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Molecular epidemiology, susceptibility profiles, outcomes and transmission dynamics in patients with extensively drug-resistant tuberculosis (XDR-TB) in two provinces of South Africa

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ABSTRACT

Background: Recent gains in TB control in South Africa are being reversed by drug-resistant tuberculosis (MDR-TB and XDR-TB), which has a high mortality, is a threat to health care workers, and is prohibitively costly to treat. MDR-TB has been supplanted by XDR-TB, resistance beyond XDR-TB, and programmatically incurable TB. Short-term treatment-related outcomes of XDR-TB patients are known to be poor. However, there are no prospective data to inform long-term treatment-related outcomes, design of effective XDR-TB treatment regimens, and public health interventions required to interrupt transmission. In particular, the utility of certain costly drugs, e.g. capreomycin, for the treatment of XDR-TB remain unclear. There are also few data about how these characteristics differ in HIV-infected persons. Finally, little is known about the experiences of patients living with XDR-TB. This thesis aims to provide best practice evidence to promote drug-resistant TB control in high burden TB and HIV syndemic countries.

Methods: We prospectively followed two cohorts of adult South African XDR-TB patients who received hospital and community treatment, which included a capreomycin and PAS-based regimen: (i) cohort A (n=107) from 3 provinces were diagnosed between August 2002 and February 2008 (retrospectively identified) and then prospectively followed up till August 2012; (ii) cohort B (n=273) from 2 provinces were prospectively identified between October 2008 and October 2012 and followed up till October 2014. Strain typing and drug susceptibility testing were performed and treatment-related outcomes were determined. In-depth interviews were conducted with therapeutically destitute patients from cohort B (n=12) and were home-discharged from hospital back to the community.

Results: Both cohorts were young (median age of 33 years), predominantly of mixed ancestry (median varying from 50% to 56%), and a slight preponderance of HIV uninfected persons [56% to 59%]. Treatment-related 60 month outcomes of cohort A were poor (11% cured, 10% failed treatment, and overall mortality was 73%). Independent predictors of net culture conversion were absence of a MDR-TB history [HR 10.21 (95% CI: 2.64-39.38), p= 0.001] and use of clofazamine [HR 0.14 (95% CI: 0.034-0.59) p=0.007], while independent predictors of survival

were net culture conversion [HR 0.14 (95% CI: 0.06-0.34), p=<0.001), treatment with clofazamine [HR 0.38 (95% CI: 0.16-0.87), p=0.021], and use of antiretroviral therapy in HIV-infected patients [HR 0.13 (95% CI: 0.03-0.50), p=0.003]. In cohort A median survival from time of discharge in 19/45 (42%) community-based treatment failures was 19.84 months (IQR 4.16-26.04), and 6/17 (35%) of these patients were smear positive at discharge. Clustering, including transmission within families containing a community-based relative who failed XDR-TB treatment, of cases was identified. The poor outcomes outlined above were confirmed in cohort B (15.8% cured; 40.3% failed treatment, defaulted, or relapsed; all-cause mortality was 68%). With respect to HIV co-infection in cohort A survival was worse only in those with CD4 <200 cells/ml. By contract in cohort B survival in HIV-infected patients was worse than in uninfected patients without a discernible impact of CD4 count. There was also a higher proportion of clinically defined primary XDR-TB in HIV-infected persons compared to uninfected patients from cohort B (p=0.02). To further interrogate the cause of poor outcomes we evaluated the utility of routinely administered empiric capreomycin in patients from cohort A and B (n=178). A high proportion of isolates [154/178 (87%)] contained a *rrs* A1401G capreomycin resistance-conferring mutation. Survival or sputum culture conversion was similar in resistant patients, whether they received capreomyicn or not, suggesting no therapeutic benefit of this drug. Therapeutically destitute patients expressed feelings of mistrust in health care, futility of treatment regimens, a need for subsistence, and a purpose in life as themes related to their treatment experience. Those who interrupted treatment cited the 'never-ending drug regimen' and negative experiences with health care as factors contributing to non-adherence. Lack of knowledge regarding TB disease and transmission, and lack of concern about transmission were evident.

Conclusion: Treatment-related outcomes in XDR-TB were poor, irrespective of HIV status. This is in part due to the unavailability of effective drugs. Given its lack of benefit resources spent on expensive drugs like capreomycin could be better used elsewhere. Therapeutically destitute community-based drug-resistant TB patients remain a major public health problem. They are of a high TB transmission risk given their smear status, longevity, and molecular clustering. Containment strategies such as community stay and palliative care facilities are urgently

needed. Furthermore, these patients have specific psycho-social and economic needs which should be taken into account when planning intervention and management programmes. Whilst prevention of drug-resistant TB is critical, the widespread roll out of new drugs and rapid diagnostic tests are urgently needed to deal with the current epidemic.

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Elize Pietersen, October 2016

ABBREVIATIONS

AFB	Acid-fast bacilli
AIDS	Acquired Immunodeficiency Syndrome
AE	Adverse Event
ARV	Anti-retro viral
ART	Anti-retro viral therapy
BCH	Brooklyn Chest Hospital
BDQ	Bedaquiline
CAS	Central-Asian family
CD4	Cluster of differentiation 4
EAI	East-African-Indian
EC	Eastern Cape Province
DoH	Department of Health, South Africa
DR-TB	Drug-resistant tuberculosis
DST	Drug Susceptibility Testing
FU-RC	Prospective follow-up of Retrospective Cohort
HIV	Human Immunodeficiency Virus
HPCA	Hospice Palliative Care Association of South Africa
INH	Isonicotinic acid hydrazide / Isoniazid
KZN	KwaZulu-Natal
LAM	Latino-American-Mediterranean
LIIU	Lung Infection and Immunity Unit
LMIC	Low middle income country
LPA	Line probe assay
MDG	Millennium Development Goals
MDR	Multi drug-resistant
MIC	Minimum Inhibitory Concentration
M.tb	*Mycobacterium tuberculosis*
NC	Northern Cape Province

NHLS	National Health Laboratory Service
PAS	*para*-aminosalicylic acid
PC	Prospective Cohort
PLWH	People Living With HIV
Pre XDR-TB	Pre extensive drug-resistant tuberculosis
PZA	Pyrazinamide
RC	Retrospective cohort
SA	South Africa
TB	Tuberculosis
USA	United States of America
UNAIDS	United Nations Programme on HIV/AIDS
VL	Viral load
WC	Western Cape Province
WHO	World Health Organisation
WPCA	Worldwide Palliative Care Alliance
XDR-TB	Extensively drug-resistant tuberculosis

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CHAPTER 1: INTRODUCTION

Context and rationale of the study

The 2006 outbreak of extensively drug-resistant TB (XDR-TB) in the Tugela Ferry, KwaZulu-Natal (KZN) [1] necessitated South African TB programme leaders, and scientific communities globally, to urgently confront the public health threat of XDR-TB. As a result, a need emerged to provide evidence for best practice healthcare delivery, including prevention of transmission of this deadly disease, in South Africa (SA) and globally. The subsequent emergence of programmatically incurable XDR-TB, and resistance beyond XDR-TB, [2] compounded this need given that drug-resistant tuberculosis (DR-TB) is responsible for ~25% of TB-related mortality globally [3] and is prohibitively expensive to treat [4, 5].

In 2014 an estimated 9.7% of ~500000 multi-drug resistant TB (MDR-TB) patients from 105 countries were diagnosed with XDR-TB [3] and a considerable burden of TB disease is confined to high-burden countries with ~60% of the DR-TB disease burden [6]. This is especially relevant in low-middle income and high-burden countries like SA where, despite the decrease in TB incidence, there has been a steady increase in the burden of DR-TB [3]. In the province of KwaZulu-Natal (KZN) for instance, the incidence of XDR-TB during the period 2010 to 2012 was 3.5/100 000 (776 XDR-TB cases) of the population, a 13% increase in incidence since 2007 [7].

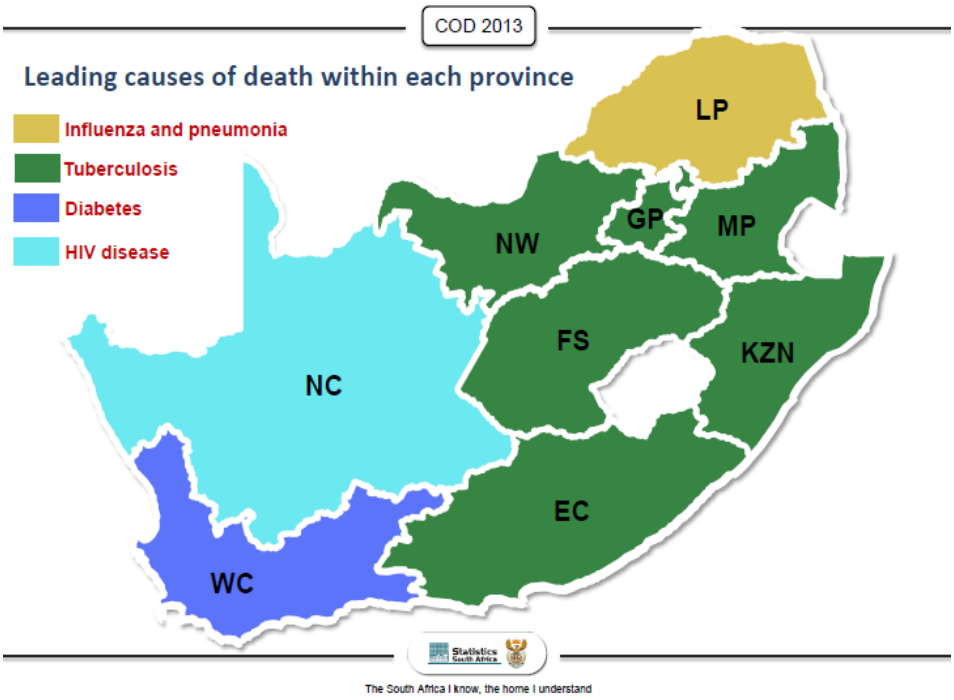
When the WHO declared TB a public health emergency in 1993 ~13 million people globally were living with human immunodeficiency virus (HIV) [8]. In 2014 12% (1.2 of 9.6 million) of

people who developed TB globally were HIV-infected and 74% of these cases were from the African region [3]. Furthermore, SA remains a high burden HIV-endemic country [3] with ~11.2% HIV-infected persons [9] with ~7.4% and ~5% residing in the Western Cape Province (WC) and Northern Cape Province (NC) respectively [10].

TB, including DR-TB, was the leading cause of death in six of the nine provinces in SA in 2014. The association between diabetes mellitus and TB is well established [11] and although, in 2014, diabetes mellitus was the leading cause of death in the WC, HIV was ranked 2nd and TB 4th while HIV and TB, ranked 1st and 2nd respectively, were the leading causes of death in the NC in 2014 (Figure 1A, B) [12].

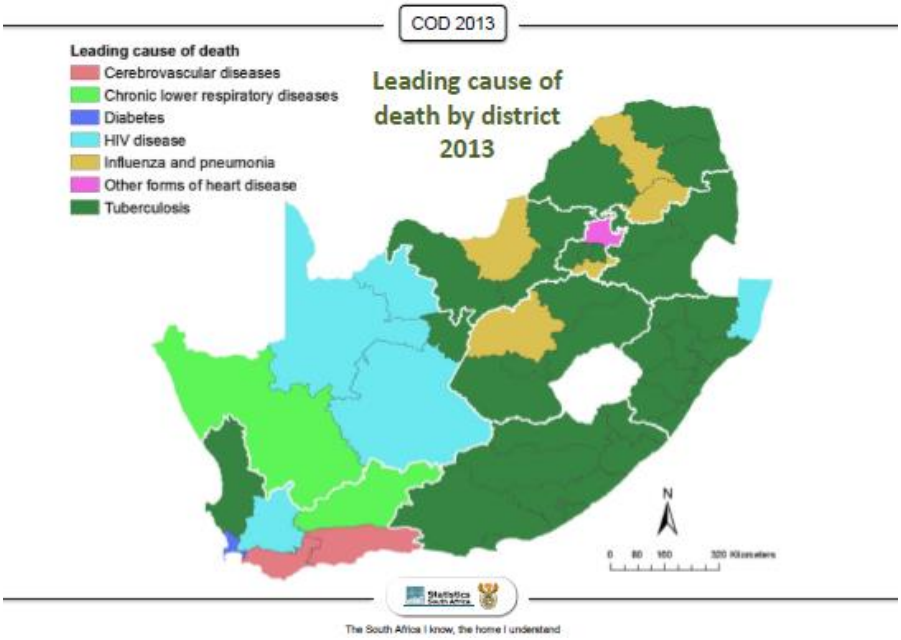
Figure 1: Leading causes of death in South Africa (SA) as per (A) provinces in SA and (B) per district in each province [13]

(A)



WC=Western Cape; NC=Northern Cape; EC=Eastern Cape; FS=Free State; KZN=KwaZulu-Natal; NW=North West; GP=Gauteng Province; MP=Mpumalanga; LP=Limpopo

(B)



In the WC and NC XDR-TB patients, in whom treatment failed, are discharged back to their families and the community. Incurable community-based XDR-TB patients are at high risk of transmitting XDR-TB, which potentially weakens efforts to eradicate DR-TB in a high burden country like SA. However, long-term treatment-related outcomes of these patients have not been studied adequately and transmission rates from this group are unknown. Furthermore published primary and acquired DR-TB transmission rates are limited [14].

There are also no prospective data that inform long-term treatment-related outcomes, design of effective XDR-TB regimens, and public health interventions required to prevent DR-TB transmission, or to inform policy guidelines and intervention strategies. There are similarly limited data regarding interactions, and subsequent treatment-related outcomes of XDR-TB

patients co-infected with HIV. Furthermore, the experiences of patients living with incurable XDR-TB are sparse. In this research project I aimed to investigate treatment-related outcomes, molecular epidemiology, susceptibility profiles, transmission dynamics and social perspectives of XDR-TB patients from the WC and NC.

Research questions

The key research questions addressed in this thesis are directed at providing evidence related to best practice healthcare required to promote drug-resistant TB control in SA, a high burden TB and HIV syndemic country. These questions include:

1. What are the clinical and demographic profiles, and outcomes of XDR-TB patients in the Western Cape and Northern Cape provinces of South Africa?
2. What are the associations between the clinical and demographic profile, sputum culture conversion, treatment-related outcomes (cure, treatment completion, death, default, treatment failure and relapse), molecular epidemiology, and transmission proxies of XDR-TB?
3. Are favourable (cure, treatment completed) and unfavourable (death, treatment failure, interrupted treatment) treatment-related outcomes associated with a particular XDR-TB regimen and/or adverse events?
4. What are the outcomes in XDR-TB treatment failures?
5. What factors are associated with community-based management of XDR-TB patients whose treatment failed them?

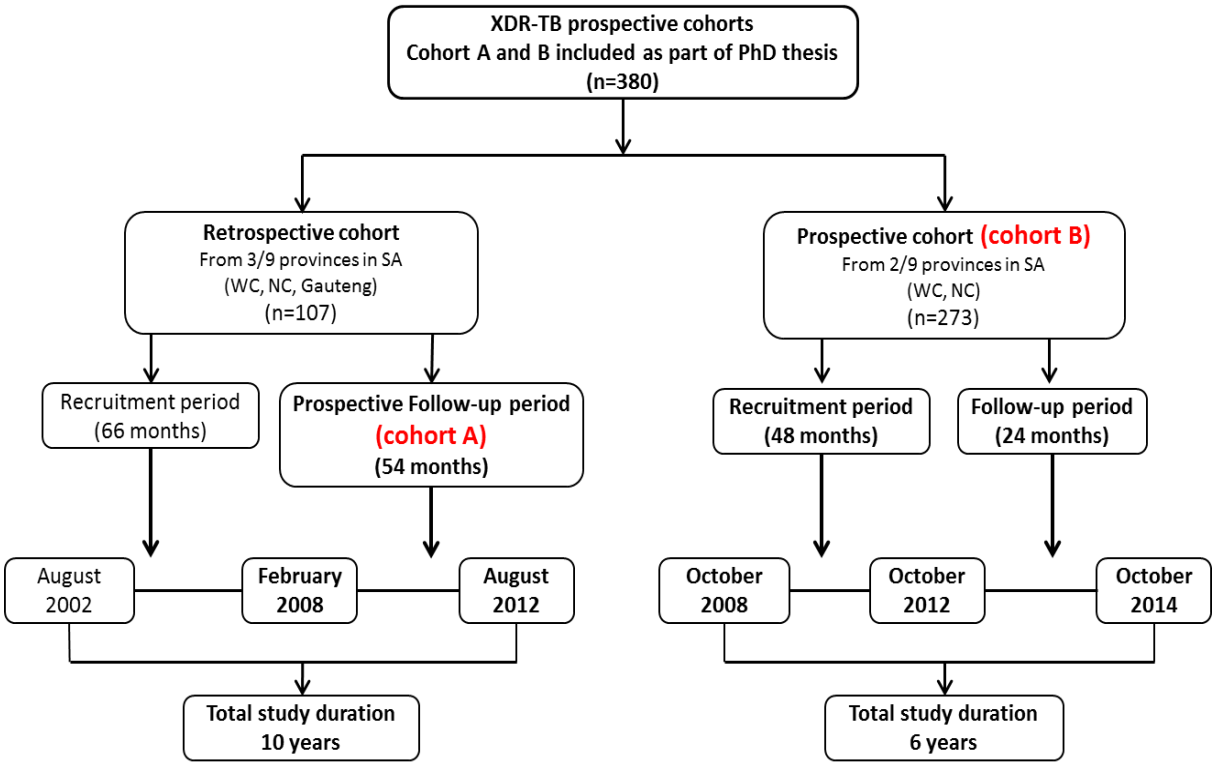
Overall project description and chronology of studies included in this thesis

The Lung Infection and Immunity Unit (LIIU) at the University of Cape Town (UCT), under the leadership of Professor Keertan Dheda, conducted a retrospective cohort study of XDR-TB patients, diagnosed between August 2002 and February 2008, from four provinces (WC, NC, Gauteng, Eastern Cape). Early treatment-related outcomes of these XDR-TB patients were reported in 2010 [15]. Starting in 2008 the LIIU prospectively recruited a separate study cohort of adult DR-TB patients diagnosed from October 2008. These patients were admitted to Brooklyn Chest Hospital (BCH) in Cape Town, WC and Harry Surti Hospital (HSH) [previously Gordonia Hospital] in Upington, NC. At 31 July 2016 a total of 1312 DR-TB patients have been recruited and included 529/1312 XDR-TB patients (other recruited subsets are not outlined in this thesis).

These two study cohorts were included in my thesis. Cohort A included XDR-TB patients diagnosed between August 2002 and February 2008 and were followed-up from March 2008 to August 2012 to determine end-of-treatment as well as long-term treatment-related outcomes. Cohort B was a subset of the prospective cohort and included 273/529 XDR-TB patients, diagnosed between October 2008 and October 2012. These patients were followed-up until October 2014 (Figure 2: cohort A+ B). [UCT ethics approval: REC REF 038/2008]. Patients included in the cohorts were admitted to BCH and HSH with a DR-TB bed capacity of ~120 and ~35 beds respectively. BCH and HSH deliver DR-TB healthcare in urban (BCH) and rural (HSH) settings and serve a South African population of ~6.2 million (11.3%) in the WC and ~1.9 million

(2.2%) in the NC [9]. In the WC, XDR-TB treatment, during the intensive phase, is hospital-based whereas in the NC treatment is either hospital or community-based.

Figure 2: Study plan: recruitment and prospective follow-up of XDR-TB patients included in the retrospective (cohort A) and prospective (cohort B) cohorts as part of this thesis



The coherence of my PhD thesis is underpinned by a unified common theme of exploring the burden of XDR-TB and XDR-TB HIV co-infection, treatment-related outcomes, molecular epidemiology, and transmission dynamics. The work completed during my PhD presents an evolution of XDR-TB, keeping track with a growing body of knowledge related to changes in management of XDR-TB, including new drug discoveries, and the molecular epidemiology of *Mycobacterium tuberculosis.*

I (Gerbrecht Elizabeth Pietersen) was the first author on all 3 of the manuscripts included as part of my PhD thesis and was the lead investigator in all of the studies reported (Table 1). I was co-involved in data collection, analysis and writing of manuscripts presented in the Appendices. Finally, all the work reported in this thesis has been undertaken with the guidance and the supervision of Professor Keertan Dheda.

Table 1: Summary of research questions and key conclusions related to manuscripts included in as part of my thesis

Research question	**Aim**	**Cohort**	**Key conclusions**	**Chapters and manuscripts**
FIRST AUTHOR MANUSCRIPTS				
Treatment outcomes of XDR-TB (Research questions 1+2+4)	Long-term outcomes of XDR-TB	Cohort A	Long-term outcomes in XDR-TB, irrespective of HIV status, are poor. XDR-TB patients, discharged into the community, whose treatment failed, are likely to transmit TB disease to the wider community. Policy makers should implement appropriate intervention to minimise TB transmission.	**Chapter 3:** Manuscript 1 First author paper [*Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study* **Pietersen**, Dheda et al Lancet 2014]
Management of XDR-TB (Research question 3)	Capreomycin treatment of XDR-TB: outcomes and resistance	Cohorts A+B	The frequency of capreomycin conferring mutations in XDR-TB is extremely high. XDR-TB patients with capreomycin resistance seem not to benefit from capreomycin usage. Second-line drug susceptibility testing in the design of a treatment regimen is essential.	**Chapter 4:** Manuscript 2 First author paper [*High frequency of resistance, lack of clinical benefit and poor outcomes in capreomycin treated South African patients with extensively drug-resistant tuberculosis* **Pietersen**, Dheda et al PlosOne 2015]
Profile of incurable XDR-TB (Research question 5)	Community-based incurable XDR-TB	Cohort B (Purposive sampling: in-depth interviews)	Current models of care are not adequately meeting the needs of uncured XDR-TB patients and relatives. Need exists for community-based palliative care, vocational facilities fostering economic opportunities, home-based infection control and improved psychological support for patients and family.	**Chapter 6:** Manuscript 3 *Joint first author paper [*Lifestyle, attitudes and needs of uncured XDR-TB patients living in the communities of South Africa: a qualitative study* Senthilingam*, **Pietersen*,** Dheda et al TMIH 2015]

CO-AUTHORED MANUSCRIPTS				
Management of XDR-TB (Research question 3)	Adverse events (AEs) in patients with XDR-TB	Cohorts A+B	Drug-associated AEs occur commonly during XDR-TB treatment. AEs are often severe, caused treatment interruption and negatively impact culture conversion.	**Chapter 4:** Appendix 2 <u>Co-authored paper</u> *Drug-associated adverse events and their relationship with outcomes in patients receiving treatment for extensively drug-resistant tuberculosis in South Africa* [Shean, Streicher, Dheda, **Pietersen** et al PlosOne 2013]
	Cost of XDR-TB management	Cohort A	DR-TB forms a small proportion of the total burden of TB cases yet consumes a disproportionate and substantial amount of the annual South African TB budget. Rational resource allocation and selection of management strategies for DR-TB in high burden settings are required.	**Chapter 4** Appendix 3 <u>Co-author paper</u> *What is the cost of diagnosis and management of drug resistant tuberculosis in South Africa?* [Pooran, Dheda, **Pietersen** et al PlosOne 2013]

Cohort A: Prospective follow-up of retrospective cohort (XDR-TB diagnosed August 2002 to February 2008). Follow-up between March 2008 and August 2012
Cohort B: Prospective cohort (XDR-TB diagnosed October 2008-October 2012). Follow-up between November 2012 and October 2014.

Outline of thesis

In **Chapter 2** I provide context and a literature review regarding the global and local burden of XDR-TB and XDR-TB HIV co-infection. The emergence of drug-resistance, factors associated with favourable and unfavourable treatment-related outcomes, molecular epidemiology and transmission dynamics are reviewed.

In **Chapter 3** I present data regarding long-term follow-up of a cohort of XDR-TB patients diagnosed between August 2002 and February 2008, with prospective follow-up to August 2012 (Figure 1: cohort A). There is a paucity of clinical evidence regarding the long-term effectiveness of XDR-TB treatment. I highlight long-term (10 year) treatment-related outcomes [**Manuscript 1: Pietersen et al Lancet 2014**] and outline molecular epidemiology and clustering related to XDR-TB. I present data highlighting XDR-TB patients with failed treatment and discharged from the hospital back into the community.

In **Chapter 4** I discuss the management of XDR-TB with specific reference to an effective XDR-TB treatment regimen, adverse events associated XDR-TB treatment, resistance to anti-TB drugs included in the XDR-TB regimen, and the cost of DR-TB treatment. The therapeutic value of capreomycin, the backbone of an XDR-TB regimen in South Africa till recently, is uncertain and the frequency of capreomycin resistance in XDR-TB patients is not well established. Capreomycin is a toxic drug and known to cause renal impairment which could be life threatening. I thus investigated [**Manuscript 2: Pietersen et al PlosOne 2015** (cohorts A+B)] the frequency of resistance, clinical benefits, and outcomes related to capreomycin treatment.

In **Chapter 5** I present the demographic and clinical profile of XDR-TB (cohort B) with specific reference to the role of HIV and ART. I explore associations between treatment-related outcomes, molecular epidemiology and transmission of XDR-TB HIV co-infection with specific reference to the community. I explore the association between previous DR-TB treatment, duration of XDR-TB treatment, and favourable versus unfavourable outcomes in XDR-TB HIV-infected and uninfected patients.

XDR-TB mortality rates are high and the majority of patients succumb while hospitalised. However, patients with failed XDR-TB treatment are no longer hospitalised indefinitely. Policy guidelines were revised and patients with failed XDR-TB treatment are now discharged from hospital without any treatment. In **Chapter 6** I investigate [**Manuscript 3: Senthilingam, Pietersen** (joint first authors) **et al TMIH 2015** (cohort B)] the needs, wants and experiences expressed by uncured community-based XDR-TB patients and their family members.

In **Chapter 7** I summarise findings across all studies included in my thesis and discuss overall conclusions. The implications of my research in terms of XDR-TB management, and TB control in general, are discussed and priorities for future research are identified.

CHAPTER 2: BACKGROUND

2.1 HISTORICAL PERSPECTIVE ON TUBERCULOSIS AND GLOBAL BURDEN OF DISEASE

Tuberculosis (TB), an ancient out-of-Africa [16, 17] mainly airborne treatable communicable infectious disease, [3, 6] re-emerged as a public health concern when the WHO declared TB a global emergency in 1993, the same year ~13 million people were living with HIV globally [8]. TB, mostly affecting the lungs (pulmonary TB), is caused by an intracellular pathogen *Mycobacterium tuberculosis,* (*M.tb*).

Hundred and thirty one years after the Nobel Prize winner, Robert Koch, discovered the tubercle bacillus *M.tb*, in 1882, as the etiological cause of TB the WHO, in 2014, estimated the global TB incidence at 9.6 million cases, an incidence that included 12% HIV-infected individuals [3]. Although the African region had a mere 28% of the global TB incidence in 2014, the population burden of TB disease in this region was double, an average TB incidence of 281/100000 per population, compared to the global average TB incidence of 133/100000 per population [3]. Annual treatment success rates of 86%, in newly diagnosed DS-TB patients, have been maintained since 2005 [3] and TB-related mortality reduced with 47% since 1990, mostly as a result of the initiation of the MDGs in 2000 [3]. Yet, an estimated 1.5 million TB-related deaths still continued in 2014 [3]. Furthermore, despite a 32% decrease in HIV/TB associated mortality since 2004 TB, together with HIV, was ranked as the leading causes of death [3].

The role of HIV, identified merely ~3 decades ago as the cause of AIDS, in the recurrence of TB is conclusive and not only led to a regeneration of interest to foster standard principles of TB

and HIV control [18-21] but also to a sevenfold increase in the incidence of TB on the African continent [19, 22]. HIV and TB, two independent infectious diseases, create a deadly synergy fuelling mortality, incidence and prevalence of either disease [23, 24].

In 2014 the majority (93%) of TB patients globally were tested for HIV. Most of those tested 61% (179756 cases), were HIV-infected and ~75%, compared to ~50% globally, and were from the African region [3]. SA had 33% of the global HIV burden, [25] the largest collective of PLWH globally [26].

Anti-TB drug development and subsequent drug-resistance

Sixty years after the discovery of *M.tb* the first anti-TB drug, streptomycin, was developed (1943) and the first randomised control trial, including streptomycin, was conducted in 1948 [27]. A pipeline of anti-TB drug development, summarised in Table 1, followed (1948–1963) [28-30] and until recently the WHO recommended most of these drugs for an XDR-TB regimen.

Drug-resistance, however, followed shortly after treatment initiation of each anti-TB drug discovered [28, 31]. Surveys to determine anti-TB drug-resistance in the United Kingdom (1955-1956) showed resistance to streptomycin (2.5%), *para*-aminosalicylic acid (PAS) (2.6%) and isoniazid (1.3%) [29]. In the United States of America (USA) drug-resistance to isoniazid increased during a 3 year period from 6.3% (1961–1964) to 9.7% (1965–1969) [28]. Modern-day understanding of MICs, genotypic drug-resistance and cross resistance between anti-TB drugs are summarised in Table 1.

Single drug TB treatment changed to combination anti-TB drug therapy and ultimately short-course TB chemotherapy of 6–8 months was initiated [28, 29]. The success of short-course combination anti-TB drug therapy led to the belief that TB was a disease of the past and the 1978 Alma-Ata declaration of 'health for all' by the year 2000 mirrored optimism for the eradication of TB among TB specialists. Funding for TB control in the USA was moved (1972) from federal sources to public health block grants and states in the USA had no specific TB control requirements to meet [31]. An unprecedented re-emergence of TB in the USA surfaced in 1985 [32].

