Epidemiological impact of HIV on second-line drug resistance in patients with multidrug resistant tuberculosis in high HIV prevalent settings in South Africa

By Ronél Odendaal

Summary

Introduction:

Multidrug resistant (MDR) Tuberculosis (TB) comprises approximately 19% of the total TB burden in the world. Although the global estimates of MDR-TB patients living with human immunodeficiency virus (HIV) are unknown due to limited information, it was estimated in 2010 that about 13% of the TB population in the world is infected with HIV. HIV infection has been associated with the development of TB and MDR-TB, thus contributing to the prevalence of MDR-TB and increasing the burden. HIV infection has also been associated with mortality in drug resistant (DR) TB patients.

Methods:

A cross-sectional analysis was performed on data from a prospective cohort study that was conducted during 2005-2008, in 4 provinces of South Africa. Drug susceptibility testing was performed and the prevalence of drug resistance in HIV sero-positive patients and HIV sero-negative patients were evaluated. Risk factors for drug resistance were also determined.

Results:

The prevalence of resistance to at least one aminoglycoside was 45% for HIV sero-positive patients not on Antiretroviral (ARV) treatment and 27% for HIV sero-positive patients on ARV treatment ($p = 0.028$). Similarly, the prevalence of resistance to all three aminoglycoside was 41% for HIV sero-positive patients not on ARV treatment and 24% for HIV sero-positive patients on ARV treatment (0.058). Province was found to be a strong risk factor for drug resistance ($p<0.001$), but HIV infection was not found to be associated with drug resistance.

Conclusion:

A relationship between HIV infection and primary resistance was not found, but unexpected findings regarding the role of ARV treatment was found in the analysis. A significant relationship was however found between the provinces and the association of resistance to second-line TB drugs.

Keywords: ARV, HIV, TB, MDR-TB, Prevalence, Resistance, South Africa

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Abbreviations

AG	Aminoglycoside
ARV	Antiretroviral Treatment
CDC	Center for Disease Control
CI	Confidence interval
CRF	Case Report Form
DR	Drug resistant
EMB	Ethambutol
FLQ	Fluoroquinolone
INH	Isoniazid
HIV	human immunodeficiency virus
MDR	Multidrug resistant
RIF	Rifampicin
Max	Maximum
Min	Minimum
MRC	Medical Research Council
M.tb	*M.tuberculosis*
SD	standard deviation
SM	Streptomycin
SLD	second-line drugs
TB	Tuberculosis
TDR	totally drug resistant
USAID	United State Agency for International Development
WHO	World Health Organization
XDR	extensively drug resistance

Ethical Approval

Ethical approval was obtained from the University of Pretoria, faculty of health sciences research ethics committee.
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Statement of Original Authorship

I declare that the dissertation titled "Epidemiological impact of HIV on second-line drug resistance in patients with multidrug resistant tuberculosis in high HIV prevalent settings in South Africa" which I hereby submit for the degree Masters of Science (Epidemiology) At the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

TUBERCULOSIS (TB) BURDEN IN SOUTH AFRICA

South Africa is home to not only the 3rd highest TB burden in the world[1] but also has one of the largest populations living with HIV. United State Agency for International Development (USAID) reported in 2011 that 5.6 million people in South Africa are living with HIV which is almost 16% of the total burden worldwide. It was estimated in 2010 that about 13% of the TB population in the world is infected with HIV[2]. South Africa has the highest TB and HIV co-infection burden in the world[1] with 60% of the TB population in South Africa being HIV infected[1].

DRUG RESISTANT (DR) TB BURDEN IN SOUTH AFRICA

Multidrug resistant-TB is defined as TB resistant to at least isoniazid (INH) and rifampicin (RIF), which are the two most important drugs in the first-line anti-TB drug regimen. Extensively drug resistant-TB is defined as MDR-TB, resistant to at least one drug within the fluoroquinolone (FLQ) and aminoglycoside (AG) drug groups classified as the two most important groups of the second-line anti-TB drugs in the treatment of MDR-TB. Multidrug-resistant -TB contributes about 19% of the total TB burden in the world [1] with South Africa carrying 19% of that burden, which is the 4th highest burden[1].

DRUG RESISTANCE TO ANTI-TB DRUGS IS INCREASING IN SOUTH AFRICA

The MDR-TB numbers in South Africa have increased from 3219 new cases in 2004 to 7386 in 2010[3], which has more than doubled in number. XDR-TB increased from 84 cases in 2004 to an alarming 741 cases in 2010. Although the global estimates of MDR-TB patients living with HIV are unknown due to limited information, the Medical

Research Council (MRC) estimated during their drug-resistance survey in 2002 that about 40% of MDR-TB cases in South Africa are also HIV infected[4]. Not only is South Africa facing an increase in MDR-TB and XDR-TB numbers, but a recent study has indicated the emergence of totally drug resistant TB (TDR-TB) which is a concept not clearly defined by the World Health Organization (WHO) yet, but in general refers to XDR-TB cases resistant to all the drugs in the FLQ and AG drug groups[5]. The latter emphasises that, not only is the number of cases being infected with resistant- TB strains increasing, but the severity or degree of resistance to anti-TB drugs is also worsening.

DEVELOPMENT OF DR TB[5]

M.tuberculosis(M.tb) naturally undergoes mutation with the consequence that organisms that are resistant to tuberculosis, mutates. This probability of mutation differs for each drug, for example the probability of mutation for isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide is 10^{-6}, 10^{-9},10^{-6}, 10^{-5},10^{-5} cell divisions respectively. The probability to be resistant to more than one or more than two etc. would be calculated by $10^{-xyz\cdots}$. In theory the probability for drug-resistant strains to emerge naturally is very unlikely, since the drugs are used in combinations and not as mono-therapy. Since natural emergence is very unlikely it is believed that when the anti-TB drugs kill off the susceptible bacilli, drug resistance occurs when resistant mutants are selected in a bacterial population. Thus when susceptible bacilli are killed rapidly then resistant mutants are able to multiply and drug resistant strains emerge. The latter is also known as acquired resistance.

TRANSMISSION OF DR STRAINS

Three ways of transmission or development of drug-resistant TB strains have been noticed occurring in South Africa.

1. Primary transmission: The patient is infected with an organism already resistant to anti-TB drugs.
2. Acquired resistance: When the original susceptible TB strain changes or evolves in to a drug-resistant TB strain due to the mutation.
3. Exogenous/Nosocomial reinfection: Transmission of drug-resistant TB strains that occur in a specific setting such as a hospital, health clinic or ward.

In the early 90's it was believed that the main way of transmission in South Africa was acquired drug-resistance. It was suggested that the reason for this was inadequate treatment and patients not adhering to treatment. Thereafter South Africa implemented the DOTS-PLUS strategy for MDR-TB treatment as a strategy to standardise MDR-TB treatment and attempt to reduce acquired drug-resistance. Patient adherence to treatment was also promoted and specific measures were put in place to help patients adhere to their treatment[6,7,8].

In recent years, studies have found that the majority of infections of drug-resistant strains are not through acquired transmission any more, but through primary infection and nosocomial infection[9,10,11,12].

Is it possible that the drug-resistant TB epidemic is changing in South Africa?
And if it is changing, what is the driving force behind the change?

HIV INFECTED POPULATION AS A VULNERABLE POPULATION TO TB

Because of the immune suppression caused by HIV, HIV infection has been found to be the strongest risk factor to develop active TB (susceptible or drug-resistant) when infected with *M.tb*[13,14,15]. It also has been found to increase the susceptibility to be infected with *M.tb* and reactivate latent TB. In HIV patients, TB is the most common opportunistic infection[16,17,18]. World Health Organization estimated that HIV infected people have a 20 times increased risk to develop TB in countries like South Africa with a high HIV burden[19].

With DR-TB numbers increasing and South Africa fighting a very challenging HIV epidemic, Wells et al. described the collision of these two epidemics by calling it the "perfect storm"[21,20].

