EXTENSIVELY DRUG RESISTANT TUBERCULOSIS IN SOUTH AFRICA

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DECLARATION

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Abbreviations

AFB	acid-fast bacilli
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
BCG	bacilli calmette-guérin
BCH	Brooklyn Chest Hospital
US CDC	United States Centers for Disease Control
CPT	co-trimoxazole preventive therapy
DOT	directly observed therapy
DOTS	directly observed therapy short course
DRS	drug resistance surveillance
DST	drug susceptibility testing
EC	Eastern Cape
FIND	Foundation for Innovative New Diagnostics
HCWs	health care workers
HIV	human immunodeficiency virus
INH	isoniazid
IQR	inter-quartile range
IRIS	immune reconstitution inflammatory syndrome
KZN	KwaZulu-Natal
LFT	liver function test
MDR-TB	multidrug-resistant tuberculosis
NDOH	National Department of Health
PZA	pyrazinamide
RFLP	restriction fragment length polymorphism
PAS	Aminosalicylic Acid
SA	South Africa
TB	tuberculosis
TSH	thyroid-stimulating hormone
TST	tuberculin skin test
WC	Western Cape
WHO	World Health Organization
XDR-TB	extensively drug resistant tuberculosis

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Abstract

Background: There are few data for treatment-related outcomes in patients with XDR tuberculosis in settings with high HIV prevalence.

Methods: We reviewed the case records of 227 consecutively diagnosed South-African patients with XDR-TB between August 2002 and February 2008 at four designated provincial treatment facilities. Mortality and conversion were stratified by HIV status. Furthermore, the records of 115 patients, from three out of the four centres, were retrospectively analysed for adverse drug reactions (ADRs), which were graded for severity (severe= therapy stopped, life threatening or death).

Results: Of 227 patients studied, 195 XDR-TB patients were included in the study and 174 (82 HIV-infected) received treatment. 36% (62/174) died during follow up. The overall culture conversion rate was 19% (33/174) and 70% of patients converted within 6 months. Mortality rates in HIV-infected patients were not significantly different from uninfected patients [41.5% (34/82) vs. 30.4% (28/92) (p=0.13)]. Treatment with moxifloxacin, prior culture-proven MDR-TB, and increasing number of drugs used were independent predictors of death. HIV-infected patients treated with HAART had lower mortality (hazard ratio = 0.31, 95% CI 0.15-0.61, p=0.01). 58% (67/115) patients experienced 161 ADR's. The offending drug was discontinued in 19/67(28%) of patients, reactions were life-threatening in 2/67(3.0%) and 6/67(9.0%) died. Patients with severe ADRs were less likely to culture-convert [2/27(7.4%) vs. 24/88(27.3%); p=0.02], and more likely to default therapy

(p=0.003). Only culture conversion and a history of MDR-TB were independent risk factors for mortality in those with severe ADRs compared to those wild mild, moderate or no ADRs. Death from an ADR was commoner in HIV-infected patients [5/6(83.3%) vs. 1/6(16.6%), p=0.01)].

Conclusions: In South Africa a significant proportion of XDR-TB patients remain HIV-unrelated, and prognosis, regardless of HIV status, poor. Nevertheless, survival in HIV-infected patients is better than previously reported, and treatment with HAART improves outcomes. The frequency of ADR's with XDR-TB treatment regimens is high, often severe, and negatively impacts on culture conversion outcomes. These data have implications for the formulation of recommendations for control programmes in resource-poor settings.

Chapter 1 Introduction

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1.1 Introduction:

Here I will review the literature about drug resistant tuberculosis (DR-TB) globally, in the African continent, and more specifically in the South- African context. Although the work presented here is specifically about extensively drug-resistant TB (XDR-TB), I will also discuss multi drug-resistant TB (MDR-TB). XDR-TB is mostly acquired as a result of failed MDR-TB treatment making an understanding of MDR-TB necessary.

1.2 Global history and Epidemiology of MDR and XDR-TB

1.2.1 Background

Despite its decline in Europe and North America, *Mycobacterium tuberculosis* (MTB) continues to be a major global killer and the most common cause of chronic pulmonary disability in the 21st century. There are 10 million cases reported annually worldwide (Corbett et al., 2006) and MTB is the leading cause of death in South Africa (statistics South Africa, 2009). Over the last two decades resistance to anti-TB drugs has resulted in the emergence of multidrug-resistant TB (MDR-TB)

and extensively-drug resistant (XDR-TB). MDR-TB has been defined as resistance to isoniazid and rifampicin and XDR-TB resistance to rifampicin, isoniazid, one of the fluoroquinolones, and at least one of the second-line injectable agents (i.e. kanamycin, amikacin or capreomycin) (CDC, 2006). In 2008 there were approximately 500 000 cases of MDR-TB globally and more alarmingly, about 5% to 10% of MDR-TB cases are thought to be XDR-TB. MDR-TB and XDR-TB threatens to destabilise TB control in several regions of the world including Africa, Eastern Europe, Russia, central Asia, India and China. This has resulted in a dire situation in South Africa, where the human immunodeficiency virus (HIV) co-infection is fuelling the spread of the epidemic.

1.2.2 Development of drug-resistant tuberculosis

The history of drug resistant tuberculosis dates back to soon after the first drug for treating TB was made available. Streptomycin was introduced in 1944, and in 1947 Pyle et al. described resistance to streptomycin (Pyle, 1947). The hypothesis of naturally existing resistant variants was confirmed in 1952. Lederberg and Lederberg showed that resistance of the tubercle bacillus to antimicrobial agents was not caused by the drug but a process where resistant variants, which pre-exist, were effectively selected for survival (Lederberg and Lederberg, 1942). About the same time it was shown that resistance to isoniazid occurred shortly after its introduction as an anti-TB drug. Bacillary population size and its ability to multiply were identified as two important pre-requisites for the emergence of bacterial resistance (Spradling and Ridzon, 2003). As new anti-tuberculosis drugs were

developed, descriptions of individual resistance and cross-resistance between different drug classes was described (Canetti, 1962, Middlebrook, 1954) (Mitchison, 1984). Although all the anti-tuberculosis drugs cause resistance, some confer resistance quicker than others, by selection of pre-existing resistant variants.

Canetti (1965) found that the ability of a single anti-tuberculosis drug to enhance the selection of resistance depended on a certain drug concentration in vivo, and the drugs ability to get rid of drug susceptible portions of the microbial population (Canetti, 1962, Canetti, 1965). Later David (1970) showed that the rate of spontaneously resistant variants in a sample of wild type mycobacterium should be 3.5×10^{-6} for isoniazid, 3.8×10^{-6} for streptomycin, 3.1×10^{-8} for rifampicin, and 0.5×10^{-4} for ethambutol. Based on these frequencies, the probability of a naturally occurring bacillus resistant to isoniazid and rifampicin is approximately 1 in 10 bacilli (David, 1970.) This work clearly demonstrated that in order for TB treatment to be successful, several anti-TB drugs, especially in the early phase of treatment when the bacillary load is at its highest and contains large enough numbers of naturally occurring drug-resistant variants, are required. Because of this, two phases of TB treatment were described: the initial phase, when numbers of organisms are high, followed by the continuation phase, when smaller numbers of bacilli need a prolonged chemotherapeutic course to ensure all bacilli are killed. This strategy continues to be a guiding principle of anti-tuberculosis therapy today (Canetti, 1962).

1.2.3 Drug resistant tuberculosis in America

A study conducted in the United States in 1991 (CAUTHEN et al., 1994) on all culture positive TB isolates with drug susceptibility testing (DST) results found resistance to one or more drugs in 14.2% of cases and resistance to isoniazid and / or rifampicin was 9.5%. This is substantially higher than published estimates in South Africa in 2002 (MRC, 2004). Another study done in New York showed that patients with MDR-TB were as likely to be foreign born as USA born. Responding to this growing public health crisis, the national MDR-TB task force recommended in 1992 that DST be performed on initial *MTB isolates* on all TB patients. This, in addition to increased drug resistant surveillance, meant that from 1993 - 2000 the number of MDR-TB cases in the Unites states declined from 485 to 141, mainly in New York (CDC, 2001) (Shah and al., 2008).

1.2.4 Emergence of drug-resistant TB in other countries

A review of 63 surveys of resistance to anti-tuberculosis drugs was carried out between 1985 and 1994 (Cohn et al., 1997). Isolates from new TB cases showed 10.6% of patients were resistant to isoniazid, 9% to streptomycin, 2.4% to rifampicin and 1.8% to ethambutol. Rates for retreatment cases were much higher and the median rate of resistance to isoniazid was 10.6%, to streptomycin 4.9%, to rifampicin 2.4%, and to ethambutol 1.8%. The rates of MDR-TB among new cases ranged from 0 to 10.8% (median, 0.5%) and among retreatment cases: Nepal 48.0%; Gujarat, India 33.8 %; New York City 30.1%; Bolivia 15.3% and Korea 4.5%.

As a result, the World Health Organisation (WHO) and United States Centers of Disease Control (US CDC) started a global project on anti-tuberculosis drug resistant surveillance in 1994. By 2000 two global surveys had been completed, the first from 1994 - 1996 (Pablos-Mendez et al., 1998) and the second from 1996 - 1999 (Espinal et al., 2001). Seventy two regions were included in at least one of the surveys and isolates from about 118 000 patients were tested. The geographic area covered 33% of the world's population. The results showed that drug resistant TB was present in all areas surveyed although in small proportions (Cegielski et al., 2002). In the second survey, the median prevalence of MDR was 1%, with a range of 0-14.1%. Seven geographic area had high levels of MDR-TB >5% among new cases; four of these sites (Estonia, Latvia, and Ivanovo and Tomsk Oblasts of the Russian Federation) were in the former Soviet Union. A report from 2002, (Dye et al., 2002), was the first comprehensive incidence estimates of the MDR-TB burden in 136 countries making up 97% of the world's population. Dye et al. estimated there were 273 000 MDR-TB cases worldwide which comprised of 3.2% of all TB cases (Dye et al., 2002).

In the fourth global report on anti-TB drug resistance (WHO/IUATLD, 2008) data on the extent of drug-resistant TB between 2002 and 2007 was reviewed. This report includes data for drug susceptibility testing (DST) of 90 726 patients from 83 countries (Wright et al., 2009). The median prevalence of MDR-TB in new cases was 1.6% (IQR 0.6-3.9) ranging from 0% in eight countries with low TB prevalence to 19.4% in Moldovia and 22.3% in Baku, Azerbaijan. The median prevalence of MDR-TB in previously treated TB cases was 11.7% (IQR 4.9-20.9) (Wright et al., 2009). Among the 17 settings reporting a prevalence of MDR-TB >25% in retreatment cases, nine were in former Soviet Union countries (WHO and IUATLD, 2008). At least one country in all of the six WHO regions reported a prevalence of approximately 3% MDR or XDR-TB cases among newly diagnosed TB cases (WHO and IUATLD, 2008, Chiang et al., 2010).

From November 2004 to November 2005 the global Supranational Reference laboratories (SRL) Network requested all *Mycobacterium tuberculosis* isolates that had undergone testing for first and second-line drug susceptibility testing between 2000 and 2004 to be reviewed. The study found that 20% of isolates met MDR-TB definitions and 2% were classified as XDR-TB. Population-based assessment showed that 4% of the cases were from the USA, 15% from South Korea and 19% from Latvia. In 2010, 58 countries from all the continents had reported at least one case of XDR-TB. Altogether 963 cases were notified globally compared with 772 cases from 28 countries in 2007. It is believed that many XDR-TB cases are never diagnosed due to lack of laboratory capacity to test for second-line drugs and that

many patients die before they are recognised as MDR or XDR-TB (M/XDR) (Shah, 2007).

In 2009 there were just over 30 000 MDR-TB cases reported globally which is just 7% of the estimated 440 000 cases (range, 390 000 - 510 000). This shows the limited availability of DST in many countries because of a lack of laboratory capacity. Twenty-seven high burden countries are responsible for 86% of all such cases. The four countries with the largest numbers of estimated MDR-TB cases were China, who reported 100 000 cases (range, 79 000 - 120 000), India with 99 000 (range 79000 – 120 000), the Russian Federation with 38 000 (range, 30 000 - 45 000) and South Africa with 13 000 (range 10 000 - 16 000). This suggests that overall, the numbers of patients diagnosed with MDR-TB and started on treatment will almost double in 2010 and 2011 compared with 2009. Although countries are taking steps to improve their surveillance systems, most available data is from “ad hoc” designed studies(Sotgiu et al., 2009);Migliori, 2008 #1436;Chiang, 2010 #1435}, which do not allow for scientific clarification.

1.2.5 Emergence of drug-resistant tuberculosis in Africa

Although data from Africa are, in general, scanty, due to weakness of the laboratory infrastructure and difficulties in performing DST, six countries reported drug resistance data to WHO for the fourth global report (WHO and IUATLD, 2008). It was estimated at this time that over 60 000 drug-resistant cases are probably occurring in Africa which accounts for 30% of the global burden; half of these 14% new cases (Chiang et al., 2010). Thirty-four countries in Africa reported MDR-TB cases and eight XDR-TB cases (WHO, 2010). Of the nine countries that were estimated to have over 5 000 incident MDR-TB cases, three of these countries, Ethiopia, Nigeria and Sudan, were from Africa. However, as only 50% of the population is represented in WHO/IUATLD studies, the true extent of drug-resistant tuberculosis is unknown and likely to be underestimated (Schaaf et al., 2009). A few MDR-TB hotspots (i.e. MDR-TB > 3% of TB cases) have been reported in Africa: these include Mozambique, Cote d'Ivoire and more recently Rwanda and Democratic Republic of Congo(Schaaf et al., 2009)}. Nine African countries rate among the 27 high burden MDR-TB countries: South Africa (4^{th}), Nigeria (9^{th}), Democratic republic of Congo (13^{th}), Ethiopia (16^{th}), Kenya (20^{th}), Mozambique (21^{st}), Zimbabwe, (23^{rd}), Cote d'Ivoire (25^{th}) and Sudan (26^{th}) (Schaaf et al., 2009). Those defending WHO data argue that national TB programmes are working well, and due to the later introduction of rifampicin to African countries, there has been less time for resistance to develop (Academies, 2009).

It has also been suggested, that the rifampicin argument (as mentioned above) may also be invalid. Mozambique started using rifampicin about 10 years after South Africa and yet their MDR-TB rate is already higher than South Africa's. The MDR- TB rate in Mozambique, which introduced rifampicin at the same time as Gambia, is 10 times higher than that in Gambia. It can be argued that African countries that are able to carry out drug resistant surveys are more likely to have well-functioning national TB programs, laboratory structure, and transport networks and therefore lower rates of MDR-TB than those countries without these resources.

The low MDR-TB rates among TB cases in Africa compared with that in regions such as Eastern Europe and Central Asia could be due to outdated studies or surveys in which the scientific rigour is questionable or coverage is not nationwide. Nevertheless, given that African countries have the highest incidence rate of TB in the world, even at low proportions of drug resistance the caseload of MDR-TB patients is very high. As a result, the rates of MDR-TB cases arising per 100 000 population in some southern African countries are five to six times higher than those of China and India. Latest estimates of WHO put the number of MDR-TB cases emerging in 2008 in Africa at 69 000 (53 000 - 110 000) (WHO, 2010). Because of the factors mentioned previously, these estimated numbers of MDR-TB cases are based on mathematical modelling rather than empirical studies. Laboratory surveillance for MDR-TB and XDR-TB should be strengthened and expanded across the region, particularly in countries with large populations where studies have never been done or are now older than five years. (Figure 1.1)

Figure 1.1 African countries with a known MDR-TB rate.

Map 1: Represents data from the 3rd global WhO/IUATLD report -2004 (WHO and IUATLD, 2004).

Map 2: Represents data from the 4th WHO/IUATLD global report, 2008. (WHO and IUATLD, 2008).

Map 3: Based on a formula developed by Zignol et al.(Zignol et al., 2006), to estimate the MDR-TB rate in countries where a survey has never been conducted.

1.2.6 A brief look at M/XDR-TB in sub saharan Africa

The number of TB cases in sub-saharan Africa has risen sharply in the past 10 years due largely to the HIV epidemic (WHO and IUATLD, 2008) (Raviglione and IM, 2007). WHO estimates that between 1990 and 2005 the incidence of tuberculosis doubled from 149 to 343/100 000 population. However, despite the high prevalence of HIV/tuberculosis co-infection the second global resistance survey measured MDR rates of only 0.8-2.6% in the sub saharan African region (WHO and IUATLD, 2004).

1.2.7 Development of drug-resistant tuberculosis in South Africa

The first cases of MDR-TB in South Africa were diagnosed in 1985 in Gauteng province and shortly thereafter in the Western Cape. A retrospective analysis of MDR-TB patients notes and their DST results revealed that XDR-TB cases were to be found as far back as 1992. In 2000 a pilot project with standardised treatment regimens for MDR-TB was implemented in all provinces by the Department of Health in collaboration with The Medical Research Council of South Africa. In 2006

the MDR-TB programme was officially incorporated into the National TB control programme.

South Africa is ranked as the fourth highest drug-resistant TB high burden country in the world, behind countries with much larger populations such as China, India and the Russian Federation. Almost 7 000 cases of M/XDR-TB were notified in 2008, most coming from KwaZulu-Natal and the Western Cape.

The true impact of drug-resistant TB in sub- saharan Africa became known with a report from a rural hospital in Tugela Ferry, KwaZulu-Natal, South Africa, (Gandhi et al., 2006a). The study showed that of the 1539 people tested for tuberculosis (Jan 2005, to March 2006), 542 had a culture positive for *M. tuberculosis* and of these 53 had XDR-TB. Median time of death from sputum collection was 16 days (range 2 - 210 days) for 52 of 53 patients who died. Even more alarming was that 26(55%) of the patients had no history of previous TB although 67% had a recent hospital admission before their TB diagnosis. These two points suggest nosocomial transmission in this outbreak of XDR-TB. It is estimated that 1.8% (range 1.5-2.3) of all new cases of TB in South Africa are MDR-TB cases and 6.7% (5.5-8.1) of retreatment cases. It is expected that in South Africa 7 301 MDR-TB patients will be treated in 2010 and 8 642 in 2011 (WHO, 2010) demonstrating continued growth of the epidemic.

PROVINCE	2007				2008				2009			
	MDR Reg	MDR Rx started	XDR Reg	XDR Rx started	MDR Reg	MDR Rx started	XDR Reg	XDR Rx started	MDR Reg	MDR Rx started	XDR Reg	XDR Rx started
Eastern Cape	932	932	171	171	797	772	135	135	1006	847	158	135
Free State	158	158	10	7	265	233	7	7	216	148	7	6
Gauteng	497	497	45	45	601	414	40	40	808	512	29	25
KwaZulu Natal	788	788	170	170	1061	1039	165	163	969	927	183	177
Limpopo	71	71	2	2	104	104	0	0	90	88	3	3
Mpumala-nga	148	148	0	0	272	272	3	3	198	198	5	5
Northern Cape	145	145	11	11	148	148	8	8	70	253	14	13
North West	156	156	4	4	159	159	1	1	175	175	9	9
Western Cape	862	439	81	64	1145	890	59	34	1201	995	86	58
South Africa	3 757	3334	494	474	4552	4031	418	391	4933	4143	494	431

Reg = Registered, Rx=treatment

Table 1.1 MDR-TB and XDR-TB cases, registered, in South Africa compared to those in whom treatment was initiated, per province, 2007-2009 (NTP)

Table 1.1 shows the large gap between the numbers of MDRs diagnosed registered and started on treatment, per province. In 2008 the National TB programme (NTP) did not start treatment in nearly 35% of all diagnosed MDR-TB patients and 32% of all diagnosed XDR-TB patients. Initiation of treatment depended on the prevalence of drug-resistance in that particular province as well as accessibility and efficiency of diagnostic and treatment services in the provinces (WHO, 2010).

1.2.8 Molecular Epidemiology

"The ability to discern the molecular "fingerprint" (GENO-type) of *M. tuberculosis* isolates has revolutionised our understanding of the transmission of tuberculosis" (Barnes and Cave, 2003). Genotyping of isolates from patients is useful in several situations:

1. About 3% of patients, from whom *M. tuberculosis* is apparently isolated in a clinical laboratory, do not have tuberculosis; the positive cultures are due to cross contamination. When clinical findings are not suggestive of tuberculosis although *M. tuberculosis* has been isolated, concurrent genotyping could suggest cross contamination and lead to anti-tuberculosis drugs being stopped.
2. Genotyping allows for isolates with different drug susceptibility which can be useful in cases where (a) the original organism developed drug resistance during or after anti-tuberculosis therapy, (b) the patient was re-infected with a different strain or (c) cross-contamination is suspected. Genotyping isolates from the patient and others done at the same time can look at all the possibilities. If the first organism developed resistance, the cause could be non adherence to therapy, lower concentrations of drugs used for treating the TB, as a result of malabsorbtion of the drugs or by drug interactions. If the cause is re-infection health authorities should attempt to find the source.

3. Genotyping can be used to identify whether a patient has been re-infected or has relapsed, using isolates from previous and present episodes. If the patient has relapsed, the susceptibility of the original isolate can be used to guide treatment and reasons for treatment failure evaluated. If the patient has been re-infected the source case should be found. It is important to distinguish between relapse and re-infection to be able to define treatment failure.
4. Genotypic methods plus epidemiologic factors can help determine whether an outbreak has occurred or whether there is a coincidental occurrence of a large number of cases. There is huge variability in the genotypes of *M. tuberculosis* isolates with epidemiologically unrelated tuberculosis, but identical in cases that have been infected by a common source (isolates with identical or closely related genotypes are known as clusters).

Spoligotyping of XDR-TB isolates collected retrospectively in four provinces identified 17 strains belonging to seven lineages during the period June 2005 to December 2006 (Mlambo, 2008). The genetic diversity and wide geographical area that these strains came from suggest that in 63% to 75% of cases, XDR-TB had been acquired. This study further showed that XDR-TB was mostly associated with the Beijing lineage 34%.

1.3 Clinical features of M/XDR-TB

The clinical presentation of MDR-TB and XDR-TB is similar to that of drug-susceptible TB. Risk factors are described in section 1.3.1 below, but it must be remembered that in up to half of, the diagnosed cases of drug-resistant TB, there may be no risk factors. In HIV-infected patients, the clinical and radiological picture may be atypical, with a more rapid disease progression especially in the setting of nosocomial transmission with virulent strains.(Gandhi et al., 2006a)

1.3.1 Risk factors

M/XDR-TB may be linked to previous TB treatment. The prevalence of drug resistance among previously treated TB cases was much higher than that among new TB cases (WHO, 2008, WHO, 2000, Chiang et al., 2010). Six common treatment errors have been identified: (1) addition of a single drug to a failing regimen, (2) an inadequate primary regimen, (3) failure to recognize initial or acquired resistance, (4) failure to recognise and deal with non-adherence, and (5) failure to provide directly observed treatment (Spradling and Ridzon, 2003). It is reported that the probability of any resistance in previously treated patients is over four-fold higher, and that of MDR-TB over ten-fold higher, then for untreated patients (Espinal et al., 2001). Various specific risk factors are listed in the table below.

Risk factor	Comments
Failure of retreatment regimen (Regimen 2)/ chronic TB patients	Chronic TB patients are defined as patients who are sputum positive at the end of the intensive phase and on completion of re-treatment regimen. These patients have the highest MDR-TB rates, often greater than 80%.
Exposure to a confirmed MDR-TB patient	Most studies have shown that close contacts of MDR-TB patients have very high rates of MDR-TB. This includes children, who should be started on MDR-TB therapy empirically until proven not to have MDR-TB. This could also include hospitalized TB patients in settings where MDR-TB is diagnosed.
Failure of treatment regimen for new patients (Regimen 1)	Failures of Regimen 1 or Regimen 3 are adult or paediatric patients, respectively, who remain positive at the end of the intensive phase or become sputum smear or culture positive 5 months or later during the course of treatment.
Relapse and default	Erratic drug intake or early relapse may point to possible MDR-TB. Relapses within the first six months post-treatment may have similar MDR-TB rates as failures. Repeated interruption of treatment can also result in selection for resistant mutants.
Use of drugs that compete with or alter the metabolism of TB drugs, resulting in reduced serum levels	Antifungal agents in the azole family interfere with rifamycins will lower azole levels. In addition ketoconazole can lower rifampicin levels by 40%-50%; protease inhibitors (Lopinivar/ritonavir (Kaletra) can affect the absorption of other drugs by the body.
Co-morbid conditions associated with malabsorption or rapid transit diarrhoea	Malabsorbtion may result in selective low serum drug levels and may occur in either HIV negative or positive patients.
HIV	Numerous MDR-TB outbreaks have been documented in HIV+ individuals as a result of the depressed immune system and high susceptibility to infection.

