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Clinical Predictors of Drug Resistance and Mortality Among Tuberculosis  
Patients in a Rural South African Hospital: A Case-Control Study

A Thesis Submitted to the  
Yale University School of Medicine  
in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Medicine

by

Jason Randolph Andrews

2007

## ABSTRACT

### CLINICAL PREDICTORS OF DRUG RESISTANCE AND MORTALITY AMONG TUBERCULOSIS PATIENTS IN A RURAL SOUTH AFRICAN HOSPITAL: A CASE-CONTROL STUDY

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The recent discovery of a high prevalence of multidrug-resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) in rural South Africa, where HIV is rampant, has provoked alarms about the future of tuberculosis control in the region. Little is known about the clinical manifestations of MDR-TB in general, and XDR-TB in particular, in the high HIV prevalence settings of Sub-Saharan Africa.

We performed a retrospective, case-control study of patients diagnosed with tuberculosis at a rural hospital in KwaZulu Natal, South Africa, where large numbers of MDR-TB and XDR-TB cases have been identified. All MDR-TB and XDR-TB patients who began treatment for TB between June 1, 2005 and August 31, 2006 and whose charts were available were included in the study. A comparison group of patients without resistance to both isoniazid and rifampicin (non-MDR-TB), matched 1:1 with the size of the MDR-TB and XDR-TB groups, was created. Clinical and laboratory data were obtained through review of hospital records, clinic registers, and the laboratory system. We compared clinical characteristics to identify risk factors for MDR-TB, XDR-TB, and mortality. Bivariate and multivariate analyses were performed.

A total of 170 patients were enrolled in the study: 52 MDR-TB, 61 XDR-TB and 57 non-MDR-TB. Greater than 75% of patients from all groups were tested for HIV; HIV prevalence among those tested was 94% in the non-MDR group, 93% in the MDR group, and 100% in the XDR-TB group ( $P=1.000$  for MDR versus non-MDR;  $p=0.089$  for XDR versus non-MDR). Forty percent of MDR-TB patients and 57% of XDR-TB patients had no previous history of TB treatment, strongly suggesting transmitted drug resistance.

Significant associations and risk factors for MDR-TB and XDR-TB in bivariate analysis included positive sputum smear ( $P=0.015$ ,  $P=0.005$ ), TB treatment in the past year ( $P<0.0001$ ,  $P<0.001$ ), and hospitalization in the past two years ( $P=0.007$ ,  $P=0.004$ ). In multivariate logistic regression, positive sputum smear remained a significant risk factor for XDR-TB (adjusted odds ratio (AOR) 2.79, 1.20-6.47), and TB treatment in the past year remained a risk factor for both MDR-TB and XDR-TB (AOR 8.33, 95% CI 1.64-42.33; AOR 7.19, 95% CI 1.35-38.17).

Mortality for the non-MDR, MDR and XDR groups was 36.8%, 73.1% and 85.3%, respectively ( $P=0.0001$  for MDR versus non MDR;  $P<0.0001$  for XDR versus non-MDR;  $P=0.109$  for XDR versus MDR); median survival from TB diagnosis was 199 days, 103 days, and 92 days, respectively ( $P<0.001$ ). In Cox Proportional Hazards model, positive sputum smear ( $P=0.003$ ), MDR-TB ( $P=0.028$ ), XDR-TB ( $P=0.002$ ), and CD4 cell count less than 200 cells/mm<sup>3</sup> ( $P=0.037$ ) were significant risk factors for mortality.

Forty of the 170 patients had sputum isolates with differing resistance patterns, and 18 moved from a lower to a higher resistance category; this increasing drug resistance appeared to be more likely the result of super-infection than amplification.

A significant proportion of MDR-TB and all XDR-TB appear to be due to primary resistance, with nosocomial transmission playing a critical role. MDR-TB and XDR-TB carry extraordinarily high mortality rates in this setting; previous hospitalization, previous TB treatment, positive sputum smear and low CD4 count may be used to target drug susceptibility testing for patients at high risk of drug resistant TB and mortality.

## ACKNOWLEDGEMENTS

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This work was for the patients at Church of Scotland Hospital, Tugela Ferry, South Africa.

How should they have given a thought to anything like plague, which rules out any future, cancels journeys, silences the exchange of views. They fancied themselves free, and no one will ever be free so long as there are pestilences. – Albert Camus

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## COMMON ABBREVIATIONS

COSH	Church of Scotland Hospital
CPX	Ciprofloxacin
DOTS	Directly Observed Therapy Short-course
DST	Drug Susceptibility Testing
EMB	Ethambutol
GLC	Green Light Committee
INH	Isoniazid
KM	Kanamycin
KZN	KwaZulu Natal
MDR-TB	Multidrug-Resistant Tuberculosis
RIF	Rifampicin
SCC	Short-course Chemotherapy
SLD	Second-line Drugs
SM	Streptomycin
XDR-TB	Extensively Multidrug-Resistant Tuberculosis

## **INTRODUCTION**

### **Tuberculosis and South Africa**

Despite the availability of effective diagnostic, preventive, and curative technologies, tuberculosis (TB) remains the number one cause of adult deaths by a curable infectious disease worldwide (1). In 2004, the last year for which global TB estimates are available, there were an estimated 9 million new cases and 14.6 million prevalent cases of tuberculosis, resulting in 3-4 million deaths (2). Control of the disease has been highly uneven across various parts of the world. While incidence has declined dramatically since the early 19<sup>th</sup> century in the industrialized world, worldwide incidence has been slowly increasing in recent years, largely as a result of dramatic rises in the former Soviet Union and Africa (2, 3).

The developing world now bears 95% of all cases of TB, of which 70% occur on two continents: Asia and Africa.(2). Countries of Sub-Saharan Africa have witnessed the worst trends in recent years. Poor health infrastructure and a scarcity of human and financial resources have combined with soaring HIV rates to effect a dramatic rise in TB incidence in this region (4-6). Eight of the top ten countries by TB incidence are in Sub-Saharan Africa, and the average incidence among these countries is 4 times the global rate and 140 times that of the United States (2). Mortality from tuberculosis is likewise disproportionately high in the developing world, in general, and in Sub-Saharan Africa, in particular. 98% of all deaths from TB occur in developing countries. Africa has the highest mortality rates and lowest treatment success rates of any region in the world (2).

Within Africa, South Africa has the second highest TB incidence and the highest number of reported TB cases. Approximately 339,000 cases of tuberculosis occur every

year, resulting in an estimated 64,000 deaths (2). Sixty percent of all incident cases occur in people infected with HIV. As TB incidence and prevalence continue to rise, DOTS case detection rate has actually been falling in recent years, making evident the struggle that the public health infrastructure is facing in meeting the demands of the growing disease burden. The DOTS treatment success rate was 67% nationwide in 2003, well below the WHO's standard of 85% for DOTS programs, and in some areas is substantially lower.

### **Review of Tuberculosis Drug Resistance**

Since the introduction of the first effective anti-TB drug, streptomycin, in the late 1940's, resistance of *Mycobacterium tuberculosis* to chemotherapeutic agents has been understood as a major problem in the management of TB disease. Clinical relapse after three to six months of improvement was observed in the earliest studies of streptomycin (7, 8). Randomized controlled trials carried out by British researchers upon the introduction of PAS in 1948 found that patients receiving combined therapy (PAS and SM) had lower rates of relapse than those receiving either drug alone (9, 10). *M. tuberculosis* has been able to acquire resistance to every effective chemotherapeutic agent used against it.

Tuberculosis drug resistance can be either primary (transmission of resistant organisms) or secondary (resistance acquired in the host related to inadequate treatment). There are four broad categories of mechanisms of acquired resistance to drugs by *M. tuberculosis*: 1) the creation of a lipid-rich cell wall that can reduce the permeability of drugs (and arrest phagosome maturation); 2) the production of enzymes that degrade or modify compounds, rendering them useless; 3) the efflux of drugs through protein



pumps, described for isoniazid and ethambutol; and 4) spontaneous chromosomal mutations that affect key drug targets (11-14). Among these, the fourth mechanism is considered to be the most important. Mobile or horizontal transmission of resistance, such as plasmid mediated resistance, does not occur in *M. tuberculosis*. Random genetic mutations occur with low but predictable frequencies in the range of one mutation per  $10^6$  to  $10^9$  organisms. The frequency of mutations conferring resistance to particular agents varies from the range of  $10^{-3}$  for many second line drugs (thiacetazone, ethionamide, capreomycin, cycloserine, and viomycin) to an intermediate level (around  $10^{-6}$ ) for some first and second line drugs (isoniazid, streptomycin, ethambutol, kanamycin, and p-amino salicylic acid) to the lowest levels for rifampicin, on the order of  $10^{-8}$  to  $10^{-10}$ . When large populations of *M. tuberculosis* are formed in a host and selective pressure is placed by a chemotherapeutic agent, the small population of *M. tuberculosis* that has evolved resistance to the agent will continue to multiply while the susceptible *M. tuberculosis* is suppressed. This enables the drug resistant organism to become the dominant organism in the host.

In order to prevent this scenario from occurring, the central strategies in therapy are to: 1) administer several chemotherapeutic agents, such that if there are organisms resistant to one or two agents, they will be killed by the other agents; 2) provide therapy for an adequate duration in order to ensure eradication of populations of *M. tuberculosis*, which evades both host immune response and drug actions by a number of intricate cellular mechanisms (14). Because the probability of two simultaneous mutations—the product of the individual probabilities of mutations—is small ( $10^{-11}$  to  $10^{-14}$ ) compared with typical bacillary loads (up to  $10^9$  in a pulmonary cavity), the sustained presence of

two or more effective drugs should eradicate the entire population of bacilli (this traditional model, while useful, is an oversimplification due to the formation of microenvironments with differing drug concentrations and activities) (14). The time period of acquired resistance under monotherapy varies between agents and has been well characterized for many of the initial anti-TB agents; in a 1952 study of isoniazid monotherapy, 11%, 52% and 71% of patients developed resistant strains after one, two and three months, respectively (15).

Not surprisingly, the most common ways in which *M. tuberculosis* drug resistance evolves or amplifies in the host involve the violation of these principles. The causes of these violations range widely, from the actions of the individuals, by nonadherence, to those of the health provider, by improper regimen selection or suboptimal dosing, to the failure of TB control programs to provide a consistent supply of necessary agents (11). Understanding of these causes has evolved considerably over the past two decades, trending towards increasing recognition of the impact of the social, economic and political environments in which therapy takes place upon the likelihood that patients will be exposed to the proper treatment for an adequate duration (16, 17).

Numerous host factors have been implicated in the facilitation of acquired drug resistance, including the development of local tissue microenvironments recalcitrant to antibiotic penetration or activity and the failure of the immune system to act in synergy with antibiotic activity (14). Compromise of the host immune response, such as that caused by infection with HIV, may be a significant risk factor for the evolution of drug resistance. This is discussed in more detail below.

More recently, the use of chemotherapeutic agents with efficacy against tuberculosis for treatment and prophylaxis of other diseases has been implicated in the development of resistance to these drugs by *M. tuberculosis*. This includes empiric use of quinolones for community acquired pneumonia, when in fact the patient is manifesting tuberculosis, or aminoglycosides for a number of diseases (18-20); both are important second line tuberculosis classes that are widely used in routine clinical practice for the treatment of other diseases.. The duration of exposure required for resistance to evolve has not been well characterized yet; nevertheless, this has led some high TB-prevalence countries to regulate empiric use of these classes of drugs. The use of rifamycins in the prophylaxis of mycobacterium avium-intracellulare disease has been associated with the development of rifampicin-resistant TB in HIV patients (21, 22).

Finally, there exists considerable cross-resistance and class-resistance to antituberculosis agents. All rifamycins have high levels of cross resistance (23, 24). Fluoroquinolones have considerable cross resistance, but in vitro data suggests that newly introduced fluoroquinolones may be effective when resistance to previous generation fluoroquinolones is present (cross resistance within earlier quinolones, such as ciprofloxacin and ofloxacin, is very high) (23, 24). Kanamycin and amikacin have almost 100% cross resistance (23, 24). Streptomycin is believed to have low levels of cross resistance with kanamycin and amikacin (23, 25).

### **Emergence of Multidrug Resistant Tuberculosis**

Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin (the two most important first-line drugs), appeared after the introduction of rifampicin in 1966. Until 1990, however, most MDR cases occurred in

patients receiving prolonged, inappropriate therapy; while sporadic outbreaks of primary transmission occurred, the magnitude and impact was relatively limited (26). In the early 1990's, several large outbreaks of MDR-TB unfolded in hospitals and institutions in the United States, announcing MDR-TB as a major public health threat (27-30). In New York City, where the largest number of MDR-TB cases were reported, as many as one in five TB cases involved MDR (28). Strong evidence of recent, primary transmission of resistant TB was established. Among patients who had never been treated before, 23% were resistant to one or more drugs (28). Molecular fingerprinting by restriction fragment length polymorphism (RFLP) implicated a single strain in 22% of MDR cases in New York City in 1992 (31). High rates of nosocomial transmission, to health care workers and HIV positive patients in particular, were documented (27, 29). Together, these circumstances demonstrated the rapidity with which MDR-TB could spread through susceptible populations. Through a massive investment of human and financial resources (estimated by some to be as high as a billion dollars), the MDR-TB epidemics in New York and elsewhere in the country were brought under control and the incidence of MDR-TB plummeted (32). Subsequent nosocomial and institutional outbreaks in Italy, Spain, Russia and Chile made it clear that MDR-TB ranked among the most serious public health issues facing the world (33-36).