Some of the implications HIV has on the DR-TB epidemic are:

- HIV contributes to the prevalence of TB and DR-TB[13,21,23,21,22,23]
- DR-TB is more complex to treat and diagnose in HIV positive patients[24]
- Delayed diagnosis can increase the spread of DR strains[27]
- HIV infection increases the risk of nosocomial transmission[13,15,23,25]
- Poorer treatment outcomes especially an increase in mortality[13,23,27,26,27,28]
- If cured, HIV positive patients have a 5 times greater chance that the disease will recur [29]

A decrease in CD4 and CD8 T cells has been associated with an increased risk of TB and multiple *M.tb* infection which could lead to super infections of drug resistant strains[30,31]. It was also found that even when the CD4 T cells in HIV infected patients were high for instance with the use of ARVs and in the early stages of disease, the risk of TB didn't reduce.[32,33] Not only does HIV infection increase the risk of TB infection, but research indicated that TB infection in HIV infected patients was associated with the clinical progression of the HIV disease.[34]

ASSOCIATION OF HIV WITH ANTI-TB DRUG RESISTANCE:

Little is known about the association between HIV and second-line anti-TB drug resistance. A few studies that were published showed evidence of association between acquired rifampicin resistance and HIV infection[35,36,37] and another study from Mozambique showed an association between HIV infection and isoniazid resistance as well as streptomycin resistance[38]. Conflicting results have been published on the association of HIV infection and MDR-TB. Some studies didn't find an association between HIV and MDR-TB[37,39,40] but these studies had very small sample sizes. Other studies did identify HIV infection as a risk factor for MDR-TB and XDR-TB.[27,39,41]

Studies in Latvia and the Ukraine have successfully succeeded in proving that HIV patients are at higher risk for primary drug resistance but could not successfully prove the relationship between HIV and XDR-TB due to the lack of second-line drug testing[42,43]. These studies have successfully demonstrated that there is a possible association between HIV infection and first-line anti-TB drug resistance as well as an association between acquired resistance to rifampicin and HIV infection.

In South Africa, three particular studies did not find any association between MDR-TB and HIV infection, but these studies had limited baseline characteristics and no statistical test results were presented. Also, only drug susceptibility testing to the first-line drugs was performed. No second-line drug susceptibility was performed so no inference could be made between the association of second-line drugs and HIV infection[44,45,46].
Another study found that HIV infection was associated with DR-TB outbreaks[23].

MOTIVATION FOR THIS ANALYSIS

Literature has suggested that the relationship between HIV and drug-resistance to anti-TB drugs are not entirely understood, especially the association to second-line anti-TB drug resistance.[23,47] Studies have suggested the importance of fluoroquinolones in the treatment of MDR-TB, and XDR-TB in HIV sero-positive patients has been seen as almost untreatable and relating to poor outcomes. Information on the prevalence of resistance to second-line drugs in South Africa could give a better idea of the adequacy of treatment[48,49].

Information on the association of HIV infection and resistance to anti-TB drugs will improve the rationale of the challenges that are faced in South Africa and enhance the knowledge of the DR-TB epidemic and the consequences of HIV co-infection. This analysis tried to establish if HIV infection is indeed a driving force behind DR-TB in the country and to what extent.

AIM AND OBJECTIVES

Aim:

The aim of this analysis was to evaluate the possible effect of HIV infection on resistance to second-line anti-TB drugs (SLD) in 4 provinces of South Africa.

Primary objective:

To evaluate the prevalence of baseline second-line drug (SLD) resistance in adults with pulmonary MDR TB, stratifying by province or HIV status.

Secondary objective:

To evaluate the risk factors for baseline second-line drug resistance in adults with pulmonary MDR TB, specifically investigating HIV infection as a risk factor.

CHAPTER 2 – METHODOLOGY

STUDY DESIGN

A cross-sectional analysis was performed on data from a prospective cohort study that was conducted during 2005-2008 in collaboration with the Centre for Disease Control (CDC) in Atlanta, USA and the MRC, Pretoria, South Africa. The study was funded by USAID.

SETTING

The study was conducted in 4 of the 9 provinces in South Africa:

- North West province, Klerksdorp MDR-TB hospital
- Mpumalanga, Witbank MDR-TB hospital
- KwaZulu-Natal, King George IV MDR-TB hospital
- Eastern Cape, Jose Pearson MDR-TB hospital and Fort Gray MDR-TB hospital

PATIENT SELECTION

Patients diagnosed with pulmonary MDR-TB, who started treatment between January 2005 and June 2008, with at least one positive culture of *M. tb* available within one month from the day they started treatment for MDR-TB were enrolled into the study.

Prisoners, pregnant women, children under the age of 18 and patients that were treated with MDR TB prior to the study were excluded from the study.

DATA COLLECTION

A standardised case report form (CRF) was used to collect and record data. The data were collected and recorded from the patient files kept at the MDR-TB hospitals by trained fieldworkers from the MDR-TB hospitals and the relevant clinics after discharge.

Data that were recorded on the CRF included demographic information, patient characteristics, clinical data, details on previous TB episodes, current MDR-TB treatment, surgery, hospital admissions, comorbidities such as HIV and diabetes, baseline and follow-up microbiology results and MDR-TB treatment outcomes. Additional information that was recorded on HIV sero-positive patients included: CD4 count at start of treatment and ARV regimen if the patient was on ARV treatment.

BACTERIOLOGY AND SECOND-LINE DRUG TESTING

Baseline and follow-up sputum samples were collected from the patients in each of the settings and cultured monthly for the duration of the patient's treatment. All positive baseline and follow-up cultures were shipped to CDC, Atlanta, USA where the baseline isolates were tested for drug susceptibility according to clinical laboratory standards[50], using the indirect agar proportions method that uses Middlebrook 7H10 agar.

The criteria for cultures to be shipped for drug susceptibility testing were that the patient had to have a positive baseline culture as well as at least one follow-up positive baseline culture.

The following drugs were tested for drug susceptibility on the baseline isolates at the laboratory of CDC, Atlanta:

- ethambutol 5.0 ug/ml
- streptomycin 2.0 ug/ml
- ofloxacin 2.0 ug/ml
- ciprofloxacin 2.0 ug/ml
- kanamycin 5.0 ug/ml
- capreomycin 10.0 ug/ml
- amikacin 4.0 ug/ml
- aminosalicylic acid 2.0 ug/ml
- ethionamide 10.0 ug/ml

Drug resistance was reported when the proportion of growth on the medium was at least 1% of that on the drug-free medium.

DATA MANAGEMENT

The paper CRFs were safely kept and stored at the MRC, Pretoria where all data were double data entered in to Epi Info, version 3.3.1 using a standardised database form. Data were thoroughly checked for inconsistencies using built in data checks.

DATA ANALYSIS

Statistical data analysis was performed using STATA (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

Dependent variables included drug susceptibility results, either individually or grouped by specific definitions.

Groups were defined as:

All first-line drugs:	isoniazid, rifampicin, ethambutol, streptomycin
fluoroquinolone drug class:	ciprofloxacin, ofloxacin
aminoglycoside drug class:	kanamycin, amikacin, capreomycin

Independent variables included demographic variables, patient characteristics, baseline clinical data and baseline microbiological data.

Baseline characteristics were summarised by presenting the numbers (n) and percentages (%) for categorical variables, and for continuous variables, the mean, standard deviation (sd), minimum (min) and maximum (max) were presented. Fisher's exact test statistic was used to test for statistical significance for categorical data and the student t-test was used to test for statistical significance for continuous variables.

Prevalence:

The prevalence of drug resistance to a specific drug or group in each province was calculated by:

$$\frac{\text{number of patients in the sample with resistance in the province}}{\text{number of patients in the sample in the province}} \times 100$$

The prevalence of drug resistance to a specific drug or group in HIV sero-positive patients was calculated by:

$$\frac{\text{number of HIV sero-positive patients in the sample with resistance}}{\text{number of HIV sero-positive patients in the sample}} \times 100$$

The prevalence of drug resistance to a specific drug or group in HIV negative patients was calculated by:

$$\frac{\text{number of HIV negative patients in the sample with resistance}}{\text{number of HIV negative patients in the sample}} \times 100$$

The prevalence of drug resistance to a specific drug or group in HIV sero-positive patients on ARV treatment was calculated by:

$$\frac{\text{number of HIV sero-positive patients in the sample on ARV treatment with resistance}}{\text{number of HIV sero-positive patients in the sample on ARV treatment}} \times 100$$

The prevalence of drug resistance to a specific drug or group in HIV sero-positive patients not on ARV treatment was calculated by:

$$\frac{\text{number of HIV sero-positive patients in the sample not on ARV treatment with resistance}}{\text{number of HIV sero-positive patients in the sample not on ARV treatment}} \times 100$$

The 95% Confidence interval (CI) was presented for all prevalence calculations.