(Adapted from: World Health Organization. *Guidelines for the Programmatic Management of drug-resistant Tuberculosis (WHO/HTM/TB/2005.361)*, World Health Organization: Geneva, Switzerland, 2008).

Table 1.2 Risk factors for MDR-TB (Health, 2010)

Other risk factors not included in the above table include:

- Immigration from high prevalence countries can increase the risk of being infected by a resistant strain, affecting not only foreign born people but native individuals as well.

- There is also strong evidence that TB is more common among children living in a household affected by TB (Chiang et al., 2010, WHO, 2000).
- Alcohol, substance abuse and other socio-economic factors such as homelessness, malnourishment and poor living conditions, or have a higher defaulter rate have been shown to have a higher prevalence of MDR-TB.

1.3.2 HIV co-infection

In 2007 it was estimated that of the 9.27 million people with new TB infections, 1.37 million of them were co-infected with HIV 14.8%, and that some 456 000 of these co-infected patients died. Of serious concern is the fact that in some countries in sub-saharan Africa, the TB/HIV co-infection rates are between 50% and 80% (WHO, 2009, Nunn et al., 2007). South Africa and Zimbabwe have the highest incidence, mortality, and HIV/TB co-infection rates among the 22 high-burden countries (Table1.3). In a systematic Review conducted by Suchindran *et al.* explored the question of whether HIV infection was a risk factor for multi-drug resistant tuberculosis. While they could not find an overall association between MDR-TB and HIV, or acquired MDR-TB and HIV, there is a possible association with primary MDR-TB and HIV (Suchindran et al., 2009). There is little data from Africa about HIV co-infection and XDR-TB, other than Ghandi's study, where almost 100%

of the XDR-TB patients were HIV-infected and the median time to death was 16 days (Gandhi et al., 2009).

22 countries with the highest-burden of TB				
Country	Incidence of TB (all types) per 100 000 population	Mortality per 100 000 population	HIV prevalence in incident TB cases, in %	MDR in new cases, in %[@]
South Africa	948	230	73	1.8
Zimbabwe	782	365	69	1.9
Cambodia	495	89	7.8	<0.0
Mozambique	431	127	47	3.5
DR Congo	392	82	5.9	2.3
Kenya	353	65	48	1.9
Ethiopia	378	92	19	1.6
Uganda	330	93	39	0.5
UR Tanzania	297	78	47	1.1
Nigeria	311	93	27	1.8
Philippines	290	41	0.3	4.0
Indonesia	228	39	3.0	2.0
Bangladesh	223	45	0	3.5
Pakistan	181	29	2.1	3.2
Vietnam	171	24	8.1	2.7
Myanmar	171	13	11	4.0
India	168	28	5.3	.8
Afghanistan	168	30	>0.05	3.3
Thailand	142	21	17	1.7
Russ. federation	110	18	16	13
China	98	15	1.9	5.0

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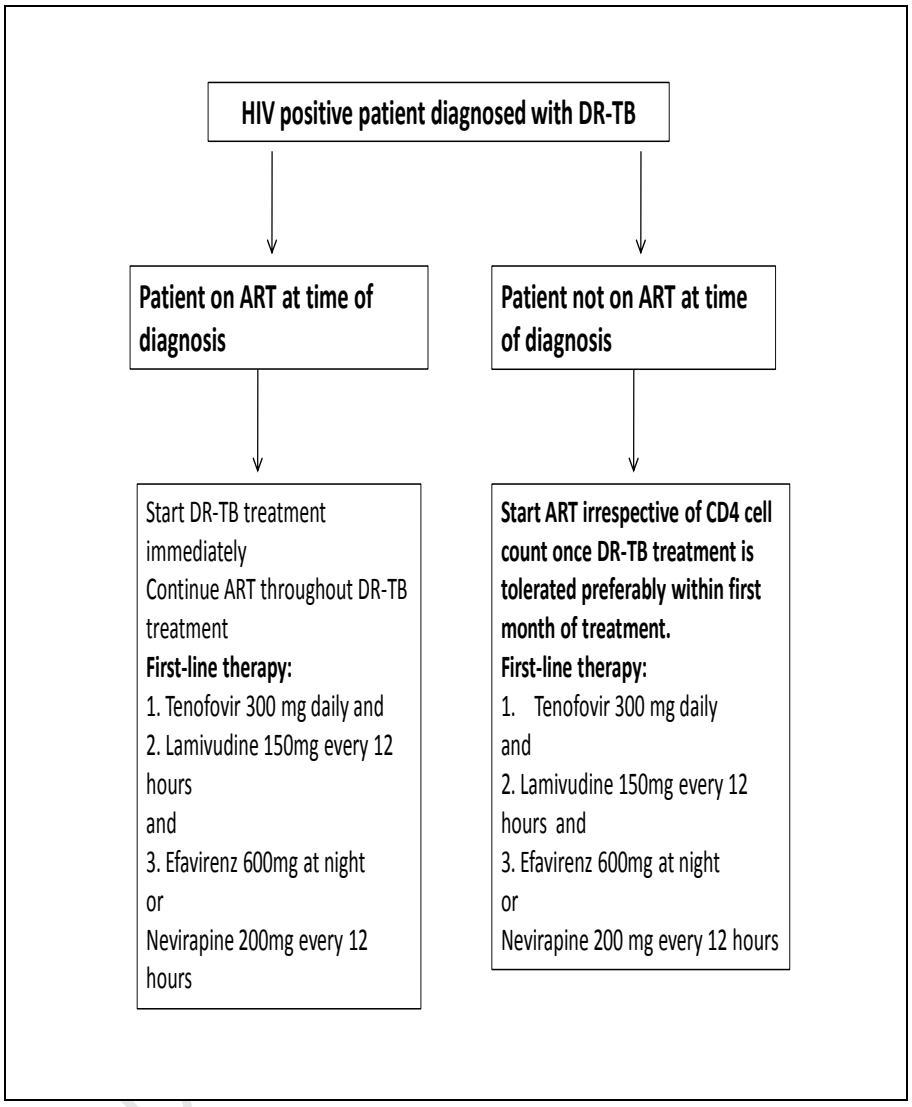
China	98	15	1.9	5.0

[@]MDR, multidrug resistance to (at least) isoniazid and rifampicin

Table 1.3 World Health Organisation estimates of the incidence and mortality of all types of tuberculosis per 100 000 population, the prevalence of HIV incident TB cases, and the MDR rate among new cases in 2007.

HIV-infected persons with latent *M. tuberculosis* infection are at a much higher risk of developing tuberculosis and of those that do get active disease; the prognosis is dire (Nunn et al., 2007). Tuberculosis cases among HIV co-infected people is also difficult to diagnose with conventional diagnostics, and patients could be started on ineffective treatment regimens, resulting in an increase in morbidity and mortality in co-infected individuals (Jassal and Bishai, 2009). DR-TB treatment is the same for HIV-infected and uninfected patients but patient management can be more complicated with more adverse drug reactions being experienced in co-infected patients. Mortality is also high during treatment especially in a patient with the advanced stages of immunodeficiency. Patients already on antiretroviral treatment when MDR- or XDR-TB is diagnosed should immediately be started on appropriate DR-TB treatment. If the patient presents with DR-TB before commencing ART the initiation should be fast tracked as soon the DR-TB treatment is tolerated (Figure 1.4) (Health, 2010). Does HAART in HIV-infected XDR-TB patients make a difference in outcomes? If so when should the HAART be initiated in relationship to the initiation of the XDR-TB treatment? What about side effects of the ARVs. and second-line drugs?

Figure 1.2 Flow chart for anti-retroviral therapy in adult patients with drug-resistant tuberculosis



1.3.3 Transmission dynamics (clinical aspects)

MDR-TB cases are generated by transmission in congregate settings and these cases are being generated faster than emerging M/XDR-TB treatment programmes can cure them. It is estimated that under 10% of MDR-TB cases are being treated globally and that as many as a half of these cases are primary, thereby indicating

transmission (WHO, 2010). It is also thought that transmission is mainly from unknown or inadequately treated persons with MDR and XDR-TB thus fuelling the global DR-TB epidemic especially where HIV rates are high. Once mutant organisms are selected by poor chemotherapy, the airborne spread needs to be curbed and the most effective way to do this is by prompt diagnosis and effective treatment. Adding to this are the limitations of Infection control practises in health care institutions. Health care facilities have both patients and health care workers (HCW's) who are HIV infected and those with DR-TB who in turn can be infected or re-infected (Joshi et al., 2006). The lack of rapid diagnostics results in transmission from unsuspected cases in the community, clinics, hospitals, prisons and other congregate settings (WHO, 2010).

Most TB facility guidelines concentrate on the known or suspected cases already on treatment but it has been known for some time that the bigger risk is from unsuspected, untreated cases (Nardell and Dharmadhikari, 2010). Investigators in Lima, Peru screened 250 patients admitted to a female medical ward (Willingham et al., 2001). They found that 40 patients who were TB culture positive, including 65% (26) smear positive and 33% (13) unsuspected TB patients. Of the 40 culture positive cases, eight had MDR-TB. Without prompt identification and effective treatment of TB and drug resistance in particular transmission from such patients continues (Becerra et al., 2010).

1.3.4 Contact Tracing

Effective contact tracing includes identifying, screening and treating all adult and child close contacts of a DR-TB patient. Close contacts are defined as "people living in the same household, or spending many hours a day together with the patient in the same indoor living space" (WHO, 2006). All contacts should be screened and if active TB is suspected a sputum specimen sent for culture and drug susceptibility testing. An empirical treatment based on susceptibility results of the index case can be started while waiting for DST. Increased morbidity and mortality and amplification of resistance can occur from any delays in diagnosis and / or treatment (WHO, 2006). Children under five, who don't have active TB, should be given prophylactic treatment for six months.

In a study by Becerra et al., 693 households where there was an index case with M/XDR-TB were investigated. Of the 4503 household contacts, 2.6% (117) had active TB at the time the index case started MDR-TB treatment. During the four-year follow up, 5.3% (242) contacts developed active TB (of the 142 patients who had DST results 90.9% had MDR-TB). In light of this high risk of disease among contacts, TB programmes should implement systematic household investigations for all household contacts of proven M/XDR-TB cases. Any contacts diagnosed as having active TB should be assumed to be drug resistant until proved otherwise (Becerra et al., 2010).

1.5 M/XDR-TB Management

1.5.1 Principles for treating M/XDR-TB

Design treatment regimens with a consistent approach based on the hierarchy of the five groups of anti-tuberculosis drugs (see Table 1.5)

1. Prompt diagnosis of DR-TB and initiation of appropriate therapy
2. Use at least four drugs with either certain, or almost certain, effectiveness
3. DST should generally be used to guide therapy, however do not depend on DST in individual regimen design for ethambutol, pyrazinamide, and group 4 and 5 drugs. (Table 1.5)
4. Do not use ciprofloxacin as an anti-tuberculosis agent
5. Design a programme strategy that takes into consideration access to high quality DST, rates of DR-TB, HIV prevalence, technical capacity and financial resources (Table 1.6)
6. Treat for 18 months after culture-conversion
7. Use adjunctive measures appropriately, including surgery, nutritional and social support
8. Aggressively treat XDR-TB whenever possible
9. Treat adverse effects immediately and adequately

The rationale for individualised regimens being used for treatment of MDR-TB is that the patient receives TB drugs to which they are susceptible. The problem is that DST results for second-lines are difficult and not as susceptible as is ideal. Results can be easily misread as the margin between the minimum inhibitory concentration and the critical concentration is small. The only accurate second-line DST tests are for kanamycin, amikacin and the fluoroquinolones. This rationale combined with a lack of second-line drugs available to treat MDR-TB treatment has led to standardised treatment regimens for MDR-TB proposed by WHO and adopted by South Africa in 2000 (MRC, 2004). Orenstein et al. (Orenstein et al., 2009) conducted a meta-analysis looking at treatment outcomes in standardised versus individualised regimens. Data was analysed from 33 studies from 20 countries with treatment outcomes for 8506 patients receiving second-line drugs for MDR-TB. The overall success rate, either treatment completion or cure was 62% (95% CI 58-67%).Among the 29 studies using individualised regimens treatment success was 64% (95% CI 59-68%) while in the 5 studies looking at standardised regimens had a lower success rate of 54% (95% CI 43-68%). This was not statistically significantly however. In 12 studies that combined the two factors with the largest effect on success (treatment length of at least 18 months and use of DOTs throughout the treatment period), saw the pooled success was 69% (95% CI 64%-73%), which was significantly higher than the pooled success estimate for the other 22 studies that did not meet both criteria (585; 95% CI 52-64%). This study highlights the importance of closely monitoring patients whom are initiated on appropriate treatment regimens and that patients should be treated for at least 18 months to attain treatment success more readily.

It is also worthy to note that in a meta-analysis carried out by Johnson *et al.* in 2009, where 36 studies were reviewed and pooled, successful outcomes occurred in 62% (95% CI 57-67) (Johnston et al., 2009), and in that by *Akcakir et al*. 50% of patients had a successful treatment outcome. Factors which increased treatment success were found to be treatment duration longer than 20 months, use of more than 3 susceptible drugs, individualized regimens, use of fluoroquinolones, and use of 2nd line agents in general (Akcakir et al., 2008). Those factors associated with worse outcome included male gender 0.61(0.46-0.82), alcohol abuse 0.49(0.39-0.63), low BMI 0.41(0.23-0.72), smear positivity at diagnosis 0.53(0.31-0.91), fluoroquinolone resistance 0.45(0.22-0.91) and the presence of an XDR-TB resistance pattern 0.57(0.41-0.80) (Johnston et al., 2009) (26 trials included with a total of 4959 patients)

Step 1	Use any available **group 1: first line oral agents** pyrazinamide ethambutol	Begin with any first-line agents that have certain, or almost certain, efficacy. If a first line agent has a high likelihood of resistance, do not use it. (E.g. most category IV regimens used in treatment failures of Cat. II do not include ethambutol because it is likely to be resistant based on treatment history).
Step 2	Plus one of these **Group 2: Injectable Agents** kanamycin (or amikacin) capreomycin streptomycin	Add an injectable agent based on DST and treatment history. Avoid streptomycin, even if DST suggests susceptibility, because of high rates of resistance with DR stra and higher incidence of ototoxicity.
Step 3	Plus one of these **Group 3; Fluoroquinolones** levofloxacin moxifloxacin ofloxacin	Add a quinolone based on DST and treatment history, In cases where resistance to ofloxacin or XDR-TB is suspected, use a higher-generation quinolone, but do not rely upon it as one of the four core drugs
Step 4	Pick one or more of **Group 4: Second line oral bacteriostatic agents** p-aminosalicylic acid cycloserine (or terizidone) ethionamide(or protionamide)	Add group 4 drugs until you have at least 4 drugs likely to be effective. Base choice on treatment history, adverse effect profile and cost. DST is not standardised for the drugs in this group.
Step 5	Consider use of these **Group 5: Drugs of unclear role in DR-TB treatment** clofazamine[a] linezolid amoxicillin/clavulanate thiocetazone[b] imipenem/cilastatin high dose isoniazid clarithromycin	Consider adding Group 5 drugs in consultation with an MDR-TB expert if there are not four drugs that are likely to be effective from Groups 1-4. If drugs are needed from this group , it is recommended to add at least two. DST is not standardised for the drugs in this group. [a]drug of choice in group 5

[b]Thiacetazone is contraindicated in HIV-infected individuals because of the serious risk of life-threatening adverse reaction

Table 1.5 Grouping of drugs, for use in M/XDR-TB treatment, and their use in M/XDR-TB regimens

1.5.2 Management challenges

Clinicians in resource poor settings face several management challenges. Once the diagnosis of MDR-TB has been made, drug-susceptibility testing facilities for second-line drugs are often very limited. In patients who fail WHO regimen 1, it is important to establish the degree of drug compliance and the correct diagnosis by considering other possibilities including alternative respiratory disorder. In HIV co-

infected patients opportunistic infections and IRIS need to be considered. A failing regimen 1 should never be replaced by regimen 2 as this represents adding a single drug to a failing regimen. Selection of an MDR-TB treatment regimen must be based on local drug susceptibility data and previous treatment history. Where compliance has been established and there is failure of a MDR-TB treatment regimen suspicion of XDR-TB should be raised. This presents an even greater challenge because several second-line drugs including moxifloxacin are often not available to clinicians in resource-poor settings. A recent meta-analysis by Jacobson (Jacobson, 2010) recently found that moxifloxacin was an independent predictor of survival in XDR-TB. Further studies are now urgently required to determine the potency of the specific quinolones against extensively-drug resistance stains of *M. tuberculosis*. High dose isoniazid is a widely available drug worthy of consideration in treatment regimens for MDR-TB and XDR-TB (Katiyar, 2008).

If a diagnosis of XDR-TB is made and there is localised disease and lung function permits, then surgery should be considered after the first few months of therapy. For the patients who fail to respond to XDR-TB treatment, the absence of dedicated hospices, long stay community treatment facilities or isolation facilities makes further management of these patients challenging. The isolation of treatment failures with no other therapeutic options and those who are recurrent defaulters also present ethical and medico-legal dilemmas(Bateman, 2007). The sheer case load of treatment failures has now outstripped bed capacity in TB hospitals in South

Africa and many of these patients are now being discharged back into the community.

A co-ordinated response in resource poor settings is urgently required to prevent further transmission and amplification of the XDR-TB problem. Recurrent defaulting of treatment also presents a dilemma. Withdrawing treatment is a last resort in patients who have recurrently defaulted, where the amplification of drug-resistance has occurred, and where any further treatment would render the patient at high risk for further amplification and transmission of disease.

1.5.3 Outcomes

Although the treatment outcomes of MDR-TB are encouraging in some settings (Mitnick et al., 2003, Yew and Leung, 2008, Leimane et al., 2010), in resource-poor settings like Africa, treatment outcomes are poorer (see table 1.6 for outcome definitions). For example, in the West Coast region of the Western Cape, South Africa, of 491 MDR-TB patients who received treatment only 49% were cured and / or completed treatment (Shean et al., 2008). Although XDR-TB shows encouraging treatment outcomes in countries such as Peru (Mitnick et al., 2008) in Africa treatment-related outcomes are poorer (Schaaf et al., 2009). In a hospital outbreak of XDR-TB in Kwazulu-Natal, the median time to survival in a population with advanced HIV, was approximately two weeks (Gandhi et al., 2009). Thus, a widely held view is that XDR-TB in Africa is mainly associated with HIV and has a dismal

prognosis. XDR-TB is extremely difficult to treat and diverts much needed resources away from treatment programmes. Prevention of XDR-TB is therefore paramount.

The first reports on XDR-TB (Migliori et al., 2007b, Migliori et al., 2007a, Kim et al., 2007, Leimane et al., 2010, Keshavjee et al., 2008) confirmed that patients generally have a poorer prognosis as well as fewer treatment options then those with MDR-TB. Most of the manuscripts reviewed showed that XDR-TB has a higher probability of death, failure, longer hospitalisation, longer treatment duration, as well as a longer time to culture-conversion rates. Mortality was almost always increased among XDR-TB patients and this may suggest that incurable patients do exist (Table 1.7) (Migliori et al., 2007b{Sotgiu, 2009 #1489). It will require aggressive case finding and treatment regimens for drug susceptible and MDR-TB to prevent a catastrophic XDR-TB-related decline of TB control in Africa.

Cure:	A patient who has completed treatment and has been consistently culture-negative for five consecutive months in the final twelve months of treatment. If one positive culture is reported during that time and there is no concomitant clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures, taken at least thirty days apart.
Treatment completed:	A patient who has completed treatment but does not meet the definition for cure due to lack of bacteriologic results (i.e. less than five cultures were performed in the final twelve months of treatment).
Death:	A patient who dies from any cause whilst on DR-TB treatment
Treatment default:	A patient who interrupts DR-TB treatment for two or more consecutive months for any reason.
Treatment failure:	A patient who has had two or more of the five consecutive cultures taken in the final twelve months are positive, or if any one of the final three cultures are positive.
Transfer out:	A patient who has been transferred to a reporting unit in another province and for whom the treatment outcome is unknown.
Treatment stopped due to adverse drug reactions	A patient who develops adverse drug reactions whilst on DR-TB and could not continue treatment in spite of the management of the adverse drug reactions as per protocols and the decision has been taken to stop treatment.
Treatment stopped due to other reasons:	A patient who could not continue on DR-TB treatment for any other medical reason than adverse drug reactions, and a decision to stop treatment was made.
Still on treatment:	A patient who for any reason is still on treatment at the time of submission of treatment outcome report.

The outcome definitions are based on bacteriological culture as a monitoring tool:

Table 1.6 treatment outcome definitions (Health, 2010)

Study	Time to conversion	Treatment success MDR vs. XDR n (%)	Failure MDR vs. XDR n (%)	Death MDR vs XDR n (%)
(Ghandi et al., 2006b), Lancet	NA	NA	NA	NA vs. 52 (98%)
(Migliori et al., 2007b), EID	SS: 41 vs 110days C: 58 vs 7.5 days (median)	45 (35.7%) Vs 0	NA	8(6.3%) Vs 4 (36.4%) P:<0.001; RR: 5.45
(Migliori et al., 2007a), ERJ	SS: 56 vs 110 days C: 60 vs 168 days (median)	165 (45.7%) vs 12 (18.7%)	32 (8.9%) Vs 12 (18.7%) P; 0.016; RR: 2.12	43 (11.9%) Vs 14 (21.9%) P: 0.03; RR: 1.84
(Kim et al., 2007), CID	NA	109 (64.9%) vs 23 (53.5%)	29 (17.3% vs 11 (25.6%) p 0.21; RR: 1.48	13 (7.7%) Vs 6 (14.0%) P: 0.20; RR: 1.8
(Mitnick et al., 2008), NEJM	C: 61 vs 90days (median)	400 (66.3%) Vs.29 (60.4%)	13 (2.1%) Vs 5 (10.4%) P:0.7; RR: 0.83	123 (20.4% Vs 11 (22.9%) P: 0.67; RR: 1.12
(Chan et al., 2008), NEJM	NA	Odds ratio (MDR) 23.4	NA	Hazard ratio (XDR) 2.5 P: 0.07
(Keshavjee et al., 2008), Lancet	C: 2 vs 2 months (median)	386 (66.7%) Vs 14 (48.3%)	49 (8%) Vs 9 (31%) P: 0.65;RR: 1.38	29 (5%) S 2 (7%) P: 0.65; RR: 1.38
(Eker et al., 2008), EID	SS: 53.5 vs 88 days C 61.5 vs 117 days (median)	105 (59.3%) Vs 4 (57.1%)	1 (0.6%) Vs 0	14 (7.9%) Vs 1 (14.3%) P: 0.5; RR 1.81
(Kwon et al., 2008), CID	NA	84 (66%) Vs 18 (67%)	NA	NA
(Lai et al., 2008) CID	NA	NA	NA	NA
(Banerjee et al., 2008), CID	C: 98.5 vs 195 days (median)	345 (66%) Vs 7 (41.2%)	NA	80 (15.3%) Vs 5(29.4%) P:0.4; RR: 1.41
(Bonilla, 2008), PlosOne	C: 3 vs 26 months (median)	ITR* 372 (75%) Vs 18 (4(%)	ITR* 50 (10%) Vs 5 (14%) P: 0.005; RR: 1.34	ITR* 39 (8%) Vs 8 (22%) P: 0.005; RR: 2.74
(Kim et al., 2008), AJRCCM	NA	615 (46.2%) Vs 22 (29.3%)	53 (4%) vs 12 (16%)	124 (9.3%) vs 20 (26.7%)
Median	SS: 53 vs 110 days C: 61 vs 117 days	-	-	-

*Individualised treatment, MDR: multidrug-resistant; XDR: extensively drug-resistant

Table 1.7 Treatment efficacy end-points in the studies reviewed (Sotgiu et al., 2009)

1.5.4 Role of surgery

The role of surgery in M/XDR-TB is controversial. After effective drugs were discovered to treat MDR-TB, surgery was used less and less until it virtually disappeared in the 1970s from case management strategies. The question now being asked is what to do in M/XDR-TB patients who are resistant to virtually all the available drugs. These patients to all intents and purposes are back in the pre-chemotherapy days.

