

TUBERCULOSIS MORTALITY, DRUG RESISTANCE, AND INFECTIOUSNESS IN PATIENTS WITH AND WITHOUT HIV INFECTION IN PERU

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Abstract. The effects of HIV co-infection and multi-drug resistant tuberculosis (MDRTB) on tuberculosis prognosis are poorly defined. Therefore, we studied infectiousness and mortality of 287 tuberculosis patients treated with standard, directly observed, short-course therapy in the Peruvian community. During 6–17 months of treatment, 49 (18%) of patients died, of whom 48 (98%) had AIDS and 28 (57%) had MDRTB; 17/31 (55%) of MDRTB-patients with AIDS died within 2 months of diagnosis, before traditional susceptibility testing would have identified their MDRTB. Most non-MDRTB became smear- and culture-negative within 6 weeks of therapy, whereas most MDRTB remained sputum-culture-positive until death or treatment completion. HIV-negative patients with non-MDRTB had good outcomes. However, MDRTB was associated with prolonged infectiousness and HIV co-infection with early mortality, indicating a need for greater access to anti-retroviral therapy. Furthermore, early and rapid tuberculosis drug-susceptibility testing and infection control are required so that MDRTB can be appropriately treated early enough to reduce mortality and transmission.

INTRODUCTION

Tuberculosis kills 1.7 million people each year.¹ Modern treatment regimens are generally associated with a good prognosis,² but HIV co-infection³ and multi-drug resistant tuberculosis (MDRTB)⁴ have poorly defined effects upon treatment outcome⁵ and infectiousness.⁶ Peru has a model tuberculosis control program² and an HIV prevalence < 0.5% but is one of the 30 highest tuberculosis-burden countries, with a tuberculosis incidence of 188/100,000 in the year 2003.² MDRTB rates have recently exceeded 3% of all new cases and 9% of infectious sputum microscopy-positive patients, making Peru one of the eight highest MDRTB-burdened countries.^{4,7,8} As in most resource-poor settings, tuberculosis drug-susceptibility testing is restricted to high-risk groups, and facilities for respiratory isolation are sparse.⁷ In this setting, a prospective cohort study was performed to study the effects of HIV and MDRTB on tuberculosis infectiousness and mortality during tuberculosis therapy.

PATIENTS

All adults who were diagnosed consecutively by the Peruvian National Tuberculosis Control Program as having laboratory-proven pulmonary tuberculosis were invited to join the study over a 17-month period at two diagnostic centers. Informed written consent was given by 287 patients (Table 1): 223 (78%) patients were diagnosed at the Maria Auxiliadora general hospital outpatient clinic, where TB was diagnosed principally by sputum microscopy; and 64 (22%) patients were diagnosed concurrently at the infectious diseases unit at Dos de Mayo Hospital, the principal referral center for

people with HIV infection in Lima, which serves an area including that of Maria Auxiliadora Hospital. National policy recommends a 12-month course of isoniazid preventive therapy for all HIV-seropositive people, and we considered this to have been taken if ≥ 3 months had been completed. Extrapulmonary tuberculosis was not studied. This project had national and international ethical approval, including A.B. Prisma, Peru, and Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

METHODS

Investigations. For initial tuberculosis diagnosis, the Ministry of Health clinical laboratory performed Ziehl-Neelsen sputum microscopy and, for patients known to be HIV-positive, sputum culture on modified Ogawa medium with drug-susceptibility testing by the proportions method.

For this study, two additional sputum samples were subjected to auramine microscopy^{9,10} and culture with both the 7H9 broth microscopic observation drug-susceptibility (MODS) assay^{11,12} and Lowenstein-Jensen slopes¹⁰ in the university research laboratory, and these results, that confirmed tuberculosis in all cases, were used for our analysis. MDRTB was defined as resistance to at least isoniazid (0.4 mg/mL) and rifampicin (1.0 mg/mL) in MODS and the microplate colorimetric assay.¹³ Chest radiographs were not studied.