Statistical models:

A logistic regression model was used to determine if there was an association between drug resistance and HIV infection, and to evaluate any other risk factors associated with drug resistance. Gender, age, weight, previous TB treatment, baseline chest radiography results and baseline smear microscopy results were evaluated as confounders or risk factors. If any of these factors were found to be significantly associated with drug resistance ($p<0.05$) in the multivariate model or if they were found to change the odds ratio with more than 15%, they were included in the model; otherwise they were excluded from the model.

SAMPLE SIZE

Sample size assumption to calculate the prevalence:
Assuming that the observed expected proportion of HIV sero-positive patients with resistance from a random sample out of the population is 0.5, then with a precision of 0.081, the sample size needed to test a single proportion using the large sample normal approximation with a two-sided 95% confidence interval is approximetely145.

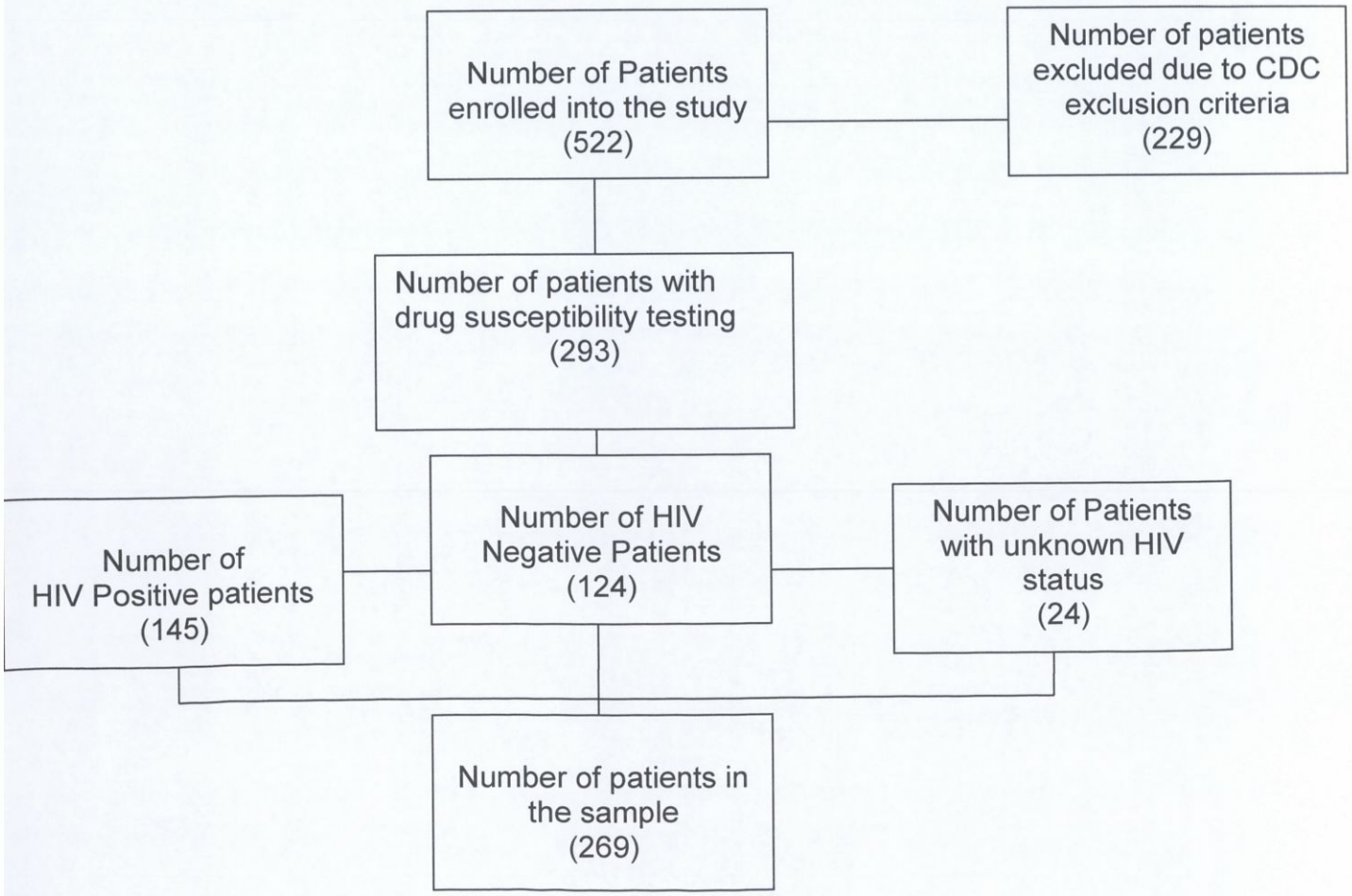
In this analysis 293 random patient records from 4 provinces were used to calculate the sample prevalence of resistance in HIV sero-positive patients, and thus larger than the above mentioned sample size and therefore sufficient.

Sample size assumptions to determine risk factors for drug resistance:
Literature suggests that 10 events per independent variable are sufficient to estimate risk factors using logistic regression modeling[51]. Assuming that a maximum of 10 risk factors will be included in the model, a sample size of 100 was concluded to be sufficient to evaluate risk factors for drug resistance. The sample of 293 in this analysis was therefore assumed to be sufficient.

CHAPTER 3 – RESULTS

The number of patients enrolled from 2006-2008 was 522 across all 4 provinces. Since CDC required that a patient had to have a baseline positive culture as well as at least one follow-up positive culture, 229 patients did not have drug susceptibility testing performed and were excluded. Of the 293 patients with drug susceptibility testing performed, 145 patients were HIV sero-positive and 124 were found to be HIV sero-negative. Twenty four patients had no HIV status and were also excluded from the sample.

Figure 1: Patient accountability



Baseline Characteristics:

Table 1: Patient Baseline Characteristics by HIV status:

Characteristic		HIV Positive n = 145 n (%)	HIV Negative n = 124 n (%)	p-value [a]
Sex	Male	60 (42.3)	82 (57.7)	<0.001
	Female	85 (66.9)	42 (33.1)	
Age (years)	mean,sd,min,max	35.6,8.4,19-60	37.0,11.7,17-62	0.243
Weight (kg)	mean,sd,min,max	50.1,10.5,33-100	51.7,11.9,25-103	0.225
Previous TB	Yes	140 (54.5)	117(45.5)	0.555
	No	5 (41.7)	7(58.3)	
Radiographic chest results	Bilateral	128 (54.0)	109 (46.0)	0.919
	Unilateral	16 (51.6)	15 (48.4)	
	Normal	1 (100.00)	0 (0.0)	
Smear Result	Positive	124(52.3)	113 (47.7)	0.188
	Negative	21 (65.6)	11 (34.4)	
Province	North West	18 (50.0)	18 (50.0)	0.674
	Mpumalanga	8 (61.5)	5 (38.5)	
	Kwazulu-Natal	54 (58.1)	39 (41.9)	
	Eastern Cape	65 (51.2)	62 (48.8)	

[a] fishers exact test

Majority (66.9%) of HIV sero-positive patients were females, had a mean age of 35.6 years and had a mean baseline weight of 50.1 kg. Most patients from both groups had bilateral chest radiographic results and positive smear results at start of treatment. The number of patients

with HIV infection was equally distributed within the provinces with just a little more than 50% of the patients being HIV sero-positive.