Global data on the prevalence of MDR-TB, however, were lacking. The first global survey of TB drug resistance was published in 1997 by the Global Project on Anti-TB Drug Resistance, a collaboration between the World Health Organization and the International Union Against Tuberculosis and Lung Disease. Two subsequent global surveys, covering the periods of 1996 to 1999 and 1999 to 2002, further elucidated the

worldwide picture of drug resistance (37). The most recent data published by the Global Project revealed that virtually all countries surveyed reported TB drug resistance and estimated that 424,000 cases of MDR-TB emerged in 2004 (38). With the exception of Botswana, which was found to have rising rates of MDR-TB, no trend data was available from Africa, a result of the poor laboratory infrastructure and surveillance on the continent. Twenty sites worldwide reported drug resistant TB prevalence in excess of 20%, and eleven sites reported rates of MDR-TB among new cases of over 6.5%. The geographic distribution of MDR-TB is highly uneven and ranges from 0.7% in new cases in established market economies, to around 2% in Africa, Southeast Asia and South America, and over 10% in some areas of the former Soviet Union and several provinces in China. Among previously treated cases, the rate of MDR is often several fold higher; by 2002, nine settings had been identified as having MDR rates of above 30% in previously treated cases.

More recently, the emergence of extensively drug resistant tuberculosis (XDR-TB), defined as TB resistant to isoniazid, rifampicin, quinolones and at least one of three injectable second line drugs (kanamycin, capreomycin, or amikacin), in every region of the world has raised further alarms about the future of TB control (see “The ‘Tugela Ferry Outbreak’ of Extensively Drug Resistant Tuberculosis,” below) (39-41). A review of global DST data conducted by researchers at the CDC found 347 isolates of XDR-TB worldwide, accounting for 2% of all TB isolates surveyed and 15% of MDR-TB isolates; data from African and Asian countries, other than South Korea, were notably lacking (39). In early 2005, the first reports emerged of an outbreak of XDR-TB at a hospital in rural KwaZulu Natal, South Africa, confirming fears about the rise of drug resistant TB

in high HIV prevalence settings (see “The ‘Tugela Ferry Outbreak’ of Extensively Drug Resistant Tuberculosis,” below) (41).

There is tremendous concern among public health practitioners that the rise of drug resistant tuberculosis will undermine the success of extent TB DOTS programs and worldwide TB control. The ability of DOTS programs to reduce transmission and incidence of both drug susceptible and drug-resistant tuberculosis is debatable; while some studies have shown successful reduction of drug resistance under the WHO strategy (42), others have demonstrated an “amplifier effect” of increasing drug resistance under DOTS-prescribed short-course chemotherapy (43-46). One study of patients receiving short course chemotherapy in a penitentiary hospital in Siberia found that over 3% of patients completing treatment, and over twenty percent of patients who began treatment with an isolate resistant to three first-line drugs, had amplified resistance over the course of therapy (47). Large scale epidemiological data is presently lacking, but mathematical models have suggested that MDR-TB hotspots could evolve in areas with successful DOTS programs due to the amplifier effect (48).

Treatment of multidrug-resistant tuberculosis with second line drugs is much more expensive and requires a longer duration of therapy. As such, it became a contentious public health issue in the past decade, pitting moral and cost-effectiveness arguments against each other in debates about global health resource allocation (49). Prior to 1999, the prices of second line drugs were exorbitantly high and no global mechanism existed for coordinating supply, negotiating drug prices, financing programs, setting treatment guidelines and standards, and overseeing program performance (50). In 1999, the WHO and its partners launched a “DOTS-Plus for MDR-TB” initiative,

followed by the “Green Light Committee” (GLC) a year later (51). Together, these bodies have increased access to second line drugs in resource poor settings and ensured that treatment of MDR-TB supplements, rather than detracts from, the success and resources of existing TB DOTS programs. Despite the success that the DOTS Plus initiative and GLC have had in scaling up MDR-TB treatment in resource poor countries, only 10,000 patients, or less than 5% of the world’s total cases, are currently receiving second line drugs (SLD) through this mechanism (24). The overwhelming majority of patients afflicted with MDR-TB in developing countries remain without access to second line drugs.

### **Multidrug-Resistant Tuberculosis in South Africa**

Tuberculosis drug resistance surveillance throughout Africa has been extremely relatively limited due to poor public health infrastructure and the paucity of laboratories with capacity to perform drug susceptibility testing (DST); nevertheless, the existing data suggest that African countries have some of the lowest rates of multidrug-resistant tuberculosis of developing countries. Reasons for this include the relatively recent introduction of rifampicin into the public sector and delays in drug resistance surveillance and reporting amidst volatile HIV and TB epidemics (52).

Compared with most Sub-Saharan African countries, South Africa has an advanced public health infrastructure and far greater capacity for drug resistance surveillance. Between 1965 and 1991, twenty-five annual surveys of drug resistance were carried out by the Tuberculosis Research Institute of the South African Medical Research Council (53). The results of these surveys found a dramatic decline in prevalence of both primary and acquired drug resistant tuberculosis. In 1995, the

Medical Research Council's TB research programme was suspended due to budgetary constraints. Several smaller drug resistance surveys, including two provincial surveys, were undertaken, but national data were not collected again until 2001. The data from these interim surveys suggested that the prevalence of MDR-TB remained low in the mid-1990s but began to rise in the latter half of the decade (50, 54-57).

The most recent national estimates of tuberculosis drug resistance in South Africa were based upon a survey covering nine provinces of the country and carried out between 2001 and 2002 by the Tuberculosis Lead Programme of the Medical Research Council in South Africa, which is part of the Global Network of Supranational Reference Laboratories for Drug Resistance Surveillance of the World Health Organization (58). Nationwide, any drug resistance was detected in 7.8% of isolates from new patients and 15.5% of isolate from retreatment cases. MDR prevalence was 1.6% among new cases and 6.6% among retreatment cases. A quarter of all MDR cases had resistance to all four first line drugs against which they were tested (isoniazid, rifampicin, ethambutol, and streptomycin). In KwaZulu Natal (KZN), the prevalence of MDR-TB among new patients and retreatment patients were marginally higher than the national average at 1.7% and 7.7%, respectively. The prevalence of HIV among pan-susceptible, any resistance and MDR patients were 63.2%, 76.1% and 76.9%. The survey in KZN suffered from the failure of most districts to submit an adequate number of specimens; only two of the eight districts met the sample number targets, and five of the eight districts reported less than 70% of the targeted number of specimens. DST for second line drugs was not performed in this survey.



Though the rates of MDR-TB among new and previously treated cases were relatively low compared with some areas of Eastern Europe, Central Asia and Russia, the absolute number of TB patients in South Africa is so high that the burden of MDR-TB is enormous. In KZN alone, the estimated number of new MDR cases per year, by these rates, was 1385 to 2616—ten to twenty times the total number of cases of MDR-TB in the entire United States in 2005 (58, 59). The declining case identification and treatment success rates in South Africa in recent years make the rise of drug resistant tuberculosis a likely and concerning possibility (2). A recent survey of drug resistance at one hospital in KZN found an MDR-TB rate of 39% among sputum culture positive cases (41). Notably, the district in which this hospital lies was not included in the drug resistance surveillance study of 2000-2002, suggesting that the survey may have failed to identify significant MDR hotspots.

### **‘The Tugela Ferry Outbreak’ of Extensively Drug Resistant Tuberculosis**

In early 2005, researchers from a joint U.S.-South African team conducting a prospective trial of HIV and TB treatment integration at a rural hospital in Tugela Ferry, KwaZulu Natal, South Africa, noted higher than anticipated rates of mortality in their cohort of subjects receiving antiretroviral therapy concomitant with antituberculosis therapy (41). Further investigation revealed that six of the 119 patients co-infected with HIV and TB were infected with *M. tuberculosis* that was resistant to all six drugs against which it was tested: isoniazid, rifampicin, ethambutol, streptomycin, ciprofloxacin, and kanamycin. Increased surveillance undertaken as a result of these findings revealed that between the period of January 2005 and March 2006, 53 patients presenting to the

hospital were infected with organisms showing this same pattern of resistance to six drugs, meeting the WHO's new criteria for XDR-TB (60). Mortality was exceptionally high (98%), with the median survival from the time of sputum collection being just 16 days. All patients for whom HIV status was known (83%) were positive. The majority (55%) of patients had never received previous treatment for tuberculosis and only 15% had failed or defaulted therapy. Genotyping of 46 of the isolates revealed that 39 (85%) were genetically similar. These findings, taken together, were considered evidence of primary drug resistance with recent transmission, and the high proportion of patients who were hospitalized in the past year was suggestive of nosocomial transmission. Further concerning was the high prevalence of MDR-TB (39%) among culture positive cases, a figure far higher than that found by a province-wide tuberculosis resistance survey undertaken three years prior (58).

The results of this investigation, presented in August of 2006 at the International AIDS Conference, provoked alarm in the public health community. The findings in Tugela Ferry represented one of the first instances of the recognition of large number of cases of highly drug resistant tuberculosis in a high HIV-prevalence setting; additional laboratory data from the provincial diagnostic mycobacteriology laboratory revealed that XDR-TB isolates had been found in 28 hospitals across the province. The World Health Organization, in collaboration with the South African Medical Research Council and U.S. Centers for Disease Control, responded by convening an expert consultation on XDR-TB in Johannesburg in September, followed by the first meeting of the Global Task Force on XDR-TB in Geneva in October, 2006 (61). The WHO Global Task Force on XDR-TB revised the definition of XDR-TB and promulgated recommendations for its prevention

and control (62). According to the revised definition of XDR-TB, the total number of XDR cases identified at this single hospital between January 2005 and December 2006 was over 200.

## **Clinical Issues in Drug Resistant Tuberculosis**

### *Laboratory and Clinical Diagnosis*

Drug susceptibility testing (DST) is the principal strategy for establishing the resistance pattern of tuberculosis to antituberculosis chemotherapeutic agents. The classic approach to DST involves inoculation of a solid media (e.g. Lowenstein-Jensen, 7H10 or 7H11 agar) impregnated with a tuberculosis drug in a defined concentration with either a concentrated specimen (direct method) or pure culture (indirect method) and then assessing growth by one of several methods: the proportions method, the absolute-concentration method, or the resistance-ratio method (63). According to the proportions method, a resistant isolate is one in which the number of colonies growing on the drug-impregnated plate is greater than or equal to 1% of the number of colonies on a non-drug impregnated plate (direct comparison between plates for colonies can be made by increasing the concentration of the isolate on the impregnated plate by 100 fold).

Additional methods include the BACTEC-460, an automated liquid medium system that assesses mycobacterial growth by detecting the consumption of radioactive ( $^{14}\text{C}$ ) mycolic acids through measuring the release of  $^{14}\text{CO}_2$  (63). More recently, a nonradioactive automated BACTEC-960 system has shown promise as an alternative for mycobacterial culture and DST in advanced laboratories. These systems are more rapid

but are expensive and require a laboratory infrastructure far beyond that available in many resource poor settings.

Molecular approaches for identifying drug resistant *M. tuberculosis*, including rapid genetic tests, are currently in use in many laboratories and are gaining interest for their speed and potential for use in areas with limited laboratory capability. The most commonly used tests assay for the *rpoB* gene associated with rifampicin resistance (11). Several large studies are currently underway to evaluate the use of rapid rifampicin tests in resource-limited settings. Finally, the recent demonstration of a rapid, low cost technique for culture and susceptibility known as “Microscopic Observation of Drug Susceptibility” (MODS) has generated considerable excitement about the potential for cheap and rapid culture and DST, particularly in resource limited settings. In demonstration and operational studies in Peru, MODS was shown to be highly sensitive and specific, cost a fraction of existing culture and DST methods, and provide results in half the time of BACTEC and less than a fifth the time of conventional Lowenstein-Jensen agar with the proportion method (64). However, the sensitivity and specificity of this method for identifying resistance to SLDs has not been studied, and biosafety concerns still present a major obstacle to the rapid introduction of this test into resource limited environments.

Criteria for drug resistance testing for most SLDs (excluding more recently introduced drugs, such as quinolones) were established nearly 50 years ago; nevertheless, their validity in terms of correlation with *in vivo* outcomes has recently come into question (65). DST for SLD is considered to be more complicated, in part because critical drug concentrations defining resistance are not as close to the minimal inhibitory

concentrations for SLDs as they are for first-line drugs (66). Furthermore, proficiency metrics, such as those obtained for first-line drug susceptibility testing, are unavailable for most SLDs (though proficiency testing exercises among supranational reference laboratories is underway).

In the vast majority of clinical settings in the developing world, where most cases of tuberculosis occur, DST for first or second line drugs is not routinely performed. Even in areas where DST is available, routine use in all newly diagnosed patients is often infeasible due to costs or the limited sample processing capacity of laboratories. Further, the existing methodologies require 2-3 months for diagnosis. As such, the clinical history plays a large part in diagnosing probable drug resistance and targeting DST. Risk factors for drug resistant tuberculosis include: failure of short-course chemotherapy (SCC), known exposure to an MDR-TB case, known exposure to a patient who died while taking SCC, relapse after default, non-adherence to SCC, co-morbid conditions associated with malabsorption, and residence in areas (or institutions) of high MDR-TB prevalence (11). The role of outcomes from previous TB treatment in predicting drug resistant TB is significant yet not fully characterized; the prevalence of TB and of drug resistant TB in the population, as well as the immune status of the patient, probably impact considerably the predictive value of this factor. In several case control studies assessing risk factors for drug resistant tuberculosis, the increased risk associated with previous TB treatment ranges from two fold to more than ten fold (67-74). However, little data from Africa or high HIV prevalence settings is available.

In patients who are receiving therapy, those who remain or become sputum positive at 4 months, have persistent fevers, or have worsening clinical or radiological

parameters should provoke a high suspicion for drug resistance. To date, however, there are no reliable clinical algorithms for accurately predicting drug resistant tuberculosis (52).