Table 1: Summary of anti-TB drugs used for treatment of *M.tb,* MIC cut-off points (ranges as per phenotypic methods applied in this thesis), genes and targeted mutation* conferring resistance and cross resistance between anti-TB drugs. [** Genotypic testing at the National Health Laboratory Service (NHLS)] Table 1 references: [28, 29, 33-43]

Drugs used in the treatment of TB	**Year discovery**	**Drug group** (applicable during study period)	**MIC cut-off** (Range) (µg/mL)	**Genes conferring drug resistance** Genotypic DST* at NHLS**	**Cross resistance between drug**	**Programmatic phenotypic DST at NHLS**	**Phenotypic DST** (applied in thesis)
Dapsone	1937 (for leprosy)	5	64	-	None	No	BACTEC 960
Streptomycin	1943	1	2 (0.25-32)	*rrs*, rpsL, gidB*	Amikacin, Capreomycin, Kanamycin	Yes (previously)	Sensititre
PAS	1948	4	2	*thyA*	None	No	Sensititre
Thiacetazone	1948	5	-	-	Ethionomide, Isonaizid	-	-
Isoniazid**	1951	1	0,2 (0.03–4)	*inhA promotor* katG**	Ethionomide (if inhA mut present)	Yes	Sensititre
Cycloserine	1952	4	25 (2–256)	*cycA*	None	No	Sensititre
PZA	1952	1	100	*pncA**	None	No	BACTEC 960
Clofazamine	1954	5	1	*Rv0678, ndh, Rv1979c, pepQ*	Bedaquiline	No	BACTEC 960
Ethionomide	1956	4	5 (0.3-40)	*ethA, inhA**	None	Yes (previously)	Sensititre
Amikacin	1957	2	5 (0.12–16)	*rrs*, eis*	Capreomycin, Kanamycin	Yes	Sensititre
Rifampicin	1957	1	1 (0.12–16)	*rpoB*, rpoC, rpoA, rpoD*	None	Yes	Sensititre
Kanamycin	1957	2	5 (0.6–40)	*rrs**	Amikacin, Capreomycin	Yes	Sensititre
Capreomycin	1960	2	2.5	*rrs*, tlyA*	Kanamycin, Amikacin	No (on request)	BACTEC 960
Ethambutol**	1962	2	5 (0.5–32)	*embB, inhA**	None	Yes (previously)	Sensititre
Ofloxacin	1963	3	2 (0.25–32)	*gyrA*, gyrB**	Between all FQs	Yes	Sensititre
Rifabutin	1975	1	0,5 (0.12–16)	*rpoB**	Rifampicin	No	Sensititre
Clarithromycin	1980s	5	8	*Erm37 (ermMT), whiB7 (whmC)*	None	No	BACTEC 960
Linezolid	1990s	5	1	*rplC, rplD, rplW, rrl*	None	No	BACTEC 960
Moxifloxacin	1996	3	2	*gyrA*, gyrB**	Between all FQs	Yes	Sensititre
Bedaquiline (TMC207, R207910)	2012	5	0.5	*Rv0678 atpE*	Clofazamine	No	-
Delamanid (OPC-67683)	2014	5	0.006- 0.012	*Rv3547, FGD, FbiA, FbiB, FbiC*	None	No	-

2.2 ACQUISITION AND TRANSMISSION OF DRUG-RESISTANT TUBERCULOSIS

Reports of rifampicin-resistant *M.tb* isolates from TB patients who received monotherapy emerged ~1960s in Europe and ~1975 in the USA [31]. Furthermore, outbreaks of multi-drug resistant TB (MDR-TB), defined as drug-resistance to at least isoniazid and rifampicin, occurred in various states in the USA (1985–1992) coinciding with the resurgence of TB and the HIV epidemic [31]. Acquired drug-resistance TB, defined as a *M.tb* isolate developing drug-resistant mutations, is thought to result from ineffective TB treatment, including monotherapy, inappropriate combination of anti-TB drugs, poor quality drugs and drug mal-absorption or non-adherence. Primary drug-resistance TB may occur via direct inhalation of bacteria from a TB patient and as the product of poor TB control, given a delay in drug susceptibility testing (DST) and/or TB treatment initiation and undiagnosed TB [19, 44-46]. Recent spread of XDR-TB, irrespective of primary drug-resistance, can be inferred from the presence of strain clusters (genotypic identical) and DR-TB in previously untreated patients. Non-identical strains (distinct genotypes) would point to possible endogenous reactivation of XDR-TB or newly acquired drug-resistance in XDR-TB patients who were previously treated with anti-TB drugs and consequently developed resistance during treatment [47, 48].

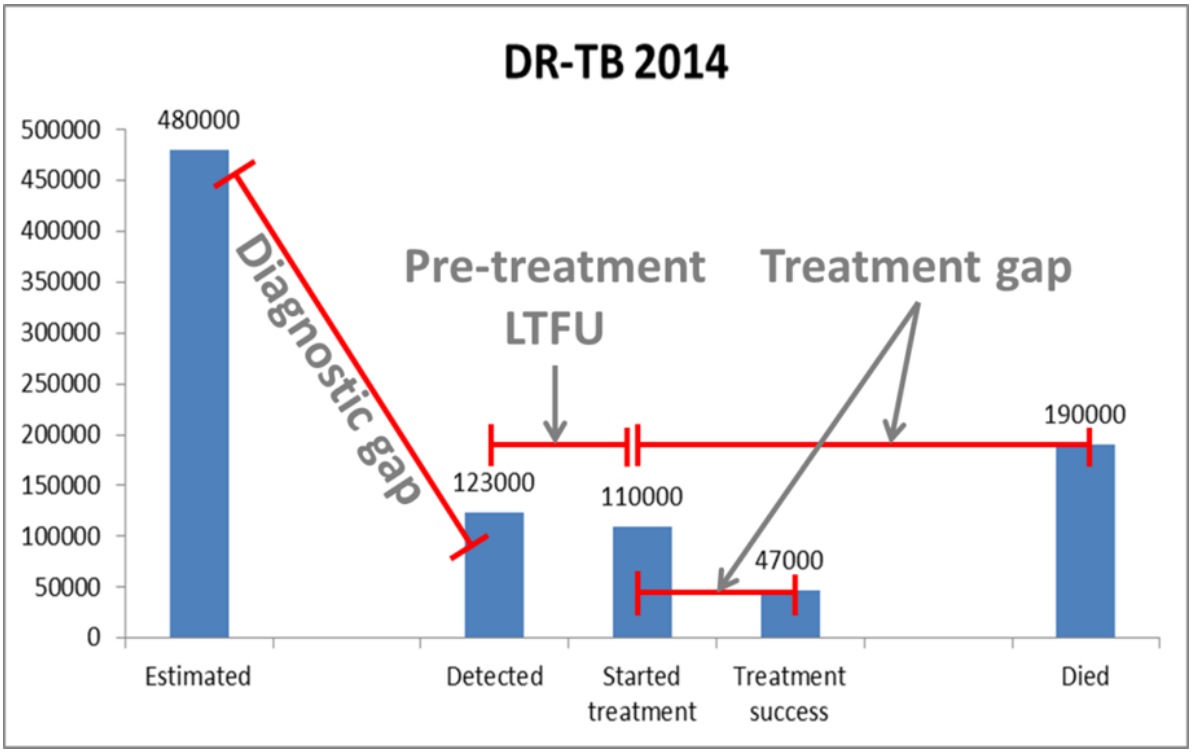
Acquired and primary drug-resistant transmissions are both important indicators of an effective national TB programme. The continued emergence of XDR-TB is, in most instances, the result of acquired drug-resistance while MDR-TB predominantly develops as a result of primary drug-resistance [44, 49-51]. Transmission of drug-resistant strains in the community escalates XDR-TB disease, and clustering of genotypes is presumed to suggest TB transmission while non-

clustered genotypes are thought of as acquisition or reactivation of previous infections [36, 47, 48, 52].

2.3 BURDEN AND EPIDEMIOLOGY OF DRUG-RESISTANT TUBERCULOSIS

The estimated global incidence of MDR-TB fails as a true reflection of the burden of diseases given that a mere 41% (123000 cases) were detected and reported in 2014. However, treatment initiation, during the same period, increased with 14% [3]. The global burden of DR-TB could be attributed to disparities regarding two crucial components of programmatic TB control, namely diagnostic uptake and treatment success. These gaps in the cascade of programmatic TB care, as diagrammatically illustrated in Figure 1, need to be addressed to reach the sustainable development goals (SDG) set for TB by 2035 [3].

Figure 1: Diagrammatic illustration of gaps in the cascade of programmatic DR-TB care as highlighted in the WHO global report [3]



Resistance beyond isoniazid and rifampicin (MDR-TB) developed and the term extensively drug resistant TB (XDR-TB) was first used in 2006, 124 years after the discovery of *M.tb* and a mere 13 years since TB became a global epidemic [53]. XDR-TB is defined as resistance to at least isoniazid and rifampicin in addition to resistance to any fluoroquinolone and at least one of three injectable second-line anti-TB drugs (amikacin, kanamycin and capreomycin) [54]. The development of resistance beyond isoniazid and rifampicin was from the outset considered a reflection on the poor quality and effectiveness of the implementation of programmatic TB control [53-55].

A considerable burden of TB disease remains in high-burden countries where ~60% of the DR-TB disease burden is gathered [6]. In 2014 the global incidence of DR-TB was estimated at 3.3% (95% CI 2.2–4.4%) of new, and 20% (95% CI 14–27%) of previously treated DS-TB cases subsequently diagnosed with MDR-TB, an insignificant change from previous years [3].

Furthermore, the high proportion (9.7%) of MDR-TB patients subsequently diagnosed with XDR-TB is critical [3]. Since resistance beyond MDR-TB emerged studies suggested that XDR-TB is predominantly driven by transmission of MDR-TB strains [44, 56]. This is especially relevant in low-middle income, high-burden countries like SA where, despite the decrease in TB incidence, there has been a steady increase in the burden of DR-TB [3]. For instance the incidence of XDR-TB in KwaZulu-Natal, South Africa, increased by 13% from 2007 to 2012 [7].

The continued increase of DR-TB in high-burden countries together with resistance beyond XDR-TB is a concern [2]. Fundamentally the threat of the burden of XDR-TB is different from that of MDR-TB especially given the sombre global XDR-TB mortality and treatment failure rates [57] of 30% and 19% respectively, [3] and the dismal proportion, 26%, XDR-TB patients who are cured following a 20-24 month XDR-TB treatment program [3].

2.4 MOLECULAR EPIDEMIOLOGY OF DRUG-RESISTANT TUBERCULOSIS

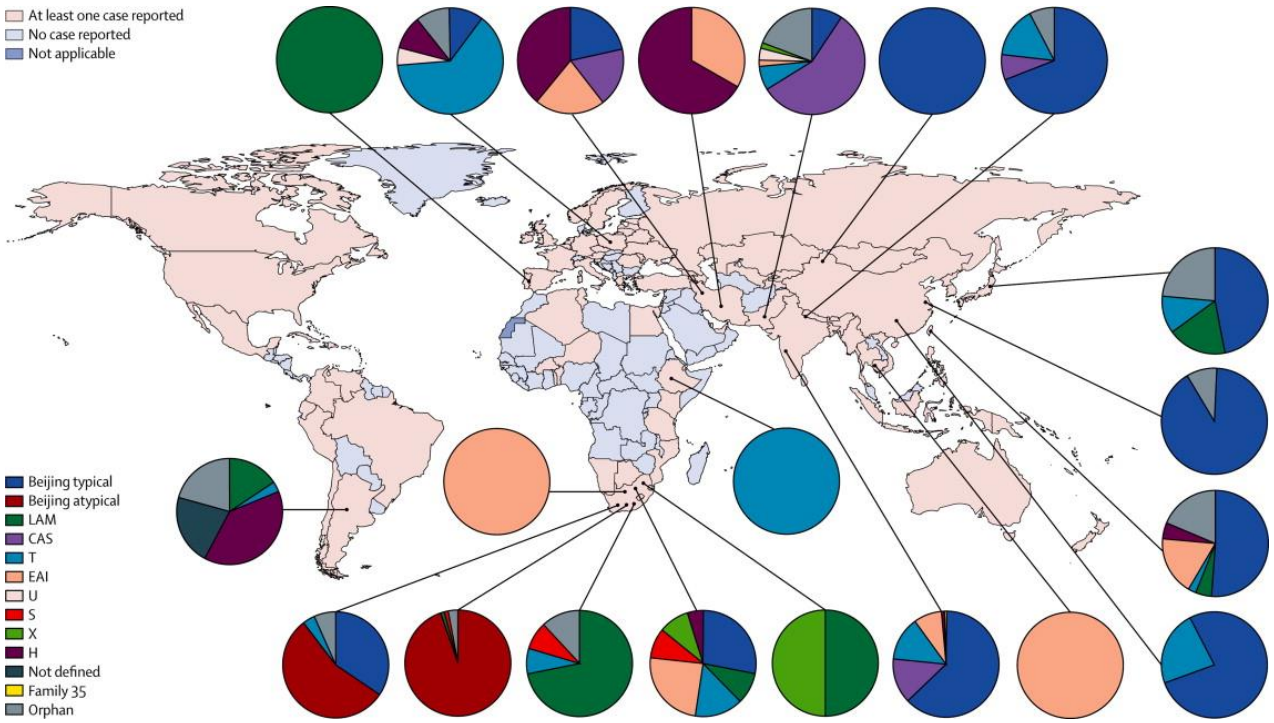
The *M.tb* Beijing genotype, which is strongly associated with drug-resistance globally [50] was first described in 1995 [58]. Mechanisms for a possible association between a Beijing genotype and higher drug-resistance are unclear. Assumptions are that Beijing genotype strains have higher mutation rates or that the nature of the cell-wall structure of the Beijing strain lead to suboptimum intracellular anti-TB drug concentrations which leads to acquired drug-resistance [59].

The Beijing genotype represents ~13% of strains globally and, compared to other genotype families, is genetically highly conserved. Furthermore it showed the largest increase the past 180 years [17, 59]. There are 7 lineages in the Beijing genotype family, from sub lineage 1, thought of as atypical (‘ancestral’) to sub lineage 7 considered as typical (‘modern’), each cluster with specific spoligotypes, distributed globally [42, 60, 61].

The co-evolution of host and bacterium genotypes is well described [16, 17, 42]. Geographical variations, as illustrated in Figure 2, in the population structure of *M.tb* strains may be the result of environmental factors, migration of human populations or even differences in TB control as certain lineages have a preferential spread in particular populations [59, 60, 62].

Figure 2: Distribution of XDR-TB genotypes for the following countries: South Africa, Ethiopia, Argentina, Portugal, Poland, Iran, Pakistan, India, Nepal, Cambodia, China, Taiwan, and Japan. The Beijing strains shown for South Africa distinguish between typical and atypical phenotypes to illustrate regional differences in the population structure of XDR-TB.

LAM=Latino-American-Mediterranean family. CAS=Central-Asian family. T=T family. EAI=East-African-Indian family. U=U family. S=S family. X=X family. H=Haarlem family. [47]



2.5 TOOLS FOR DIAGNOSIS AND MONITORING OF DRUG-RESISTANT TUBERCULOSIS TREATMENT

Smear microscopy

Sputum smear microscopy, assessment of acid-fast bacilli (AFB) (~10 000 TB CFU/ml of sputum to be positive) of *M.tb* is a low cost front-line diagnostic test analysing bacterial load predominantly regarding DS-TB. Furthermore, smear microscopy is a widely used marker for assessing clinical outcomes and infectiousness [63-65]. Data suggest that a minority of TB

patients are highly infectious and responsible for the spread of TB disease while lack of smear conversion is mostly followed by DR-TB [65]. HIV-infected persons predominantly have smear negative microscopy results [66, 67].

Drug susceptibility testing

Phenotypic DST

Phenotypic drug susceptibility testing (DST), recommended by the WHO as the reference diagnostic method for detecting XDR, [68] involves culturing of *M.tb* in the presence of an anti-TB drug to detect growth (drug-resistance) or inhibition (drug susceptibility) of the drug tested [33]. However, phenotypic DST, by virtue of the natural growth rate of *M.tb,* is a slow process (6–8 weeks to obtain results) delaying rapid diagnosis and effective XDR-TB treatment initiation [35, 69]. Furthermore, uncertainty about conventional minimum inhibitory drug concentration breakpoints limits the use of phenotypic DST to establish accurate susceptibility of second-line drugs [70, 71]. Phenotypic DST tests laboratory methods (Sensititre MYCOTB plate and MGIT960 BACTEC), used to analyse isolates during the study period of the thesis, followed manufacturer guidelines. Reference TB laboratories in SA offered phenotypic DST for isoniazid, rifampicin, amikacin, kanamycin, ethambutol and ofloxacin and targeted DNA sequencing investigating mutations related to isoniazid (*katG*) and ethambutol (*inhA*) during the study period of the thesis.

GENOTYPIC METHODS

Genotypic testing

Genotypic DST relies on molecular-based tests to detect genetic determinants of resistance. The WHO endorsed, in 2010, a cartridge-based, fully automated, rapid point of care molecular diagnostic test, XpertMTB/RIF, as the preferred test to detect MDR-TB cases and SA introduced XpertMTB/RIF point of care testing ~2011. The XpertMTB/RIF assay uses real-time PCR to identify *M.tb* complex and rifampicin associated mutations in 2 hours [33]. In addition to replacing microscopy as the routinely used diagnostic test, XpertMTB/RIF point of care testing equally led to an increase in TB testing and case detection [7, 72].

The WHO furthermore approved the MTBDR*sl* line probe assay (LPA), a genotypic laboratory based rapid turn-around-time test. The MTBDR*sl* determines resistance to fluoroquinolones (*gyrA* mutation) and second-line injectable anti-TB drugs (*rrs* mutation) [73]. Same-day rapid diagnosis of XDR-TB is thus available through combined use of XpertMTB/RIF and LPA tests.

However, compared to the scope of phenotypic DST targeted DNA sequencing, although offering rapid test results, does not uncover the full range of mutations conferring drug resistance, summarised in Table 1 (p 20), essential for the management of XDR-TB [68]. Reporting targeted DNA sequencing results, like offering MIC ranges using phenotypic DST, has the value of offering a clinician genotypic results that would allow for optimising an effective XDR-TB treatment regimen taking low or high level resistance into account [33]. For instance, the current practice of NHLS in the Western Cape in reporting *kat*G and *inh*A mutations allow

clinicians to optimise an XDR-TB regimen by including high-dose isoniazid categorised as a group 5 drugs during the study period, to the XDR-TB regimen [33].

XDR-TB regimens thus could be optimised based on extensive DST results [35]. The existing range of anti-TB drugs susceptibility testing available at reference laboratories, especially in LMIC, limits optimising a regimen for XDR-TB cases in which a WHO approved programmatic XDR-TB regimen failed. Furthermore, dismal treatment-related outcome in XDR-TB patients [15, 74] is evidence for the need for the further development, and implementation, of rapid diagnostic DST. Yet, although rapid point of care molecular TB diagnostic tests is currently the norm in most countries culture based testing of sputum remains the WHO reference standard for bacteriological confirmation of DR-TB [3, 33]. However, sputum culture testing prolongs diagnostic delays which consequently amplify potential transmission of DR-TB [34, 35].

2.6 MANAGEMENT OF DRUG-RESISTANT TUBERCULOSIS

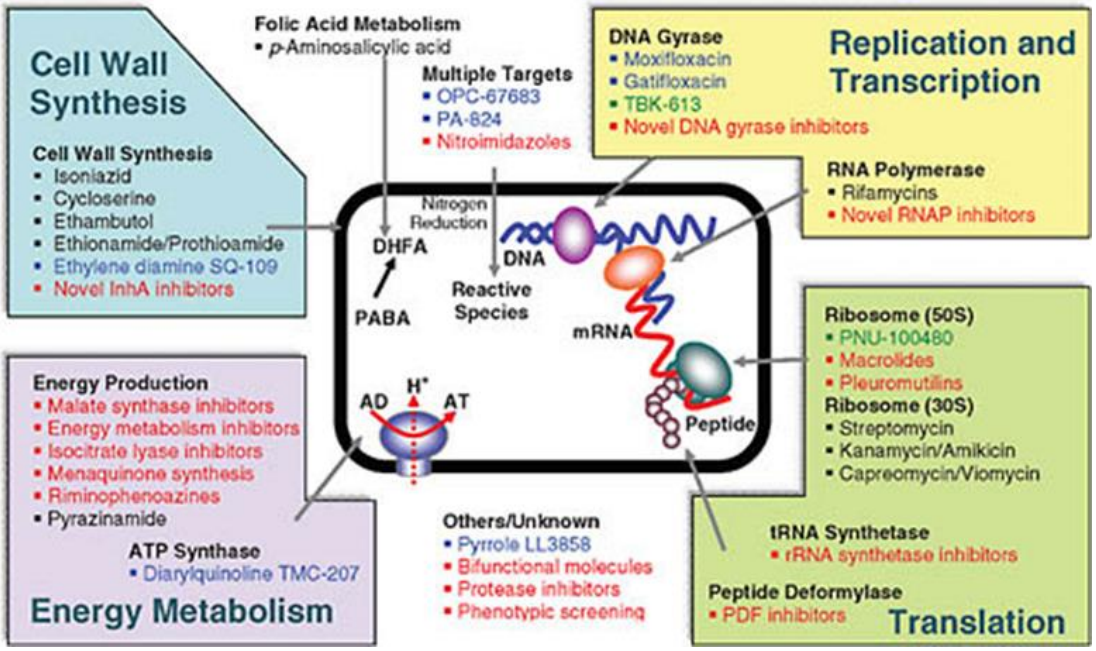
Limited data exists to inform best clinical practice in terms of XDR-TB management and clinical management of MDR-TB do not necessarily apply equally to management of XDR-TB [33]. The step-wise approach applicable to the constructing of a standardised MDR-TB regimen [33] is less clear when constructing an XDR-TB regimen. While best practice would be to construct an individualised XDR-TB regimen, taking a variety of patient-related factors and DST results into account a ‘standardised’ XDR-TB regimen could be prescribed even in cases with no conclusive DST results [33].

Various additional drug-related and patient-related considerations need to be taken into account when constructing an individualised optimum XDR-TB regimen. Factors pertaining to efficacy of drugs, drug susceptibility profile, previous anti-TB treatment, genotypic level of drug-resistance, cross resistance between drugs, drug-drug interactions, contra-indications between anti-TB and ARV drugs, extend of lung disease, HIV status and HIV disease progression. Furthermore, socio-economic factors like predictive patient behaviour in terms of adherence to a regimen, social support available to the patient and distances from health care facilities should also be taken into consideration when managing an XDR-TB patient [33].

Extensively drug-resistant tuberculosis treatment

The gold standard not to treat a TB patient with a single anti-TB drug, and never to add a single drug to a failing regimen, still prevails [28, 29, 36]. The advantage of combination anti-TB drug treatment is based on the premise that different anti-TB drugs have unrelated drug-resistant genetic loci and the probability of advancement to drug-resistance would thus be negligible [37]. A variety of anti-TB drugs used in combined therapy occupies a broad physicochemical space thus populating distinct chemical areas (Figure 3).

Figure 3: Schematic representation of anti-TB drug targets [38]
[http://www.nap.edu/read/12570/chapter/9#83 last accessed 20.3.16]



During the study period, XDR-TB patients encountered exposure to anti-TB drugs with ambiguous bactericidal and sterilising capacities, a limited inventory of anti-TB drug options and a collection of drugs that were restricted in those who received previous TB treatment. Furthermore, second-line anti-TB drugs, available ~60 years, [Table 1 (p 20) and 2 below] are fundamentally inadequate in an XDR-TB regimen in terms of efficacy, toxicities and cost [4, 37, 38]. Besides, the ability of drug-resistant *M.tb* strains to remain in the host for prolonged periods culminate in an exceptionally long DR-TB treatment duration [38] while *M.tb* survival mechanism(s) result in the development of resistance to drugs included in the XDR-TB regimen [37].

Table 2: WHO recommended anti-TB drugs mostly prescribed in XDR-TB regimens (2008-2012) in the Western Cape and Northern Cape and available in the respective provinces during the study period

Reference used regarding information in Table 2: [33, 75, 76]

Drug groups + Drug classes	Drugs	Chemical description	Efficacy	Adverse event associated with drug
Group 1 **First-line oral drugs**	PZA	Nicotinamide derivative	Bactericidal	Hyperuricemia, arthralgia, hepatotoxicity, rash, photosensitivity, gastrointestinal distress
	Ethambutol	Ethylene diimino di-1-butanol	Bacteriostatic	Neuritis
	Isoniazid	Nicotinic acid hydrazide	Bactericidal	Hepatitis, peripheral neuropathy, hypersensitivity reactions, optic neuritis, arthralgia, CNS changes, drug induced lupus, diarrhoea
Group 2 **Second-line injectable drug** (during intensive phases)	Capreomycin	Cyclic polypeptide	Bactericidal	Nephrotoxicity, ototoxic, electrolyte abnormalities (hypokalaemia, hypocalcaemia, hypomagnesaemia)
Group 3 **Second-line oral drugs**	Moxifloxacin (4^{th} generation FQ, used from ~2010)	8-Methoxy-fluoroquinolone	Bactericidal	Nausea, headache, dizziness
	Olfloxacin (1^{st} generation FQ, used up to ~2010)	Fluoroquinolone	Bacteriostatic	Hyper- or hypoglycaemia, QTc prolongation, insomnia, drowsiness, headache, confusion, tremors, seizure (occasionally), photo toxicity, diarrhoea
	Levofloxacin (3^{rd} generation FQ, used selectively from ~2012)			
Group 4 **Second-line oral drugs**	Terizidone / Cycloserine	Serine derivative	Bacteriostatic	CNS toxicity: seizure, depression, psychosis, suicidal ideation. Skin problems including Stevens-Johnson syndrome
	para-amino salicylic acid (PAS)	*para*-amino salicylic acid	Bacteriostatic	Gastrointestinal distress, reversible hypothyroidism
	Ethionomide	Isonicotinic acid derivative	Bacteriostatic	Gastrointestinal problems and anorexia. Endocrine: gynaecomastia, hair loss, acne, impotence, menstrual irregularity. Reversible hypothyroidism. Neurotoxic
Group 5 **Third-line oral drugs**	Amoxycillin-clavulanate	β-Lactam with β-lactamase inhibitor	Bactericidal	Diarrhoea, nausea + vomiting, abdominal discomfort
	Clarithromycin	Macrolide	Bactericidal / bacteriostatic	Diarrhoea, nausea, abnormal taste, dyspepsia, abdominal pain/discomfort, headache
	Clofazamine	Iminophenazine derivative	Bacteriostatic	Discoloration of skin, conjunctiva, cornea, body fluids. Gastrointestinal intolerance, photosensitivity, skin reactions
	Dapsone	diamino-diphenyl-sulfone	Bacteriostatic	Nausea, vomiting, loss of appetite, dizziness, blurred vision, tinnitus, headache, insomnia, or increased sensitivity of the skin to sunlight.
	Amoxil	β-Lactam aminopenicillin	Bactericidal	Skin rash, itching or hives, swelling of the face, lips, or tongue. Breathing problems. Redness, blistering, peeling

				or loosening of the skin, including inside the mouth.
	Linezold	Oxazolidinone derivative	Bactericidal	Myelosuppression, diarrhoea, nausea, optic neuropathy, peripheral neuropathy, lactic acidosis
	Bedaquiline	Diaryquionline	Bactericidal	Diarrhoea, nausea + vomiting, abdominal discomfort, QT prolongation, hyperuricemia, phospholipidosis
	High dose Isonaizid	Nicotinic acid hydrazide	Bactericidal	Hepatitis, peripheral neuropathy, hypersensitivity reactions, optic neuritis, arthralgia, CNS changes including psychosis, drug induced lupus, diarrhoea
	Thiacetazone	Thiosemicarbazone derivative	Bacteriostatic	No longer recommend for use in TB treatment

Although most TB patients, in the absence of a concomitant disease, absorb anti-TB drugs well, [77] an XDR-TB regimen ideally would include a shorten treatment duration, minimal drug-drug interactions, dosing frequency allowing for optimal bio-availability, combination drugs that will reduce pill burden and low cost drugs [37, 78]. Previous WHO XDR-TB treatment recommendations included drugs from all drug groups (Table 2) despite recognising that group 4 and 5 drugs were less effective [33]. The therapeutic benefit of capreomycin, presumed as the backbone second-line injectable in an effective XDR-TB regimen, was however questionable. XDR-TB treatment outcomes remained dismal despite a capreomycin-based XDR-TB regimen [15, 79] and the likelihood of totally drug resistant became an attractive option [2, 34, 53, 80, 81]. Furthermore, an understanding of XDR-TB as a disease of heterogeneous *M.tb* populations, each possibly requiring specific chemotherapeutic management, highlighted a lack of understanding of XDR-TB management of subpopulations of *M.tb* with available anti-TB drugs [78].

Treatment success

The WHO's target of treatment success in DR-TB is ≥75%. In 2014 the global MDR-TB treatment success was 50% and a mere 43/127 countries reached the WHO target of >75% DR-TB

treatment success [3]. Although more XDR-TB patients globally received XDR-TB treatment in 2014, compared to previous years, treatment success in XDR-TB patients was only 26% globally, mirroring the continued low treatment success of previous years.

Adverse events

Adverse events (AE), defined as any unfavourable or unintended sign, symptom or disease associated with, yet not related to, the use medical treatment are graded 1-5 based on common terminology criteria for AE (CTCAE) [82]. Grade 1-2 denotes mild AEs, grade 3 severe, and grades 4-5 life threatening and death related AEs. AEs commonly associated with anti-TB drugs are summarised in Table 2 (p 29+30) [76].

No distinct AE can be attributed to a particular anti-TB or ARV drug when used concurrently. Overlapping AEs, and toxicity profiles, are however increasing and remains a concern in terms of the safe use of either drug [30, 78] despite evidence that drug toxicities might have limited effect in TB HIV treatment [83]. TB drug-induced liver injury, especially in HIV-infected patients, is the most common AE requiring TB treatment interruption [76, 84] while nephrotoxicity, a life threatening AE during treatment with capreomycin, is of concern [85]. Furthermore, the question remains whether an XDR-TB regimen is more toxic in patients living with HIV [6] especially given the extent of probable adverse events accompanying both XDR-TB and ART regimens [85]. Most studies regrettably only report observational data regarding AEs experienced by DR-TB patients [15, 85-87].

Treatment adherence

Adherence, generally measured as the extent to which a patient keeps to a prescribed treatment dose and frequency, is an important requirement, especially in a ~24 month XDR-TB treatment programme, to reach treatment success and ultimately for TB control as optimal treatment adherence reduce drug-resistance and TB transmission [15, 43, 68, 88-92]. Furthermore, poor treatment adherence, even during hospitalisation, remains a prominent aspect in the pathogenesis of TB [47]. However, conversely, drug-resistance could develop despite proven adherence to treatment [93].

Newer generation and repurposed drug-resistant tuberculosis treatment

Bedaquiline (BDQ) the first anti-TB drug approved the past 18 years [94] became available, in 2013, in ~43 countries as MDR-TB treatment, and in SA for compassionate use in XDR-TB [3, 78]. BDQ, a novel diarylquinoline [75] inhibits mycobacterial F1F0 proton denosine triphosphate (ATP) synthase which leads to ATP depletion [43, 94] and is highly active against MDR-TB strains [43]. Previous WHO DR-TB treatment guidelines were subsequently updated [95] to accommodate newer anti-TB treatment combinations and efficacy and safety of a shorter DR-TB regimen. In Table 3 I contrast the WHO drug groups and classes recommended for use in an XDR-TB regimen during the period of this thesis and the 2016 updated WHO treatment guidelines.

Table 3: Summary of WHO approved drug groups and classes applicable during study period compared to 2016 updated WHO guidelines of drug groups [95]

WHO recommended drug groups and classes during study period	**Anti-TB drugs available during study period**	**WHO updated 2016 recommended drug groups**
Group 1 *(first-line oral drugs)*	PZA	**Group D1**
	Ethambutol	**Group D1**
	Isonaizid	*Not included in 2016 update*
Group 2 (second-line injectable drug)	Capreomycin	**Group B**
Group 3 (second-line oral drugs)	Moxifloxacin	**Group A**
	Levofloxacin	**Group A**
	Ofloxacin	*Not included in 2016 update*
Group 4 (second-line oral drugs)	Terizidone / cycloserine	**Group C**
	Ethionomide	**Group C**
	para-amino salicylic acid (PAS)	**Group D3**
Group 5 (third-line oral drugs)	Clofazamine	**Group C**
	Linezolid	**Group C**
	High dose Isonaizid	**Group D1**
	Bedaquiline	**Group D2**
	Amoxycillin-clavulanate	**Group D3**
	Thiacetazone	**Group D3**
	Clarithromycin	*Not included in 2016 update*
	Dapsone	
	Amoxil	

While principles applicable to constructing a DR-TB regimen, [96] including new and or repurposed anti-TB drugs, might be seemingly uncomplicated in patients with no previous TB history the challenge remains in constructing a regimen applicable to XDR-TB patients who failed a WHO recommended regimen. Susceptibility to new, and repurposed drugs, in these patients, a substantial number in low-income high-burden countries, [3] cannot be assumed [94, 96].