Table 2: Patient Baseline Characteristics by ethambutol drug resistance:

Characteristic		ethambutol resistant n = 161 n (%)	ethambutol susceptible n = 108 n (%)	p-value [a]
Sex	Male	87 (61.3)	55 (38.7)	
	Female	74 (58.3)	53 (41.7)	0.621
Age (years)	mean,sd,min,max	36.4;10.9;18-62	36.2;9.2;17-61	0.837
Weight (kg)	mean,sd,min,max	51.0;10.8;25-103	51.0;11.4;30-100	0.989
Previous TB	Yes	153 (59.5)	104(40.5)	
	No	8 (66.67)	4 (33.3)	0.767
Radiographic chest results	Bilateral	147(62.0)	90(38.0)	
	Unilateral	13(41.9)	18(58.1)	
	Normal	1(100.0)	0(0.0)	0.050
Smear Result	Positive	144(60.8)	93(39.2)	
	Negative	17(53.1)	15(46.9)	0.445

[a] fishers exact test

When the characteristics sex, age (years), weight (kg), previous TB history, baseline radiographic results and baseline smear results were compared between patients with ethambutol resistance and patients with ethambutol susceptibility, no significant difference were found (Table 2).

Table 3: Patient Baseline Characteristics by streptomycin drug resistance:

Characteristic		streptomycin resistant n = 196 n (%)	streptomycin susceptible n = 73 n (%)	p-value
Sex	Male	97(68.3)	45(31.7)	
	Female	99(78.0)	28(22.1)	0.099
Age (years)	mean,sd,min,max	36.0; 10.1;17-62	37.2; 10.7;18-61	0.641
Weight (kg)	mean,sd,min,max	50.3; 11.2;25-103	52.7; 10.3;33-91	0.182
Previous TB	Yes	185 (72.0)	72(28.0)	
	No	11(91.7)	1(8.3)	0.190
Radiographic chest results	Bilateral	177(74.7)	60(25.3)	
	Unilateral	18(58.1)	13(41.9)	
	Normal	1 (100.0)	0 (0.0)	0.095
Smear Result	Positive	169 (71.3)	68(28.7)	
	Negative	27(84.4)	5(15.6)	0.141

[a] fishers exact test

When the characteristics sex, age (years), weight (kg), previous TB history, baseline radiographic results and baseline smear results were compared between patients with streptomycin resistance and patients with streptomycin susceptibility, no significant difference were found between the two groups(Table 3).

Table 4: Patient Baseline Characteristics by fluoroquinolone drug resistance:

Characteristic		fluoroquinolone resistant n = 34 n (%)	fluoroquinolone susceptible n = 235 n (%)	p-value [a]
Sex	Male	12 (8.5)	130(91.6)	
	Female	22 (17.3)	105 (82.7)	0.042
Age (years)	mean,sd,min,max	36.7; 10.2; 18- 58	36.3; 10.3; 17- 62	0.360
Weight (kg)	mean,sd,min,max	49.9; 11.2; 30- 85	51.1; 11.0; 25-103	
Previous TB	Yes	32 (12.5)	225(87.6)	
	No	2 (16.7)	10 (83.3)	0.653
Radiographic chest results	Bilateral	30(12.7)	207(87.3)	
	Unilateral	4 (12.9)	27(87.1)	
	Normal	0 (0.0)	1 (100.0)	1.000
Smear Result	Positive	27(11.4)	210(88.6)	
	Negative	7(21.9)	25(78.1)	0.150

[a] fishers exact test

When the characteristics, age (years), weight (kg), previous TB history, baseline radiographic results and baseline smear results were compared between patients with resistance to at least one fluoroquinolone and patients with fluoroquinolone susceptibility, no significant difference were found between the two groups(Table 4). The comparison between sex and fluoroquinolone resistance were significantly different (p=0.042) and sex was identified as a possible confounder to be included in the model.

Table 5: Patient Baseline Characteristics by aminoglycoside drug resistance :

Characteristic		aminoglycoside resistant n = 82 n (%)	aminoglycoside susceptible n = 187 n (%)	p-value [a]
Sex	Male	39 (27.5)	103 (72.5)	
	Female	43 (33.9)	84 (66.1)	0.289
Age (years)	mean,sd,min,max	37.2; 10.2; 18- 60	36.0; 10.3; 17- 62	0.217
Weight (kg)	mean,sd,min,max	49.7; 10.7; 25- 85	51.5; 11.1; 30- 103	0.213
Previous TB	Yes	79(30.7)	178(69.3)	
	No	3(25.0)	9(75.0)	1.000
Radiographic chest results	Bilateral	5 (16.1)	26(83.9)	
	Unilateral	76(32.1)	161(67.9)	
	Normal	1 (100.0)	0 (0.0)	0.054
Smear Result	Positive	66 (27.9)	171(72.2)	
	Negative	16(50.0)	16 (50.0)	0.014

[a] fishers exact test

When the characteristics, sex, age (years), weight (kg), previous TB history, baseline radiographic results and baseline smear results were compared between patients with resistance to at least one aminoglycoside and patients with aminoglycoside susceptibility, no significant difference were found between the two groups(Table 5). However the comparison between baseline radiographic chest results and aminoglycoside resistance were significantly different (p=0.054) as well as baseline smear results (0.014). Baseline radiographic chest results and baseline smear results were identified as possible confounders to be included in the model.

Prevalence of baseline drug resistance

Table 6: Prevalence and 95% CI of drug-resistance by province

Drug resistance	North West n = 36	Mpumalanga n =13	Kwazulu-Natal n =93	Eastern Cape n =127	Overall n =269	P-Value [a]
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	
Ethambutol	13 (36) (22,54)	11 (85) (55,98)	45 (48) (37.9,59.0)	92(72.4) (36.8,80.0)	161 (59.0) (53.7,65.8)	<0.001
Streptomycin	15 (42) (26,59)	9 (69) (38.6,90.9)	63 (68) (57.3,77.1)	109 (85.8) (78.5,91.4)	196 (72.9) (67.1,78.1)	<0.001
All first-line drugs	8 (22) (10,39)	9 (69) (38.6,90.9)	32 (34) (24.9,45.0)	82 (64.6) (55.6,72.8)	131 (48.7) (42.6,54.8)	<0.001
At least one FLQ	1 (3) (0,15)	2 (15) (1.9,45.4)	6 (7) (2.4,13.5)	25 (19.7) (13.2,27.7)	34 (12.6) (8.9,17.2)	0.005
At least one AG	2 (6) (1,19)	2 (15) (1.9,45.4)	11 (12) (6.1,20.2)	67 (52.8) (43.7,61.7)	82 (30.48) (25.0,36.4)	<0.001
All AG	1 (3) (0,15)	1 (8) (0.2,3.6)	8 (9) (3.8,16.2)	65 (51.2) (42.2,60.1)	75 (27.8) (22.6,33.6)	<0.001

[a] fishers exact test

Eastern Cape had the highest prevalence of ethambutol resistance (72.4%), streptomycin resistance (85.8%), resistance to all first-line drugs (INH, RIF, EMB and SM) (64.6%), resistance to at least one FLQ (ciprofloxacin or ofloxacin) (19.7%), resistant to at least one AG (amikacin, kanamycin or capreomycin) (52.8%), and resistance to all three AG (51.2%). Overall resistance was high (59.9 %) for EMB and 72.9 % to SM.

Table 7: Prevalence and 95% CI of drug-resistance among HIV positive and HIV negative patients

	HIV Positive n = 145	HIV Negative n = 124	Overall n = 269	p-value [a]
	n (%) (95% CI)	n (%) (95% CI)	n (%) (95% CI)	
Ethambutol	77(53.1) (44.6,61.43)	84 (67.7) (58.8,75.9)	59.9 (53.7,65.8)	0.018
Streptomycin	105 (72.4) (64.4,79.5)	91 (73.4) (64.7,80.9)	72.9 (67.1,78.1)	0.891
All first-line drugs	63 (43.4) (35.2,51.9)	68 (54.8) (45.7,63.8)	48.7 (42.6,54.8)	0.067
At least one FLQ	15 (10.3) (5.9,16.5)	19 (15.3) (9.4,22.9)	12.6 (8.9,17.2)	0.270
At least one AG	48 (33.1) (25.5,41.4)	34 (27.4) 2(19.8,36.2)	30.5 (25.0,36.4)	0.353
All AG	44 (30.3) (23.0, 38.5)	32(25.0) (17.7,33.6)	27.9 (22.6,33.6)	0.343

[a] fishers exact test

The prevalence of drug resistance to the first-line drugs (ethambutol and streptomycin) was higher in HIV sero-negative patients, 67.7% (p=0.018) and 73.4% (p=0.891) respectively. Prevalence to the second-line drugs (FLQ and AG) was higher in HIV sero-positive patients, 10.3% (p= 0.270) in at least one FLQ, 33.2% (p=0.353) in at least one AG and 30.3% (p=0.343) to all three AG's. Only ethambutol resistance was found to be statistically significant between HIV sero-positive and HIV sero-negative patients (p = 0.018).

