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Acquired anti-tuberculosis drug resistance over the course of treatment for multidrug-resistant tuberculosis in Arkhangelsk oblast, Russia

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Acquired anti-tuberculosis drug resistance over the course of treatment for multidrug-resistant tuberculosis in Arkhangelsk oblast, Russia

By Sarah E Smith

Introduction: The rise of multidrug-resistant tuberculosis (MDR TB), defined as *Mycobacterium tuberculosis* (*M tb*) with *in vitro* resistance to at least rifampin and isoniazid, poses an enormous threat to global TB control and prevention. The Green Light Committee (GLC), a subcommittee of the International Working Group on MDR TB, was created to evaluate, lend guidance and approve MDR TB programs for access to reduced price, quality-assured second-line drugs, the drugs used to treat MDR TB [1]. Unfortunately, even MDR TB programs that follow GLC guidelines observe unacceptable percentages of poor treatment outcomes leading one to suspect that *M tb* may be acquiring additional drug resistance over the course of treatment [2]. Naturally, the question arises what is different about patients whose isolates acquire additional drug resistance over the course of MDR TB treatment? Is the number of effective drugs at the beginning of treatment for MDR TB associated with less acquired resistance and better treatment outcomes?

Methods: To address these questions, demographic, clinical and laboratory follow up data from Arkhangelsk, Russia, a GLC-approved TB program site, were analyzed via multiple logistic regression modeling techniques to identify risk factors associated with acquired drug resistance while controlling for potential confounding.

Results: Patients who were treated with at least 4 effective drugs at the beginning of current MDR TB treatment had a 0.22 risk odds of acquiring drug resistance during treatment compared to patients who did not receive treatment with at least 4 effective drugs while controlling for the number positive follow-up cultures and days spent in the hospital during intensive phase (95% Conf. limit: 0.07, 0.71).

Discussion: Treatment with at least 4 effective drugs from the start of treatment had protective effects against acquiring drug resistance compared to treatment with fewer than 4 effective drugs. In other words, patients who were treated with fewer than 4 effective drugs had a significantly increased risk of acquired drug resistance compared to patients who were treated with at least 4 effective drugs. Future research should incorporate other aspects of drug effectiveness including dosage and drug quality.

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INTRODUCTION

Tuberculosis (TB) is a disease of poverty; 95% of cases and 99% of deaths due to TB occur in low and middle income countries representing 85% of the world's population [3]. The World Health Organization (WHO) estimates that one third of the world's population is infected with *Mycobacterium tuberculosis (M tb)*, the infectious agent causing TB disease [3]. TB is curable with available anti-TB drugs but treatment efficacy is compromised when drug resistance is present. The rise of multidrug-resistant (MDR) TB, defined as *M tb* with *in vitro* resistance to at least rifampin (RIF) and isoniazid (INH), poses an enormous threat to global TB control and prevention. Treatment of TB without RIF or INH, the two most effective anti-TB drugs, extends the duration of treatment to at least 2 years as opposed to 6 months for drug-susceptible TB, increasing the chances of acquiring additional drug resistance. Furthermore, the drugs used to treat MDR TB, second-line anti-TB drugs (SLDs), are more costly, more toxic, and less effective than first-line anti-TB drugs. The Green Light Committee, a subcommittee of the International Working Group on MDR TB, was created to evaluate, lend guidance and approve MDR TB programs for access to reduced price, quality-assured SLDs [1]. Programs are encouraged to use strategies outlined in the WHO guidelines for programmatic management of MDR TB [4]. These DOTS-Plus programs are designed to improve treatment outcomes and minimize the risk of acquired drug resistance for MDR TB patients.

Unfortunately, even programs that follow DOTS-Plus guidelines observe unacceptable percentages of poor treatment outcomes leading one to suspect that *M tb* may be acquiring additional drug resistance over the course of treatment [2]. With the limited treatment options for MDR TB patients, acquiring additional resistance complicates treatment even more and leads to less treatment success [5]. Naturally, the question arises what is different about patients whose isolates acquire additional drug resistance over the course of MDR TB treatment? Is the number of effective drugs at the beginning of treatment for MDR TB associated with less acquired resistance and better treatment outcomes? To address these questions, demographic, clinical and laboratory follow up data from Arkhangelsk, Russia, a GLC-approved TB program site, were analyzed via multiple logistic regression modeling techniques to identify risk factors associated with acquired drug resistance while controlling for potential confounding.

BACKGROUND

TB is a disease of poverty; 95% of cases and 99% of deaths due to TB occur in low and middle income countries representing 85% of the world's population [3]. The WHO estimates that one third of the world's population is infected with *M tb*, the infectious agent causing TB disease [3]. Of those infected with *M tb* who do not receive treatment for latent TB infection, about 5% to 10% will develop TB disease over their lifetime; however, this rate is amplified to about 10% per year for those with HIV infection [6]. In countries with the highest TB burden, the combination of low economic resources coupled with high case load has overloaded existing health systems. Global efforts have been made to aid these countries in TB control and prevention; however, these efforts have been compromised by drug-resistant TB.

TRANSMISSION AND DIAGNOSIS

Pulmonary TB is spread through airborne droplet nuclei, particles 1 to 5μm in diameter containing *M tb* [7]. When someone with active pulmonary TB disease of the lungs coughs, sneezes, shouts or sings, droplets of phlegm are launched into the air in the person's immediate vicinity. The moisture content evaporates rapidly and what remains are called "droplet nuclei." Droplet nuclei can remain suspended in the air for hours. People who breathe in air containing droplet nuclei can become infected. Transmission of TB is preventable by good airborne infection control practices. Infection control techniques range both in price and complexity: the cheapest and easiest solution is as simple as opening windows to allow fresh air to enter an enclosed area. However, even

this strategy is often not an option for places with uncomfortable outside air temperatures. Without good infection control practices in place, drug-resistant *M tb* may spread from a contagious patient to other individuals [8]. Poverty creates a breeding ground for TB infection due to overcrowded living conditions, poor access to medical care, and lack of education on disease transmission.

Patients are typically diagnosed with pulmonary TB by a combination of clinical symptoms and signs, chest radiography, and bacteriological laboratory tests [9]. There are three levels of TB laboratories which perform bacteriological laboratory tests. Resource limited settings, areas with low population density, or areas with few cases rely primarily on level I laboratories to perform acid-fast bacilli (AFB) smear microscopy examination of sputum specimens [10]. Typically, a patient with a high bacillary load, reflecting a high likelihood of being contagious, will have a positive smear result. AFB smears produce results relatively fast (same day), are inexpensive and require modest technical skills to perform. However, this test is not specific to just *M tb*, but instead identifies all acid-fast bacilli such as non-TB mycobacteria, resulting in false positive diagnoses. In addition, sputum must contain at least 10,000 AFB/milliliter to be able to detect at least one bacillus in 100 oil-immersion (1000x) microscope fields. In countries with greater resources the most common laboratory diagnostic is mycobacterial culture, defining Level II laboratories [10]. *M tb* can be cultured on solid media (egg based, Löwenstein–Jensen (LJ), or agar based, Middlebrook7H10 (MB) or 7H11 medium) and liquid media (BACTEC or MB 7H9). Culture results in liquid media are observed in 1-3 weeks, compared with 3-8 weeks on solid media [11]. Species identification and drug

susceptibility testing (DST) requires Level III laboratories with an even higher level of sophistication and capability. Therefore, DST is not performed initially in most countries. In these settings, the effectiveness of treatment is assessed and monitored by microscopy of serial sputum specimens during treatment. Converting to smear (or culture) negative status is an indicator of adequate treatment as long as the sputum specimens remain negative. If the smears/cultures fail to convert to negative or if they become positive after a series of negative results, DST may be performed at that point in many countries. There are four conventional methods of detecting drug resistance in mycobacterium: method of absolute concentrations, method of proportions, method of proportions modified for liquid media, and resistance ratio method [10].

TREATMENT AND TREATMENT OUTCOMES

TB is a curable disease with currently available anti-TB drugs; however, drug resistance reduces the efficacy of treatment. INH and RIF are the two most effective drugs currently available that, together with pyrazinamide (PZA) and ethambutol (EMB), are used to cure drug-susceptible TB. However, after the development and widespread use of INH and then RIF, TB cases were observed caused by organisms with *in vitro* resistance to RIF and INH. Resistance to at least these two drugs is defined as MDR TB [12]. Later through similar mechanisms, a subgroup of MDR TB, named extensively drug-resistant (XDR) TB, was defined in which the patient's isolates had additional resistance to fluoroquinolones and second-line injectable drugs, the two most important classes of SLDs used to treat MDR TB patients [13].

Treatment of TB requires at least 4 drugs and takes from 6 months to 2 years depending on susceptibility of the *M tb* isolate. The WHO recommends that new TB cases are prescribed 4 first-line, anti-TB drugs (RIF, INH, EMB, and PZA) for 2 months (intensive phase), then 2 of the 4 drugs (RIF and INH) are continued for 4 more months (continuation phase) under direct observation [14]. TB patients are treated in phases according to the populations of bacilli within the patient. The 4-drug regimen used in the beginning quickly reduces the bacillary load to a non-contagious level by killing the actively replicating bacilli. The continuation phase is necessary to eradicate so-called "persisters" that grow slowly or intermittently. Anti-TB drugs are grouped into first- and second-lines according to their *in vivo* efficacy and safety against *M tb*. Second-line treatment for patients with multidrug-resistant TB is often lengthy and complex; however, prompt initiation of effective treatment is associated with sputum culture conversion to negative in over half of all patients within 3 months as well as overall improved outcomes [15]. Second-line anti-TB drugs are grouped by their chemical structures into 6 classes: fluoroquinolones, aminoglycosides, cyclic polypepties, thioamides, para-aminosalicylic acid (PAS) and cycloserine/terizidone. Aminoglycosides and cyclic polypeptides (collectively called second-line injectable drugs) and fluoroquinolones are the two most important classes of second-line drugs due to their superior efficacy relative to the other groups of second-line drugs [16, 17].

TB treatment outcomes are classified according to a combination of clinical and microbiological characteristics. Drug-susceptible TB treatment outcomes are defined below according to WHO standards [18].

Cure: A patient whose sputum smear or culture was positive at the beginning of the treatment but who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.

Treatment completed: A patient who successfully completed treatment but who does not have a negative sputum smear or culture result in the last month of treatment and on at least one previous occasion.

Treatment failure: A patient whose sputum smear or culture is positive at 5 months or later during treatment. Also included in this definition are patient found to harbor a multidrug-resistant (MDR) strain at any point of time during the treatment, whether they are smear-negative or –positive.

Death: A patient who dies for any reason during the course of treatment.

Default: A patient whose treatment was interrupted for 2 consecutive month or more.

Transfer out: A patient who has been transferred to another recording and reporting unit and whose treatment outcome is unknown.

Treatment outcome definitions for cure, treatment completed and treatment failure differ for patients treated for MDR TB [12].

Cure: An MDR TB patient who has completed treatment according to country protocol and has been consistently culture-negative (with at least five results) for the final 12 months of treatment. If only

one positive culture, and there is no concomitant clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures, taken at least 30 days apart.

Treatment completed: An MDR TB patient who has completed treatment according to country protocol but does not meet the definition for cure or treatment failure due to lack of bacteriologic results.

Treatment failure: Treatment will be considered to have failed if two or more of the five cultures recorded in the final 12 months are positive, or if any one of the final three cultures is positive (treatment will also be considered to have failed if a clinical decision has been made to terminate treatment early due to poor response or adverse events).

TB treatment outcome definitions are an essential part of the recording and reporting system in most national TB control programs (NTPs) allowing for the assessment of program performance over time and comparisons across countries [3].

ANTI-TB DRUG RESISTANCE

Drug-resistant TB occurs by two mechanisms: primary resistance or secondary resistance. Primary resistance occurs when an individual is infected initially with a drug-resistant strain. Secondary resistance, or acquired resistance, refers to the emergence of a drug-resistant population of bacilli during treatment of an individual who initially had drug-susceptible TB. Tubercle bacilli naturally develop drug resistance via spontaneous

genetic mutation [19]. Inadequate treatment regimens or noncompliance with treatment regimens kills susceptible bacilli and selects for resistant bacilli. However, in areas with high rates of transmission and poor infection control a second infection with a different strain may occur during treatment. Advances in technology and research into the *M tb* genome have allowed for the capacity to detect many of the DNA mutations associated with drug resistance via gene sequencing. If the *M tb* strain is in fact the same strain as that initially recovered and the DST results change, then one supposes there was acquired resistance. Conversely, if the *M tb* strain is found to be a different strain from the initial isolate then the change in drug resistance was caused by a different *M tb* strain or by laboratory cross-contamination.