2.7 BURDEN OF TUBERCULOSIS IN SOUTH AFRICA

TB remains the leading cause of death in SA and maintained first rank, the same ranking as the previous 3 years, despite a decline in TB related deaths from 10.7% (2011) to 8.8% (2013) [97]. Furthermore TB remained the leading cause of HIV-related deaths in SA [18, 97]. In 2012 HIV-related deaths in SA ranked 6th however escalated to 3rd rank in 2013, with the total number of HIV-related deaths reaching 5.1%, as a result of the 21% increase in HIV-related deaths during 2012-2013 [97].

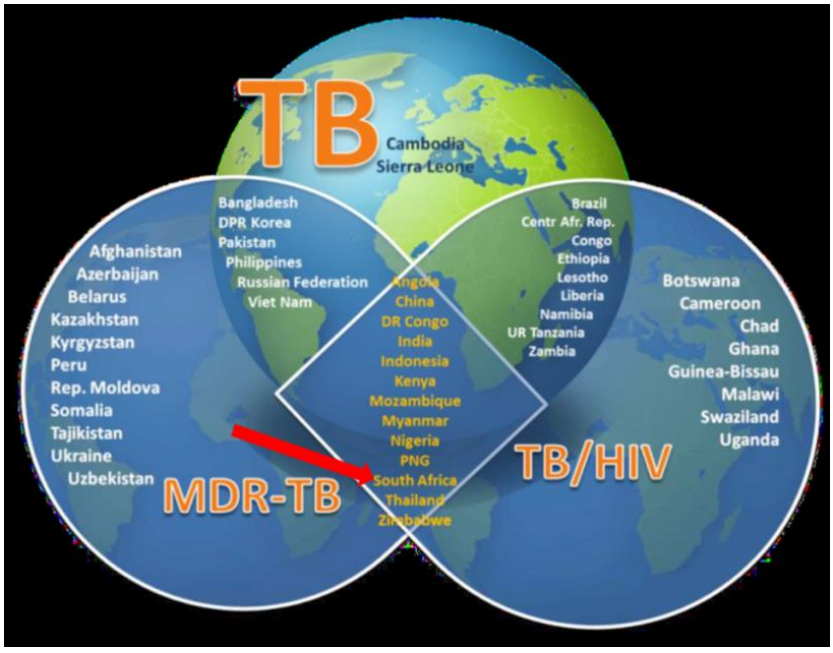
In 2013 ~6.3 million (11.2%) South Africans lived with HIV and 61% of TB patients were HIV-infected [3, 6, 25]. HIV prevalence in SA remains high despite the decline in the incidence, since 2008, of HIV [98]. In 2014 18734 cases had laboratory confirmed DR-TB ranking SA as second in Africa, with the largest burden of TB/HIV disease [3] and a treatment success rate in TB HIV-infected patients of 73%, compared to 88% in HIV-uninfected patients [3]. A person infected with both XDR-TB and HIV is living with the predicament of one body required to control two diseases, a viral infection and a bacterial infection, and health care professionals need to direct treatment and care of these patients from this framework [99].

During 1987-1988, prior to combination anti-TB drug therapy in the Western Cape, 30% (103/343) DS-TB patients were diagnosed with resistance to either isoniazid or rifampicin and 70% (240/343) with MDR-TB [100]. Dismal 5-year treatment-related outcomes, mortality of 49% and 33% cure, were reported for the province and a specialist MDR-TB management clinic was initiated at BCH (~1990) [86, 100]. In the Eastern Cape Province 34.1% (107/314) previously

treated DS-TB patients were diagnosed with MDR-TB during September 2003–May 2004, a substantially higher burden at the time, to the estimated 8% MDR-TB cases in the province [101]. In 7/9 provinces of SA a total of 63% (440/699) patients were diagnosed with MDR-TB between June 2005-December 2006 [49] and in KwaZulu-Natal, during 2008–2010, a total of 1549 adults were diagnosed with MDR-TB and 75% of the patients tested for HIV were HIV-infected [102].

SA was since 2002 known as a high-burden TB, and HIV country [103]. Yet, with the introduction of SDGs, and the End TB Strategy, SA now furthermore is one of thirteen high-burden TB, TB/HIV and MDR-TB countries that will receive considerable targeted global action during the period 2016 to 2035 (Figure 4). End TB Strategy and UNAIDS targets and milestones will to help build and sustain funding and political commitment [103] in these countries with the ultimate aim to meet the newly established SDGs.

Figure 4: End TB Strategy high-burden TB, TB/HIV and MDR-TB target countries [103]



Resistance beyond multi drug-resistant tuberculosis

In 2006 SA experienced an outbreak of XDR-TB in the Tugela Ferry region of KwaZulu-Natal when 24% (53/221) MDR-TB patients were diagnosed with XDR-TB [1]. Mortality in this cohort was 98% (52/53) in a median survival of 16 days (IQR 6-37). All patients tested were HIV-infected and 85% (39/46) isolates had similar genotype strains [1].

XDR-TB was similarly uncovered in the rest of SA. In 1992 the first XDR-TB patient was recorded at BCH, ~7 years after MDR-TB treatment became available in the Western Cape. Furthermore, retrospective diagnosis of 48/2919 MDR-TB cases, predominantly HIV-negative with a median survival of 10.8 months, admitted to BCH during 1990-2002 uncovered a diagnosis of XDR-TB [104]. During June 2005–December 2006 a total of 41/699 patients were diagnosed with XDR-TB in 7/9 provinces in SA [49] while in the Eastern Cape Province, during October 2006–January 2008, 274 patients were diagnosed with XDR-TB.

XDR-TB treatment success in SA declined since 2007 which was, in 2012, a discouraging 20%, compared to 26% globally [3]. Furthermore the WHO estimated XDR-TB mortality in SA at 47% (2014), compared to 30% globally, which is allegedly associated with the high level of HIV-infection [3].

Acquisition and transmission of drug-resistant tuberculosis

How drug-resistance developed, and an understanding of factors facilitating drug-resistance in SA, remain unresolved [43]. One model suggests the evolution of different XDR-TB strains based

on the use of non-standardized drug regimens prior to 2002, thus driving on-going transmission of MDR-TB and pre XDR-TB genotypes [44]. The subsequent implementation of standardised MDR-TB regimens, however, could further have promoted spread of resistant strains, which essentially originated from an era when TB treatment was not controlled in SA, ~1950's, and against which the subsequent newly adopted standardised MDR-TB regimen was not effective. Specific resistant strains, programmatically selected, thus became dominant over time in MDR-TB cases as [44] acquired drug-resistance was proven in XDR-TB patients from Western Cape and KwaZulu-Natal [1, 48, 50].

Molecular epidemiology

The introduction of the Beijing genotype to SA is a recent event, [60] possibly as a result of the sea trade route via Indonesia from east Asia to Europe ~400 years ago [105] and is described as a significant part of the East-Asian lineage [59]. Although not endemic to Africa, ~30% of XDR-TB cases in SA have a Beijing genotype [50, 59]. During 2001-2002 the Beijing genotype was reported in 28% of XDR-TB TB cases and during 2005-2006 in ~34-37% XDR-TB cases countrywide [49, 50, 106]. However, during 2008-2009 a total of 94.3% (181/192) XDR-TB patients in the Eastern Cape Province were reported with atypical Beijing genotype strains contrasting the 24.9% (70/218) rate of Beijing genotype strains in MDR-TB isolates [34].

XDR-TB patients in the Tugela Ferry region predominantly had LAM strains (or called KZN strains at the time) [36, 107]. During 2000-2010 LAM was identified in 72% (18/25) XDR-TB patients from KwaZulu-Natal while during the same period the Beijing genotype was identified in 89.5%

(94/105) XDR-TB patients from the Western Cape and 94.7% (89/94) from the Eastern Cape Province [62]. Atypical Beijing genotypes detected in isolates from the Western Cape were suggestive of *M.tb* lineages from TB patients who migrated from the Eastern Cape to the Western Cape, presumably to pursue TB treatment in the Western Cape [48, 62]. Beijing strains are likewise presumed to be transmitted within urban areas in the Western Cape [48].

Distribution of *M.tb* strains families in the remaining areas of SA are a mix of EAI (East-African-Indian), LAM (Latino-American-Mediterranean family), CAS (Central-Asian family), T family, U family, S family, X family and the Haarlem family [47, 59].

2.8 HOME-DISCHARGED EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS PATIENTS

A diagnosis of XDR-TB, a life-limiting and life-threatening infection, introduces an extended disease and management trajectory that demands comprehensive care, including home-based care, and support to patients and family members. The outbreak of XDR-TB, for many a fatal disease, not only necessitated rapid effective worldwide measures to restrain transmission but also required pragmatic sanctioned patient restriction measures. Furthermore, the demand for community stay facilities and palliative care facilities, especially for therapeutically destitute XDR-TB patients, has been ongoing since XDR-TB became a public health concern [15, 57, 74, 108].

The SA Department of Health (DoH), in compliance with Siracusa principles [109] and grounded in the bill of rights in the Constitution of SA (1996) [110] has a legal responsibility in terms of

health care towards XDR-TB patients, the community, health care providers and TB control in general. In 2010 the SA DoH reported a hospital bed capacity shortage, with respect to DR-TB, of 37% in the Western Cape, 57% in the Northern Cape and 31% in KwaZulu-Natal and decentralised care for XDR-TB patients, irrespective of treatment success or failure, originated [111]. These health care resource constraints have thus directly put the onus on home-discharged XDR-TB patients to co-operate and maintain ethical acceptable TB prevention measures when in contact with the public [112]. An ethical dilemma related to therapeutically destitute, incurable home-discharged XDR-TB patients, residing with their families in the community, as a means to increase bed capacity for newly diagnosed XDR-TB patients, who are probably more likely to achieve XDR-TB treatment success transpired [57].

Uncured community-based XDR-TB patients, both victims of and probably vectors of the disease, [108] likely have a direct influence on the continued burden of disease, which was man-made in the first instance [28, 108]. Furthermore, the DoH provide DR-TB health services from the premise that an XDR-TB patient's freedom should be 'carefully restricted' especially when alternative approaches to prevent the spread of DR-TB are most likely ineffective. Limiting an individual's freedom of choice may therefore, from a health care perspective, be essential to protect all people and entire communities [110].

The patient charter for TB care [113] refers to 'care and dignity' which incorporates palliative care. However, palliative care, in the context of TB, continues to be an emerging concept particularly given the prevailing perception of palliative care as health care reserved for the

dying. A study conducted in 11 public sector hospitals in the Cape Town metropole found that 9.6% (138/1443) of patients, occupying acute-care hospital beds, who required palliative care were diagnosed with HIV and/or TB while >50% of all patients in acute-care hospital beds, who required palliative care, had associated co-morbid diseases like hypertension, diabetes, HIV or TB [114].

Furthermore, the perception of palliative care, and community stay facilities, as care of the dying has not significantly changed since the WHO's declaration on palliative care for DR-TB [115-117]. This declaration urged multi-disciplinary teams to consider palliative care, including end-of-life care, as part of TB health care service delivery and thus to be incorporated in the management of XDR-TB [118]. The Hospice Palliative Care Association (HPCA) of SA, on behalf of the SA national TB programme, delivers community-based and hospice-based palliative care to TB patients and their families [118, 119]. Incurable XDR-TB patients in the Western Cape, however, do not qualify to be admitted to a hospice-based care facilities as appropriate infection control measures are not integral to these facilities. Furthermore, structural changes, to current community stay facilities, and resources required to provide comprehensive health care to XDR-TB patients might not be sufficient to reduce the surge of XDR-TB [108].

The slippery slope, and circular arguments, regarding ethical and human rights, and violations, in terms of hospitalisation, community-based palliative care or hospice-based community stay facilities, palliative care in general, and ultimately XDR-TB control, are manifold and these contentious issues are yet to be resolved [74, 112, 118, 120]. Furthermore, although studies

reported rates of XDR-TB treatment failure little is known about the growing problem of community-based incurable DR-TB likely driving TB transmission [121-124].

CHAPTER 3: LONG-TERM TREATMENT-RELATED OUTCOMES IN PATIENTS WITH EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS AND PROGRAMMATICALLY INCURALBE TUBERCULOSIS

MANUSCRIPT 1: Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a prospective cohort study

Elize Pietersen, Elisa Ignatius, Elizabeth M Streicher, Barbara Mastrapa, Xavier Padanilam, Anil Pooran, Motasim Badri, Maia Lesosky, Paul van Helden, Frederick A Sirgel, Robin Warren, Keertan Dheda [Published: Lancet 2014, 383 (9924), page 1230-1239]

PhD context

Long-term treatment-related outcomes in patients with XDR-TB have been poorly studied. Most studies had, hitherto, only reported short-term-related outcomes [122, 125, 126]. These data are required to inform clinical management and resource allocation. In Chapter 3 I report on an initially retrospective selected cohort which we previously reported on [15] and which we prospectively followed up from 2008 to 2012. The fate of XDR treatment failures has been poorly studied. Whilst a number of studies have reported on the frequency of XDR treatment failures within specific cohorts [122, 127-129] none had reported on what happened to these individuals after outcome designation, whether in hospital or when discharged into the community. It was also unknown for how long treatment failures lived in the community. It was previously assumed that they survived for a short period of time (days to a couple of weeks) but the exact fate of these patients in the community remained unknown. Furthermore, it was unclear whether they had the capacity to transmit disease given the dogma that XDR-TB strains have attenuated virulence. Additionally, there was hardly any data about long-term outcomes in HIV-infected versus uninfected patients.

Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study

Elize Pietersen, Elisa Ignatius*, Elizabeth M Streicher, Barbara Mastrapa, Xavier Padanilam, Anil Pooran, Motasim Badri, Maia Lesosky, Paul van Helden, Frederick A Sirgel, Robin Warren, Keertan Dheda*

Summary

Background Long-term treatment-related outcomes in patients with extensively drug-resistant (XDR) tuberculosis are unknown. We followed up a cohort of patients to address knowledge gaps.

Methods Between March, 2008, and August, 2012, we prospectively followed up a cohort of 107 patients from three provinces in South Africa, who had been diagnosed with XDR tuberculosis between August 2002, and February, 2008. Available isolates from 56 patients were genotyped to establish strain type and used for extended susceptibility testing.

Findings All patients were treated empirically as inpatients with a median of eight drugs (IQR six to ten). 44 patients (41%) had HIV. 36 (64%) of 56 isolates were resistant to at least eight drugs, and resistance to an increasing number of drugs was associated with the Beijing genotype (p=0·01). After 24 months of follow-up, 17 patients (16%) had a favourable outcome (ie, treatment cure or completion), 49 (46%) had died, seven (7%) had defaulted (interruption of treatment for at least 2 consecutive months), and 25 (23%) had failed treatment. At 60 months, 12 patients (11%) had a favourable outcome, 78 (73%) had died, four (4%) had defaulted, and 11 (10%) had failed treatment. 45 patients were discharged from hospital, of whom 26 (58%) had achieved sputum culture conversion and 19 (42%) had failed treatment. Median survival of patients who had failed treatment from time of discharge was 19·84 months (IQR 4·16–26·04). Clustering of cases and transmission within families containing a patient who had failed treatment and been discharged were shown with genotypic methods. Net sputum culture conversion occurred in 22 patients (21%) and median time to net culture conversion was 8·7 months (IQR 5·6–26·4). Independent predictors of probability of net culture conversion were no history of multidrug-resistant tuberculosis (p=0·0007) and use of clofazamine (p=0·0069). Independent overall predictors of survival were net culture conversion (p<0·0001) and treatment with clofazamine (p=0·021). Antiretroviral therapy was also a predictor of survival in patients with HIV (p=0·003).

Interpretation In South Africa, long-term outcomes in patients with XDR tuberculosis are poor, irrespective of HIV status. Because appropriate long-stay or palliative care facilities are scarce, substantial numbers of patients with XDR tuberculosis who have failed treatment and have positive sputum cultures are being discharged from hospital and are likely to transmit disease into the wider community. Testing of new combined regimens is needed urgently and policy makers should implement interventions to minimise disease spread by patients who fail treatment.

Funding European and Developing Countries Clinical Trials Partnership, South African National Research Foundation (SARChI), and the South African Medical Research Council.

Introduction

Tuberculosis remains a major global problem: the estimated number of new cases in 2011 was almost 9 million.[1] Sustained control is undermined by the growing threat of drug-resistant tuberculosis. Of 12 million cases of tuberculosis worldwide in 2010, 650 000 (5·4%) were estimated to be of multidrug-resistant disease.[2] 5–10% of the cases of multidrug-resistant tuberculosis are thought to be extensively drug-resistant (XDR) disease—defined as multidrug-resistant disease with resistance to a fluoroquinolone and either capreomycin, amikacin, or kanamycin.[3]

In South Africa—where the incidence of tuberculosis is 948 per 100 000 individuals per year[1]—surveys indicated that the percentage of tuberculosis cases that were multidrug-resistant disease increased in the country from 3·1% in 2002, to 9·6% in 2008.[4–6] In 2011, more than 8000 culture-confirmed cases of multidrug-resistant tuberculosis were identified, of which about 500 were culture-confirmed XDR disease.[1,7] According to South African guidelines,[8] patients with XDR tuberculosis should be admitted to designated treatment facilities and empirically treated with a para-aminosalicylic acid and capreomycin-based regimen (until 2010, capreomycin resistance profiling was not available in the public sector).

The issue of drug-resistant tuberculosis is important because it predominantly affects economically productive young adults and is associated with a high mortality.[9] Additionally, the high treatment-related costs are unsustainable in the low-income and middle-income countries where it is most prevalent. For example, in 2010, despite drug-resistant tuberculosis being officially responsible for less than 3% of the total case load in South Africa, it consumed almost 45% of the national tuberculosis budget of about US$280 million.[10] Such disproportionate

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and prohibitive costs threaten to destabilise tuberculosis control programmes in South Africa and other countries with similar resource constraints. Cogent intervention strategies and public policies are needed to control XDR tuberculosis, but rational planning of interventions and allocation of public health resources are hampered by the scarcity of long-term outcome data relating to mortality, cure, and treatment failure.

In view of the long treatment duration and scarcity of outcome data, how treatment failure should be defined is also unknown. The present practice, by which patients who do not achieve culture conversion after 12 months are deemed to have failed treatment, is not well supported by robust evidence. Few data are available for the proportion of patients with XDR tuberculosis who fail treatment, their outcomes after treatment failure, and their long-term potential for disease transmission. Therefore, long-term outcomes of XDR tuberculosis and the fate of patients with treatment failure, and how these outcomes differ by HIV status, remain unknown. To address these knowledge gaps, and particularly those from a high burden African setting, we prospectively followed up a cohort of patients, and now report the long-term treatment outcomes.

Methods

Participants

We previously reported the retrospective analysis of short-term outcomes of 114 adults (aged >16 years) with culture-proven XDR tuberculosis diagnosed between August, 2002, and February, 2008,[9] and who were initiated on XDR tuberculosis treatment with an empirical capreomycin and para-aminosalicylic acid-based regimen (other drugs used were at the discretion of the attending physician, outlined in table 1, and guided by susceptibility data where relevant). From the censor date of the previous study (February, 2008),[9] we prospectively followed up 107 of these patients from three provinces in South Africa (three were lost to follow-up and four transferred out [ie,

	Whole cohort (n=107)	Patients with HIV (n=44)	Patients without HIV (n=63)	p value*
Age at diagnosis (years)	33 (27–43)	33 (28–40)	32 (25–46)	0·66
Male sex	58 (54%)	19 (43%)	39 (62%)	0·08
Mixed ancestry	54 (50%)	9 (20%)	45 (71%)	<0·0001
Ever smoker†	48 (53%)	14 (36%)	34 (65%)	0·01
CD4 count (cells per mL)‡	..	365 (157–414)	..	..
Previous diagnosis of culture-confirmed multidrug-resistant tuberculosis	95 (89%)	35 (80%)	60 (95%)	0·03
Number of drugs used	8 (6–10)	8 (7–9)	9 (6–10)	0·51
Weight (kg)§	49 (40–58)	50 (42–60)	48 (40–52)	0·46
Drugs used				
High-dose isoniazid (10 mg/kg)	30 (28%)	16 (36%)	14 (22%)	0·17
Isoniazid	39 (36%)	17 (39%)	22 (35%)	0·85
Pyrazinamide	83 (78%)	33 (75%)	50 (79%)	0·77
Ethambutol	48 (45%)	18 (41%)	30 (48%)	0·62
Ethionamide	68 (64%)	26 (59%)	42 (67%)	0·55
Ofloxacin	39 (36%)	16 (36%)	23 (37%)	1
Ofloxacin and moxifloxacin	8 (7%)	5 (11%)	3 (5%)	0·37
Streptomycin	1 (1%)	0	1 (2%)	..
Amikacin	4 (4%)	2 (5%)	2 (3%)	1
Capreomycin	98 (92%)	40 (91%)	58 (92%)	1
Dapsone	43 (40%)	12 (27%)	31 (49%)	0·04
Amoxicillin plus clavulanic acid	65 (61%)	22 (50%)	43 (68%)	0·09
Para-aminosalicylic acid	96 (90%)	41 (93%)	55 (87%)	0·51
Clofazamine	22 (21%)	14 (32%)	8 (13%)	0·03
Azithromycin	9 (8%)	8 (18%)	1 (2%)	0·01
Rifabutin	1 (1%)	0	1 (2%)	..
Amoxicillin	38 (36%)	10 (23%)	28 (44%)	0·04
Moxifloxacin	16 (15%)	12 (27%)	4 (6%)	0·01
Clarithromycin	80 (75%)	29 (66%)	51 (81%)	0·12
Terizidone (cycloserine derivative)	100 (93%)	43 (98%)	57 (90%)	0·27

Data are median (IQR) or n (%), unless otherwise stated. *Comparison between patients with and without HIV, calculated with Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. †Data available for 91 patients in whole cohort, 39 patients with HIV, and 52 patients without HIV. ‡CD4 counts available at diagnosis for only 21 patients with HIV. §Weight at diagnosis available for only 68 patients in whole cohort, 29 patients with HIV, and 39 without HIV.

Table 1: **Characteristics of the cohort**

left the region and treatment outcome unknown]) from March, 2008, to August, 2012, from three designated XDR tuberculosis facilities in South Africa (Brooklyn Chest Hospital, Cape Town, Western Cape; Gordonia Hospital, Upington, Northern Cape; and Sizwe Tropical Diseases Hospital, Johannesburg, Gauteng province). All patients were admitted to one of the facilities until culture conversion, death, or discharge because of treatment failure. At discharge, treatment in the continuation phase was directly observed. Data were captured on a quarterly basis by a trained researcher using a standardised case record form. CD4 counts in patients infected with HIV were recorded at the time of initial diagnosis for the purposes of outcome analysis. All patients had smear microscopy and culture at monthly intervals. Ethics approval was obtained from the human research ethics committees at the University of Cape Town and the University of Witwatersrand.

Procedures

Information about regimens, start and stop dates of treatment, adverse events, and treatment outcomes were recorded. Isolates were obtained every month to establish smear and culture status. Phenotypic routine testing for susceptibility to rifampicin, isoniazid, ofloxacin, amikacin, and ethionamide was done at the discretion of each patient's doctor in the centralised National Tuberculosis Programme reference laboratory (National Health Laboratory Service) as previously described.[11] WHO does not recommend susceptibility testing for para-aminosalicylic acid in routine diagnostic laboratories because this method has not been standardised.[12] To establish the profile and extent of drug resistance, available isolates from a subset of patients biobanked at diagnosis (n=56; from the Western Cape) were also genotyped to establish strain type by spoligotyping[13] and IS*6110* DNA fingerprinting,[14] and targeted DNA sequencing of the *inhA* promoter and the *katG*, *rpoB*, *embB*, *pncA*, *gyrA*, and *rrs* genes was used to identify mutations conferring resistance.[15] Resistance to para-aminosalicylic acid was determined with a culture-based method[16] (see appendix for detailed methods).

Outcomes

Early treatment outcomes (ie, within 12 months of treatment initiation) were sputum culture conversion and reversion. Late treatment outcomes (ie, after 24 months) were treatment completion, treatment cure, all-cause mortality (not necessarily secondary to progression of tuberculosis), default (interruption in treatment for at least 2 consecutive months for any reason), treatment failure, and transfer out (appendix).[17] Culture conversion was defined as two consecutive negative sputum cultures at least 30 days apart. Culture reversion was defined as two consecutive positive sputum cultures at least 30 days apart after initial sputum culture conversion. Patients were deemed to have achieved net conversion if their last sputum culture event (conversion or reversion) during follow-up was conversion, even if they had had one (or occasionally more than one) previous episode of reversion. Patients were deemed to be net reverters if their last sputum culture event was reversion. Patients were deemed to have failed treatment if at least two of five sputum cultures were positive in the previous 12 months, if any of the final three sputum cultures in the previous 12 months were positive, or if their treatment was stopped earlier than suggested by national programmatic guidelines because of inadequate response or adverse events.

See Online for appendix

	Genotypic resistance	Phenotypic resistance
Rifampicin (*rpoB*)	55/56 (98%)	56/56 (100%)
Isoniazid (*katG*)	38/56 (68%)	56/56 (100%)
Isoniazid (*katG* plus *inhA* promoter)	56/56 (100%)	..
Aminoglycosides, kanamycin, and capreomycin (*rrs*)	46/56 (82%)	56/56 (100%)
Ofloxacin (*gyrA*)	50/56 (89%)	56/56 (100%)
Pyrazinamide (*pncA*)	47/56 (84%)	..
Ethambutol (*embB*)	49/56 (88%)	..
Ethionamide (*inhA* promoter)	39/56 (70%)	..
Para-aminosalicylic acid	..	3/48 (6%)

Data are number resistant/isolates tested (%). Genes and promoters sequenced given alongside drugs.

Table 2: **Number of biobanked isolates with drug-specific genotypic and phenotypic resistance**

	24 months	36 months	48 months	60 months
Died	49 (46%)	61 (57%)	74 (69%)	78 (73%)
Treatment default*	7 (7%)	6 (6%)	5 (5%)	4 (4%)
Treatment cure	7 (7%)	6 (6%)	5 (5%)	5 (5%)
Treatment failure	25 (23%)	19 (18%)	14 (13%)	11 (10%)
Treatment completion	10 (9%)	10 (9%)	6 (6%)	7 (7%)
Insufficient information	9 (8%)	5 (5%)	3 (3%)	2 (2%)

Data are n (%). *Interruption of treatment for at least 2 consecutive months for any reason; once patient classified as a default, the classification remained unless individual died; all other categories (except died) needed a minimum of 24 months' treatment.

Table 3: **Outcomes of 107 patients with extensively drug-resistant tuberculosis after treatment, by duration of follow-up**

	Treatment failure (n=19)	Achieved culture conversion (n=26)
Smear microscopy positive	6 (35%)*	1 (5%)†
Unfavourable outcome	17 (89%)	13 (50%)
Died	14 (74%)	6 (23%)
Treatment failure	3 (16%)	2 (8%)
Defaulted	0	4 (15%)
Readmitted to hospital	0	1 (4%)
Favourable outcome	2 (11%)	13 (50%)
Cured	2 (11%)	13 (50%)

Data are n (%). *Smear microscopy requested for 17 patients. †Smear microscopy requested for 19 patients.

Table 4: **Outcomes of the 45 patients who were discharged into the community**

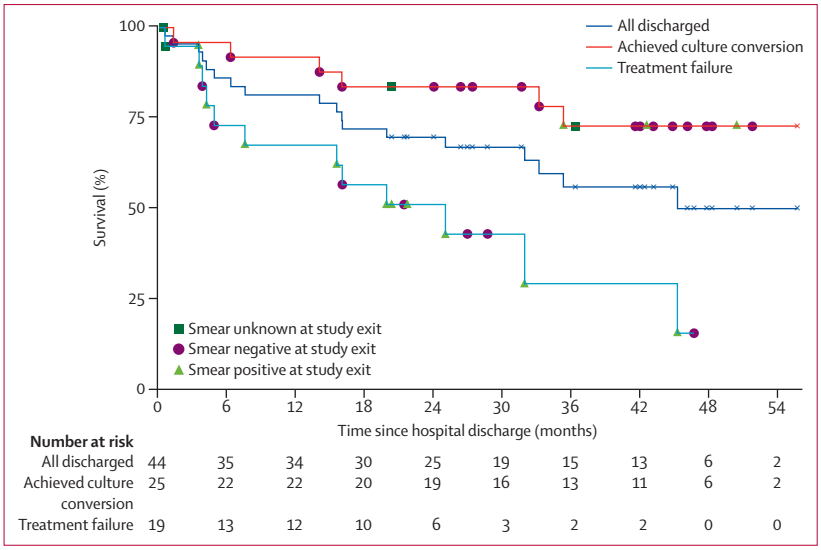

Figure 1: **Kaplan-Meier for probability of survival since discharge from hospital**
Crosses indicate censoring.

	No conversion (n=68)	Net conversion (n=22)	Net reversion (n=17)
Time from treatment start date to outcome or last sputum culture event (months)	16·8 (4·5–32·5)	8·7 (5·6–26·4)	23·5 (15·2–27·6)
With HIV	30 (44%)	10 (45%)	4 (24%)
Favourable outcome	1 (1%)	15 (68%)	1 (6%)
Completed treatment	1 (1%)	7 (32%)	1 (6%)
Cure	0	8 (36%)	0
Unfavourable outcome	67 (99%)	7 (32%)	16 (94%)
On treatment	1 (1%)	1 (5%)	1 (6%)
Defaulted	2 (3%)	2 (9%)	0
Treatment failure	3 (4%)	0	5 (29%)
Died	61 (90%)	4 (18%)	10 (59%)

Data are median (IQR) or n (%).

Table 5: **Treatment-related outcomes by conversion status**

Statistical analysis

Continuous variables were summarised by median and IQR, using Wilcoxon rank sum for p values, categorical variables by counts and percentages using Fisher's exact test for p values. Variables with a large percentage of missing data are noted. Durations were calculated in days and converted to number of months by days/30·4 (as the number in days of an average month) for simplicity. Univariate Cox proportional hazards models were used to assess the relation between explanatory variables and time-to-event outcomes (including time to death, time to net sputum culture conversion, and time to net sputum culture reversion). Variables considered included HIV status (positive or negative), combined HIV and antiretroviral therapy status (HIV negative, HIV positive on antiretroviral therapy, or HIV positive not on antiretroviral therapy), sex, ethnic origin (mixed ancestry or black), treatment outcome (positive or negative), adverse drug reactions, cavitation (yes or no), bilateral disease (yes or no), strain family (Beijing or other), smoking (yes or no), cohort (Western Cape, Northern Cape, or Siswe), weight less than 50 kg at diagnosis, weight (kg) at diagnosis, age at diagnosis, number of drugs prescribed, history of tuberculosis (none, confirmed, or unknown), history of multidrug-resistant tuberculosis (yes or no), and a binary variable for each of the drugs prescribed. Unless otherwise noted, the time to event was taken as days from treatment start date. Kaplan-Meier curves were estimated for probability of survival by various strata. Tests between strata were done by the log-rank test. Cumulative incidence curves under competing risks assumptions were estimated for cumulative probability of net conversion and net reversion. Cause-specific Cox proportional hazard regression models in competing risk were fitted to risk of net conversion and reversion with death as a competing risk. Multivariate Cox proportional hazards models for mortality included variables that were significantly associated with outcome ($p<0·05$) and additional prespecified variables (eg, sex). In some cases, important variables (eg, weight) were omitted from multivariate models because of the high percentage of missing data; these cases were noted. All statistical analysis was done in R (version 3.0) and graphics generated with the package ggplot2. A simple cost analysis was done to determine the cost relative to net gain in culture conversion rate when longer treatment duration was used as a cutpoint to define treatment failure (see appendix for methods, assumptions, and detailed results).