At the general hospital, ELISA HIV antibody testing was done after counseling for 176 (79%) patients and was positive in 8 (5%) cases. The remaining 47 (21%) patients at the general hospital stated that they had no behavioral risk factors for HIV infection and did not have HIV tests. Because HIV is relatively uncommon in Peru, infecting 0.5% of the population and 1.7% of tuberculosis patients,⁷ these 47 patients were considered HIV-seronegative for all analyses. All 64 patients at the infectious diseases unit were HIV-seropositive by ELISA with confirmatory Western blot assay, but HIV clinical staging data, CD4 counts, and viral load measurements were not available for this study.

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TABLE 1
Clinical characteristics of the study population at diagnosis according to clinic

Feature	All participants (n = 287)	General clinic (n = 223)	ID unit (n = 64)	P value comparing hospitals
HIV seropositivity	72 (25)	8 (5)	64 (100)	< 0.001
MDRTB at time of diagnosis	51 (18)	21 (9)	30 (47)	< 0.001
Outpatient at time of diagnosis	257 (90)	223 (100)	34 (53)	< 0.001
Sputum smear positivity	274 (96)	223 (100)	51 (80)	< 0.001
Previous TB treatment	60 (21)	37 (17)	23 (36)	0.001
Known TB exposure at home/work	103 (36)	83 (37)	20 (31)	ns
Previous TB preventive therapy	18 (6)	3 (1)	15 (23)	< 0.001
Hospitalization in last 2 years	45 (16)	23 (10)	22 (34)	< 0.001
Male sex	182 (63)	130 (58)	52 (81)	0.001
BCG scar(s)	257 (90)	199 (89)	58 (91)	ns
Age in years: mean (SD)	29 (11)	28 (11)	31 (8)	ns

Note: The number (percentage) of patients with relevant clinical characteristics are shown for the whole study population and divided according to the hospital at which tuberculosis was diagnosed. Tuberculosis was treated within the same community health posts.

Abbreviations: HIV, human immunodeficiency virus; MDRTB, multi-drug resistant tuberculosis; TB, tuberculosis; BCG, Bacillus Calmette-Guerin; SD, standard deviation; ID, infectious diseases; ns, no significant difference.

Treatment. All patients received the standard Peruvian treatment regimen with direct observation of tablet consumption at local health posts.² Treatment was therefore unaffected by recruitment center. This study was not involved in patient care or treatment, but all laboratory results were rapidly communicated to patients and their clinicians, including the results of first-line drug susceptibilities. In line with Peruvian national policy, all patients received a complete trial of first-line tuberculosis therapy. Most patients were treated with 2 months of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) for 6 days/week, followed by 4 months of H and R for 2 days/week (2HRZE₆/4HR₂). Patients known to be co-infected with HIV and tuberculosis received 2HRZE₆/7HR₂. If tuberculosis had been treated previously, then streptomycin (S) was added: 1SHRZE₆/2HRZE₆/5HRE₂. Only 12 patients received second-line drugs for a short term, due to cost and availability and these were administered in addition to the standard treatment regimen: ciprofloxacin alone in 5 cases, with ethionamide ± kanamycin and amoxicillin-clavulanate in 4 cases, and other regimens in 3 cases.

Follow-up. Recruitment took place from February 1999 to July 2000, and follow-up continued for each patient until completion of therapy (April 2001). After diagnosis and recruitment to the study, medical care and treatment were identical for all patients in the same community DOTS program, irrespective of the place of recruitment. Tuberculosis treatment adherence and survival at least to treatment completion were determined for 224 (78%) participants, and 217/224 (97%) completed tuberculosis treatment. Survival analysis was based upon the interval from tuberculosis treatment initiation until the first occurring date of: treatment completion, death; default from therapy; loss to follow-up; or end of study.

Infectiousness during follow-up was analyzed for both tuberculosis microscopy and culture as the time from initiation of therapy until the mid-point between the last positive result and the first of 2 consecutive negative sputum specimens, or the last positive result if there was no conversion.¹⁴ All participants provided diagnostic sputum samples, and we collected additional samples 2 weeks later from 108 (38%); after 1 month of treatment from 259 (90%); after 2 months treatment from 249 (86%); after 4 months treatment from 229 (78%); and later in treatment from 166 (54%).

