, resistance present at baseline.

[a] The concentration of Mtb (ordinal scale, 1–4) seen on smear miscroscopy.
[b] Time to positive culture result was determined using the BACTEC 460 test system for most patients.
[c] Serial culture results show that resistance was acquired in 2 steps: resistance was first acquired to INH and EMB, then to RMP.
[d] Time to positive culture result was determined using the BACTEC MGIT 960 test.
[e] Patient reported missing a few doses during 1 month of treatment.

investigated for an association with the acquisition of drug resistance: DST pattern, extent of lung disease on chest radiograph, presence of cavities on chest radiograph, time to a positive culture result, body mass index, HIV infection status, and for HIV-infected patients, CD4 cell count and use of antiretroviral drugs; we also studied treatment compliance and time to sputum culture conversion (table 4). The risk of acquiring drug resistance was independently associated with rifampicin or isoniazid resistance at baseline (adjusted HR, 10.0; 95% CI, 2.2–44.8), MDR TB (adjusted HR, 10.2; 95% CI, 2.5–42.3), and a delay in sputum culture conversion after >5 months of treatment (adjusted HR, 25.0, 95% CI, 3.0–207.8). HIV infection, presence of cavities on chest radiograph, and having more-advanced TB on chest radiograph were not associated with the acquisition of drug resistance.

## DISCUSSION

In this study, 28% of treatment-experienced patients with TB presented with TB resistant to at least 1 first-line drug, and MDR TB was found in 12.7% of patients. Surveys in African countries performed from 1999 through 2002 demonstrated a median prevalence of resistance to at least 1 drug among previously treated patients with TB of 16.7% (range, 0%–30.3%) [23]. The median rate of MDR TB among previously treated patients with TB was 5.9% (range, 0%–13.7%). A recent national survey in Rwanda found that 9.4% of treatment-experienced patients with TB had MDR TB [24]. In a referral center in Addis Ababa, Ethiopia, the prevalence of MDR TB among previously treated patients with TB was 12% [25]. Most recently, the WHO reported that 16% of recurring cases in Senegal involve MDR TB, but this estimate was based on a small sample size [26].

We observed only 2 patients with isolates that demonstrated monoresistance to rifampicin, and thus, we conclude that rifampicin resistance is an excellent marker for MDR TB, as suggested elsewhere [25, 27]. The unavailability of second-line drugs in Uganda presumably accounts for the low levels of resistance to second-line drugs among patients with MDR TB. The considerable levels of resistance to ethionamide may be attributable to cross-resistance with isoniazid [28] or laboratory error [20]. Overall resistance to ofloxacin was low; among MDR isolates, we observed a 5.8% rate of resistance to ofloxacin. These results are encouraging because of the widespread use of fluoroquinolones in the treatment of conditions other than TB.

In agreement with previous studies, we found that MDR TB at baseline was associated with a history of treatment failure [2, 29], an increased number of previous TB episodes [30], and the presence of cavities on chest radiograph [31]. In the human lung, the selection of drug-resistance mutations in *M. tuberculosis* occurs predominantly within cavities, a site of high bacterial loads, active mycobacterial replication, reduced levels of

**Table 4. Crude and adjusted hazard ratios (HRs) for characteristics associated with amplification of drug resistance in previously treated patients with tuberculosis.**

| Characteristic | No. (%) of patients with acquired resistance ($n$ = 13) | No. (%) of patients without acquired resistance ($n$ = 237) | Crude HR[a] (95% CI) | Adjusted HR[b] (95% CI) | $P$ |
|---|---|---|---|---|---|
| Baseline drug resistance | | | | | |
| No resistance or other pattern | 3 (23) | 203 (86) | Reference | Reference | |
| Rifampicin or isoniazid resistance | 4 (31) | 27 (11) | 10.4 (2.3–46.4) | 10.0 (2.2–44.8) | .003 |
| Rifampicin and isoniazid resistance | 6 (46) | 7 (3) | 46.1 (11.5–185.0) | 10.2 (2.5–42.3) | .001 |
| Time to culture conversion, months | | | | | |
| ⩽5[c] | 1 (8) | 191 (81) | Reference | Reference | |
| >5 or never[d] | 12 (92) | 46 (19) | 42.4 (5.5–325.7) | 25.0 (3.0–207.8) | .003 |