Almost all of the guidelines and recommendations mention the role of surgery, although in a very secondary role (American Thoracic Society et al., 2003). Surgery is however recommended in those patients who meet the following three criteria: (1) localised disease with good chances of complete or nearly complete resection and adequate expected post operative lung function; (2) adequate pulmonary function and (3) enough available drugs to formulate an acceptable drug regimen to ensure post-surgery stump healing (Caminero, 2006).

Surgical interventions together with individualised chemotherapy regimens have shown good results of >90% in a number of studies but there is little data on surgical interventions on XDR-TB patients. A study of adjunctive surgical intervention in XDR-TB patients, conducted by *Dravniece et al.*, showed that despite failure of pharmacological treatment in 15 out of 17 patients, eight (47%) of these patients were cured following surgery (Dravniece et al., 2009).

1.5.5 Adverse drug reactions

Virtually all patients will report adverse effects to second-line drugs and it is known that second-line drugs are more toxic then first-line drugs. Close monitoring of patients is essential. Table 1.8 below lists all the important side effects and the monitoring thereof.

Second line drug	Adverse effect*	Tests to monitor
Amikacin Kanamycin Capreomycin	Ototoxicity (cumulative dose important) Nephrotoxicity	Audiology (hearing test) Monthly if possible Levels monthly Serum creatinine and potassium, monthly high-risk patients more often
Fluoroquinolones	Gastrointestinal disturbance Insomnia Arthralgia	Clinical observation Serum uric acid if used with pyrazinamide
Ethionamide (or protionamide)	Gastrointestinal disturbance Hepatotoxicity Hypothyroidism	Clinical observation. Prevent by initially splitting dose or increasing dose or increasing dose gradually Jaundice. Serum alanine transferase and bilirubin Thyroid-stimulating hormone levels (freeT4). At least 6 monthly
Cycloserine (or terizadone)	Psychosis, seizures, parasthesia depression	Clinical observation. All patients to receive preventative pyridoxine
Para-Aminosalicylic acid (PAS)	Gastrointestinal disturbance Hypothroidism	Clinical observation Thyroid –stimulating hormone levels (free T4).
Linezolid	Myelosuppression Lactic acidosis Peripheral neuropathy Pancreatitis (abdominal pain) Optic neuritis	Full blood count. Weekly at first, then monthly Serum lactate level Clinical observation Clinical and serum amylase as indicated Vision testing

*Adverse drug effects of first-line drugs and antiretroviral drugs in patients infected with HIV often have overlapping adverse drug effects

Table 1.8 Important adverse drug effects of second-line drugs, and tests to monitor them (Schaaf, 2010)

Adverse effects should be treated early and failure to do so could lead to poor adherence or irreversible problems such as hearing loss or peripheral neuropathy and ultimately death in extreme cases (e.g. acute renal failure in capreomycin). Adverse drug reactions of first-line drugs and antiretroviral drugs in patients infected with HIV often have overlapping adverse effects with second-line drugs, such as gastrointestinal disturbance (almost all), hepatitis (INH, d4T, ddl.), central nervous system effects (INH, efavirenz), pancreatitis (d4T, ddl) and lactic acidosis (d4T, ddl, AZT, 3TC)(WHO, 2008).

1.6 Study objectives and rationale

1.6.1 **Background/ motivation:**

The rise in DR-TB is a global public health concern, particularly in resource-limited countries with a high burden of TB. Multidrug-resistant TB (MDR-TB), with resistance to the two first-line TB drugs isoniazid (INH) and rifampicin (RIF), has been recognized as a major problem in TB management and control.

Recently, a joint global survey by the WHO and the US CDC identified XDR-TB in all regions of the world, thereby recognising this as a new threat to TB control (Centers for Disease control and Prevention (CDC) 2006). This concern was further heightened with the identification of an XDR-TB outbreak involving fifty-three cases in South Africa (Gandhi et al., 2006a)(Gandhi et al. 2006). This outbreak showed exceptionally high mortality among HIV co-infected cases. Subsequently, this research group suggested that

in the absence of DST the evolution of MDR-TB and XDR-TB was inevitable (Pillay & Sturm 2007). Scarce drug resistant data from Africa on prevalence of resistant TB patterns, while lower then Asia and Europe is increasing and together with HIV can fast track XDR-TB into an uncontrollable epidemic.

XDR-TB threatens to destabilize TB control in South Africa. However, there are few data that inform and guide the rational implementation and planning of control strategies, and treatment services, for XDR-TB in this country. Current tools to diagnose tuberculosis have poor susceptibility and results are not available for several weeks, whilst tools used for the diagnosis of drug-resistant TB are cumbersome and not widely available. Treatment options, because of the high level resistance, are severely limited in patients with XDR-TB. Adverse drug reactions (ADRs) may be an important reason for treatment interruption in XDR-TB and influences patient perceptions about toxicity and hence compliance. There are hardly any data about the risk factors for ADRs in patients with XDR-TB, whether ADRs impact on treatment-related outcomes, and how drug regimens should be monitored. The main aim of this study was to evaluate treatment-related outcomes, DST patterns and ADR's in extremely drug-resistant TB.

1.6.2 Aims and objectives

1. To evaluate treatment-related outcomes (early outcomes and ADRs) and DST patterns in XDR-TB patients in South Africa.

   Sub aim 1: What are the early outcomes (conversion and death) in XDR-TB patients?

   Sub aim 2: What proportion of XDR-TB is due to primary and acquired resistance?

   Sub aim 3: What is the relationship between patient-related factors (demographic, clinical and laboratory) and treatment outcome (sputum conversion and death) in XDR-TB.

   surgery?

   Sub aim 4: What is the morbidity and mortality, as well as treatment outcome in HIV-infected XDR-TB patients compared to uninfected patients (and in those HIV-infected persons who access and receive treatment with HAART vs. no HAART)? In the latter group when should HAART be initiated in relation to the initiation of the XDR-TB treatment and what are the side effects of the ARVs in relation to second-line drugs?

   Sub aim 5: What is the profile of XDR-TB patients who undergo surgery?

2. To evaluate extent, severity and toxicity of drugs used for XDR-TB

Sub aim 1: How well tolerated are XDR-TB regimens?

Sub aim 2: Do HIV-infected people have more severe adverse drug reactions than those whom are HIV-uninfected?

Sub aim 3: How are treatment outcomes affected by severe adverse drug reactions?

Chapter 2 Methodology

In this chapter I will detail the design of my study, how the study was constructed and executed, and a description of the study sites and participants.

2.1 Study setting and participants

2.1.1 Gordonia Hospital

2.1.2 Sizwe Hospital

2.1.3 Jose Pearson Hospital

2.1.4 Brooklyn Chest Hospital

2.2 Diagnosis of XDR-TB

2.3 Treatment regimens

2.4 Outcomes

2.5 Strain typing

2.6 Data handling and statistical analysis

Figures

Figure 2.1 Outlines the centre-specific dates from which study enrolment started and ended (1 February 2008 was the enrolment censor date and 1 September 2008 was the follow-up censor date so that there could be at least a six-month follow up on all patients included in the analysis).

Figure 2.2 Study plan

2.1 Study setting and participants

The case records of 227 patients diagnosed with XDR-TB between August 2002 (the start of the DOTs plus programme in South Africa i.e. a standardised treatment regimen for MDR-TB patients) and the end of February 2008 were retrospectively reviewed. Patients had to be 18 years or older to be included in the study. This was a retrospective observational cohort study.

The 227 patients included in this study had either: (i) been admitted as an inpatient in one of the 4 dedicated XDR-TB inpatient facilities in South Africa, or (ii) had died during the interval between sputum acquisition date and sputum results confirming the diagnosis of XDR-TB or (iii) the patient died while awaiting admission to the specialised hospital for treatment initiation, or (iv) the patient died shortly after being admitted before treatment commencement. The patients were admitted to the following hospitals:

2.1.1 Gordonia Hospital, in the town of Upington in the Northern Cape Province is a level one, 189-bedded hospital, consisting of 5 wards, one of which is used for housing patients with MDR and XDR-TB. This region has amongst the highest documented incidence of tuberculosis (TB) in the world at 800 cases per 100 000 people. (Beth Engelbrecht).

Sixteen of the study patients were registered at Gordonia and the HIV rate was 12.5% (2/16).

2.1.2 Sizwe Hospital, just outside Johannesburg, in the Gauteng Province, has 260 beds and provides high–security isolation facilities for a range of

highly infectious haemorrhagic viral diseases. It is also Gauteng's referral hospital for inpatient care of MDR and XDR-TB patients. (AVERT). 35 study patients were registered at Sizwe and the HIV rate was 66% (23/35).

2.1.3 Jose Pearson in Port Elizabeth, Eastern Cape is a South- African National Tuberculosis Association (SANTA) centre dedicated to the inpatient treatment of MDR and XDR-TB. Currently it has 250 beds for the inpatient treatment of MDR-TB patients as well as 134 beds for XDR-TB. Fifty-nine study patients were from Jose and the HIV rate was 57.6% (34/59).

2.1.4 Brooklyn Chest Hospital (BCH) in Cape Town is the referral centre for XDR-TB patients in the Western Cape province who are admitted until sputum culture-conversion or until they are deemed (by the provincial review board) to be a treatment failure. Drug susceptible and MDR-TB patients are also admitted to BCH. Eighty-nine study patients were registered at BCH, and HIV rates were 23/37(62%).

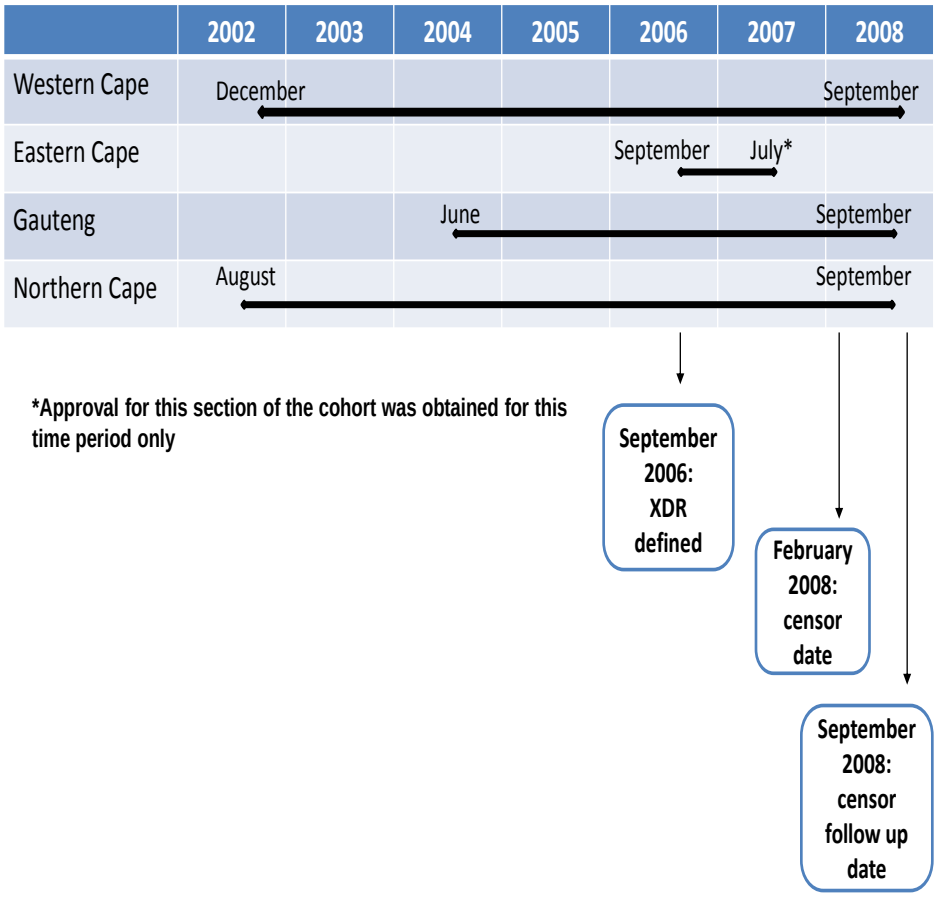


Figure 2.1 Outlines the centre-specific dates from which study enrolment started and ended. The first of February 2008 was the enrolment censor date and 1 September 2008 was the follow-up censor date so that there could be at least a six-month follow up on all patients included in the analysis.

Documents which were carefully reviewed included hospital folders, the notes written by the doctors and nurses, clinic folders where available, a data base in use at BCH (TB data) and the patient's paper-based MDR-TB forms which have been in use since the early 1990's. Data was entered into case record forms (CRF's - Appendix 1). Data captured included demographics, previous TB episodes,

treatment taken and outcomes for each TB episode. Concomitant illnesses, HIV status and history, history of any surgery - thoracic and /or other, adverse drug reactions and all the patients laboratory results, were also recorded.

The standardised CRF was completed by myself in the Western and Northern Cape. At Sizwe the data was captured by a clinician working at the hospital and the CRF's were then couriered down to the Lung Infection and Immunity Unit (LIIU) in Cape Town. The data from Jose Pearson was captured, on a separate database, by a trained health care worker working for the South- African Medical Research Centre (SAMRC). This data was then merged with the other data. All data was entered onto an Excel spreadsheet by a trained data capture person.

A summary of the study plan and details of the treatment facilities is outlined in Figure 2.2 All patients diagnosed with XDR-TB were admitted to the relevant facility until sputum smear conversion or until they were deemed a treatment failure as decided by the relevant provincial review boards.

Twenty-eight of the 227 enrolled patients were excluded from the study: Two participants were excluded as they were children under the age of 18. In eight patients the sputum culture result was unknown at XDR-TB treatment initiation and the one-month sputum culture was negative so it was not clear if these patients had thus converted on MDR or XDR-TB treatment. One patient who had been

transferred from the Eastern Cape to the Western Cape had been entered into the database twice. In four patients there was insufficient data for them to be included and in 13 patients the HIV status was unknown or the patient had refused testing. This left 199 enrolled patients for further analysis (Figure 2.2).

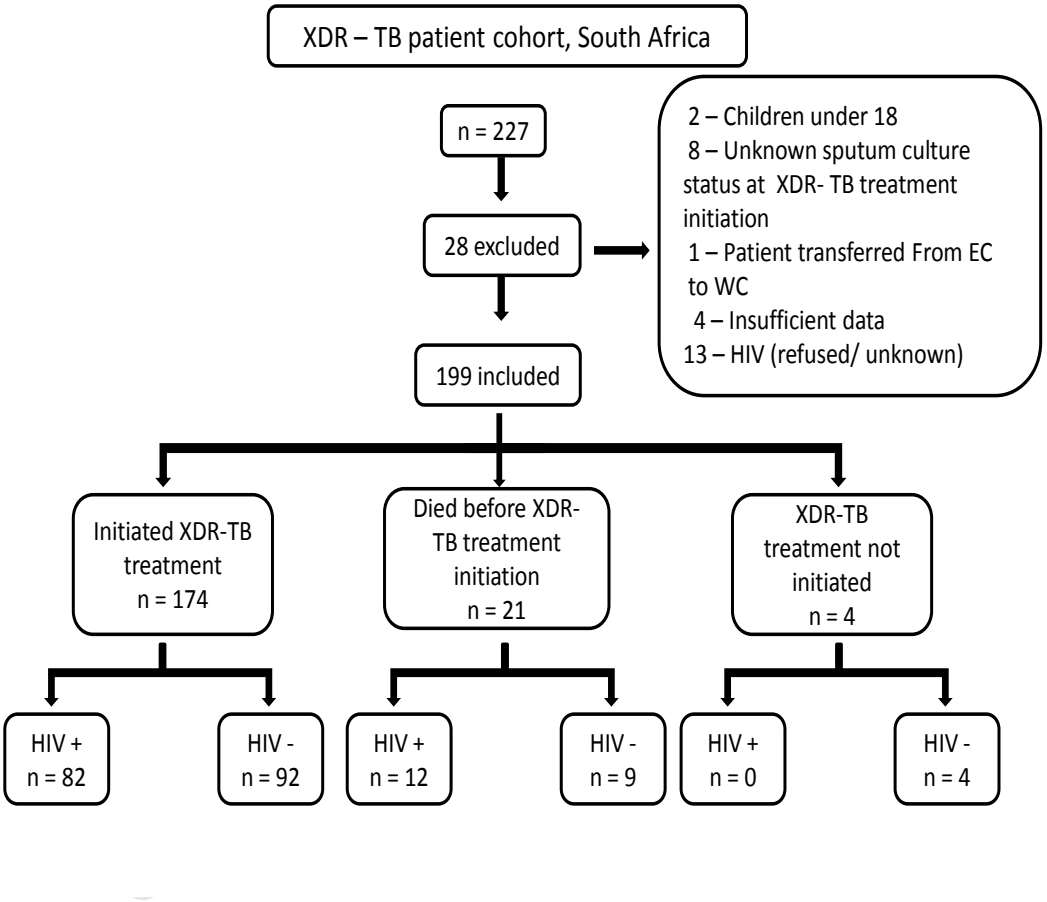


Figure 2.2 Study plan

Ethical approvals were obtained for each site. The Lung Infection and Immunity Unit (LIIU) obtained ethical approval from the University of Cape Town, Sizwe from the University of the Witwatersrand University, and the South- African Medical Research Council from their own ethics board. Provincial approvals were also obtained from the different Provincial Research boards.

2.2 Diagnosis of XDR-TB

All new TB cases entering the health system were diagnosed and treated based on smear microscopy results. The National TB policy recommended that culture and DST for isoniazid, rifampicin and ethambutol be performed in all new TB cases who had not converted by month three, in all retreatment cases at presentation and in high risk patients. High risk patients include prisoners, health care workers and contacts of MDR and XDR-TB patients. As part of the DOTs-plus strategy, MDR-TB patients who were still culture positive after nine months of treatment had sputum sent for second-line testing as part of the DOTs plus strategy. XDR-TB patients were retrospective diagnosed, prior to the XDR-TB definition, based on the nine-month culture and DST results.

Susceptibility to INH and rifampicin was done by the HAIN probe assay if the smear microscopy was positive. If the smear microscopy was negative, the isolate was cultured by conventional methods and then DST done by polymerase chain reaction (PCR). Second-line testing was performed using the indirect method on solid media (7H11) at a dedicated Provincial TB laboratory (Cape Town, Port Elizabeth, Kimberley and Johannesburg) using standardised reagents and methodologies. This method did not change during the course of the study.

Sputum isolates which showed resistance to isoniazid and rifampicin were automatically sent for second-line testing to ethionamide, ofloxacin, amikacin or kanamycin) Testing to the first line injectable, streptomycin (an amino glycoside) and pyrazinamide (PZA) was also done in selected centres. In Kimberley (Northern Cape) DST was done for kanamycin, whereas in the other provinces testing was done for amikacin under the premise that the cross resistance between kanamycin and amikacin is high (Kruuner et al., 2003, Tsukamura, 1975, Tsukamura, 1980).

In this study patients whose isolates were resistant at diagnosis (time of initial sputum collection) to at least isoniazid, rifampicin, a fluoroquinolone and at least one of the second-line injectable agents (i.e. amikacin, kanamycin or capreomycin) were deemed to have XDR-TB. In early 2007, with the newly adopted definition of XDR-TB and revised guidelines second-line DST became more widely available. Thus, XDR-TB was only widely diagnosed and treated from late 2006 or early 2007 with a limited number of cases identified retrospectively from the pre-2006 period. DST to capreomycin, terizidone and the newer generation fluoroquinolones was not available within the national health laboratories services. However, to gain further insight into resistance patterns DST was performed on capreomycin in 56 isolates obtained from patients in the Western Cape in accordance with the US CDC guidelines (Isenberg, 2004.). This testing was done genotypically at the Molecular and Cellular Biology Department at the Health Science Faculty, University of Stellenbosch.

2.3 Treatment regimens

Prior to the diagnosis of XDR-TB any previous episodes of MDR-TB were generally treated with a standardised regimen comprising of kanamycin, ofloxacin, ethionamide, ethambutol and pyrazinamide (terizidone was substituted for ethambutol in patients demonstrating resistance to ethambutol). Hospitalisation for initiation of MDR-TB treatment and monitoring until culture-conversion was recommended but lack of bed capacity often precluded this.

For initiation of treatment for XDR-TB treatment hospital admission was mandatory as specified in the MDR-TB national guidelines. Treatment was individualised with the use of capreomycin and para-aminosalycilic acid (PAS) as the anchor drugs. These two drugs were released in South Africa specifically for the treatment of XDR-TB. Other first and second-line drugs were used at the discretion of the attending clinician or those to which the organism demonstrated susceptibility while taking into account the patient's previous treatment history.

Linezolid is currently unavailable through the National TB Programme. Clofazamine was used in Sizwe for inclusion in an XDR-TB regimen and moxifloxacin at Jose Pearson. Patients from Khayelitsha, in the Cape Metropole, received moxifloxacin as part of a community treatment project. The drug was sponsored by Médecins Sans Frontières (MSF). Highly active antiretroviral therapy (HAART) was offered to all HIV-infected patients at the discretion of the attending clinician.

2.4 Outcomes

The date on which the sputum sample was taken and from which XDR-TB was cultured was deemed the date of diagnosis of XDR-TB. There was a measurable delay between diagnosis and XDR-TB treatment initiation due to the length of time it takes to perform culture and DST. Conversion was defined as to have occurred when two consecutively negative cultures were obtained, one month apart, and where there was proven culture positivity at treatment initiation. Conversion is then measured in days from treatment initiation date until the take date of the first negative culture.

Widespread and targeted screening for XDR-TB was only available in many centres from late 2006 to early 2007. Therefore the majority of the cohort had a relatively short follow-up period and we thus focussed on early outcomes [conversion (Chapter 4) mortality (Chapter 5) and ADR's (Chapter 6)].

2.5 Strain typing

To see whether there was any additional resistance acquisition during XDR-TB treatment, a subset of 52 XDR-TB isolates, were obtained from the NHLS, where they had been stored, from patients from the Western Cape and were genotyped by IS*6110* DNA fingerprinting (Warren, 2006) spoligotyping (Kamerbeek, 1997) and DNA sequencing of the *inhA* promoter and the *katG*, *rpoB*, *embB*, *pncA*, *gyrA* and *rrs* genes. Transmission chains were defined by isolates having identical IS*6110* DNA fingerprints, spoligotypes and mutations conferring resistance.

2.6 Data handling and Statistical analysis

A data risk management tool, including double data entry, was used to ensure data integrity. Categorical variables were compared using the chi-square test or Fisher exact test for smaller samples (i.e. all expected frequencies not ≥ 5) and continuous variables because data does not follow normal distribution, the non-parametric Mann-Whitney or Kruskal-Wallis tests were used.

Kaplan-Meier's method (event over time analysis) was used to calculate probabilities of events at different time points and the Log-rank test was used to compare these probabilities by group. Our approach to survival data were the Cox proportional hazards regression models which were fitted to determine risk factors associated with outcomes in a time-to-event based analyses. Variables found to be significantly associated with the outcome ($p<0.05$) were included in the final model. The proportionality assumption of the Cox models was tested using –ln [–ln (survival)] curves and regression of scaled Schoenfeld residuals on functions of time. The assumption of uninformative censoring was examined by plotting observed survival times against the values of the explanatory variables included in the model. In addition, sensitivity analyses were conducted to account for patients excluded from the analysis, and for those lost to follow-up.