THE DOTS-Plus PROGRAM

In April 1993 the WHO declared TB a global public health emergency and within a year launched an adapted version of Dr. Karel Styblo's program for control of TB later named Directly Observed Therapy, Short-course (DOTS) - a strategy with five key components to control TB in resource-limited settings [20]. The DOTS strategy includes (1) political commitment with increased and sustained financing, (2) case detection through quality-assured bacteriology, (3) standardized treatment with supervision and patient support, (4) an effective drug supply and management system, and (5) a systematic monitoring and evaluation [20]. This strategy has proven to be effective in controlling drug-susceptible TB in many countries [3]. Since 1995, 46 million patients have been successfully treated and up to 6.8 million lives have been saved through DOTS and the Stop TB strategy [3]. Unfortunately, the original DOTS strategy was not effective against drug-resistant TB

because diagnosis was based on microscopy and treatment was standardized for drug-susceptible TB [21]. Due to global increases in MDR TB, the International Working Group on MDR TB was created to bring together clinical, program, and laboratory experts to develop guidelines for MDR TB case management in resource limited settings. In 1999 the working group developed DOTS-Plus for MDR-TB, expanding each of the 5 facets of DOTS to address the complexities of diagnosis and treatment associated with MDR-TB [4]. More specifically, the strategy includes culture and DST, treatment strategies that use second-line drugs under proper management conditions, an uninterrupted supply of quality assured second-line drugs, and a recording and reporting system modified specifically for DOTS-Plus programs.

One of the key elements of DOTS-Plus is the use of DSTs to guide treatment with SLDs under proper management conditions, in other terms, individualized treatment. The importance of individual treatment in areas with a high burden of drug-resistant TB has been supported by many studies [5, 22]. Individualized treatment (based on DST) can reduce the risk of acquired drug resistance and subsequent treatment failure [15]. In countries with a high prevalence of pre-existing drug-resistant TB, the use of standard DOTS regimens has amplified first-line drug resistance. A study in Tomsk oblast, Russia found that 17% of TB strains from patients infected with polyresistant (resistance to more than one anti-TB drug) TB amplified their drug resistance when treated with standard short-course chemotherapy [5]. Furthermore, a study of patients in whom standardized retreatment failed in Lima, Peru demonstrated that 83% of patients had MDR-TB strains,

acquiring resistance to at least one additional drug (including second-line anti-TB drugs) during the course of standardized retreatment [22].

In the context of drug-resistant TB, the consequences of under-investment in basic activities to control TB, poor management of the supply and quality of anti-TB drugs, improper treatment of TB patients, and transmission of the disease in congregate settings are even more detrimental than with drug-susceptible TB. The emergence of drug-resistant strains of *M tb* threatens global TB control programs due to the decreased effectiveness, high cost and toxicity of SLDs as compared to drugs used to cure fully susceptible TB. In countries with low resources, the treatment of patients with MDR-TB is absent or inadequate as a result of the high cost of diagnosis and treatment of drug-resistant TB. Consequently, public health leaders debated the value of treating MDR-TB cases, for example, whether it diverted resources from key DOTS programs in these poorer areas of the world [23]. The substantial consequences to health systems of ignoring drug-resistant TB have become apparent; therefore, countries affected most by drug-resistant TB have adopted programs to prevent, control and treat drug-resistant TB [24].

THE GREEN LIGHT COMMITTEE

Expanding access to expensive, second-line drug treatment for drug-resistant TB is critical, but increasing use of these drugs, especially in programs with the most drug-resistant TB, carries with it the risk of increasing resistance to these same drugs. To increase global access to quality-assured second-line drugs at reduced prices while at the

same time preventing the emergence of even worse resistance, the WHO and the Stop TB Partnership formed the Green Light Committee (GLC), a subgroup of the International Working Group on MDR TB [1]. The GLC is an independent group of experts in programmatic, scientific, and clinical aspects of TB that serves WHO in a technical advisory capacity. Countries that wanted to launch DOTS-Plus programs applied to the GLC and the GLC carefully reviewed which programs were prepared to utilize the discounted second-line drugs in accordance with its aim to increase access to these TB drugs while at the same time preventing the emergence of resistance [25]. By 2005, there was substantial evidence from GLC-approved programs of the cost-effectiveness, effectiveness and feasibility of MDR TB management under programmatic conditions in middle- and low-income countries. This led to the expansion of existing programs and encouragement for programs of all sizes to apply to the GLC [26]. Programs were approved to receive the discounted second-line drugs if they had the capacity and expertise to diagnose and treat MDR TB effectively, including laboratory capacity and infection control strategies. The principles used by the GLC when considering applications were described in the WHO document *Guidelines for Establishing DOTS-Plus Projects for the Management of Multidrug-Resistant Tuberculosis* as guidelines to be utilized by both small-scale and national MDR TB programs [4].

TUBERCULOSIS IN ARKHANGELSK, RUSSIA

WHO estimates that almost half of all TB cases in countries of the former Soviet Union involve resistance to at least one drug and that one in five cases involve MDR-TB [27]. Furthermore, MDR-TB cases in this region have more extensive resistance patterns and

the highest prevalence of XDR-TB compared to the rest of the world [27]. In Russia rates of MDR-TB in new cases vary from 8.8% to 15% in different regions [27]. Within Russia, the highest rates of MDR-TB in new (13.5%) and previously treated (60%) cases have been observed in Arkhangelsk oblast, located in the northwestern part of Russia [28]. DOTS has been in effect in Arkhangelsk since 1998 [28]. Before 1998, patients were treated with non-standardized regimens of three drugs, often including second-line drugs (usually ethionamide or kanamycin). Severely ill patients were prescribed four drugs. There was no supervision to ensure treatment was taken. The oblast experienced a problem with irregular supply of second-line drugs in the 1990s when the economic crisis in Russia resulted in insufficient financial support of the healthcare system [28]. The emergence of drug resistance stemmed from interruption of treatment due to noncompliance, irregular drug supply and inadequate treatment regimens [29]. Furthermore, the large reservoir of latently *M tb* infected individuals in combination with the spread of HIV has increased TB disease [30]. Only within the past 15 to 20 years has it been possible for Russia to collaborate in the field of TB with international organizations. In 2003, Arkhangelsk applied for a pilot program and was approved by the GLC to receive second-line anti-TB drugs [31].

The Preserving Effective Tuberculosis Treatment Study (PETTS), launched in 2004, aims to evaluate the extent to which the GLC has achieved its goal of preventing acquired drug resistance. The study includes 9 countries; with 26 clinical sites. The protocol required a duplicate of all positive mycobacterial cultures from enrolled patients to be sent to CDC for centralized DST and genotyping. Due to unexpected political challenges, the

specimens from Arkhangelsk could not be sent to CDC laboratories for analysis and therefore could not be included in PETTS. Consequently, CDC investigators arranged for Arkhangelsk isolates to be tested at the State Research Center for Applied Microbiology and Biotechnology (SRCAMB) in Obolensk, Russia. Arkhangelsk enrolled two cohorts, one just after GLC approval and another 4 years after receiving drugs through the GLC.

Unfortunately, even programs that follow the DOTS-Plus guidelines observe unacceptable percentages of poor treatment outcomes leading one to suspect that *M tb* is acquiring additional drug resistance over the course of treatment [2]. With the limited treatment options for MDR TB patients, acquiring additional resistance complicates treatment even more and leads to less treatment success [5]. Naturally, the question arises, what is different about patients who acquire additional drug resistance over the course of MDR TB treatment in DOTS-Plus programs? Furthermore, is the number of effective drugs at the beginning of treatment for MDR TB associated with less acquired resistance and better treatment outcomes? To address these questions, demographic, clinical and laboratory follow up data from Arkhangelsk, Russia, a GLC-approved TB program site, were analyzed via logistic regression modeling techniques to identify risk factors significantly associated with acquired drug resistance while controlling for confounding.

METHODS

NULL HYPOTHESIS

There is no difference in the frequency of acquired second-line anti-TB drug resistance over the course of treatment of patients who were treated with at least 4 effective drugs at the start of treatment compared with patients who were treated with fewer than 4 effective drugs.

STUDY DESIGN

This project is a secondary analysis of data collected from 2 sequential prospective cohorts of MDR TB patients treated in Arkhangelsk. Standardized clinical and demographic data were recorded on paper forms created for this purpose. The data were double-entered into an Epi Info database in Arkhangelsk then sent to the International Research and Programs Branch (IRPB) of the Division of Tuberculosis Elimination of the Centers for Disease Control and Prevention. Laboratory data were sent to IRPB from the SRCAMB laboratory in Obolensk, Russia. This analysis is the first analysis performed on this subset of PETTS data.

The protocol for the PETTS study was approved by CDC and Russian IRB. This secondary analysis was exempt from further IRB approval by Emory IRB (Appendix B).

STUDY POPULATION

Two cohorts of consecutive, consenting adult patients with locally confirmed pulmonary MDR TB were enrolled in Arkhangelsk oblast, Russia: 81 individuals in 2005-2006 (Cohort I) and 121 in 2007-2008 (Cohort II). All those enrolled were starting treatment with 2nd-line drugs at the time of enrollment. Pulmonary TB and MDR TB case definitions were consistent with those outlined in WHO guidelines [14]. To be included, patients had to have at least one positive culture for *M tb* within one month (before or after) of the day of starting second-line drugs for the treatment for MDR-TB and to continue 2nd-line treatment for at least one month.

Patients were excluded from analysis if:

1. They had only extrapulmonary TB without pulmonary involvement.
2. They were documented to have been exogenously reinfected by another patient.
3. Prisoners, pregnant women, and children under 18 years of age.

DATA COLLECTION

Patient clinical data were collected from patient charts during the course of treatment using standardized forms. Data on patient demographics, diagnostics, treatment, outcome, comorbidities, and social characteristics were collected. Baseline and follow-up sputum specimens were cultured in the Arkhangelsk oblast TB laboratory, and positive cultures were sent to the State Research Center for Applied Microbiology and Biotechnology (SRCAMB) in Obolensk, Russia for 1st- and 2nd-line DST, genotyping, and DNA sequencing.

LABORATORY TESTING

Sputum samples were collected from each patient at the start of treatment and monthly until treatment was complete. The first isolate was from a specimen collected within 30 days, before or after, the patient started 2nd line TB drugs and is considered the baseline isolate. The Arkhangelsk Regional TB Dispensary shipped to the State Research Center for Applied Microbiology & Biotechnology (SRCAMB; Obolensk, Russia) 245 *M tb* isolates derived from 41of 81 patients (about 5 isolates per patient) in Cohort I and 495 *M tb* isolates (in thawed liquid nutrient medium) obtained from the 128 patients (range of 1 to 16 isolates per patient) in Cohort II. Forty of 81 patients in cohort I had only a single positive baseline culture and no follow-up cultures.

As soon as the isolates were delivered to SRCAMB, they were unpacked and registered. Dates of isolation were confirmed. Registration forms were copied both to Arkhangelsk and Atlanta.

In the SRCAMB lab, each isolate was cultured in ~6 ml of MB 7H9 broth to an optical density of ≥1.0 McFarland standard, as well as on LJ medium. Five aliquots of 1 ml each for each viable culture were prepared. Two aliquots were frozen at -70C. Three aliquots were kept at +4°C for study purposes.

Drug susceptibility for the first (baseline) and the last follow-up isolates from each patient were determined for the following anti-TB drugs: isoniazid, rifampin, ethambutol, streptomycin, kanamycin, amikacin, capreomycin, ofloxacin, ethionamide, and para-

aminosalicylic acid. Drug susceptibility was determined by method of proportions according to the CDC Protocol [10]. Isolates were genotyped by MIRU-VNTR and RFLP-IS6110 to determine genotype for first and last isolates which differed in drug susceptibilities. When DST data differed for the first and last isolates, and genotypes were the same, DST was repeated for all of the intermediate isolates to identify a point in time when differences among the isolates appeared.

DATA MANAGEMENT

The de-identified clinical and demographic dataset from Arkhangelsk was received in Microsoft® Access. The laboratory results from SRCMB were received as a Microsoft® Word Document from which the data were imported into Microsoft® Excel. The two datasets were merged by patient study ID number in SAS.

STATISTICAL ANALYSIS

Exposure variables: The main exposure variable was treatment with at least 4 drugs which the local baseline DST results reported as susceptible. This was coded as a binary variable: RX4SUS=1 if the patient was treated with at least 4 drugs which the baseline DST results reported as susceptible within one month (30 days) from the reported results, and RX4SUS=0 if not.

Outcome variables: The main outcome of interest was acquired resistance. This binary variable was coded as "acquired resistance" if the final DST results showed decreased susceptibility *in vitro* compared with the first DST result and genotyping confirmed it

was the same strain. The variable was coded as "not acquired resistance" (1) if there was no change in DST results, (2) there was a change in DST results but from resistant to susceptible, (3) there was change in DST but confirmed by genotyping to be a different strain, or (4) the patient died, defaulted or converted to culture negative after the baseline DST and there were no further positive cultures. The secondary outcome of interest was TB treatment outcome as defined by the WHO and noted above [12]. Treatment outcome was coded as a nominal variable into four categories. Cure and treatment completed were grouped to represent treatment success. The other three treatment outcome categories were treatment failure, death and default. All other treatment outcomes (transfer, continuing treatment, and unknown) were set to missing.