### *Fitness and Transmissibility*

The debate surrounding the relative virulence or fitness of drug resistant strains compared with drug sensitive strains is an unresolved one. Early animal models suggested that the development of drug resistance may be associated with a loss of virulence factors, such as catalase activity, by bacilli (75, 76). Subsequent epidemiological studies, looking at cluster development of drug resistant versus drug susceptible cases and the differential development of secondary cases among contacts, affirmed this hypothesis by showing lower infection rates among groups exposed to drug resistant TB (77, 78). However, other studies, including those of the “strain W” responsible for much of the New York City outbreak in the 1990s, have found drug resistant TB to be at least as virulent and transmissible (79, 80); “strain W”, in particular, was shown to be catalase positive. In all likelihood, there is heterogeneity in virulence among strains of drug resistant TB.

Whether or not drug resistant tuberculosis is less fit or transmissible, cases of MDR-TB are likely to generate more secondary cases due to the prolonged infectious period associated with delayed identification, inadequate treatment, longer time to culture conversion once on SLD, and lower cure rates. Moreover, mathematical models suggest that, even where strains of MDR-TB are less fit than pan-susceptible strains, hot zones of MDR-TB can develop, largely as a result of low cure rates and amplification probabilities (48).

### *HIV and TB Drug Resistance*

The associations between HIV infection and the development of multi-drug resistant tuberculosis have not yet been fully clarified. Among the most important unresolved questions is whether HIV infection is an independent risk factor for the development of multi-drug resistant tuberculosis. Several studies have shown increased rates of drug resistant TB among HIV infected individuals (81-83), while other data, including the results of the Global Anti-TB Drug Resistance surveys, have failed to confirm this finding (37, 56, 84-86). Higher rates of drug resistant TB found in the smaller studies could, in part, be due to the fact that recently circulating strains are more likely to be drug resistant, and HIV-infected patients manifest TB disease more rapidly, such that they are disproportionately represented in the early stages of outbreaks of drug resistant TB.

While the population-level impact of HIV on drug resistance remains to be established, several clinical observations have been studied concerning the relationship between HIV disease and the development of drug resistance. Patients with HIV have been observed to have higher rates of rifampicin monoresistance, further associated with diarrhea, prior rifabutin use (e.g. for MAI treatment/prophylaxis), positive AFB smear, nonadherence to therapy, severe immunosuppression, and antifungal therapy (22, 87). Patients co-infected with HIV have varying degrees of intestinal absorption of TB drugs (88-90) and of treatment failure with standard regimens (91-93) both potentially increasing the risk of acquiring or amplifying TB drug resistance.

In addition to its role as a determinant or co-determinant of drug resistance, HIV disease and its management have clinical implications for the diagnosis and treatment of MDR-TB. HIV-infected individuals undergoing treatment for MDR-TB have lower rates of treatment success and higher mortality than uninfected patients (94, 95). Diagnosis of any form of TB, including MDR-TB, is more challenging in the presence of HIV disease, in that sputum smear and radiographic findings are less sensitive and extrapulmonary disease is more common than in uninfected patients (96-102). Certain second line drugs are more toxic in patients infected with HIV, while the use of antiretrovirals concomitantly with SLDs may result in problematic drug-drug interactions (11, 24).

Finally, regardless of whether HIV is an independent risk factor for the development of MDR-TB at the individual level, the increased pool of susceptible patients who serve as both hosts and vectors for all forms of TB, including MDR-TB, is certain to increase the absolute burden of MDR-TB at a population level. Moreover, at a programmatic level, the HIV epidemic, particularly in Africa, has overwhelmed and disrupted the established TB control programs, causing rising treatment failure rates and increasing the opportunity for drug resistant TB to emerge and spread.

#### *Treatment of MDR-TB*

Compared with therapy for drug susceptible tuberculosis, treatment of MDR-TB requires a longer duration, is considerably more complicated, expensive, and toxic, and treatment success rates are typically lower. Various treatment strategies have been employed, including the use of standardized treatment regimens based upon representative local susceptibility patterns, empirical treatment based upon previous



treatment history and local DST patterns, and individualized treatment designed on the basis of individual DST results (24). It is recommended that regimens include at least four drugs that are certain, or expected, to be effective and that the duration be a minimum of 18 months beyond sputum conversion. Injectable agents should be used for a minimum of 6 months.

Management of patients receiving second line drugs requires fairly intensive monitoring for drug toxicities and treatment failure. While some cohorts have found high rates of treatment interruption due to side effects and toxicities, well designed programs have demonstrated that, in spite of the high frequency of adverse effects, life-threatening adverse events are uncommon and management in resource limited settings can be done successfully (103-105). Sputum culture conversion typically occurs between one to two months after the initiation of therapy, while smear conversion may take longer as it does not distinguish between viable and nonviable organisms (106). Patients who have persistently positive sputum smears or cultures after three months of therapy with SLD should raise concerns for either poor adherence or improper regimen choice, and further evaluation including DST may be indicated (11).

Treatment outcomes among MDR-TB cases have varied widely; a recent survey of five GLC-approved sites in resource-limited countries found treatment success rates of 70% (107). A number of factors have been associated with treatment failure and death. In the aforementioned survey of GLC-approved sites in resource-limited countries, treatment success and death rates were 77% and 3.5%, respectively, in new cases and 68.5% and 14% in previously treated cases. Patients infected with HIV have consistently been found to have higher rates of mortality during MDR-TB treatment than HIV

uninfected individuals (94, 108). One case series in South Africa found MDR-TB treatment success rates of 38% in HIV infected individuals, compared with 47% in those who were uninfected (109). In another cohort in Peru, low baseline hematocrit and body mass index were each independently associated with decreased time to death, while the inclusion of pyrazinamide and ethambutol in the regimen (in patients with DST-documented susceptible organisms) was independently associated with favorable treatment outcomes (106). A review of MDR-TB treatment outcomes in Latvia found treatment success of 76% among HIV uninfected patients and of 56% among infected patients; resistance to ofloxacin was independently associated with a much slower time to culture conversion and an increased risk of poor outcomes (110).

At a programmatic level, the World Health Organization recommends that treatment of multidrug-resistant tuberculosis be based upon principles closely related to those of its general DOTS strategy for TB control: sustained political commitment; a rational case-finding strategy including accurate, timely diagnosis through quality-assured culture and DST; appropriate treatment strategies that use second-line drugs under proper case management conditions; uninterrupted supply of quality-assured antituberculosis drugs; standardized recording and reporting system (24). While these components can be expensive and require substantial investment of human and laboratory resources, the experience from multiple countries is that addressing drug resistant tuberculosis strengthens, rather than detracts from, national tuberculosis programmes. Moreover, data from Peru suggests that treatment of MDR-TB is cost-effective (111). To date, however, DOTS Plus for MDR has not been implemented on a large scale in Africa, and data on outcomes from the region are limited.

### *Need for Rapid Identification of MDR and XDR-TB Patients*

The timely identification of patients with MDR-TB and XDR-TB is challenging and yet has significant implications on the outcomes for individual patients and their potential for spread to others. Among the first 53 patient reported with XDR-TB in Tugela Ferry, the median survival from the time of sputum collection was 16 days; however, the results of sputum culture and DST by conventional methods (as are available KwaZulu-Natal) typically takes three to six weeks, meaning most patients had died before their diagnosis of XDR-TB was made. Moreover, many patients are hospitalized while awaiting sputum culture and DST results; in Tugela Ferry, patients with unknown TB resistance patterns share a single, large room with approximately 40 other patients. Such circumstances make nosocomial transmission of drug resistant tuberculosis highly probable.

While the introduction of rapid culture and DST techniques is needed in this setting, the requisite infrastructure and resources are not yet available. As such, there is an urgent need for the further elucidation of the clinical characteristics of MDR-TB and XDR-TB patients and the identification of clinical predictors of drug resistant TB. Such information may facilitate the early identification of patients with MDR-TB and XDR-TB, hopefully leading to improved treatment outcomes and reducing community and nosocomial transmission. In settings without access or with limited access to DST, which include most of the developing world, clinical assessment of TB for drug resistance is the primary tool for diagnosis, making the development of clinical predictors of drug resistant TB particularly needed.

## **METHODS**

### **Setting**

This study was carried out at the Church of Scotland Hospital (COSH), a 360 bed provincial government district hospital in the Msinga subdistrict of KwaZulu Natal, South Africa, a rural region of approximately 2000 km<sup>2</sup>. COSH provides medical services to a catchment population of approximately 250,000 Zulu people, living in traditional family compounds dispersed widely through areas with few roads, poor transportation infrastructure, sparse electricity and a dearth of potable water. Unemployment rates are estimated to be in excess of 60%. The prevalence of HIV infection among inpatients and women presenting to the maternity ward are 40% and 20%, respectively. There are ten full-time physicians working at the hospital at any given time, and laboratory infrastructure exists for sputum microscopy for acid-fast bacilli but no culture or other microbiological tests.

At present, there are four TB wards: two general TB wards (one male, one female), each containing approximately 35 beds; and two TB “isolation” wards (one male, one female), each containing 10 beds. The male isolation ward is a separate building, whereas the female isolation ward is a partitioned room of the female TB ward that has no door and an open airflow with the general female TB ward. Both wards are almost always over capacity, forcing a number of patients to sleep on the floor. In addition there is one male and one female internal medicine ward, a surgical ward, pediatrics ward, and obstetrics wards. HIV testing is offered to all patients with TB, and around 70% of patients accept testing.

COSH has been a site for a government-sponsored TB DOTS program since 1993. Diagnosis is made by sputum microscopy, x-ray, and/or clinical criteria, according to the South African National Tuberculosis Guidelines (112). Each year, approximately 1800 cases of tuberculosis are diagnosed and managed by COSH and its surrounding satellite clinics. All patients receive free directly observed therapy, administered at home by community health workers or in nearby clinics. A standard regimen is used, consisting of isoniazid, rifampicin, ethambutol and pyrazinamide for a two month intensive phase, followed by a 4-month continuation phase with isoniazid and rifampicin. These are given as fixed dose combination pills containing 4 drugs (intensive phase) or 2 drugs (continuation phase), reducing pill burden. Since June 2005, sputum samples for all patients with signs and symptoms of tuberculosis have been collected and sent for culture and drug susceptibility testing at the provincial diagnostic mycobacteriology laboratory in Durban. Results are transmitted back to COSH via paper copies once weekly and are also available to COSH staff on an intranet-based laboratory reporting system. The details of the culturing and drug susceptibility testing methods are described in the “Mycobacterial Culture and Drug Susceptibility Testing Methods” section.

### **Study Design**

This was a retrospective, case-control control study of patients diagnosed with tuberculosis at a rural hospital in KwaZulu Natal, South Africa. This site was chosen due to the recognition of an outbreak of XDR-TB in 2005 (see **INTRODUCTION**). The primary objective of the study was to identify clinical predictors of tuberculosis drug resistance and mortality among this population. The study was designed as a case-control study comparing three groups of patients: patients with XDR-TB, patients with MDR-TB but

not XDR-TB, and patients with non-MDR-TB. Cases were defined according to drug susceptibility testing of sputum cultures, described below. XDR-TB was defined as resistance to at least isoniazid, rifampicin, ciprofloxacin and kanamycin. Ciprofloxacin and Kanamycin were the only second line drugs against which *M. tuberculosis* is currently tested at the referral laboratory for COSH, the provincial diagnostic mycobacteriology laboratory at the Inkosi Albert Luthuli Central Hospital (See **Mycobacterial Culture and Drug Susceptibility Testing Methods** below). MDR-TB was defined as resistance to at least isoniazid and rifampicin. Non-MDR-TB was defined as susceptibility to either rifampin or isoniazid or both (irrespective of susceptibility to other drugs). While XDR-TB is a subgroup of the category of MDR-TB (all XDR isolates must have resistance to isoniazid and rifampicin), the MDR-TB group in this paper refers to patients who had only MDR-TB and did not meet the criteria for XDR-TB.

The study period was from June 1, 2005 through August 31, 2006. The starting point of this period was selected because from this point forward, the hospital adopted a policy whereby sputum samples for culture and drug susceptibility testing were collected for all patients suspected of having tuberculosis. This reduced bias in patients having sputum cultures, in that, rather than targeting patients suspected of failing treatment for DST, clinicians sought to acquire DST for all patients.

The primary outcomes of interest were risk factors for MDR-TB and XDR-TB. Secondary outcomes were risk factors for death. Variables or factors examined included demography, treatment outcomes, mortality, HIV status, number and duration and timing of hospitalizations prior to and subsequent to treatment, time elapsed between initiation

of treatment and death, time elapsed between treatment initiation and DST, time elapsed between DST and death, use of second line drugs, recorded symptoms, weight changes, use of antiretroviral therapy, CD4 count, laboratory results (hemoglobin and ESR) at diagnosis and on therapy, and sputum smear and culture results.

### **Selection of Subjects**

Selection of subjects for inclusion in the study was carried out in the following way. First, a list of patients with MDR and XDR-TB was compiled based upon a registry kept and maintained by the staff at COSH. Charts were obtained from the hospital's TB DOTS office and the hospital's central file storage room. All patients diagnosed by sputum drug susceptibility testing as having MDR or XDR-TB between the period of June 1, 2005 and August 31, 2006 for whom charts could be located were included. Patients who were diagnosed with TB before June 1, 2005 were excluded because prior to this date, sputum for culture and DST were only targeted at patients with treatment failure. Since June 1, 2005, the hospital policy has been to obtain sputum for culture and DST on all newly diagnosed TB patients.

Once review of charts was completed, patients whose current episode of TB was diagnosed and treatment initiated before June 1, 2005 were excluded from the study. These were patients who began treatment prior to June 1, 2005 but whose diagnostic sputum culture was collected after June 1, 2005. This excluded 13 patients with XDR-TB and 10 patients with MDR-TB, leaving 52 patients in the MDR-TB group and 61 patients in the XDR-TB group.