Statistical analysis. Person-years of observation were calculated by recording the total number of surveillance days for

each participant. For individuals lost to follow-up, person-time denominator data were censored at the time of the last visit. Continuous variables were compared using the Student's *t* test or the Mann-Whitney *U* test. Categorical variables were compared using the χ^2 test or Fisher's exact test. Kaplan-Meier survival analysis was performed to evaluate time to conversion and time to death, and Cox proportional hazards regression analysis was performed to evaluate risk factors associated with conversion and mortality. The magnitude of associations of potential risk factors was expressed as hazard ratios (HR). Risk factors with *P* < 0.15 in univariate analyses were included in multiple regression analysis. All *P* values were two-sided. All statistical analyses were carried out using SPSS, version 13.0 (SPSS Corp., Chicago, IL).

RESULTS

Survival. The survival curves shown in Figure 1 demonstrate the effects of HIV and MDRTB on mortality: 49 (17%) of patients who were followed-up, died; 48 (67%) of 72 HIV-infected patients died compared with 1 (0.5%) of 215 HIV-negative patients (*P* < 0.0001), who died of hepatitis. MDRTB was diagnosed in 18% of all patients and in 71% of the patients who died.

The major predictor of mortality was HIV infection and there were also significant associations with: MDRTB; previous tuberculosis treatment; and lack of known exposure to a tuberculosis case at home or work (implying a greater probability of tuberculosis transmission outside of the home, including nosocomial transmission) (Table 2). HIV and MDRTB co-infection caused 55% mortality 2 months after diagnosis, and only 16% survived to complete 9 months of therapy. Considering each center separately, 45/64 (70%) of the patients diagnosed at the infectious diseases unit died during follow-up. In contrast, 4/223 (1.8%) of patients recruited at the general clinic died, 3 of whom were HIV-positive. However, in multiple regression analysis, the site of patient recruitment was not independently associated with mortality. This difference in mortality between hospitals was explained by HIV and MDR prevalence: prognosis was similarly poor for HIV-positive tuberculosis patients recruited from either hospital (Table 2).

Infectiousness. Durations of sputum smear and culture positivity are shown in Figure 2. Most patients whose sputum initially grew MDRTB remained smear- and culture-positive

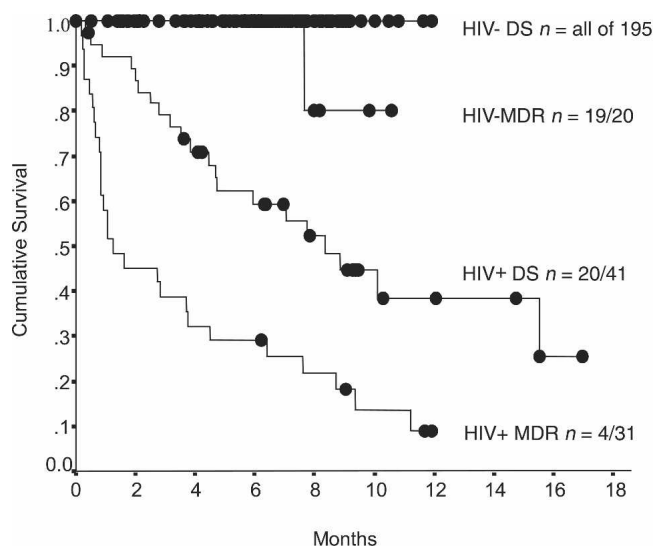


FIGURE 1. Kaplan-Meier analysis of mortality following commencement of tuberculosis therapy. The study population is divided according to HIV serology (HIV negative or positive) and tuberculosis drug resistance at the time of enrollment (MDR, strains resistant to isoniazid and rifampicin; DS, drug-susceptible, non-MDR strains); *n*, number of patients alive at the end of follow-up/the number of patients recruited to that diagnostic group; ●, patient whose follow-up ended at that time because of completion of or default from follow-up.