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

By August, 2012, 79 patients (74%) had died, of whom 32 (41%) had HIV infection. 17 patients (16%) were still alive, of whom seven (41%) had HIV infection. The other 11 patients (10%) were lost to follow-up, of whom five (45%) had HIV infection. 63 patients (59%) were not infected with HIV. 35 (80%) of 44 patients with HIV infection were on ART (a combination of lamivudine, stavudine, efavirenz, nevirapine, zidovudine, and lopinovir–ritonavir; frequency and dosing of ART is outlined in detail by Shean and colleagues[18]). The median follow-up from diagnosis to mortality or censor date was 28·5 months (IQR 13·8–49·3) and the median duration of in-patient stay was 13·29 months (8·27–23·89).

All participants were treated as inpatients with directly observed therapy. Median treatment duration was 22·1 months (8·8–34·3). Because linezolid was unavailable, access to capreomycin susceptibility testing

was poor, and second-line phenotypic susceptibility testing was unreliable, patients were treated empirically with a combination of drugs (table 1). The three most frequently prescribed drugs were capreomycin, para-aminosalicylic acid, and terizidone (a cycloserine derivative; table 1).

Isolates from all 107 patients were phenotypically resistant to rifampicin, isoniazid, amikacin, and ofloxacin. According to national policy, isolates resistant to isoniazid and rifampicin are sent for some second-line testing (resistance to ethionamide, ofloxacin, amikacin, and occasionally streptomycin). We did extended molecular and phenotypic susceptibility testing for the 56 biobanked isolates. 20 isolates (36%) had genotypic resistance (phenotypic resistance to para-aminosalicylic acid) to up to seven drugs, 18 (32%) had resistance to eight drugs, 17 (30%) to nine drugs, and one (2%) to all ten drugs tested (resistance beyond XDR tuberculosis or so-called totally drug-resistant tuberculosis).[15,19–21] We recorded some discordance between genotypic and phenotypic resistance patterns (table 2). In a multivariable model, resistance to an increasing number of drugs was associated with the Beijing genotype (odds ratio 2·66, 95% CI 1·18–17·35; p=0·01), but not with mortality or non-conversion status (appendix).

At 60 months, few patients had a favourable outcome, many had died, and a tenth had failed treatment (table 3). The persistently high frequency of treatment failure represents patients who exhausted treatment options and were discharged from inpatient care to reside in the community. 56 patients died before discharge from hospital and six transferred out of the region. The remaining 45 patients were discharged into the community, of whom 26 (58%) had achieved sputum culture conversion. Notably, more than a third of patients deemed to have failed treatment were smear microscopy positive at discharge and almost 90% had an unfavourable outcome (>80% died; table 4). Median survival of patients who had failed treatment from time of discharge was 19·84 months (IQR 4·16–26·04). Patients discharged after culture conversion had significantly longer survival (36·1 months, IQR 21·23–46·25; p=0·0015; figure 1). Notably, two female patients who had failed treatment at discharge had a favourable outcome despite withdrawal of therapy: both are alive, well, and fulfilling duties in the community.

Net sputum culture conversion occurred in 22 patients (21%; table 5). More than half of these patients achieved net sputum culture conversion by 9–12 months (table 6), although the overall mortality of this group—six of whom had HIV infection—was 27% (appendix). Favourable outcomes were more common in patients who achieved net culture conversion than in those with net culture reversion or who did not achieve culture conversion (table 5). Time to culture conversion was not associated with improved outcome (appendix). Independent predictors of probability of net culture conversion were no history of multidrug-resistant tuberculosis and use of clofazamine (table 7). Culture reversion occurred in 17 (44%) of 39 initial converters (appendix) and 94% of net reverters had an unfavourable outcome (table 5).

A separate analysis of treatment costing showed that substantial cost would be expended for little gain in

	First conversion (n=39)		Net conversion (n=22)		Net reversion (n=17)	
	During timeframe	Cumulative	During timeframe	Cumulative	During timeframe	Cumulative
<2 months	1 (3%)	1 (3%)	1 (5%)	1 (5%)	0	0
2–4 months	3 (8%)	4 (10%)	1 (5%)	2 (9%)	0	0
4–6 months	6 (15%)	10 (26%)	4 (18%)	6 (27%)	1 (6%)	1 (6%)
6–9 months	9 (23%)	19 (49%)	5 (23%)	11 (50%)	1 (6%)	2 (12%)
9–12 months	4 (10%)	23 (59%)	1 (5%)	12 (55%)	1 (6%)	3 (18%)
12–18 months	7 (18%)	30 (77%)	2 (9%)	14 (64%)	3 (18%)	6 (35%)
18–24 months	3 (8%)	33 (85%)	2 (9%)	16 (73%)	3 (18%)	9 (53%)
≥24 months	6 (15%)	39 (100%)	6 (27%)	22 (100%)	8 (47%)	17 (100%)

Follow-up time is calculated from treatment start date and all periods are up to and including the upper limit.

Table 6: **Number of patients achieving first culture conversion, net conversion, or net reversion, by follow-up time**

	Full cohort (n=107)		Patients with HIV (n=44)	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Net conversion				
Age at diagnosis	0·99 (0·95–1·04)	0·87	0·95 (0·88–1·04)	0·26
Male sex	1·48 (0·58–3·78)	0·39	0·76 (0·21–2·82)	0·69
Mixed ancestry	0·59 (0·14–2·58)	0·5	1·59 (0·27–9·24)	0·61
HIV infection	1·48 (0·50–4·39)	0·46	..	..
Antiretroviral therapy	..	..	0·93 (0·10–8·82)	0·95
No history of multidrug-resistant tuberculosis	10·21 (2·64–39·38)	0·0007	1·61 (0·37–6·96)	0·53
Clofazamine	0·14 (0·034–0·59)	0·0069	..	..
Mortality				
HIV infection	1·51 (0·87–2·63)	0·147	..	..
Antiretroviral therapy	..	..	0·13 (0·03–0·50)	0·003
Male sex	0·86 (0·54–1·37)	0·526	0·42 (0·15–1·17)	0·096
Mixed ancestry	0·66 (0·37–1·18)	0·159	0·46 (0·16–1·29)	0·138
Net conversion	0·14 (0·06–0·34)	<0·0001	0·12 (0·03–0·53)	0·005
Net reversion	0·24 (0·12–0·48)	<0·0001	0·36 (0·07–1·99)	0·242
Age at diagnosis	0·98 (0·95–1·00)	0·052	0·95 (0·89–1·01)	0·113
Clofazamine	0·38 (0·16–0·87)	0·021	2·26 (0·57–8·99)	0·247
Azithromycin	..	..	0·53 (0·11–2·62)	0·434
Para-aminosalicylic acid	..	..	0·68 (0·14–3·36)	0·638
Ethambutol	..	..	3·12 (1·01–9·67)	0·048
Ofloxacin plus moxifloxacin	..	..	2·70 (0·78–9·32)	0·115

Many values for weight at diagnosis were missing in the full cohort (36%) and in patients with HIV (34%), so this variable was omitted from the final multivariate models. CD4 cell count at diagnosis was not included in multivariate models for patients with HIV because many values (52%) were missing. A complete case model was run including CD4 cell count (n=21; omitting azithromycin and clofazamine because used in few patients), ethnic origin, antiretroviral therapy, net conversion, and ethambutol, and remained significant at p<0·05. CD4 cell count had a hazard ratio of 0·99 (95 %CI 0·99–1·00), p=0·045.

Table 7: **Cox cause-specific hazards model of time from treatment start to net conversion (under competing risk of death) and multivariate Cox proportional hazards model for risk of death from treatment start**

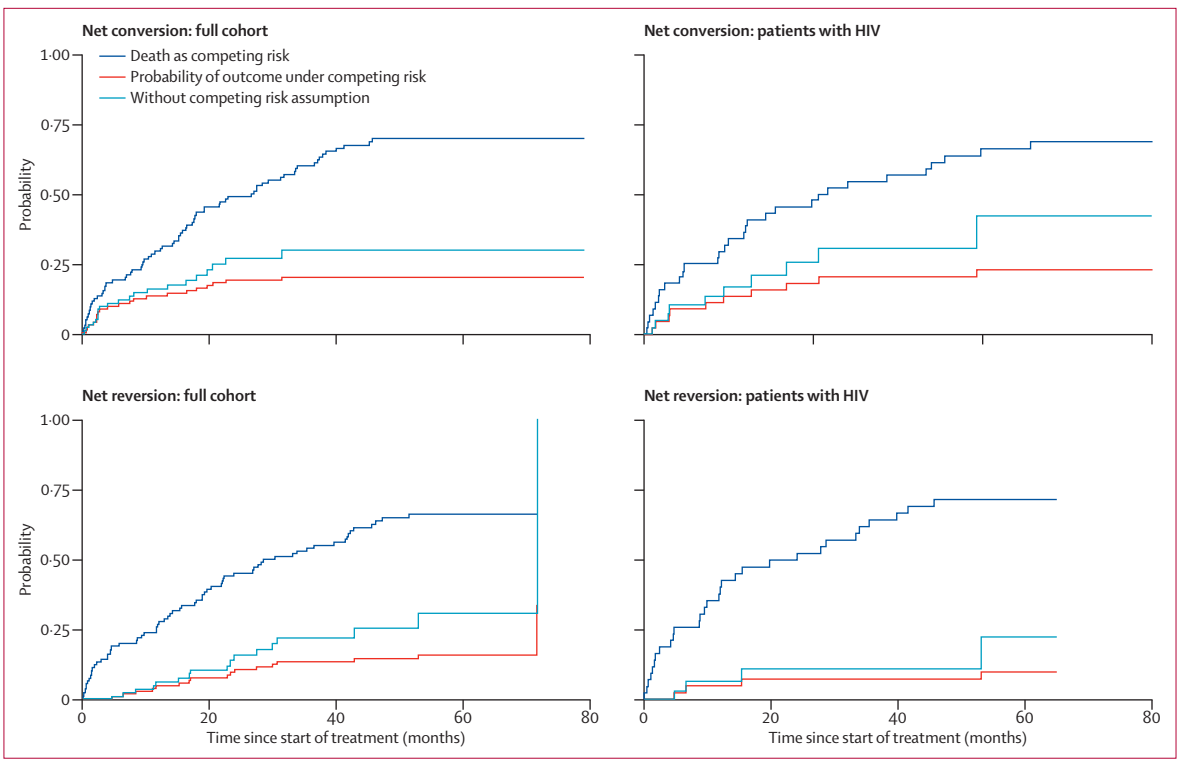

Figure 2: **Cumulative incidence estimates under competing risks assumptions of culture conversion and culture reversion, and cumulative incidence estimates without assumption of competing risks**

sputum culture conversion rates: in a hypothetical cohort of 100 patients with XDR tuberculosis, the cost of treatment would be US$363 886 if it were extended to 18 months (rather than 12 months) and an additional 2·2 patients would achieve sputum culture conversion by 18 months. Cost would increase to $726 760 if treatment were extended to 24 months, and an additional 4·4 patients would achieve sputum culture conversion (appendix).[10]

When plotting cumulative incidence estimates under competing risks, the probability of net culture conversion rises to about 20% in the first 24 months and stabilises (figure 2). Probability of net culture reversion increases more slowly, needing about 40 months to stabilise (figure 2). Without the competing risk assumption, the probability of net culture conversion would be significantly overestimated (see appendix for univariate analysis).

Almost half the patients in the cohort died within 24 months of treatment (figure 3A, table 3). 24 (69%) of 35 patients living with HIV and receiving antiretroviral therapy had died by the end of follow-up, compared with 46 (73%) of 63 patients not infected with HIV (p=0·77) and all nine patients with HIV not taking antiretroviral therapy (p=0·0069; figure 3B). CD4 count of <200 cells per mL was also associated with increased mortality (p=0·069; figure 3C).

In a multivariate Cox proportional hazards model, net culture conversion status and treatment with clofazamine were associated with decreased risk of death in the overall cohort (table 7; see appendix for univariate analysis). In patients with HIV, net culture conversion and use of antiretroviral therapy were associated with improved survival (table 7). Net culture reversion was a predictor of survival in the full cohort despite the high frequency of unfavourable outcomes in patients with net culture reversion, because unfavourable outcomes were even more common in patients who did not achieve conversion (table 5; appendix).

We identified three families in which at least three members had been diagnosed with any type of drug-resistant tuberculosis in the previous 10 years (appendix). Strain typing and parallel mutational analysis of available isolates in one family showed evidence of transmission from one individual—B28, a treatment failure residing in the community—to his brother who also eventually died in the community as a treatment failure.

Discussion

We have shown that long-term outcomes in patients with XDR tuberculosis are poor, irrespective of HIV status (panel), although antiretroviral therapy has improved survival in patients with HIV. Many patients in our cohort who were discharged from hospital had positive sputum cultures, had failed treatment, and had no further therapeutic options. These patients survive for long periods living in the community and are likely to

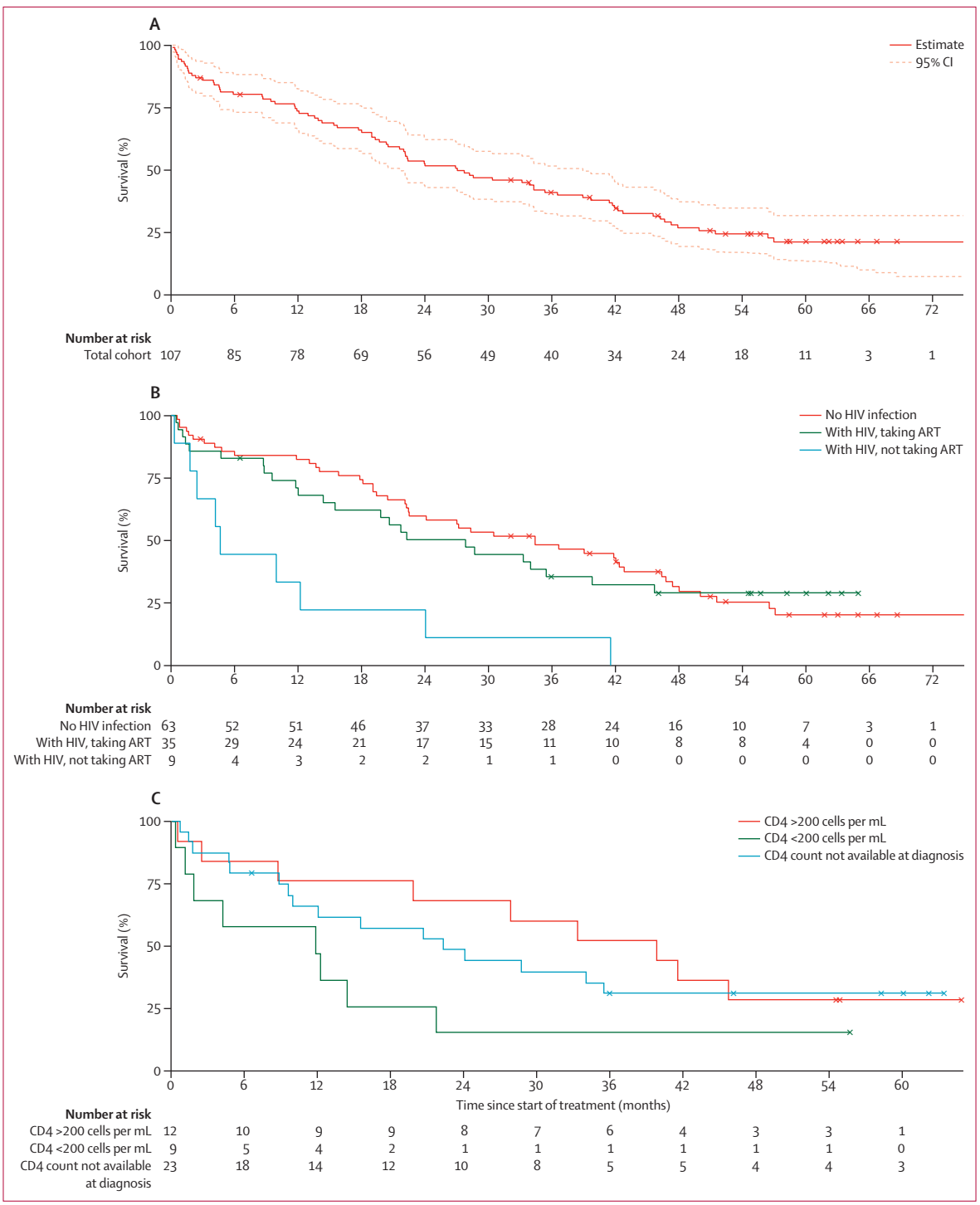

***Figure 3*: Kaplan-Meier survival estimates**
(A) Probabilities of survival in all patients. (B) Probabilities of survival stratified by HIV infection and ART. (C) Probabilities of survival in patients with HIV stratified by CD4 cell count. Crosses indicate censoring. ART=antiretroviral therapy.

contribute to community-based spread of XDR tuberculosis. Overall net culture conversion was fairly infrequent and only two-thirds of patients who achieved net conversion had a favourable outcome. Therefore, some initial responders subsequently relapse. Additionally, only slightly more than half of patients who achieved sputum culture conversion did so by 12 months. The proportion increased slightly to 24 months, but a cost analysis indicated that additional gains that would result from an extension would be small and costly.

***Panel:* Research in context:**

Systematic review

We searched PubMed for reports published in English before Sept 1, 2013, that presented results for treatment outcomes in patients with extensively drug-resistant (XDR), extremely drug-resistant, or totally drug-resistant tuberculosis. We combined search terms for drug-resistant tuberculosis ("MDR-TB", "XDR-TB", "XXDR-TB", and "TDR-TB") with those indicating outcome ("outcome", "mortality or death", "culture conversion"), molecular epidemiology ("strain type", "DNA fingerprinting", "spoligotyping", "IS*6110*", "mutational analysis", and "Beijing"), and drug susceptibility. Including systematic reviews, we identified 97 studies of treatment outcome, 51 related to molecular epidemiology, and 159 related to drug susceptibility. We identified no studies in which the frequency or long-term outcomes of patients with XDR tuberculosis who fail treatment was assessed.

Interpretation

To our knowledge, ours is the first prospective study in which 60-month treatment-related outcomes are recorded in patients with XDR tuberculosis, with further investigation of the frequency and outcomes of patients who failed treatment. We prospectively confirmed that in a setting where tuberculosis is endemic, long-term outcomes of patients with XDR tuberculosis are poor, irrespective of HIV status and despite an intensive injectable-based regimen. Alarmingly, we have shown for the first time that, by contrast with sporadic and isolated cases of treatment failure and near total or totally drug-resistant cases that have been reported in several countries, therapeutic failure is occurring systematically on a country-wide level. Patients who fail treatment, many of whom have high transmission potential, are being discharged back into the communities. Often survival is for months to years with substantial potential for disease transmission. Our data provide important information to policy makers to allow them to design appropriate interventional strategies. Although prevention of further cases is mandatory through improved regimens for multidrug-resistant tuberculosis and better functioning programmes, and fast-tracking of new drug regimens and improved diagnostics, the growing pool of treatment failures needs to be addressed with a coordinated strategy that involves supported home care interlinked with urgent building of long-term community stay and palliative care facilities.

Overall predictors of mortality included conversion status and treatment with clofazamine; in patients with HIV, an additional predictor was use of antiretroviral therapy. Survival of patients with HIV taking antiretroviral therapy was similar to that of patients without HIV infection, confirming previous findings[8] that antiretroviral therapy is mandatory in patients with HIV and XDR tuberculosis.

Isolates from patients in our cohort had a high level of resistance (almost two-thirds of the cohort had resistance to at least eight drugs) and we have confirmed the emergence of totally drug-resistant disease, as previously described.[15] Capreomycin resistance as defined by the *rrs* 1401 mutation[22] was common, but empirical treatment with capreomycin was given to most patients, despite its toxicity.[18] The Beijing strain type was associated with advanced resistance in our cohort.

Overall, despite apparently good adherence and intensive inpatient therapy with an empirical regimen of capreomycin and para-aminosalicylic acid, 5-year outcomes in our cohort were worse than previous estimates of short-term and 24-month outcomes in settings with intermediate-burden[23,24] and those with high burden, like South Africa.[9,25–27] These poor outcomes were probably due to high-grade resistance, meaning that no effective drugs were available to treat the disease.[18] One of the isolates showed resistance beyond XDR tuberculosis, a concerning clinical entity previously described in India, Iran, Italy, and more recently South Africa.[15,19–21] Only para-aminosalicylic acid and clofazamine were likely to be effective in these patients and active disease is unlikely to respond to only two active agents (both of which are fairly weak mycobacteriostatic drugs from the WHO class 4 category of drugs). New drugs such as linezolid, which could be effective against XDR tuberculosis,[28] are not yet available in the South African national tuberculosis programme. Other drugs approved by the US Food and Drug Administration (eg, bedaquiline[29,30]) have not yet been approved for use in South Africa. Therefore, linezolid should be introduced into the South African national tuberculosis programme. Studies assessing new multidrug regimens for XDR tuberculosis are urgently needed, although enthusiasm is tempered by concerns about reductions in their effective lifespan through use in regimens in which they are least protected (ie, drug resistance is most likely to develop). Other factors that could be driving poor outcomes include nutritional status, drug absorption, and host immunity, including HIV.

Consistent with other evidence,[31] the Beijing strain type was associated with increased resistance in our cohort. More detailed gene-based studies are now needed to identify the mechanisms by which this increased resistance might develop.

Many patients who fail treatment are being discharged back into the community because little bed space is available in designated tuberculosis hospitals, alternative long-term residential and palliative care facilities are scarce, and resources to support proper home-based care when appropriate are inadequate. A third of discharged patients were smear microscopy positive at discharge, suggesting high transmission potential. The identification of epidemiological clusters and primary spread by strain typing and mutational analysis in the initial cohort[9] and in our follow-up study suggest that community-based spread of drug-resistant tuberculosis is likely. There is an urgent need to connect home-based care with palliative care and long-term sheltered community stay facilities (modernised sanatoriums) where such patients can voluntarily reside, thus minimising continuing transmission.[32] Clearly, preventive strategies and testing of new drug regimens for XDR tuberculosis are also urgently needed. The findings we have outlined are likely to be relevant in several settings where XDR or totally drug-resistant tuberculosis has been described, such as Iran, India, Italy, Russia, and eastern Europe.[15,19–21] As described in prechemotherapeutic times, some patients who fail treatment were cured after discharge and withdrawal of therapy.

Our data challenge previous findings (in the context of short-term outcomes)[9] and the widespread national

policy of treatment withdrawal if patients do not achieve culture conversion after 12 months of treatment. Our findings further indicate that treatment of 100 patients with XDR tuberculosis for a further 6 months leads to only an additional two culture conversions at a substantial cost. We also noted that almost 20% of initial converters subsequently reverted, and 90% of patients with net reversion have an unfavourable outcome. These data will be useful for policy makers when defining measures of treatment failure and criteria for withdrawal of treatment.

Overall predictors of survival included net culture conversion and use of clofazamine (also a predictor of culture conversion). The association with use of clofazamine has not previously been described in XDR tuberculosis, but intuitively makes sense because clofazamine is a mycobacteriostatic agent not widely used in treatment regimens for multidrug-resistant tuberculosis in South Africa. A systematic review[33] suggested a potential beneficial effect of clofazamine in XDR tuberculosis and our data suggest that it should be a key drug in any regimen. Interestingly, the mortality benefit was not recorded in patients infected with HIV. This finding could represent type 2 error due to small sample size, although increased mortality with clofazimine has been shown in patients with HIV during treatment of *Mycobacterium avium* complex.[34] Other studies[9,35] showed that moxifloxacin was associated with survival in patients with XDR tuberculosis despite ofloxacin resistance, and minimum inhibitory concentrations for local XDR tuberculosis isolates are often below the achievable serum concentrations of moxifloxacin.[36] However, the fact that we did not show an outcome advantage might be related to the relatively small numbers of patients on moxifloxacin or could be related to the changing susceptibility profile of isolates in South Africa. With continuing treatment and primary spread, the proportion of isolates that are sensitive to moxifloxacin could have decreased substantially.

Our study has several limitations. The initial cohort that we followed up was derived retrospectively and so mortality and unfavourable outcomes have probably been underestimated because of selection bias. Indeed, about 21% of patients with XDR tuberculosis in the original cohort died before starting treatment, most of whom had HIV infection,[9] suggesting that outcomes are worse than recorded in this study. However, the frequency of default was low and our intention was to document the long-term treatment-related outcomes to inform future cost-conscious public health interventions. We did not distinguish between deaths related to tuberculosis and those ascribable to HIV or other medical causes. We assessed only a proportion of isolates for susceptibility testing and did not test for drugs such as cycloserine, because methods for testing of such drugs are unreliable and not well defined. Furthermore, phenotypic testing methods have several limitations,[12] and we therefore resorted to genotypic testing. Our findings apply to patients treated across South Africa where previous multidrug-resistant tuberculosis is common, HIV co-infection is frequent, and empirical capreomycin is used widely. Therefore, our findings might not be applicable to other settings including localised or extended outbreaks, low and intermediate burden settings, and those with a low HIV prevalence including other parts of Africa.

In conclusion, our data suggest that even with a multidrug capreomycin-based regimen and fairly good adherence, long-term treatment outcomes in patients with XDR tuberculosis are poor. These patients also frequently fail treatment and are discharged with positive smear microscopy, and therefore are likely to transmit disease. Collectively, our data underscore the urgent need for testing of new combined regimens for XDR tuberculosis and institute interventions such as community and palliative care facilities to minimise disease spread by patients who fail treatment.[32] At the same time, preventive strategies are important, such as strengthening of the national tuberculosis programme, reductions in the overall burden of disease through HIV control and poverty, and management of overcrowding.

Contributors

KD conceived and designed the study. EP, EMS, BM, XP, AP, MB, ML, PvH, FAS, RW, and KD collected data. EI, ML, and KD analysed data. All authors interpreted data. EP, EI, RW, and KD wrote the first draft of the report.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

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THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Pietersen E, Ignatius E, Streicher EM, et al. Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study. *Lancet* 2014; published online Jan 17. http://dx.doi.org/10.1016/S0140-6736(13)62675-6.

ONLINE SUPPLEMENT

Incurable TB: Long-term outcomes in treatment failures with XDR-TB and resistance beyond XDR-TB from South Africa (a 10-year cohort study)

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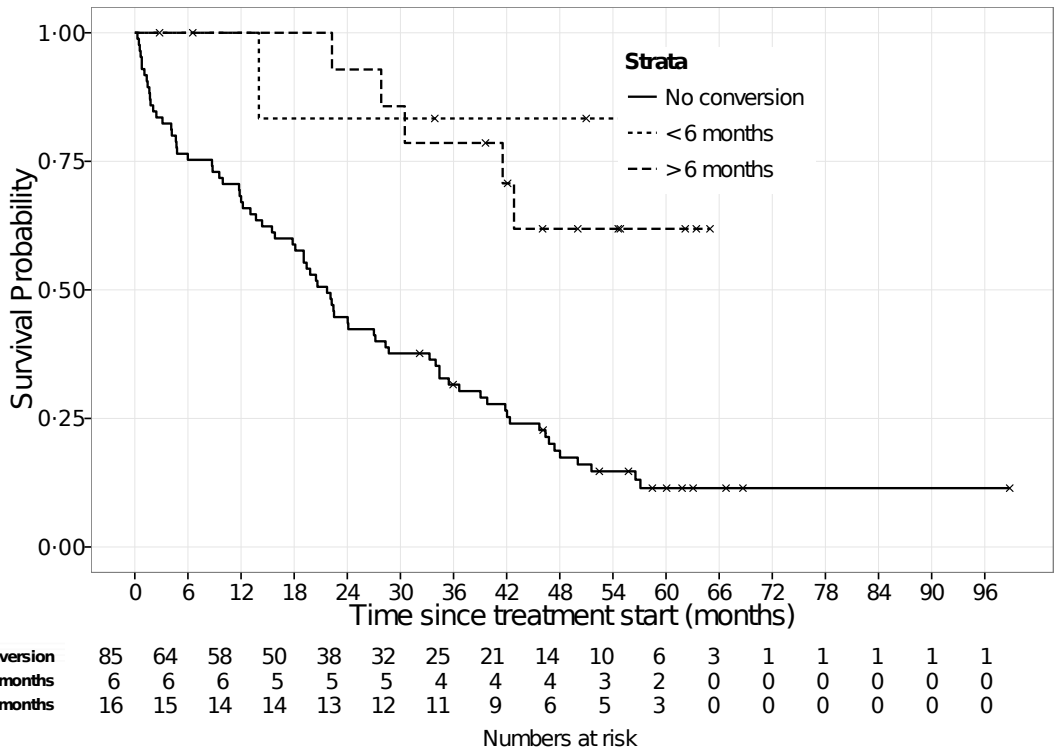
Definitions of outcomes (additional detail)
Treatment cure was defined as 24 months of treatment completed and with consistent negative sputum cultures with at least five negative sputum cultures in the last 12 months of treatment. One positive sputum culture in the final 12 months of treatment was permissible provided there were no other indications of disease recurrence or clinical worsening, and there were three subsequent negative cultures. Treatment completion was defined as those who culture-converted without reversion and completed at least 18 months of treatment post-conversion but did not meet the definition of cure due to missing laboratory data. Treatment default was defined as those who missed more than 2 months of treatment. Patient follow-up continued after discharge into the community. Patients who were transferred to treatment centres other than the three included in the study were defined to be "transfers" and all patients for whom no other treatment outcome could be defined were considered lost to follow-up.

Statistical analysis
Median, inter-quartile range (IQR), counts and percentages were calculated when possible. Variables with a large percentage of missing data are noted. We compared categorical variables by use of Fisher's exact test, and we compared continuous variables via the Wilcoxon rank sum test. Durations were calculated in days and converted to number of months by days/30·4 (as the number in days of an "average" month) for simplicity. Univariate Cox proportional hazards models were used to evaluate the relationship between explanatory variables and various time to event outcomes. Unless otherwise noted, the time to event was taken as days from treatment start date. Kaplan-Meier curves were estimated for probability of survival by various strata. Tests between strata were done by the log rank test. Cumulative incidence curves under competing risks assumptions were estimated for cumulative probability of net conversion and net reversion. Cause-specific Cox proportional hazard regression models in competing risk were fit to risk of net conversion and reversion with death as a competing risk. Multivariate models for mortality were Cox proportional hazards, including variables that were significantly associated with outcome ($p < 0{\cdot}05$) and additional pre-specified variables (eg Gender). In some cases, important variables (eg. weight) were omitted from multivariate models due to the high percentage of missing data; these cases were noted. All statistical analysis was performed in R (R Core Team, Vienna, Austria, 2012) and graphics generated using the package ggplot2 (Wickham, Springer, New York, 2009).