The control group of non-MDR patients was compiled by reviewing the TB DOTS registry, which contains all patients diagnosed with tuberculosis at COSH, and by cross-checking with the Kwazulu-Natal Department of Health laboratory server. All patients diagnosed with TB at COSH after June 1, 2005 were eligible for inclusion. The first 60 patients with a positive sputum culture, a non-MDR-TB first resistance pattern, and an available medical chart were entered into the study. Three patients were later excluded due to the absence of chart information, leaving 57 patients in the non-MDR-TB group.

### **Classification of Patients with Multiple Resistance Patterns**

Many patients had two or more *M. tuberculosis* isolates with different drug susceptibility patterns, some of which fell in two or more different drug resistance groups. For comparative purposes, it was necessary to group patients according to drug susceptibility pattern, but to do so in a cross-sectional manner, without assigning patients to multiple groups. This presented a challenge in categorizing these patients into drug resistance groups without biasing the groups. For example, if the highest level of resistance was used to classify patients, then all patients who had a pan-susceptible sputum first and then developed MDR-TB or XDR-TB would be classified in the latter two groups; when analyzing the outcomes of the pan-susceptible group, it would appear as though no patients went on to develop MDR-TB or XDR-TB. If the first sample found in the time period under examination was used to classify patients, no patients with MDR-TB or XDR-TB could have a prior non-MDR-TB sample, which would obscure acquired and transmitted resistance.



To address this problem and minimize biases in clinical predictors and outcomes, patients who had two or more *M. tuberculosis* isolates with different drug susceptibility patterns were treated in the following manner during subject selection.

For all groups, if the differing isolates were collected within three days of one another, the patient was grouped according to the highest level of drug resistance. For example, if sputum samples from one day showed an isolate with pan-susceptible tuberculosis and another isolate within 3 days had XDR-TB, the patient was placed in the XDR-TB group.

For the creation of the MDR and XDR groups, if the isolates with differing DST patterns were collected on separate days more than 3 days apart, the patient was grouped according to the highest level of resistance found.

The creation of the non-MDR-TB group was done systematically through the review of DOTS registers, so the first (non-MDR) sputum found was used to classify patients. If these patients went on to develop MDR-TB or XDR-TB, they were nevertheless kept in the non-MDR-TB group, because the development of MDR-TB or XDR-TB in these patients was considered an important outcome.

Finally, patients who were first classified into the MDR and XDR TB groups but who were selected randomly through the TB register as non-MDR-TB patients were moved to the non-MDR-TB group.

Patients who had multiple susceptibility patterns were included in the bivariate and multivariate analyses to examine clinical predictors and outcomes and were also examined separately as a case series.

## **Data Collection**

There were five sources of data utilized in this study: the TB DOTS register; the hospital medical record; laboratory data stored on the Department of Health's server and accessible through an intranet; the registry of MDR and XDR-TB patients in the TB DOTS clinics; and spreadsheets maintained by the COSH ARV Clinic.

The TB DOTS register contained treatment category (new patient, retreatment after cure, re-treatment after interruption, etc), treatment regimen, sputum smear information at 0, 2 and 6 months, and treatment outcomes (cure, treatment completion, treatment interruption, transferred out, death).

The patient's hospital medical record contained the majority of information, including demographics, HIV status, clinical and laboratory details from outpatient visits and hospitalizations, previous episodes of tuberculosis, date of transfer to a tertiary facility for second line drugs, and death certificate. The registry of MDR and XDR-TB patients maintained in the TB DOTS clinic contained information on the tracing of patients found to have MDR and XDR-TB, including whether the patient was alive or deceased at the time of tracing. Spreadsheets maintained by the COSH ARV Clinic contained antiretroviral therapy related information including CD4 counts, viral loads, and date of initiation of therapy.

Data collection from each of these sources was performed by the author and entered directly onto a standardized electronic data collection form linked to a Microsoft Access 2002 (Microsoft Corp, Redmond, WA, USA) database created by the author and a co-investigator (NRG). Data collected concluded on November 15, 2006; mortality and other outcomes were included up until this date. For patients who were not confirmed to

have died, the survival or observation period was taken to conclude at the last point in which the patient was documented to have visited the hospital or clinic.

### **Mycobacterial Culture and Drug Susceptibility Testing Methods**

Sputum samples were obtained from all patients for mycobacterial culture and drug susceptibility testing as part of routine clinical care and disease surveillance at COSH. Some of the sputa were collected at the time of TB diagnosis as directed by hospital procedures during this time; others were collected after the initiation of therapy, either due to suspected treatment failure or the lack of sputum collection at the time of diagnosis. The sputum collection, culture, and drug susceptibility testing methods have been previously described and published (41):

Typically, one to three samples were taken per patient. The samples were not induced and were taken at any time of day. Sputum specimens were stored at 4°C for up to 3 days until transport to the provincial diagnostic mycobacteriology laboratory in Durban. Digestion and decontamination was done with the N-acetyl-L-cysteine-sodium hydroxide method. An auramine-stained smear was made and the remaining deposit was inoculated in one mycobacteria growth indicator tube (MGIT) broth and on one Middlebrook 7H10 agar plate. The broths were incubated at 37°C in an automated incubator. Agar plates were sealed in CO<sub>2</sub>-permeable plastic bags and incubated in 5% CO<sub>2</sub> at 37°C. Acid-fast microscopy was done on each positive MGIT broth when a positive reading was obtained. Those containing acid-fast bacilli were subcultured on Middlebrook 7H10 agar. Primary Middlebrook agar plates were read weekly for 3 weeks or until growth was observed. Microscopy was done to confirm the presence of acid-fast bacilli. All positive cultures were identified as *Mycobacterium tuberculosis* by means of niacin and nitrate reductase tests. The risk of cross-contamination was minimized by processing samples individually in real time, rather than batching. Quality assurance was done weekly by the UK National External Quality Assessment Service programme, where ten consecutive isolates were fingerprinted to rule out cross-contamination. Susceptibility tests were done on all isolates using the 1% proportional method on Middlebrook 7H10 agar. All isolates were tested for susceptibility to isoniazid (1 mg/L), rifampicin (2 mg/L), ethambutol (5 mg/L), streptomycin (2 mg/L), kanamycin (16 mg/L) and ciprofloxacin (2 mg/L). Susceptibility testing to pyrazinamide and the remaining four classes of second

line drugs—ethionamide, cycloserine, capreomycin, and para-aminosalicylic acid—are not routinely done.

### **Statistical Analysis**

Bivariate analyses were undertaken to compare clinical characteristics between drug resistance groups and identify significant associations with mortality among all drug resistance groups. T tests and Wilcoxon tests were used to compare means and medians, respectively, and all dichotomous and nominal data were analyzed by Chi-square analysis and Fischer's exact tests. Kaplan-Meier survival curves were produced to express differences in survival between drug resistance groups. Comparisons in each analysis were made between 2 of the 3 groups at a time: non-MDR vs. MDR, MDR vs XDR, and non-MDR vs. XDR.

Multivariate logistic regression was used to determine factors independently associated with drug resistance. Similar to the bivariate analysis, comparisons were made between 2 of the 3 drug-resistance groups at a time. Independent factors tested were: age, sex, CD4 cell count, hospitalization in the previous 2 years, TB treatment in the previous year, and sputum smear. Cox proportional hazards modeling was used to assess factors independently associated with mortality. Independent factors tested included: age, sex, CD4 cell count less than 50, CD4 cell count less than 200, MDR-TB, non-MDR-TB, XDR-TB, TB treatment in the previous year, and hospitalization in the previous year. A subset of patients who had changes in resistance patterns over the survey period were analyzed separately and descriptively as a case series. All analysis was performed by the author and a co-investigator (NRG) and done in SAS version 9.1 (SAS Institute Inc, Cary, NC, USA).

### **Ethics and Institutional Review**

The study was approved by the Human Investigation Committee at the Yale School of Medicine and the Research Ethics Committee at the Nelson Mandela School of Medicine.

## RESULTS

### Demographics and Clinical Characteristics by Drug Resistance Group

Data for 193 patients were reviewed for the study. After excluding patients the most recent episode of TB was diagnosed and treatment initiated prior to June 1, 2005, there were 170 patients remaining in the study. Fifty-seven patients were classified into the non-MDR-TB group, 52 patients were classified into the MDR-TB group, and 61 patients were classified in the XDR-TB group. The demographic information and baseline clinical characteristics are depicted in Table 1. Of note, women comprised more than half of the XDR-TB group (55.7%) and less than half of the non-MDR and MDR groups (40.4%, 34.6%). The difference in sex between the XDR group and the MDR group was statistically significant ( $P=0.025$ ). Ages were similar between all three groups. The median and range of age for the non-MDR, MDR and XDR groups were 32.6 (14.7-70.6), 33.7 (19.9-56.9) and 34.5 (15.4-61.4), respectively.

The sputum smear positive rates were 47.4%, 71.2% and 73.8% for non-MDR, MDR and XDR patients, respectively. The differences between the non-MDR and MDR and XDR group were statistically significant ( $P=0.015$ ,  $P=0.005$ ). XDR and MDR patients had slightly higher rates of extrapulmonary TB (29.5%, 30.8%) compared with non-MDR patients (24.6%), but the differences were not statistically significant ( $P=0.469$  for MDR versus non-MDR,  $P=0.546$  for XDR versus non-MDR). At baseline, most patients in all three groups had moderate-severe anemia, with median hemoglobin in the non-MDR, MDR and XDR groups of 9.0 g/dL, 9.3 g/dL and 9.3 g/dL. Median erythrocyte sedimentation rate (ESR) was elevated in all three groups and higher in the non-MDR group (115.5 mm/h) than in the MDR (95 mm/h) and XDR groups (100

mm/h), but not significantly so ( $P=0.187$ ,  $P=0.272$ ). Median weights were slightly higher for both men and women in the non-MDR groups (53.0 kg, 52.0 kg) compared with the MDR only (47.5 kg, 51.0 kg) and XDR groups (49.25 kg, 50.5 kg), but the differences between all groups were not statistically significant. There were no significant differences in any groups in terms of reported symptoms of weight loss, cough, night sweats, or fever.

The HIV-related characteristics of patients by drug resistance group are described in Table 2. Compared with patients in the MDR group, XDR patients were more likely to have been tested for HIV (93.4% versus 76.9%,  $P= 0.012$ ). Among non-MDR patients, 82.5% were tested for HIV, which was not statistically different from the MDR and XDR groups. The HIV prevalence in the non-MDR, MDR, and XDR groups (among tested patients) was 93.6%, 92.5%, and 100%, respectively. The differences in HIV prevalence were not statistically significant. Among individuals co-infected with HIV, 25.0%, 24.3% and 33.3% of patients in the non-MDR, MDR and XDR groups received antiretrovirals at any time; more patients in the XDR group were on antiretroviral therapy (ART) at the time of TB diagnosis (14.0%) than in the non-MDR (4.5%) and MDR groups (5.4%). None of the differences in ART use were statistically significant.

Fewer than half of all HIV infected patients had a viral load performed around the time of TB diagnosis, and the median log viral load did not differ between the non-MDR, MDR and XDR groups (5.6, 5.2 and 5.1). Of patients on ART at the time of TB diagnosis, five patients in the XDR group and none in the MDR and non-MDR groups had a viral load result. Of the five patients in the XDR group on ART, three had achieved viral suppression (viral load < 25 copies/mL).

Among HIV-infected patients, median CD4 cell count at diagnosis (70.5 cells/mm<sup>3</sup>) was higher in the non-MDR group than in the MDR only (57 cells/mm<sup>3</sup>) and XDR groups (56 cells/mm<sup>3</sup>), but the differences were not statistically significant. When CD4 counts were stratified into three groups—less than 50 cells/mm<sup>3</sup>, 50 to 200 cells/mm<sup>3</sup>, and >200 cells/mm<sup>3</sup>—the proportion of XDR patients with CD4 cell counts over 200 (14.3%) was less than that of MDR and non-MDR patients (20.8%, 18.5%), but not significantly.

Patients in the MDR and XDR groups were more likely than the non-MDR-TB group to have any previous TB treatment and TB treatment in the past year (Table 3). Among non-MDR, MDR and XDR patients, 26.3%, 56.9% and 42.6% had been previously treated for TB. The difference between MDR and non-MDR patients was statistically significant (P<0.001; OR 4.13, 95% CI 1.84-9.28). MDR-TB patients were more likely than XDR-TB patients to have had any previous TB treatment, but this difference did not reach statistical significance. (P=0.072; OR 1.99, 95% CI 0.94-4.21). However, both MDR and XDR patients were significantly more likely to have had TB treatment in the past year compared with non-MDR-TB patients (P<0.0001, OR 13.36, 95% CI 2.91-61.39; P<0.001, OR 10.63, 95% CI 2.33-48.48). The difference between the MDR and XDR groups for TB treatment in the previous year were not statistically significant (P=0.577).

Compared with non-MDR patients, MDR and XDR patients were significantly more likely to have had any previous hospitalization (42.3% and 41.0% versus 21.1%; P=0.017 and P=0.020; OR 2.75 95% CI 1.19-6.38 and OR 2.6 CI 1.15-5.89) and hospitalization in the past two years (36.5% and 37.7% versus 14.0%; P=0.007 and



P=0.004; OR 3.53, 95% CI 1.38-9.00 and OR 3.71, 95% CI 1.49-9.20). The proportion of patients with previous hospitalizations and hospitalizations in the past two years were similar for MDR and XDR groups.