throughout follow-up. In contrast, most non-MDRTB became smear- and culture-negative in the first 6 weeks of therapy. Cox regression analysis revealed MDRTB to be the main risk factor for prolonged positive sputum smear (HR = 3.6, 95% CI = 1.9–6.6, $P < 0.001$) and culture (HR = 3.8, 95% CI = 2.1–6.9, $P < 0.0001$). As patient age increased, so did the duration of positive sputum smear ($P = 0.04$) and culture ($P = 0.05$). Although HIV co-infection had borderline associations with prolonged positive sputum smear and culture in univariate analysis, neither approached statistical significance in multiple regression analysis that included the effect of MDRTB. Mortality was also associated with prolonged positive sputum smear (HR = 14, 95% CI = 6.7–27, $P < 0.001$) and culture (HR = 8, 95% CI = 3.9–15, $P < 0.001$).

MDRTB. Fifty-one (18%) of 287 patients had MDRTB, and 31/51 (61%) of them were HIV-positive. Of all 72 HIV-

positive patients, 31 (43%) had MDRTB. In addition to the drug-susceptibility data from our university laboratory, susceptibilities were also available from the Ministry of Health clinical laboratory for 32 (50%) of patients at the infectious diseases unit, and there was complete concordance in all except 3 cases. Twelve (30%) of the 40 MDRTB patients became culture-negative on treatment, despite 6 of them receiving only standard first-line tuberculosis therapy and 8 of them being HIV-negative.

Risk factors for MDRTB. MDRTB was associated in multiple regression (all $P \leq 0.01$) with previous tuberculosis treatment (HR = 2.8, 95% CI = 1.3–5.9), previous tuberculosis preventive therapy (HR = 7.9, 95% CI = 2.3–27), and current tuberculosis diagnosis made at the infectious diseases unit (HR = 4.9, 95% CI = 2.4–10). To evaluate the independent effect of recruitment center, data were also analyzed separately according to recruitment clinic. The general hospital had 21 (9%) of the MDRTB patients, but none of their recorded characteristics was significantly associated with drug resistance (all $P > 0.1$). At the infectious diseases unit, where all patients were HIV-positive, 30 (47%) of them had MDRTB. Multiple regression analysis revealed that MDRTB was associated with previous tuberculosis preventive therapy (HR = 16, 95% CI = 2.8–85, $P = 0.002$) and recent hospitalization (HR = 6.8, 95% CI = 1.9–24, $P = 0.003$).

DISCUSSION

This study demonstrates the profound impact of HIV infection and multi-drug resistance on survival and infectiousness in tuberculosis patients. Survival throughout treatment was almost universal in HIV-negative individuals, irrespective of drug susceptibilities. However, most HIV-positive patients co-infected with tuberculosis died during therapy, and most of those co-infected with MDRTB died in the first 2 months of treatment. Conventional drug-susceptibility testing used throughout the less-developed world would have identified MDRTB before death in only a minority of these cases, whereas inexpensive, recently reported techniques could have more rapidly provided this information and allowed appropriate therapy to be instituted.^{7,12,15} Most patients with MDRTB remained infectious throughout therapy, which is a concern because facilities for respiratory isolation of inpatients or outpatients are limited in less-developed countries.

TABLE 2
Risk factor analysis for mortality

Feature	Kaplan-Meier		Cox regression		
	Log-rank test	<i>P</i> value	Hazard ratio	95% CI	<i>P</i> value
HIV seropositivity	130	< 0.001	99	13–730	< 0.001
MDRTB at time of diagnosis	65	< 0.001	2.5	1.4–4.5	0.002
Previous TB treatment	16	0.001	2.1	1.1–3.7	0.02
Known exposure to TB at home/work	4.5	0.03	0.5	0.3–1.0	0.05
Diagnosed in the infectious diseases unit	118	< 0.001			ns
Previous TB preventive therapy	29	< 0.001			ns
Hospitalization in last 2 years	26	< 0.001			ns
Male sex	4.9	0.03			ns
BCG scar(s)	0.14	ns	–	–	–
Age in years	–	–			ns

Note: Relative risk of mortality associated with each patient's initial clinical characteristics is shown for Kaplan-Meier survival analysis and in a Cox regression model, together with 95% confidence intervals.