Chapter 3: Diagnosis, susceptibility patterns and transmission dynamics in patients with XDR-TB

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3.1 Introduction

South Africa has a population of nearly 48 million people, an HIV prevalence of 11% in 2008, a tuberculosis notification rate of 948 per 100 000 population in 2007, and a notification of more than 7000 MDR cases in 2007 (WHO, 2010). National policy recommends that drug-susceptibility testing should be done for rifampicin, isoniazid, and ethambutol in all new cases of tuberculosis who have not culture-converted by two to three months, all retreatment cases at presentation, and high-risk patients (e.g. prisoners, contacts of MDR-TB patients, HCW's etc.) at presentation (Health, 2010).

Rapid diagnosis of XDR-TB has several important benefits. Firstly, optimal treatment can be quickly initialised (before routine culture and DST results become available), thus reducing the length of time that inappropriate and sub-optimal therapies are administered. This is crucial to reduce the period of time on inappropriate therapy, which can lead to the selection of further drug resistance and possible development of totally drug-resistant tuberculosis. Secondly, earlier diagnosis and isolation of patients will reduce the likelihood of transmission of drug resistant TB to contacts. Several rapid techniques for drug susceptibility testing have become available, (Dheda et al., 2010a, Schaaf et al., 2009, Morgan et al., 2005, Pai et al., 2005) which make it possible to consider intensifying detection of patients with XDR-TB, and which would enable results to be reported to clinicians in "real time" impacting directly on initial treatment decisions and timing.

Molecular strain typing techniques like RFLP have revolutionised epidemiological investigation of TB cases. It is useful for large-scale epidemiological studies to see if strains are part of a true cluster or a local outbreak and to investigate laboratory cross contamination. There are few data about whether XDR-TB in the Western Cape is due to primary or acquired resistance.

XDR-TB was only widely diagnosed and treated from late 2006 / early 2007, with a limited number of cases identified retrospectively from the pre-2006 period. As no international consensus on management of XDR-TB patients existed at that time, the WHO advocated the use of the same general principles as those for treatment of MDR-TB. While we know that MDR-TB treatment outcomes are poor there is little data on treatment outcomes in XDR-TB (Chapters 4 and 5), what drugs are being used in regimens and the adverse side effect profiles of these drugs (Chapter 6).

A grossly neglected area in the management of XDR-TB is the screening of close contacts. In many high burdened under-resourced countries there is no capacity to perform active case finding. This is a missed opportunity to identify and treat patients and thereby assisting in bringing down morbidity and mortality rates (WHO, 2006, Cain, 2008, March 31).

Overcrowding, lack of isolation facilities and basic infection control within prisons exacerbates transmission of tuberculosis, and remains a global problem. Russia has been an example of this in recent years where the prison system has been identified as helping in fuelling the TB epidemic. In 1997 notification rates among the 1.1 million incarcerated people was 4 000/100 000 several times higher than among the general population where the rate was 81.3/100 000 (Academies, 2009). At the beginning of 2007 it was estimated that there were about 166 000 people incarcerated in 237 operational South- African prisons where actual capacity was 115 000, some 40% over capacity. It is also estimated that 20.5% of these prisoners are HIV-infected (2009). However, there is little data from South- African prisons about TB prevalence and the presence of XDR-TB.

Finally, although surgical intervention for patients who have run out of chemotherapeutic options, or are likely to relapse, or who remain consistently sputum culture positive, is considered a suitable adjunct, (Van Leuven et al., 1997, Mohsen et al., 2007) there are few data about surgical outcomes in XDR-TB. Moreover, most surgical studies were performed in MDR-TB patient cohorts and further studies need to be performed in patients with XDR-TB (Van Leuven et al., 1997, Mohsen et al., 2007).

3.2 Methodology

3.2.1 Study setting and participants

We retrospectively reviewed the case records of 227 patients, over the age of 18 years, diagnosed with culture proven XDR-TB. In the final analysis we analysed data from 174 patients who started XDR-TB treatment within a hospital setting. These patients were diagnosed between August 2002, at the start of DOTs-plus and February 2008. They were diagnosed at four of nine dedicated provincial facilities for the treatment of XDR tuberculosis in South Africa.

3.2.2 Diagnosis of XDR tuberculosis

According to national policy, isolates resistant to isoniazid and rifampicin would undergo second-line testing (kanamycin or amikacin and ofloxacin), only if requested. Previously in line with DOTs-plus guidelines DST to second-line drugs was done at nine months although there were clinicians who still performed DST to second-line drugs if the patient's culture was still positive at the end of the intensive phase (i.e. at the end of the four months of an injectable agent). In early 2007, with the newly adopted definition of XDR tuberculosis and revised guidelines, second-line drug-susceptibility testing became widely available with the policy changing so that all isolates demonstrating resistance to isoniazid and rifampicin would automatically be sent for second-line DST.

Second-line testing was done by use of the indirect method on solid media (Middlebrook 7H11, Becton Dickinson, Le Pont de Claix, France) at dedicated provincial tuberculosis laboratories in Cape Town and Port Elizabeth. Liquid media (The BACTEC MGIT 960 System, Becton Dickinson) was used in Johannesburg. Both of these methods were used with standardised reagents and methods, which did not change during the study.

DST to capreomycin, terizidone and fluoroquinolones other then ofloxacin was not available within the Provincial laboratories. However, to gain further insight into prevailing patterns we performed DST to capreomycin in 56 isolates obtained from patients in the Western Cape in accordance with Centers US CDC guidelines (Isenberg, 2004.).

3.2.3 Treatment regimens

The XDR-TB drug regimens included three to four drugs with known efficacy and to which the patient had not been previously exposed. All XDR-TB regimens included an injectable and other medication added based on the susceptibility profile and the patient's previous TB drug history. Group 5 drugs (Table 18, pg 55) were not recommended for routine use in treatment of M/XDR-TB as the efficacy against *Mycobacterium tuberculosis* had not been proved. These drugs were used, however, if the available drugs in the person's regimen were inadequate and the

regimen needed to be strengthened. Drugs with known unmanageable side effect profiles or to which the patient was allergic were omitted from the regimens.

Capreomycin and para-aminosalicylic acid (PAS) were introduced into the National TB Programme in early 2007 specifically for the use in XDR-TB regimens. Linezolid is currently unavailable through the National TB Programme and clofazamine (despite being the drug of choice in group 5 (Table 1.8 page…55) and moxifloxacin was being used only in selected centres on a limited basis. Moxifloxacin was only accessible at Jose Pearson in the Eastern Cape, and through a nongovernmental organisation (MSF) on a restricted basis in the Western Cape (Khayelitsha). HAART was offered to all HIV-infected patients at the discretion of the attending physician. The national TB policy was recently amended and terizidone now replaces ethambutol in the standardised MDR-TB regimen.

3.2.4 Contact Tracing

Effective contact tracing includes identifying, screening and treating, all adult and child, close contacts of a drug-resistant TB patient already diagnosed. Close contacts were defined as “people living in the same household, or spending many hours a day together with the index patient in the same indoor living space” (WHO, 2006). All contacts should be screened and if active TB is suspected sputum sent off for culture and susceptibility. Increased morbidity and mortality and amplification of resistance can occur from any delays in diagnosis and / or treatment. The policy

in South Africa with regards to contact screening of XDR-TB patients was that once a patient was diagnosed as having XDR-TB, the house was visited, an assessment done, and all household contacts screened (WHO, 2006).

3.3 Results

3.3.1 Baseline demographic, clinical and laboratory characteristics

In the final analysis we analysed data from 174 patients who started XDR-TB treatment within a hospital setting. The patients were generally young with a median age of 33 years, (IQR 26-45). Whilst there was only one white patient identified, 39.6% (69/174) of the XDR-TB patients were of mixed ethnicity and 59.8% (104/174) of black ethnicity. Gender was roughly equal with 51% of patients being female (89/174) and 49% males (85/174). Interestingly, in this study, HIV infected and uninfected rates were similar [47% (82/174) vs. 53% (92/174)]. Demographic variables of the whole cohort of 199 patients stratified by HIV status are shown in **(Table 3. 1).**

Characteristic	HIV status		P-value
	Negative	Positive	
Total number (%)	105(52.8)	94 (47.2)	
Gender			**0.002**
Female	42 (40.0)	58(51.7)	
Male	63 (60.0)	36(38.3)	
Age, median (IQR)	32.1(25.9– 44.6)	36.1(28.0–45.8)	**0.11**
Ethnicity			**<0.0001**
Black	34(32.4)	80(85.1)	
Mixed origin	70(66.6)	14(14.9)	
White	1(1)	0(0)	
Employment			**0.68**
Employed	15(14.3)	10(10.6)	
Unemployed	80(76.2)	73(77.7)	
Unknown	10(9.5)	11(11.7)	
Smoking history			**0.09**
Yes	37(35.2)	20(21.3)	
No	27(25.7)	31(33.0)	
Unknown	41(30.0)	43(45.7)	
Smear status			**0.60**
Positive	16(15.2)	17(18.1)	
Negative	89(84.8)	77(81.9)	
XDR-TB treatment status			**0.94**
Treated	92(87.6)	82 (87.2)	
Not treated	13(12.4)	12(12.8	
History of prison contact			**0.20**
Current	5(4.8)	3 (3.2)	
Previous	12 (11.4)	5 (5.3)	
None	32 (30.5)	40 (42.6)	
Unknown	56 (53.3)	46 (48.9)	
Days from sputum acquisition to result, median (IQR)	53(20.0 – 77)	39(16.5 – 82.5)	**0.40**
Days from result to treatment initiation, median (IQR)	20(8.0 – 39.8)	19(5.5 – 23.5)	**0.20**
Weight (kg) at start of treatment, median (IQR)(n=72)	48(39.9-53.3)	49.7(42-60)	**0.46**
Number of prior sensitive episodes, median(range)	1(1 – 5)	1(1 – 4)	**0.35**
History of MDR TB			

For all variables n= 199, unless otherwise stated.

*P-value: χ^2 test used for all categorical variables, and Mann-Whitney test for all continuous variables.

± significant inter-group difference because the HIV-infected group had minimal dispersion about the median compared to HIV-uninfected group, which had a positively skewed spread.

Table 3.1: Demographic and clinical characteristics of the cohort (n=199) stratified by HIV status

3.3.2 Diagnosis

Fourteen percent or 25 of the 199 documented XDR-TB patients did not start treatment. Twenty-one of these patients either died prior to confirmation of the diagnosis (DST result) or prior to being traced and admitted to a suitable facility to initiate therapy. The remaining four patients were admitted to the specialised XDR-TB unit but treatment was not initiated due to the unsatisfactory condition of the patient on admission. Sputum "take" date to DST result acquisition ranged from 16.5 to 82.5 days, with a median time of 53 days in HIV-uninfected and 39 days in HIV-infected patients. Days from sputum DST result acquisition to treatment initiation ranged from 8 - 39 days. The median length of time from sputum collection (primary sputum used to make the XDR-TB diagnosis) to treatment initiation was 75 days (range 26-122).

3.3.3 Strain typing

Seventy four percent of XDR-TB patients had previous culture-confirmed MDR-TB (**Table 3.1**), suggesting acquisition of additional resistance during this treatment period. To test this notion genotypic data from 52 patient isolates in the Western Cape were analysed. Seventeen XDR-TB patients had an *M. tuberculosis* isolate with a unique IS*6110* genotype, while the remaining 35 XDR-TB cases had an *M. tuberculosis* isolate, which could be grouped into one of six clusters. Of these clustered cases only 19% (10) showed identical resistance causing mutations to both first and second-line drugs, suggesting that resistance to second-line drugs was acquired in 81% of the patients from the Western Cape.

3.3.4 Treatment regimens

One hundred and seventy-four patients initiated treatment with a regimen containing a backbone of capreomycin and para-aminosalicylic acid (PAS), and received a median of 7 (IQR 6-8) drugs. The most common drugs used in the regimens in this cohort were (the denominator in this analysis is 115 as Jose Pearson, in the Eastern Cape was omitted, due to several fields not being captured): terizadone 91.3% (105/115), followed by capreomycin 90.4% (104/115), PAS at 87% (101/115), clarithromycin and PZA both 69% (80/115). Capreomycin was given until the attainment of at least 3 negative cultures, after which it was stopped and patient was discharged. The other drugs were continued for the remainder of the treatment period or were stopped at the discretion of the treating medical officer or according to ADR side effects. Of the 82 HIV co-infected patients who received

XDR-TB treatment, 63% (52/82) received highly active antiretroviral therapy (the commonest regime used in 50% of the patients was zidovudine, lamivudine, and efavirenz).

	Patients given drug N=174 N(%)	Treatment duration	Resistance pattern (number of patients resistant to treatment/number of susceptibility tests done in treated patients
Ethambutol	103 (59)	6.8(4.0-11.4)	62/117(53%)
Pyrazinamide	140 (80)	7.2(3.3-11.5)	ND
Amikacin	3(2)	4.2(2.1-5.8)	124/124(100%)
Capreomycin	162(93)	7.2(3.1-12.1)	22/42(52%0
Kanamycin	4(2)	13.4(4.8-15.6)	50/50 (100%)
Moxifloxacin	14(8)	12.1(10.4-18.3)	ND
Ofloxacin	31(18)	8.1(4.2-12.0)	174/174(100%)
Terizidone	147(84)	9.2(3.5-12.1)	ND
Ethionamide	107(61)	8.0(3.2-11.9)	59/167(35%)
Para-aminosalicylic acid	156(90)	7.3(3.7-12.2)	ND
Amoxicillin-clavulanate	66(38)	7.1(3.1-12.2)	ND
Clarithromycin	77(44)	8.6(3.7-13.9)	ND
Clofazamine	28(16)	8.7(4.3-14.3)	ND
Dapsone	95(55)	6.6(3.1-11.6)	ND
Azithromycin	11(6)	7.9(2.8-12.1)	ND
Isoniazid /thiacetazone	2(1)	7.8(1.9-13.7)	ND
Rifabutin	1(<1)	NA	ND
Streptomycin	1(<1)	NA	ND

Data are number (%), unless otherwise indicated. ND = not done. NA = not applicable

Table 3.2: Specific drugs and the dosages used in XDR-TB treatment regimens

The median duration of follow-up from treatment initiation to event (death, loss to follow-up or the censor date) was 6.9 months (IQR 3.1 to 12.0) and duration of treatment with injectable drugs was 13.4 months (IQR 4.7 - 15.5). At the time of data analysis, three out of the 13 culture converters had completed treatment, two of whom were HIV infected. Two patients remained culture-negative and on treatment and one patient died after culture-conversion – (these cases were all from the Western Cape for whom there was follow-up data after the study).

3.3.5 Drug susceptibility testing:

Overall drug susceptibility profiles did not differ with HIV status or when HIV-infected patients were stratified by CD4-cell count. Resistance, to ethambutol was high at 55.8%, and resistance to ethionamide 42.8% (Table 3.2). 58% (33/57) of available isolates that were retrospectively tested for susceptibility to capreomycin were resistant, and 74% (42/57) of patients were given the drug after the isolate was harvested for susceptibility testing. Only 48% (20/57) of these patients had isolates that were capreomycin-susceptible.

3.3.6 Contacts

Fifty percent (33/66) had documented contacts who had a history of previous active TB. A more detailed TB contact history was taken from 33 of the 66 XDR-TB patients who were admitted at BCH for initiation of XDR-TB. The total number of contacts among these 33 index patients was 55, (median, range) of 1.6 (1-5) contacts per patient. Of these contacts 16.4% (9/55) had drug susceptible TB, 60% (33/55) had MDR TB and 23.6% (13/55) XDR-TB. The vast majority (all except four) of the contacts were family members. In two cases the relationship to the patient was unknown and two contacts were prisoners both of whom had a diagnosis of XDR-TB.

3.3.7 Prisoners.

Of the cohort of 174 XDR-TB patients, 55.7% (97/174) had a recorded prison history. 25.8% (25/97) had a history of incarceration. Eight patients 8.% (8/97) were under correctional service at the time of the history taking, and the remaining 17.5% (17/97) had been previously incarcerated.

3.3.8 Surgical outcomes

Tarek Mohsen et al., in a seven year study of surgical interventions in Egypt, found that 34% of the patients had post operative complications with a mortality rate of 4.3%. These results compare very favourably to those in a study we conducted in

1997 where we found 23% morbidity and 1.6% mortality rates. From these results we can see that post operative complication rates are acceptable and, overall mortality low.

Of 135 XDR-TB patients assessed at a centre with thoracic surgery facilities 7.4% (10/135) were deemed suitable for possible surgery and referred for assessment by the cardio-thoracic surgeons. Of these 10 patients only three, all HIV-uninfected, underwent surgery (two undergoing pneumonectomies and one a lobectomy). Four patients refused surgery and three others were deemed unsuitable for surgery. Of those who underwent surgery, one patient converted and continued treatment but later relapsed. The other two patients died post-operatively at 12 and 24 days, respectively. Four patients with XDR-TB had previously undergone surgery for MDR-TB.

3.4 Discussion

Urgent implementation of rational control policies are required to counter the threat of XDR-TB. However, there are few data from Africa on which to base such recommendations, including those relevant to diagnosis, case finding, and treatment. We therefore attempted to address these issues using data gathered from our experience of treating patients with XDR tuberculosis at four centres in South Africa.

We found that the median time for an XDR-TB patient to be started on treatment was 75 days, but patients could wait as long as 122 days (roughly four months) to initiate treatment. *Bonilla et al*. reported that XDR-TB cases treated on the basis of DST results available within 31 days of starting treatment had much better outcomes then those XDR-TB patients whose DST results were only available after more than 30 days (Bonilla, 2008); however no reasons were given for this difference. In a study carried out in Hlabisa, KwaZulu-Natal, Heller et al. reported a median time from sputum acquisition to treatment start date of 84 days, in a community management programme, vs. 106.5 days in a hospital based treatment initiation model, some twenty-two and a half days earlier than the hospital-based cohort. This could possibly be due to delays in getting patients hospitalised due to unavailability of beds and the logistics of getting patients to the hospital. Culture-conversion was also earlier in the community based programme (85 days vs. 119 days) than in the more traditional hospital-based model. Therefore outcome results, particularly that of culture-conversion could be affected by the length of time a patient waits to be diagnosed, traced and initiated on treatment (Heller et al., 2010).

The inordinately long time which patients and HCWs waiting for sputum results makes the use of rapid diagnostics crucial in reducing the time to diagnosis and treatment initiation in XDR-TB patients (Dowdy, Uys et al., 2009). Given that existing nucleic acid amplification tests perform less efficiently in smear-negative TB, (Ling DI, 2008), it would be rational to apply rapid DST to *all* smear positive

patients at first presentation to treatment facilities. A favourable cost benefit analysis of such an approach is supported by mathematical models (Dowdy). Alternative technologies that may perform well in smear-negative TB also require urgent development and validation (Migliori, 2008a, Pai M, 2006). Rapid diagnostic testing to second-line drugs need to be further developed and utilised (Ling DI, 2008).

An interesting finding in our study was that HIV uninfected patients had a longer delay than HIV-infected patients in initiating treatment. One possibility to account for these findings could be that patients attending HIV services are likely to be screened for TB earlier and sputum results carefully followed up. The possibility of survival and selection bias in explaining these results cannot be excluded.

Given these results it is clear that a proportion ~15-20% of XDR-TB is primary. We thus found, in terms of transmission dynamics, that genotypically 80% of the patients in our study had acquired XDR-TB. Mlambo et al. also showed in a previous South- African-based study, that 26 out of the 41 XDR-TB patients 63% had a strain that was unique to the geographical settings from where the patients originated, suggesting acquisition of extensive drug resistance. Fifteen patients (37%) in this study, from five clinics/hospitals, showed isolates with clustered spoligotypes, indicative of primary XDR-TB transmission attributed to higher

numbers of XDR-TB cases in South Africa. These findings, therefore, could be indicative of the early stages of an evolving epidemic (Mlambo, 2008).

As part of his meta-analysis Sotgiu et al. found that the proportion of retreatment, in nine studies, was 49-98% in MDR-TB (higher in Tomsk (Russia), Peru and Korea than in Germany, Italy and in the four European countries study). The proportion of retreatment cases among XDR-TB was consistently higher at 75-100% (Sotgiu et al., 2009). Similarly, in our studies over 70% of subjects had previous MDR-TB. This suggests, in keeping with the DNA fingerprinting data, that suboptimal treatment and drug management may have contributed to the amplification of drug resistance. There is an opportunity, therefore, to prevent further drug resistance through better clinical practises. Cognisance should therefore be paid to a patients TB history, patients with a history of previous MDR-TB should have a bolstered regimen and their clinical progress carefully monitored. In patients who are failing treatment, serious consideration should be given to stopping treatment to prevent amplification of drug resistance whereby strains could then be transmitted.

The high capreomycin resistance in our study 58% is cause for concern since this antibiotic is the mainstay of regimens for the treatment of XDR tuberculosis that are used globally but not previously widely used in South Africa. The reasons for these high resistance rates are not known, but might partly indicate the unreliability of capreomycin susceptibility testing, or the potentially high cross resistance with

aminoglycosides like kanamycin (Jugheli, 2005, Via et al., 2010). The high resistance rates to ethambutol, coupled with the fact that patients have already been exposed to this drug in regimens 1 and 2, were behind the rationale to change from ethambutol to terizidone in standardised MDR-TB regimens.

Although we had small numbers of XDR-TB patients undergoing surgery in our study (three patients), other studies have demonstrated surgery to be a useful adjunct to chemotherapeutic treatment: *Tarek Mohsen et al*., in a seven-year study of surgical interventions on patients in Egypt, it was found that 34% had post operative complications with a mortality rate of 4.3%. These results compare very favourably to those in a study we conducted, in 1997, where we had 23% morbidity and 1.6% mortality rate. From these results we can see that post-operative complication rates are acceptable and overall mortality low (Van Leuven et al., 1997, Takeda et al., 2005) (Mohsen et al., 2007, Wang et al., 2008).

Sotgiu et al., in his systematic review reviewed seven studies, which reported on surgical outcomes. In two studies, better success rates were linked to surgery among XDR-TB patients (Kim et al., 2007, Mitnick et al., 2008, Chan et al., 2008, Eker et al., 2008, Kwon et al., 2008, Keshavjee et al., 2008). Thoracic resectional surgery (e.g. lobectomy or pneumonectomy) is an acceptable intervention given the limited choices available to treat XDR-TB patients and is considered if lung disease is

localised and high-grade resistance present (Iseman et al., 1990). However, this strategy is not evidence based and the good outcomes may reflect selection bias.

Our study found that, among those patients, where it was recorded, prison history among XDR-TB patients was high at 25.8% (25/97). In Russia TB rates in the penitentiary system are more than four-fold higher than in the general population (Academies, 2009). The nosocomial spread of tuberculosis in prisons to fellow prisoners and staff, as well as the spill over into the community when inmates are released, is of growing concern. Many prisoners have social and clinical risk factors for tuberculosis, which poses a challenge to TB control. The management of tuberculosis among prisoners is further complicated by high rates of loss to follow-up care and poor treatment outcomes.

In summary diagnosis of XDR-TB takes several weeks, treatment initiation after diagnosis is equally delayed, transmission of XDR-TB in the WC (2002 - 2008) is mainly acquired, rates of capreomycin resistance are high and primary transmission of XDR-TB in the community and prisons are an increasing concern as the epidemic evolves. These data call for: (i) rational control policies, to counter the XDR-TB threat including, (ii) infection control in congregate settings, (iii) appropriate treatment regimens for susceptible as well as MDR-TB, (iv) close monitoring of patients to prevent amplification of drug resistance, (v) introduction of community based programmes to reduce pressure on hospital beds, and (vi) for efficient early

initiation of therapy. (vii) Rapid diagnostics (especially technologies for smear negative DR-TB, and susceptibility testing to second line drugs). (viii) Surgical intervention where appropriate and feasible.