Covariates: The covariates initially considered were determined from reviewing literature and from data available in the PETTS dataset. Basic demographic (sex, age, marital status, employment, and educational background), patient characteristics (history of imprisonment, high risk occupation, alcohol abuse, housing status, tobacco smoking, and illicit drug use), and clinical (comorbidities prior to enrollment, site of current MDR TB disease, radiographic extent of pulmonary disease, body mass index (BMI) at diagnosis of current MDR TB episode) variables were considered. Age was grouped into quartiles (AGE4): 18-34 years, 35-41 years, 42-48 year, and 49 or older. Marital status was regrouped as single/never-married, currently married, separated/divorced/widow(er)ed, or other. An overall comorbidity variable (COMORB) was created where 0=no known comorbidities and 1=at least one known comorbidity. Comorbidities included HIV infection, active hepatitis/cirrhosis, diabetes mellitus, chronic renal insufficiency,

vomiting/diarrhea, gastric or duodenal ulcer, seizure disorder, major psychiatric or mental disorder, or immunosuppressive diseases (each coded as 0=no, 1=yes, 2=not tested, or 9=unknown). BMI was calculated by the patient's weight (in kilograms) divided by height (in meters) squared and categorized (BMIcat) as underweight (<18.5), normal weight (18.5-24.9), overweight (25-29.9) and obese (30 and above) according to CDC adult BMI definitions [32]. The patients TB history was also of interest. More specifically, patients were classified by prior treatment history and prior treatment outcome. The cohort in which the patient was enrolled was also considered: cohort I enrolled in 2005-2006 and cohort II enrolled in 2007-2008. Number of positive follow-up cultures was recategorized into tertiles (<2, 2-3, and 4 or more) as was number of days spent in the hospital during intensive phase of treatment (<117, 117-232, and 233 or more).

Analysis: The analysis consisted of a traditional epidemiologic analysis for a prospective cohort study with one main exposure and one main outcome of interest. Logistic regression modeling was used to determine the effect of the main exposure of interest (treatment with at least 4 drugs initially susceptible) on a dichotomous measure of acquired resistance as defined above controlling for other factors.

First, univariate distributions of each variable in the analysis were examined for shape, missing values, and outliers. Continuous and categorical variables were recategorized as described above.

Second, bivariate associations were examined. Covariates were screened for possible confounders first by assessing their statistical association with exposure and with outcome (among the unexposed) with the chi-squared test and Fisher's exact test under the null hypothesis of no association. Continuous variables were assessed with the t-test and the Wilcoxon rank sum test when assumption of normality was violated. Covariates that were not associated with either exposure or outcome at $\alpha=0.1$ level were dropped from further analysis. Cut points to categorize continuous and ordinal variables were re-examined to create categories which evenly divided the population and contained sufficient numbers of TB cases for further analysis.

Third, the crude odds ratio (OR) with 95% confidence limits was calculated between acquired drug resistance and treatment with four or more effective drugs and then five or more effective drugs.

Fourth, remaining covariates were assessed for confounding by comparing logistic regression models: a simple bivariate model containing only the main exposure and outcome, and a model containing in addition the covariate. Covariates were assessed for confounding by comparing the crude and adjusted odds ratios for the main effect for meaningful differences.

Fifth, the final logistic regression models were executed which included outcome (0,1) as the dependent variable, exposure (0,1) as the main independent variable, and the important covariates as determined per the preceding paragraph. Crude and adjusted odds

ratios were compared for meaningful differences and the ratio of the upper 95% confidence limit over the lower 95% confidence limit was compared for meaningful changes in precision.

Finally, sensitivity analysis was performed by considering different cut points of number of effective drugs with treatment with less than 4 effective drugs as the referent and the association with acquired drug resistance.

In addition, polytomous logistic regression was performed which included treatment outcome as the dependent variable (treatment success as the referent group) and acquired drug resistance as the independent variable. Polytomous logistic regression with initial effective treatment as the independent variable was also considered.

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

There were 202 MDR TB patients enrolled in Arkhangelsk oblast, Russia: 81 patients enrolled between January 1, 2005 and December 31, 2006 (cohort I) and 121 patients enrolled between January 1, 2007 and December 31, 2008 (cohort II). Of these 202 patients, 16 (7.9%) had *M tb* specimens which acquired resistance over the course of treatment for MDR TB confirmed by changes in DST, genotyping and gene sequencing performed by SRCAMB.

Descriptive statistics were performed on variables to describe the study sample (Tables 1 and 2). There were 31 females (15.4%) and 171 males (84.7%) enrolled. The average age at diagnosis was 41.8 years (sd: 9.5 years). Most patients were normal weight at diagnosis of the current MDR TB episode (n=138, 68.2%), 45 were underweight (22.3%) and 19 were overweight (9.4%). Zero patients were obese at current diagnosis. Thirty-two (16.1%) patients were employed prior to the current MDR TB episode, 89 (44.7%) were unemployed, 13 (6.5%) were students, and 65 (32.7%) were disabled. Only 4 (2.0%) patients were health care workers prior to enrollment. About half of enrolled patients were single or never married at enrollment (n=104, 52.3%), 47 (23.6%) were married, 24 (12.1%) were separated, divorced or widowed/widowered, and 24 (12.1%) had other marital status classifications. Almost half of the enrolled patients completed secondary education as their highest level of education (49.2%) while 13.2% completed higher levels of education (university or technical) and 37.6% completed lower levels of

education (none or primary). The average number of total years of education completed was 9.6 years (sd: 2.0 years; n=196). Over half of enrolled patients had a history of imprisonment (50.5%), 66.7% abused alcohol, and 91.5% smoked tobacco at the time of enrollment. Only one patient was recorded to use illicit drugs at the time of enrollment. Only 7 patients (3.5%) had a history of high risk occupational exposure to TB. Most patients (94.1%) lived in fixed housing immediately prior to enrollment. Eighty-nine patients (44.1%) had at least one documented comorbidity prior to enrollment. Zero were HIV positive while one had unknown HIV status. Most patients reported any prior episodes of TB (n=172; 85.2%) with a median of 1 prior TB episode per patient (min=0, 25%ile=1, 75%ile=2, max=7) however only 33 (16.3%) reported the current MDR TB episode as the first time treated for MDR TB. Eighty-three patients (41.5%) were classified as new MDR TB case, 51 (25.5%) as MDR TB patients previously treated with first-line drugs only, and 66 (33.0%) as MDR TB patients previously treated with SLDs according to prior treatment history. Patients were also classified by prior treatment outcome: 79 (39.5%) new MDR TB cases, 37 (18.5%) treatment after relapse, 24 (12.0%) treatment after failure, 32 (16.0%) treatment after default, and 28 (14.0%) change to category IV for MDR TB. Only seven (3.5%) patients had extrapulmonary involvement in addition to pulmonary TB. Of the 189 patients with follow-up culture results, patients had a median of 3 positive follow-up cultures (min=1, 25%ile=2, 75%ile=5, max=17). Of the 116 patients with follow-up smear results, patients had a median of 2 positive follow-up smears (min=1, 25%ile=1, 75%ile=3, max=12). Patients had an average of 628.4 days (almost 2 years) on treatment for the current episode of MDR TB (n=200; sd=253.8). Patients spent a median of 169 days (min=0, 25%ile=86, 75%ile=281, max=799) in the

hospital during intensive phase of MDR TB treatment and a median of 0 days (min=0, 25%ile=0, 75%ile=0, max=591) during continuation phase.

AMPLIFICATION OF DRUG RESISTANCE

Without controlling for confounding, patients taking at least 4 effective anti-TB drugs at the beginning of MDR TB treatment had 0.33 (95% Conf. Limit: 0.12, 0.94) risk odds of any acquired drug resistance over the course of MDR TB treatment compared with patients who were treated with fewer than 4 effective anti-TB drugs at the beginning of MDR TB treatment (Table 3).

To further investigate the association between effective treatment and acquired drug resistance, covariates were assessed for potential confounding (Tables 4 and 5). Acquired drug resistance was significantly associated with age at diagnosis (*P*-value = 0.1), previous treatment with PAS (*P*-value = 0.04), number of days in the hospital during intensive phase of treatment (*P*-value = 0.008), and number of positive follow-up cultures (*P*-value = 0.002). Treatment with at least 4 effective drugs was associated with age at diagnosis (*P*-value = 0.04), BMI at diagnosis (*P*-value = 0.07), health care worker (*P*-value = 0.04), prior episodes of TB (*P*-value = 0.04), enrollment cohort (*P*-value = 0.0005), number of days in hospital during intensive phase of treatment (*P*-value = 0.04) and previous treatment with ethambutol (*P*-value = 0.07), isoniazid (*P*-value = 0.08), levofloxacin (*P*-value = 0.03), prothionamide (*P*-value = 0.07), pyrazinamide (*P*-value = 0.1) and rifampin (*P*-value = 0.03). Thus, age at diagnosis and number of days in the hospital during intensive phase was associated with both outcome and exposure.

Using these results, logistic regression was performed to model the effect of effective treatment at the beginning of current MDR TB treatment on acquired drug resistance during MDR TB treatment (Table 6). Using $\alpha=0.1$ for Wald chi-square test statistic for variable inclusion in the model, 3 covariates were considered as possible confounders in the full model. After considering meaningful changes in ORs and precision and Wald chi-square test statistics for individual variables, model 12 was chosen as the best model ($\alpha=0.1$). Therefore, patients who were treated with at least 4 effective drugs at the beginning of current MDR TB treatment had a 0.22 risk odds of acquiring drug resistance during treatment compared to patients who did not receive treatment with at least 4 effective drugs while controlling for the number of positive follow-up cultures and days spent in the hospital during intensive phase (95% Conf. limit: 0.07, 0.71).

To further investigate the association of effective drugs on acquired drug resistance, a sensitivity analysis was performed by comparing risk odds ratios of acquired drug resistance with different cut points in terms of the number of effective drugs with less than 4 effective drugs as the referent group (Table 7). The risk odds ratios of acquired drug resistance decreased from the use of 4 effective drugs to 5 effective drugs (OR=0.60 and OR=0.13, respectively). The risk odds ratio of 6 effective drugs (OR=0.16) was slightly greater than 5 effective drugs. Finally, the risk odds ratio of treatment with 7 or more effective drugs was the highest but still under the null value (OR=0.91).

TB TREATMENT OUTCOMES

The crude effect of acquired drug resistance during current MDR TB treatment on TB treatment outcome was assessed using polytomous logistic regression (Table 8). Those who acquired any drug resistance had a 7.07-fold (95% Conf. Limit: 2.17, 22.97) increased risk odds of treatment failure versus treatment success compared with those who did not acquire any drug resistance, but death during treatment (versus treatment success) was not associated with acquired drug resistance.

The crude effect of treatment with at least 4 effective drugs at the beginning of current MDR TB treatment on TB treatment outcome was assessed using polytomous logistic regression (Table 8). Those who received at least 4 effective drugs had a 0.31 (95% Conf. Limit: 0.12, 0.83) risk odds of treatment failure versus treatment success than those who did not receive at least 4 effective drugs. Neither death during treatment versus treatment success nor default during treatment versus treatment success had a significant association with treatment with at least 4 effective drugs.

DISCUSSION

With the limited treatment options currently available for MDR TB patients, acquiring additional drug resistance complicates treatment even more and leads to proportionally less treatment success [5]. The present study found that patients who acquired any drug resistance during current treatment for MDR TB had a 7.07-fold increased risk odds of treatment failure versus treatment success compared with those who did not acquire any drug resistance, supporting the association between acquired drug resistance and poor treatment outcomes. Because there is such a strong association between acquired drug resistance and treatment failure, it is essential to examine possible risk factors for acquired drug resistance to improve MDR TB programs and increase rates of treatment success.

One of the key facets of DOTS-Plus programs for the management of MDR TB is the use of second-line drugs under proper management conditions [4]. This includes having the capacity to provide correct drug dosing, ensuring patients are in fact taking the drugs, and reliance on quality-assured mycobacteriology results (specifically, DST) to modify treatment. By performing these tasks, DOTS-Plus programs decrease the chances that a patient's *M tb* flora will acquire further drug resistance during treatment, increasing the patient's chances of treatment success. Treatment with at least 4 effective drugs from the start of treatment had protective effects against acquiring drug resistance compared to treatment with fewer than 4 effective drugs. In other words, patients who were treated with fewer than 4 effective drugs had a significantly increased risk of acquired drug

resistance compared to patients who were treated with at least 4 effective drugs. These findings support current WHO recommendations of treating MDR TB patients with at least 4 drugs (1 injectable and 3 other SLDs) to which the patient's *M tb* illustrates *in vitro* susceptibility [12].

The same protective effect against acquired drug resistance was observed when sensitivity analysis was performed by comparing treatment with different cut points of effective drugs to the referent group of less than 4 effective drugs. The protective effect became greater as the number of effective drugs increased from 4 to 5 and stayed relatively the same from 5 to 6. However, the protective effect lessened for 7 or more effective drugs. The reasons for the decreased protective effect are difficult to identify. One might suppose that increased numbers effective anti-TB drugs would decrease the chances that *M tb* would have the ability to acquire additional drug resistance due to the amount of different bactericidal mechanisms being implemented *in vivo*. However, the effective treatment variable did not consider the length of time a patient was receiving effective treatment. If the patient had adverse reactions and subsequently stopped receiving the drugs or some of the drugs, then the few bacilli that had acquired resistance to these drugs would likely flourish. In addition, because of the relatively small number of patients who acquired resistance, the odds ratio estimates may have fluctuated substantially by chance.

One limiting step of effective treatment is the length of time it takes to receive DST results; taking anywhere from 4 weeks to 2 months depending on the DST method used.