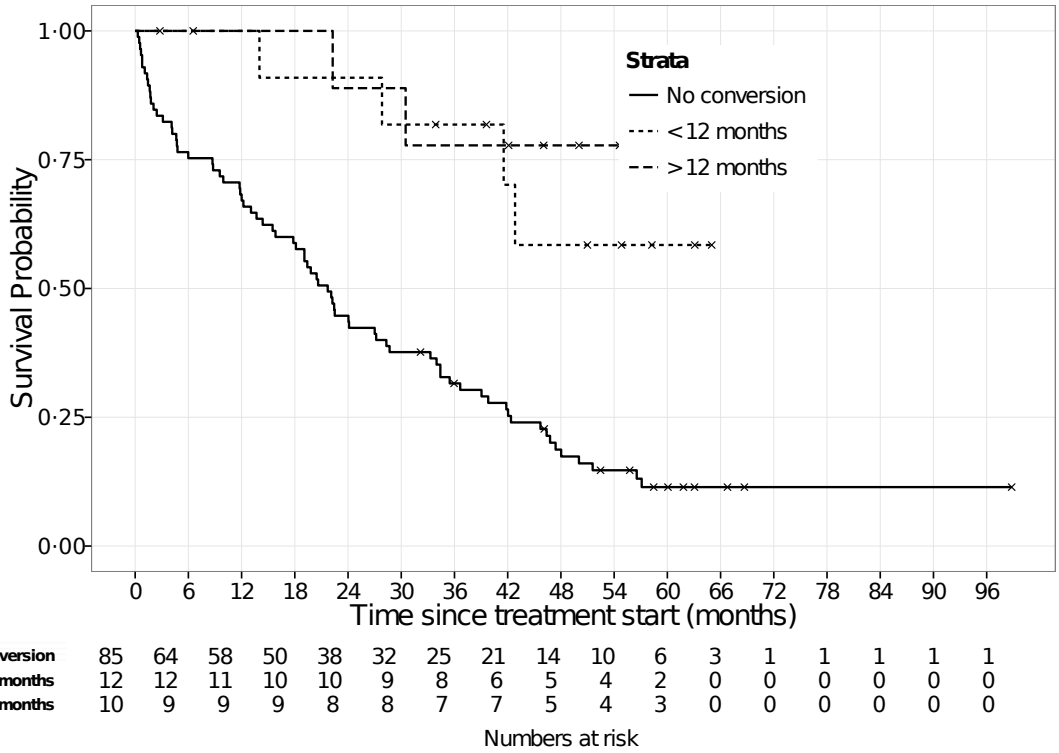
Figure S1. Kaplan-Meier with difference between non-converters and net converters [<6 months vs > 6 months, and <12 months vs >12 months]

A



Numbers at risk																	
No conversion	85	64	58	50	38	32	25	21	14	10	6	3	1	1	1	1	1
<6 months	6	6	6	5	5	5	4	4	4	3	2	0	0	0	0	0	0
>6 months	16	15	14	14	13	12	11	9	6	5	3	0	0	0	0	0	0

B



Numbers at risk																	
No conversion	85	64	58	50	38	32	25	21	14	10	6	3	1	1	1	1	1
<12 months	12	12	11	10	10	9	8	6	5	4	2	0	0	0	0	0	0
>12 months	10	9	9	9	8	8	7	7	5	4	3	0	0	0	0	0	0

Figure S2. Flow diagram summarising time to conversion and reversion events, numbers of individuals experiencing conversion and reversion events and summary of outcomes. The far left column gives the median (IQR) months from treatment start to the conversion and reversion events. The centre left gives numbers (%) of individuals experiencing events from treatment start as well as the median (IQR) months between events. The centre right column summarises the number of individuals and median (IQR) months to the three possible categories - no conversion, net conversion, net reversion and the far right column summarises the mortality, HIV and treatment outcomes for each category. Note that treatment outcome may or may not coincide with mortality/last follow-up, hence the differing counts for deceased.

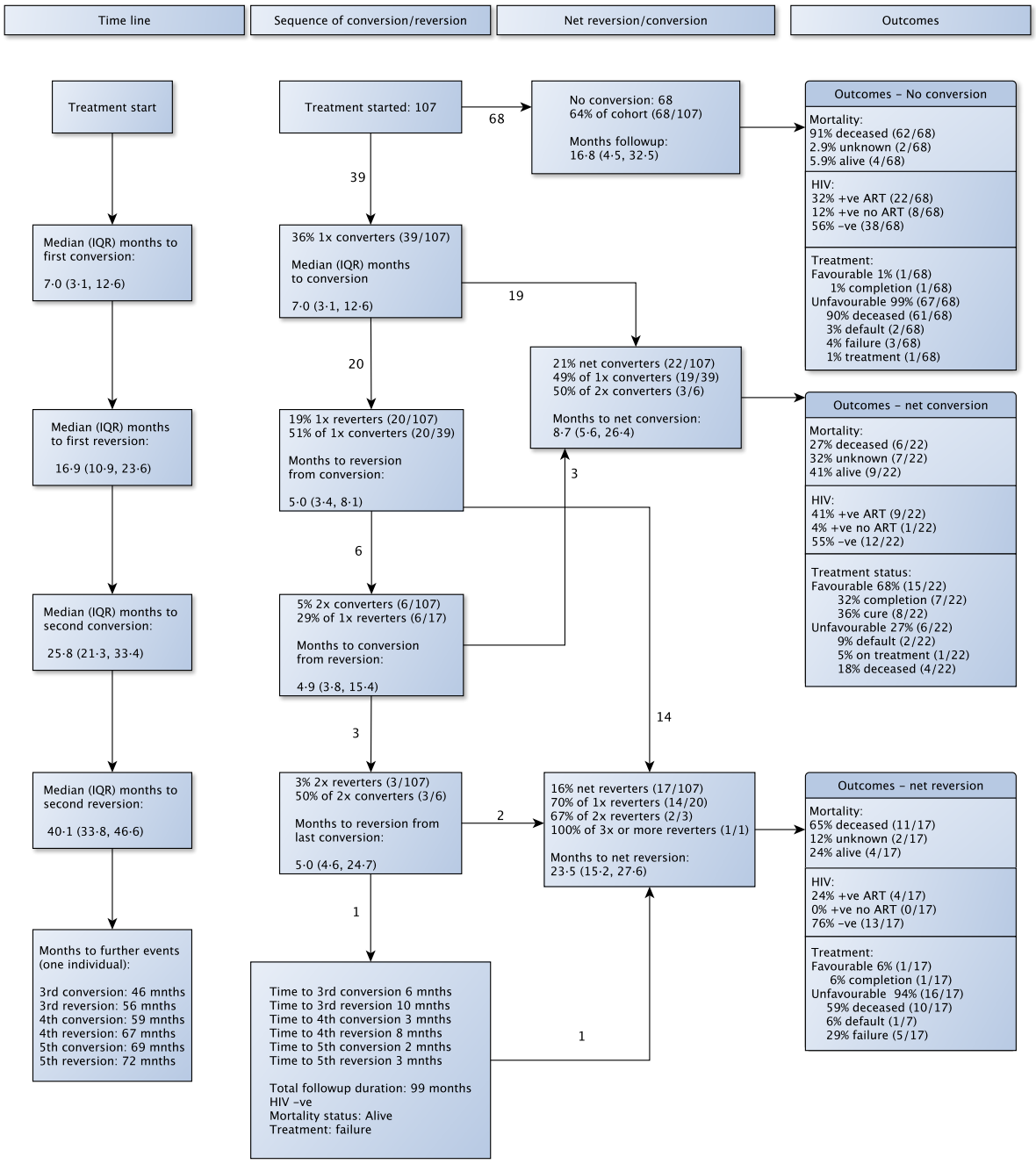
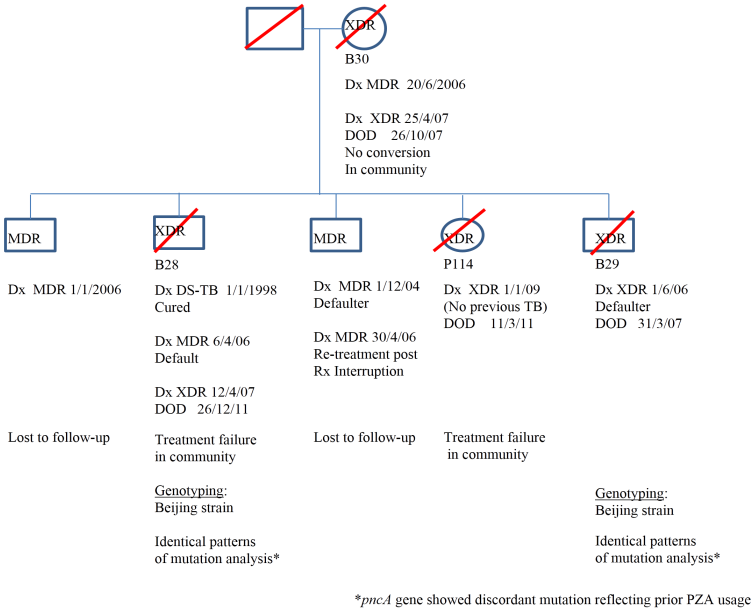

Time line
Sequence of conversion/reversion
Net reversion/conversion
Outcomes
Treatment start
Median (IQR) months to first conversion:
7·0 (3·1, 12·6)
Median (IQR) months to first reversion:
16·9 (10·9, 23·6)
Median (IQR) months to second conversion:
25·8 (21·3, 33·4)
Median (IQR) months to second reversion:
40·1 (33·8, 46·6)
Months to further events (one individual):
3rd conversion: 46 mnths
3rd reversion: 56 mnths
4th conversion: 59 mnths
4th reversion: 67 mnths
5th conversion: 69 mnths
5th reversion: 72 mnths
Treatment started: 107
68
No conversion: 68
64% of cohort (68/107)
Months followup:
16·8 (4·5, 32·5)
Outcomes – No conversion
Mortality:
91% deceased (62/68)
2.9% unknown (2/68)
5.9% alive (4/68)
HIV:
32% +ve ART (22/68)
12% +ve no ART (8/68)
56% -ve (38/68)
Treatment:
Favourable 1% (1/68)
1% completion (1/68)
Unfavourable 99% (67/68)
90% deceased (61/68)
3% default (2/68)
4% failure (3/68)
1% treatment (1/68)
39
36% 1x converters (39/107)
Median (IQR) months to conversion
7·0 (3·1, 12·6)
19
21% net converters (22/107)
49% of 1x converters (19/39)
50% of 2x converters (3/6)
Months to net conversion:
8·7 (5·6, 26·4)
Outcomes – net conversion
Mortality:
27% deceased (6/22)
32% unknown (7/22)
41% alive (9/22)
HIV:
41% +ve ART (9/22)
4% +ve no ART (1/22)
55% -ve (12/22)
Treatment status:
Favourable 68% (15/22)
32% completion (7/22)
36% cure (8/22)
Unfavourable 27% (6/22)
9% default (2/22)
5% on treatment (1/22)
18% deceased (4/22)
20
19% 1x reverters (20/107)
51% of 1x converters (20/39)
Months to reversion from conversion:
5·0 (3·4, 8·1)
3
6
5% 2x converters (6/107)
29% of 1x reverters (6/17)
Months to conversion from reversion:
4·9 (3·8, 15·4)
14
3
3% 2x reverters (3/107)
50% of 2x converters (3/6)
Months to reversion from last conversion:
5·0 (4·6, 24·7)
2
16% net reverters (17/107)
70% of 1x reverters (14/20)
67% of 2x reverters (2/3)
100% of 3x or more reverters (1/1)
Months to net reversion:
23·5 (15·2, 27·6)
Outcomes – net reversion
Mortality:
65% deceased (11/17)
12% unknown (2/17)
24% alive (4/17)
HIV:
24% +ve ART (4/17)
0% +ve no ART (0/17)
76% -ve (13/17)
Treatment:
Favourable 6% (1/17)
6% completion (1/17)
Unfavourable 94% (16/17)
59% deceased (10/17)
6% default (1/7)
29% failure (5/17)
1
Time to 3rd conversion 6 mnths
Time to 3rd reversion 10 mnths
Time to 4th conversion 3 mnths
Time to 4th reversion 8 mnths
Time to 5th conversion 2 mnths
Time to 5th reversion 3 mnths
Total followup duration: 99 months
HIV -ve
Mortality status: Alive
Treatment: failure
1

Figure S3. All patients with red line are deceased. A- Genogram of a XDR-TB family cluster, mother and 5 children (four sons + one daughter), diagnosed with DR-TB. There was molecular epidemiological evidence of transmission between brothers [B28, B29] and subsequent evolution of pyrazinamide resistance, as demonstrated by hetero resistance in both cases. B- Genogram of a family cluster, three siblings (two sisters + one brother), and their offspring also diagnosed with DS-TB and DR-TB. C- Genogram of family cluster, an extended family (two sisters + one husband) with their offspring also diagnosed with DR-TB. (Unknown day and month are recorded as 01/01).

A



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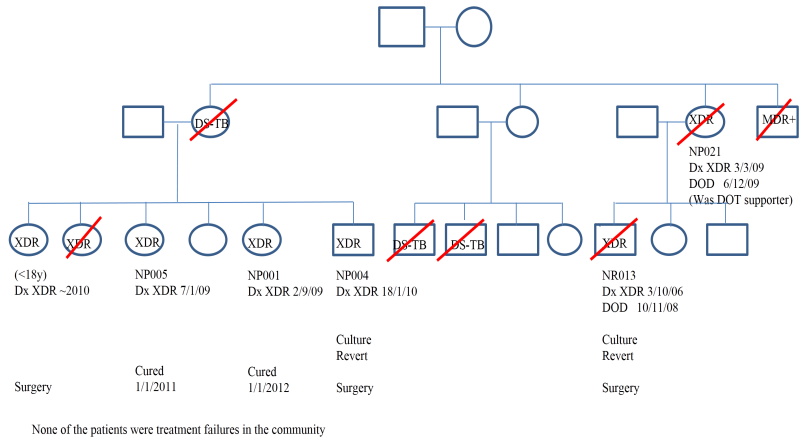

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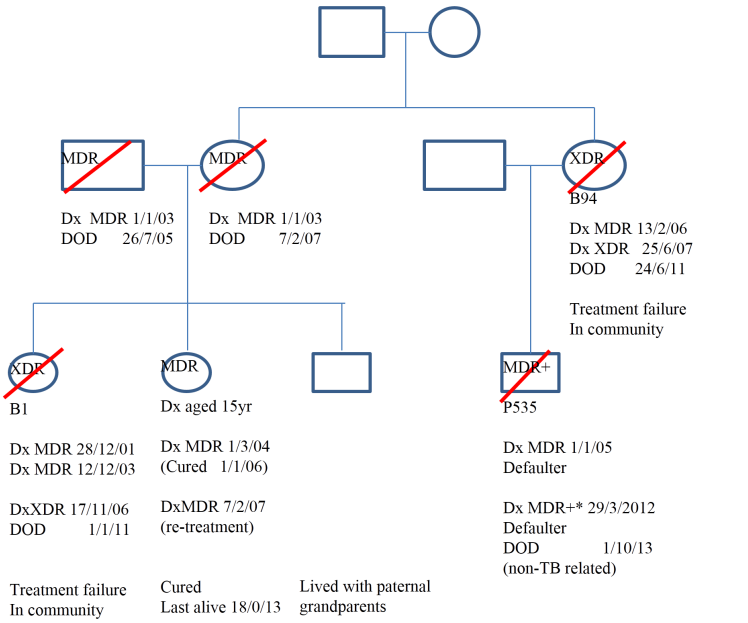


* MDR+ : resistance to R,I plus resistance to FQ or AG

Table S1. (A) Proportion isolates genotypically resistant to 5 or less, 6 or 7 drugs for selected patient characteristics and outcomes and (B) factors and outcomes associated with WHO TB drug groups. P-values by Fisher's exact test.

A

	Number of drugs genotypically resistant to			
	≤5 drugs (n=18)	**6 drugs (n=17)**	**7 drugs (n=18)**	**p-value**
Characteristic				
Gender (male)	33·3% (6/18)	64·7% (11/17)	61·1% (11/18)	0·16
Race (coloured)	66·7% 12/18)	76·5% (13/17)	50% (9/18)	0·27
HIV +ve	2·2% (4/18)	35·3% (6/17)	50% (9/18)	0·23
ART	75% (3/4)	66·7% (4/6)	77·8% (7/9)	1
Previous MDR	94·4% (17/18)	100% (17/17)	83·3% (15/18)	0·31
Beijing	88·9% (16/18)	76·5% (13/17)	94·4% (17/18)	0·27
Outcome				
Mortality	88·9% (16/18)	88·2% (15/17)	66·7% (12/18)	0·25
Conversion	27·8% (5/18)	41·2% (7/17)	44·4% (8/18)	0·6
Net conversion	27·8% (5/18)	11·8% (2/17)	22·2% (4/18)	0·6
Net reversion	0%	29·4% (5/17)	22·2% (4/18)	0·038

B

	XDR SLID + ≤G1 (n = 16)	**XDR SLID + ≤G1 + G4* (n = 37)**	**p-value**
Characteristic			
Race (coloured)	93·8% (15/16)	1·4% (19/37)	0·004
Gender (male)	68·8% (11/16)	45·9% (17/37)	0·15
Adverse events	81·3% (13/16)	94·6% (35/37)	0·16
HIV +ve	31·3% (5/16)	37·8% (14/37)	0·77
Previous MDR	100% (16/16)	89·2% (33/37)	0·3
Beijing	68·8% (11/16)	94·6% (35/37)	0·021
Weight ≤50	33·3% (5/15)	54·2% (13/24)	0·17
Outcome			
Mortality	93·8% (15/16)	75·7% (28/37)	0·25
Conversion	43·8% (7/16)	35·1% (13/37)	0·56
Net conversion	18·8% (3/16)	21·6% (8/37)	1

* XDR SLID: XDR (RIF, INH, OFX) Second Line Injectable Drugs (KANA, AMI, CAPREO) ; G1 (EMB, PZA); G4 (ETH)

Note: In a multivariate model including race, HIV status and Beijing genotype, only the latter was independently associated with increasing drug resistance (OR= 2·66; 95%CI= 1·18-17·35)

Table S2: Cox proportional hazards regression models of factors associated (univarate analysis) with risk of net conversion (from treatment start) in all patients and in HIV-infected patients only.

	Full cohort (n = 107)			**HIV +ve (n = 44)**	
Characteristic	**Hazard ratio (95% CI)**	**p-value**		**Hazard ratio (95% CI)**	**p-value**
HIV +ve	1·564 (0·672, 3·64)	0·3			
HIV +ve ART	1·619 (0·68, 3·85)	0·276	CD4	1·004 (0·999, 1·008)	0·108
HIV +ve no ART	1·18 (0·15, 9·3)	0·875	ART	1·276 (0·156, 10·465)	0·82
Gender	1·054 (0·45, 2·467)	0·904		1·25 (0·36, 4·345)	0·725
Race	0·46 (0·193, 1·1)	0·081		0·784 (0·165, 3·717)	0·759
Positive treatment outcome	14·01 (5·697, 34·467)	< 0·0001		4·818 (1·372, 16·912)	0·014
ADR	0·935 (0·366, 2·392)	0·888		1·071 (0·222, 5·16)	0·931
Cavitation	1·068 (0·225, 5·068)	0·934		0·58 (0·066, 5·053)	0·622
Bilateral disease	0·809 (0·171, 3·827)	0·789		0·652 (0·075, 5·646)	0·697
Strain typing	1·357 (0·286, 6·437)	0·7		2·247 (0·203, 24·928)	0·51
Smoking	0·273 (0·104, 0·715)	0·008		0·184 (0·023, 1·484)	0·112
Cohort - Northern Cape	0·302 (0·039, 2·342)	0·252		-	
Cohort - Siswe	2·188 (0·926, 5·171)	0·074		1·193 (0·344, 4·141)	0·781
Weight <50kg	0·674 (0·249, 1·818)	0·436		0·291 (0·06, 1·412)	0·126
History of TB (confirmed)	0·293 (0·067, 1·273)	0·101		0·801 (0·097, 6·586)	0·836
History of TB (unknown)	1·999 (0·325, 12·317)	0·455		2·673 (0·232, 30·846)	0·431
History or MDR-TB	0·207 (0·075, 0·571)	0·002		0·467 (0·12, 1·817)	0·272
Weight at XDR diagnosis	1·01 (0·971, 1·05)	0·615		1·015 (0·966, 1·067)	0·558
Number of Drugs	0·91 (0·768, 1·079)	0·277		0·998 (0·716, 1·39)	0·989
Age at diagnosis	1·031 (0·994, 1·069)	0·103		1·046 (0·973, 1·124)	0·221
Drugs Used					
Amikacin	1·032 (0·139, 7·691)	0·975		-	-
Amoxil	0·839 (0·351, 2·008)	0·694		0·86 (0·212, 3·483)	0·832
Augmentin	0·444 (0·191, 1·031)	0·059		0·401 (0·103, 1·567)	0·189
Azithromycin	2·258 (0·763, 6·679)	0·141		2·604 (0·733, 9·253)	0·139
Capreomycin	1·98 (0·266, 14·761)	0·505		-	-
Clarthromycin	0·518 (0·217, 1·24)	0·14		0·325 (0·09, 1·169)	0·085
Clofazamine	3·398 (1·462, 7·901)	0·004		1·913 (0·545, 6·713)	0·311
Dapsone	0·271 (0·091, 0·805)	0·019		0·609 (0·127, 2·924)	0·535
Ethambutol	0·747 (0·313, 1·786)	0·512		0·892 (0·226, 3·529)	0·87
Ethionomide	0·833 (0·355, 1·95)	0·673		1·169 (0·328, 4·166)	0·81
Isoniazid	1·712 (0·737, 3·979)	0·211		1·169 (0·337, 4·063)	0·806
Kanamycin	0·724 (0·245, 2·143)	0·559		1·135 (0·241, 5·36)	0·872
Ofloxacin	0·784 (0·329, 1·872)	0·584		2·303 (0·646, 8·207)	0·198
Ofloxacin + Moxifloxacin	0·667 (0·089, 4·978)	0·693		1·308 (0·159, 10·783)	0·803
Pyrazinamide	0·439 (0·187, 1·03)	0·059		0·626 (0·174, 2·246)	0·472
Para-aminosalicylic acid	0·859 (0·2, 3·688)	0·838		-	-
High dose Isoniazid	1·72 (0·735, 4·027)	0·211		0·964 (0·271, 3·432)	0·954
Moxifloxacin	1·241 (0·419, 3·673)	0·697		0·956 (0·245, 3·74)	0·949

Table S3: Cox proportional hazards regression models of factors associated (univarate analysis) with risk of net reversion (from treatment start) in all patients and in HIV-infected patients only.

	Full cohort (n = 107)			**HIV +ve (n = 44)**	
Characteristic	**Hazard ratio (95% CI)**	**p-value**		**Hazard ratio (95% CI)**	**p-value**
HIV +ve	0·557 (0·178, 1·742)	0·315			
HIV +ve ART	0·608 (0·194, 1·906)	0·394	CD4	0·992 (0·972, 1·011)	0·384
HIV +ve no ART		0·998	ART	-	-
Gender	1·148 (0·417, 3·58)	0·79		1·08 (0·152, 7·675)	0·939
Race	0·926 (0·345, 2·482)	0·878		-	-
Positive treatment outcome	0·125 (0·016, 0·973)	0·047		0·532 (0·04, 7·128)	0·634
ADR	0·701 (0·243, 2·02)	0·511		0·301 (0·042, 2·158)	0·232
Cavitation	0·484 (0·107, 2·188)	0·346		-	-
Bilateral disease	0·463 (0·089, 2·396)	0·359		-	-
Strain typing	0·547 (0·068, 4·411)	0·571		-	-
Smoking	1·659 (0·498, 5·529)	0·41		2·638 (0·346, 20·126)	0·349
Cohort - Northern Cape	1·693 (0·513, 5·585)	0·387		23·596 (0·979, 569·068)	0·052
Cohort - Siswe	0·607 (0·163, 2·255)	0·456		2·276 (0·206, 25·154)	0·502
Weight <50kg	0·565 (0·14, 2·275)	0·422		-	-
History or MDR-TB	1·477 (0·194, 11·235)	0·706		0·682 (0·068, 6·78)	0·744
Weight at XDR diagnosis	1·057 (0·998, 1·119)	0·057		1·166 (0·976, 1·392)	0·09
Number of Drugs	1·026 (0·836, 1·26)	0·805		0·623 (0·309, 1·259)	0·187
Age at diagnosis	0·979 (0·936, 1·024)	0·357		0·862 (0·711, 1·044)	0·129
Drugs Used					
Amikacin	-	-		-	-
Amoxil	1·218 (0·452, 3·277)	0·697		0·602 (0·061, 5·977)	0·665
Augmentin	2·95 (0·839, 10·371)	0·092		3·455 (0·359, 33·248)	0·283
Azithromycin	1·141 (0·257, 5·058)	0·862		2·631 (0·364, 19·03)	0·338
Capreomycin	1·4 (0·185, 10·623)	0·745		-	-
Clarthromycin	1·366 (0·389, 4·802)	0·627		0·207 (0·021, 1·988)	0·172
Clofazamine	0·186 (0·025, 1·415)	0·104		0·553 (0·057, 5·328)	0·609
Dapsone	1·219 (0·453, 3·281)	0·695		-	-
Ethambutol	1·302 (0·481, 3·525)	0·603		0·855 (0·085, 8·628)	0·895
Ethionomide	1·002 (0·363, 2·762)	0·997		0·258 (0·027, 2·494)	0·242
Isoniazid	0·347 (0·111, 1·088)	0·069		1·099 (0·154, 7·815)	0·925
Kanamycin	1·784 (0·614, 5·186)	0·287		1·723 (0·178, 16·66)	0·639
Ofloxacin	0·814 (0·295, 2·246)	0·692		0·4 (0·041, 3·857)	0·428
Ofloxacin + Moxifloxacin	1·232 (0·162, 9·375)	0·84		-	-
Pyrazinamide	1·076 (0·346, 3·343)	0·899		0·437 (0·061, 3·136)	0·41
Para-aminosalicylic acid	-	-		-	-
High dose Isoniazid	0·438 (0·124, 1·542)	0·198		1·392 (0·196, 9·915)	0·741
Moxifloxin	0·64 (0·145, 2·826)	0·556		0·601 (0·062, 5·783)	0·659

Table S4: Cox proportional hazards regression models of factors associated (univarate analysis) with risk of death in all patients and in HIV-infected patients only.

	Full cohort (n = 107)			**HIV +ve (n = 44)**	
Characteristic	**Hazard ratio (95% CI)**	**p-value**		**Hazard ratio (95% CI)**	**p-value**
HIV +ve	1·341 (0·856, 2·098)	0·2			
HIV +ve ART	1·088 (0·664, 1·782)	0·739	CD4*	0·998 (0·995, 1·001)	0·179
HIV +ve no ART	3·737 (1·791, 7·791)	< 0·0001	ART	0·353 (0·161, 0·776)	0·01
Gender	0·676 (0·435, 1·051)	0·082		0·606 (0·298, 1·235)	0·168
Race	1·15 (0·736, 1·797)	0·541		0·806 (0·349, 1·861)	0·614
Positive trt outcome	0·032 (0·004, 0·228)	0·001		-	0·997
ADR	1·114 (0·665, 1·868)	0·682		0·83 (0·384, 1·793)	0·635
Net conversion	0·186 (0·081, 0·428)	< 0·0001		0·164 (0·049, 0·547)	0·003
Net reversion	0·487 (0·256, 0·924)	0·028		0·461 (0·11, 1·929)	0·289
Cavitation	1·641 (0·666, 4·043)	0·282		1·67 (0·369, 7·553)	0·505
Bilateral disease	0·761 (0·311, 1·861)	0·549		1·155 (0·147, 9·052)	0·891
Strain typing	0·975 (0·409, 2·321)	0·954		1·363 (0·373, 4·978)	0·639
Smoking	1·129 (0·693, 1·842)	0·626		1·275 (0·593, 2·74)	0·534
Cohort - Northern Cape	0·893 (0·477, 1·672)	0·724		1·583 (0·361, 6·938)	0·542
Cohort - Siswe	0·526 (0·287, 0·965)	0·038		0·644 (0·308, 1·347)	0·242
Weight <50kg***	2·699 (1·461, 4·983)	0·002		4·992 (1·87, 13·316)	0·001
History of TB (confirmed)	1·138 (0·358, 3·622)	0·827		1·198 (0·284, 5·053)	0·806
History of TB (unknown)	1·429 (0·319, 6·398)	0·641		1·694 (0·31, 9·272)	0·543
History or MDR-TB**	0·942 (0·433, 2·052)	0·881		0·871 (0·358, 2·117)	0·76
Weight at XDR diagnosis	0·96 (0·936, 0·985)	0·002		0·963 (0·93, 0·997)	0·033
Number of Drugs	0·964 (0·879, 1·058)	0·439		0·917 (0·752, 1·117)	0·39
Age at diagnosis	0·977 (0·957, 0·998)	0·032		0·934 (0·887, 0·983)	0·01
Amikacin	0·904 (0·284, 2·881)	0·864		1·034 (0·245, 4·362)	0·963
Amoxil	0·864 (0·548, 1·363)	0·529		0·447 (0·183, 1·091)	0·077
Augmentin	1·01 (0·636, 1·603)	0·968		1·16 (0·586, 2·298)	0·671
Azithromycin	0·448 (0·164, 1·226)	0·118		0·311 (0·095, 1·021)	0·054
Capreomycin	0·696 (0·334, 1·449)	0·332		0·675 (0·235, 1·939)	0·465
Clarthromycin	0·935 (0·557, 1·568)	0·798		1·415 (0·672, 2·977)	0·361
Clofazamine	0·428 (0·214, 0·858)	0·017		0·532 (0·239, 1·182)	0·121
Dapsone	1·182 (0·759, 1·84)	0·46		1·016 (0·471, 2·188)	0·968
Ethambutol	1·524 (0·979, 2·373)	0·062		2·651 (1·311, 5·36)	0·007
Ethionomide	1·218 (0·757, 1·958)	0·416		1·583 (0·766, 3·274)	0·215
Isoniazid	0·486 (0·296, 0·796)	0·004		0·521 (0·247, 1·097)	0·086
Kanamycin	1·065 (0·628, 1·804)	0·816		0·83 (0·319, 2·158)	0·702
Ofloxacin	0·914 (0·582, 1·436)	0·697		0·758 (0·372, 1·543)	0·445
Ofloxacin + Moxifloxacin	1·822 (0·835, 3·975)	0·132		2·856 (1·051, 7·76)	0·04
Pyrazinamide	1·504 (0·844, 2·68)	0·166		1·425 (0·618, 3·287)	0·406
Para-aminosalicylic acid	0·698 (0·336, 1·454)	0·337		0·233 (0·067, 0·816)	0·023
High dose Isoniazid	0·619 (0·362, 1·06)	0·081		0·624 (0·296, 1·314)	0·214
Moxifloxacin	0·855 (0·452, 1·618)	0·63		0·833 (0·387, 1·793)	0·641

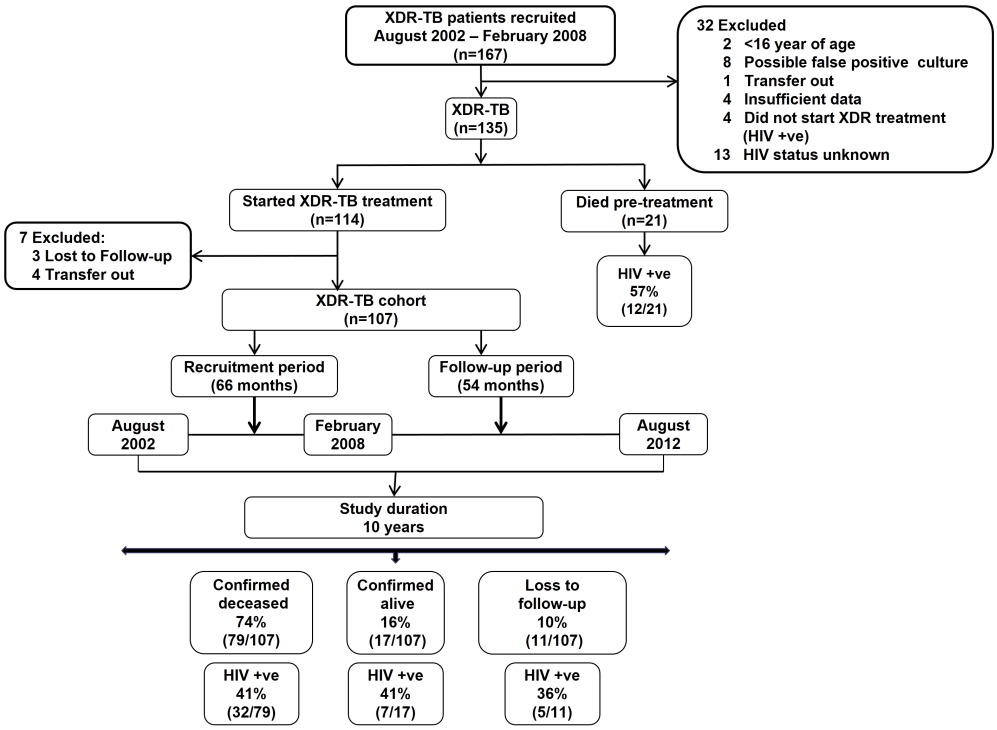
* CD4 was available for 21/44 (48%) HIV +ve; ** Culture proven MDR; *** Weight at diagnosis was available for 68/107 (64%) of cohort

Table S5. Multivariate Cox proportional hazards model for risk of death from treatment start for whole cohort and HIV-infected persons including weight at diagnosis.