Chapter 4: Conversion

Figures

Tables

Table 4.3 Socio-demographic and clinical characteristics of XDR-TB patients who initiated treatment (n=174) comparing converters to non converters

4.1 Introduction

The success of a person's anti-tuberculosis treatment is related to the bacteriological status while on treatment (Holtz et al., 2006). In South Africa, as in many other resource limited countries, the diagnosis of first time tuberculosis is made primarily on sputum smear microscopy although many individuals are HIV co-infected and may be smear negative (2003). A culture is not performed for first time smear positive individuals.

According to the WHO guidelines, following an initial positive smear if the sputum smear remains negative at subsequent testing during six months of treatment and there is clinical improvement, a sputum culture is not performed. If, however, the smear microscopy is positive at the end of the two-month intensive treatment phase, a culture with drug susceptibility testing should be requested. If drug resistance is present treatment should be altered appropriately. Sputum smear microscopy, culture and DST are also requested on those with suspected TB who have been previously treated for TB and in individuals at high risk for MDR TB including health care workers, contacts of MDR-TB patients and prisoners (WHO, 2006). The rationale and validity of these guidelines are questionable. The Centres for Disease Control and Prevention (US

CDC) includes sputum culture-conversion as one of the few follow-up measures for national TB surveillance (Holtz et al., 2006, dela Cruz).

Previous WHO TB treatment guidelines have recommended the continuation of the intensive phase in newly treated TB patients if the two month smear is positive (WHO, 2003). There has, however, been a lack of evidence to support this. Horne (Horne et al., 2010) conducted a systematic review and meta-analysis to look at the accuracy of the sputum examination. In this Meta analysis both culture and smear had low positive predictive values (PPV) (9-18%) at two months and high negative predictive values (NPV) i.e. 93%. These findings suggested that it was improbable that sputum microbiology can correctly predict failure or relapse but that a negative sputum result makes relapse or failure unlikely (Horne et al., 2010).

A summary of the two-month sputum smear and culture results from several studies included in Horne's Meta analysis is shown in (Table 4.1). In smear positive TB (11 studies) the median percentage of two-month positive sputum was 13% range (3%-50%). In those studies that looked at positive cultures (seven studies) the percentage was 17% with a range of (2%-63%). Another study conducted by Wang et al., showed a two-month sputum smear positivity rate of 11.1% (Wang et al., 2009).

Study	Month of sample	Smear pos. n(%)	Smear neg. n(%)	Culture pos. n(%)	Culture neg. n(%)
Benator, Lancet 2002	2	44 (11%)	369 (89%)	72 (17%)	346 (83%)
Cao, IJTLD 1998	2	56 (19%)	240 (18%)	--	--
Cao, IJTLD 1998	3	12 (21%)	44 (79%)	--	--
Chang, AJRCCM 2004	2-3	113 (33%)	226 (67%)	113 (33%)	226 (67%)
Nettles, CID 2004	2	--	--	45 (12%)	316 (88%)
Picon, JBP 2007	>=4	43 (7%)	567 (93%)	--	--
Ramarokoto, IJTLD 2002	2	117 (50%)	117 (50%)	--	--
Tam, IJTLD 2002	2	14 (8%)	158 (92%)	14 (8%)	153 (92%)
Tam, IJTLD 2002	3	6 (3%)	166 (97%)	4 (2%)	163 (98%)
Thomas, IJTLD 2005	2	100 (20%)	403 (80%)	--	--
Van Deun, IJTLD 2006	2	1098 (13%)	7132 (87%)	--	--
Wilkinson, IJTLD 1998	2 or 3	31(10%)	289 (90%)	--	--
Zierski, ARRD 1980	1	--	--	222 (63%)	130 (37%)
Zierski, ARRD 1980	2	--	--	112 (32%)	240 (68%)

Table 4.1 Number and percentage of patients with susceptible, multidrug-resistant versus extensively drug resistant-tuberculosis who smear and culture convert (Horne et al., 2010)

In contrast to drug susceptible TB there are few studies looking at culture-conversion amongst XDR-TB patients starting treatment in high burden areas. O'Donnell et al. reported very low conversion rates of 20% in KwaZulu-Natal (O Donnell et al., 2009). These low rates of conversion might, however, be a reflection of the high TB burden together with the HIV epidemic and poor access to health facilities. How do conversion rates in XDR-TB differ from those of MDR-TB and what

are the factors underpinning this? Holtz et al. found that in MDR-TB patients who did not convert were more likely to have a history of previous

treatment for MDR-TB (29% vs. 11%; p=0.004), have a history of incarceration (45%

vs. 26%; p= 0.003), and to be resistant to more drugs (6 vs. 5; p=0.008).

In a systematic review undertaken by TBNET, *Sotgiu et al*. found that longer time to sputum conversion was experienced in the majority of the MDR and XDR-TB studies looked at (n=7). The median time to sputum culture-conversion in MDR-TB ranged from 58-99 days, while in XDR-TB this range was 60-195 days (Sotgiu et al., 2009).

In the cohorts that Horne used in his systematic review and meta-analysis, **(shown below in Table 4.2**) conversion of susceptible TB culture was 56.6 days. In MDR-TB the smear conversion median was days (range) 53.5(41-56), and culture-conversion 61.2(56-98.5) days, which is similar to Sotgiu's findings. In XDR-TB smear conversion occurred at 110(88-110) days and culture-conversion at 107.5 (52-195) days.

	Smear	Culture	Smear	Culture	Smear	Culture
Migliori, EID 2007	--	--	41	56	110	97.5
Migliori, ERJ 2007	--	--	56	60	110	168
Eker, EID	--	--	53.5	61.5	88	117
Banerjee, CID 2008	--	--	--	98.5	--	195
Leimane, RIT	--	--	--	62 (34-109) (n=979)	--	52 (28-118) (n=48)
Mitnick, NEJ	--	--	--	61(59-67)	--	90 (57-115)
dela Cruz, PJIM 2003	--	56.5	--	98.9	--	--

Table 4.2: Median days and range, where available to smear and culture-conversion, in drug susceptible, multidrug-resistant versus extremely drug resistant-tuberculosis (Horne et al., 2010)

Another important question, relates to the proportion of patients who convert but still have an unsuccessful treatment outcome. Holtz et al. documented "re-conversion" in MDR-TB patients. These were patients who initially converted to sputum culture negative but subsequently reverted back to culture positive 11% (14/129). Median time for these patients to revert was 179 days with a range of 70-746 days. Interestingly all except one of these patients "re-converted" back to negative, after between 2 and 7 positive cultures, and successfully completed treatment (Holtz et al., 2006).

Conversion also helps to define the definition for treatment failure, which hitherto, remains obscure in XDR-TB. Given the paucity of data about culture-conversion related outcomes in XDR-TB we undertook a study to investigate the conversion rates among XDR-TB patients in four provinces/regions of South Africa. Furthermore we wanted to explore the factors contributing to a patient's non-conversion or factors contributing to a delayed time to conversion.

4.2 Methodology

4.2.1 Patients

We retrospectively studied pulmonary XDR-TB patients diagnosed between 2002 and 2008 in four provinces of South Africa (Western Cape, Eastern Cape, Northern Cape and Gauteng). Pulmonary XDR-TB was defined as a positive sputum culture for *M. tuberculosis* with *in vitro* resistance to at least: isoniazid, rifampicin, one of the second-line injectable agents (kanamycin, amikacin or capreomycin) and a fluoroquinolone. XDR-TB patients were hospitalised until sputum conversion and, at least in the Western Cape, patients were hospitalised until they had had four or five negative cultures before they were discharged for community treatment.

4.2.2 Bacteriological Examination

XDR-TB patients had monthly sputum smear microscopy and culture; results were reported according to international standards (WHO, 1998). Both smear microscopy and culture were used to monitor patients throughout therapy (WHO, 2006).

Up until 2006 all second-line testing was being done by the Lowenstein-Jensen on which growth of TB organisms is very slow. This method was phased out and has been replaced by one of the liquid medium Bactec systems.

Culture and sensitivities were performed either by using Bactec MGIT (Mycobacteria Growth Indicator Tube) 960 fluorometric system (Sizwe and Gordonia) or the indirect proportion method on Middlebrooks 7H11 (Brooklyn and Jose Pearson).

4.2.3 Definitions

In order for a patient to be considered culture and / or sputum smear positive at treatment initiation, the following criteria had to be met: at least one positive pre-treatment culture taken less than 30 days before or within seven days after initiation of XDR-TB treatment (WHO, 2006). In this XDR-TB study the definition used for the pre-treatment or “0” month sputum was a sputum sample that was taken up to two weeks before or after treatment was initiated.

Culture-conversion was defined as: two consecutively negative sputum cultures obtained 30 days apart, following proven culture positivity at treatment initiation. Time to culture-conversion was calculated in days from treatment initiation until the date on which the first of two negative cultures was taken (Laserson et al., 2005).

Patients who converted to sputum culture negative could also subsequently revert (i.e. become culture positive again). Date of reversion was calculated in days from the take date of the first of the two negative sputum's (denoting culture-conversion), to the date on which the first positive culture thereafter was taken.

4.2.4 Statistical Analysis

A univariate Cox regression analysis (i.e. one predictor variable) was performed to look at predictor variables associated with culture-conversion versus no culture-conversion and the p values for comparison between groups evaluated by using the chi-square test or Fisher exact test. Statistical tests were two-sided and a p value less than 0.05 was considered statistically significant. Kaplan Meier curves were used for time to event analysis. Multivariate modelling was conducted on positive associations identified in the univariate models.

	Converted	Did not convert	P
	n(%)	n(%)	value
Total number of patients	33(19)	141(81)	
Age, median(IQR)	33.2(26.3-044.7)	33.4(26.3-44.4)	0.99
HIV status			0.83
Positive	15(44.5)	67(47.5)	
Negative	18(54.5)	74(52.5)	
Gender			0.47
Female	15(45.5)	67(47.5)	
Male	18(54.5)	74(52.5)	
Smoking History			
Yes	9(27.3)	40(28.4)	
No	14(42.4)	35(24.8)	
Unknown	10(30.3)	66(46.8)	
Previous MDR TB			0.11
Yes	20(60.6)	105(74.5)	
No	13(39.4)	36(25.5)	
Weight(kg), median (IQR), (n=65)	54.5(49.8-61.3)	48.0(39.0-52.5)	0.007
Drugs used:			
Ofloxacin	7(21.2)	23(16.3)	0.50
Capreomycin	32(97.0)	130(92.2)	0.33
Ethionamide	19(57.6)	86(61.0)	0.72
Ethambutol	14(42.4)	87(61.7)	0.40
PAS	32(97.0)	124(87.9)	0.13
Moxifloxacin	1(3.0)	13(9.2)	0.24

Table 4.3 Socio-demographic and clinical characteristics of XDR-TB patients who initiated treatment (n=174) comparing converters to non converters.

4.3 Results

Culture-conversion occurred in 19% (33/174) of patients who initiated treatment (**Table 4.3)** and this did not differ by HIV status, 45.5% (15/33) HIV-infected vs. 54.5% (18/33) HIV-uninfected; p=0.57, (**Figure 4.1 A and 4.1B**). Of these 33 patients, conversion occurred in 70%, 85% and 91% by 6, 9 and 12 months, respectively. Before the censor follow up date of 30 September 2008, 6% (2/33) of the XDR-TB patients who had converted had reverted back to culture positivity. Clinical and laboratory characteristics stratified by conversion status are shown in (**Table 4.3).**

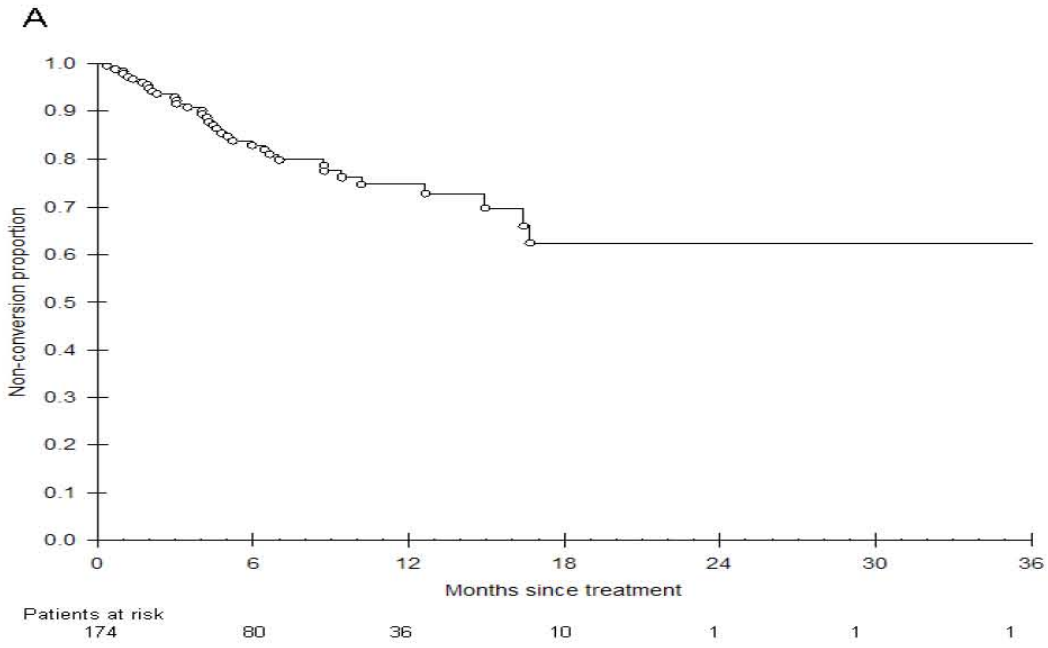
The number of days from the sputum acquisition (i.e. the date the sputum was taken) until the sputum report was 46 days (IQR 16.5-82.5), and from sputum report to treatment initiation a median of 20 days (IQR 5.5-39.8). The length of time from sputum acquisition until the date XDR-TB treatment was initiated was significantly longer in converters versus non-converters [91(61-116) vs. 59 (43-86); p= 0.001]. In addition low weight (<50kg) prior to treatment was associated with failure to convert (RR=3.31, 95%CI 1.08-10.1, p=0.04).

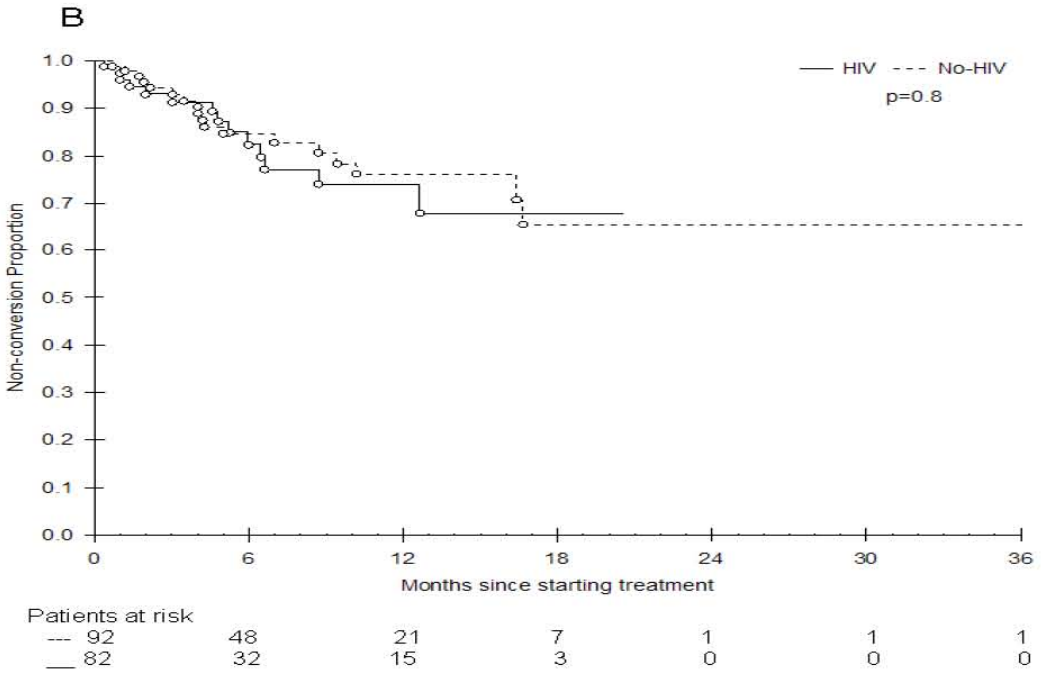
Conversion rate was unaffected by gender, ethnicity or the number of prior MDR-TB episodes median (range) of 1 (1-2) in converters vs. non converters 1 (1-2) for the number of MDR-TB episodes. There was no significant difference in the duration of previous MDR median (IQR) in converters vs. non-converters 9 (6-14) vs. 6 (4-10) months respectively, (p= 0.37) and similarly, the median (IQR) number of prior drug susceptible TB episodes converters 1(1-2vs. non-converters 1(1-2), p=0.2.

Figure 4.1 Kaplan-Meier probabilities of XDR-TB culture-conversion in patients whom received treatment for XDR-TB.

A whole cohort of patients receiving treatment

B HIV-infected and HIV-uninfected patients receiving treatment.





Discussion

Overall culture-conversion rates for sputum positive XDR-TB patients were disappointingly low at 19% (33/174). These rates are, however, similar to those documented by O'Donnell et al., whose study in KwaZulu-Natal, showed culture-conversion rates of 20% (12/60). Results from four studies in intermediate or high-burden settings with smaller cohorts had better culture-conversion rates than those found in this study (Mitnick et al., 2008, Kwon et al., 2008, Keshavjee et al., 2008, Kim et al., 2008, O Donnell et al., 2009). Mitnick and colleagues, in Peru, showed much higher conversion rates among XDR-TB patients, 67% (32/48) of patients with XDR-TB had converted by four months (median time to culture-conversion 90 days). In this study moxifloxacin, a newer generation fluoroquinolone, formed a standard part of the treatment regimen (Jacobson, 2010). In our study very few subjects received a fluoroquinolone, which could be one of the reasons for our poor conversion rates.

Determining the length of time to sputum culture-conversion assists programme managers in defining when patients could be considered treatment failures. In this study 85% of patients had converted by month 9 and only 6% (2/33) further patients converted thereafter. Thus patients can be considered a treatment failure after 12 months of adherent and observed treatment. Based on DOTs-plus data and reaffirmed in our study findings this definition is now being used in the Western Cape Province.

Holtz et al. has previously shown that culture-conversion is a valuable interim measure to evaluate patient progress (Holtz et al., 2006). He showed that patients who convert before two months had better treatment related outcomes. Although we did not find this in our study, Holtz et al. found that previous MDR treatment was a predictor for delayed culture-conversion. A reason for this could be that patients with previous MDR-TB treatment have been treated for a longer time period have more extensive bilateral disease and have a lower body weight (Holtz et al., 2006). Our data supports this with the univariate regression analysis showing a relative risk of failure to convert of 3.3% in those with low body weight at treatment initiation. We were not able in this retrospective review to accurately document the extent of disease on chest X-ray (CXR).

HIV status did not affect culture-conversion rates with approximately 50 percent of both HIV positive and HIV negative patients converting to sputum culture negative. O'Donnell et al. also found that HIV status was not a predictor of culture-conversion in KwaZulu-Natal (O Donnell et al., 2009). ……Given the experience of the Tugela Ferry outbreak and high mortality rates in severely immune-compromised individuals it is conceivable that HIV positive subjects with severe disease may have died before diagnosis. In addition HIV status did not affect survival in this study and thus only the relatively immune intact HIV patients survived and thus no effect of HIV status was seen. The findings are also

in keeping with those in drug susceptible patients where culture-conversion is independent of HIV status (Senkoro et al., 2010, Bwire et al., 1999).

Culture reversion occurred in 6% (2/13) but by the end of the study and at the time of writing a further four patients had reverted to sputum culture positive 54% (7/13). The reasons why patients convert and then revert back to culture positivity needs to be explored. Possible reasons could include the stopping of capreomycin, because at the time of discharge from hospital following sputum conversion capreomycin is stopped. In addition due to the high pill burden adherence is likely to be poorer in the outpatient compared to in hospital treatment administration. Poor sterilising ability of XDR TB drugs and residual disease not communicating with the airways may give false indications of conversion by sputum culture. Ongoing mycobacterial replication and subsequent erosion into airways may thus result in reversions.

Early diagnosis and initiation of XDR-TB treatment, particularly in patients in whom we can predict a longer time to culture-conversion and poorer treatment outcomes, is crucial. In this study the average length of time from sputum acquisition to treatment initiation was 75 days (IQR 43-116). However, delay in treatment initiation of nearly four months is unacceptable. It is imperative that the time to treatment initiation be reduced as it may lead to poor conversion rates and ultimately treatment failure. Rapid TB diagnostic and susceptibility

tests will undoubtedly reduce the time to diagnosis of XDR-TB. However, even then the performance of the Hain MDR-TBplus assay is suboptimal in smear negative compared with smear positive patients. As many of the patients in this study were smear negative, better polymerase chain reaction (PCR) based tests will be required. The performance of the Hain-SL version remains to be evaluated in clinical samples and in smear negative TB. If rapid diagnostics are coupled to good clinic and laboratory infrastructure along with prompt communication of results, time to initiation of therapy is likely to be reduced. The ultimate effect on treatment outcomes remains to be seen.

There are several limitations to this study inherent to its retrospective nature (outlined in the last chapter and restated here; furthermore, several of these limitations will apply to subsequent chapters). We tried to limit bias by excluding from the analysis, patients with incomplete treatment and microbiological data, subjects whose sputum culture status was unknown at treatment initiation, and cross-checking patients against clinical and laboratory databases. Given that all cases of XDR-TB were detected passively, it is likely that several cases were not documented. This is due to patients remaining undiagnosed, dying prior to susceptibility testing, not accessing health care, or in whom appropriate susceptibility-testing was not undertaken due to capacity limitations. Although this would not alter the conversion rates of those on treatment it would impact on the total cases of XDR-TB in the country. Another limitation is that our study does not contain long-term treatment outcome data such as completion and

cure. However, as capreomycin-based regimes and second-line DST only became widely available in several South- African centres in 2007 these data are not yet available. Nevertheless, our study did encompass mortality data and culture-conversion which correlates well with late treatment outcomes in drug-resistant TB (Holtz et al., 2006).

XDR-TB patients who have a low body weight at diagnosis may well benefit from intensive nutritional support (e.g. high protein diets, meal supplements and vitamins) and close monitoring of treatment response. Newer rapid diagnostic methods, with appropriate infrastructural and communication support, need to be implemented to reduce the time taken for culture-conversion and DST results to be available to managing clinicians. This will in turn allow for earlier treatment initiation or modification with a better chance for a successful treatment outcome. Given the poor treatment outcomes in XDR-TB aggressive preventative measures to limit the creation of MDR-TB and XDR-TB must be taken, and new drugs for the treatment of XDR-TB are urgently needed.

Chapter 5: Mortality

Figures

Tables

Table 5.2: Cox proportional hazards regression model of factors associated with risk of death in all patients receiving XDR-TB treatment (n=174) (2-A), and in HIV-infected patients only (2B).

5.1 Introduction

Tuberculosis kills an estimated 1.3 million people per year worldwide, with an estimated 150 000 from MDR-TB (WHO, 2010). In 2008 the number of cases of MDR-TB in Africa (primary and acquired) was estimated at 69 000, ~ 30% of the global estimate, with an estimated annual mortality of 22 000 cases. This mortality rate may in fact be higher as in many parts of Africa where laboratory facilities are limited and many cases may never be diagnosed or adequately treated with second-line drugs. This makes accurate mortality rates due to MDR-TB challenging. In South Africa the number of MDR-TB cases was estimated at 13 000 cases in 2008 (WHO, 2010).

In case-based surveillance data from over 40 000 TB patients treated in 17 European Union countries MDR was strongly associated with the risk of dying from any cause (adjusted OR=3.9, 95% CI 3.3–4.6). This surveillance although it had many limitations, showed that drug resistance was an important risk for death among TB patients even in developed countries (WHO, 2010).