The clinician treating a patient with MDR TB will not know the patient's *M tb* drug resistance pattern for several weeks. Therefore, the date local DST results were reported was used to create the effective treatment variable instead of the date the specimen was collected. Even though the *M tb* could potentially acquire drug resistance during the time between specimen collection and reported results, using the specimen collection date would only illustrate the acquired drug resistance timeline. Using specimen collection date would not give insight into effective patient management since treatment regimens before DST report date would only rely on the underlying drug resistance pattern in the population, previous treatment history and contact history. By allowing 30 days from the time the DST results were reported for the clinician to change the regimen to an effective regimen, any reasonable time lag of the reported results reaching the clinician would be accounted for. Any amount of time longer than 30 days would likely be a result of poor patient management or other breakdowns in the TB control strategy.

There is debate concerning how many drugs to which the *M tb* are susceptible are necessary to successfully treat MDR TB without acquiring drug resistance during treatment. To address these concerns, the present study performed a sensitivity analysis of the effects of treatment with different cut points of effective drugs at the beginning of treatment on acquired drug resistance during treatment of MDR TB. There did not seem to be any clear advantage to using increased numbers of effective drugs as compared to using at least 4 effective drugs at preventing acquired drug resistance.

STRENGTHS AND LIMITATIONS

Even though the analysis of the data from Arkhangelsk provides insight into the impact on acquired drug resistance of treating with at least 4 effective drugs, there are important limitations to these results. First, the small number of outcomes and small overall sample size led to difficulties in analysis. Few covariates were detected to have any significant association with the exposure or outcome variable at $\alpha=0.1$ let alone at the commonly utilized $\alpha=0.05$ level. Small sample sizes often lead to smaller or undetectable effects. On the other hand, the covariates that were found to be significantly associated with either variable can be stated with the confidence that the association was not a product of effect inflation due to large sample size. Furthermore, sensitivity analysis using a continuous variable or dummy variables of number of effective drugs could not be performed because of small numbers. Second, susceptibility testing of *M tb* to SLDs is difficult and not well standardized [28]. This could have caused patient misclassification both for the treatment with effective drug variables and acquired drug resistance variable since both variables included some form of DST results. There is less concern of patient misclassification of the acquired drug resistance variable due to the high quality of the SRCAMB laboratory in Obolensk, Russia. Third, the exposure variable of treatment with effective drugs did not consider dosage –an important component of effective treatment. Furthermore, the length of time the patient was on the effective regimen was not incorporated into the variable. The variable only considered if the patient was recorded to have been prescribed a drug to which the baseline DST results reported susceptibility from the date the results were reported to 30 days after the reported results. The effective

treatment variable would not have been able to detect if the patient was subsequently changed to an ineffective regimen after the 30 day period.

By participating in PETTS, the data collection tool and data collection method for the present analysis was piloted and standardized across multiple study sites. Standardizing data collection methods allows for comprehensive comparisons of results between the study sites. Even though the SRCAMB laboratory performed the DST, genotyping and gene sequencing instead of the CDC laboratory for this particular study site, the SRCAMB laboratory uses CDC level III laboratory guidelines for *M tb* DST. However, inter- and intra-laboratory variability should still be considered when comparing results. Because the data was collected prospectively and the outcome of acquired drug resistance is relatively rare, odds ratios calculated from these data can be interpreted as risk ratios. Odds ratios are commonly more difficult for most people to completely understand than risk ratios. Being able to estimate risk of acquired drug resistance contributes to the interpretability of the results and overall strength of the study.

POSSIBLE FUTURE DIRECTIONS

The present study addresses a few issues of MDR TB management however many questions and concerns are left unanswered. A similar analysis on all PETTS data would eliminate concerns and limitations of small sample sizes. Utilizing the larger sample size to further investigate the effects of the number of drugs treating a patient with MDR TB on acquired drug resistance and treatment outcomes could shed light on common confounders across study sites. More specifically, investigating the effects of smaller numbers of effective drugs (1 drug versus more than 1, 2 or fewer drugs versus more than 2, etc.) on acquired drug resistance and treatment outcomes could potentially have implications for MDR TB management or further support the WHOs current recommendation of 4 effective drugs. Furthermore, an ordinal variable of number of effective drugs for effects on acquired drug resistance and treatment outcomes should be analyzed. This analysis was the first analysis of data from the PETTS study on acquired drug resistance and on treatment outcomes, and experience with the present analysis can be used to inform the analysis of the parent study. In addition, the SAS code could be easily adapted to the larger study. The next analysis should also investigate dosage and drug quality when comparing treatment regimens and the effects on acquired drug resistance.

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TABLES

Table 1: Distribution of categorical covariates		
Variable	Category	n (%)
Sex	Female	31 (15.4)
	Male	171 (84.7)
Body Mass Index	Underweight	45 (22.3)
	Normal	138 (68.3)
	Overweight	19 (9.4)
Employment	Employed	32 (16.1)
	Unemployed	89 (44.7)
	Student	13 (6.5)
	Disabled	65 (32.7)
Health care worker	Yes	4 (2.0)
	No	198 (98.0)
Marital status	Single/Never married	104 (52.3)
	Married	47 (23.6)
	Separated/Divorced/Widow(er)	24 (12.1)
	Other	24 (12.1)
Education	None	1 (0.5)
	Primary	73 (37.1)
	Secondary	97 (49.2)
	University/Technical	26 (13.2)
History of imprisonment	Yes	102 (50.5)
	No	100 (49.5)
History of high risk occupation	Yes	7 (3.5)
	No	194 (96.5)
Currently abuse alcohol	Yes	134 (66.7)
	No	67 (33.3)
Currently smoke tobacco	Yes	184 (91.5)
	No	17 (8.5)
Currently use illicit drugs	Yes	1 (0.5)
	No	191 (99.5)
Current housing status	Fixed housing/apartment	190 (94.1)
	Other	12 (5.9)
Any comorbidities prior to PETTS enrollment	Yes	89 (44.1)
	No	113 (55.9)
Any prior episodes of TB	Yes	172 (85.2)
	No	30 (14.8)
MDR TB classification by prior treatment history	New MDR TB case	83 (41.5)
	MDR TB patient previously treated with 1st-line drugs	51 (25.5)
	MDR TB patient previously treated with SLDs	66 (33.0)
MDR TB classification by prior treatment outcome	New MDR TB case	79 (39.5)
	Treatment after Relapse	37 (18.5)
	Treatment after Failure	24 (12.0)
	Treatment after Default	32 (16.0)
	Change to category IV - MDR TB	28 (14.0)
Site of disease	Pulmonary only	195 (96.5)
	Pulmonary + Extrapulmonary	7 (3.5)
First time treated for MDR TB	Yes	169 (83.7)
	No	33 (16.3)

Table 2: Distribution of continuous covariates			
Variable	n	mean (sd)	median
Age	202	41.77 (9.47)	42.00
Total years of education completed	196	9.64 (1.97)	10.00
Number of inpatient days during intensive phase	202	195.24 (143.16)	168.50
Number of inpatient days during continuation phase	202	29.34 (89.60)	0.00
Number of times hospitalized during present episode	201	1.65 (1.08)	1.00
Number of all prior TB episodes	180	1.65 (0.98)	1.00
Body Mass Index	202	20.83 (2.98)	20.58
Number of positive cultures	189	3.92 (3.34)	3.00
Number of positive AFB smears	116	2.55 (2.10)	2.00
Number of days on treatment for current episode	200	628.35 (253.78)	728.00

Table 3: Crude risk odds ratio of effective treatment for those who acquire drug resistance compared to those who do not acquire drug resistance

Initial treatment	n	no acquired resistance n (%)	acquired resistance n (%)	OR	95% Confidence Limits
< 4 effective drugs	45	38 (84.4)	7 (15.6)		
≥ 4 effective drugs	157	148 (94.3)	9 (5.7)	0.33	(0.12, 0.94)

Table 4: Bivariate association of covariates with acquired drug resistance				
Categorical Variable		No acquired resistance n (%)	Acquired resistance n (%)	*P*-value*
Sex	Female	28 (90.3)	3 (9.7)	
	Male	158 (92.4)	13 (7.6)	0.7
Highest education level	None	1 (100.0)	0 (0.0)	
	Primary	68 (93.2)	5 (6.8)	
	Secondary	88 (90.7)	9 (9.3)	
	University/technical	24 (92.3)	2 (7.7)	0.9
Age at diagnosis (years)	18-34	41 (83.7)	8 (16.3)	
	35-41	47 (95.9)	2 (4.1)	
	42-48	49 (96.1)	2 (3.9)	
	49+	49 (92.5)	4 (7.5)	0.1
BMI at diagnosis	Underweight	39 (86.7)	6 (13.3)	
	Normal	130 (94.2)	8 (5.8)	
	Overweight	17 (89.5)	2 (10.5)	0.2
Comorbidity	None	102 (90.3)	11 (9.7)	
	At least one	84 (94.4)	5 (5.6)	0.3
Illicit drug use	No	178 (93.2)	13 (6.8)	
	Yes	1 (100.0)	0 (0.0)	1.0
Alcohol abuse	No	61 (91.0)	6 (9.0)	
	Yes	124 (92.5)	10 (7.5)	0.7
Smoke tobacco	No	16 (94.1)	1 (5.9)	
	Yes	169 (91.9)	15 (8.1)	1.0
Employment status	Employed	30 (93.8)	2 (6.2)	
	Unemployed	79 (88.8)	10 (11.2)	
	Student	12 (92.3)	1 (7.7)	
	Disabled	62 (95.4)	3 (4.6)	0.5
Health care worker	No	182 (91.9)	16 (8.1)	
	Yes	4 (100.0)	0 (0.0)	1.0
Housing status upon enrollment	Fixed housing/apartment	11 (91.7)	1 (9.3)	
	Other	175 (92.1)	15 (7.9)	1.0
History of incarceration	No	93 (93.0)	7 (7.0)	
	Yes	93 (91.2)	9 (8.8)	0.8
Marital status	Single/never married	93 (89.4)	11 (10.6)	
	Married	44 (93.6)	3 (6.4)	
	Separated/divorced/Widowed	23 (95.8)	1 (4.2)	
	Other	24 (100.0)	0 (0.0)	0.4
History of high risk occupation	No	178 (91.8)	16 (8.2)	
	Yes	7 (100.0)	0 (0.0)	1.0
MDR TB classification by prior treatment history	New MDR TB case	76 (91.6)	7 (8.4)	
	MDR TB patient previously treated with 1st-line drugs	49 (96.1)	2 (3.9)	
	MDR TB patient previously treated with SLDs	60 (90.9)	6 (9.1)	0.6
Any prior episodes of TB	No	29 (96.7)	1 (3.3)	
	Yes	157 (91.3)	15 (8.7)	0.5
Site of disease	Pulmonary only	179 (91.8)	16 (8.2)	
	Pulmonary + Extrapulmonary	7 (100.0)	0 (0.0)	1.0
MDR TB classification by prior treatment outcome	New MDR TB case	74 (93.7)	5 (6.3)	
	Treatment after Relapse	35 (94.6)	2 (5.4)	
	Treatment after Failure	21 (87.5)	3 (12.5)	
	Treatment after Default	28 (87.5)	4 (12.5)	
	Change to category IV - MDR TB	26 (92.9)	2 (7.1)	0.6

First time treated for MDR TB	Yes	157 (92.9)	12 (7.1)	
	No	29 (87.9)	4 (12.1)	0.3
Cohort	I	77 (95.1)	4 (4.9)	
	II	109 (90.1)	12 (9.9)	0.3
Positive follow-up cultures	<2	55 (100.0)	0 (0.0)	
	2-3	70 (94.6)	4 (5.4)	
	4 or more	61 (83.6)	12 (16.4)	0.002
Time spent in hospital during intensive phase (days)	<117	64 (95.5)	3 (4.5)	
	177-232	65 (97.0)	2 (3.0)	
	233 or more	57 (83.8)	11 (16.2)	0.008
Previous treatment with amikacin	No	158 (92.4)	13 (7.6)	
	Yes	4 (80.0)	1 (20.0)	0.3
Previous treatment with capreomycin	No	153 (92.7)	12 (7.3)	
	Yes	9 (81.8)	2 (18.2)	0.2
Previous treatment with ciprofloxacin	No	157 (91.8)	14 (8.2)	
	Yes	5 (100.0)	0 (0.0)	1.0
Previous treatment with cycloserine or terizidone	No	142 (92.2)	12 (7.8)	
	Yes	20 (90.9)	2 (9.1)	0.7
Previous treatment with ethambutol	No	9 (100.0)	0 (0.0)	
	Yes	153 (91.6)	14 (8.4)	1.0
Previous treatment with ethionamide	No	158 (91.9)	14 (8.1)	
	Yes	4 (100.0)	0 (0.0)	1.0
Previous treatment with isoniazid	No	9 (100.0)	0 (0.0)	
	Yes	153 (91.6)	14 (8.4)	1.0
Previous treatment with kanomycin	No	95 (92.2)	8 (7.8)	
	Yes	65 (91.5)	6 (8.5)	0.9
Previous treatment with levofloxacin	No	159 (92.4)	13 (7.6)	
	Yes	3 (75.0)	1 (25.0)	0.3
Previous treatment with ofloxacin	No	141 (93.4)	10 (6.6)	
	Yes	21 (84.0)	4 (16.0)	0.1
Previous treatment with PAS	No	138 (93.9)	9 (6.1)	
	Yes	24 (82.8)	5 (17.2)	0.06
Previous treatment with prothionamide	No	133 (93.7)	9 (6.3)	
	Yes	29 (85.3)	5 (14.7)	0.2
Previous treatment with pyrazinamide	No	7 (100.0)	0 (0.0)	
	Yes	154 (91.7)	14 (8.4)	1.0
Previous treatment with rifampin	No	10 (100.0)	0 (0.0)	
	Yes	152 (91.6)	14 (8.4)	1.0
Previous treatment with streptomycin	No	133 (91.1)	13 (8.9)	
	Yes	23 (95.8)	1 (4.2)	0.7
Previous treatment with any injectable	No	87 (92.6)	7 (7.5)	
	Yes	76 (91.6)	7 (8.4)	1.0
Previous treatment with any fluoroquinolone	No	134 (93.7)	9 (6.3)	
	Yes	28 (84.9)	5 (15.2)	0.1
Continuous variable - T test		mean (sd)	mean (sd)	*P*-value
Years of education completed		9.6 (2.0)	9.8 (1.6)	0.7
Number of days on treatment for current episode		626.4 (252.8)	650.6 (271.8)	0.7
Continuous variable - Wilcoxon rank sums		mean score	mean score	*P*-value
Days spent in the hospital during continuation phase		102.1	94.4	0.4
Number of all prior TB episodes		90.1	95.1	0.7
Number of positive follow-up AFB smears		57	69.4	0.2