In multivariable analysis comparing the MDR and non-MDR groups (Table 4), TB treatment in the previous year was the only significant independent predictor of MDR-TB (adjusted OR 8.33, 95% CI 1.64-42.33). In multivariable analysis comparing XDR and non-MDR groups (Table 5), significant independent differences were found for TB treatment in the past year (adjusted OR 7.19, 95% CI 1.35-38.17) and sputum smear positive (adjusted OR 2.79, 95% CI 1.20-6.47). Sex was the only significant independent factor in the multivariable model comparing the XDR and MDR groups (adjusted for male sex OR 0.41, 95% CI 0.19-0.89) (Table 6). Hospitalization in the past two years and patient age were not statistically significant risk factors for any of the groupings under this analysis.

## **Mortality**

The overall mortality rates in the non-MDR, MDR and XDR groups were 36.8%, 73.1% and 85.3%, respectively. Survival by drug resistance group from time of TB treatment start is depicted by Kaplan-Meier curve in Figure 1. Median survival times for the non-MDR, MDR and XDR groups under Kaplan-Meier analysis (limiting survival time among censored to the follow-up period) were 199 days, 103 days, and 92 days, respectively. The median follow-up period from time of TB diagnosis among surviving patients was shorter in the non-MDR group (median 52.5 days, interquartile range (IQR) 10-262.5 days) than in the MDR only group (median 119 days, IQR 99-282 days) and XDR group (median 161 days, 84-181 days). Survival by drug resistance group from

time of collection of diagnostic sputum (for culture and DST) is shown in Figure 2.

Median survival times for the non-MDR, MDR and XDR groups from sputum collection were 190 days, 22 days, and 14 days, respectively.

Table 7 shows demographic and baseline clinical characteristics among patients surviving and those who died. In bivariate analysis, there were no differences in sex or age between patients who died and those who survived or whose outcome was unknown. Patients with combined extrapulmonary and pulmonary TB were more likely to die than those with pulmonary TB alone, though the difference was not statistically significant ( $P=0.095$ , OR 1.88, 95% CI 0.89-3.97); mortality was 75% with patients with extrapulmonary TB (36/48 patients). Patients who died were more likely to be sputum smear positive (71.2% versus 50.8%,  $P=0.007$ ). Median hemoglobin and ESR at baseline were not significantly different. Median weight at baseline was lower for both men and women among patients who died, but the differences were not significant (51.0 kg versus 52.0 kg,  $P=0.40$ ; 48.3 kg versus 53.0 kg,  $P=0.111$ ).

There was no difference between the proportion of patients tested for HIV between those who died and survived (85.6%, 83.1%;  $P=0.662$ ) (Table 8). The HIV prevalence was slightly higher, but not statistically so, among patients who died (96.8% versus 93.9%;  $P=0.409$ ). The proportion of patients on ART at the time of TB diagnosis did not differ significantly between those who died and those who did not (9.8% versus 6.5%,  $P=0.522$ ), but the proportion of those who ever received ART was higher in those who survived (39.1% versus 22.8%,  $P=0.049$ ). Median log viral load at time of TB diagnosis did not differ between those who died and survived (5.23 versus 5.28,  $P=0.82$ ).

Viral load measurements on ART at the time of TB diagnosis were available for five patients total; among four who died, two had viral load suppression.

A little over half of patients in both outcome groups had CD4 counts within 120 days of TB diagnosis; median CD4 count was lower in the group who died, but the difference was not significant (53.5 cells/mm<sup>3</sup> versus 104 cell/mm<sup>3</sup>; P=0.552). Patients who died were more likely to have a CD4 count less than 50 cells/mm<sup>3</sup> (48.1% versus 28.0%, P=0.004) and less than 200cells/mm<sup>3</sup> (90.7% versus 64.0%, P=0.004). The odds ratios of death among patients with <50 cells/mm<sup>3</sup> or 50-200 cells/mm<sup>3</sup> compared with >200 cells/mm<sup>3</sup> were 6.69 (95% CI 1.69-26.45) and 4.60 (95% CI 1.21-17.52) respectively.

The relationship between previous TB treatment and previous hospitalization and mortality is shown in Table 9. The proportion of patients who had ever been treated for TB did not differ between those who died and survived (44.1% versus 39.0%, P=0.517); the proportion with treatment in the last year was higher, but not significantly, among those who died (25.2% versus 13.6%, P=0.076). There was a trend indicating that patients who died were more likely to have been hospitalized ever (39.6% versus 25.4%, P=0.064) or in the previous year (27.9% versus 15.3%, P=0.064).

Under the Cox Proportional Hazards model (Table 10), mortality from TB treatment start was independently and significantly associated with positive sputum smear (HR 2.09, P=.003), MDR-TB (HR 2.00, P=0.028), XDR-TB (HR 2.50, P=0.002), and CD4 cell count less than 200 cells/mm<sup>3</sup> (HR 3.23, P=0.037). In the Cox model examining mortality from time of diagnostic sputum collection (Table 11), all of these associations were greater in magnitude and statistical significance: positive sputum smear

(HR 2.36, P<0.001); MDR-TB (HR 3.09, P<0.001); XDR-TB (HR 4.31, P<0.0001); and CD4 cell count less than 200 cells/mm<sup>3</sup> (HR 4.69, P=0.006). Patient age, sex, extrapulmonary tuberculosis, treatment in the previous year, admission in the previous year, and CD4 cell count less than 50 cells/mm<sup>3</sup> all failed to achieve statistical significance in this model.

### **Drug Resistance Patterns Among Isolates**

Of the 170 patients included in the study, 125 (74%) had a single drug-susceptibility testing (DST) pattern which remained unchanged over their treatment course. Of the remaining 45 patients, 34 had isolates with two different DST patterns, 10 had three DST patterns, and one had four DST patterns.

A total of 229 isolates with differing DST patterns were cultured from the 170 patients in this study (Table 12). These consisted of 74 non-MDR isolates, 70 MDR isolates and 85 XDR isolates. Among the 74 non-MDR isolates, 66 (89%) were fully susceptible, 7 (9%) were mono-resistant (5 to isoniazid (INH), 2 to rifampicin (RIF)), and one (1%) was poly-resistant (INH and streptomycin (SM)). Among the 70 MDR isolates, 17 (24%) were resistant to only INH and RIF. Resistance to INH, RIF and SM (42/70, 60%) was the most common MDR resistance pattern. None had resistance to only INH, RIF and ethambutol (EMB), and 6 had resistance to INH, RIF, EMB and SM.

Of the 85 XDR-TB isolates, 21 (26%) were resistant to INH, RIF, ciprofloxacin (CPX) and kanamycin (KM) only. Six (7.5%) were resistant to INH, RIF, CPX, KM and EMB but not SM, and 24 (30%) were resistant to INH, RIF, CPX, KM and SM but not EMB. Thirty-four (42.5%) were resistant to all six drugs tested.

## Changes in Drug Resistance Patterns

Forty-five patients had multiple sputum susceptibility patterns in the study period, of which 30 patients had sputum isolates that fell into two or more resistance categories (MDR-TB, XDR-TB or non-MDR-TB) (Figure 3). Of the thirty patients with sputum isolates in more than one resistance group, 18 went from a lower to a higher resistance group over time (e.g. non-MDR to MDR), 8 had isolates from the same week in two different resistance groups, and 4 went from a higher to a lower resistance group (e.g. XDR to MDR).

Among the 18 patients who had initial isolates in a lower resistance category followed by later isolates in a higher resistance category, 7 went from non-MDR to MDR, 8 went from non-MDR to XDR, 1 went from MDR to XDR, and 2 went from non-MDR to simultaneous MDR and XDR (Table 13). Among the seven patients having a non-MDR isolate and a subsequent MDR isolate, the mean time between isolates was 146 days (range 69-222 days). Among those having a non-MDR isolate followed by a XDR isolate, the mean time between isolates was 109.5 days (range 69-183 days). For the two patients who had a non-MDR isolate followed by simultaneous isolates of MDR and XDR-TB, the mean time was 191 days (153, 299 days).

For the single patient who had an MDR-TB isolate followed by an XDR-TB isolate, the duration between the isolates was 260 days; the patient received INH,RIF,EMB,SM and pyrazinamide (PZA) for three and a half weeks, followed by second line drugs (ofloxacin, ethionamide, pyrazinamide, amikacin, ethambutol ). SLD were given two months after the isolate for MDR-TB (INH, RIF resistance) and five

months before the isolate for XDR-TB (INH, RIF, SM, CPX, KM resistance) was collected.

Six of the seven patients with a fully susceptible TB in their first isolate and MDR in their subsequent isolate had resistance to SM as well, despite never having received SM during that period. All of the patients whose DST pattern changed from non-MDR to XDR were hospitalized at some point during the period between collection of the isolates, suggesting the possibility of exogenous super-infection with a more drug-resistant strain.

Two patients with MDR had increasing drug resistance: one patient had INH and RIF resistance at baseline and INH, RIF, SM, CPX and KM (XDR-TB) after 260 days; the other patient had INH and RIF resistance at baseline, followed by INH, RIF, SM and KM resistance (still MDR-TB) after 33 days.

Seventy-four percent (52/70) of MDR-TB isolates had SM resistance, and 60% (42/70) of all MDR-TB isolates had the same DST pattern of resistance to INH, RIF and SM, despite the fact that few patients had exposure to SM. Only 5 patients with MDR-TB had prior streptomycin exposure (all had resistance to SM at baseline). An additional nine patients with MDR-TB received SM as part of initial therapy during the study period, among whom only four had SM resistant isolates at baseline. Of these four, two of the sputa were collected within two weeks of treatment start (2 days, 11 days), making acquired resistant highly improbable. The other two were collected 55 and 68 days after start of treatment. No patients receiving streptomycin were observed to have a previously streptomycin susceptible sputum followed by a streptomycin resistant sputum.

**Table 1** Demographics and Baseline Clinical Characteristics and Symptoms by Drug Resistance Group

	Non-MDR	MDR	XDR	P Values		
Total, N	57	52	61	MDR / Non-MDR	XDR / Non-MDR	XDR / MDR
Sex						
Women, n (%)	23 (40.4)	<b>18 (34.6)</b>	<b>34 (55.7)</b>	0.537	0.095	<b>0.025</b>
Age, years						
Mean (S.D.)	34.5 (10.1)	35.4 (8.3)	35.1 (7.8)			
Median (range)	32.6 (14.7-70.6)	33.7 (19.9-56.9)	34.5 (15.4-61.4)	0.420	0.350	0.947
Sputum Smear						
Positive, n (%)	<b>27 (47.4)</b>	<b>37 (71.2)</b>	<b>45 (73.8)</b>	<b>0.015</b>	<b>0.005</b>	0.756
Extrapulmonary TB§						
Present, n (%)	14 (24.6)	16 (30.8)	18 (29.5)	0.469	0.546	0.884
Hemoglobin (g/dL), n	45	43	53			
Median (IQR)	9.0 (7.5-10.5)	9.3 (8.0-11.4)	9.3 (7.8-10.8)	0.237	0.435	0.602
ESR (mm/h), n	28	32	37			
Median (IQR)	115.5 (74-130)	95.0 (53.5-126.5)	100.0 (55-123)	0.187	0.272	0.834
Weight (kg)						
Women, n	15	12	16			
Median (IQR)	53.0 (46.0-60.0)	47.5 (45.0-54.8)	49.25 (43.6-54.0)	0.338	0.252	0.945
Men, n	24	21	22			
Median (IQR)	52.0 (48.0-54.5)	51.0 (45.0-54.0)	50.5 (47.0-60.0)	0.455	0.870	0.301
Weight Loss						
Yes, n (%)	35 (61.4)	33 (63.5)	36 (59.0)	0.918	0.783	0.707
Cough						
Yes, n (%)	46 (80.7)	39 (75.0)	49 (80.3)	0.365	0.802	0.497
Night Sweats						
Yes, n (%)	18 (31.6)	16 (30.8)	23 (37.7)	0.878	0.529	0.440
Fever						
Yes (n,%)	25 (43.9)	19 (36.5)	27 (44.3)	0.392	0.967	0.405

§ All patients had pulmonary TB as well. Extrapulmonary TB patients had pulmonary and extrapulmonary disease.  
Statistically significant values are in bold.

**Table 2** HIV-related Clinical Information By Drug Resistance Group

	Non-MDR	MDR	XDR	MDR / Non-MDR	XDR / Non-MDR	XDR / MDR
Total, N	57	52	61			
				P Values		
Tested For HIV n (%)	47 (82.5)	<b>40 (76.9)</b>	<b>57 (93.4)</b>	0.472	0.065	<b>0.012</b>
HIV Positive n (% of tested)	44 (93.6)	37 (92.5)	57 (100)	1.000	0.089	0.067
On ART						
Before TB Diagnosis						
n (% of HIV+)	2 (4.5)	2 (5.4)	8 (14.0)	1.000	0.180	0.306
Ever						
n (% of HIV+)	11 (25.0)	9 (24.3)	19 (33.3)	0.944	0.334	0.323
VL at Diagnosis*						
n	8	13	20			
Log Median (IQR)	5.6 (5.0-6.1)	5.2 (4.6-6.0)	5.1 (3.2-5.7)	0.690	0.177	0.269
n on ART (VL<25)	0	0	5 (3)			
CD4 at Diagnosis*						
n	24	27	28			
median (IQR)	70.5 (36-131.5)	57 (34-104)	56 (19-156.5)	0.461	0.344	0.561
<50 (n,%)	9 (37.5)	12 (44.4)	12 (42.9)	0.777	0.781	1.000
50-200 (n,%)	10 (41.7)	10 (37.0)	12 (42.9)	0.780	1.000	0.785
>200 (n,%)	5 (20.8)	5 (18.5)	4 (14.3)	1.000	0.716	0.729

VL = Viral Load; OR = Odds Ratio; IQR = Interquartile Range; CI= Confidence Interval; ART = Antiretroviral Therapy

\*Values were included if performed within 120 days of start of TB treatment

CD4 counts are cells / mm<sup>3</sup>; Viral Load is expressed in copies per mL

Statistically significant values are in bold.