However, there are few data about XDR-TB mortality globally and in Africa. The first indication of the potential magnitude of the mortality rate attributable to XDR-TB in

Africa was the Tugela Ferry XDR-TB outbreak reported in 2006. In this hospital-acquired XDR-TB outbreak involving 53 XDR-TB patients there was an almost 100% mortality with the average time to death of 14 days (Gandhi et al., 2010). Although these patients had not received XDR-TB treatment and hardly any Highly active anti-retroviral therapy (HAART), this news was received with shock by the global community and set a precedent for just what TB control programmes could expect from efforts to treat this highly resistant form of tuberculosis (Mitnick et al., 2008). Other studies have also described the scenario of untreatable strains of tuberculosis (Chauhan, 2007, Masjedi et al., 2006).

Thus, a widely held perception was that XDR-TB in Africa occurs predominantly in HIV positive patients who are more susceptible to infection and whose survival is dismal. It has however been shown that XDR-TB existed in South Africa prior to this outbreak in most provinces, with 4% of MDR-TB cases in fact being undiagnosed XDR-TB cases (Mlambo, 2008).

In another study in KwaZulu-Natal, Gandhi et al. showed extremely high death rates from MDR and XDR-TB, particularly in the first 30 days after sputum acquisition, when 40% of MDR and 51% of XDR-TB cases died. The one year mortality in this study was 71% for MDR and 83% for XDR-TB with HIV rates of 90% in both the MDR and XDR groups (Gandhi et al., 2006b). It is likely that HIV co-infection in MDR and XDR-TB results in high early mortality.

In a study conducted by *Mitnick et al*. (Mitnick et al., 2008)in Peru, 0.2% (7/651) of patients died before treatment could be initiated. 14.3% (1/7) of these patients were XDR, and 85.7% (6/7) were MDR. None of these patients were HIV-infected. The hazard ratio for death in XDR-TB patients as compared to MDR was 1.1% (95% CI, 0.59 to 2.02, p=0.79) and the death rate was 10%. MDR mortality rates were higher but the risk of death did not differ significantly between the two groups of patients 22.9% (11/48) in XDR-TB and 20.4% (123/603). Although causes of death were unknown, more drug exposure was associated with the risk of death in this study.

The large numbers of people who die soon after sputum collection and before treatment initiation highlights the importance of timely diagnosis of resistant TB and early initiation of effective anti-tuberculosis treatment and antiretrovirals (ART) (ARVs) in co-infected patients. (Gandhi et al., 2006a) In a systematic review and meta-analysis on XDR-TB outcomes the summary estimate of patients who died was 20.8% (95% CI, 14.2%-27.35). This was higher than the death rates reported for MDR-TB providing further proof of the higher mortality of those patients with XDR-TB (Jacobson, 2010).

Despite having the highest TB caseloads there have been relatively few XDR-TB cases identified from high burden settings, including those with a high HIV prevalence. Thus, the mortality of XDR-TB in Africa, outside of an outbreak setting

remains largely unknown. It also remains unclear whether a similar mortality rate was prevalent in other South- African provinces where different strains and predictors of outcomes were prevalent. To address these questions we evaluated mortality in XDR-TB patients recruited from four provinces, other than KwaZulu-Natal, in South Africa.

5.2 Methodology

5.2.1 Patients

A total of 227 patients diagnosed with XDR-TB were identified. Twenty eight were excluded: (two children under the age of 18, eight participants with unknown sputum culture results at XDR-TB treatment initiation, one patient who was transferred from the Eastern Cape to the Western Cape, four patients whom had incomplete data and 13 without a documented HIV status.) Of the remaining 199 XDR-TB patients 174 patients initiated treatment for XDR-TB, 21 who died before treatment initiation and four in whom XDR-TB treatment was never initiated.

5.2.2 Definition

The definition of death, adapted from that defining MDR-TB mortality, was based on that used by Laserson et al. “an XDR-TB patient who died for any reason during the course of XDR-TB treatment” (Laserson et al., 2005) .

5.2.3 Statistical Analysis

A univariate Cox regression analysis (i.e. one predictor variable) was performed to look at predictor variables associated with death versus no death and the p values for comparison between groups evaluated by using the chi-square test, or Fisher exact test. Statistical tests were two-sided and a p value less than 0.05 was considered statistically significant. Kaplan Meier curves were used for time to event analysis, both from the time of diagnosis and that of treatment. No multivariate model was conducted owing to lack of association in the univariate models performed. Cox proportional hazards regression models were fitted to determine risk factors associated with outcomes in a time-to-event based analyses. Variables found to be significantly associated with the outcome ($p<0.05$) were included in the final model. The proportionality assumption of the Cox models was tested using –ln[–ln (survival)] curves and regression of scaled Schoenfeld residuals on functions of time. The assumption of uninformative censoring (i.e. more censored observations at an earlier time in one group compared to another or a greater proportion of censored survival times in patients with a particular range of values of the explanatory variables) was examined by plotting observed survival times against the values of the explanatory variables included in the model.

5.3 Results

In total 42.2% (84/199) of the XDR-TB patients died during a median follow-up period of 6.9 (3.1-12.0) months. 10.5% (21/199) patients died before treatment was initiated. Overall and treatment-initiated one year mortality rates were 42% (84/199) and 36% (62/174), respectively (Figure 5.1D and Table 5.1); 33, 3% (28/84) of all deaths occurred before treatment initiation (figure 5.1C). Of the total of 174 subjects who initiated treatment, the mortality rate was 41.5 % (34/82) in HIV infected and 30.4 % (28/92) in HIV un-infected patients, respectively; (p=0.13;

	Mortality		
	Alive n(%)	Died n(%)	P
Total number of patients	112(64.4)	62(35.6)	
Age, median(IQR)	32.1(25.8-42.8)	37.2(28.5-47.1)	0.07
HIV status			0.13
Positive	48(42.9)	34(54.8)	
Negative	64(57.1)	28(45.2)	
Gender			0.59
Female	59(52.7)	30(48.4)	
Male	53(47.3)	32(51.6)	
Smoking History			0.004
Yes	39(34.8)	10(16.1)	
No	34(30.4)	15(24.2)	
Unknown	39(34.8)	37(59.7)	
Previous MDR TB			<0.0001
Yes	70(62.5)	55(88.7)	
No	42(37.5)	7(11.3)	
Weight(kg), median (IQR), (n=65)	50.0(44.0 – 60.0)	44.0(39.0–49.0)	0.004
Drugs used			
Ofloxacin			0.02
Yes	25(22.3)	5(8.1)	
No	87(77.7)	57(91.9)	
Capreomycin			0.43
Yes	103(92.0)	58(95.8)	
No	9(8.0)	3(4.8)	
Ethionamide			0.61
Yes	66(58.9)	39(62.9)	
No	46(41.1)	23(37.1)	
Ethambutol			0.01
Yes	57(50.9)	44(71.0)	
No	55(49.1)	18(29.0)	
PAS			0.41
Yes	102(91.1)	54(87.1)	
No	10(8.9)	8(12.9)	
Moxifloxacin			0.02
Yes	13(11.6)	1(1.6)	
No	99(88.4)	61(98.4)	

Table 5.1: Mortality in XDR-TB patients who initiated treatment (n=174) stratified by socio-demographic and clinical characteristics.

Of the deaths prior to treatment initiation 75% (21/28) died before a diagnosis of XDR-TB had been made (i.e. during the lapse of time between sputum acquisition date and sputum report date or time to trace the patient once the result was known). By contrast, 25% (7/28) of deaths occurred in patients waiting for a bed at the XDR-TB facility or shortly after admission but before treatment could be commenced. In 4 patients it was decided, on admission to the XDR-TB treatment facility, that the patient's clinical condition was so poor it precluded them from XDR-TB treatment initiation.

Demographic variables which were stratified by mortality are shown in Table 1. There was no difference in the number of deaths ($p>0.05$) when these outcomes were stratified by sex, ethnic origin, and the number of previous episodes of MDR tuberculosis (median 1 episode [1–1] each in those who survived and died, 1 episode [1–2]. There was no significant difference in the duration of previous treatment for MDR tuberculosis in patients who survived versus those who died (median 8.0 months [5.0–12.0] vs. 5.5 months [4–8], respectively; $p=0.7$). Similarly, the median number of previous drug-susceptible episodes of tuberculosis in the same groups was not different (survivors *vs.* non-survivors: 1 [1–2] vs. 1 [1–2], respectively, $p=0.1$ vs. 1 [1–2], respectively, $p=0.2$). The time from sputum acquisition to start of treatment was significantly longer in those who survived than in those who died (78 days [53–107] vs. 57 days [36–67]; $p=0.001$).

There was no significant difference in the median (IQR) duration (months) of prior MDR treatment in those that survived versus those that died [8 (5-12) vs. 5.5 (4 -8); p=0.7]. Similarly, the median number of prior drug susceptible episodes of TB was no different in the same groups (2 vs. 2; p=0.1 and 2 vs. 2; p=0.2, respectively).

The length of time from sputum acquisition (i.e. the date the sputum was taken) until the date XDR-TB treatment was initiated was significantly longer in those who survived versus those who died [78 (53-107) days vs. 57 (36-67) days; p= 0.001]. The median duration of follow-up from treatment initiation to event (death, loss to follow-up or the censor date) was 6.9 months (IQR 3.1 to 12.0). The median (IQR) time (days) from sputum acquisition to treatment initiation was significantly longer in those who survived versus those who died [78 (53-107) vs. 57 (36-67); p=0.001].

The results were similar when survival was calculated from diagnosis rather than treatment initiation date and outcomes were similar prior to and after March 2007 when capreomycin and PAS were introduced into the programme.

It was not possible to ascertain the cause of death but in those who died prior to treatment initiation HIV-infected patients had a significantly higher mortality in the time to event analysis compared to HIV-uninfected patients (Figure 5.1C). 174 (82 HIV-infected and 92 HIV-uninfected) received treatment. Of these 36% (62/174) died during follow up (Figure 1D). Mortality rates between HIV-infected and HIV-

uninfected patients were not significantly different: [(34/82(41.5%) vs. 28/9(30.4%); p=0.13)] (Table 5.1 and Figure 5.1E)

In the sub-analysis conducted in HIV-infected patients, only HAART and moxifloxacin usage were independent predictors of survival (Table 5.2B). Mortality was significantly lower in HIV-infected individuals who received HAART compared to those who did not (adjusted risk ratio= 0.31, 95% CI 0.15-0.61, p=0.01) and 12 month mortality was 26% vs. 76%, p=0.02 in the same groups; (Figure 5.1F; Table 5.2B). Survival did not differ by CD4 count but the analysis was based upon a small number of participants with verifiable CD4 counts specifically at the time of treatment initiation [n=35/82; median (IQR) CD4 count of 273 (169-396) cells/mm^3].

Table 2A

Factor	Univariate analysis		Multivariate analysis	
	Odds Ratio (95%CI)	P-value*	Odds Ratio (95%CI)	P-value
Isoniazid	0.20(0.08-0.49)	< 0.0001	0.76(0.24-2.51)	0.68
Moxifloxacin	0.12(0.02-0.89)	0.03	0.11(0.01-0.82)	0.03
Ethambutol	1.87(1.08-3.26)	0.03	1.49(0.69-3.23)	0.31
Dapsone	2.19(1.25-3.84)	0.006	1.79(0.84-3.85)	0.13
Clofazamine	0.30(0.11-0.82)	0.02	0.54(0.14-2.08)	0.37
Clarithromycin	0.55(0.32-0.94)	0.03	1.46(0.61-3.52)	0.40
Terizidone	0.48(0.27-0.84)	0.01	1.35(0.71-2.55)	0.37
Number of drugs used	0.74(0.61-0.88)	0.001	0.59(0.45-0.78)	<0.0001
Previous MDR treatment	3.73(1.69-8.22)	0.001	5.21(1.93-14.1)	0.001

Table 2B

Factor	Univariate analysis		Multivariate analysis	
	Odds Ratio (95%CI)	P-value*	Odds Ratio (95%CI)	P-value
HAART	0.31(0.15-0.61)	0.001	0.38(0.18-0.80)	0.01
Isoniazid	0.17(0.05-0.56)	0.005	0.41(0.06-2.96)	0.39
Moxifloxacin	13(0.02-0.92)	0.006	0.08(0.01-0.61)	0.02
PZA	4.1(1.00-17.5)	0.04	3.48(0.74-16.5)	0.12
Clofazamine	0.21(0.05-0.86)	0.03	1.37(0.14-13.82)	0.79
Previous MDR treatment	7.46(1.79-31.2)	0.006	4.50(0.83-24.4)	0.08
Number of drugs used	0.74(0.56-99)	0.04	0.87(0.58-1.26)	0.43

Table 5.2: Cox proportional hazards regression model of factors associated with risk of death in all patients receiving XDR-TB treatment (n=174) (2-A), and in HIV-infected patients only (2B).

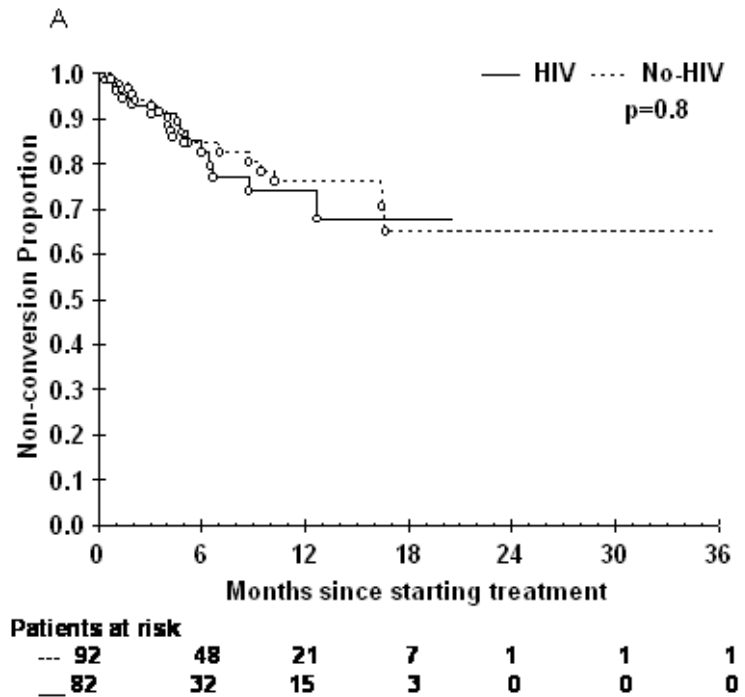
Figure 5.1: Kaplan-Meier probabilities of death:

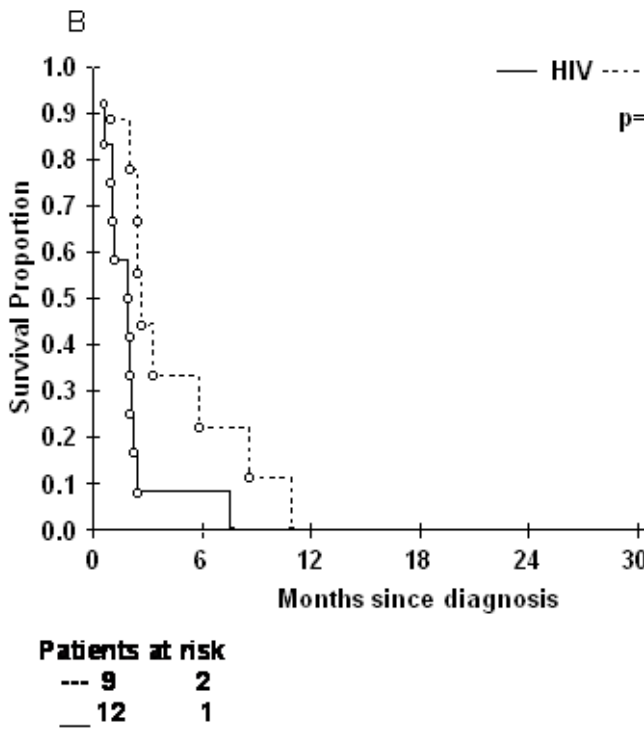
(A) Probabilities of culture conversion in patients with and without HIV infection given treatment

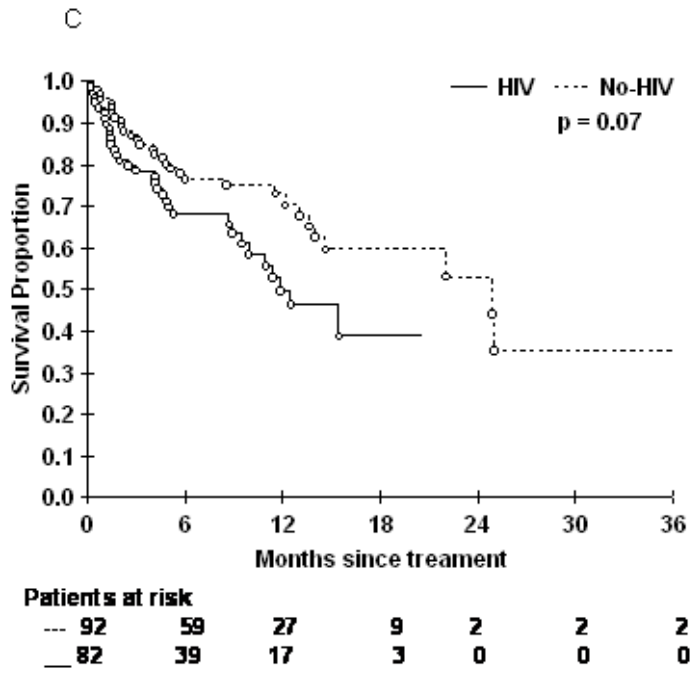
(B) Probabilities of death in patients who died before initiation of treatment stratified by HIV status

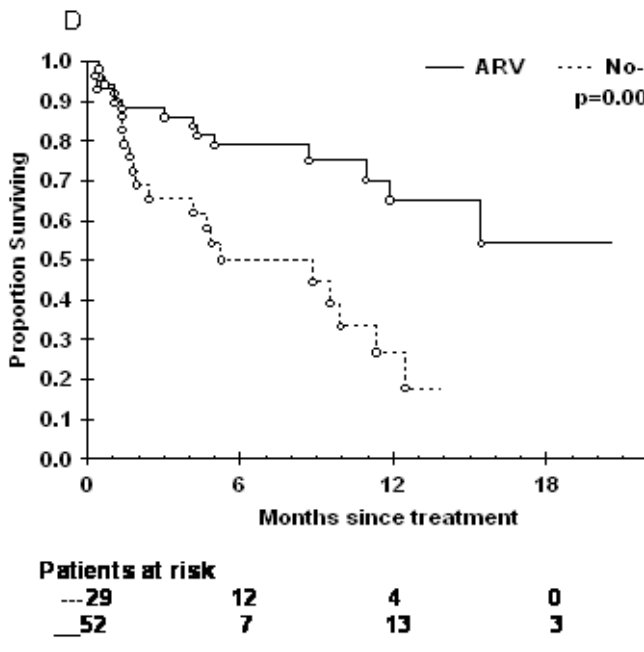
(C) Probabilities of death in patients with and without HIV infection given treatment from date of treatment initiation

(D) Probabilities of death in patients with HIV infection given treatment stratified according to use of highly active antiretroviral therapy









5.4 Discussion

Available XDR-TB-related data from Africa indicate that in a setting of probable nosocomial transmission XDR-TB almost 100% of patients were HIV-infected and died at a median of two weeks after treatment initiation (Gandhi et al., 2006b). By contrast, our study showed that a large proportion of XDR-TB patients were HIV-uninfected and that there is no difference in mortality rates when comparing HIV-infected versus HIV non-infected individuals. Furthermore, O'Donnell et al. found in his study at King George V hospital in Durban, KwaZulu-Natal that HIV status did not predict death or culture-conversion (O Donnell et al., 2009).

Moreover, we have shown that survival in African XDR-TB HIV co-infected patients recruited from a wider setting is substantially longer (12 month mortality of 42%) than previously recognised. It has also shown for the first time that HAART, despite its overlapping toxicity and side effects with anti-TB drugs and the high pill burden, substantially improves survival in HIV positive patients and was generally well tolerated. O'Donnell et al. demonstrated a trend towards better survival in HIV-infected patients with CD4 counts less than 200 cellss/mm^3 on HAART compared to those not on HAART (OR 0.094,95% CI 0.007-1.22) (O Donnell et al., 2009). Our data suggests that HAART should be used at an early stage in all co-infected patients with MDR and XDR-TB.

A key finding of this retrospective case note review was that overall prognosis was poor regardless of HIV status despite supervised in-patient multi-drug treatment; and access, when available, to surgical resection of diseased lung. Possible reasons for the poorer survival seen in this cohort compared with that seen in the meta analysis conducted by Jacobson et al. (Jacobson, 2010) may include the long duration of previous drug-resistant TB and the predominantly non moxifloxacin containing regimens used. The national TB programme, at least in the Western Cape, had not as yet released moxifloxacin for use in XDR-TB regimens at the time of this study.

Nevertheless, mortality rates did not differ in centres with a high urbanised population and easy medical access (Gauteng and Western Cape) compared with more rural populations with limited access (Eastern Cape and Northern Cape). Although we found a survival benefit in moxifloxacin-treated patients, we could not correlate this to quinolone-specific resistance profiles because of the lack of specific DST data. It should also be emphasised that our findings are only generalisable to a resource-poor high HIV prevalence setting like South Africa. Outcomes in low HIV prevalence settings and those in even more severely resource-constrained settings may be different.

HIV-infected XDR-TB patients treated with appropriate anti-TB regimens together with HAART had a lower mortality than in those who were treated with appropriate TB treatment but not started on HAART and thus survival in HIV-infected patients is better than that previously reported (Gandhi et al., 2009).

There are several limitations of our study (in addition to those already mentioned in the previous chapter). The WHO definition of death is any death during treatment and does not take into account patients dying after treatment failure or interruption. Furthermore, deaths in our study were not categorised into causes of death due to drug resistant TB or other causes. Selection bias, particularly in the HIV-infected sub-group, may have led to an underestimate of the true mortality as survivors would have been more likely to have been included in our study.

In summary, given the currently available diagnostic tools, case finding strategies, available resources and co-morbidities, treatment-related outcomes in patients with XDR-TB in South Africa are poor. Death rates from XDR-TB are high but not necessarily linked to HIV infection as has been suggested by studies from KwaZulu-Natal. Nevertheless the addition of moxifloxacin to regimens can reduce mortality. Studies examining the efficacy of newer generation fluoroquinolones are now urgently needed.

Furthermore, survival in HIV co-infected subjects is better than previously reported and may be substantially improved with the provision of HAART. Active case finding

is now needed to identify M/XDR-TB cases early and before they transmit disease. Rapid diagnostics and early initiation of treatment and ART, regardless of CD4 counts are imperative for better treatment outcomes. Community-based M/XDR-TB models are also need to be explored to reduce the risk of nosocomial transmission of patients within hospitals.

Chapter 6 Adverse Drug reactions

Figures

Tables

6.1 Introduction

Over the last two decades the entity multi-drug resistant tuberculosis (MDR-TB i.e. resistance to at least isoniazid and rifampicin) has emerged. In 2008 there were approximately 450 000 cases of MDR-TB globally. Between 5 to 10% of MDR-TB cases are thought to be due to extensively drug resistant tuberculosis (XDR-TB i.e. resistance to rifampicin, isoniazid, fluoroquinolones and one of the 2^{nd} line injectable agents i.e. kanamycin, amikacin or capreomycin). MDR-TB and XDR-TB now threaten to destabilise TB control in several regions of the world including Africa, Eastern Europe, Russia, central Asia, India and China (Dheda et al., 2010b).