*p-values are from Chi-square tests of association unless any expected cell value was less than 5 at which point Fishers exact p-values were used

Table 5: Bivariate association of covariates with effective treatment				
		< 4 effective drugs	≥ 4 effective drugs	
Categorical Variable		n (%)	n (%)	*P*-value*
Sex	Female	8 (25.8)	23 (74.2)	
	Male	37 (21.6)	134 (78.4)	0.6
Highest education level	None	0 (0.0)	1 (100.0)	
	Primary	18 (24.7)	55 (75.3)	
	Secondary	23 (23.7)	74 (76.3)	
	University/technical	4 (15.4)	22 (84.6)	0.7
Age at diagnosis (years)	18-34	9 (18.4)	40 (81.6)	
	35-41	18 (36.7)	31 (63.3)	
	42-48	10 (19.6)	41 (80.4)	
	49+	8 (15.1)	45 (84.9)	0.04
BMI at diagnosis	Underweight	11 (24.4)	34 (75.6)	
	Normal	26 (18.8)	112 (81.2)	
	Overweight	8 (42.1)	11 (57.9)	0.07
Comorbidities prior to enrollment	None	21 (18.6)	92 (81.4)	
	At least one	24 (27.0)	65 (73.0)	0.2
Illicit drug use	No	45 (23.6)	146 (76.4)	
	Yes	0 (0.0)	1 (100.0)	1.0
Alcohol abuse	No	14 (20.9)	53 (79.1)	
	Yes	31 (23.1)	103 (76.9)	0.7
Smoke tobacco	No	3 (17.6)	14 (82.4)	
	Yes	42 (22.8)	142 (77.2)	0.8
Employment status	Employed	11 (34.4)	21 (65.6)	
	Unemployed	17 (19.1)	72 (80.9)	
	Student	2 (15.4)	11 (84.6)	
	Disabled	15 (23.1)	50 (76.9)	0.3
Health care worker	No	42 (21.2)	156 (78.8)	
	Yes	3 (75.0)	1 (25.0)	0.04
Housing status upon enrollment	Fixed housing/apartment	2 (16.7)	10 (93.3)	
	Other	43 (22.6)	147 (77.4)	1.0
History of incarceration	No	23 (23.0)	77 (77.0)	
	Yes	22 (21.6)	80 (78.4)	0.8
Marital status	Single/never married	23 (22.1)	81 (77.9)	
	Married	14 (29.8)	33 (70.2)	
	Separated/divorced/Widowed	2 (8.3)	22 (91.7)	
	Other	6 (25.0)	18 (75.0)	0.2
History of high risk occupation	No	42 (21.6)	152 (78.4)	
	Yes	3 (42.9)	4 (57.1)	0.2
MDR TB classification by prior treatment history	New MDR TB case	22 (26.5)	61 (73.5)	
	MDR TB patient previously treated with 1st-line drugs	11 (21.6)	40 (78.4)	
	MDR TB patient previously treated with SLDs	12 (18.2)	54 (81.8)	0.5
Any prior episodes of TB	No	11 (36.7)	19 (63.3)	
	Yes	34 (19.8)	138 (80.2)	0.04
Site of disease	Pulmonary only	45 (23.1)	150 (76.9)	
	Pulmonary + Extrapulmonary	0 (0.0)	7 (100.0)	0.4
MDR TB classification by prior treatment outcome	New MDR TB case	19 (24.1)	61 (75.9)	
	Treatment after Relapse	6 (16.2)	31 (83.8)	
	Treatment after Failure	7 (29.2)	17 (70.8)	
	Treatment after Default	7 (21.9)	25 (78.1)	
	Change to category IV - MDR TB	4 (14.3)	24 (85.7)	0.6

First time treated for MDR TB	Yes	35 (20.7)	134 (79.3)	
	No	10 (30.3)	23 (69.7)	0.2
Cohort	I	8 (9.9)	73 (90.1)	
	II	37 (30.6)	84 (69.4)	0.0005
Positive follow-up cultures	<2	12 (21.8)	43 (78.2)	
	2-3	18 (24.3)	56 (75.7)	
	4 or more	15 (20.6)	58 (79.5)	0.9
Time spent in hospital during intensive phase (days)	<117	22 (32.8)	45 (67.2)	
	117-232	11 (16.4)	56 (83.6)	
	233 or more	12 (17.7)	56 (82.4)	0.04
Previous treatment with amikacin	No	35 (20.5)	136 (79.5)	
	Yes	1 (20.0)	4 (80.0)	1.0
Previous treatment with capreomycin	No	33 (20.1)	131 (79.9)	
	Yes	3 (25.0)	9 (75.0)	0.7
Previous treatment with ciprofloxacin	No	34 (19.9)	137 (80.1)	
	Yes	1 (20.0)	4 (80.0)	1.0
Previous treatment with cycloserine or terizidone	No	29 (19.0)	124 (81.0)	
	Yes	6 (26.1)	17 (73.9)	0.4
Previous treatment with ethambutol	No	4 (44.4)	5 (55.6)	
	Yes	32(19.1)	136 (80.9)	0.07
Previous treatment with ethionamide	No	35 (20.7)	134 (79.3)	
	Yes	0 (0.0)	7 (100.0)	0.3
Previous treatment with isoniazid	No	4 (44.4)	5 (55.6)	
	Yes	32 (19.1)	136 (80.9)	0.08
Previous treatment with kanomycin	No	19 (18.8)	82 (81.2)	
	Yes	15 (20.6)	58 (79.4)	0.8
Previous treatment with levofloxacin	No	32 (18.6)	140 (81.4)	
	Yes	3 (75.0)	1 (25.0)	0.03
Previous treatment with ofloxacin	No	27 (18.4)	120 (81.6)	
	Yes	8 (27.6)	21 (72.4)	0.3
Previous treatment with PAS	No	28 (19.1)	119 (80.9)	
	Yes	7 (24.1)	22 (75.9)	0.5
Previous treatment with prothionamide	No	24 (17.1)	116 (82.9)	
	Yes	11 (30.6)	25 (69.4)	0.07
Previous treatment with pyrazinamide	No	3 (42.9)	4 (57.1)	
	Yes	33 (19.4)	137 (80.6)	0.1
Previous treatment with rifampin	No	5 (50.0)	5 (50.0)	
	Yes	31 (18.6)	136 (81.4)	0.03
Previous treatment with streptomycin	No	29 (20.6)	112 (79.4)	
	Yes	5 (17.2)	24 (82.76	0.7
Previous treatment with any injectable	No	18 (19.6)	74 (80.4)	
	Yes	18 (21.2)	67 (78.8)	0.8
Previous treatment with any fluoroquinolone	No	25 (18.0)	114 (82.0)	
	Yes	10 (27.0)	27 (73.0)	0.2
Continuous variable - T test		mean (sd)	mean (sd)	*P*-value
Years of education completed		9.7 (1.4)	9.6 (2.1)	0.8
Number of days on treatment for current episode		612.5 (253.6)	633.0 (254.5)	0.6
Continuous variable - Wilcoxon rank sums		mean score	mean score	*P*-value
Days spent in the hospital during continuation phase		103.5	94.4	0.2
Number of all prior TB episodes		91.5	86.7	0.6
Number of positive follow-up AFB smears		58.7	57.8	0.9

*p-values are from Chi-square tests of association unless any expected cell value was less than 5 at which point Fishers exact p-values were used

Table 6: Adjusted risk odds ratios of effective treatment with at least 4 drugs for those who acquire drug resistance compared with those who do not acquire drug resistance controlling for confounding

Model		OR	95% Conf. Limits	Wald chi-square	*P*-value
Crude model	RX4SUS	0.33	(0.12, 0.94)	4.28	0.04
Model 1	RX4SUS	0.34	(0.12, 0.99)	3.93	0.05
	age4	0.70	(0.43, 1.15)	1.95	0.2
Model 2	RX4SUS	0.21	(0.07, 0.67)	7.04	0.008
	ppas	3.10	(0.92, 10.50)	3.30	0.07
Model 3	RX4SUS	0.24	(0.08, 0.73)	6.32	0.01
	hpdyint3cat	2.79	(1.33, 5.89)	7.28	0.007
Model 4	RX4SUS	0.28	(0.09, 0.84)	5.10	0.02
	cntcx3cat	5.27	(1.85, 14.99)	9.71	0.002
Model 5	RX4SUS	0.32	(0.11, 0.92)	4.49	0.03
	BMIcat	0.63	(0.25, 1.55)	1.03	0.3
Model 6	RX4SUS	0.29	(0.10, 0.85)	5.12	0.02
	priortb	3.62	(0.45, 29.42)	1.45	0.2
Model 7	RX4SUS	0.38	(0.13, 1.12)	3.11	0.08
	cohort	1.66	(0.49, 5.61)	0.68	0.4
Model 8	RX4SUS	0.22	(0.07, 0.70)	6.58	0.01
	plev	1.86	(0.16, 21.47)	0.25	0.6
Model 9	RX4SUS	0.23	(0.07, 0.71)	6.49	0.01
	ppro	1.90	(0.57, 6.35)	1.09	0.3
Model 10	RX4SUS	0.13	(0.04, 0.48)	9.62	0.002
	ppas	2.87	(0.77, 10.65)	2.49	0.1
	hpdyint3cat	3.56	(1.47, 8.59)	7.95	0.005
Model 11	RX4SUS	0.15	(0.04, 0.53)	8.68	0.003
	ppas	2.41	(0.65, 8.87)	1.74	0.2
	cntcx3cat	5.73	(1.73, 20.17)	8.02	0.005
Model 12	**RX4SUS**	**0.22**	**(0.07, 0.71)**	**6.42**	**0.01**
	hpdyint3cat	2.10	(0.96, 4.60)	3.45	0.06
	cntcx3cat	4.39	(1.49, 12.88)	7.23	0.007
Full model	RX4SUS	0.11	(0.03, 0.42)	10.20	0.001
	ppas	2.24	(0.59, 8.52)	1.39	0.2
	hpdyint3cat	2.76	(1.09, 7.01)	4.54	0.03
	cntcx3cat	4.87	(1.32, 17.95)	5.65	0.02

Table 7: Sensitivity analysis of treatment with effective drugs					
Exposure	n	No acquired resistance n (%)	Acquired resistance n (%)	OR	95% Conf. Limits
3 or less effective drugs	45	38 (84.4)	7 (15.6)	ref	ref
4 effective drugs	10	9 (90.0)	1 (10.0)	0.60	(0.07, 5.54)
5 effective drugs	43	42 (97.7)	1 (2.3)	0.13	(0.02, 1.10)
6 effective drugs	69	67 (97.1)	2 (2.9)	0.16	(0.03, 0.82)
7 or more effective drugs	35	30 (85.7)	5 (14.3)	0.91	(0.26, 3.14)

Table 8: Risk odds ratios of TB treatment outcome using polytomous logistic regression with treatment success as referent

Exposure	Comparison	OR	95% Confidence Limit
Any acquired drug resistance	failure vs success	7.07	(2.17, 22.97)
	death vs success	0.76	(0.09, 6.49)
	default vs success	0.36	(0.04, 3.02)
≥ 4 effective drugs	failure vs success	0.31	(0.12, 0.83)
	death vs success	0.54	(0.19, 1.56)
	default vs success	0.71	(0.30, 1.67)

APPENDIX A

PETTS Patient Data Form

IDENTIFICATION AND ENROLLMENT*

1. Patient Identification Number __ __ __ __ __
02000=Estonia 03000=Latvia 04000=Peru 05000=Philippines
06000=Arkhangelsk 07000=Vladimir 08000=Orel 09000=Tomsk
11000=South Africa 12000=South Korea 13000=Thailand

2. Date of Enrollment* __ __/__ __/__ __ __ __ (*dd/mm/yyyy)*

3. Site __ __
es=Estonia la=Latvia pe=Peru pi=Philippines
ar=Arkhangelsk vl=Vladimir or=Orel to=Tomsk
sa=South Africa sk=South Korea th=Thailand

Facility Name* _____

Given Name(s)* _____

Middle Name(s)* _____

Family Name(s)* _____

4. Date of Birth* __ __/__ __/__ __ __ __ *(dd/mm/yyyy)*

.....