	Full cohort (n = 67*)			**HIV +ve (n = 29*)**	
	Hazard ratio (95% CI)	**p-value**		**Hazard ratio (95% CI)**	**p-value**
HIV +ve	1·733 (0·848, 3·543)	0·132	ART	0·044 (0·005, 0·426)	0·007
Gender (Male)	1·553 (0·757, 3·187)	0·229	Gender (Male)	0·34 (0·058, 1·99)	0·231
Race (Coloured)	0·698 (0·335, 1·452)	0·336	Race (Coloured)	0·525 (0·112, 2·45)	0·412
Net converter	0·232 (0·082, 0·654)	0·006	Net converter	0·063 (0·009, 0·424)	0·004
Net reverter	0·391 (0·153, 1·001)	0·05	Net reverter	0·142 (0·007, 2·861)	0·203
Age at diagnosis	0·992 (0·96, 1·025)	0·644	Age at diagnosis	0·912 (0·816, 1·019)	0·105
Weight at diagnosis	0·965 (0·931, 1·001)	0·055	Weight at diagnosis	1·032 (0·962, 1·106)	0·383
Clofazamine	0·149 (0·044, 0·498)	0·002	Clofazamine	0·632 (0·033, 11·941)	0·759
			Azithromycin	2·13 (0·163, 27·827)	0·564
			Para-aminosalicylic acid	0·487 (0·083, 2·872)	0·427
			Ethambutol	3·246 (0·739, 14·268)	0·119
			Ofloxacin + Moxifloxacin	11·803 (1·853, 75·189)	0·009

Figure S5. Study plan indicating follow-up duration and summary of outcomes (1A), and outcomes in patients who survived till hospital discharge (1B).

1A



1B

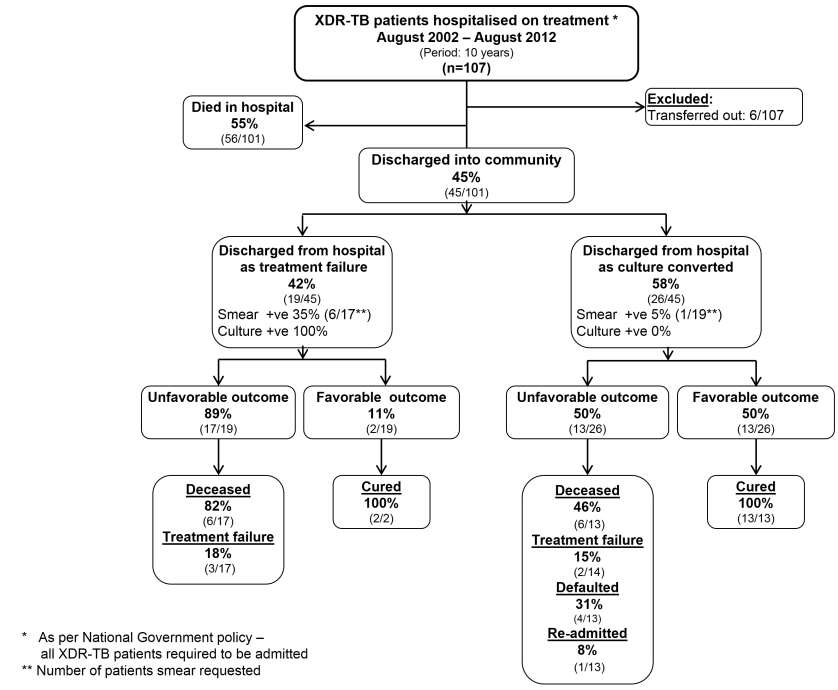
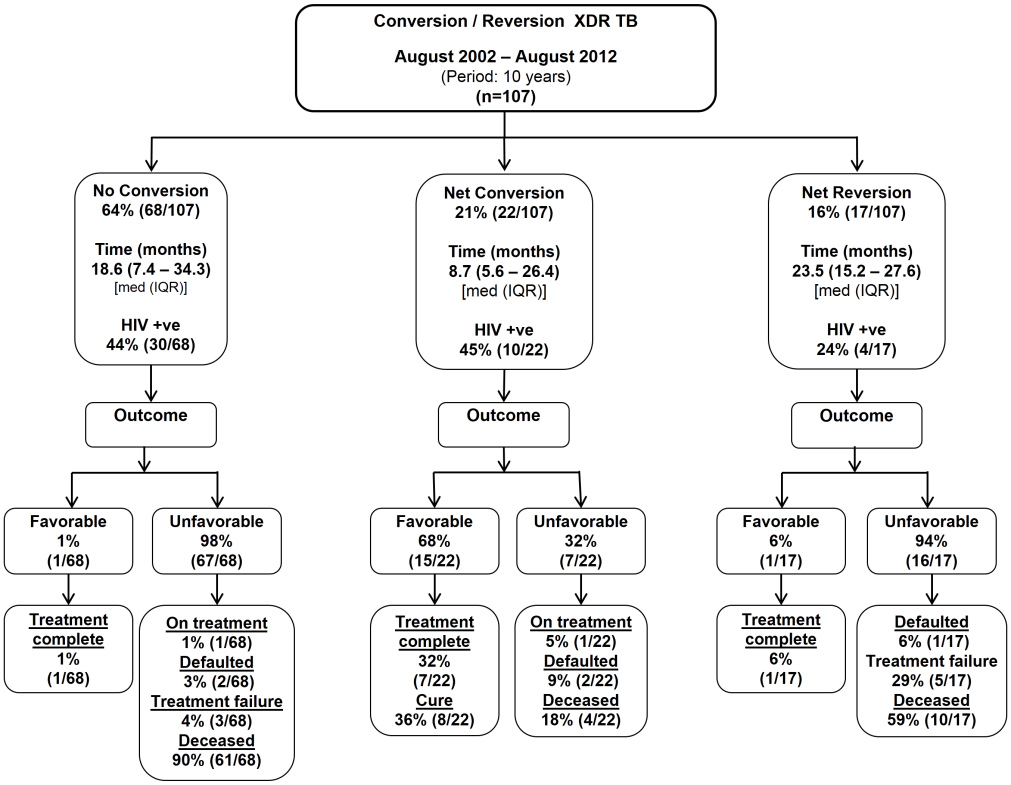


Figure S6. Treatment-related outcomes according to conversion status in 107 patients with XDR-TB during a follow-up period of 120 months



CHAPTER 4: MANAGEMENT OF EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS: TREATMENT, ADVERSE EVENTS AND COST ANALYSIS

MANUSCRIPT 2: High Frequency of Resistance, Lack of Clinical Benefit, and Poor Outcomes in Capreomycin Treated South African Patients with Extensively Drug-Resistant Tuberculosis

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PhD context

Prior to the availability of bedaquiline and linezolid in the National TB Programme (NTP) (until very recently) patients were treated with a capreomycin and/or PAS-based regimen. I wanted to investigate why the outcomes, outlined in chapter 3, were so poor despite a capreomycin and PAS-based regimen. The extent of capreomycin resistance in the South African context, and in other contexts, had not hitherto been reported. The NTP was spending a considerable amount of money on a capreomycin-based regimen. A 6-month capreomycin-based regimen cost approximately R15000, [Appendix 3 [4]] not to mention the painful injections that patients received and level of toxicity associated with capreomycin [Appendix 2 [85]]. However, it was unclear whether capreomycin was conferring any benefit? It is well known that there is significant cross-resistance between capreomycin and aminoglycosides but capreomycin achieves high serum concentration relative to MIC, and thus it was unclear whether capreomycin had therapeutic benefit despite in-vitro resistance. I thus investigated the frequency of capreomycin resistance in XDR-TB isolates, and also evaluated whether there was any therapeutic benefit of capreomycin despite microbiological resistance. These data informed

decisions by the NTP to move away from a capreomycin-based regimen. It also explained, in part, why the outcomes were so poor.

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RESEARCH ARTICLE

High Frequency of Resistance, Lack of Clinical Benefit, and Poor Outcomes in Capreomycin Treated South African Patients with Extensively Drug-Resistant Tuberculosis

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Abstract

Background

There are limited data about the epidemiology and treatment-related outcomes associated with capreomycin resistance in patients with XDR-TB. Capreomycin achieves high serum concentrations relative to MIC but whether capreomycin has therapeutic benefit despite microbiological resistance remains unclear.

Methods

We reviewed the susceptibility profiles and outcomes associated with capreomycin usage in patients diagnosed with XDR-TB between August 2002 and October 2012 in two provinces of South Africa. Patients whose isolates were genotypically tested for capreomycin resistance were included in the analysis.

Results

Of 178 XDR-TB patients 41% were HIV-infected. 87% (154/178) isolates contained a capreomycin resistance-conferring mutation [80% (143/178) *rrs* A1401G and 6% (11/178) were heteroresistant (containing both the *rrs* A1401G mutation and wild-type sequences)]. Previous MDR-TB treatment, prior usage of kanamycin, or strain type was not associated with capreomycin resistance. 92% (163/178) of XDR-TB patients were empirically treated with capreomycin. Capreomycin resistance decreased the odds of sputum culture conversion. In capreomycin sensitive and resistant persons combined weight at diagnosis was the only independent predictor for survival (p=<0.001). By contrast, HIV status and use of

co-amoxicillin/clavulanic acid were independent predictors of mortality (p=<0.05). Capreomycin usage was not associated with survival or culture conversion when the analysis was restricted to those whose isolates were resistant to capreomycin.

Conclusion

In South Africa the frequency of capreomycin conferring mutations was extremely high in XDR-TB isolates. In those with capreomycin resistance there appeared to be no therapeutic benefit of using capreomycin. These data inform susceptibility testing and the design of treatment regimens for XDR-TB in TB endemic settings.

Introduction

Multi-drug resistant tuberculosis (MDR-TB) is a burgeoning problem worldwide with an estimated ~480 000 cases recorded globally in 2014 [1]. About 5–10% of cases of MDR-TB have extensively drug-resistant TB (XDR-TB) and some strains have evolved to resistance beyond XDR-TB (XXDR-TB or totally drug-resistant TB) [2–4]. Treating drug-resistant TB consumes almost 45% of the total budget of the South African National TB Programme (NTP) [5] and this scenario has the potential to destabilise successful TB treatment programmes in many high burden countries. Initial optimism about reasonably good outcomes [6, 7] have been supplanted by more dismal data from high burden setting(s) [8–12], indicating a high mortality and culture conversion rates of less than 20%. The factors underpinning the poor outcomes in high burden settings compared to intermediate burden settings, are not well understood.

Patients with XDR-TB are resistant to four potent anti-TB drugs (rifampicin, isoniazid, fluoroquinolones and aminoglycosides) and in South Africa, resistance to the latter two drugs is mostly acquired (i.e. a high proportion of cases have been infected with a circulating MDR-TB strain). This in part is due to a weakened MDR-TB regimen because of the unrecognized high level of ethionamide resistance [13]. Given that alternative drugs like linezolid are not available to resource poor national TB programmes, therapeutic options are severely limited, and capreomycin forms the backbone of a presumed effective empiric regimen. Although capreomycin has been used since 2006 in South Africa, capreomycin susceptibility testing only became more widely available after 2010 and thus the overall levels of resistance to this drug, despite empiric use, has been poorly studied [14, 15]. Given the above-mentioned considerations we reasoned that capreomycin resistance might be significant, be associated with prior aminoglycoside usage, and may explain the poor treatment outcomes [16, 17]. Furthermore, given that peak serum levels attained with capreomycin are well above the minimum inhibitory concentration (MIC) [18, 19], we hypothesised that capreomycin could still have a therapeutic benefit despite the presence of the *rrs* A1401G mutation conferring *in vitro* resistance, according to the WHO defined critical concentration (2.5ug/ml) in MGIT media [20]. By contrast, lack of benefit is also likely to inform patient management as we recently showed that capreomycin is a toxic drug with significant morbidity and mortality [21], and an expensive drug that may be inappropriately diverting resources away from effectively functioning segments of the NTP [5]. Thus, defining the context-specific risk-benefit ratio of capreomycin is critical. Such data also inform advocacy efforts to accelerate the development of new anti-TB drugs and trial of immunotherapeutic options in patients with XDR-TB.

To address these unanswered questions, we reviewed the susceptibility profiles, associated risk factors, and treatment outcomes of patients with XDR-TB in whom bio-banked isolates were available for *rrs* genotyping.

Materials and Methods

Setting and patients

We retrospectively reviewed the case records of 310 patients (>18 years) with culture proven XDR-TB diagnosed between August, 2002 and October 2012 at two of nine dedicated provincial facilities for the treatment of XDR-TB in South Africa. Data including regimens, treatment start and stop dates, adverse-events, and treatment outcomes were recorded

Definitions and diagnosis of MDR-TB, Pre-XDR TB and XDR-TB

Pre-XDR TB is defined as resistance to rifampicin, isoniazid and either a fluoroquinolone or a second line injectable drug (amikacin, kanamycin or capreomycin). Standard definitions for MDR-TB and XDR-TB are outlined in the online supplement (S1.1 in S1 Definitions and Methods).

Outcomes

Early treatment outcomes were sputum culture conversion and reversion and late treatment outcomes were treatment cure/completion, death, default, treatment failure or transfer out. Death was the primary outcome measure in this study. Culture conversion was defined as two consecutive negative sputum cultures at least 30 days apart. Culture reversion was defined as two consecutive positive sputum cultures at least 30 days apart after initial sputum culture conversion. Death was all-cause mortality, not necessarily secondary to TB progression.

Drug susceptibility testing

Isolates underwent routine phenotypic drug susceptibility testing (DST) (rifampicin, isoniazid, ofloxacin, amikacin and ethionamide) in the centralised NTP-designated reference laboratory (NHLS) as previously described [22]. Drug susceptibility testing to terizidone, fluroquinolones other than ofloxacin and para-aminosalicylic acid is unavailable within the provincial laboratories. Targeted DNA sequencing of the *inhA* promoter and the *katG*, *rpoB*, *embB*, *pncA*, *gyrA*, and *rrs* genes was used to identify mutations conferring resistance [2]. Based on data from a previous study about the frequency of mutations conferring phenotypic resistance to capreomycin in the *rrs* gene (A1401G, G1484T) and *tylA* gene in clinical MDR-TB, Pre-XDR TB and XDR-TB isolates from the Eastern Cape, South Africa, the *rrs* A1401G mutation was selected to identify genotypic resistance to capreomycin [15]. Thus, isolates harbouring this mutation, including heteroresistance (presence of both the *rrs* A1401G mutation and wild-type *rrs* sequences), were designated as resistant. Targeted DNA sequencing of the *rrs* gene was used to identify the *rrs* A1401G mutation [15] (See online supplement (S1.2 in S1 Definitions and Methods) for full details). DST of the clinical isolates against capreomycin was carried out according to the standard proportion method on Middlebrook 7H10-agar as suggested by the National Committee on Clinical Laboratory Standards at a critical concentration of 10.0 μg/ml [23]. Stock solutions of the drug were prepared in distilled water and sterilised by filtration through a Millex-GV 108 syringe-driven filter with a membrane pore size of 0.22 μm. Aliquots of stock solutions were then stored at -80°C in screw-cap polypropylene cryovials up to 6 months. Further dilutions were made in sterile distilled water as required.

MIC testing against capreomycin was done using the MGIT 960 system with EpiCenter TB eXiST software on a subset of isolates which were susceptible to capreomycin according to the standard proportion method. A MIC equal to the critical concentration was reported as susceptible, while MICs $\geq$2.5 μg/ml [15] was considered resistant [18, 20]. More detailed methods are outlined in the online supplement (S1.3 in S1 Definitions and Methods).

Molecular epidemiology

A subset of 126 isolates from patients from the Western Cape Province was genotyped by use of spoligotyping [22].

Statistical analysis

We compared categorical variables by use of the χ^2 or Fisher exact test where appropriate, and we compared continuous variables using the Mann-Whitney U or Kruskal-Wallis tests. Univariate and multivariate logistic regression analysis was used to control for confounding and identify associates of capreomycin genotypic resistance, XDR-TB mortality and sputum culture conversion. See online supplement (S1.4 in S1 Definitions and Methods) for further details.

Ethics Statement

Ethical approval was obtained from the University of Cape Town human research ethics committee. Patient information was anonymised and de-identified prior to analysis.

Results

Study cohort and demographic data

Between August 2002 and October 2012 57% (178/310) of the XDR-TB patients who were commenced on treatment were genotyped for the *rrs* A1401G mutation. No differences in the age, gender, ethnicity and HIV serostatus was noted between genotyped included and non-genotyped excluded XDR-TB patients (data not shown). The median age of genotyped patients was 33 years (IQR 27–41 years), 55% were male, 47% were of mixed ancestry and the median weight at diagnosis was 50.4kg (Table 1). 41% of the cohort was HIV-infected with a median CD4 count of 193 (IQR 99–379 cells/mm^3) and 87% were receiving antiretroviral therapy (ART) at XDR-TB diagnosis. Of the 126 isolates spoligotyped, 83% were the Beijing genotype (Table 1). 60% had a previous diagnosis of MDR-TB and 13% were Pre-XDR TB. 67% of the cohort had previous exposure to either amikacin or kanamycin. The median total number of drugs in the regimen was 8 (IQR 7–9) and 92% of regimens were inclusive of capreomycin (Table 1). Capreomycin resistance as per the presence of the *rrs* A1401G mutation was present in the isolates from 87% (154/178) of patients who were predominantly HIV-uninfected [58% (90/154)] (Fig 1). The demographic profile and clinical characteristics were not significantly different when comparing isolates from patients with *rrs* A1404G mutation compared to wild type (Table 1).

Capreomycin susceptibility data

We compared capreomycin phenotypic (via agar proportions method) and genotypic susceptibility for 51% (91/178) of isolates (Table 2). There was concordance for capreomycin susceptibility in 79% (72/91) of isolates. 16 of the 19 discordant isolates were available for further analysis and discordance was consolidated using the MGIT 960 system (MIC shown in Table 3). 100% of these isolates, excluding three with contaminated or non-viable MGIT 960 cultures, were reclassified as resistant (Table 3). Resistance in the 3 isolates found to be

Table 1. Demographic profile, clinical characteristics and treatment related outcomes in patients with XDR-TB stratified by *rrs* A1401G mutation status.

Patient characteristic	All (N = 178) n (%)	*rrs* A1401G mutation (N = 154) n (%)	*rrs wild type* (N = 24) n (%)	P-value
Demographics				
Age, yrs, median (IQR)	33 (27–41)	33 (26–41)	31 (28–40)	n/s
Male	97 (55)	85 (55)	12 (50)	n/s
Mixed ancestry	84 (47)	72 (47)	12 (50)	n/s
HIV-infected	73 (41)	64 (42)	9 (38)	n/s
CD4 count, cells/μl, median (IQR) #	193 (99–379)	193 (99–365)	213 (112–486)	n/s
Receiving anti-retroviral therapy at diagnosis	61/70 (87)	52/61 (85)	9/9 (100)	n/s
Weight at diagnosis, kgs, median (IQR)	50.4	50.3	50.9	n/s
	(44.4–60)	(44.4–61.1)	(44–57.9)	
TB history—sputum culture proven				
Previous MDR-TB	103 (60)	85 (57)	18 (75)	n/s
Previous Pre-XDR TB†	23 (13)	21 (14)	2(8)	n/s
TB strain				
Beijing genotype strain	105/126 (83)	93/109 (85)	12/17 (71)	n/s
Treatment exposure—ever				
SLID§	119 (67)	101 (68)	18 (75)	n/s
Capreomycin	163 (92)	141 (92)	22 (92)	n/s
Ofloxacin	110 (62)	97 (63)	13 (54)	n/s
Moxifloxacin	29 (16)	26 (17)	3 (13)	n/s
Number of drugs in regimen, median (IQR)	8 (7–9)	8 (7–9)	8 (7–9)	n/s
XDR-TB treatment outcomes ¶				
Converted	53 (31)	42 (28)	11 (42)	n/s
Reverted	18/53 (34)	16/42 (38)	2/11 (19)	n/s
Mortality	93 (53)	78 (52)	15 (63)	n/s

#4/73 HIV-infected patients missing CD4 cell count data and 3/73 patients missing ARV data.

†Previous Pre-XDR TB is defined as MDR-TB plus resistance to either FQ or second line injectable drugs.

§SLID: Second-line injectable drug (either kanamycin or amikacin).

¶4/178 and 3/178 missing accurate conversion and mortality data respectively.

Abbreviations: IQR: interquartile range, n/s: not significant (p = >0.05), n/c: not calculated.

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phenotypically resistant with no *rrs* A1401G mutation present could potentially be explained by mutations outside the *rrs* region (unknown mechanisms conferring resistance) or an underlying population not detected by DNA sequencing.

Relationship between capreomycin susceptibility and treatment-related outcomes

The overall cohort mortality was 53% (93/175) with three patients missing data, culture conversion was 31% (53/174) with four patients missing data, and culture reversion was 34% (18/53 patients that converted). 92% (141/154) of isolates with the *rrs* A1401G mutation received capreomycin as part of their XDR-TB regimen while 92% (22/24) of patients with wild type *rrs* isolates received capreomycin (Fig 1). When not stratified by capreomycin treatment status, there were no significant differences in the above-mentioned short-term clinical outcomes between those with an isolate with an *rrs* A1404G mutation versus those with the wild type *rrs*

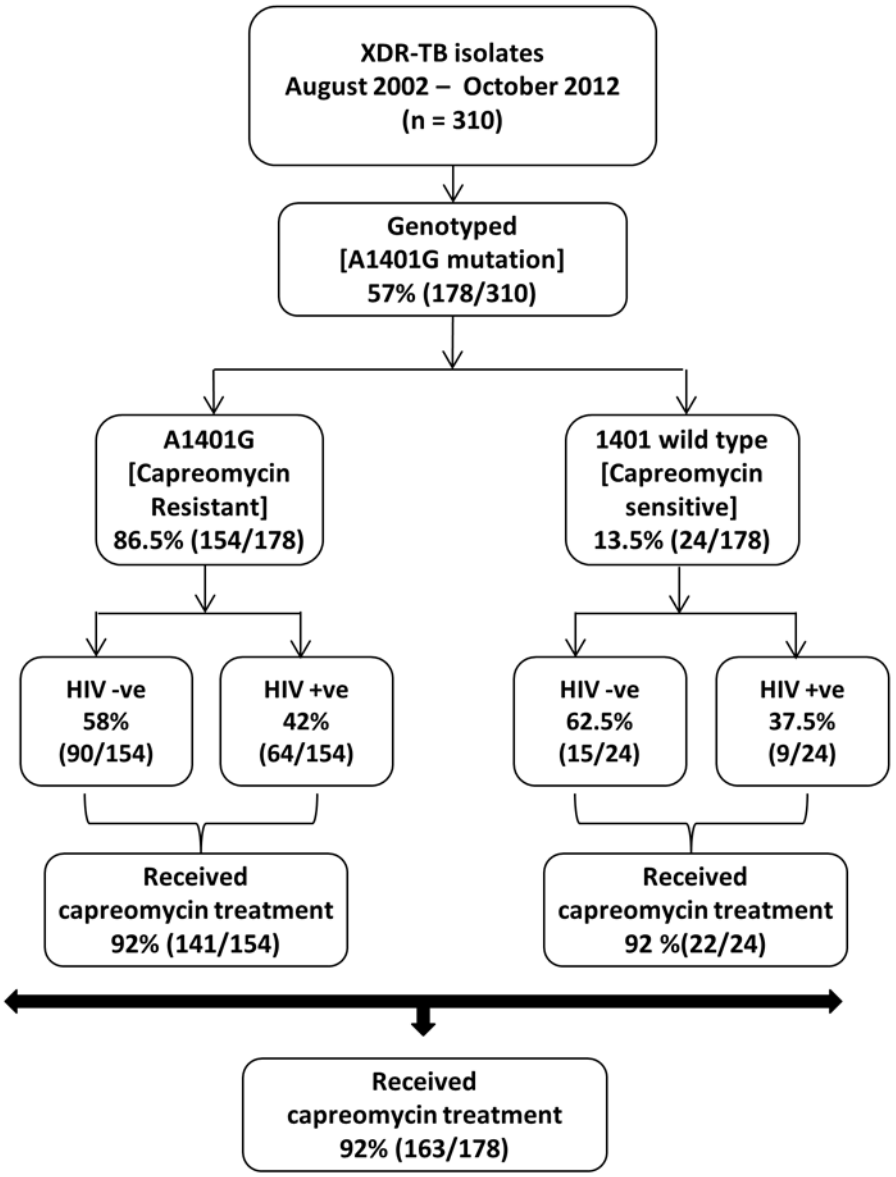

Fig 1. Study plan showing the relationship between capreomycin genotypic susceptibility profile (wild type, *rrs* A1401G mutation), HIV status, and proportion of participants who received capreomycin.

doi:10.1371/journal.pone.0123655.g001

genotype. (Table 1) However, in an analysis uncorrected for potential confounders, the mortality was significantly higher in patients (resistant and sensitive capreomycin genotype combined) that did not receive capreomycin treatment compared to those that did [86% (12/14) versus 50% (81/161), p = 0.01]. Restricted to patients with genotypic resistance mortality was also higher in patients who did not receive capreomycin compared with those who did [83% (10/12) versus 49% (68/139) p = 0.022] (Table 4; there were 2 patients with missing data).

Sputum culture conversion and reversion was not significantly different in any of the capreomycin-specific susceptibility categories irrespective of capreomycin treatment (Table 4).

Table 2. Comparison of capreomycin phenotypic and genotypic drug susceptibility.

Phenotypic DST[ζ]	Capreomycin genotype		Total
	rrs A1401G	*rrs* wild type	
Sensitive	16[†]	10	26
Resistant	62	3*	65
Total	78	13	91

[ζ]Phenotypic DST on Middlebrooks 7H10-agar was performed using the current critical concentration for capreomycin of 10μg/ml (1). Phenotypic results were only available for 91/178 patients with *rrs* genotyping results.

[†]16/19 discordant isolates that were available were re-tested using MGIT (see Table 3).

*3/19 discordant isolates may be due to mutations outside the *rrs* region or an underlying population not detected by DNA sequencing.

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Impact of strain type and HIV co-infection on capreomycin resistance and treatment outcomes

Strain type by spoligotyping and HIV infection were not associated with capreomycin genotypic resistance (Table 1). Mortality in HIV-infected patients was significantly higher than in HIV-uninfected patients [62% (44/71) versus 47% (49/104) p = 0.05]. Mortality in HIV-infected patients whose isolates were capreomycin resistant was not significantly different when compared to isolates from HIV-uninfected patients. Sputum culture conversion did not vary significantly, when stratified by HIV sero-status and when comparing HIV-infected patients whose isolates were capreomycin resistant versus those whose isolates were capreomycin sensitive.

Table 3. Minimum inhibitory concentrations (MIC) using the MGIT 960 phenotypic drug-susceptibility method for initially genotypic resistant, phenotypic sensitive (agar-based) discordant capreomycin isolates.

rrs genotype	Initial phenotypic classification using ECOFF for capreomycin (10μg/ml) on 7H10 agar	MGIT 960 MIC (μg/ml)	Phenotypic reclassification
A1401G	S	10	R
A1401G/A	S	5	R
A1401G	S	>10	R
A1401G	S	5	R
A1401G	S	Contaminated	n/r
A1401G	S	5	R
A1401G	S	10	R
A1401G	S	5	R
A1401G	S	5	R
A1401G	S	5	R
A1401G	S	5	R
A1401G	S	10	R
A1401G	S	5	R
A1401G	S	Contaminated	n/r
A1401G	S	5	R

MGIT: Microscopic growth in-tube (BD Biosciences, USA); S: Sensitive, R: Resistant, n/r: no result

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Table 4. Mortality, sputum culture conversion and sputum culture reversion in XDR-TB patients classified by capreomycin genotype and treatment status.

Capreomycin genotype	Mortality (n/N, %)	Sputum culture conversion (n/N, %)	Sputum culture reversion (n/N, %)
Capreomycin treatment given (N = 163)			
Resistant and sensitive combined (n = 163)	81/161 (50.3)* [1]	50/163 (30.7)	17/50 (34.0)
rrs A1401G wild type (sensitive) (n = 22)	13/22 (59.1)	10/22 (45.5)	1/10 (10.0)
rrs A1401G (resistant) (n = 141)	68/139 (48.9)* [2]	40/141 (28.4)	16/40 (40.0)
No capreomycin treatment given (N = 15)			
Resistant and sensitive combined (n = 15)	12/14 (85.7)* [1]	3/11 (27.3)	1/3 (33.3)
	*1p = 0.011		
rrs A1401G wild type (sensitive) (n = 2)	2/2 (100.0)	1/2 (50.0)	1/1 (100.0)
rrs A1401G (resistant) (n = 13)	10/12 (83.3)* [2]	2/9 (22.2)	0/2 (0)
	*[2]p = 0.022		

*p-values are for χ^2 testing between proportions for different capreomycin treatment status but similar genotypic DST results. Only significant p-values shown (p = <0.05).

[1] mortality in all patients (resistant and sensitive) treated and not treated with capreomycin

[2] mortality only in patients with *rrs* A1404G mutation treated and not treated with capreomycin

The following numbers of patients were missing mortality outcome data: i) 2/141 of isolates from patients with *rrs* A1401G mutations receiving capreomycin treatment, and ii) 1/13 isolates from patients with *rrs* A1401G mutations not receiving capreomycin treatment. 4/13 isolates from patients with *rrs* A1401G mutations not receiving capreomycin treatment are missing sputum conversion data.

doi:10.1371/journal.pone.0123655.t004

Multivariate analysis and factors associated with outcomes

Mortality. In a multivariate analysis looking at predictors of mortality in the genotyped study population, only weight at diagnosis [OR 0.935 (95% CI 0.902–0.969), p = <0.001] and HIV infection [OR 2.9 (95% CI 1.3–6.3), p = 0.007] was associated with decreased and increased odds of mortality, respectively. Neither capreomycin genotype resistance (p = 0.32) nor capreomycin treatment (p = 0.16) had a significant impact controlling for confounders. Similar significant predictors of mortality were noted when restricting the multivariate analysis to i) only patients receiving capreomycin treatment and ii) only patients with capreomycin resistance, with the addition of co-amoxicillin/clavulanic acid treatment as a significant predictor of mortality in both sub-group analyses (Table 5).