In high burden settings treatment outcomes of MDR-TB are disappointing with only ~50% of patient's successfully completing treatment (Shean et al., 2008). Outcomes in XDR-TB are poorer. We and others have recently shown that, in contrast to low and intermediate burden settings (Mitnick et al., 2008), less that 20% of patients with XDR-TB culture-convert in high burden settings (Migliori et al., 2008, Dheda et al., 2010b). Treatment options, because of the high grade of drug resistance are severely limited and the higher the total number of appropriate drugs used in a regimen the better the outcome(Dheda et al., 2010b). Thus, treatment interruption due to any cause may potentially subvert successful outcome in patients with XDR-TB. Failure to identify and manage adverse drug reactions (ADRs) may also have serious implications for patient perceptions about toxicity versus benefit, and thus may impact on compliance. Even in compliant inpatients, we and others have

recently shown that (ADRs) are common in patients with XDR-TB (Dheda et al., 2010b, O Donnell et al., 2009).

However, data about whether ADRs impact on treatment-related outcomes in patients with drug-resistant TB are scarce. It is also unclear how the *M. tuberculosis* strain phenotype and host factors such as HIV co-infection impact on the frequency and severity of ADRs, and associated clinical outcomes. Given that capreomycin modulates outcomes and is a vital backbone of most XDR-TB treatment regimens (Migliori, 2008b), the frequency of ADRs to capreomycin and their temporal relationship to treatment initiation are of interest. Collectively, these data can inform on several aspects of management including the design and monitoring of treatment regimens for XDR-TB and formulating strategies to prevent treatment interruption, thus facilitating compliance and minimising treatment failure. To address these gaps in our knowledge and, in particular, to evaluate the impact of ADRs on outcomes we reviewed the case records of 115 patients treated for XDR-TB at three treatment centres in South Africa.

6.2 Methodology

6.2.1 Study setting and participants

We retrospectively reviewed the case records of 115 laboratory-confirmed XDR-TB patients diagnosed between August 2002 and February 2008 at three designated XDR-TB treatment centres in South Africa (see Figure 6.1 for the study outline). Case records were comprehensively reviewed by a trained researcher for ADRs

(duration, type and severity), regimen used (dose, indication, route of administration), culture-conversion and mortality outcomes, and HIV status. Associated demographic and clinical information were also captured. Ethical approval was obtained from the University of Cape Town, and the University of Witwatersrand's human research ethics committees.

6.2.2 Diagnosis of XDR-TB

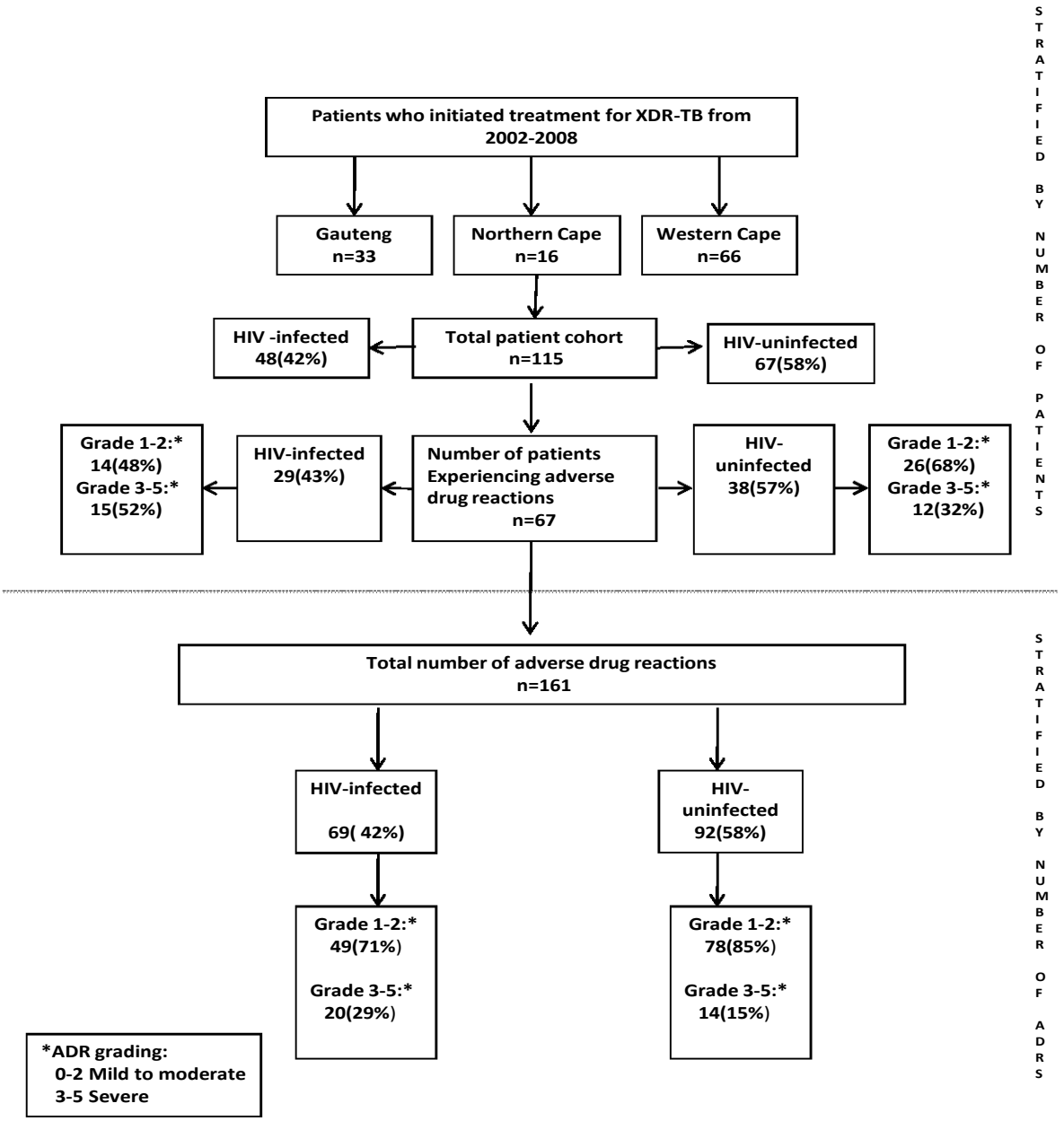
From 2002 sputum culture and susceptibility testing for second-line agents, amikacin, ofloxacin, and ethionamide was performed as previously described (Dheda et al., 2010a). Isolates of *M. tuberculosis* that were resistant at diagnosis (time of sputum collection) to at least isoniazid, rifampicin, a fluoroquinolone, and at least one of the second-line injectable drugs (amikacin, kanamycin, or capreomycin) were judged to be XDR-TB. Drug-susceptibility testing to capreomycin, cycloserine, terizidone (a derivative containing a double molecule of cycloserine), and fluoroquinolones other than ofloxacin was unavailable within the provincial laboratories.

6.2.3 Treatment regimens

XDR-TB treatment was only initiated and administered in hospital with the use of capreomycin and para-aminosalycilic acid (PAS) as the anchor drugs, which became available in South Africa in late 2006 / early 2007. **The drugs used in the treatment regimens are shown in Table 4.** Third-line drugs (clarithromycin, dapsone,

amoxicillin/clavulanate and azithromycin) were used at the discretion of the attending clinician and those to which the organism demonstrated susceptibility. High-dose INH was administered at a dose of 10mg/kg. Linezolid was unavailable through the National TB Programme and clofazimine and moxifloxacin was used in selected centres on a limited basis. HAART was offered to all HIV co-infected patients at the discretion of the attending physician and irrespective of the patients CD4 count.

Figure 6.1 Study plan stratified according to treatment site, HIV status and severity of adverse drug reactions.



6.2.4 Definition of adverse drug reactions

ADRs were defined as unintended adverse responses occurring at therapeutic doses resulting in either death, drug withdrawal, change in the administration of the frequency or dose of the drug, or, no action being taken (Guidelines). These were graded according to the modified American National Institute of Health (common terminology criteria for adverse events [CTCAE]. For the purposes of analysis grades 1 and 2 ADRs were considered mild to moderate and grade 3 to 5 severe. Multiple events of the same ADR were counted separately. The definitions used to identify and classify adverse drug reactions are outlined in (Table 6.1).

A. Grading of Adverse drug reactions[1]	
grade 0	no ADR
grade 1	mild adverse event i.e. described in the patient's management records but no action was taken
grade 2	moderate ADR resulting in either changing the dose or frequency of the offending drug or another drug(s) was added to manage the adverse event
grade 3	the side effect was severe enough for the offending drug to be stopped
grade 4	the ADR was life threatening or disabling
grade 5	the adverse event caused the death of the patient
B. Definitions used to identify and classify adverse drug reactions.	
Nausea, vomiting, diarrhoea. Other GI symptoms: abdominal pain, dyspepsia, epigastric discomfort and cramps	As documented by the physician or nursing staff
Dizziness/disorientation/confusion	
Body aches /pains/cramps	
Headache	
Sore tongue / throat	
Generalised itchiness	
Fatigue	
Numbness of extremities	Symptoms and findings consistent with neuropathy, e.g. pain or numbness of the distal extremities diagnosed by a physician.
Skin reaction	A dermatological reaction felt to be related to anti-tuberculosis medications as documented by the physician or dermatologist
Hypokalemia	< 3.5 meq/L (normal range: 3.5 - 5.5 meq/L)
Hypothroidism	At least one thyroid stimulating hormone(TSH) result >4.94 IU/ml (normal 0.35 - 4.94)
Depression/psychosis	As diagnosed by the TB physician and/ or psychiatrist, based on international classification of diseases (ICD)-10 criteria
Visual disturbance	Diagnosed by the physician / eye specialist as being related to the TB drugs
Arthralgia	Painful joints as reported by patient and documented by physician or nurse
Ototoxicity	Hearing loss confirmed by audiometry and/or physical examination
Renal impairment /renal failure	Creatinine > 100 µmol/L
Hepatotoxicity	Raised bilirubin or elevated transaminases > 3 times the upper limit of normal, and ascribable to a specific drug

[1]These were graded according to the modified American National Institute of Health common terminology criteria for adverse events [CTCAE]

Table 6.1 Definitions used to grade, identify and classify adverse drug reactions**.**

6.2.5 Outcomes

The date of diagnosis of XDR-TB was taken as the date on which the sputum sample was taken, and from which the organism was cultured. Conversion was judged to have occurred when two consecutively negative cultures were obtained, one month apart, and providing that a culture taken at initiation of XDR-TB treatment was positive.

6.2.6 *Mycobacterium tuberculosis* strain typing

A subset of 53 XDR-TB isolates from patients from the Western Cape were genotyped by IS*6110* DNA fingerprinting (Warren, 2006) spoligotyping (Kamerbeek, 1997). Strains were categorised as Beijing or non-Beijing according their spoligotype signature {STREICHER, 2007 #1588}.

6.2.7 Data handling and statistical analysis

Refer to methodologyin the other chapters

6.3 Results

6.3.1 Demographic and clinical characteristics

ADR's were reported in 58% (67/115) of patients. The breakdown by severity of ADR and HIV status, stratified by patient number and total number of ADRs, is shown in Figure 1. We could not identify any demographic and clinical variables that were specifically associated with the development of

ADRs (grade 1 to 5) compared to those who did not develop an ADR (grade 0). We then evaluated demographic and clinical characteristics stratified by the severity of ADRs (group 0, 1 and 2 vs. group 3, 4, and 5; outlined in Table 6.2). Patients with severe ADRs (grade 3 to 5), when compared to those with mild, moderate or no ADRs (grade 0, 1 and 2), were more likely to be female, have had previous MDR-TB or drug susceptible TB, and had fewer drugs in their treatment regimens (Table 6.2). In the multivariate analysis only a history of previous MDR-TB was independently associated with the patient developing severe ADRs; p=0.009. The median CD4 count in HIV-infected persons was 204 (range 13 – 893) cells/mm^3. The drugs used in the treatment regimens are shown in Table 6.2 and Table (Chapter 3 pg, 55).

	Grade: 0-2 none/mild/moderate adverse drug reactions n=88(% unless otherwise stated)	**Grade 3-5 severe/life threatening /death adverse drug reactions n=27(% unless otherwise stated)**	**P value** p =
Sex			0.014
male	53(60.2)	9(33.3)	
female	35(39.8)	18(66.7)	
Ethnicity			0.969
mixed origin	46(52.3)	14(51.9)	
black	42(47.7)	13(48.1)	
HIV status			0.096
infected	33(37.5)	15(55.6)	
uninfected	55(62.5)	12(44.4)	
HAART			0.917
yes	23(71.9)	11(40.7)	
no	9(28.1)	4(14.8)	
Number of previous sensitive TB episodes (IQR)	1(1-2)	1(1-2)	0.033
Previous MDR-TB episodes			0.009
yes	47(53.4)	22(81.5)	
no	41(46.6)	5(18.5)	
Previous MDR-TB episodes (IQR)	1(1-1)	1(1-2)	0.467
Number of drugs in the treatment regimens (IQR)	6(5-7)	5(4-6)	0.001
Smoking			0.454
current	37(42)	12(44.4)	
non	36(40.9)	13(48.1)	
previously	15(17)	2(7.4)	
Weight at diagnosis of XDR-TB (IQR)	48(44-59)	48(36-59)	0.626
Age at diagnosis of XDR-TB (range in years)	31.0(26.4-42.0)	36.8(25.0-46.2)	0.892
Died			0.003
yes	17(19.3%)	13(48.1%)	
no	71(80.7%)	14(51.9%)	
Conversion			0.035
yes	24(27.3%)	2(7.4%)	
no	64(72.7%)	25(92.6%)	

Table 6.2 Socio-demographic and clinical characteristics of 115 patients who initiated treatment for extensively drug-resistant tuberculosis (XDR-TB)

6.3.2 Frequency and severity of ADRs

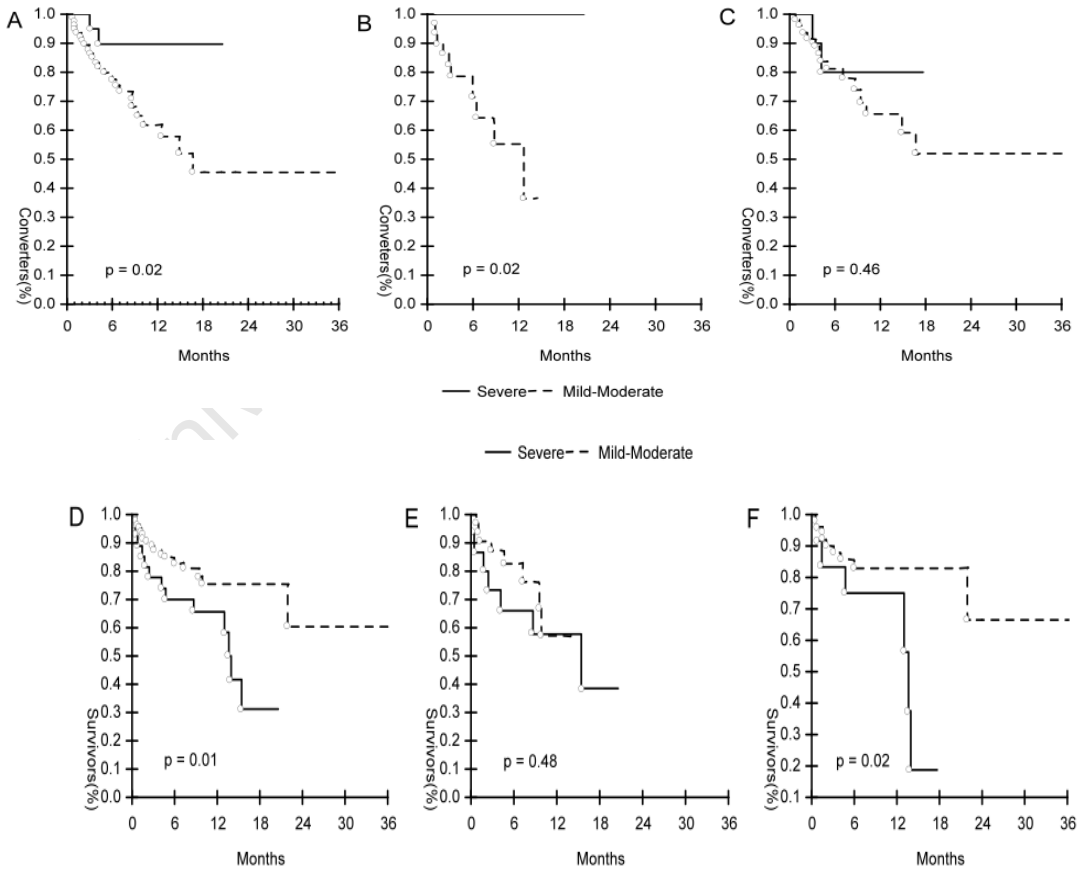
161 ADRs were experienced by 67/115 (58%) patients (Figure 1; upper panel). When the results were stratified by the number of patients: 17/67 (25%) patients required no intervention; 23/67 (34%) required modification of treatment, the offending drug was discontinued in 27/67 (40%) of patients; reactions were life * threatening in 2/67(3.0%), and 6/67(9.0%) died. When the results were stratified by ADRs (Figure 1; lower panel): in 58/161 (36%) instances an ADR was described but there was no intervention; 69/161 (43%) required modification of treatment in either the dose or frequency of the drug being taken, or, the prescription of an additional drug to treat the adverse event; the offending drug was withdrawn in 34/161 (21%); the ADR was life threatening in 2/161 (1.2%) instances (both ADRs were due to renal failure), and death was associated with 6/161 (4%) of ADRs. All 6 deaths were related to capreomycin (hypokalaemia in 1 patient and renal failure in 5 others), and these patients died at a median of 14 days (range of 9-73 days) after starting capreomycin therapy. There was no association between the severity of ADR and frequency and duration with which the drug was used, or, the resistance pattern of the drug.

Treatment regimen characteristics:						
duration of treatment — months (IQR)					**7.3 (3.12-12.6)**	
duration of treatment with injectable agents - months (IQR)					**4.4 (1.6-7.3)**	
number of drugs in regimens among all available agents – median (IQR)					**7 (6-8)**	
Drug	Number of patients who received a drug (%)		Duration of treatment in months Median in months (range)		Number of resistant isolates/ total number of susceptibility tests (%)	
	none/mild moderate ADR (0-2)	severe/life threatening/ death (3-5)	none/mild moderate ADR (0-2)	severe/life threatening/ death (3-5)	none/mild/ moderate ADR (0-2)	severe/life threatening/ death (3-5)
Ethambutol	**27(58.7)**	**19(41.3)**	**7.3(4.2-12.1)**	**8.7(2.4-12.1)**	**53/84(63)**	**9/27(33.3)**
Pyrazinamide	**56(70)**	**24(30)**	**7.3(3,4-10.9)**	**9.6(2.9-11.1)**	**Not Tested**	**Not Tested**
Amikacin	**3(100)**	**-**	**4.2(0.03-5.8)**	**-**	**3/3(100)**	**Not Tested**
Capreomycin	**78(75)**	**26(25)**	**7.4(3.1-12.4)**	**10.1(3.8-13.7)**	**13/27(48)**	**9/15(60)***
Kanamycin	**4(100)**	**-**	**13.4(4.8-15.5)**	**-**	**46/47(98)**	**4/4(100)**
Moxifloxacin	**2(100)**	**-**	**4.6(1.9-2.3)**	**-**	**Not Tested**	**Not Tested**
Ofloxacin	**23(79.3)**	**6(20.7)**	**6.5(3.0-12)**	**8(8.0-12.0)**	**88/88(100)**	**27/27(100)**
Terizidone	**80(76.2)**	**25(23.8)**	**6.8(3.4-12)**	**10(3.5-13.6)**	**Not Tested**	**Not Tested**
Ethionamide	**45(68.2)**	**21(31.8)**	**7.6(3.5-11.8)**	**10(2.1-13.6)**	**44/85(52)**	**4/27(15)**
PAS	**77(76.2)**	**24(23.8)**	**7.6(3.5-12.5)**	**10.1(3.3-13.9)**	**Not Tested**	**Not Tested**
Amoxicillin–clavulanate	**46(70.8)**	**19(29.2)**	**6(2.2-11.1)**	**10.394.8-14)**	**Not Tested**	**Not Tested**
Clarithromycin	**57(77)**	**23(23)**	**7.4(3.1-13.9)**	**10.7(4.6-14.3)**	**Not Tested**	**Not Tested**
Clofazimine	**25(89.3)**	**3(10.7)**	**8.7(4.4-14.2)**	**13.6(2.8-17.1)**	**Not Tested**	**Not Tested**
Dapsone	**27(75)**	**9(25)**	**7.3(3-14.4)**	**5.8(3-11.9)**	**Not Tested**	**Not Tested**
Azithromycin	**10(91)**	**1(9)**	**6.7(2.8-12.3)**	**-**	**Not Tested**	**Not Tested**

Table 6.3 Individualised regimens prescribed for 115 Patients with XDR-TB stratified by duration of treatment, severity of the ADR, and drug resistance profiles.

Figure 6.2

Kaplan-Meier probabilities of XDR-TB culture-conversion in: (A) The whole cohort of patients who experienced ADRs stratified by severity score i.e. none or mild to moderate (grade 0, 1 and 2), and severe (grade 3 to 5); (B) HIV-infected patients whom experienced ADRs stratified by stratified by severity score; (C) HIV-uninfected patients who experienced ADRs stratified by stratified by severity score, and Kaplan-Meier probabilities of death: (D) The whole cohort of patients from the date of treatment-initiation, (E) HIV-infected patients who experienced ADRs stratified by severity score, and (F) HIV-uninfected patients who experienced ADRs stratified by ADR categories



6.3.3 Outcomes

Culture conversion occurred in 26/115 (22.6%) of patients. Patients with grade 3-5 ADRs had a lower sputum culture conversion rate compared with those with grade 0-2 ADRs [2/27(7.4%) vs. 24/88(27.3%); p=0.02; Figure 2A]. In the whole cohort the hazard ratio for ADR as a risk factor for culture conversion was 0.22 (0.05-0.95); p = 0.04. There were no other significant variables associated with culture conversion.

In contrast to HIV-uninfected patients (Figure 2C], HIV-infected patients (Figure 2B) with severe ADRs (grade 3-5) had a significantly lower sputum culture conversion rate than those with grade 0-2 ADRs [0/15 (0%) vs. 10/33 (30.3%), p=0.02].

Of the 115 patients in the cohort, 30(26.1%) died. Overall, those who survived experienced fewer ADRs than those who died [48/67 (71.6%) vs. 19/67 (28.3%); p= 0.01] and patients with grade 3-5 ADRs had a higher death rate compared with those with grade 0-2 ADRs [13/27(48.1%) vs. 17/88(19.3%); p=0.003; Figure 2D]. However, when risk factors for death were analysed by multivariate analysis in the whole cohort only culture conversion and previous MDR-TB (but not ADR) were independently associated with death (Table 3).

In HIV-infected patients mortality rates were higher in those with grade 3-5 (severe) ADRs compared to those with grade 0-2 ADRs [7/15 (46.7%) vs. 8/33 (24.2%); p=0.12; Figure 2D, 2E, 2F]. Similarly, in the HIV-uninfected patients those with

severe ADRs had a higher death rate compared to those without severe ADRs [6/12(50.0%) vs. 9/55(16.4%); p=0.012; Figure 2F]. Of the 13 all-cause deaths occurring in the severe ADR group 6 were due to an ADR itself (5 due to renal failure and 1 due to hypokalemia - all ascribable to capreomycin). These patients were not terminally or critically ill and there was a clear temporal relationship between the initiation of the drug and the patient's death. Five out of the 6 patients who died were HIV-infected.

25/115 (21.7%) of patients defaulted from the inpatient facilities. There was no difference in the proportion of patients with severe ADR in those that defaulted compared to those who were non-defaulters [7/25 (28%) vs. 27/90 (30%); p= 0.96].