**Table 3** Previous TB Treatment and Previous Hospitalizations by Drug Resistance Group

	Non-MDR	MDR	XDR	MDR / Non-MDR	XDR / Non-MDR	XDR / MDR
Total, N	57	52	61			
				<b>OR (95% CI)</b>		
Previous TB Treatment						
Any, n (%)	<b>15 (26.3)</b>	<b>31 (59.6)</b>	26 (42.6)	<b>4.13 (1.84-9.28)</b>	2.08 (0.96-4.53)	0.50 (0.24-1.07)
Past Year, n (%)	<b>2 (3.5)</b>	<b>17 (32.7)</b>	<b>17 (27.9)</b>	<b>13.36 (2.91-61.39)</b>	<b>10.63 (2.33-48.48)</b>	0.80 (0.36-1.78)
Previous Hospitalization						
Any, n (%)	<b>12 (21.1)</b>	<b>22 (42.3)</b>	<b>25 (41.0)</b>	<b>2.75 (1.19-6.38)</b>	<b>2.6 (1.15-5.89)</b>	0.95 (0.45-2.01)
Past 2 Years, n (%)	<b>8 (14.0)</b>	<b>19 (36.5)</b>	<b>23 (37.7)</b>	<b>3.53 (1.38-9.00)</b>	<b>3.71 (1.49-9.20)</b>	1.02 (0.47-2.20)

Previous hospitalizations were not limited to admissions for TB.  
Statistically significant values are in bold.

**Table 4** Risk Factors for MDR-TB in Logistic Regression Model (comparison with non-MDR-TB)

	Unadjusted OR	95% CI	Adjusted OR	95%CI
Male Sex	1.28	0.59-2.78	1.24	0.52-3.00
TB Treatment in Last Year	<b>13.36</b>	<b>2.91-61.39</b>	<b>8.33</b>	<b>1.64-42.33</b>
Hospitalized in Last 2 Years	<b>3.53</b>	<b>1.38-9.00</b>	1.69	0.56-5.09
Sputum Smear Positive	<b>2.74</b>	<b>1.24-6.06</b>	1.59	0.66-3.81

Statistically significant values are in bold.

**Table 5** Risk Factors for XDR-TB in Logistic Regression Model (comparison with non-MDR-TB)

	Unadjusted OR	95% CI	Adjusted OR	95%CI
Male Sex	0.54	0.26-1.12	0.45	0.20-1.03
TB Treatment in Last Year	<b>10.63</b>	<b>2.33-48.48</b>	<b>7.19</b>	<b>1.35-38.17</b>
Hospitalized in Last 2 Years	<b>3.71</b>	<b>1.49-9.20</b>	1.81	0.62-5.25
Sputum Smear Positive	<b>3.13</b>	<b>1.44-6.76</b>	<b>2.79</b>	<b>1.20-6.47</b>

Statistically significant values are in bold.

**Table 6** Risk Factors for XDR-TB in Logistic Regression Model (comparison with MDR-TB)

	Unadjusted OR	95% CI	Adjusted OR	95%CI
Male Sex	0.42	0.20-0.90	0.41	0.19-0.89
TB Treatment in Last Year	0.8	0.36-1.78	0.79	0.31-2.00
Hospitalized in Last 2 Years	1.02	0.47-2.20	1.19	0.49-2.86
Sputum Smear Positive	1.14	0.50-2.61	1.39	0.56-3.42

Statistically significant values are in bold.

**Table 7** Demographics and Baseline Clinical Characteristics by Outcome

	<b>Died</b>	<b>Alive/Unknown</b>	<b>P Values</b>
Total, N	111	59	
Sex			
Female, n (%)	50 (45.0)	25 (42.4)	0.738
Age			
Median (IQR)	33.9 (29.4-39.9)	33.4 (27.9-39.1)	0.480
Extrapulmonary TB			
Present, n (%)	36 (32.4)	12 (20.3)	0.095
Sputum Smear			
Positive, n (%)	<b>79 (71.2)</b>	<b>30 (50.8)</b>	<b>0.007</b>
Hemoglobin (g/dL), n	95	46	
Median (IQR)	9.1 (7.8-10.6)	9.3 (7.5-11.2)	0.958
ESR (mm/h), n	63	34	
Median (IQR)	99 (57-123)	114 (60-130)	0.354
Weight (kg)			
Women, n	26	17	
Median (IQR)	48.3 (45-53.5)	53 (46-60)	0.111
Men, n	44	23	
Median (IQR)	51.5 (46-54.5)	52 (45-56)	0.400

Statistically significant values are in bold.

**Table 8** HIV-related Clinical Information by Outcome

	Died	Alive/Unknown	P Values	OR (95% CI)
Total, N	111	59		
Tested for HIV n (%)	95 (85.6)	49 (83.1)	0.662	
HIV Positive n (% of tested)	92 (96.8)	46 (93.9)	0.409	2.00 (0.39-10.30)
On ART Total n (% of HIV+)	<b>21 (22.8)</b>	<b>18 (39.1)</b>	<b>0.049</b>	<b>0.44 (0.21-0.95)</b>
Before TB Diagnosis n (% of HIV+)	9 (9.8)	3 (6.5)	0.522	1.50 (0.39-5.83)
CD4 at TB Diagnosis* n (% of HIV+)	54 (58.7)	25 (54.3)		
Median (IQR)	53.5 (25-95)	104 (41-225)	0.552	
<50	<b>26 (48.1)</b>	<b>7 (28.0)</b>	<b>0.004</b>	<b>6.69 (1.69-26.45)</b>
50-200	<b>23 (42.6)</b>	<b>9 (36.0)</b>	<b>0.021</b>	<b>4.60 (1.21-17.52)</b>
>200	<b>5 (9.3)</b>	<b>9 (36.0)</b>	<b>0.004</b>	<b>Referent</b>
VL at TB Diagnosis* N	29	12		
Log Median	5.23 (4.63-5.89)	5.28 (2.45-5.82)	0.82	
n on ART (VL < 25)	4 (2)	1 (1)		

VL = Viral Load; OR= Odds Ratio; CI= Confidence Interval; IQR = Interquartile Range

\*Values were included if performed within 120 days of start of TB treatment

CD4 counts are cells / mm<sup>3</sup>; Viral Load is expressed in copies per mL

**Table 9** Previous TB Treatment and Previous Hospitalizations by Outcome

	<b>Died</b>	<b>Alive/Unknown</b>	<b>P Values</b>	<b>OR (95% CI)</b>
Total, N	111	59		
Previous TB Treatment				
Any, n (%)	49 (44.1)	23 (39.0)	0.517	1.24 (0.65-2.35)
Past Year, n (%)	28 (25.2)	8 (13.6)	0.076	2.15 (0.91-5.08)
Previous Hospitalization*				
Any, n (%)	44 (39.6)	15 (25.4)	0.064	1.93(0.96-3.87)
Past Years, n (%)	31 (27.9)	9 (15.3)	0.064	2.15(0.95-4.90)

\*Previous hospitalizations were not limited to admissions for TB.

**Table 10** Risk Factors for Mortality from Start of TB Treatment in Cox Proportional Hazards Model

	HR	95% CI	P value
Male Sex	0.90	0.45-1.35	0.658
TB Treatment in Last Year	1.60	1.08-2.12	0.077
Hospitalized in Last Year	1.57	1.08-2.06	0.073
Sputum Smear Positive	<b>2.09</b>	<b>1.60-2.58</b>	<b>0.003</b>
XDR-TB	<b>2.50</b>	<b>1.92-3.08</b>	<b>0.002</b>
MDR-TB	<b>2.00</b>	<b>1.38-2.62</b>	<b>0.028</b>
CD4 less than 200/mm3	<b>3.23</b>	<b>2.13-4.33</b>	<b>0.037</b>

Statistically significant values are in bold.

**Table 11** Risk Factors for Mortality from Time of Diagnostic Sputum Collection in Cox Proportional Hazards Model

	HR	95% CI	P value
Male Sex	1.09	0.64-1.54	0.718
Treatment in Last Year	1.29	0.78-1.80	0.321
Hospitalized in Last Year	1.24	0.76-1.72	0.378
Sputum Smear Positive	2.36	1.88-2.84	<0.001
XDR-TB	4.31	3.71-4.91	<0.0001
MDR-TB	3.09	2.47-3.71	<0.001
CD4 less than 200/mm3	4.69	3.59-5.79	0.006

Statistically significant values are in bold.

**Table 12** Drug Resistance Patterns Found and Their Frequencies

Resistance Class	Drug Resistance Pattern	Frequency, n*
Drug Susceptible	--	66
Monoresistant	INH	5
	RIF	2
Polyresistant	INH, SM	1
Multidrug-Resistant	INH, RIF	17
	INH, RIF, SM	42
	INH, RIF, KM	1
	INH, RIF, SM, KM	2
	INH, RIF, EMB, SM	6
	INH, RIF, EMB, SM, CPX	2
Extensively Drug-Resistant	INH, RIF, CPX, KM	21
	INH, RIF, CPX, KM, EMB	6
	INH, RIF, CPX, KM, SM	24
	INH, RIF, CPX, KM, EMB, SM	34
Total		229

INH = Isoniazid; RIF = Rifampicin; EMB = Ethambutol;  
SM = Streptomycin; CPX = Ciprofloxacin; KM = Kanamycin

\* Multiple isolates from the same patient with the same resistance pattern were only counted once

**Table 13** Drug Resistance Patterns and Timing of Isolate Collection for Patients with Multiple Resistance Patterns Conferring Changes in Resistance Category

Pt	Outcome	Isolate 1 Resist.	Days	Isolate 2 Resist.	Days	Isolate 3 Resist.	Days	Isolate 4 Resist.	Days	Isolate 5 Resist.
			Iso 1 to Iso 2		Iso 2 to Iso 3		Iso 3 to Iso 4		Iso 4 to Iso 5	
<i>non-MDR to MDR Only</i>										
1	death	none	137	HRS	--	--	--	--	--	--
2	death	none	175	HRES	--	--	--	--	--	--
3	death	none	78	HRS	--	--	--	--	--	--
4	death	none	69	HRS	--	--	--	--	--	--
5	alive	none	187	HR	--	--	--	--	--	--
6	death	none	6	none	222	HRS	--	--	--	--
7	death	none	154	HRES	0	HRS	--	--	--	--
<i>non-MDR to XDR</i>										
8	death	none	94	HRCK	--	--	--	--	--	--
9	death	none	101	HRSCK	1	HRSCK	0	HRESCK	--	--
10	death	none	69	HRESCK	--	--	--	--	--	--
11	unknown	none	48	none	132	HRESCK	72	HRESCK	0	HRSCK
12	Alive	none	80	HRCK	--	--	--	--	--	--
13	Death	none	183	HRESCK	--	--	--	--	--	--
14	Death	none	32	none	68	HRCK	--	--	--	--
15	Death	none	6	none	63	HRSCK	--	--	--	--
<i>non-MDR to MDR/XDR</i>										
16	Death	none	153	HRS	0	HRSCK	--	--	--	--
17	Death	none	229	HRK	0	HRESCK	--	--	--	--
<i>MDR to XDR</i>										
18	alive	HR	260	HRSCK	--	--	--	--	--	--

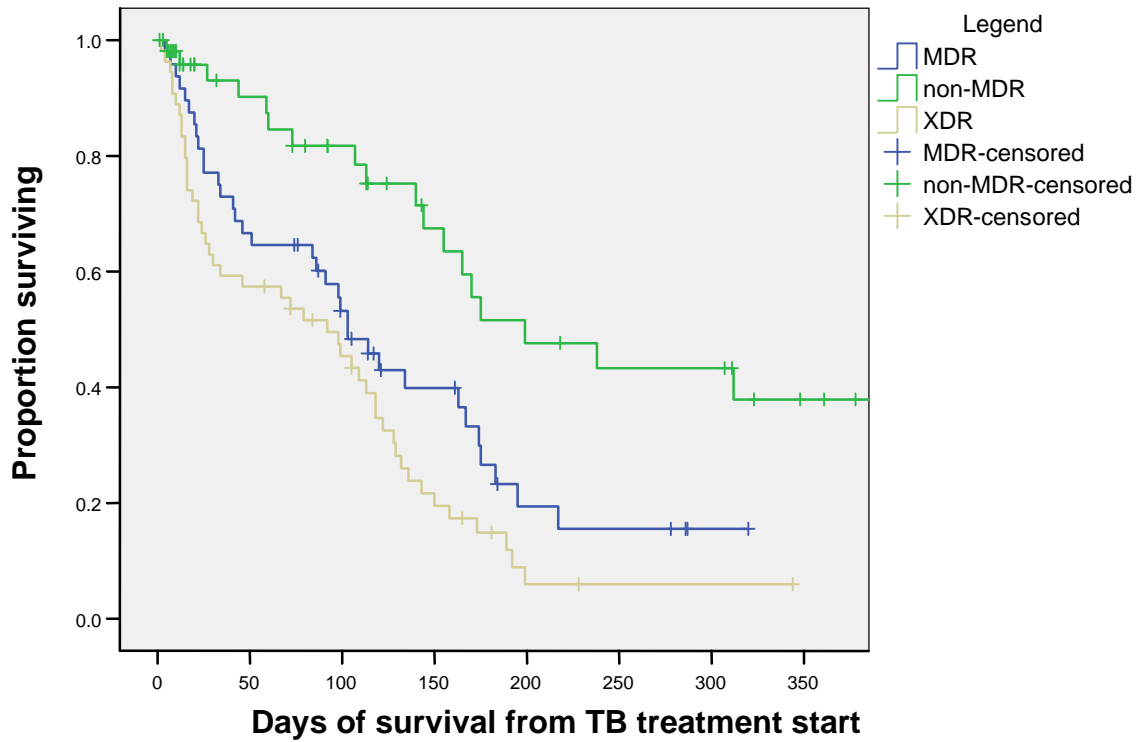
Pt = Patient; Iso = Isolate; Resist. = Resistance