SITE-DEFINED VARIABLES* (SDV) PIN 77777

SDV1 _____

SDV2 _____

SDV3 _____

*** Instructions on back**

Instructions for Identification and Enrollment

Confidentiality of patient information: This page is the only page on which the patient's name appears together with the study code number. **After data collection is complete** for the full follow up period this page should be separated from the rest of the form and kept in a separate, secure file for the PETTS data form Identification and Enrollment page ("face sheet"). These face sheets should be kept together in a locked file**. After analysis is complete**, the "face sheets" will be destroyed.

2. Date of Enrollment: Date patient signed informed consent form. This should be the same date the Patient ID number assigned.

Names - ***Facility and patient names will not be entered into database***

Middle Name(s): In CIS countries, use this space for patronymic; in the Philippines, for mother's maiden name

Family Name(s): In Latin America, record both maternal and paternal last names

4. Date of Birth: enter as much as known. Enter 9s for missing parts of date

Difficulties with dates: Every effort should be made to determine a precise date. If part of the date cannot be determined, then as much as can be determined *with certainty* should be recorded in the space provided. The missing parts should be recorded as 9's, for example, 99/May/2004, or 99/99/2001. If the month and year cannot be determined for certain, several methods enable an approximate date to be determined.

- The patient can be prompted by relating the date of interest to an important historical event such as a change in government, a period of civil unrest, an important holiday or to an important personal event such as a change in jobs or residence, a birth, death, illness, graduation, marriage, or divorce.
- Show the patient a calendar with important events marked on it.
- It may be possible to determine a period of time during which the date of interest must have occurred, rather than a specific date. Examples would include, "between June and August, 2004," or for more distant episodes, "after 1991 but before 1995."
- It may be possible to deduce the period or the date from information such as the timing of other events.

Approximate dates should not be recorded in the data entry field, but next to the data field along with a note to the investigator explaining how the date was approximated.

Instructions for Site-Defined Variables

Each site may use these spaces to record any information that is not recorded elsewhere on these forms, for example, a code number, a physician's name, an accounting code or any other data. This information will not be entered into the electronic database unless the site so desires. The database will be set up to received these data, and it can be entered into the computer if the site wants it to be, but it does not have to be. *To retain these data when the face sheets are destroyed, divide the page along the dotted line or photocopy the lower half of the page*

DEMOGRAPHICS

5. Sex ___ *(1=male 0=female)*

6. Age at diagnosis of current MDR TB _____

7. Employment prior to current MDR TB ____

0=employed 1=unemployed 2=retired 3=student 4=disabled 5=housewife 9=unknown

8. Health Care Worker ___ *(0=no 1=yes 9=unknown/not asked)*

8a. Marital Status at enrollment ____

1=single/never married 2=now married 3=separated/divorced 4=widow/er 5=engaged to be married 6=cohabiting 9=unknown/not asked 0=other

8b. Any children ____ **8c. Any children living with patient** ____

0=no 1=yes 9=unknown

9 . Education highest level completed ____

1=primary 2=secondary 3=university or professional 4=technical school
5=other 9=unknown 0=none (no formal education)

9b. Total years completed ____

PATIENT CHARACTERISTICS

10. Contact with any TB patient ____ *(0=no 1=yes-type unspecified 2=yes-family/household/living quarters; 3=yes-work/school; 9=unknown)*

11. Contact with MDR TB patient ____ *(0=no 1=yes-type unspecified 2=yes-family/household/living quarters; 3=yes-work/school; 9=unknown)*

12. Has the patient EVER had the following? *(For <u>each</u> item, write 0, 1, or 9 in the corresponding blank, where 0=no, 1=yes, 9=unknown)*

12a. History of Imprisonment ____

12b. Homelessness* ____*(*Definition of homelessness-see back of page)*

12c. High risk occupation ____

12d. What occupation(s)? _____

(Health care worker, mine worker, prison worker, etc.)

13. Does the patient currently *(0=no 1=yes 9=unknown for each)*

13a. abuse alcohol? ____

13b. smoke tobacco? ____

13c. use illicit drugs? ____

14. Current Housing status ____ *(0=homeless; 1=fixed housing/apartment 2=other 3=hospital 4=housing facility for TB patients 9=unknown)*

14b. If other, specify: _____

Instructions For Determining Homelessness

Homelessness is defined by where the person sleeps, including sleeping outside, in vacant buildings, in vehicles, in shelters that do not require payment. Homelessness does not include sleeping in the residence of relatives/friends. Also, it does not include sleeping in temporary quarters that require payment such as a hotel, motel, boarding house, rooming house, or dormitory.

CLINICAL INFORMATION

15. Co-morbidities prior to PETTS enrollment ____
(For each of the following, record: 0=no 1=yes 2=not tested 9=unknown)

15a. HIV/AIDS ___ **-->***(IF YES, complete HIV/AIDS supplemental data box below)*

15b. Active hepatitis/cirrhosis ___ **15c. Diabetes mellitus** ___
15d. Chronic renal insufficiency ___ **15e. Vomiting/diarrhea** ___
15f. Gastric or duodenal ulcer ___ **15g. Seizure disorder** ___
(epilepsy, convulsions, fits)

15h. Major psychiatric or mental disorder ___
(for example, depression, schizophrenia, or mental retardation)

15i. Immunosuppressive diseases ___**-->Specify** _____
(for example, malnutrition, leukemia, lymphoma, head & neck cancer, renal failure, gastric resection, intestinal bypass, alcoholism, injecting drug abuse)

15j. Immunosuppressive drugs ___ **--> Specify** _____

16. Weight at diagnosis of this episode of MDR TB *(kilograms)* _____

17. Height at diagnosis of this episode of MDR TB *(centimeters)* ____

18. Is patient hospitalized at time of enrollment in PETTS? ___
(0=no 1=yes 9=unknown)

For patients <u>without</u> HIV/AIDS (15a≠1), skip to item 19 on page 7.

SUPPLEMENTAL DATA FOR PATIENTS WITH HIV/AIDS

HIV1. HIV/AIDS diagnosed by _____ *(Record all that apply: 0=Clinical criteria, 1=1 ELISA, 2=2 ELISAs, 3=Western Blot, 4=Serology unspecified, 8=other, 9=unknown)*

HIV2. HIV/AIDS diagnosis date __ __/__ __ __ __ *(mm/yyyy)*

HIV3. CD4 count _____ *(Use nearest to PETTS enrollment date)*

HIV4. Date of CD4 count __ __/__ __ __ __ *(mm/yyyy)*

HIV5. HIV-associated illnesses *(Record all HIV-associated diseases & start--end dates. Do not put TB history here.)*
Opportunistic infections _____
Malignancies_____

HIV6. Antiretroviral drugs *(Record all ARV regimens & start--end dates)*
_____

HIV7. Cotrimoxazole preventive treatment ____ *(0=no 1=yes 9=unknown)*

7a. IF YES, Start--end dates _____*(mm/yy--mm/yy)*

HIV8. TB preventive treatment ____ *(0=no 1=yes 9=unknown)*

8a. IF YES: What drug? _____**Start--end date** _____*(mm/yy--mm/yy)*

What constitutes an episode of TB?

For patients who had TB in the past, the TB HISTORY may be one of the more difficult and time consuming sections of the data form. Yet, it is crucial because prior treatment is the most important risk factor for drug resistance. Each drug selects for microbes resistant to itself. The ***extent of exposure*** of a patient's population of microbes to a specific drug is the strongest risk factor for development of resistance to that drug. Determining the frequency and causes of acquired drug resistance are the goals of PETTS.

The term **episode of TB** implies a distinct period of time during which an individual had active TB disease. This period is determined by its start and end dates. For PETTS, an ***episode of TB*** is defined as the occurrence in an individual of ***active TB disease*** with ***identifiable start and end dates.*** In case the exact date is not easily identified, a method for determining the next best proxy is suggested below.

A **prior episode of TB** is defined as one in which the patient reached a defined end-point according to the standard WHO treatment outcomes.[12] Prior episode is synonymous with previous episode and past episode. The end of the episode is the date the patient first met one of these outcome definitions. A recent addition to the 6 classic, standard WHO outcome definitions that takes into account MDR TB and Category IV is described below[*].

The **start of an episode** is defined as the ***date of diagnosis*****,** specifically***; the date the first specimen was obtained*** that provided bacteriological confirmation.

1) *In case the specimen date cannot be determined*, the date it was received by the lab, the date of the smear result, or the date of the culture result, in that sequence of preference, should be recorded. For any date other than the specimen date, a note should be recorded on the data form to specify which date was recorded.
    a) For routine diagnosis of Category I, II, and III patients, sputum microscopy demonstrating AFB is considered sufficient by WHO. If the specimen must be transported to the lab, microscopy may be delayed by several days or more. In smear positive cases, the patient had TB at the time the specimen was collected and throughout the period of waiting for the smear result.
    b) Culture results may require 4 to 6 weeks longer than the smear result, more if the specimen had to be transported to a culture lab. Thus, in smear-negative, culture-positive cases, the patient had TB at the time the specimen was collected and throughout the period of waiting for the culture result.
    c) For diagnosis of drug-resistance, the DST result may require an additional 4-6 weeks longer than the culture result, more if the specimen or culture must be transported to a reference lab. Therefore, confirmation of drug-resistant TB may be delayed by 2 to 3 months or more. Nevertheless, the patient had drug-resistant TB at the time the specimen was collected and throughout the period of waiting for the DST results.
    d) Any date other than the specimen date should be accompanied by a note recorded on the data form to specify which date was recorded.
2) *In case TB is not bacteriologically confirmed*, the date of diagnosis is the date the responsible clinician (or committee) decided the patient has active TB and should be treated for it (regardless of the availability of drugs).
3) *In case it is not possible to identify the date of diagnosis in the medical record*, the patient can be asked when they were first told by their medical provider – in relation to that specific episode – that they had TB.
4) *In case it is not possible to determine the date of diagnosis* by any of these methods use the earlier of the following dates for the start of the episode:
    a) Treatment start date
    b) Case registration date
    c) Record a note on the form explaining which date is recorded and how it was determined.

---

[1] WHO. Treatment of tuberculosis: guidelines for national programmes, 3rd ed. Geneva, World Health Organization, 2003 (WHO/CDS/TB/2003.313), pp. 21-26, 30-35, 39-44, 53-56.

[2] WHO. Guidelines for the programmatic management of drug-resistant tuberculosis. Geneva, World Health Organization, 2006 (WHO/HTM/TB/2006.361), pp. 18-23.

TB HISTORY (not including current diagnosis of MDR TB)

19. Has the patient had prior episodes of TB? ___ --> If NO, skip to #23 *(0=no 1=yes 9=unknown*

20. Number of all prior TB episodes, if known ___

> ***What constitutes an episode of TB? The explanation appears on pages 6, 8, 10 (the backs of data pages 5, 7, 9), including episode of TB, prior episode of TB, start of an episode, end of an episode. Examples of each are provided.***

21. SURGERY: Has patient ever had thoracic surgery to treat TB or complications of TB prior to the current episode of MDR TB? ____
IF NO, go to #22 *(0=no 1=yes 9=unknown)*

21a. If yes, how many times? _____ *Date format dd/mm/yyyy)*

21b. Procedure _____ 21c. Date ____/____/_____

21d. Procedure _____ 21e. Date ____/____/_____

21f. Procedure _____ 21g. Date ____/____/_____

22. DRUG TREATMENT: Has patient ever had the following medications in previous TB episodes (not including current episode of MDR TB)?