Factor	Univariate analysis		Multivariate analysis	
	Hazard Ratio (95%CI)	P-value	Hazard Ratio (95%CI)	P-value
Adverse drug reaction				
Grade 3-5	2.4(1.1-5.0)	0.02	1.4 (0.7-3.1)	0.35
Grade 0-2	1		1	
Previous MDR TB				
Yes	3.3(1.3-8.0)	0.01	2.9 (1.2-7.4)	0.02
No	1		1	
6 month culture-conversion				
Yes	0.1(0.01-0.6)	0.02	0.1 (0.01-0.8)	0.03
No	1		1	

Table 6.4 Univariate and multivariate analysis of factors associated with mortality in 115 patients with XDR-TB

21.7% (25/115) of patients defaulted from the inpatient facilities. The proportion of patients who experienced an ADR was no different in those who defaulted compared to those who did not 40% (10/25) vs. 60% (15/25), p=0.33. However, default was more likely in those that had a severe ADR compared to those who did not [27/34 (79.4%) vs. 7/34 (20.6%); p=0.003].

6.3.4 ADRs in HIV-infected patients

Outcome-independent data in HIV-infected patients

The number of persons with ADRs, the number of total ADRs per person, and the number of severe ADRs was not significantly different in HIV-infected versus uninfected persons [29/48 (60.4%) vs. 38/67 (58%); p=0.26)] vs. [2.37 ADRs per person vs. 2.42 ADRs per person] vs. [20/69(29.4%) vs. 14/92(15%); p= 0.31], respectively. Thus, the type, frequency and severity of the number of ADRs was similar in HIV-infected and uninfected patients. However, those who died of an ADR were more likely to be HIV-infected than HIV un-infected [5/6(83.3%) vs. 1/6(16.7%), p=0.01)].

34/48 (71%) HIV-infected patients were on HAART (highly active anti-retroviral therapy) but this did not impact on the frequency of ADRs and was generally well tolerated. 23/34 (68%) of patients were on a combination of lamivudine (3TC), stavudine (D4T) and efavirenz. The specific role of overlapping toxicities between HAART and anti-TB drugs could not be evaluated but the number of patients experiencing an ADR were significantly different in those taking HAART compared to HIV un-infected patients [29/34(85%) vs. 38/67(56.7%) p=0.008]. However, the number of patients experiencing a severe ADR were not significantly different in those taking HAART compared to HIV un-infected patients [11/34(32.3%) vs. 12/67(17.9%); p=0.17].

6.3.5 Drug-specific adverse events

Of the total number of drugs stopped (n=34) capreomycin (Capstat; Pharmacare Johannesburg) was the most common drug withdrawn in 15/34 (44.1%) of cases, followed by PAS in 8/34 (23.5%) of cases, and ethionamide 7/34 (20.6%) of cases (Table 4). The withdrawal of capreomycin due to an ADR occurred at a median of 73 days range (9-485) days after initiation of therapy.

The most common reactions causing grade 3-5 ADRs were vomiting (29% of severe ADRs) and renal failure (21%). Some patients experienced multiple ADRs. These were frequently clustered in the gastro-intestinal subgroup. Thus, of those who had diarrhoea 15/22 (68.2%) also experienced nausea and vomiting, and 10/22 (45%) nausea and vomiting together with abdominal pain and dyspepsia. ADR clustering was also evident in the neurological category (overlapping symptoms of headaches, dizziness, generalised aches and pains etc).

For each of the 18 drugs used in the XDR-TB treatment regimens there was no relationship between the severity of ADR and the number of patients who received each drug , total duration of treatment (months), and the proportion of resistant isolates (susceptible vs. resistant expressed as a %).

6.3.6 ADRs stratified by *Mycobacterium tuberculosis* strain type

Of the 115 patients with XDR-TB, isolates were available for genotyping in 53 of the patients from the Western Cape. Significantly more patients had a Beijing compared to a non-Beijing strain [81% (43) vs. 18.8% (10); p=0.0001]. The severity of ADRs was not significantly different in the Beijing and non-Beijing families (Table 6.4).

Strain type	Beijing N (%)		Non-Beijing N= (%)	
Severity of ADR	**ADRs 0-2**	**ADRs 3-5**	**ADRs 0-2**	**ADRs 3-5**
HIV-infected	**9/14 (64.3)**	**5/14 (35.7)**	**1/3 (33.3)**	**2/3 (66.7)**
HIV-uninfected	**20/29 (69.0)**	**9/29(31.0)**	**5/7 (71.4)**	**2/7 (28.6)**
Sub-totals	29/43 (67.4)	14/43 (32.6)*	6/10 (60)	4/10 (40)
Total	43/53(81)		10/53(19)**	

*P= 0.03 (severe vs. mild to moderate ADRs)

**p=0.0001 (total Beijing versus non-Beijing)

Table 6.4 Effect of TB strain type on ADRs stratified by Beijing strains and non-Beijing strains

6.4 Discussion

This is the first comprehensive report of ADRs and their impact on outcomes in a large cohort of patients with XDR-TB. Our key findings were that: (i) the frequency of ADRs with XDR-TB treatment regimens is high (~60%), and in 20% of cases the ADR was associated with interruption therapy or fatal consequences; (ii) HIV-infected patients with XDR-TB were more likely to die from a severe ADR and thus greater vigilance is required in this group; (iii) those with severe ADRs have poorer culture conversion but not higher mortality thus underscoring the need for careful treatment monitoring for early detection of ADRs, and (iv) capreomycin was the most common cause of drug withdrawal (44% of all withdrawals) and thus careful monitoring of this drug is mandatory.

A fundamental finding of this study is that XDR-TB patients with severe ADRs had poorer culture-conversion outcomes This is in keeping with the findings of *O'Donnell et al* (O Donnell et al., 2009) but in contrast to patients with MDR-TB from Turkey (Torun et al., 2005) and Russia where ADRs were common (~ 70% of patients) but were not associated with unfavourable outcomes (Shin et al., 2007). Thus, in contrast to MDR-TB, in XDR-TB patients the consequences of ADR-associated interruption of individual drugs impacts on culture-conversion outcomes. This most likely represents discontinuation of crucial drugs like capreomycin.

The high capreomycin toxicity seen in our study (almost half of all drug withdrawals due to an ADR) is in keeping with the findings of a Peruvian study where 31% of 115 MDR-TB patients had hypokalaemia, which was independently associated with the administration of capreomycin (Shin et al., 2007). Based on our findings we suggest weekly checks of renal function and electrolytes in the 1st 4 weeks of therapy, and then every 2 weeks for the next two months, and monthly thereafter. Active monitoring for ADRs, correct dosing by body weight, correction of dehydration, and regular monitoring of renal function and electrolytes is required particularly in those with risk factors (hypertension, diabetes, HIV-associated nephropathy, vomiting and diarrhoea, dehydration, electrolyte abnormalities, diuretic usage, alcohol abuse, and use of potentially nephrotoxic drugs such as tenofovir, cotrimoxazole etc). This has implications for the out-patient management of XDR-TB, which is currently being rolled out in high burden settings due to the sheer burden of cases that have overwhelmed designated facilities (Mitnick et al., 2008). Our data inform on resource allocation by national TB programmes in high burden settings that will need to take into account provision of monitoring and laboratory infrastructure when planning decentralised and nurse-led services for drug-resistant TB. Given the associated poorer outcomes in XDR-TB patients with ADRs, health care workers should be educated about the recognition of ADRs, and patients should be followed up more closely and be offered appropriate counselling to ensure drug adherence. Thus, our recommendations are easily implementable and do not detract from providing decentralised MDR treatment services in resource-poor settings.

Nausea and vomiting, in keeping with the findings of Shin et al. (Shin et al., 2007), was the most common reason for suspension of drug therapy (in any severity category) and needs to be managed with patient counselling, anti-emetics, and/or splitting of the dose to improve tolerability. ADRs although frequent were not more common in HIV-infected patients unlike observations that we (Marks et al., 2009) and others (Yee) have documented in patients with drug-susceptible TB. The reasons for this are unclear but could reflect poorer absorption of second line drugs and hence lower serum levels, or, ascertainment bias as HIV-infected patients may have died prior to diagnosis. Nevertheless, HIV-infected patients were more likely to die from severe ADRs and increased vigilance and correct dosing by body weight is required in this group.

The frequency of ADRs in this study (~60%) are similar to those that evaluated ADRs to second line drugs in the context of XDR-TB (58%)(O Donnell et al., 2009) and MDR-TB (73.3% in Tomsk, Russia (Shin et al., 2007), 69,2% in Istanbul Turkey (Torun et al., 2005)and 79% in Latvia (Bloss et al., 2010)but twice that of ADRs to first line drugs in those with drug-susceptible TB (Migliori et al., 2008). Suspension of any agent (16% in our study) occurred at a similar frequently compared to a Peruvian study in patients with MDR-TB (14%) (Furin et al., 2001) but less frequently than in a large multi-centric study in patients with MDR-TB (30%) (Nathanson et al., 2004), in Turkish patients with MDR-TB (55%) (Torun et al., 2005), and in patients with XDR-TB(O Donnell et al., 2009).

We found no significant association between strain phenotype and the frequency or severity of ADRs. This may reflect a true lack of association or type 2 error given the small numbers of isolates that were accessible for genotyping. Recent data suggest that DR-TB strains have in addition to resistance conferring mutations hundreds of compensatory mutations that may alter the structure and hence antigenic properties of the organism (Desouza et al.). This may impact host immune profiles and hence interaction with drug compounds. Further and larger studies are required to clarify this issue.

Similar to findings in earlier studies in drug-susceptible TB (Javadi et al., 2007); (Yee, 2003, Marra F, 2007), there was the higher number of women experiencing ADRs. The reason for this remains unclear. Similar to the findings in the context of drug-susceptible TB (Javadi et al., 2007), the higher rate of ADRs in those with prior MDR-TB, may reflect prior sensitisation, higher drug levels in patients with a lower body weight, and the generally poorer health status in keeping with chronic disease.

There are several limitations of our study. These include the retrospective study design, ascertainment bias due to data capture from medical notes, inability to calculate drug-specific ADR rates and ADR rates per person months of exposure, or to definitively delineate ADRs from disease-related morbidity in HIV-infected patients. However, this is difficult to calculate even in prospective studies because of the inability to ascribe a particular ADR to a specific drug in a multidrug regimen. Nevertheless, the patients were consistently seen by a small group of experienced

clinicians who based assessments on their clinical judgement and temporal relationship to symptoms, signs, and laboratory data, and we only extracted variables that could be confidently ascertained. We were also reliant on the judgement and investigative evaluation of clinicians who ascribed renal failure to capreomycin rather than dehydration, vomiting and diarrhoea etc. Thus, our analysis may reflect this clinical bias. Survivor selection bias may have led to an underestimate of the true mortality in the HIV-infected sub-group whilst late detection and delayed management of ADRs could have contributed to mortality given that severe ADRs occurred on average about 2 months after mild to moderate ADRs. Only a prospective study will be able to address this hypothesis. Our findings are only generalisable to a resource-poor high HIV prevalence setting like South Africa where there is a high rate of prior MDR-TB and of concomitant substance abuse.

In conclusion, the frequency of ADRs with XDR-TB treatment regimens is high and often severe. Those with severe ADRs have poorer treatment-related outcomes. Early detection and monitoring of ADRs is thus crucial, and XDR-TB patients with ADRs should be closely monitored for the remainder of their therapy. Assays to monitor serum levels of second line drugs and less toxic drugs are urgently needed. These data inform on the management and monitoring of patients being treated for XDR-TB, factors that impact on patient compliance, and the provision of resources within national TB programmes that seek to offer decentralised and nurse-led care for patients with XDR-TB.

Chapter 7 Discussion

XDR-TB has now been identified in over 59 countries and, given recent changes in international travel and migration patterns, the threat is global. Treatment of XDR-TB results in modest survival and treatment-related outcomes in intermediate and low burden settings (WHO, 2010). However, despite having the highest TB and TB/HIV caseload in the world, there have been scant data on M/XDR-TB from high burden settings in sub-saharan Africa. This is not due to absence of drug-resistant cases of TB but due to lack of data, which cannot be collected because of the absence of drug-testing facilities or the capacity to perform the required diagnostic studies. Thus, for economic and logistical reasons, routine drug susceptibility testing (DST) testing services are not available in high TB endemic countries, and any M/XDR-TB incidence and prevalence data from African countries may be a gross underestimate. Our work, recently published in the Lancet, indicates that XDR-TB inflicts a high mortality, causes chronic pulmonary disability, and diverts ~40% of TB-devoted resources away from existing treatment programmes. The key findings outlined in this thesis are:

(i) Survival in HIV-infected patients is better than that previously reported by the KwaZulu-Natal outbreak study.

(ii) HIV-infected XDR-TB patients treated with highly active anti-retroviral therapy (HAART) had lower mortality than untreated patients.

(iii) Moxifloxacin was an independent predictor of survival.

(iv) Previous culture-proven MDR tuberculosis and high number of drugs used in a regimen were predictors of survival and culture conversion.

(v) The outcome of management of patients with XDR tuberculosis in South Africa is poor, despite good adherence and availability of drugs.

(vi) Diagnosis occurred more than two months after presentation because of the lack of rapid diagnostic testing facilities

(vii) Side effects of TB drugs used in the management of XDR-TB patients may interfere with treatment completion.

(viii) XDR-TB among health care workers is now an urgent issue and the magnitude of the problem, including implementation of infection control measures in health care facilities, needs to be defined.

One of the key findings in our study, as discussed in chapter three, was the 11% (21/195) of patients who died before starting treatment, which indicated that the patients either, had late access to treatment or inappropriate delivery of health services. Our data emphasises the need to reduce delays in diagnosis and initiation of treatment through intensified case finding, {Basu, 2009 #201}, improved patient access, diagnostic reporting systems {Loveday, 2008 #1506;Storla, 2008 #1378}, and improvement in the rollout of rapid diagnostic tests for XDR tuberculosis as has been laid out in chapter three (Ling DI, 2008). Development of alternative methods that could be useful for detection of sputum-smear-negative tuberculosis are also urgently needed as well as validation of newly developed tests such as the Hain SL, and GeneXpert tests. (Migliori, 2008a, Pai M, 2006).

The high capreomycin resistance, also highlighted in chapter three, is a cause for concern since this antibiotic is the mainstay of regimens for the treatment of XDR-TB globally, and has not been widely used in South Africa previously. The reasons for this high resistance are not known, but it might partly indicate the unreliability of capreomycin susceptibility testing, or the potentially high cross-resistance with aminoglycosides like kanamycin and further work in this area needs to be undertaken {Jugheli, 2009 #1610;Via, 2010 #1366}.

With our findings, in Chapter 3, as well as those from other studies looking at MDR tuberculosis {Leimane, 2005 #1517;Mitnick, 2008 #1497}, we suggest that patients with a weight of less than 50 kg should be given nutritional supplementation, intensive follow-up, and a bolstered regimen (Podewils et al., 2011). These recommendations, which also have importance for the design of treatment programmes, need to be prospectively validated

Also covered in chapter three is a section on transmission dynamics. The KwaZulu-Natal strain showed different pathogenic properties due to its high capacity to cause reinfection, and clonal expansion through transmission, compared with the findings in the Western Cape, where we know that resistance was mostly acquired (81%) (Andrews et al., 2007){Ioerger`, 2009 `#1514}. The 19% primary transmission we have reported on is of growing concern, and the scenario of infectious patients awaiting sputum results, or a bed in the XDR-TB facility or alternatively when the patient is sent back to the community once treatment has failed is a reality. Palliative care facilities are urgently needed for end stage patients. Good

administrative measures for a rapid turnaround of sputum results and earlier treatment initiation need to be in place. The high incidence of M/XDR-TB in HCW's as described by us {O'Donnell, 2010 #1596} highlights that administrative measures, in particular the importance of appropriate infection control, need to be rolled out , especially for HCW's who are immune-compromised.

In chapter four we address the low conversion rates (19%) which were not, however, affected by HIV status, possibly because the HIV-infected patients were dying before diagnosis, or treatment initiation. Of the patients who converted, 85% had done so by month nine, with only 6% (2/33) converting thereafter. This has implications for TB programmes in defining XDR-TB treatment failure. The average time from sputum acquisition to treatment start was 75 days (see chapter 3) which also contributes to the low conversion rates, and once again highlights the need for roll out of rapid diagnostics. The low conversion rates also underscore the necessity and urgency of developing new drugs for treatment of XDR-TB. Patients, whom reconvert back to positive or never convert in the first place, thus defined as a treatment failure, are discharged back into the community where transmission of XDR-TB continues. The poor conversion rates also underscore the need to urgently develop and prospectively validate new diagnostic tests, treatment and prevention interventions for XDR-TB. Other preventative measures are described in the last paragraph. The next step then was for us to look at our mortality findings.

Looking at the available data for XDR tuberculosis from Africa we saw that this disease was almost exclusively associated with HIV infection, and that prognosis in

patients with both diseases was poor with a high 30-day mortality rate {Ghandi, 2006 #177;Gandhi, 2009 #90}. By contrast, we have shown in chapter 5, that substantial proportions (53%) of patients in our study were HIV-negative. Survival in patients with HIV infection in our study cohort, from a wider South African setting, is substantially longer (50% died at 12 months *vs.* 83–100% then those seen in KwaZulu-Natal {Ghandi, 2006 #1386;Gandhi, 2009 #90}. Although not small, this proportion is substantially better (Gandhi et al., 2009, Gandhi et al., 2010). The reasons are unclear, as elucidated in chapter 5, but might include patients presenting at different stages of their illness, the degree of immune-suppression, different drug-susceptibility profiles of isolates, lack of availability of capreomycin before 2006, nutritional status, and local differences in strain virulence. XDR tuberculosis in South Africa is therefore not predominantly associated with HIV infection or more than 80% mortality within a few weeks as perceived from studies done in KwaZulu-Natal in 2006 and 2009 {Ghandi, 2006 #177;Gandhi, 2009 #90}. Although the numbers of deaths in our study were high, we noted no difference in treatment outcomes (mortality and culture-conversion status) when comparing patients who were HIV-positive with those who were HIV-negative. This finding is important for the design of policy guidelines and programmes, and de-stigmatises XDR-TB in HIV-infected persons.

These poor outcomes draw attention to the need to urgently develop and prospectively validate new interventions for XDR tuberculosis (Rook and Hernandez-Pando, 2007).

Independent of HIV status, possible reasons for the poorer survival in our cohort of patients as cited in chapters 4 (conversion) and 5 (mortality), include the long duration of drug-resistant and drug-susceptible tuberculosis before initiation of treatment for XDR tuberculosis; delays in initiation of such treatment; malnutrition; co-exposures such as smoking, alcohol, and illicit drug use; different *M. tuberculosis* strains (their pathogenic characteristics and resistance patterns); lack of treatment with moxifloxacin and low weight (<50kg). These poor outcomes underscore the need to urgently develop and prospectively validate new diagnostic, treatment and prevention interventions for XDR-TB.

The outcome data as discussed in chapters 4 and 5, (conversion, and mortality) and the high proportion of acquired resistance to second-line drugs in this study and nationally as addressed in chapter 3 {Mlambo, 2008 #91;Pillay, 2007 #106}, suggest that the national tuberculosis control programmes needs to aggressively ensure treatment adherence with effective regimens to restrict the spread of resistance. This prevention of drug resistance can be achieved by programme strengthening to prevent system failures, {Padayatchi, 2008 #1595;Loveday, 2008 #1506}, integration of treatment for drug-resistant tuberculosis and HIV/AIDS, (Gandhi et al., 2009), intensive counselling and follow-up, and surgery where it is indicated and feasible.

We also show, in Chapter 5 that highly active antiretroviral therapy, despite its overlapping toxicity and adverse drug effects with anti-tuberculosis drugs, and the high pill burden, substantially improves survival in patients with concomitant HIV/AIDS and XDR tuberculosis, and was generally well tolerated. Our data suggest

that highly active antiretroviral therapy should be used at an early stage, regardless of CD4 count, in patients with HIV infection and XDR tuberculosis. These findings are important for advocacy purposes because they support the notion that treatment for XDR tuberculosis in patients with HIV infection is not without hope, and thus counteracts the stigmatisation of this group of patients.

Another very important finding also discussed in chapter 5 is that treatment with moxifloxacin was an independent predictor of survival in ofloxacin-resistant patients with XDR tuberculosis. Jacobson et al. in their meta analysis also found that moxifloxacin was a predictor of survival (Jacobson, 2010). Evidence suggests that the incomplete cross-resistance within the quinolone class can be explained by differential drug-specific binding to DNA gyrase {Kam, 2006 #1512}. Unlike the cohort in Mitnick and colleagues' study, (Mitnick et al., 2008) with 72% (34/47) patients ever treated with moxifloxacin, our programme does not provide this drug nationally. Prospective clinical and in-vitro studies are urgently needed to investigate how best to use newer generation fluoroquinolones for treatment of XDR-TB. Meanwhile, we recommend that, in the absence of specific results of drug-susceptibility testing, moxifloxacin should be used in treatment regimens for XDR-TB unless contraindicated. This is an extremely important finding because there are no new licensed drug options for XDR-TB in high-burden settings.

Our data, as discussed in chapter 6, shows a high incidence (60%) of adverse events associated with drugs used for the treatment of XDR-TB and this was unaffected by HIV status. ADR's impact negatively in conversion (discussed in chapter 4), but not

mortality (chapter 5). In 40% of ADR's treatment was modified and in 20% of cases the drug was discontinued, interrupting therapy, sometimes with fatal consequences. The most serious adverse effects were caused by capreomycin-associated renal dysfunction, which can occur at any time during the course of treatment; thus monitoring of capreomycin-based regimens, even in resource-poor settings is mandatory. Capreomycin was the most common cause of drug withdrawal in 44% of patients, and was responsible for all deaths attributed to a severe ADR. ADR's need to be vigilantly monitored, and major global funders need to input sufficient resources so the epidemic doesn't progress.

Limitations (as set out in chapters 3-6) of our findings are due to the retrospective study design. We have tried to reduce bias by excluding patients with incomplete treatment and microbiological data, by excluding individuals who had culture converted before initiation of treatment for XDR-TB, or whose sputum status was unknown at treatment initiation, by crosschecking patients against clinical and laboratory databases, and by using methods to ensure data integrity. The use of different data collectors at the various sites could have added further bias. Furthermore, the patients who died are included in the study but were excluded from the specific analysis of treatment-related outcomes as they did not receive therapy. HIV-infected and uninfected patients may have died due to failure to treat (before they sought diagnosis, or diagnosis was late or inappropriate, or due to the natural history of their disease, incorrect treatment etc).

Conclusion

In summary, while survival is better than previously reported, prognosis is still poor. HIV-infected patients treated with HAART had a lower mortality and moxifloxacin was an independent predictor of survival. Prior culture-proven MDR-TB and increasing number of drugs used in a regimen were predictors of survival and culture-conversion. The significant mortality rates reported brings to light the question of variations in *M.tuberculosis* strain virulence and their transmission patterns. Diagnosis occurs more than two months after presentation, and treatment initiation some one to two months thereafter. Adverse side-effects of TB drugs used in the management of XDR-TB patients are high, often severe and can lead to disability and even death. High XDR-TB rates among health care workers are a growing concern. These data call for: (i) The design, development and prospective validation of policy guidelines and programmes especially in the following areas: (ii) management policies, (iii) tools to intensify case finding, (iv) strategies to improve patient access to treatment, (v) new treatment and prevention interventions (vi) intensive counselling and follow-up, training and advocacy for HCW's, (vii) appropriate infection control measures in health care facilities and (viii) the exploration and roll out of community based, nurse initiated treatment models. (ix) Improvement in the rollout of rapid diagnostic tests as well as diagnostic reporting systems and rapid testing facilities, (x) development of alternative methods that could be useful for detection of sputum-smear-negative tuberculosis, (xi) validation and implementation of newly developed tests such as the Hain SL, and GeneXpert tests. (xii) Patients with a weight (<50kg) should be given nutritional supplementation, intensive follow-up, and a bolstered regimen. (xiii) Development

of new licensed drug options for XDR-TB, (xiv) in the absence of specific results of drug-susceptibility testing moxifloxacin should be used in treatment regimens for XDR-TB, (xv) and monitoring of capreomycin-based regimens. (xvi) Surgery where it is indicated and feasible. (xvii) Major global funders need to provide sufficient funding for resources to ensure that the XDR-TB epidemic is halted at all costs.

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