Table 8: Prevalence and 95% CI of drug-resistance among HIV positive patients on ARV's and not on ARV's

	ARV's n = 94	Not on ARV's n = 51	
	% (95% CI)	% (95% CI)	p-value [a]
Ethambutol	49 (39,60)	61 (46,74)	0.233
Streptomycin	72 (62,81)	72 (58,84)	1.000
All first-line drugs	42 (31,52)	47 (33,62)	0.599
At least one FLQ	10 (5,17)	12 (4,24)	0.777
At least one AG	27 (18,37)	45 (31,60)	0.028
All AG	25 (16,34)	41 (28,59)	0.058

[a] fishers exact test

By stratifying the HIV sero-positive patients on whether they were on ARV treatment at the start of their MDR-TB treatment or not, it was found that the prevalence of ethambutol resistance was 61% for HIV patients not on ARV treatment compared to 48% for HIV positive patients on ARV treatment, however was not found to be statistical significant (p = 0.233) (table 8). Prevalence of streptomycin resistance was the same whether the patients were on ARV treatment or not (p=1.000). The prevalence of resistance to at least one AG was 45% for HIV patients not on ARV treatment and 27% for patients on ARV treatment (p = 0.028), which was statistical significant. Similarly, the prevalence of resistance to all three AG was 41% for HIV positive patients not on ARV treatment and 24% for patients on ARV treatment(0.058).

Risk Factors:

Gender, age (years), weight (kg), previous TB treatment and smear microscopy, radiographic results weren't found to be significant risk factors or confounders for drug resistance and did not contribute significantly to the models after inclusion.

Table 9: Factors associated with ethambutol resistance

	EMB Resistance (n=137)	Total* (n=220)	OR (95% CI)	p-value
HIV Negative	73	101	**Reference**	
HIV Positive on ARV's	39	76	0.42 (0.2,0.8)	0.008
HIV Positive not on ARV's	25	43	0.53 (0.2,1.1)	0.107
Kwazulu-Natal	45	93	**Reference**	
Eastern Cape	92	127	2.7 (1.5,4.9)	0.001

* Data from North West Province and Mpumalanga were excluded due to the small numbers in the sample

Human immunodeficiency virus patients who received ARV's had significantly lower rates of EMB resistance (OR=0.42; p=0.008) compared to HIV sero-negative patients (table 9). Province was a significant risk factor for ethambutal resistance and patients from Eastern Cape were much more associated with being resistant to EMB compared to Kwazulu-Natal (OR=2.7; p=0.001).

Table 10: Factors associated with streptomycin resistance

	SM Resistance (n=172)	Total* (n=220)	OR (95% CI)	p-value
HIV Negative	77	101	**Reference**	
HIV Positive on ARV's	63	76	1.71 (0.8,3.7)	0.177
HIV Positive not on ARV's	32	43	0.94 (0.4,2.2)	0.880
Kwazulu-Natal	63	93	**Reference**	
Eastern Cape	109	127	3.02 (1.5,5.9)	0.001

*Data from North West Province and Mpumalanga were excluded due to the small numbers in the sample

Human immunodeficiency virus positive patients on ARVs and not on ARVs were not significantly associated with SM resistance compared to HIV sero-negative patients (table 10).

(OR=1.71; p=0.177 and OR=0.94; p=0.880 respectively). Province was a strong risk factor for streptomycin resistance and patients from the Eastern Cape province had a significantly higher associationof being SM resistant compared to Kwazulu-Natal (OR = 3.02; p=0.001).

Table 11: Factors associated with all first-line drugs resistance

	Resistant to all first-line drugs (n=114)	Total* (n=220)	OR (95% CI)	p-value
HIV Negative	59	101	Reference	
HIV Positive on ARV's	35	76	0.65 (0.4,1.2)	0.179
HIV Positive not on ARV's	20	43	0.62 (0.3,1.3)	0.208
Kwazulu-Natal	32	93	Reference	
Eastern Cape	82	127	3.42 (1.9,6.0)	<0.001

*Data from North West Province and Mpumalanga were excluded due to the small numbers in the sample

Human immunodeficiency virus status was not associated with being resistant to all first-line drugs (OR = 0.65, p = 0.179; or = 0.62, p = 0.208) but again province was found to be a strong risk factor and the resistance rates in Eastern Cape was much higher than those in Kwazulu-Natal (OR=3.42; p<0.001) (table 11).

Table 12: Factors associated with resistance to at least one fluoroquonolone

	Resistant to at least one FLQ (n= 31)	Total* (n= 220)	OR (95% CI)	p-value
HIV Negative	16	101	Reference	
HIV Positive on ARV's	9	76	0.78 (0.3,1.9)	0.582
HIV Positive not on ARV's	6	43	0.89 (0.3,2.5)	0.822
Kwazulu-Natal	6	93	Reference	
Eastern Cape	25	127	3.50 (1.4,8.9)	0.009

*Data from North West Province and Mpumalanga were excluded due to the small numbers in the sample

Human immunodeficiency virus status was not associated fluoroquinolone resistance (OR = 0.78, p = 0.582; OR = 0.89; p = 0.822) but again province was found to be a strong risk factor and the resistance rates in Eastern Cape was much higher than those in Kwazulu-Natal (OR=3.50; p=0.009) (table 12).

Table 13: Factors associated with resistance to at least one aminoglycoside

	Resistant to at least one AG (n=78)	Total* (n=220)	OR (95% CI)	p-value
HIV Negative	32	101	Reference	
HIV Positive on ARV's	24	76	1.2 (0.6,2.4)	0.607
HIV Positive not on ARV's	22	43	3.0 (1.3,7.0)	0.010
Kwazulu-Natal	11	93	Reference	
Eastern Cape	67	127	9.12 (4.3,19.2)	<0.001

*Data from North West Province and Mpumalanga were excluded due to the small numbers in the sample

Human immunodeficiency virus positive patients not on ARV's had much higher rates of resistance to at least one AG compared to HIV sero-negative patients (OR=3.0; p=0.010). No significant difference was found between the rates of AG resistance between HIV negative patients and HIV sero-positive patients on ARV treatment (OR = 1.2; p = 0.607). Province was a strong risk factor for AG resistance and patients from the Eastern Cape province had a significantly higher rates of AG resistance compared to Kwazulu-Natal (OR=9.12; p<0.001) (table 13).

CHAPTER 4 – DISCUSSION AND LIMITATIONS

The high prevalence of resistance found to some of the most important drugs was evident from this study (table 6-8) and support literature that suggests that the prevalence of drug resistance is on the increase[5,6,7].

Overall resistance to the first-line drugs, the fluoroquinolone drug class and aminoglycosides drug class was high, with Eastern Cape carrying the highest burden, with alarming resistance to the aminoglycoside drug class (Table 6). Province was also found to be the strongest risk factor associated with resistance to the individual drugs; and drug groups as defined in chapter 2 (Table 9 -13). From literature we know that the most common strain in Eastern Cape is the East Asian-Beijing strain[7]. Literature has also suggested that this strain is possibly more aggressive and more prone to resistance and recurrence, which could explain the high resistance in the Eastern Cape [7,52,53].

A significant association between HIV infection and drug resistance could not be indicated through this study and support the findings of the other studies[37,43,44,46,48,49,50]. However, these studies did not take ARV treatment into consideration and it was evident from our study that ARV treatment could have an effect on the risk of drug resistance (Table 9-13). Patients with HIV and receiving ARV treatment at the time of diagnosis, was significantly less likely to be resistant to ethambutol compared to HIV negative patients (Table 9)No literature was found to support this finding.

Human immunodeficiency virus patients not on ARV treatment were significantly at much higher risk compared to HIV negative patients to be resistant to aminoglycosides (Table 13). No statistical significant difference in the association to aminoglycoside resistance compared to HIV sero-negative patients were found in HIV sero-positive patients on ARV treatment.