0=no, never received drug or for <1 month; 1=yes, received drug for ≥ 1 month; 2=yes, received drug unknown duration; 9=unknown

Drug name	Received?	Drug name	Received?
22a. isoniazid		22j. ethionamide	
22b. rifampicin		22k. prothionamide	
22c. ethambutol		22l. ofloxacin	
22d. pyrazinamide		22m. ciprofloxacin	
22e. streptomycin		22n. levofloxacin	
22f. thioacetazone		22o. p-aminosalicylic acid	
22g. kanamycin		22p. cycloserine	
22h. amikacin		Terizidone	
22i. capreomycin		22q. Other _____	
Other _____		22r. Other _____	
Other _____		Other _____	

What constitutes an episode of TB? *(continued)*

1) The **<u>end of an episode</u>** is defined as *the point in time when a patient reaches one of the standard treatment outcomes* as defined by WHO - cure, treatment completion, death, failure, default, and transfer out[34]. In other words, when the patient meets a specific outcome definition, the episode is over.
2) The definitions of default and death are the same for all registration categories: Default = 2 months without treatment. Death = death from any cause during treatment. The definitions of cure, completion, and failure differ slightly for categories I, II, and III. For Category IV, the definitions of cure, completion, and failure differ greatly.
3) Patients who have sputum collected for culture and DST at the start of treatment may have to wait weeks or months for the results. In 2003 and again in 2006, WHO codified the practice of changing case registration and treatment categories based on DST results that show MDR TB, including an outcome category of "***Change to Category IV – MDR TB***"[56]. How this should be done depends on the initial case registration and treatment.
   a) **<u>Patients registered and treated as Category I/II/III</u>**: After the DST results are reported as showing MDR TB, treatment with Category I/II/III regimens is no longer appropriate. The patient should be changed to Category IV. ***The Category I/II/III TB case should be closed in the (DOTS) TB register and the outcome recorded as "Change to Category IV – MDR TB." This is the end of that episode of TB. The end date is the date the case is closed. Then the patient is registered (in the DOTS-Plus register) as a Category IV patient. Registration as Category IV defines a different episode of TB. The start of the episode is still the date of diagnosis as above.***
      i) What about patients who continue treatment with only 1st line drugs despite DST results showing MDR TB? How should the recording and reporting be managed? <u>It depends on the reason</u>:
      ii) In case the clinician thinks the DST results may be wrong, then the patient should continue Cat. I/II/III without change in registration. ***The episode does not end, the same episode continues.***
      iii) In case the clinician thinks the DST results may be correct, but continues only 1st-line drugs – either because 2nd-line drugs are not available or because 1st-line treatment has been effective (e.g., based on clinical, radiographic and subsequent bacteriological examinations) – case registration should be changed to Category IV (irrespective of the drug regimen) as described above in 4a (bold/italics). ***The episode of Category I/II/III TB ends. A new episode of Category IV TB starts.*** (The clinician may continue treating with 1st-line drugs, but the case registration will be corrected).
   b) **<u>Patients registered and treated as Category IV</u>**: A fraction of patients may receive a Category IV treatment regimen empirically based on risk factors for MDR TB before the DST results are reported. These patients should be registered as Category IV initially. Subsequent decisions depend on subsequent treatment.
      i) In cases in which 2nd-line drugs are continued (for example, DST results confirm MDR TB), the patients should continue in registration Category IV with no change in registration. ***This same episode of Category IV TB continues***. Treatment may be adjusted based on DST results, but as long as treatment includes 2nd-line drugs, Category IV is appropriate.
      ii) On the other hand, in cases in which treatment is changed to Category I, II, or III (for example, DST shows susceptibility to 1st-line drugs), the patient should be removed from Category IV retroactive to the initial diagnosis and treatment. The DOTS-Plus register is marked (legibly, not erased), and the patient is registered in the regular (DOTS) register as Category I, II, or III whichever is appropriate. *The TB episode is considered (retrospectively) to have begun at the time of the initial diagnosis and initial empiric treatment.* ***This patient will not be eligible to continue in the PETTS study.***

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[3] Ibid. WHO/CDS/TB/2003.313, pp. 21-26, 30-35, 39-44, 53-56.

[4] Ibid. WHO/HTM/TB/2006.361, pp. 18-23.

HISTORY OF PRIOR EPISODES OF TB (PRIOR TO PRESENT EPISODE OF MDR TB)*				
Questions refer to episode named in column heading	**1st EPISODE**	**2nd EPISODE**	**3rd EPISODE**	**4th EPISODE**
Date of first TB diagnosis for the episode *dd/mm/yyyy*				
Site of disease for the episode *1=pulmonary only 2=extrapulmonary only 3=both pulmonary & extrapulmonary 9=unknown*				
Patient's sputum smear status for the episode *1=smear-positive 2=smear-negative 3=unknown 4=not done*				
Was the episode the first time patient was treated for TB? *1=yes 2=no 9=unknown*				
Was the episode MDR TB? *(initial diagnosis/registration) 0=no 1=yes 9=unknown*				
Was episode drug-resistant TB other than MDR? *(initial diagnosis/registration) 0=no 1=yes 9=unknown*				
Was episode treated under DOTS (or DOTS-Plus) strategy? *0=no 1=yes 2=partly 9=unknown*				
Was episode treated in private sector? *0=no 1=yes 2=partly 9=unknown*				
Drugs used in episode > 1 month				
Treatment outcome for the episode: *1=cure, **2**=treatment completed, **3**=treatment failure, 4=death, **5**=default, **6**=transferred out, 7=Changed to Cat.IV-MDR TB, 8=Changed to Cat.IV for any other reason (e.g.other types of drug esistance, chronic TB), 9=unknown*				

Examples of determining Episodes of TB

Example – Episode 1: A new, sputum smear-positive (Category I) patient starts treatment on January 1st. He interrupts treatment beginning March 15th. On May 15th he meets the definition of default. ***This episode of TB (Cat. I) ends May 15th*** *with the outcome classified as default.*

Example – Episode 2: The same patient returns to clinic and has a positive sputum smear on May 31st, 5 months after initially starting treatment and 2½ months after interrupting treatment. His outcome of Category I treatment remains default (on May 15th), not treatment failure, because default came first. *As of May 31st, he is registered and treated as a Category II patient.* ***A new episode of TB (Cat. II) begins on May 31st.***

Example – Episode 3: The May 31st sputum specimen is cultured, and the mycobacterial isolate is sent to the reference lab for DST. On September 1st, the lab reports resistance to INH, RIF, and SM. The patient returns to clinic and his Cat. II treatment is stopped. *His case is closed in the TB register as of September 1st with the outcome recorded as Change to Category IV – MDR TB.* ***That episode (#2) ends September 1st.*** He is sent to the referral hospital where he is registered in Category IV and starts treatment with 2nd-line drugs. ***A new episode of TB (#3) began on the date of diagnosis as defined above.*** **This patient now qualifies for enrollment in PETTS (MDR TB + 2nd-line drug treatment). This episode (#3) should be recorded not in the TB History (pp. 7, 9) but in the current MDR TB episode (pp. 11-20). Although the dates of episodes #2 and #3 overlap, the situation will be clear based on subsequent items on the PETTS data form.**

Correctly determining the beginning and end of each episode of TB depends on thoroughly knowing and applying the two standard WHO references.

Standard WHO outcome definitions for Category I, II, and III TB patients (TB History)

- **Cured**=Sputum smear positive patient who is sputum-smear negative in the last month of treatment and on at least one previous occasion
- **Treatment completed**=Treatment completed without meeting the criteria to be classified as cure or failure
- **Failed**=sputum-smear positive at or more than 5 months during treatment or smear negative patients who become sputum positive after more than 2 months of treatment
- **Died**=patient dies for any reason during the course of treatment
- **Transfered**=patient is transferred to another facility and treatment outcome is unknown
- **Unknown**= Outcome unknown or undocumented

Standard WHO outcome definitions for Category IV

• **Cured**. Patient has completed treatment according to the programme's protocol and has at least 5 consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment. If a single scanty positive (<10 colonies) culture is reported during that time, and there is no clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures taken at least 30 days apart.
• **Treatment completed**. Patient completed treatment according to the programme's protocol but does not meet the definition for cure because of lack of bacteriological results (i.e. fewer than five cultures were performed in the final 12 months of treatment).
• **Failed**. Two or more of the 5 cultures in the final 12 months of therapy are positive or any one of the final 3 cultures is positive. (Also, treatment will be considered to have failed if a clinical decision has been made to terminate treatment early because of poor response or adverse events. These latter failures can be indicated separately for the purposes of sub-analysis.)
• **Died**. Patient died for any reason during the course of MDR-TB treatment.
• **Defaulted**. Patient's treatment was interrupted for two or more consecutive months for any reason.
• **Transferred**. Patient transferred to another recording and reporting unit and treatment outcome is unknown.

CLASSIFICATION OF MDR TB CASE BY PRIOR TREATMENT HISTORY

23. Which one of the following best describes this patient at the start of the current episode of MDR TB? ***(see instructions on back)***

___ 1=New MDR TB case

___ 2=MDR TB patient previously treated with first-line drugs only

___ 3=MDR TB patient previously treated with second-line drugs

___ 4=Unknown/missing

CLASSIFICATION OF MDR TB CASE BY PRIOR TREATMENT OUTCOME

24. Which one of the following best describes this patient at the start of the current episode of MDR TB? ***(see instructions on back)***

___ 1=New MDR TB case

___ 2=Treatment after Relapse

___ 3=Treatment after Failure

___ 4=Transfer in

___ 5=Chronic

___ 6=Treatment after default.

___ 7=Change to Category IV – MDR TB

___ 8=Other (prior treatment outside of DOTS strategy)

___ 9=Unknown

MDR TB CLINICAL CLASSIFICATION – CURRENT EPISODE

25a. Site of disease ___ *1=pulmonary only 2=extrapulmonary only 3=both pulmonary & extrapulmonary 9=unknown*

25b. What was the patients smear status for this MDR TB episode? ____
1=smear-positive 2=smear-negative 9=unknown

25c. Was this the first time patient was treated for MDR TB?___
1=yes 2=no 9=unknown

26a. Date of chest radiograph closest to MDR TB treatment initiation
__ __/__ __/__ __ __ __ (*dd/mm/yyyy*)

26b. Radiographic extent of disease at treatment initiation _____
0=normal 1=unilateral TB disease 2=bilateral TB disease 3=abnormal with TB side(s) unknown 4=abnormalities not TB 9=unknown

26c. Was there a cavity on chest radiograph at treatment initiation?____ *0=no 1=yes-unilateral 2=yes-bilateral 3=yes-side(s)unknown 9=unknown*

Instructions for MDR TB case classification by prior treatment history

Definition of First-line drugs: isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin

Definition of Second-line drugs: kanamycin, amikacin, capreomycin, ethionamide, prothionamide, ofloxacin, ciprofloxacin, levofloxacin, other fluoroquinolones, para-aminosalicylic acid (PAS), cycloserine, terizidone, clofazimine

Thioacetazone may be classified as 1st- or 2nd-line according to how it is used in your country

Definition of New MDR TB case: MDR TB patient who has never received anti-TB treatment or who has received anti-TB treatment for less than one month. ***NB: Patients who had a specimen taken for DST at the start of a Category I regimen and then, based on the DST results, are changed to a regimen for MDR TB should be classified in this group, even if they received more than one month of Category I treatment.***

Definition of MDR TB patient previously treated with first-line drugs only. MDR TB patient who has been treated for one month or more with first-line drugs only.

Definition of MDR TB patient previously treated with second-line drugs. MDR TB patient who has been treated for one month or more with one or more second-line drugs, with or without first-line drugs.

Instructions for MDR TB case classification by prior treatment outcome

New MDR TB case: MDR TB patient who has never received anti-TB treatment or who has received anti-TB treatment for less than one month. *NB: Patients who had a specimen taken for DST **at the start** of a Category I regimen and then, based on the DST results, are changed to a regimen for MDR TB should be classified in this group, even if they received more than one month of Category I treatment.*

Treatment after Relapse: A TB patient who previously received treatment and was declared cured (or who successfully completed treated but did not have bacteriological examination at the end of treatment) AND has once again developed bacteriologically positive pulmonary TB.

Treatment after Failure: A TB patient who while on treatment remained smear/culture positive OR, after turning smear/culture negative, once more became smear/culture positive at the 5th month or later during the course of treatment OR who was initially smear/culture negative before treatment and became smear/culture positive after the 2nd month of treatment

Transfer in: A TB patient already registered for treatment in one recording and reporting unit who transfers to another unit and continues treatment.

Chronic: A patient who remained sputum smear-positive after completing a directly-observed re-treatment (Category II) regimen

Treatment after default. A patient who stopped treatment for at least 2 months for any reason, then returns to be treated again.

Change to Category IV – MDR TB. A patient who is changed to Cat.IV treatment before the number of months have elapsed in the definition of treatment failure. *NB: Patients who had a specimen taken **more than 1 month** after the start of treatment and then, based on the DST results, are changed to an MDR TB regimen should be classified in this group. Such patients who are changed to MDR TB treatment because of clinical deterioration before DST results are reported may be classified in this group as long as the DST results confirm MDR TB.*

LABORATORY RESULTS AT MDR TB DIAGNOSIS AND PETTS ENROLLMENT

Sputum sample used to make MDR TB diagnosis

27. Date sputum sample for culture collected____/____/_____*(dd/mm/yyyy)*

28. Result of smear microscopy _____*(0=neg. 1=pos. 2=1-9AFB 3=cont.9=unk.)*

29. Date culture result reported ____/____/_____ *(dd/mm/yyyy)*

30. Result of culture _____ *(0=neg. 1=pos. 2=scanty[<10col.] 3=cont.9=unk.)*

Date 1st DST results reported* ____/____/_____ *(dd/mm/yyyy)*

1st DST results*: SUSCEPTIBLE _____ **RESISTANT** _____

Date 2nd DST results reported* ____/____/_____ *(dd/mm/yyyy)*

2nd DST results*: SUSCEPTIBLE _____ **RESISTANT** _____

Sputum sample used for PETTS enrollment

In case the same sample is used for MDR TB diagnosis as for PETTS enrollment, mark X here _____, and please rewrite the information below

31. Date sputum sample for culture collected ___/___/_____ *(dd/mm/yyyy)*

32. Result of smear microscopy _____*(0=neg. 1=pos. 2=1-9AFB 3=cont.9=unk.)*

33. Date culture result reported ___/___/_____ *(dd/mm/yyyy)*

34. Result of culture _____*(0=neg. 1=pos. 2=scanty[<10col.] 3=cont.9=unk.)*

Date 1st DST results reported* ___/___/_____ *(dd/mm/yyyy)*

1st DST results*: SUSCEPTIBLE _____ **RESISTANT** _____

Date 2nd DST results reported* ___/___/_____ *(dd/mm/yyyy)*

2nd DST results*: SUSCEPTIBLE _____ **RESISTANT** _____

35. Were 2 or more cultures inoculated? _____ *(0=no 1=yes 9=unknown)*

36. Did 2 or more cultures grow? ____ *(0=no 1=yes 3=n/a* 9=unknown)*

36a. If only 1 culture grew, what method was used to duplicate the culture prior to shipping to CDC?