Abbreviations: HIV, human immunodeficiency virus; MDRTB, multi-drug resistant tuberculosis; TB, tuberculosis; BCG, Bacillus Calmette-Guérin; SD, standard deviation; ID, infectious diseases; CI, confidence interval; ns, no significant difference; "–" signifies that analysis of that variable was not appropriate (see methods).

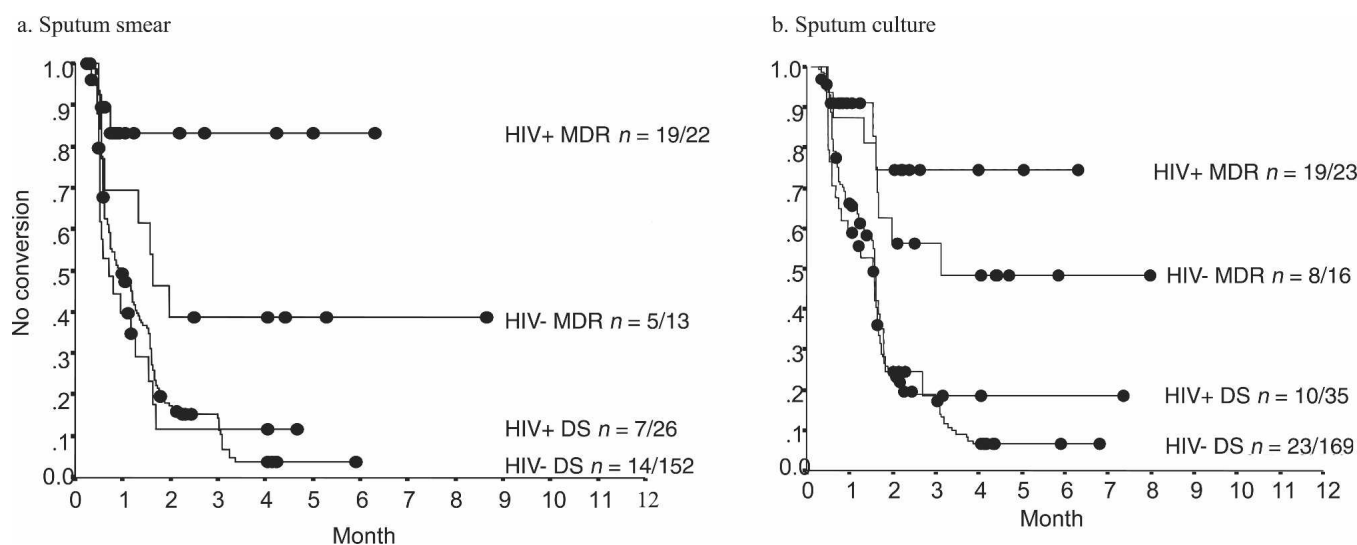


FIGURE 2. Kaplan-Meier analysis of infectiousness following commencement of TB therapy. Infectiousness is assessed as sputum smear positivity in **a** and as sputum culture positivity in **b**. The study population is divided according to HIV serology (HIV negative or positive) and tuberculosis drug resistance (“MDR” refers to strains resistant to isoniazid and rifampicin; “DS” refers to all other strains) at the time of enrollment; *n*, number of patients known to be sputum-smear- or culture-negative by the end of follow-up/the number of patients recruited to that diagnostic group; “•” signifies end of follow-up because of completion of or default from the study.

Mortality. Two-thirds of the HIV-positive tuberculosis patients died during follow-up, which is a rate that is greater than in most reports, even after allowing for differences in follow-up^{3,16} and MDRTB.^{17,18} However, there are reports of similarly high mortality,¹⁹ and Peruvian mortality during treatment of first episodes of AIDS-associated tuberculosis approached this level, falling from 63% to 39% from 1996 to 1999.^{20,21} The cause of death in these patients could not be confirmed to be AIDS-associated tuberculosis in these published studies nor in the present research because autopsies were not performed; it is therefore possible that other AIDS-related diseases contributed to these high mortality rates. Delayed diagnosis,^{22,23} minimal access to anti-retroviral drugs,²⁴ and co-existent infections¹⁷ may also have contributed. However, the median survival time of these patients at the infectious diseases unit was 61 months from the time of HIV diagnosis but < 6 months from the diagnosis of tuberculosis co-infection, indicating the major risk associated with a tuberculosis diagnosis in HIV-positive people in this setting.