From these results it is possible that ARV treatment could potentially lower the risk from having primary resistance, especially aminoglycosides (amikacin, kanamycin and capreomycin) resistance and this finding supports literature that encourage ARV treatment as

soon as possible [13,16,20,21,23,30]. More research is needed on the genetic mechanisms of ARVtreatment and their interaction with TB drugs; however, literature has shown that aminoglycosides and quinolones are effective inhibitors of HIV-1 replication[54,55,56].

Streptomycin is also part of the aminoglycoside class, yet our findings showed that the association of being resistant to SM in HIV sero-positive patient receiving ARV treatment are much higher than an HIV negative patient (OR=1.71; p=0.177),yet not significant, which is the opposite effect found from the other aminoglycosides (table 10; table 13). Literature has found that patients could be resistant to streptomycin and not resistance to the other drugs in the aminoglycoside class (amikacin, kanamycin and capreomycin), because the only shared gene involved in resistance to streptomycin, amikacin and kanamycin is the *rrs* gene, and other genes responsible for streptomycin resistance that amikacin/kanamycin do not share are the *rps*L and gidB -genes[57].

This analysis investigated baseline drug resistance, and thus primary drug resistance. The high primary resistance to the drugs used for the treatment of drug susceptible TB as well as MDR-TB raises a lot of concern and necessity for new drug regimens and more aggressive approaches of infection control. Although acquired drug resistance was not investigated in this study, literature did illustrate that acquired drug resistance and nosocomial infection has been found to be risk factors for infection[11,12,13,14,15], which in fact could increase the burden on top of the high primary drug resistance found.

In summary, a relationship between HIV infection and primary resistance was not found, but unexpected findings regarding the role of ARV treatment was found in the analysis. A significant relationship was however found between the provinces and the risk of resistance.

At this stage only speculation is possible to explain the different findings between HIV infection, ARV treatment and the resistance to specific drugs in the TB regimens, and more research in this area is definitely needed.

There are limitations to our findings. This was a cross-sectional study, and more effective evidence based designs should be used to investigate these findings more thoroughly. Another limitation to this study was that only 50% of the samples were tested by CDC for drug resistance due to the exclusion criteria of a positive baseline culture plus at least one positive

follow up culture, and this could have introduced some bias to the findings. That said, significant relationships have been found in this analysis which is supported by previous studies, with potential research questions in to how we can optimise the use of ARV treatment in conjunction with TB treatment to enhance treatment and improve treatment outcomes as well as to prevent further resistance to ARV drugs as well as TB drugs.

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Appendix 1

Protocol

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Epidemiological impact of HIV on second-line drug resistance in patients with multi-drug resistant tuberculosis in high HIV prevalent settings in South Africa

Degree: MSc Epidemiology

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EXECUTIVE SUMMARY

Multi-drug resistant tuberculosis (MDR- TB) makes up about 19% of the total TB burden in the world. Although the global estimates of MDR-TB patients living with HIV is unknown because limited information is available, it was estimated in 2010 that about 13% of the TB population in the world is infected with HIV. HIV infection has been associated with the development of TB and MDR-TB, thus contributing to the prevalence of MDR TB. HIV infection has also been associated with mortality in drug-resistant tuberculosis (DR TB) patients.

In South Africa, we have the 4th highest MDR-TB and HIV burden and the highest TB/HIV burden in the world, and with the emergence of extensively drug resistant tuberculosis (XDR-TB) and totally drug resistant tuberculosis (TDR-TB), it is becoming more of a challenge to treat these patients.

Acquired drug resistance to 1st line anti-TB drugs and nosocomial transmission of drug resistant TB strains has been associated with HIV infection, but little is known about the association of HIV infection and baseline 2nd line anti-TB drug resistance.

In this analysis, the prevalence of resistance to 2nd line- anti TB drugs in HIV positive and HIV negative patients will be evaluated to see if there indeed is a difference and risk factors associated with the resistance of 2nd line anti TB drugs will also be evaluated.

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1. DEFINING THE RESEARCH PROBLEM

In South Africa, we have the 4th highest MDR-TB and 4th highest HIV burden in the world as well as the highest TB/HIV burden worldwide. With the emergence of XDR-TB and TDR TB, it has become a necessity to understand the epidemic especially where HIV infection plays a role.

The question which will be answered in this analysis is: Does the prevalence of drug resistance differ between HIV positive and HIV negative patients, and what are the risk factors associated with second-line anti TB drug resistance.

2. LITERATURE OVERVIEW AND MOTIVATION

South Africa is home to not only the 3rd highest TB burden in the world[1] but also has one of the largest populations living with HIV. USAID reported in 2011 that 5.6 million people in South Africa are living with HIV which is almost 16% of the total burden worldwide. It was estimated in 2010 that about 13% of the TB population in the world is infected with HIV[2]. South Africa has the highest TB and HIV co-infection burden in the world[1] with 60% of the TB population in South Africa being HIV infected[3].

Multidrug resistant TB (MDR-TB) is defined as TB resistant to at least isoniazid and rifampicin which is the two most important drugs in the first-line anti-TB drug regimen and extensively drug-resistance TB (XDR-TB) is defined as MDR-TB, resistant to at least one drug within the fluoroquinolone and injectable drug groups classified as the two most important groups of the second-line anti-TB drugs in the treatment of MDR-TB.
MDR-TB makes up about 19% of the total TB burden in the world [4] with South Africa carrying of that 19%, the 4th highest burden[1].

The MDR TB numbers in South Africa have increased from 3219 new cases in 2004 to 7386 in 2010[5], which is almost double in numbers. XDR TB increased from 84 cases in 2004 to an alarming 741 cases in 2010. Although the global estimates of MDR TB patients living with HIV is unknown due to limited information, the Medical Research Council estimated during their drug-resistance survey in 2002 that about 40% of MDR TB cases in South Africa are also HIV infected[6]. Not only is South Africa facing an increase in MDR TB and XDR TB numbers, but a recent study has indicated the emergence of totally drug resistant TB (TDR TB) which is a concept not clearly defined by the World Health Organization (WHO) yet, but in general refers to XDR TB cases resistant to all the drugs in the fluoroquinolone and injectable drug groups[7] . The latter emphasises that, not only is the number of cases being infected with resistant- TB strains increasing, but the severity or degree of resistance to anti-TB drugs is also worsening.

Three ways of transmission or development of drug-resistant TB strains have been noticed occurring in South Africa.

1. Primary transmission: The patient is infected with an organism already resistant to anti-TB drugs.
2. Acquired resistance: When the original susceptible TB strain change or evolve in to a drug-resistant TB strain due to the mutation.

3. Exogenous/Nosocomial reinfection: Transmission of drug-resistant TB strains that occur in a specific setting such as a hospital, health clinic or ward.

In the early 90's it was believed that the main way of transmission in South Africa was acquired drug-resistance. It was suggested that the reason for this was inadequate treatment and patients not adhering to treatment. Thereafter South Africa implemented the DOTS-PLUS strategy for MDR-TB treatment as a strategy to standardise MDR-TB treatment and attempt to reduce acquired drug-resistance. Patient adherence to treatment was also promoted and specific measures were put in place to help patients adhere to their treatment[8,9,10].
In recent years, studies have found that the majority of infections of drug-resistant strains are not through acquired transmission any more, but through primary infection and nosocomial infection[11,12,13,14,15].

Could it be possible that the drug-resistant TB epidemic is changing in South Africa? And if it is changing, what is driving the change?

Because of the immune suppression caused by HIV, HIV infection has been found to be the strongest risk factor to develop active TB (susceptible or drug-resistant) when infected with *M.tuberculosis*[16,17,18]. It also has been found to increase the susceptibility to be infected with *M.tuberculosis* and reactivate latent TB. In HIV patients, TB is the most common opportunistic infection[19,20,21]. WHO estimated that HIV infected people have a 20 times increased risk to develop TB in countries like South Africa with a high HIV burden[22].

With drug-resistance TB numbers increasing and South Africa fighting a very challenging HIV epidemic, Wells et al described the collision of these two epidemics by calling it the "perfect storm"[21,23].