Culture conversion. In the genotyped study population weight at diagnosis [OR 1.063 (95% CI 1.027–1.101), p = 0.001] was associated with an increased odds of sputum culture conversion. By contrast, capreomycin resistance [OR 0.64 (95% CI 0.11–0.68), p = 0.007] and previous MDR-TB treatment [OR 0.45 (0.21–0.97), p = 0.04] were associated with decreased odds of sputum culture conversion. Capreomycin in the regimen and usage of ≥8 drugs was not significantly associated with culture conversion. Restricting the analysis to only those receiving capreomycin treatment, weight at diagnosis [OR 1.059 (95% CI 1.023–1.097), p = 0.001] was associated with an increased odds of sputum culture conversion; Capreomycin resistance [OR 0.28 (95% CI 0.10–0.78), p = 0.02] was associated with a decreased odds of culture conversion (Table 6).

On multivariate analysis, strain type, CD4 count and use of ART (the latter 2 co-variates included in HIV-infected individuals only) did not impact the outcomes of mortality and culture conversion.

Table 5. Multivariate logistic regression analysis for predicators† of XDR-TB mortality in the study cohort with resistance to *rrs* A1401G.

	rrs A1401G mutation present versus *rrs* wild type	
Variable	**Odds ratio (95% CI)**	**P-value**
All XDR-TB patients (n = 158/178)*		
Weight at diagnosis (kgs)	0.935 (0.902–0.969)	<0.001
HIV-infected	2.9 (1.34–6.3)	0.007
Capreomycin *rrs* resistance (A1401G mutation	0.59 (0.21–1.65)	0.32
TB drugs impacting mortality		
Capreomycin	0.27 (0.04–1.67)	0.16
Moxifloxacin	0.39 (0.14–1.05)	0.06
Co-amoxicillin/clavulanic acid	3.1 (1.4–6.6)	0.004
Only XDR-TB patients treated with capreomycin (n = 151/163)*		
Weight at diagnosis (kgs)	0.94 (0.91–0.98)	0.001
HIV-infected	2.7 (1.2–5.9)	0.01
Capreomycin *rrs* resistance (A1401G mutation	0.64 (0.22–1.83)	0.4
TB drugs impacting mortality		
Moxifloxacin	0.4 (0.15–1.11)	0.08
Co-amoxicillin/clavulanic acid	3.2 (1.4–7.2)	0.004
Capreomycin resistant (defined by *rrs* A1401G mutation) n = 136/154)*		
Weight at diagnosis (kgs)	0.950 (0.917–0.984)	0.005
HIV-infected	2.6 (1.2–5.8)	0.02
TB drugs impacting mortality		
Capreomycin	0.28 (0.04–1.87)	0.19
Moxifloxacin	0.49 (0.18–1.32)	0.16
Co-amoxicillin/clavulanic acid	3.3 (1.5–7.1)	0.003
Only *rrs* wild type (capromycin sensitive) (n = 21/24)*		
Weight at diagnosis (kgs)	0.758 (0.589–0.976)	0.03
HIV-infected	22.3 (0.29–1698.2)	0.16
TB drugs impacting mortality		
Moxifloxacin	0.01 (0.00–12.7)	0.2
Co-amoxicillin/clavulanic acid	6.9 (0.26–181.5)	0.25

†A priori variables included in both the univariate and mulitivariate analysis included i) Demographic and clinical: Age, gender, ethnicity, weight at diagnosis, HIV status, CD4 cell count and ART (in HIV-infected), previous MDR-TB treatment, previous pre-XDR diagnosis; ii) *M. tuberculosis* strain typing and drug-susceptibility testing: Beijing/non-Beijing strain, phenotypic DST for second line injectables (amikacin, kanamycin, capreomycin, streptomycin), ofloxacin, ethambutol, ethionamide, and capreomycin genotypying for *rrs* A1401G mutant, iii) XDR-TB drug treatments: amikacin, kanamycin, capreomycin, ciprofloxacin, ofloxacin, moxifloxacin, co-amoxicillin/clavulanic, ethambutol, ethionamide, pyrazinamide, PAS, clofazime, dapsone, thioacetone, terizidone/cycloserine.

*The total numbers of observations included in each of the multivariate outputs. Numbers differ from previous patient group totals due to missing data. Multiple imputation was not used.

doi:10.1371/journal.pone.0123655.t005

Discussion

There are currently limited data on the frequency and factors associated with capreomycin resistance in high burden settings. The key findings of our study were (i) a high rate (87%) of capreomycin resistance in capreomycin-naive patients with XDR-TB despite the drug not

Table 6. Multivariate logistic regression analysis for predicators† of XDR-TB sputum culture conversion in the study cohort with resistance to *rrs* A1401G.

	rrs A1401G mutation present versus *rrs* wild type	
Variable	**Odds ratio (95% CI)**	**P-value**
All XDR patients (n = 160/178)*		
Weight at diagnosis (kgs)	1.063 (1.027–1.101)	0.001
Previous MDR-TB treatment	0.45 (0.21–0.97)	0.04
HIV-infected	0.93 (0.43–2.01)	0.86
Capreomycin *rrs* resitance	0.64 (0.11–0.68)	0.007
Capreomycin	0.64 (0.12–3.50)	0.6
Only XDR patients treated with capreomycin (n = 153/163)*		
Weight at diagnosis (kgs)	1.059 (1.023–1.097)	0.001
Previous MDR-TB treatment	0.48 (0.22–1.03)	0.06
HIV-infected	0.92 (0.42–2.01)	0.84
Capreomycin *rrs* resitance	0.28 (0.10–0.78)	0.02

†A priori variables included in both the univariate and mulitivariate analysis included i) Demographic and clinical: Age, gender, ethnicity, weight at diagnosis, HIV status, CD4 cell count and ART (in HIV-infected), previous MDR-TB treatment, previous pre-XDR diagnosis; ii) *M. tuberculosis* strain typing and drug-susceptibility testing: Beijing/non-Beijing strain, phenotypic DST for second line injectables (amikacin, kanamycin, capreomycin, streptomycin), ofloxacin, ethambutol, ethionamide, and capreomycin genotypying for *rrs* A1401G mutant, iii) XDR-TB drug treatments: amikacin, kanamycin, capreomycin, ciprofloxacin, ofloxacin, moxifloxacin, co-amoxicillin/clavulanic, ethambutol, ethionamide, pyrazinamide, PAS, clofazime, dapsone, thioacetone, terizidone/cycloserine.

*The total numbers of observations included in each of the multivariate outputs. Numbers differ from previous patient group totals due to missing data. Multiple imputation was not used.

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previously being used in this population, (ii) capreomycin usage in patients whose isolates were resistant to the drug produced no detectable clinical benefit (conversion or mortality); rather, capreomycin resistance was a marker of conversion failure, (iii) capreomycin resistance was not associated with prior aminoglycoside usage, and (iv) despite an intensive in-patient multi-drug regimen treatment-related outcomes of patients with XDR-TB were poor.

In the cohort as a whole capreomycin use appeared to have a survival benefit despite genotypic resistance. However, after correcting for potential confounders, including HIV status, moxifloxacin and co-amoxicillin/clavulanic usage and previous MDR-TB, capreomycin usage was not associated with beneficial treatment-related outcomes (mortality and culture conversion) both overall and in those that were capreomycin resistant. We have recently shown that capreomycin toxicity is associated with serious morbidity and mortality in patients with XDR-TB [21]. All the drug-related adverse-event deaths were due to capreomycin (renal failure) and capreomycin made up more than half of all the drugs withdrawn [21]. Our data suggest, given the substantial costs of the drug and attendant toxicity, that capreomycin DST be routinely implemented in the NTP and those resistant to capreomycin should not be given the drug. Given that capreomycin is universally used in XDR-TB regimens in South Africa, this will also enable the channelling of substantial resources to more effective drugs like linezolid. Cost awareness is critical as we recently showed that DR-TB, despite comprising <3% of the caseload, already consumes almost 45% of the NTP budget which is not sustainable [5].

In patients with capreomycin sensitive TB, our study was not powered to make a definitive conclusion about mortality or culture conversion, as sample numbers were small. A retrospective

study in a European population found that capreomycin was favourably associated with outcomes in patients with XDR-TB [17]. Although capreomycin is likely to have benefit in such patients, impact may be limited in the African setting as there are few additional effective drugs to which isolates are susceptible. Isolates in our study were already resistant to rifampicin, isoniazid, aminoglycosides and fluoroquinolones, and given the rate of previous MDR-TB, most were also resistant to ethionamide and terizidone (a compound of cycloserine). Thus, even in patients with capreomycin-sensitive TB it is likely that a single potentially effective drug would have limited impact on successful outcomes in patients with extensive lung disease and few other effective drugs to which the isolate is susceptible (linezolid is not an option as this is not available to the national TB programme).

It is unclear why the de novo capreomycin resistance rates in this capreomycin naive population were so high. Capreomycin is known to be associated with aminoglycoside resistance given that mutations conferring resistance to both classes of drugs are encoded by the *rrs* gene [14]. However, capreomycin resistance in this cohort seemed independent of aminoglycoside usage (25% of the cohort did not receive prior aminoglycosides). Thus, it is possible that transmission of capreomycin-resistant strains led to their amplification at the community level in the Western and Northern Cape provinces. We think this hypothesis is tenable given that a high proportion of patients who had no prior aminoglycoside usage also had high rates of capreomycin resistance and that person-to-person spread is the primary modality of transmission in MDR-TB with aminoglycoside resistance (80% of MDR-TB in South Africa is now due to primary transmission) [13].

Our findings on capreomycin susceptibility and associated outcomes were independent of HIV-status though this may represent a type 2 error. Degree of host immunosuppression in HIV-infected patients, as measured by CD4 count, did not appear to be an important risk factor for capreomycin resistance or treatment related outcomes. Similarly, the strain type did not impact results.

There are several limitations to our findings, including those inherent in a study with a retrospective design. There is a selection bias as those without capreomycin drug susceptibility data were excluded from the analysis. However, a sensitivity analysis showed that the excluded population had similar characteristics to those included in the study. A second major limitation is that there were only small numbers of patients who were capreomycin-sensitive and received the drug. Thus, we were underpowered to directly answer this question and we can make limited deductions about how effective the drug is in sensitive versus resistant patients (given survival benefit in the cohort as a whole). Although this is speculative, we feel that, even in capreomycin-sensitive patients, only one active drug is unlikely to have a sustained impact in patients who have extensive disease and where there are no other effective therapeutic options (although these patients also receive drugs like co-amoxicillin/clavulanic acid, clarithromycin and clofazimine however, these are of dubious value).

In conclusion, in this retrospective study we found that there was a high rate of capreomycin resistance, even in a population where there was no prior usage of capreomycin, and also in those who had not received prior aminoglycosides. Further, capreomycin usage in those resistant to capreomycin did not produce any beneficial therapeutic effect. Collectively, these data suggest that rapid genotypic testing (e.g Genotype®MTBD*sl* that is available) should be routinely made available to the South African NTP, and that capreomycin, given its costs and toxicity profile, should not be used for perceived therapeutic benefit in patients resistant to the drug (in contradistinction to current practice). Additional studies in other high burden settings are needed to confirm our findings. These data have important implications for the optimal design of drug regimes in high burden settings, and suggest that capreomycin should not be used in patients who have genotypic resistance to this drug. This may have important implications

for resource allocation and costs borne by national TB programmes in high burden settings. These data also intensify the urgency with which new anti-TB drugs need to be developed.

Supporting Information

S1 Dataset.
(XLSX)

S1 Definitions and Methods. High frequency of resistance, lack of clinical benefit, and poor outcomes in capreomycin treated South African patients with extensively drug-resistant tuberculosis.
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: KD. Performed the experiments: ES PVH FS RW. Analyzed the data: JP. Contributed reagents/materials/analysis tools: RW. Wrote the paper: KD NR EP RW JP MD. Data collection of clinical samples: BM JTR EP.

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S1 - ONLINE SUPPLEMENT: Definitions and methods

High frequency of resistance, lack of clinical benefit, and poor outcomes in capreomycin treated South African patients with extensively drug-resistant tuberculosis.

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S1.1 Definitions and diagnosis of MDR-TB, Pre-XDR TB and XDR-TB

MDR-TB is defined at resistance to rifampicin and isoniazid. Pre-XDR TB is defined as resistance to rifampicin, isoniazid and either a fluoroquinolone or a second line injectable drug (amikacin, kanamycin or capreomycin). In this study patients with 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 drug susceptibility testing was performed in almost all patients; capreomycin susceptibility was only available in a minority of patients) were judged to have extensively drug-resistant tuberculosis (XDR-TB).

S1.2 Genotyping

DNA sequencing of the region encompassing nucleotide 1401 of the *rrs* gene (amplification product nucleotide 1339 to 1528). Briefly, a 200 µl aliquot of the MGIT culture was heat inactivated by incubating at 100°C for 30 min to generate a crude DNA lysate. PCR amplification was done in a reaction mixture containing 2 µl crude DNA template, 5 µl Q-Buffer, 2.5 µl 10 x Buffer, 2 µl 25 mM MgCl2, 4 µl 10 mM dNTPs, 1 µl of the *rrs* primer set (Forward 5'-GTAATCGCAGATCAGCAAC-3' and Reverse 5'- GTGATCCAGCCGCACCTT -3'), 0.125 µl HotStarTaq DNA polymerase (Qiagen, Germany) and made up to 25 µl with dH2O. Amplification was initiated by incubation at 95°C for 15 minutes, followed by 35 - 45 cycles at 94°C for 45 seconds, 62°C for 45 seconds, and 72°C for 45 seconds. After the last cycle, the samples were incubated at 72°C for 10 minutes. To minimize laboratory cross-contamination the preparation of the PCR reaction mixes, the addition of the DNA and the PCR amplification were conducted in physically separated rooms. Negative controls (water) were included to detect reagent contamination. Amplification was confirmed by electrophoretic fractionation in 1% agarose containing TBE pH 8.3. Amplification products were sequenced using the ABI3130XL genetic analyzer and the resulting chromatograms were analysed using Chromas software

S1.3 Capreomycin MICs

MIC testing against capreomycin was done using the MGIT 960 system with EpiCenter TB eXiST software on a subset of isolates which were susceptible to capreomycin according to the standard proportion method. Briefly, the isolates were tested against serial twofold dilutions of capreomycin ranging from 0.125 to 10 µg/ml. *M. tuberculosis* strain H37Rv (ATCC 27294) was included as a capreomycin susceptible control. The MIC was defined as the lowest concentration of drug that inhibited more than 99% of the bacterial population, relative to the 1:100 diluted drug-free controls of the corresponding strains (1% proportional method). A critical concentration of 2.5 µg/ml was adopted to differentiate between capreomycin susceptible and resistant strains as suggested in previous publications (1). A MIC equal to the critical concentration was reported as susceptible, while MICs ≥2.5 µg/ml was considered resistant (2, 3).

S1.4 Statistical analysis

A risk management strategy for data, including double data entry, was used to ensure the integrity of the data. Descriptive statistics were used for the demographic and clinical characteristics of the study population. The genotyped study population was compared with overall XDR-TB cohort to ensure that the sample was representative. We compared categorical variables by use of the χ^2 or Fisher exact test where appropriate, and we compared continuous variables, because of the non-normal distribution of the analysed variables, using the Mann-Whitney U or Kruskal-Wallis tests. Univariate and multivariate logistic regression analysis was used to control for confounding and identify associates of capreomycin genotypic resistance, XDR-TB mortality and sputum culture conversion. All statistical tests were 2-sided at α=0.05. STATA IC, version 11 (Stata Corp, Texas, USA) was used for all statistical analyses.

CHAPTER 5: TREATMENT-RELATED OUTCOMES IN HIV-INFECTED PERSONS WITH PROGRAMMATICALLY INCURALBE TUBERCULOSIS

Chapter 5 is not in the style of a manuscript that has been published or been submitted for publication. This part of the thesis is presented as a traditional chapter.

ABSTRACT

Background: Prospective data about XDR-TB in HIV-infected persons are urgently needed to inform effective treatment and intervention strategies, and for rational allocation of resources in this subgroup.

Methods: 273 South African adult XDR-TB patients from two provinces, diagnosed and treated between October 2008 and October 2012, were followed until October 2014 and evaluated by HIV status. For patients from the Western Cape Province drug susceptibility including a panel of 18 drugs and strain phenotype were investigated.

Findings: All patients were treated with a median of 9 (IQR 8, 10) anti-TB drugs and isolates were resistant to a median of 10 (IQR 8, 11) drugs. The number of drugs used and the frequency of drug resistance did not differ in HIV-infected and uninfected persons. 119/272 (44%) persons (one refused HIV testing) were HIV-infected of whom 114/119 (95.8%) received ART. Median CD4 count at XDR-TB diagnosis was 199 (IQR 85, 323) cells/mm^3. HIV-infected persons had a poorer probability of survival (p=0.02) compared to uninfected persons. Predictors of survival for HIV-infected persons included (i) weight >50kg at XDR-TB diagnosis [HR 0.57 (95% CI 0.34, 0.95) p=0.03] (ii) number of drugs in the XDR-TB regimen [HR 0.78 (95% CI 0.66, 0.92) p=0.003] and (iii) time to culture positivity [HR 0.96 (95% CI 0.93, 0.99) p=0.02]. These were the same factors that predicted survival in the whole cohort (HIV-infected and uninfected). Clinically defined primary XDR-TB was more common in HIV-infected versus uninfected persons [49/119 (41%) vs 42/153 (27.4%), p=0.02]. Overall adverse events rates were high (grade 3-5 adverse events occurred in >40%) but did not differ by HIV status. The Beijing strain phenotype did not differ in HIV-infected compared to uninfected persons. 70/172

(41%) HIV-infected persons were discharged from hospital of whom 38/104 (36.5%) had an unfavourable outcome. Discharge and unfavourable outcomes were similar in HIV-infected and uninfected persons. Probability of survival in home-discharged patients was similar in HIV-infected and uninfected persons, irrespective of whether they had failed treatment. Smear positivity rates at discharge did not differ by HIV status.

Conclusion: Although HIV-infected persons had marginally poorer survival, post-discharge survival was similar for all patients. HIV-infected persons had more clinically defined primary XDR-TB, however adverse event rates, smear positivity at discharge, and percentage Beijing strain were similar in HIV-infected and uninfected patients. However, XDR-TB treatment failure, and hence programmatically incurable TB, is also a problem in HIV-infected persons who have substantial longevity post discharge. The data inform resource allocation and interventional strategies to contain the TB epidemic.

5.1 BACKGROUND AND INTRODUCTION

The double burden of DR-TB and HIV were addressed in Chapter 2. In Chapter 5 I distinguish factors associated with effective treatment and treatment-related outcomes with specific reference to XDR-TB HIV co-infected persons. [Chapter 1, Figure 2: cohort B]. As background I address key elements concerning HIV-infection and XDR-TB.

Worldwide, people living with HIV are 26 times more likely to develop active TB compared to HIV-uninfected patients with LTBI who progress to active TB during the course of their lifetime [3]. By contrast, HIV-infected adults have an estimated 5-15% annual risk of developing active TB in their lifetime compared to HIV-uninfected adults [6, 22, 23].

The worldwide occurrence of XDR-TB in 2006 commanded a global response to the epidemic of HIV and XDR-, which from the start looked like an untreatable form of TB with limited treatment options and prolonged infectious periods [31, 112, 130]. Furthermore, XDR-TB continues to resurface in high burden HIV settings [122, 127]. HIV-infected persons are however at a disadvantage due to the lack of sensitivity and specificity of TB symptom screening, including AFB microscopy and chest radiography [131, 132]. Conversely, the question regarding the level of toxicity of an XDR-TB regimen in persons living with HIV remains ambiguous [6].

HIV should, however, not be presented as an isolated extenuating factor in the upsurge of TB, despite the fact that the deadly XDR-TB outbreak emerged in a HIV epicentre of South Africa

(SA) [112]. Human societal factors, and correspondingly failures, equally played essential roles in the rising of the drug-resistant TB prevalence [133, 134]. Nevertheless, HIV has led to a sevenfold increase in the incidence of TB on the African continent [19, 22].

By the end of 2015 SA had 33% of the global HIV burden [25] the largest collective of people living with HIV globally [26]. Furthermore, TB together with HIV was ranked as the leading causes of death in SA [13, 18]. XDR-TB related mortality was estimated at 47%, compared to 30% globally, which can partly be attributed to high levels of HIV infection in SA [3].

Limited anti-TB drug treatment options for HIV-infected persons, periods of prolonged dual infection, and transmission of DR-TB is cause for concern [31, 112, 130]. However, anti-retroviral therapy (ART) resulted in a ~60% reduction in TB incidence in people living with HIV (PLWH), irrespective of the duration of HIV infection [25]. The use of ART by PLWH is thus not only essential to reduce mortality and morbidity [135-137] but central to population-based HIV and TB HIV-infection treatment and prevention [8, 25, 137, 138].

Overlapping adverse events, and toxicity profiles, between anti-retroviral (ARV) and anti-TB drugs in terms of the safe use of drugs from either regimen remain a concern [30, 78, 139, 140] and the selection of the specific combination of anti-TB and ARV drugs in TB HIV-infected patients become an undertaking. Furthermore, drug-drug interactions could lead to sub-therapeutic drug levels regarding either regimen [30]. While it has been established that a NNTRI-based ART regimen has minimal drug-drug interactions when first-line anti-TB drugs are

prescribed [78] little is known about drug-drug interaction between second-line anti-TB drugs, used to treatment XDR-TB, and ARVs [78]. Additionally, drug-disease interaction resulting from physiological and immunological changes from either disease also needs to be considered [77]. These changes have effects on drug absorption as HIV disease progression could reduce anti-TB drugs absorption. Furthermore, advanced HIV disease can modify drug metabolism [77].

Juxtaposed against benefits of early or concurrent initiation and treatment with ARVs in XDR-TB HIV-infected patients [83] are clinical management considerations that need careful consideration. Factors like uncertainty whether an XDR-TB regimen is more toxic in PLWH, [6] treatment-limiting toxicity from both regimens, increased pill-burden resulting in reduced adherence to either or both XDR-TB and ART regimens, drug-drug interactions and immune reconstitution inflammatory syndrome in XDR-TB HIV-infected patients could threaten treatment success of either disease. Similarly, adherence to ART, and anti-TB drugs, and retention in a health care system is essential as non-adherence can lead to viral rebound, on-going HIV transmission and HIV drug-resistance [25].

ART is associated with the reduction of the incidence of TB in PLWH irrespective of the CD4 count [141] while early initiation of ART, at a CD4 count ≤350 cells/mm^3, improves survival and reduces risk of opportunistic infections [142]. A study conducted in the United Kingdom reported that PLWH, with a CD4 count ≥350 cells/mm^3 and suppressed viral load (VL), have the same life expectancy as the general population [143]. In SA the increase in life expectancy at birth of all people, including PLWH [in males from 52.6 (2002) to 60.6 years (2015) and in

females from 56.4 (2002) to 64.3 years (2015)], could be attributed to the intensified rollout of ART since 2004 [9, 26]. HIV-infected persons who received ART for 24 months have a 15-20% higher life expectancy compared to those who newly started ART [144]. Furthermore, HIV persons in typical urban settings who initiate ART at a CD4 count of ≥200 cells/mm^3 have ~80% of the life expectancy of the overall population [144].

The risk of TB in HIV-infected persons who, despite receiving ART, do not reach optimal virological suppression, or have poor immunological recovery, however, remains unanswered [145-148] and is an important factor to explore in XDR-TB. A potential suboptimal effect of ART [149] is one plausible explanation for the continued burden of TB HIV disease in high-burden countries. One measure of the potential suboptimal effect of ART is UNAIDS recommended estimates regarding median time from HIV infection to death. Estimates for the general population are 10.5 years for South African men and 11.5 years for women [9] while life expectancy of HIV persons who initiated ART is modelled at ~10 years.

In Chapters 3 and 4 we reported poor long-term treatment-related outcomes in XDR-TB patients treated with a WHO recommended capreomycin-based regimen [74, 150]. Treatment outcomes in South African XDR-TB HIV co-infected patients [122] from high HIV burden areas, HIV prevalence in 2012 of 16.9% [10], have been reported. During the same year the prevalence of HIV in the Western Cape and Northern Cape Provinces was 5% and 7.4% respectively [10]. Rational planning of health care resource allocation and intervention strategies, however, similarly requires data from low HIV prevalence settings. To address the scarcity of data related

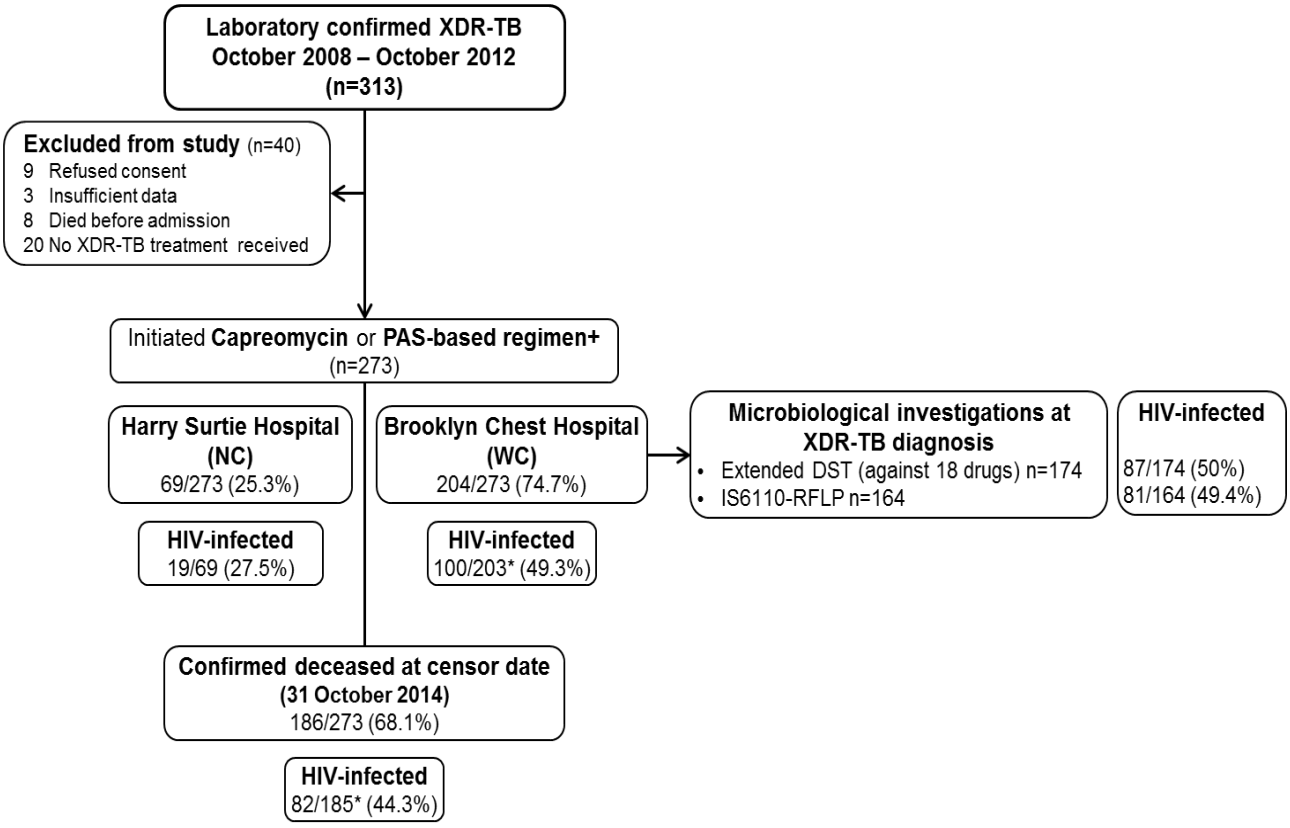
to treatment-related outcomes in XDR-TB HIV co-infected persons we prospectively followed a cohort of South African HIV-infected and uninfected XDR-TB patients from 2 provinces.

5.2 MATERIALS AND METHODS

Participants

We recruited 273 South African adult patients with XDR-TB [Chapter 1, Figure 2: cohort B]. Patients were diagnosed with microbiological confirmed XDR-TB between October 2008 and October 2012, and followed until death or 31 October 2014 (Figure 1). All patients were admitted to specialised TB treatment facilities: Brooklyn Chest Hospital in the Western Cape Province (WC) or Harry Surtie (previously Gordonia) Hospital in the Northern Cape Province (NC) and received directly observed XDR-TB treatment. Terizidone and cycloserine were prescribed interchangeably during the study period, based on availability in the NTP, and analysed together as terizidone.

Figure 1: Study plan for recruitment and prospective follow-up of XDR-TB patients, stratified by HIV status



*One patient refused HIV testing.

+Initiation of capreomycin or PAS defined as: a) ≥7 days of treatment, b) treatment initiated ≤6 months prior to or after XDR-TB diagnosis (except where the indication for capreomycin or PAS was due to Pre-XDR TB)

Definitions applied

Treatment-related outcomes, defined in Table 1A, were adapted from the 2013 WHO definitions and reporting framework guidelines [151]. Definitions specific to HIV and non-treatment related concepts are summarised in Table 1B and 1C respectively.

Table 1A: Definitions applied regarding treatment-related outcomes, as adapted from the 2013 WHO revised definitions and reporting framework for TB guidelines [151]

Treatment outcome		**Definition**
Favourable outcome	Cured	Treatment completed, as recommended by the National TB programme, without evidence of failure. Three or more consecutive negative sputum cultures, taken at least 30 days apart, after the intensive phase (up to 12 months from the initiation of treatment).
	Completed treatment	Treatment completed, as recommended by the National TB programme, without evidence of failure however no record of three or more consecutive negative sputum cultures, taken at least 30 days apart, after the intensive phase (up to 12 months from the initiation of treatment).
Unfavourable outcome	Treatment failure	Treatment terminated (stopping of two or more drugs), or the need for permanent regimen change of at least two anti-TB drugs because of one or more of the following: i) lack of sputum culture conversion, ii) bacteriological net sputum culture reversion in the intensive or continuation phase after initial sputum culture conversion, iii) evidence of additional acquired resistance to capreomycin, iv) adverse events (AEs).
	Died while on treatment	A patient who died for any reason while on any TB treatment, or within 7 days of termination of treatment. For post treatment time-specific outcome all-cause mortality will be used. Death supersedes any treatment outcome at a specific time point.
	Relapsed	Two or more consecutive positive sputum cultures, at least 30 days apart, subsequent to the outcome of 'Cure' or 'Treatment Complete'.
	Defaulted	A patient, who interrupted treatment for 2, or more, consecutive months and who did not restart treatment but remained hospitalised or traceable in the community.
Indeterminate	On-going treatment	A patient for whom no treatment outcome can be assigned due to on-going treatment in accordance with the National TB programme.

	Not assigned (transfer out or lost to follow-up)	A patient for whom no treatment outcome can be assigned. Includes cases 'transferred out' to another treatment facility or whose treatment outcome is unknown.