In contrast, there was minimal mortality in HIV-negative patients, including those with MDRTB, reflecting the renowned Peruvian tuberculosis program. For > 10 years, this program has provided > 99% of the Peruvian population all five components of the World Health Organization DOTS policy: government commitment to sustained TB control; bacteriological diagnosis; standardized short-course chemotherapy regimens under close supervision; a proper drug supply system; and recording/reporting of cases and results.^{2,5,18}

It is noteworthy that most of the HIV-positive and HIV-negative participants were outpatients at the time that tuberculosis was diagnosed, and only a minority had been hospitalized in the past 2 years (Table 1), implying that tuberculosis was not acquired during hospitalization for other terminal diseases.

Infectiousness. Although HIV status was the principle determinant of survival, MDRTB was the main determinant of infectiousness. Of those with MDRTB, approximately half of

the HIV-negative and almost all of the HIV-positive patients remained sputum smear- and/or culture-positive throughout treatment. Follow-up sputum culture data are rarely available in less-developed countries, although most tuberculosis occurs there. It is significant that sputum cultures almost exactly mirrored sputum smears, implying that positive sputum smears did not generally result from mycobacteria that had been killed by treatment. When sensitive broth culture is used, sputum smear-positivity usually indicates culture-positivity.²⁵ This implies that sputum smears are reliable indicators of the duration of infectiousness.

HIV co-infection did not significantly influence the duration of infectiousness during treatment.¹⁴ This contrasts with countries with very little MDRTB, where prolonged culture-positivity is infrequent.⁴ Peru has high tuberculosis and MDRTB prevalences.^{2,7,26–30} In this setting, conventional treatment of MDRTB for immunocompetent people was associated with good survival but frequently maintained infectiousness. Although we did not study secondary tuberculosis in contacts or radiographic data to assess disease severity, it is well established that sputum smear and culture positivity are risk factors for tuberculosis and MDRTB dissemination.⁶ As a result of this research, the infectious diseases unit is now providing same-day sputum tuberculosis microscopy for HIV-positive patients with respiratory symptoms, rapid initiation of therapy after diagnosis, respiratory isolation of smear-positive patients, and increased use of personal respirator masks.

MDRTB rates. The MDRTB rates found for HIV co-infected patients in the present study were similar to recent Lima-based research,³⁰ but MDRTB rates were higher than nationally,²⁰ even in the general clinic where we recruited unselected, predominantly HIV-negative residents. However, in much of the less-developed world, including Peru, drug-susceptibility testing is restricted to high-risk groups, including HIV co-infection and those who have failed several months of first-line tuberculosis therapy. Our results imply

that many patients with MDRTB and unrecognized HIV co-infection die before they meet these criteria, so MDRTB may be under-diagnosed.

In this study, patients with initially unrecognized MDRTB completed a trial of first-line therapy despite MDRTB being diagnosed during first-line therapy. These MDRTB results were provided routinely by the Ministry of Health for all new tuberculosis diagnoses in HIV-positive and some other patients and by our research for all patients. This initial trial of first-line therapy is explained by the observations that (1) first-line therapy includes at least two drugs to which most MDRTB is susceptible and consequently much MDRTB appears to be cured by first-line therapy^{5,31–33}; (2) second-line MDRTB therapy is expensive and toxic³⁴; (3) failure of first-line therapy reliably predicts MDRTB³⁵; and (4) conventional drug-susceptibility testing identifies MDRTB too late to influence the first months of therapy.¹⁰ Hence, the patients with MDRTB in this study first had to survive failing 6–9 months of first-line therapy before receiving specific MDRTB therapy. This was associated with mortality and prolonged MDRTB infectiousness, in contrast to the excellent prognoses for MDRTB treated with specific therapy in Peru.^{34,36} The present results imply that rapid, early MDRTB testing is a priority and that, when MDRTB is diagnosed, appropriate therapy should be instituted urgently, without awaiting completion of a trial of second-line therapy. Second-line antibiotics for MDRTB and highly active anti-retroviral therapy have both become much more widely available in Peru,³⁴ and rapid MDRTB tests,¹⁵ including the MODS assay that we used,^{11,12} have become approved components of the Peruvian National Tuberculosis Control policy.³⁷