Some of the implications HIV has on the DR-TB epidemic are:

- HIV contribute to the prevalence of TB and DR-TB[13,21,23,24,25,26]
- DR-TB is more complex to treat and diagnose in HIV positive patients[27]
- Delayed diagnosis can increase the spread of DR strains[27]
- HIV infection increases the risk of nosocomial transmission[13,15,23,28]
- Poorer treatment outcomes especially an increase in mortality[13,23,29,30,31,32]
- If cured, HIV positive patients have a 5 times greater chance that disease will recur [33]

Little is known about the association between HIV and second-line anti-TB drug resistance. A few studies that were published showed evidence of association between acquired rifampicin resistance and HIV infection[3435,36], and another study from Mozambique showed an association between HIV infection and isoniazid resistance as well as streptomycin resistance[37]. Conflicting results have been published on the association of HIV infection and MDR TB. Some studies didn't find an association between HIV and MDR TB[37,38,39] but these studies had very small sample sizes, other studies did identify HIV infection as a risk factor for MDR TB and XDR TB.[27,39,40]

Studies in Latvia and the Ukraine have successfully succeeded in proving that HIV patients are at higher risk for primary drug resistance but could not successfully prove the relationship between HIV and XDR TB due to the lack of second-line drug testing[41, 42].

These studies have successfully demonstrated that there is a possible association between HIV infection and 1st line anti-TB drug resistance as well as an association between acquired resistance to rifampicin and HIV infection.

In South Africa three particular studies did not find any association between MDR TB and HIV infection, but these studies had limited baseline characteristics and no statistical tests results were presented. Also only drug resistance to the first-line drugs was performed. No second-line drug susceptibility was performed so no inference could be made between the association of second-line drugs and HIV infection[43,44,45].
Another study found that HIV infection was associated with DR-TB outbreaks[23].

Literature has suggested that the relationship between HIV and drug-resistance to anti-TB drugs are not entirely understood, especially the association to second-line anti-TB drug resistance.[23,46] Studies have suggested the importance of fluoroquinolones in the treatment of MDR-TB, and XDR-TB in HIV positive patients has been seen as untreatable. Information on the prevalence of resistance to second-line drugs could give us a better idea of the adequacy of treatment[47,48].

Information on the association of HIV infection and resistance to anti-TB drugs will improve the rationale of the challenges we are facing in South Africa and enhance the knowledge of the DR TB epidemic and the consequences of HIV co-infection. The sub-analysis will try to establish if HIV infection is indeed the driving force behind DR TB in the country and to what extent. The results of this sub analysis will also give a good rough estimate of the prevalence of certain drug resistance in the country with 9 second-line anti-TB drugs tested on a cohort from 4 different provinces.

3. AIM AND OBJECTIVES

Aim:

To evaluate the impact that HIV has on resistance to second-line anti-TB drugs (SLD) in 4 provinces of South Africa

Objective:

1. Evaluate the prevalence of baseline second-line drug (SLD) resistance at the start of treatment in HIV positive and HIV negative adults with pulmonary MDR TB.

2. Evaluate the risk factors for baseline second-line drug resistance at the start of treatment in HIV positive and HIV negative adults with pulmonary MDR TB.

4. METHODS

4.1. STUDY DESIGN

The dataset that will be used in the secondary data analysis is data from a prospective cohort study that was conducted during 2005-2008 in collaboration with CDC and MRC. The study was funded by USAID.

4.2. SETTING

The study was conducted in 4 of the 9 provinces in South Africa:

- North West province, Klerksdorp MDR TB hospital
- Mpumalanga, Witbank MDR TB hospital
- KwaZulu-Natal, King George IV MDR TB hospital
- Eastern Cape, Jose Pearson MDR TB hospital and Fort Gray MDR TB hospital

4.3. PATIENT/RESEARCH OBJECT SELECTION

Patient inclusion criteria:

- Patients diagnosed with pulmonary MDR TB who started treatment between January 2005 and June 2008 and had
- At least one positive culture of *M. tuberculosis* within one month from the day they started on treatment for MDR TB was available.

Patient exclusion criteria:

- Prisoners
- Pregnant women
- Children under the age of 18
- Patients treated for MDR TB prior to study

4.4. MEASUREMENTS

A standardised case report form (CRF) was used to collect and record data (see Annexure).

The data was collected and recorded by trained fieldworkers. Data that was recorded on the CRF included the following:

- Demographic information
- Patient Characteristics
- Clinical data
- Details on previous TB and current MDR TB treatment
- Surgery
- Hospital admissions
- Comorbidities such as HIV and diabetes
- Microbiology results (baseline and follow-up)
- Baseline chest radiography results
- MDR TB Treatment outcomes

Additional information that was recorded for HIV-positive patients:

- CD4 count at start of treatment
- ARV regimen if patient was on ARV's
- CPT information if the patient was on CPT
- TB preventive treatment information if the patient was on TB preventive treatment

All data was double data entered in to Epi Info, version 3.3.1 and thoroughly checked for inconsistencies.

Baseline and follow-up sputum samples were collected from the patients and cultured monthly for the duration of the patient's MDR TB treatment. All Positive baseline and follow-up cultures were shipped to CDC, Atlanta, USA where the baseline isolates were tested for drug susceptibility according to clinical laboratory standards[49], using the indirect agar proportions method that uses Middlebrook 7H10 agar.

The following drugs were tested for drug susceptibility on the baseline isolates at the laboratory of CDC, Atlanta:

- ethambutol 5.0 ug/ml
- streptomycin 2.0 ug/ml
- ofloxacin 2.0 ug/ml
- ciprofloxacin 2.0 ug/ml
- kanamycin 5.0 ug/ml
- capreomycin 10.0 ug/ml
- amikacin 4.0 ug/ml
- aminosalicylic acid 2.0 ug/ml
- ethionamide 10.0 ug/ml

Drug resistance was reported when the proportion of growth on the medium was at least 1% of that on the drug-free medium.

4.5. DATA ANALYSIS

Statistical data analysis will be performed using STATA 12.

Dependent variables:

- each drug-susceptibility result individually
- groups of specific pooled drug-susceptibility results

Independent variables:

- demographic variables
- patient characteristics
- clinical data
- microbiological data

Continuous variables:
Data summary will employ means, standard deviances, medians, IQR's and 95% confidence intervals which will be reported by group. The two groups will be compared with student's t test in univariate analysis when the assumption of normality is met, else nonparametric tests such as the Mann-Whitney ranksum test statistic will be used.

Categorical variables:
Each categorical variable will be tabulated against each drug susceptibility result or pooled group and Pearson's chi-square test statistic or Fisher's exact test statistic will be used to test for statistical significance.

Prevalence and risk factors:

Separate analysies for the prevalence and the risk factors will be performed for each dependent variable using a logistic regression model. Analysis for the prevalence and the risk factors will also be performed for HIV positive and HIV negative patients' separately using a logistic regression model. A p value of 0.05 will be considered significant and a p value of less than 0.001 will be considered highly significant.

Model selection:

Potential risk factors with a univariate p value of 0.2 will be analysed as possible risk factors in the multivariate model. Continuous variables will be considered as both continuous variables or as categorical variables in the model e.g age and age categories. Forward step-wise logistic regression will be used to include variables in the model and variables with the highest p value will be removed from the model. A Change of more than 15% in a coefficient will be investigated for possible confounding and Interaction will also be investigated.

Akaike's information criteria (AIC) will be used to choose the best model fit.

4.5.1. SAMPLE SIZE

When the sample size is 145, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation will extend 0.081 from the observed proportion for an expected proportion of 0.5. Our sample size is 293 and thus larger than the above mentioned sample size and therefore will be sufficient to estimate the prevalence.

Literature suggests that 10 events per predictor variable are sufficient to estimate risk factors using logistic regression modeling[50]. A maximum of 10 predictors will be included in the model, and thus the sample of 293 will be sufficient to estimate the risk factors.

5. ETHICAL CONSIDERATIONS

Ethical approval was obtained 29 March 2005 (see annexures). Secondary data analysis will be performed on a **closed** dataset and **no additional information will be collected**. Consent was given by all the participants included in this dataset using a standard consent form (see annexures).

6. BUDGET (including Funding obtained)

Secondary data analysis will be performed and therefore no funding will be necessary.