___*1 subculture*
___*2 original culture media was cut*
___*3 original (only) culture*
___*4 other* _____

37. Was the culture isolate shipped to CDC? ___ *(0=no 1=yes 9=unknown)*
What date was it shipped to CDC? ___/___/_____ *(dd/mm/yyyy)*

Each isolate MUST be labeled correctly with the Patient ID Number

**** n/a = not applicable. In this case, less than 2 cultures were inoculated.***

**** Further instructions on back***

WHO/IUATLD Standard Sputum Smear Microscopy Results Reporting System (for reference only)

No AFB in at least 100 fields	0/negative
1 to 9 AFB in 100 fields	Actual AFB counts
10 to 99 AFB in 100 fields	+
1 to 10 AFB per fields	++
> 10 AFB per field	+++

Instructions for recording drug susceptibility test results (DST)

1st DST results, 2nd DST results: Record results from the same specimen reported at different times, e.g. #1, DST to 2nd line drugs performed after 1st line DST results show resistance, e.g. #2, isolates sent to a reference lab for 2nd line DST or for repeat testing after initial DST done locally.

Write "not done" next to the date field if that specific DST was not peformed.

Record DST results for **all drugs tested** by writing abbreviated name of drug in appropriate blank. For drugs to which isolate is susceptible, write drug name on line next to "SUSCEPTIBLE." For drugs to which isolate is resistant, write drug name on line next to "RESISTANT."

Use abbreviations for the drug names according to the following standardized abbreviations:

A, AMK	- Amikacin	MOX	- Moxifloxacin
CIP	- Ciprofloxacin	OFL	- Ofloxacin
CFZ	- Clofazimine	PAS	- para-aminosalicylic acid
Cm, CAP	- Capreomycin	PTA	- Prothionamide
Cs, CYS	- Cycloserine	RBT	- Rifabutin
E, EMB	- Ethambutol	R, RIF	- Rifampicin
ETA	- Ethionamide	S, Sm, STM	- Streptomycin
H, INH	- Isoniazid	Th, TB1	- Thioacetazone
Km, KAN	- Kanamycin	Trz	- Terizidone
LEV	- Levofloxacin	Z, PZA	- Pyrazinamide

Instructions For Recording TB Treatment (Table Beginning P. 15)

Record drugs received by patient for current episode of MDR TB, including drugs received prior to enrollment in PETTS.

In addition, in patients in whom the most recent prior episode of TB "rolled over" into the current episode of MDR TB **with an interruption of less than 2 months**, record drugs for that episode too (regardless of DST results or treatment category). This includes mainly patients in the following categories: Treatment After Relapse, Treatment After Failure, and Change To Category IV.

Treatment Changes: When drugs are changed, up to 3 changes for each drug can be recorded in this same table, with the corresponding dates, **reasons for stopping** *(see codes below)*, doses, manufacturer, procurement. through GLC.

Short interruptions: Record stop dates whenever a drug is interrupted > 2 weeks. Also, record restart date if the same drug is used again after an interruption of 2 weeks or more. In terms of recording treatment changes and stop/start dates, disregard interruptions < 2 weeks.

Reasons for Stopping Codes:

1=treatment complete 2=patient interruption > 2 weeks 3=adverse effects or drug interactions
4=drug no longer available 5=dose adjustment 6=therapeutic change (based on lab results)
7=other **(explain)** 8=change to continuation phase 9=unknown

TB TREATMENT – see instructions on page 14					
38. Date treatment in this table started ____/____/_____(*dd/mm/yyyy*)					
Drug used? *0=no 1=yes 9=unknown*	**a.GLC drug?** *(Y/N)*	**b.Start Date** *(dd/mm/yy)*	**c.Stop Date d.Reason for Stopping***	**e.Dose** *d/w x mg/d*	**f.Manufacturer*** *(company & country)*
___ **isoniazid**	a1___	b1__/__/__	c1___/___/___ d1 _____	e1____	f1 _____ _____
	a2___	b2__/__/__	c2___/___/___ d2 _____	e2____	f2 _____ _____
	a3___	b3__/__/__	c3___/___/___ d3 _____	e3____	f3 _____ _____
___ **rifampicin**	a1___	b1__/__/__	c1___/___/___ d1 _____	e1____	f1 _____ _____
	a2___	b2__/__/__	c2___/___/___ d2 _____	e2____	f2 _____ _____
	a3___	b3__/__/__	c3___/___/___ d3 _____	e3____	f3 _____ _____
___ **ethambutol**	a1___	b1__/__/___	c1___/___/___ d1 _____	e1____	f1 _____ _____
	a2___	b2__/__/___	c2___/___/___ d2 _____	e2____	f2 _____ _____
	a3___	b3__/__/___	c3___/___/___ d3 _____	e3____	f3 _____ _____
___ **pyrazinamide**	a1___	b1__/__/___	c1___/___/___ d1 _____	e1____	f1 _____ _____
	a2___	b2__/__/___	c2___/___/___ d2 _____	e2____	f2 _____ _____
	a3___	b3__/__/___	c3___/___/___ d3 _____	e3____	f3 _____ _____
___ **streptomycin**	a1___	b1__/__/___	c1___/___/___ d1 _____	e1____	f1 _____ _____
	a2___	b2__/__/___	c2___/___/___ d2 _____	e2____	f2 _____ _____
	a3___	b3__/__/___	c3___/___/___ d3 _____	e3____	f3 _____ _____
___ **thio-acetazone**	a1___	b1__/__/___	c1___/___/___ d1 _____	e1____	f1 _____ _____
	a2___	b2__/__/___	c2___/___/___ d2 _____	e2____	f2 _____ _____
	a3___	b3__/__/___	c3___/___/___ d3 _____	e3____	f3 _____ _____

* Reason for stopping codes – see page 14.

TB TREATMENT – CONTINUED					
___ **kanamycin**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____
___ **amikacin**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____
___ **capreomycin**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____
___ **ethionamide**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____
___ **pro-thionamide**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____
___ **ofloxacin**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____
___ **cipro-floxacin**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 _____ c2___/___/___ d2 _____ c3___/___/___ d3 _____	e1_____ e2_____ e3_____	f1 _____ _____ f2 _____ _____ f3 _____ _____

TB TREATMENT – CONTINUED					
___ **levo-floxacin**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1 ____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ _________
___ **para-amino-salicylic acid**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1_____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ _________
___ **cycloserine**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1_____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ _________
___ **terizidone**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1_____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ _________
___ **Other________**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1_____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ _________
___ **Other________**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1_____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ _________
___ **Other________**	a1___ a2___ a3___	b1___/___/___ b2___/___/___ b3___/___/___	c1___/___/___ d1 ___________ c2___/___/___ d2 ___________ c3___/___/___ d3 ___________	e1_____ e2_____ e3_____	f1 _________ _________ f2 _________ _________ f3 _________ __________

USE ADDITIONAL TREATMENT PAGES AS NEEDED – INSERT HERE

TREATMENT COURSE AND OUTCOME

57. Date MDR TB treatment started ____/____/_____ *dd/mm/yyyy*

58. Date intensive phase completed ____/____/_____ *dd/mm/yyyy*

59a.Date continuation phase started ____/____/_____ *dd/mm/yyyy*

59b.Date continuation phase completed ____/____/_____ *dd/mm/yyyy*

60. Was directly observed therapy (DOT) used: _____
1=DOT throughout the whole treatment regimen, 2=DOT during the initial phase only and then combination of DOT and self administered during the continuation phase, 3=DOT during the initial phase only and then only self administered during the continuation phase, 4=Combination self-administered and DOT throughout the regimen regardless of treatment phase, 5=Self-administered, 6=Other --> specify #doses DOT____/Total #doses____ (or % doses DOT ____), 9=Unknown

61. Treatment outcome ____
1=cure 2=treatment completed 3=treatment failure 4=death
5=default 6=transfer out 7=continuing treatment 9=unknown
(outcome definitions on p. 10)

62. Did the patient have thoracic surgery to treat his/her MDR TB or to treat the complications of TB during treatment with second-line TB drugs? *(0=no 1=yes 9=unknown)* ___ *(if no, skip to #64)*

63a If yes, how many times? ___ ___

63b Procedure type _____
63c Date of procedure ____/____/_____ *dd/mm/yyyy*

63d Procedure type _____
63e Date of procedure ____/____/_____ *dd/mm/yyyy*

63f Procedure type _____
63g Date of procedure ____/____/_____ *dd/mm/yyyy*

64. Number of times hospitalized during present episode _____
Dates of hospitalization: From ____/____/_____ **To** ____/____/_____
From ____/____/_____ **To** ____/____/_____
From ____/____/_____ **To** ____/____/_____
From ____/____/_____ **To** ____/____/_____
From ____/____/_____ **To** ____/____/_____

65. Total number of inpatient days during intensive phase _____

66. Total number of inpatient days during continuation phase _____

FOLLOW-UP LAB RESULTS DURING MDR TB TREATMENT Follow-up month # ____
1. Date sputum sample for culture collected ____/____/_____ *(dd/mm/yyyy)*
2. Result of smear microscopy_____ *(0=neg. 1=pos. 2=1-9AFB 3=cont. 9=unk.)*
3. Date culture result reported ____/____/_____ (dd*/mm/yyyy)*
4. Result of culture _____ (0*=neg. 1=pos. 2=scanty 3=cont. 9=unk.)*
5. Date 1st DST results reported ____/____/_____ *(dd/mm/yyyy)*
6. 1st DST results*: SUSCEPTIBLE_____ RESISTANT_____
7. Date 2nd DST results reported ____/____/_____ *(dd/mm/yyyy)*
8. 2nd DST results*: SUSCEPTIBLE_____ RESISTANT_____
9a. Were ≥2 cultures inoculated from this specimen?____*(0=no 1=yes 9=unknown)*
9b. Did ≥ 2 cultures grow? ____ *(0=no 1=yes 3=N/A (<2cultures) 9=unknown)*
9c. If only 1 culture grew, what method was used to duplicate the culture prior to shipping to CDC? ___*1 subculture* ___*2 original culture media was cut* ___*3 original (only) culture* ___*4 other* _____
9d. Was the culture isolate shipped to CDC? ____ *(0=no 1=yes 9=unknown)*
9e. What date was it shipped to CDC? ____/____/_____ *(dd/mm/yyyy)*

FOLLOW-UP LAB RESULTS DURING MDR TB TREATMENT Follow-up month # ____
1. Date sputum sample for culture collected ____/____/_____ *(dd/mm/yyyy)*
2. Result of smear microscopy _____ *(0=neg. 1=pos. 2=1-9AFB 3=cont. 9=unk.)*
3. Date culture result reported ____/____/_____ *(dd/mm/yyyy)*
4. Result of culture _____ (0*=neg. 1=pos. 2=scanty 3=cont. 9=unk.)*
5. Date 1st DST results reported ____/____/_____ *(dd/mm/yyyy)*
6. 1st DST results*: SUSCEPTIBLE_____ RESISTANT_____
7. Date 2nd DST results reported ____/____/_____ *(dd/mm/yyyy)*
8. 2nd DST results*: SUSCEPTIBLE_____ RESISTANT_____
9a. Were ≥2 cultures inoculated from this specimen?____*(0=no 1=yes 9=unknown)*
9b. Did ≥ 2 cultures grow? ____ *(0=no 1=yes 3=N/A (<2cultures) 9=unknown)*
9c. If only 1 culture grew, what method was used to duplicate the culture prior to 0shipping to CDC? ___*1 subculture* ___*2 original culture media was cut* ___*3 original (only) culture sent* ___*4 other* _____
9d. Was the culture isolate shipped to CDC? ____ *(0=no 1=yes 9=unknown)*
9e. What date was it shipped to CDC? ____/____/_____ *(dd/mm/yyyy)*

APPENDIX B

EMORY UNIVERSITY | Institutional Review Board

November 8, 2011

Sarah Smith
Principal Investigator
Public Health

RE: **Exemption of Human Subjects Research**
IRB00053623
Acquired antituberculosis drug-resistance over the course of drug-resistant tuberculosis treatment in Arkhangelsk oblast, Russia

Dear Principal Investigator:

Thank you for submitting an application to the Emory IRB for the above-referenced project. Based on the information you have provided, we have determined on 11/08/2011 that although it is human subjects research, it is exempt from further IRB review and approval.

This determination is good indefinitely unless substantive revisions to the study design (e.g., population or type of data to be obtained) occur which alter our analysis. Please consult the Emory IRB for clarification in case of such a change. Exempt projects do not require continuing renewal applications.

This project meets the criteria for exemption under 45 CFR 46.101(b)(4). Specifically, you will looking at de-identified data from SRCAMB.

Please note that the Belmont Report principles apply to this research: respect for persons, beneficence, and justice. You should use the informed consent materials reviewed by the IRB unless a waiver of consent was granted. Similarly, if HIPAA applies to this project, you should use the HIPAA patient authorization and revocation materials reviewed by the IRB unless a waiver was granted. CITI certification is required of all personnel conducting this research.

Unanticipated problems involving risk to subjects or others or violations of the HIPAA Privacy Rule must be reported promptly to the Emory IRB and the sponsoring agency (if any).

In future correspondence about this matter, please refer to the study ID shown above. Thank you.

Sincerely,

Olga Dashevskaya, JD
Research Protocol Analyst
This letter has been digitally signed

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