Recently, a community-based MDRTB treatment program achieved a cure rate of 73% among 75 patients.³⁴ Although this represents a breakthrough in MDRTB care, antibiotic costs were US dollars (USD) 15,681 per patient, a price beyond the reach of most programs. Also, this selected patient group had survived a median of 4 years of intermittent therapy before this therapy, demonstrating chronic disease. Similarly, a standardized regimen of MDRTB treatment administered by the Peruvian tuberculosis program cured 48% of MDRTB patients.³⁶ Together, these results indicate potential to prevent mortality where resources exist for rapid testing and specific MDRTB therapy.

MDRTB is associated with previous tuberculosis treatment,^{4,30} and we also found an association with previous isoniazid preventive therapy, unlike other studies.^{30,38–40} This could be explained by a covariate, such as duration of AIDS, increasing the likelihood of both preventive therapy and nosocomial MDRTB exposure. Alternatively, isoniazid preventive monotherapy could have increased the risk of subsequent isoniazid and rifampicin resistance. A recent study of the risk factors for MDRTB for HIV-positive and negative patients in Lima³⁰ and our present study both demonstrated associations between MDRTB and past tuberculosis treatment, hospitalizations, and care in centers for people with HIV. This recent case-control study was not designed to test for HIV effects on MDRTB risk, and therefore it is important to note that in the present study HIV co-infection was not independently associated with MDRTB risk.

Reliability. Studies of this kind can be confounded by laboratory cross-contamination,⁴¹ although care was taken to prevent this, including using closed culture wells.¹¹ Evidence that

sputum results diagnosed disease rather than contamination was provided by (1) association with mortality; (2) agreement between drug-susceptibility testing in separately collected samples processed in different laboratories; and (3) concordance between independent microscopy and culture in our laboratory.

Implications. Most patients with MDRTB remained infectious throughout suboptimal therapy designed for drug-susceptible tuberculosis. Greater use of drug-susceptibility testing would allow appropriate therapy, when available, to improve prognosis^{34,42} and potentially reduce MDRTB transmission. Early MDRTB diagnosis may also prevent therapy with inappropriate regimes from causing MDRTB strains to develop additional resistance.⁴³ Rapid culture and drug-susceptibility testing would be required for appropriate therapy to be instituted before most mortality occurred. Techniques suitable for less-developed countries, such as the MODS assay that we used, can provide drug susceptibilities in 5–14 days for USD 1–2 per sample,^{11,12} early and inexpensive enough to potentially reduce mortality. Another priority is improved infection control to reduce nosocomial transmission. Anti-retroviral drugs may considerably improve²⁴ the grave prognoses that we identified for patients with tuberculosis and HIV co-infection, and availability has recently increased significantly in Peru.

CONCLUSION

This research demonstrated excellent prognoses for HIV-negative individuals and rapid resolution of infectiousness for patients with non-MDRTB. However, MDRTB patients had prolonged infectiousness and, with HIV co-infection, more than 50% mortality in the first 2 months of therapy. This study therefore demonstrates the importance of early and rapid drug-susceptibility testing, improved infection control measures, and early anti-retroviral therapy so that patients with MDRTB and HIV can receive appropriate therapy early enough to reduce their mortality and potential to infect others.

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Conflict of Interest: All authors declare that they have no conflict of interest in relation to this work. Dr. Eduardo Ticona was previously head of the Peruvian National Tuberculosis Control Program.

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