7. TIME LINES AND PROJECT MANAGEMENT

Activity	Date Completed
Data Analysis	1 Month
Introduction and Methods	2 Months
Final review of Dissertation	2 Months

8. CONTRIBUTORS AND AUTHORSHIP

Name	Department	Contribution	Author or acknowledgement
Prof Paul Rheeder	UP-SHSPH	Supervisor	Author
Prof Martie van der Walt	MRC-TEIRU	Co-Supervisor	Author
CDC Coordination team	CDC, Atlanta	Principal investigators	Author
SA MRC Coordination team	MRC, Pretoria	Study coordinators	Author

9. REPORTING

Results from this analysis will be published in a suitable academic journal and presented at conferences. The results will also be reported back to Department of Health and the participating facilities.

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Appendix 2

University of Pretoria Ethics Approval

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 13/04/2011 and Expires 13/04/2014.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

27/06/2013

Approval Notice
New Application

Ethics Reference No.: 269/2013

Title: Epidemiological impact of HIV on second-line drug resistance in patients with multi-drug resistant tuberculosis in high HIV prevalent settings in South Africa

Dear Ms Ronel Odendaal

The **New Application** for your research received on the 20/05/2013, was approved by the Faculty of Health Sciences Research Ethics Committee on the 26/06/2013.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year.
- Please remember to use your protocol number (269/2013) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
Standard Conditions:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

We wish you the best with your research.

Yours sincerely

pp

Professor Werdie (CW) Van Staden
MBChB MMed(Psych) MD FCPsych FTCL UPLM
Chairperson: Faculty of Health Sciences Research Ethics Committee

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Appendix 3

PETTS Ethics approval

MRC
SOUTH AFRICA
The Medical Research Council

Ethics Committee

P.O. Box 19070, Tygerberg 7505, South Africa.
Francie van Zijl Drive, Parow Valley 7500, Cape Town.
Tel:+27 (0)21 938 0341, Fax:+27 (0)21 938 0201
E-mail: adri.labuschagne@mrc.ac.za
http:// www.sahealthinfo.org/ethics/ethics.htm

29 March 2005

Dr M van der Walt
Tuberculosis Operational and Policy Research
MRC Pretoria

Dear Dr Van der Walt

RE: Increasing access to drugs for the treatment of multidrug-resistant tuberculosis while preventing the emergence of further resistance (PETTS study)

Thank you for your response to the Committee, dated 31 January 2005. The resubmitted patient information sheet and consent form have been found to be acceptable. I am pleased to inform you that ethics approval is now granted for the study.

Wishing you well with your research.

Yours sincerely

PROF. D DU TOIT
CHAIRPERSON: MRC ETHICS COMMITTEE

Appendix 4

Informed Consent Form

DOTS-PLUS FOR MULTIDRUG-RESISTANT TUBERCULOSIS PATIENTS IN SOUTH AFRICA

PATIENT INFORMATION SHEET

Hello,

We are researchers from the Medical Research Council (MRC) who work on a type of TB called "multidrug-resistant (MDR)" TB. You have been found to have multidrug-resistant (MDR) TB, which cannot be treated with the usual TB drugs. Instead, you will have to be treated at the MDR Centre where you will receive special drugs. These drugs are very expensive and are not available outside the MDR Centre. You will have to take them for a long period (up to 22 months) and it is very important that you do not stop at any time. These drugs are provided free of charge to you by the Department of Health and the Doctor in charge at the MDR Centre will explain to you how your MDR-TB will affect your life and what needs to be done to try to get you well.

The Medical Research Council (MRC) is evaluating the treatment of MDR-TB in South Africa in a research project and the MDR Centre where you will be treated is taking part in the research. We are interested to see how well the MDR-TB drugs are working. We are asking you to be part of this research. If you agree to this, we would need to use the medical information that will be collected at the MDR Centre as part of your treatment and we would like to ask your permission to do this. All your information will be kept strictly confidential by the researchers and will not in any way affect your care at the MDR Centre. Also, your name will not be made known if the research results are published.

You are free to choose not to make your medical information available to the MRC. This will not n any way affect your medical care at the MDR Centre. If you do decide to let the MRC use your information and change your mind later, you only have to tell the Doctor in charge and s/he will let us know immediately. Also, if you have any questions, the Doctor in charge at your MDR Centre will discuss them with you. You are welcome to phone Dr Karin Weyer of the Medical Research Council in Pretoria, at telephone number (012) 339-8550.

If you are happy to let the MRC use your medical information for the research study, please read and sign the attached consent form.

Thank you

Dr Karin Weyer

Medical Research Council, Pretoria

DOTS-PLUS FOR MULTIDRUG-RESISTANT TUBERCULOSIS PATIENTS IN SOUTH AFRICA

PATIENT CONSENT FORM

DOTS-Plus NUMBER | | | | | / | | | | |

FULL NAME _____

AGE | | | RACE | 1 | 2 | 3 | 4 | 9 | GENDER | M | F |

Physician/Nurse _____ Centre _____

Dr/Mr/Ms _____ has explained my disease to me, and has explained the nature and commitment it requires from me for the next 16 to 22 months. S/he has also told me that the Medical Research Council (MRC) is evaluating the treatment of MDR tuberculosis as part of a research study and that the MRC would want to use my medical results to do this.

Dr/Mr/Ms _____ has assured me that all medical information will be kept in the strictest confidence and that I will not be identified by name when the results are published. S/he has also assured me that, if I choose not to make my medical information available to the MRC, it will not affect my medical care.

I understand everything that was explained to me and agree to have the MRC use my medical information for the research study.

Signature of patient _____ Date _____

Signature of physician/nurse _____ Date _____

Signature of witness _____ Date _____

Appendix 5

Case Report Form

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** ____ 9b. **Total years completed** ____
1=primary 2=secondary 3=university or professional 4=technical school
5=other 9=unknown 0=none (no formal education)

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 each 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 without HIV/AIDS (15a≠1), skip to item 19 on page 7.

SUPPLEMENTAL DATA FOR PATIENTS WITH HIV/AIDS (15a=1).

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 dates** _____ *(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.[1][2] 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 **end of an episode** 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) **Patients registered and treated as Category I/II/III**: 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? It depends on the reason:
      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) **Patients registered and treated as Category IV**: 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.***

Examples of determining episodes of TB follow on page 10, back of the next data page.

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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=contam. 9=unk.)*

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

30. Result of culture _____ *(0=neg. 1=pos. 2=scanty{<10col.} 3=contam. 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=contam. 9=unk.)*

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

34. Result of culture _____ *(0=neg. 1=pos. 2=scanty{<10col.} 3=contam. 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 e.g.3x900*	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___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____
___ amikacin	a1___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____
___ capreomycin	a1___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____
___ ethionamide	a1___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____
___ pro-thionamide	a1___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____
___ ofloxacin	a1___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____
___ cipro-floxacin	a1___	b1 ___/___/_____	c1___/___/___ d1 _____	e1 _____	f1 _____ _____
	a2___	b2 ___/___/_____	c2___/___/___ d2 _____	e2 _____	f2 _____ _____
	a3___	b3 ___/___/_____	c3___/___/___ d3 _____	e3 _____	f3 _____ _____

TB TREATMENT - CONTINUED					
___ levo-floxacin	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	f3 ________ ________
___ para-amino-salicylic acid	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	f3 ________ ________
___ cycloserine	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	f3 ________ ________
___ terizidone	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	f3 ________ ________
___ Other________	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	f3 ________ ________
___ Other________	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	f3 ________ ________
___ Other________	a1___	b1 ___/___/_____	c1___/___/___ d1 ________	e1 ________	f1 ________ ________
	a2___	b2 ___/___/_____	c2___/___/___ d2 ________	e2 ________	f2 ________ ________
	a3___	b3 ___/___/_____	c3___/___/___ d3 ________	e3 ________	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=contam. 9=unk.)
3. Date culture result reported	____/____/_____ (dd/mm/yyyy)
4. Result of culture _____	(0=neg. 1=pos. 2=scanty{<col.} 3=contam. 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 # ____
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FOLLOW-UP LAB RESULTS DURING MDR TB TREATMENT	**Follow-up month # ____**
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9d. Was the culture isolate shipped to CDC? ____ 9e. What date was it shipped to CDC? ____/____/_____	*(0=no 1=yes 9=unknown)* *(dd/mm/yyyy)*

USE ADDITIONAL FOLLOW-UP LAB RESULTS PAGES AS NEEDED - INSERT HERE