[a] The following characteristics were also studied: age, sex, treatment compliance, extent of lung disease and presence of cavitations on baseline radiograph, baseline body mass index, baseline time to positive culture (tested using BACTEC system), HIV status, and for HIV-infected patients, CD4 cell count and antiretroviral use.
[b] Adjusted for baseline drug resistance and time to culture conversion.
[c] One hundred ten (57%) of those who experienced culture conversion by month 5 had converted by month 2 of treatment.
[d] Thirty-one patients (53%) did not provide a sputum sample at month 5 and were assumed to be culture positive at this time; all of them provided a sputum sample at a later time point and were thus included in this analysis.

anti-TB drugs, and diminished exposure to host defense mechanisms [32, 33]. Taken together, these findings suggest that the selection of resistance occurs over the course of several treatments, a process which culminates with MDR TB leading to treatment failure. However, our study design is unable to distinguish between the proposed cause-effect relationship and the possibility of reverse causality, in which treatment failure ultimately leads to the generation of MDR TB. We did not find an association between HIV and MDR TB. This supports previously reported data from East Africa [34, 35] and other developing countries [11, 30, 36] but contrasts with studies from the United States, in which HIV-associated MDR TB clusters have been linked to nosocomial transmission [37].

Our study has several limitations. Because DST is not routinely performed in Uganda, we were unable to determine whether MDR resulted from primary resistance or from resistance acquired during a previous TB episode. Our eligibility criteria may have excluded a disproportionate number of HIV-infected patients who are more likely to have TB with negative acid-fast bacilli smear results [38]. However, of the 138 patients with negative smear results that we excluded, only 9 (7%) had positive culture results. Also, our study population may not be representative of all recurring cases in Uganda, because of selection and referral biases. A national survey is required to provide a more accurate picture of drug resistance in Uganda.

In this cohort of previously treated patients with TB, ~5% of patients developed drug resistance during or after therapy despite compliance to treatment; one-third of these patients developed incident MDR TB. The risk of acquired resistance was highest among patients who presented with existing drug resistance and among those whose sputum was slow to sterilize during treatment. Both of these factors have been shown to predict relapse [39] but have not been previously associated with an increased risk of acquired drug resistance. Although the proportion of patients that acquired resistance in this study may appear to be low, our report only reflects a single cycle of treatment in a heavily treated cohort of patients (22% had received ⩾2 treatment cycles before enrollment), and thus, our results may underestimate the degree of drug-resistance amplification. In addition, extrapolating our results to the rest of Uganda, where 10%–22% of the 41,000 notified cases in 2005 [14] were cases of recurring TB, indicates that 80–170 new MDR TB cases are generated each year—a considerable public health problem. Our results and those reported elsewhere [40] suggest that individuals who were previously treated for TB may be acting as a persistent and growing reservoir of drug resistance and as a source of contagion to the general population. This study suggests that the continued almost-exclusive focus of TB control programs on new TB cases is no longer judicious and supports the recent WHO recommendation for national programs to urgently address the growing problem of drug resistance [5].

Our analysis of the acquisition of drug resistance has limitations. Mixed infections were not excluded [41]; however, it is generally agreed that mixed infections are rare [42]. Our analysis of acquired drug resistance was limited to patients with a suboptimal clinical or microbiological response to treatment. The development of drug resistance in other patients would have gone unnoticed. Finally, the small number of outcomes observed resulted in broad 95% CIs.

The burden of drug resistance among previously treated patients with TB in Uganda is large and growing. With currently recommended treatment regimens, the risk of generating further drug resistance is significant. There is an urgent need to focus more attention on previously treated patients with TB in low-income countries.

## Acknowledgments

We thank Grace Nyakoojo, the study home health visitor; the Mulago tuberculosis ward nurses; Annette Mugenyi, project administrator; and Nancy Reilly, Karen Morgan, Kevin Fennelly, Robert Wallis, Leigh Anne Shafer, Francis Adatu-Engwau, Jimmy Whitworth, and Heiner Grosskurth, for their expert advice and support.

***Financial support.*** The Wellcome Trust (063410/ABC/00/Z).

***Potential conflicts of interest.*** All authors: no conflicts.

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