Days Iso 1 to Iso 2 = Days between collection of isolate 1 and isolate 2

H = Isoniazid; R = Rifampicin; E = Ethambutol; S = Streptomycin; C = Ciprofloxacin; K = Kanamycin

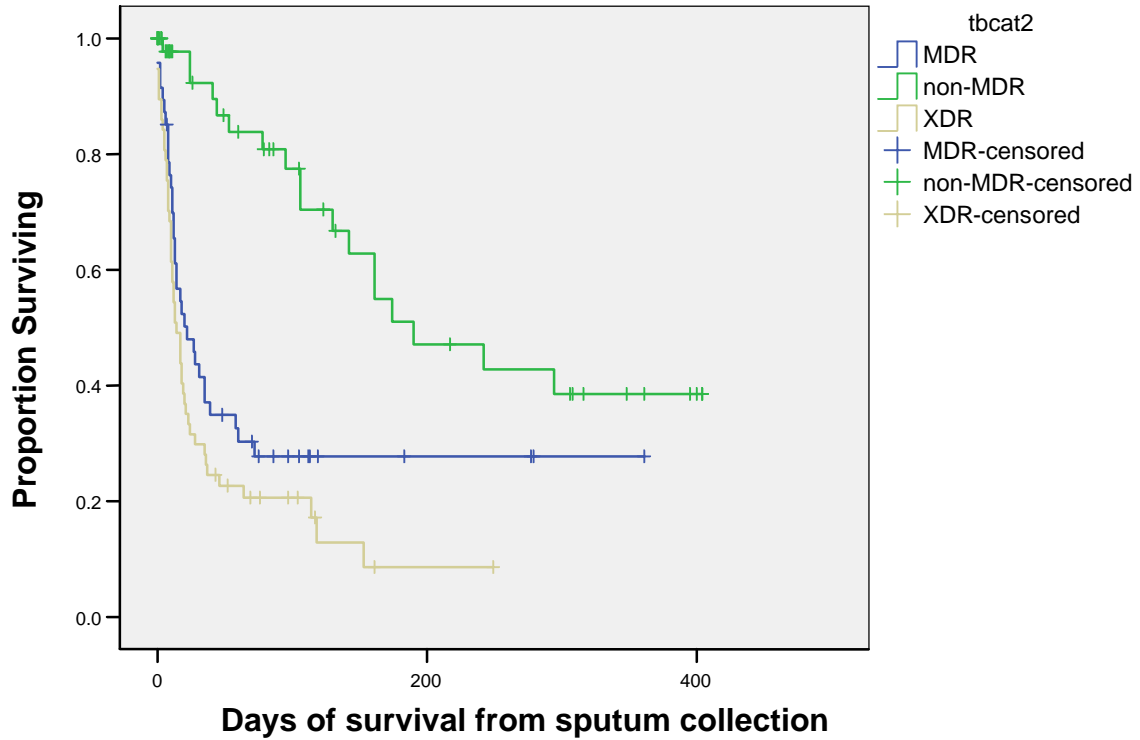


**Figure 1 Survival by Drug Resistant Group from TB Treatment Start\***



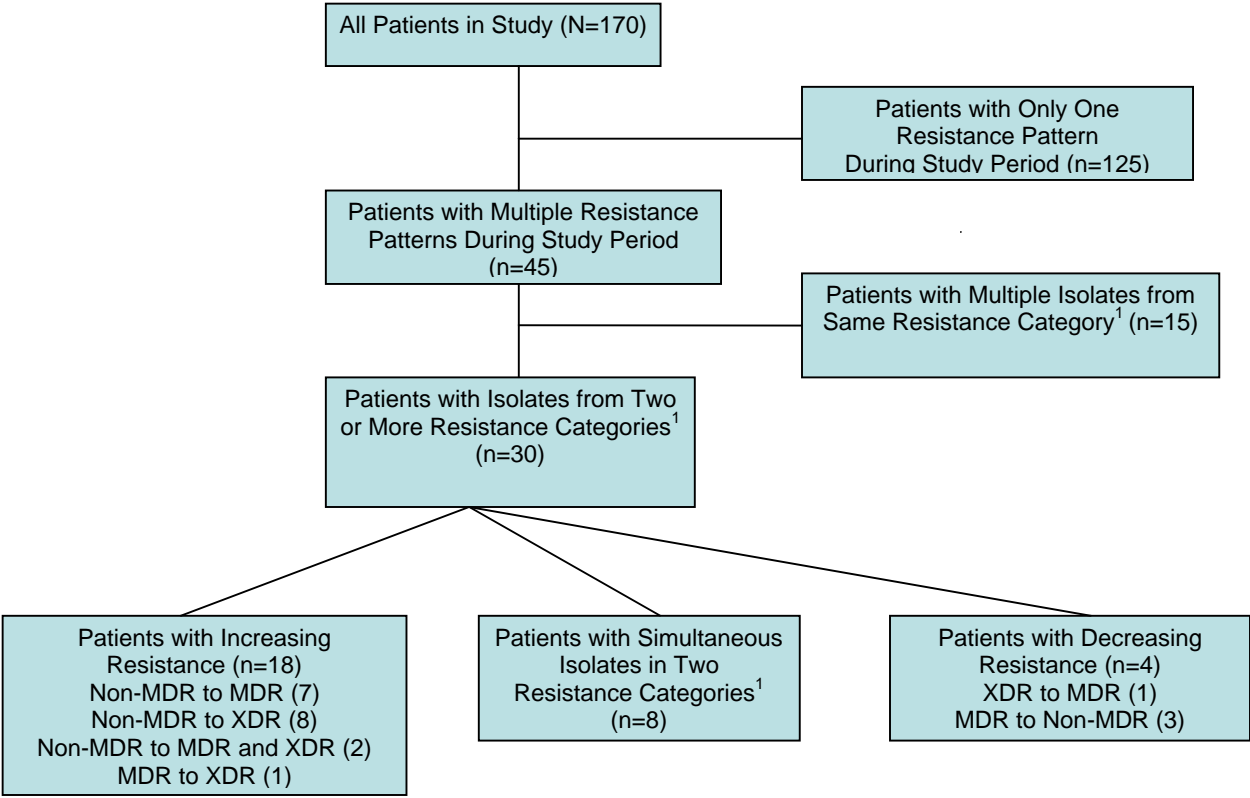
\*Kaplan-Meier survival analysis. Patients were censored (no longer represented) in the survival curve at the last point in time in which they were seen by a health provider.

**Figure 2 Survival by Drug Resistance Group from Sputum Collection Time\***



\*Kaplan-Meier survival analysis. Patients were censored (no longer represented) in the survival curve at the last point in time in which they were seen by a health provider. Sputum collection refers to the collection of the sputum for culture and DST that was used for assignment to a drug resistance group (non-MDR, MDR or XDR).

**Figure 3 Patients with Multiple Resistance Patterns During Study Period**



<sup>1</sup>Resistance categories were MDR-TB, XDR-TB and non-MDR-TB as previously defined.

## DISCUSSION

In this study in rural KwaZulu Natal, South Africa, we performed detailed analysis of cases of drug sensitive and drug resistant TB. In our previous report of XDR-TB in this setting (41), we found near universal mortality, occurring in a median of 16 days after sputum was obtained for TB culture. Many patients received the diagnosis *post-mortem* and most had the opportunity to transmit resistant organisms to others before diagnosis was made. These observations illustrated the critical need for early identification of patients with drug resistant TB and prompted this study. Due in part to the paucity of laboratory capacity for DST in Sub-Saharan Africa, there have been few reports of clinical predictors of MDR-TB in the high TB prevalence and high HIV prevalence settings of this region. We sought to further characterize the clinical manifestations and outcomes of XDR-TB and MDR-TB with comparison to non-MDR-TB in order to identify characteristics that might result in earlier diagnosis. Our results have provided some information which might be of clinical utility in this regard. We confirmed the high mortality associated with XDR-TB in this setting and found high rates of mortality among patients with MDR-TB as well. More than ninety percent of tested patients in all groups were HIV-infected, and a positive sputum smear was independently associated with both drug resistance and mortality in this population. Previous TB treatment in the last year and hospitalization in the past two years predicted drug resistant TB. MDR-TB appeared to be more often due primary drug resistance, including super-infection of fully susceptible TB, than acquisition of drug resistance. Amplification of resistance among patients with MDR-TB and XDR-TB was not widely observed. This study and the findings noted above represent the first description of clinical

characteristics of patients with extensively drug-resistant tuberculosis and the first comparative examination of risk factors and outcomes for non-MDR, MDR and XDR-TB.

### **Clinical Predictors of MDR-TB and XDR-TB**

Clinical predictors of MDR-TB and XDR-TB did not differ significantly, and apart from the interesting association with positive sputum smear, were consistent with previously described risk factors for drug resistant tuberculosis. The strongest clinical predictors of drug resistant tuberculosis (both MDR-TB only and XDR-TB) were TB treatment in the previous year, hospitalization in the previous two years and positive sputum smear. In numerous studies of risk factors for drug-resistant TB, previous TB treatment—particularly in the context of treatment default, treatment failure, or relapsed TB—has been consistently found to predict MDR-TB (67-74). Studies comparing drug susceptible TB and MDR-TB have found previous TB treatment to be associated with odds ratios ranging from 2 to more than 10. Data on outcomes of previous TB treatment were not available for this study. However, studies have found that even among MDR-TB patients found to be cured under short-course chemotherapy, 30% subsequently relapsed (113), suggesting that previous treatment cure may not reliably exclude drug-resistant TB.

While there was a strong association between previous TB treatment and drug-resistant TB, it is of great importance to note our finding that 40% of MDR-TB patients and nearly 60% of XDR-TB patients had no previous TB treatment. Moreover, the majority of MDR-TB patients had streptomycin resistance without previous exposure to

streptomycin. Together, these findings suggest that much—if not the majority—of MDR-TB in this setting is due to transmitted (or primary) drug resistance.

Previous treatment for MDR-TB may not only represent acquisition of drug resistant TB (by incomplete or inappropriate treatment), the sense in which previous treatment is usually assumed to be a risk factor in many of the aforementioned studies. Rather, this association between previous treatment and MDR-TB may, in part, be understood similarly to the association between previous treatment and XDR-TB (the proportion with previous treatment in the MDR-TB and XDR-TB groups were not statistically different), which can be explained as follows. Since no XDR-TB patients had prior exposure to second-line drugs (SLD), XDR-TB was likely all primary resistance. The association with previous treatment was likely due to two causes: many XDR-TB patients were treated with first-line drugs at first TB diagnosis because DST wasn't performed; and previous TB diagnosis resulted in hospitalization, which was a risk factor for super-infection with XDR-TB. While some MDR-TB may have been attributable to acquired drug resistance from previous failed treatment, much of the association with previous treatment may be explained by this other mechanism.

Previous hospitalization and hospitalization in the past two years were significant risk factors for MDR-TB and XDR-TB on bivariate analysis but were not found to be significant independent risk factors on multivariate analysis. The association between previous hospitalization and development of drug resistant tuberculosis may be expected due to the favorable conditions for nosocomial spread of drug resistant TB at COSH; however, the independence of previous hospitalization from previous TB treatment as risk factors might not have emerged due to the fact that the greatest risk for nosocomial

transmission is on the TB wards, and for hospitalized patients to be at great risk of acquiring MDR or XDR-TB, they would have to be undergoing treatment for TB (on these and not other wards). Notably, among the ten patients with pan susceptible isolates who later had an XDR-TB isolate (Table 13), all were hospitalized between the collection of the two isolates.

Overall, 88% of patients were hospitalized at the time of admission, and median hospitalization for non-MDR patients was 13 days. Even during this relatively short stay, these patients might have encountered drug resistant TB. At present, there is inadequate hospital space to provide for isolation of patients with unknown TB resistance patterns, so patients with drug resistant TB (whose status is usually unknown initially) are first admitted to the general TB ward with all other TB patients. Reserving hospital admission to TB wards for patients who are critically ill and shortening hospitalization times may reduce the risk of nosocomial transmission of drug resistant TB.

Female sex was a statistically significant independent risk factor for XDR-TB compared with MDR-TB. There is a lack of additional social analysis necessary to fully explore this association; simple potential explanations that may have contributed to this finding include higher rates of XDR-TB transmission on the Female TB wards, documented higher rates of HIV among women in this region, and the high rates of seasonal labor migration among men, which could either cause them to be disproportionately represented in non-MDR-TB patients while providing a protective factor of being away from a community or hospital with high levels of XDR-TB transmission.

The proportion of patients with a positive sputum smear was higher among XDR-TB and MDR-TB patients than among non-MDR-TB patients. This differential rate may be in part due to period in time between start of TB treatment and collection of sputum for smear and culture (median 7.5 days, third quartile 68 days); patients with non-MDR-TB would be likely to have a reduction in their bacillary load through standard first-line therapy during this time, while patients with MDR-TB and XDR-TB would be less likely to achieve this suppression. Sputum smear detection typically requires 5,000-10,000 organisms per milliliter, while culture can detect as low as 10-100 viable organisms per milliliter (114). Therefore culture could still be positive in the non-MDR-TB patients while smear may have already turned negative. The proportion of patients with positive smear in the MDR and XDR-TB groups in this study were higher than values reported in the literature for HIV patients, and 90% of patients in both groups were HIV positive. Elliott et al. found 43% and 24% of HIV-infected and uninfected patients with culture proven TB to be sputum smear negative (98). Other studies have found higher proportions of sputum negative TB among HIV-infected patients compared with HIV uninfected patients, but not necessarily in the context of culture positive TB (for example, due to extrapulmonary TB) (115). Finally, this finding may represent more severe disease (e.g. cavitary disease) among MDR-TB and XDR-TB patients, many of whom had prior TB treatments and may have been identified later in their course of disease.

The prevalence of HIV did not differ greatly between the three drug resistance groups and for all groups was slightly higher than the reported prevalence of HIV among TB patients in the province; not all patients were tested for HIV, however, and geographic variability of HIV prevalence within the province may partially explain these



findings. There were no HIV negative XDR-TB patients in the study. While the literature has failed to confirm HIV as an independent risk factor for MDR-TB, higher rates of HIV among XDR-TB patients would be expected if we assume all of this to be recent, primary transmission of drug resistant TB. Because HIV infected individuals are more likely to manifest TB disease within the first year after infection, they would be over-represented in the early phases of this emerging drug resistant-TB epidemic compared to non-HIV-infected individuals, who have a lower chance of progressing to primary disease following initial infection. Moreover, HIV infected patients are overrepresented on hospital wards and are more likely to be admitted with TB disease than HIV-uninfected patients; this puts them at increased risk for nosocomial acquisition of XDR-TB.

### **Predictors of Mortality**

Mortality was high for all three drug resistance groups. While mortality was greater and median survival shorter for XDR-TB patients than MDR-TB only patients, the difference was not significant, and the two groups had remarkably similar survival trajectories on Kaplan-Meier analysis (see Figure 1). This may be in part due to the fact that survival prior to receipt of SLD may be very similar for MDR compared to XDR-TB patients. For XDR-TB patients with susceptibility to ethambutol, first line therapy would contain an equal number of active agents against this form of XDR-TB as would be available to those with MDR-TB (ethambutol and possibly pyrazinamide).

In our previous report, median survival for XDR-TB patients was 16 days from time of sputum collection (41). In this study, median survival from sputum collection was 14 days for XDR-TB, 22 days for MDR-TB and 190 days for non-MDR-TB.

Therefore, for MDR-TB and XDR-TB patients, median survival is still shorter than the period of time required for DST results to be available; that is, most patients died before their diagnosis was made. Median survival from time of TB treatment start was much longer for MDR-TB and XDR-TB patients; there are two explanations for this. One is that, despite the hospital's policy of collecting sputum for culture and DST on all patients at time of diagnosis, there was a lag time between TB diagnosis and sputum collection. The median time between treatment start and diagnostic sputum collection was 14 days and 34 days for MDR-TB and XDR-TB patients, respectively. The second explanation is that some patients with MDR-TB and XDR-TB had drug susceptible TB at the start of treatment and acquired MDR-TB or XDR-TB during their initial hospitalization (we found evidence of this in the limited number of patients with multiple sputum isolates). The survival from sputum diagnosis in these patients would more accurately reflect the course of disease for MDR and XDR-TB.

Significant independent predictors of mortality included MDR-TB only group, XDR-TB group, CD4 cell count less than 200 cells/mm<sup>3</sup> and positive sputum smear. Neither previous TB treatment in the past year nor previous hospitalization in the past year were statistically significant predictors of mortality on bivariate or multivariate analysis, though both were higher among those who died, with the effect just above the level of statistical significance. The hazard ratio of mortality from sputum collection for patients with CD4 cell count less than 200 cells/mm<sup>3</sup> was 4.67, highlighting the importance of treating HIV disease as a strategy for blunting the impact of tuberculosis and drug-resistant tuberculosis. Studies have shown that antiretroviral therapy,

particularly if initiated early, can reduce mortality from TB, as well as TB incidence (116, 117).

The fact that positive sputum smear was found to be a significant risk factor for mortality on bivariate analysis was not unexpected given the finding of higher rates of positive sputum smear among MDR and XDR patients; however, it was surprising to see it emerge as a risk factor in the multivariate model. Smear negative tuberculosis has been associated with higher rates of mortality, particularly among HIV-infected individuals and in the context of severe immune compromise (5, 118). This finding may again reflect severity of disease, whereby smear positive patients were more likely to have been identified later in their course of illness; moreover, given that most sputa for smear were collected after initiation of therapy, a negative sputum smear may have reflected early smear conversion in some cases. Chest radiograph review was not performed, but may help in further clarifying this association.

Patients in the surviving group were significantly more likely to receive ART; however, it is difficult to discern whether this represents an actual survival benefit accorded by the use of antiretrovirals or the fact that those surviving had a longer period in which to access antiretrovirals. In patients with a new diagnosis of TB and a CD4 count over 50 cells/mm<sup>3</sup>, ART is often deferred for two months, until the end of intensive phase of tuberculosis therapy. Those surviving for more than two months would thus have a greater chance of receiving ART.

The mortality rate among MDR-TB only patients (73.1%) was high in comparison to values in the literature (107). Only 19% of MDR-TB patients received second-line drugs, due in part to early mortality and loss to follow-up of patients. All patients are

provided SLD through the public hospital system; however, given that the median time to sputum collection was 2 weeks, DST results take three to six weeks, and the median survival among all MDR patients was around three months, many patients died before accessing SLD. Even among patients receiving SLD for MDR-TB, a case series from South Africa found treatment success rates of 47% for HIV negative patients and 38% for HIV positive patients, both well under the norm for WHO DOTS Plus programs (107).

The mortality rate for non-MDR-TB patients (36.8%) was consistent with the values in the literature for mortality (around 40%) among TB patients with untreated HIV in the region (5). However, the follow-up was poor for non-MDR patients in particular, with subsequent hospitalization or death being the most common reasons for having follow-up data on these patients, thereby biasing the Kaplan-Meier curves towards mortality by having little follow-up among surviving patients. The high mortality and similarity to that of untreated HIV disease may be related to the fact that only 20% received ART, as many patients were lost to follow-up. Furthermore, two patients from the non-MDR-TB group went on to develop MDR-TB, and both died; four patients from this group went on to develop XDR-TB, with three dying. Therefore, 5 of the 21 deaths in non-MDR-TB patients, or almost one quarter, involved subsequent development of drug resistant tuberculosis. As later sputum DST were not available for most patients, additional initially drug sensitive patients could well have acquired drug resistant TB. Therefore this figure is a lower limit for the contribution of drug resistant TB to mortality in patients presenting with non-MDR-TB.

### **Observed Drug Resistance Patterns and Changes in Resistance**

Eighty-nine percent of non-MDR isolates were fully susceptible; only seven (9%) isolates were mono-resistant and just one (1%) had poly-resistance. No ethambutol (EMB), streptomycin (SM), kanamycin (KM) or ciprofloxacin (CPX) mono-resistant strains were found. Several small studies have provided indirect and limited evidence that empiric use of quinolones, for pathogens and diseases unrelated to TB or to exclude a diagnosis of pneumonia in cases where a TB diagnosis is possible, may lead to acquired resistance of tuberculosis to quinolones (18-20). The health governance in the Philippines, for example, has taken steps to limit the empiric use of quinolones citing this concern. However, in this study, there were no instances of monoresistance to ciprofloxacin. Except in two isolates, CPX resistance was only seen in the context of KM resistance (likewise, except in 3 isolates, KM resistance was only seen along with CPX resistance), suggesting that primary transmission of a strain with resistance to both drugs, rather than empiric use of either drug, is responsible for resistance seen in this setting.

The predominance of SM-resistant strains among MDR-TB isolates, particularly in light of the fact that few patients received SM, suggests that primary (transmitted) drug resistance accounted for much—perhaps the majority—of MDR-TB in this population. This was evidenced among the small number of patients with multiple sputum isolates: seven patients with fully susceptible tuberculosis had MDR-TB with streptomycin resistance at a later date, and none had received SM during this period; this is strongly suggestive of primary, transmitted resistance. In contrast, only one patient acquired INH and RIF resistance without acquiring SM resistance. Moreover, one permutation—resistance to INH, RIF, and SM—accounted for 60% of all (non-XDR) MDR-TB,

suggesting that a single drug resistant strain may be spreading quickly in this population, perhaps the result of a relative fitness. Genetic analysis (e.g. spoligotyping, restriction fragment length polymorphism) is planned and may further clarify the genetic similarity of these isolates.

Only one patient had an initial isolate of MDR-TB and subsequent isolate of XDR-TB; this patient had exposure to second line drugs for five months between the collection of the two isolates, making it possible that this change represented amplification of resistance.

Among XDR-TB strains, there was heterogeneity of resistance patterns found. The most frequently observed strain (resistance to all six drugs) accounted for less than half (42.5%) of all isolates. It is therefore likely that multiple XDR strains with varying resistance patterns are circulating in this setting; while amplification of resistance among XDR patients was not observed in this study, it is likely to happen when XDR patients with EMB or SM susceptibility are exposed to first line or re-treatment therapy (including SM) for long durations. A subset of patients with resistance to all six drugs had DST performed for pyrazinamide, and PZA-resistance was observed (119). First line therapy or re-treatment therapy used in patients with XDR-TB may thus contain at most only one or two active drugs, which may result in the development of resistance to these agents. Early identification of XDR-TB patients is thus particularly crucial for the preservation of these agents for use in combination with SLD.

### **Study Limitations**

There were several important limitations to this study. Many of these stemmed from the fact that this was a retrospective study in a resource-limited setting based on

chart review; as such, a limited number of charts were available and important data was missing from many charts and other data sources.

Analysis of clinical predictors of drug resistance was limited by missing data for variables identified as potential predictors, including previous TB treatment outcomes, weight changes over the initial months of treatment, two month sputum smear, baseline chest radiograph and two month chest radiograph. Because of the lack of differences in many baseline clinical characteristics, close examination of these clinical predictors in the initial period of TB treatment may provide more useful tools for distinguishing drug susceptible from drug resistant TB. Such data would be more systematically obtained through prospective investigation.

The second major limitation of the study was the short follow-up period and lack of TB outcomes data for non-MDR-TB patients, both of which may have biased the mortality analyses (underestimating mortality in this group by failing to include patients who died at home or elsewhere); this data is currently being collected for patients from this group. However, given that the mortality among this group was very similar to the literature value for TB among patients with untreated HIV disease, it is unlikely that mortality among these patients was significantly underestimated.

A third limitation is that aminoglycoside and fluoroquinolone use for reasons other than tuberculosis therapy were not recorded in the database. It is possible that exposure to these drugs for other purposes impacted resistance; however, given the absence of observed monoresistance for SM, KM or CPX, it is unlikely that short-term exposure to fluoroquinolones or aminoglycosides played a large role in TB drug resistance in this setting.

Drug susceptibility testing by the proportions method is subject to false positives and false negatives; most laboratories report an error of around 1-3% due to variations in performance and interpretation of the test, making clinical judgment an important factor in determining management of drug resistant TB patients. The laboratory reporting results for this study underwent weekly external quality assessment as described in the **Methods**; the risk of cross contamination affecting results was therefore limited.

While assumptions can be made about the role of transmitted drug resistance among MDR-TB and XDR-TB patients in this study given the findings of resistance to drugs in the absence of their exposure, genotype data will further clarify the epidemiology and solidify these findings. Genotyping is underway and analysis of the results with reference to this clinical data is planned.

The final important limitation of these related to the assignment of patients into TB categories. Four patients in the MDR-TB only group and five patients in the XDR group had non-MDR-TB isolates only at the time of TB diagnosis. For clinical predictors and calculations of survival from the time of TB diagnosis, these patients may have been more similar to non-MDR patients than to other MDR only or XDR patients. We felt it was important not to exclude these patients or reassign them to the non-MDR-TB group as they were not selected randomly like the non-MDR-TB group and therefore would have biased outcomes of that group. Additionally, excluding them from the MDR-TB and XDR-TB group would remove all patients with prior fully susceptible TB, biasing those groups away from transmitted resistance. Moreover, it is possible that these patients had mixed infection at the time of diagnosis and under first-line therapy, the drug susceptible strain was suppressed; mixed infections at the time of diagnosis were very



common—ten patients had isolates at the time of diagnosis belonging to two drug resistance groups (more may have been mixed infections in which both strains were not isolated).

Despite these limitations, this represents the largest study of clinical characteristics of drug-resistant tuberculosis in a HIV prevalence setting, and many important observations emerged. Larger, prospective studies are needed to confirm and clarify these findings.

## **Conclusion**

The results of this study indicate that MDR-TB and XDR-TB carry a high mortality in this high HIV-prevalence setting. Because patients did not have prior exposure to SLD and the majority of XDR-TB patients had no previous TB treatment, this study adds evidence to our previous report suggesting that XDR-TB in this setting was predominantly, if not entirely, the result of primary, transmitted drug resistance. Moreover, these results suggest that primary MDR-TB is responsible for much—if not the majority—of MDR-TB in this setting, as well. The median survival for both MDR and XDR-TB patients in this study was approximately three months from TB diagnosis and two to three weeks from sputum collection. This short period leaves only a small window for identification of drug resistance and intervention. Further, the majority of patients are admitted to large, common TB wards before drug resistance diagnosis is made. Thus large numbers of patients are exposed to drug resistant organisms while hospitalized. Together, these highlight the critical need for improved infection control and clinical and laboratory tools for the early recognition of drug resistant TB. These include rapid culture and resistance testing techniques and clinical algorithms. Although

much attention is now focused on the development of sophisticated molecular diagnostics for drug resistance, these are expensive and will likely remain unavailable for most patients in Sub-Saharan Africa. Moreover, currently few sites in Africa can perform DST in the conventional manner, with attendant delay in results. Therefore, further research is needed to elucidate clinical predictors of drug resistant TB, which may help with targeting DST and could result in early identification and treatment in drug resistant patients. We hope that the current work is a step in the development of such procedures.

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