Table 1B: Definitions related to HIV, including anti-retroviral combinations [152]

Immunological failure	CD4 count falls to the baseline (or below) or Persistent CD4 levels below 100 cells/mm3
Suppressed viral load	Viral load <50 copies RNA
Unsuppressed viral load	2 consecutive viral load results of >1000 copies RNA, at least 8 weeks apart.
HIV-infected prior to XDR diagnosis Concurrent HIV and XDR-TB diagnosis HIV-infected post XDR-TB diagnosis	HIV diagnostic date ≥2months before XDR-TB diagnostic date HIV and XDR-TB diagnostic dates within 0-2months of each other HIV diagnostic date ≥2 months after XDR-TB diagnostic date
<u>Anti-retroviral drug combinations applied during the study period</u> Due to programmatic ART combination changes during the period 2008-2012 SA National guidelines regarding ART combinations, considered as first line therapy, could not be followed for all patients. We consequently created a category called 'Modified first-line therapy'	<u>FIRST-LINE THERAPY</u> A combination of any 3 of the following ARVs including 2 NRTI and 1 NNRTI drugs • 3TC, NVP, EFV, D4T, TDF <u>MODIFIED FIRST-LINE THERAPY</u> Any of the first line therapy combinations where a single drug was replaced by either AZT, FTC <u>SECOND-LINE THERAPY</u> A minimum of 3 drugs which must include either LPV/R or DDI or ABC

Table 1C: Non-treatment related terms defined

Primary XDR-TB (clinical definition)	Any patient diagnosed with XDR-TB with one or more of the following: (i) No prior TB episode (ii) Prior cured DS-TB, >12 months prior to XDR-TB (iii) First empiric DS-TB treatment within 9 months prior to XDR-TB diagnosis. (iv) MDR-TB diagnostic result(s) available within, no more than 6 months prior to XDR-TB diagnosis.
Conversion	Two consecutive negative sputum cultures at least 30 days apart, with an upper limit of 4 months.
Reversion	Two consecutive positive sputum cultures at least 30 days apart, after conversion, with an upper limit of 4 months.
Net conversion	Two consecutive negative sputum cultures at least 30 days apart, with an upper limit of 4 months, in patients who previously reverted and without further reversion.
Net reversion	Two consecutive positive sputum cultures at least 30 days apart, with an upper limit of 4 months, in patients who previously converted and without further conversion.
Criteria for assignment of a treatment outcome	All patients who started XDR-TB treatment (initiation of capreomycin or PAS for 7 or more days and no more than 6 months prior to or after XDR-TB diagnosis) were assigned a treatment outcome (except where the indication for capreomycin or PAS was due to Pre-XDR TB). In patients who started capreomycin or PAS due to a diagnosis of pre-XDR-TB the date of XDR-TB diagnosis is the XDR-TB treatment start date.

Extended drug susceptibility testing

Phenotypic drug susceptibility testing was performed on 174/204 isolates from the Western Cape Province collected at XDR-TB diagnosis. We used Sensititre^{TM} MYCOTB plates to establish minimum inhibitory concentrations (MIC) for: rifampicin, isoniazid, amikacin, kanamycin,

streptomycin, cycloserine, ethionomide, ethambutol, moxifloxacin, ofloxacin and para-aminosalicylic acid. Resistance to capreomycin, dapsone, clarithromycin, clofazamine, linezolid, pyrazinamide and rifabutin was determined using the BD BACTEC^{TM} MGIT^{TM}. Drug susceptibility testing was performed according to manufacturer guidelines. 164/204 XDR-TB diagnostic isolates were subjected to IS*6110*-RFLP fingerprinting [153] and targeted mutation analysis [*inh*A and *kat*G] were performed on 142 of these isolates.

Adverse events

Adverse events (AEs) were graded according to the USA department of health and human service common terminology criteria for adverse events [82]. Grading were defined as grade 1: mild (AE just noted); grade 2: moderate (treatment dose or frequency of medication adjusted or medication prescribed to treat AE); grade 3: severe (drug stopped); grade 4: life threatening and grade 5: death.

Statistical analysis

All statistical analyses, and graphics generated, were done in STATA. Continuous variables were summarised by median and IQR, using Wilcoxon rank sum for p values; categorical variables by counts and percentages using Fisher's exact test for p values. Variables with missing data are noted. Durations were calculated in days and converted to number of months by days/30.4 (as the number in days of an average month). Univariate Cox proportional hazards models were used to assess the relation between explanatory variables and time-to-event outcomes. Variables considered included HIV status, combined HIV and antiretroviral therapy status (HIV-uninfected, HIV on antiretroviral therapy, or HIV not on antiretroviral therapy), sex, ethnic

origin (mixed ancestry or black), treatment-related outcome (favourable or unfavourable), adverse drug reactions, strain family (Beijing or other), smoking (yes or no), weight (kg) at diagnosis, age at diagnosis, number of drugs prescribed and a binary variable for each of the anti-TB drugs prescribed. Unless otherwise noted, the time to event was taken as days from treatment start date. Kaplan-Meier curves were estimated for probability of survival by various strata. Tests between strata were done by the log-rank test. Multivariate Cox proportional hazards models for mortality included variables that were significantly associated with outcome ($p<0{\cdot}05$) and additional pre-specified variables (eg, sex).

5.3 RESULTS

Demographic, clinical and molecular traits of 273 XDR-TB patients included in the study are summarised in Table 2. One person refused HIV testing and 119/272 (44%) patients were HIV-infected. HIV-infected patients were predominantly Black African (p=<0.0001); had no smoking history (p=<0.0001); an increased weight at XDR-TB diagnosis (p=0.003); a shorter duration of follow-up (p=0.002) and were predominantly diagnosed with clinically defined primary XDR-TB (p=0.02) (Table 2). 7/26 (26.9%) patients with a concomitant diagnosis of diabetes mellitus at XDR-TB diagnosis were HIV-infected.

Table 2: Demographic, clinical and microbiological characteristics of XDR-TB patients diagnosed between 1 October 2008 and 31 October 2012, stratified by HIV status

Note: Data are median (IQR) or N (%) unless otherwise stated. P values for comparison between patients with and without HIV calculated with Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables.

		HIV-uninfected n=153 (56%)	**HIV-infected** n=119 (44%)	**Total** n=272#	**p value**
Deceased		103 (67.3)	82 (68.9)	185 (68)	0.79
Clinical defined primary XDR-TB*		42 (27.4)	49 (41.2)	91 (33.5)	0.02
Female		60 (39.2)	59 (49.6)	119 (43.8)	0.11
Mixed ancestry≠		115 (75.2)	37 (31.1)	152 (55.9)	< 0.0001
Age at XDR-TB diagnosis		32.6 (24.2, 46.2)	35.5 (29.1, 39.8)	34.3 (26.5, 42.9)	0.35
Education	Primary	34 (22.2)	23 (19.3)	57 (21)	0.78
	Secondary	69 (45.1)	53 (44.5)	122 (44.9)	
	Unknown	50 (32.7)	43 (36.1)	93 (34.2)	
Smoked	No	53 (34.6)	59 (49.6)	112 (41.2)	< 0.0001
	Yes	75 (49)	35 (29.4)	110 (40.4)	
	Unknown	25 (16.3)	25 (21)	50 (18.4)	
Weight at XDR-TB diagnosis$		48.7 (42.7, 55.8)	52.5 (46.4, 64)	50.3 (43.5, 58.8)	0.003
	Weight < 50kg	85 (56.3)	46 (39)	131 (48.7)	0.01
Number of drugs in XDR-TB regimen		9 (8, 10)	9 (8, 10)	9 (8, 10)	0.12
Follow-up from XDR-TB diagnosis (months)		22.5 (11.2, 29.8)	15.6 (6.2, 26.1)	20.3 (9.6, 27.8)	0.002
Follow-up from XDR-TB treatment start (months)		20.5 (9.6, 28.06)	14.6 (4.1, 23.9)	17.9 (8.3, 25.23)	0.002
Time to positivity (days)		15 (11, 22)	16.5 (12, 23)	16 (11, 22)	0.24
Number of drugs isolate resistant to anti-TB drugs (n=167)		9 (7, 11)	10 (8, 11)	10 (8, 11)	0.32
Smear status at XDR-TB diagnosis (n=237)**	Positive	62 (45)	41 (41.4)	103 (43.5)	0.69
Strain type at XDR-TB diagnosis (n=164)**	Beijing	67 (80.2)	72 (88.9)	139 (84.8)	0.19
	Other	16 (19.3)	9 (11.1)	25 (15.2)	
Beijing family at XDR-TB diagnosis (n=139)**	Atypical	35 (52.2)	48 (66.7)	83 (59.7)	0.09
	Typical	32 (47.8)	24 (32.3)	56 (40.3)	
inhA15 mutation at XDR-TB diagnosis (n=142)**	No	36 (45.8)	29 (46)	65 (45.8)	1
	Yes	43 (54.4)	34 (54)	77 (54.2)	
katG mutation at XDR-TB diagnosis (n=142)**	No	22 (27.9)	16 (25.4)	38 (26.8)	0.85
	Yes	57 (72.2)	47 (74.6)	104 (73.2)	

* Clinically primary XDR-TB defined in Table 1B
≠Only black and mixed ancestry race in cohort
#One patient refused HIV testing
$In total cohort: three patients with no weight recorded at XDR-TB diagnosis
**Denominator correlates to available samples

HIV diagnosis and anti-retroviral therapy

105/119 (88.2%) persons became HIV-infected prior to an XDR-TB diagnosis; 12/119 (10%) were concurrently diagnosed with HIV and XDR-TB and two persons became HIV-infected post XDR-TB diagnosis. 114/119 (95.8%) HIV-infected patients received ART. Those who did not receive ART either refused ART or died before initiation of ART. Due to programmatic ARV drug changes during the study period we were unable to categorise first-line ART for a number of HIV-infected persons. Thus, for these patients we created a category called 'modified first-line therapy' (Table 1C). During XDR-TB treatment 81/117 (72.3%) HIV-infected patients received first-line, 21/117 (18.8%) modified first-line and 11/117 (9.8%) received second-line ART.

7/11 (63.6%) persons who received second-line ART were HIV-infected >24 months prior to XDR-TB diagnosis and 5/7 (74.1%) of these patients had a history of previous TB. During the course of XDR-TB treatment 22/114 (19.3%) patients changed to an alternative first-line, or a modified first-line ART regimen and one patient changed to a salvage ART regimen.

CD4 count and viral load

The median CD4 count at XDR-TB diagnosis was 199 (IQR 85, 323) cells/mm^3 and at censor date 264 (IQR 110, 456) cells/mm^3. There was no difference in CD4 count when stratified by mortality or clinically defined primary versus acquired XDR-TB. 35/117 (29.9%) HIV-infected patients had immunological failure at XDR TB diagnosis. 17/35 (48.6%) of these patients remained immunologically compromised at censor date and 15/17 (88.2%) died. At XDR-TB diagnosis 75/98 (76.5%) HIV-infected patients had a suppressed viral load (VL). 61/74 (82.4%)

patients had a suppressed VL at censor date. In patients with a CD4 count <200 versus >200 cells/mm^3 there was no difference in VL (p=0.64).

XDR-TB treatment received and phenotypic resistance to anti-TB drugs

We analysed drug groups and classes as recommended prior to the 2016 updated WHO guidelines on drug groups. We summarised 2016 changes in drug groups in Chapter 2: Table 3. Updated drug group guidelines were not incorporated in the analysis.

A larger proportion of patients [67% (183/273)] received DR-TB treatment prior to an XDR-TB diagnosis. Prior DR-TB treatment included group 1 [(172/273; 63%)], group 2 [(177/273; 64.8%)], group 3 [(169/273; 61.9%)], group 4 [(175/273; (61.4%)] and group 5 [(27/273; 9.9%)] anti-TB drugs.

XDR-TB patients were treated with a median of 9 (IQR 8, 10) anti-TB drugs (Table 2). There was no difference in anti-TB drugs included in the XDR-TB regimen comparing HIV-infected and uninfected persons. HIV-infected and uninfected patients predominantly received anti-TB drugs from drug groups 1-4 (Table 3). At XDR-TB diagnosis phenotypic resistance, irrespective of HIV status, to most anti-TB drugs were high with the exception of resistance to PAS (6.43%) and group 5 anti-TB drugs (Table 4).

Table 3: Summary of XDR-TB treatment received during the study period, grouped according to WHO anti-TB drug group categories prior to 2016 and stratified by HIV status

Drug groups*	XDR-TB treatment received	HIV-uninfected n=153 (%)	HIV-infected n=119 (%)	Total n=273 (%)	p value
Group 1	PZA	147 (96.1)	114 (95.8)	261 (96)	1
	Ethambutol	111 (72.5)	92 (77.3)	203 (74.6)	0.4
	Isoniazid	102 (66.7)	82 (68.9)	184 (67.7)	0.79
Group 2	Capreomycin	143 (93.5)	113 (95)	256 (94.1)	0.8
Group 3	Moxifloxacin	90 (58.8)	79 (66.4)	169 (62.1)	0.21
	Ofloxacin	96 (62.8)	67 (56.3)	163 (59.9)	0.32
	Levofloxacin	1 (0.6)	1 (0.8)	2 (0.7)	1
	Ciprofloxacin	11 (7.2)	NA	11 (4)	< 0.0001
Group 4	Terizidone	151 (98.7)	117 (98.3)	268 (98.5)	1
	PAS	149 (97.4)	111 (93.3)	260 (95.6)	0.14
	Ethionomide	142 (92.8)	117 (98.3)	259 (95.2)	0.04
	Cycloserine	2 (1.3)	NA	2 (0.7)	0.51
Group 5	Augmentim	86 (56.2)	58 (48.7)	144 (52.9)	0.22
	Clarithromycin	68 (44.4)	20 (16.8)	88 (32.4)	< 0.0001
	Clofazamine	45 (29.4)	44 (37)	89 (32.7)	0.2
	Dapsone	22 (14.4)	19 (16)	41 (15.1)	0.74
	Amoxil	13 (8.5)	6 (5)	19 (7)	0.34
	Linezold	4 (2.6)	2 (1.7)	6 (2.2)	0.7
	Azithromycin	1 (0.6)	3 (2.5)	4 (1.5)	0.32
	Bedaquiline	1 (0.6)	1 (0.8)	2 (0.7)	1
	Thiacetazone	NA	1 (0.8)	1 (0.4)	0.44

* Drug group categories applicable during the study period. The 2016 updated WHO drug groups are summarised in Chapter 2: Table 3. This review did not focus on the 2016 updated drug groups.

Table 4: Summary of phenotypic resistance to anti-TB drugs included in XDR-TB regimen, stratified by HIV status

Phenotypic drug susceptibility test done	Number of XDR-TB patients with phenotypic resistance, at time of XDR-TB diagnosis, to anti-TB drug tested*					
	Whole cohort		HIV-uninfected		HIV-infected	
PZA	153/167	91.62%	81/87	93.10%	71/79	89.87%
High dose INH	101/115	87.83%	52/58	89.66%	49/57	85.96%
Capreomycin	145/164	88.41%	75/84	89.29%	70/79	88.61%
Ofloxacin	86/113	76.11%	46/64	71.88%	39/48	81.25%
Moxifloxacin	53/85	62.35%	23/35	65.71%	30/50	60.00%
Ethambutol	87/168	51.79%	47/88	53.41%	40/79	50.63%
Ethionomide	86/174	49.43%	42/87	48.28%	44/86	51.16%
Terizidone	66/175	37.71%	29/89	32.58%	37/85	43.53%
Dapsone	2/27	7.41%	1/14	7.14%	1/12	8.33%
PAS	11/171	6.43%	7/88	7.95%	4/82	4.88%
Clarithromycin	1/32	3.13%	1/22	4.55%	0/9	0.00%
Clofazamine	1/53	1.89%	1/23	4.35%	0/30	0.00%
Linezolid	0/1	0.00%	0	0.00%	0	0.00%

*There were no significant differences comparing HIV-infected and uninfected persons

Adverse events

88/192 (45.8%) HIV-infected persons reported adverse events (AEs). These patients experienced a total of 320/622 (51.4%) AEs, predominantly graded as mild-moderate [264/320 (82.5%)] (Table 5). AEs reported included general malaise, pain in extremities, gastro-intestinal symptoms, renal impairment, skin reaction, hearing and vision disturbances, thyroid dysfunction and mental impairment. 9/12 (75%) patients who experienced renal related AEs were HIV-infected. Capreomycin was implicated as the plausible drug that caused renal

impairment in 6/9 (67%) of these HIV-infected patients. One HIV-infected patient died while receiving capreomycin.

Table 5: XDR-TB patients, stratified by HIV status, who experienced an adverse event and number of adverse events, graded 0-5, that were experienced

	Number of XDR-TB patients who reported an adverse events 192/272# (70.6%)*	
	HIV-infected	**HIV un-infected**
Total number of patients with AE	88/192 (45.8%)	104/192 (54.2%)
Patients with grade 0-2 AE	80/88 (91%)	92/104 (88.5%)
Patients with grade 3-5 AE	38/88 (43.2%)	42/104 (40.4%)
	Number of adverse events experienced by XDR-TB patients (n=622)*	
Total number of AEs	320/622 (51.4%)	302/622 (48.6%)
Number of grade 0-2 AEs	264/320 (82.5%)	236/302 (78.1%)
Number of grade 3-5 AEs	56/320 (17.5%)	66/302 (21.9%)

#One patient refused HIV testing

*There were no significant differences comparing HIV-infected and uninfected persons

Exposure to community-based tuberculosis

46/273 XDR-TB patients reported community-based exposure to individuals diagnosed with drug susceptible TB (10 contacts), MDR-TB (18 contacts), XDR-TB (12 contacts) and an undisclosed TB diagnosis (29 contacts). Most XDR-TB patients with community-based exposed to TB were diagnosed with clinically defined primary XDR-TB [33/46 (71.7%)]. 26/33 (78.8%) of these patients were in contact with a relative who was diagnosed with TB. HIV-infected persons who were exposed to community-based TB were diagnosed with both clinically defined primary XDR-TB [14/33 (42.4%)] and acquired XDR-TB [13/13 (100%)] (Figure 2).

Molecular epidemiology

There were no differences at XDR-TB diagnosis, stratified by HIV status, in strain phenotype [164/204, p=0.19] or mutations analysed [(142/204): *inh*A (p=1); *kat*G (p=0.85)] in isolates from the Western Cape Province (Table 2).

Treatment-related outcomes

At 60 months follow-up 43/273 (15%) patients had a favourable treatment-related outcome of cure and unfavourable outcomes occurred in 91/273 (33%) who died while on treatment; 67/273 (24.5%) who failed treatment and 46/273 (16.9%) who defaulted/relapsed (Table 6A). Although treatment-related outcomes in HIV-infected and uninfected persons were comparable (Table 6B) HIV-uninfected patients predominantly failed treatment [49/153 (32%) p=0.002)] (Table 6B). Sputum culture conversion mostly did not differ when stratified by HIV status (Table 6C). However, 44/101 (43.6%) HIV-uninfected XDR-TB patients who failed to sputum culture convert had failed XDR-TB treatment (p=0.003) (Table 6C).

Table 6: Treatment-related outcomes in XDR-TB patients (n=273) diagnosed between October 2008 and October 2012 with follow-up to October 2014, stratified by (A) time-specific cumulative outcomes (B) HIV status at end of follow-up and (C) sputum culture conversion at end of follow-up [Data presented as n (%)]

(A)

	Year of follow-up				
Treatment-related outcome	**Year 0-1**	**Year 0-2**	**Year 0-3**	**Year 0-4**	**Year 0-5**
Cured or completed treatment	0 (0)	24 (9)	36 (13)	41 (15)	43 (16)
Died whilst on treatment	66 (24)	83 (30)	86 (32)	89 (33)	90 (33)
Treatment failed/ default/ relapsed	26 (10)	72 (26)	95 (35)	110 (40)	113 (41)
Transferred out or lost to follow-up	6 (2)	14 (5)	20 (7)	23 (8)	24 (9)
On-going treatment	175 (64)	80 (29)	36 (13)	10 (4)	3[A] (1)
Death irrespective of initial outcome[B]	77 (32)	130 (55)	164 (69)	179 (76)	186 (78)

[A]Three patients had a treatment outcome of on-going treatment after three years of follow-up [B]Number (%) of individuals who died in the given year of follow-up irrespective of the treatment outcome

(B) Stratified by HIV status at end of follow-up

Treatment-related outcome	**HIV-infected n=119**	**HIV-uninfected n=153**	**p value**
Cure/Completed (n=43)	21 (17.7)	22 (14.4)	0.57
Died while on treatment (n=90)	43 (36.1)	47 (30.7)	0.42
Default/Relapse (n=43)	25 (21)	18 (11.8)	0.057
On-going treatment (n=5)	2 (1.7)	3 (2)	NA
Treatment failure (n=67)	18 (15.1)	49 (32)	0.002
Not evaluated (TO / LTFU) (n=24)	10 (8.4)	14 (9.2)	1

(C) Stratified by culture conversion at end of follow-up *

Treatment-related outcome	Net conversion 68/266 (25.6)		Net reversion 26/266 (9.8)		No conversion 172/266** (64.5)	
	HIV-uninfected 34 (50)	HIV-infected 34 (50)	HIV-uninfected 14 (54)	HIV-infected 12 (46)	HIV-uninfected 101 (59)	HIV-infected 71 (41)
Cure/Completed	21 (61.8)	21 (61.8)	0	0	0	0
Died while on treatment	2 (5.9)	2 (5.9)	1 (7.1)	2 (16.7)	44 (43.6)	39 (54.9)
Defaulted	5 (14.7)	7 (20.6)	6 (42.9)	4 (33.3)	7 (6.9)	13 (18.3)
Treatment Failed	0	0	5 (35.7)	3 (25)	44 (43.6)	15 (21.1) [A]
On-going treatment/relapse	1 (2.9)	2 (5.9)	2 (14.3)	0	0	0
Not evaluated (TO/LTFU)	5 (14.7)	2 (5.9)	0	3 (25)	6 (5.9)	4 (5.6)

* Culture conversion not determined for 6/272 [HIV-infected 2/6] patients who had insufficient data

** Patient who refused HIV testing died and did not culture convert

[A] HIV-infected compared to uninfected patients with no conversion who failed treatment (p=0.003)

Mortality

HIV-infected persons had an increased risk of death [HR 1.62 (95% CI 1.15, 2.26) p=0.005] (Table 7A). Predictors of survival for HIV-infected persons included (i) weight >50kg at XDR-TB diagnosis [HR 0.57 (95% CI 0.34, 0.95) p=0.03] (ii) number of drugs in the XDR-TB regimen [HR 0.78 (95% CI 0.66, 0.92) p=0.003] and (iii) time to culture positivity [HR 0.96 (95% CI 0.93, 0.99) p=0.02] (Table 7B). These predictors were similar for the whole cohort (HIV-infected and uninfected) (Table 7A). Longevity in HIV-uninfected patients was extended (p=0.02) (Figure 3A). However, survival in HIV-infected persons who received ART was similar to HIV-uninfected patients (p=0.06) (Figure 3B). Survival in HIV-infected patients was not associated with a CD4 count <200 cells/mm^3 (p=0.26) (Figure 4).

Table 7: Multivariate Cox proportional hazards model for risk of death in (A) all XDR-TB patients and (B) XDR-TB HIV-infected patients

(A)

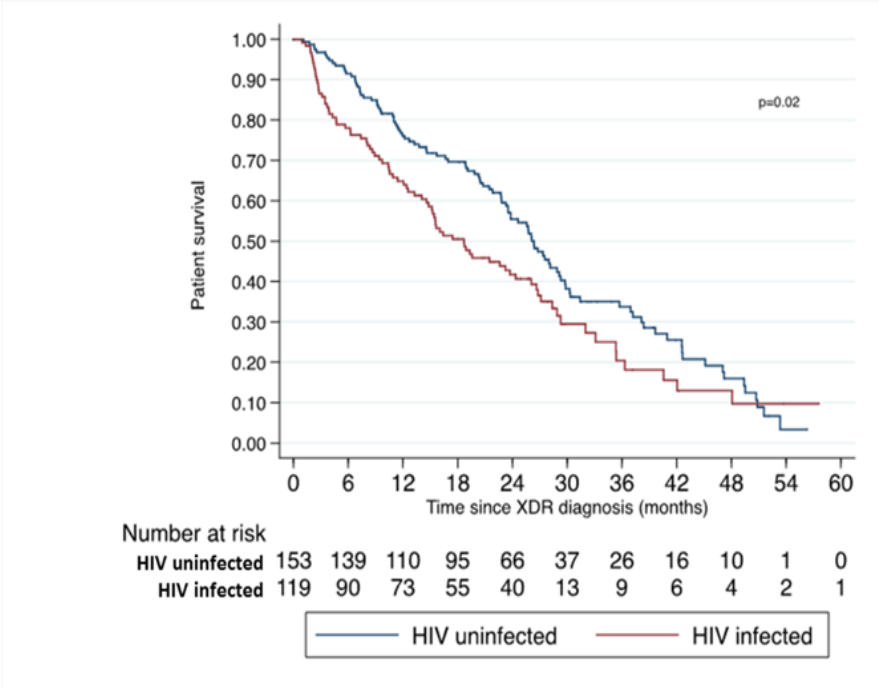
		Hazard	**p value**
Gender	Male	1.05 (0.77, 1.15)	0.77
Race Mixed Ancestry	Mixed ancestry	0.89 (0.63, 1.26)	0.51
HIV status	Infected	1.62 (1.15, 2.26)	0.005
Age at XDR-TB diagnosis		1.02 (1.01, 1.04)	0.002
Weight at XDR-TB diagnosis	>50kg	0.51 (0.36, 0.71)	<0.001
Number of drugs in XDR-TB treatment		0.85 (0.77, 0.93)	0.001
Time to culture positivity (TTP)		0.96 (0.94, 0.98)	<0.001
Clinical defined primary XDR-TB		1.2 (0.86, 1.66)	0.28

(B)

		Hazard	**p value**
Gender	Male	0.75 (0.47, 1.23)	0.25
Race	Mixed ancestry	1.14 (0.67, 1.94)	0.64
Age at XDR-TB diagnosis		1.02 (099, 1.05)	0.31
Weight at XDR-TB diagnosis	>50kg	0.57 (0.34, 0.95)	0.03
Number of drugs in XDR-TB treatment regimen		0.78 (0.66, 0.92)	0.003
Time to culture positivity (TTP)		0.96 (0.93, 0.99)	0.02
Clinical defined primary XDR-TB		1.36 (0.85, 2.17)	0.21
CD4 count <200		1.37 (0.85, 2.19)	0.20

Figure 3: Probability of survival in (A) XDR-TB patients, stratified by HIV status and (B) XDR-TB HIV co-infected patients who received ART compared to HIV un-infected patients

(A)



(B)

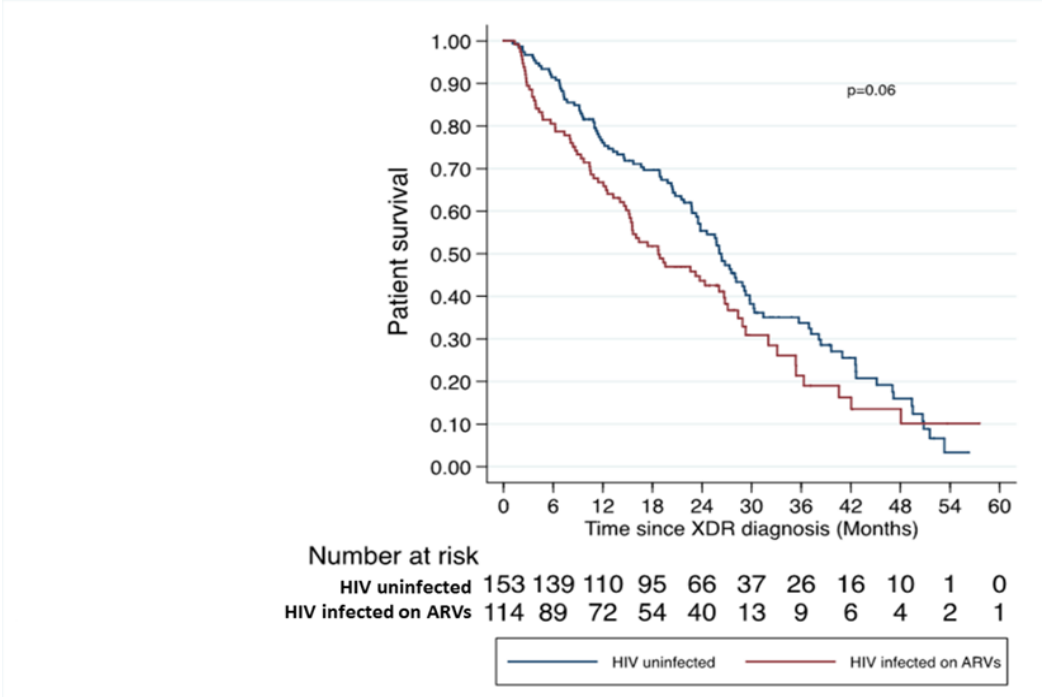
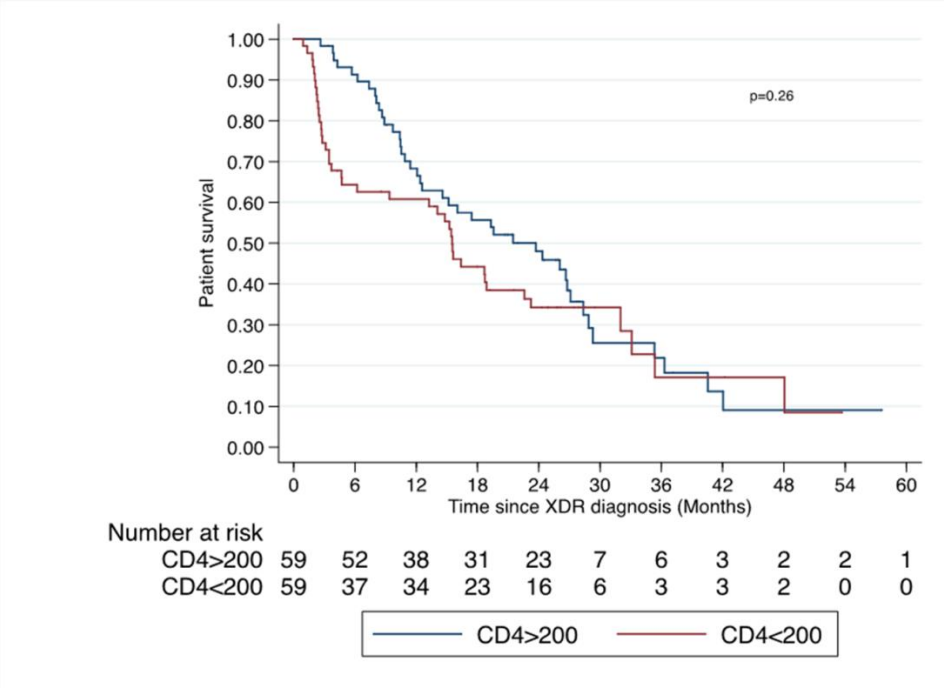


Figure 4: Probabilities of survival in XDR-TB HIV-infected patients stratified by CD4 cell count <200 versus >200 cells/mm^3 (CD4 count at time of diagnosis)



Home-discharged patients

Cumulative treatment-related outcomes in the 172/273 (63%) XDR-TB patients who were discharged from hospital back to the community are summarised in Table 8. 104/172 (60%) home-discharged patients had unfavourable treatment-related outcomes and 35/104 (34%) of these patients had a positive smear result at discharge. When stratified by HIV status, we found no differences in clinical features or smear positivity at discharge in home-discharged patients (Table 9A), or in home-discharged patients with unfavourable treatment-related outcomes (Table 9B). Survival in home-discharged HIV-infected patients were similar to HIV-uninfected patients (p=0.89) (Figure 5A), irrespective of failed treatment (p=0.08) (Figure 5B).

Table 8: Cumulative treatment-related outcomes in home-discharged XDR-TB patients (n=172)
[Data provided as n (%)]

	Duration of follow-up				
Treatment outcome in home-discharged XDR-TB patients	**Year 0-1**	**Year 0-2**	**Year 0-3**	**Year 0-4**	**Year 0-5[A]**
Cured or completed treatment	1 (1)	10 (6)	20 (12)	36 (21)	42 (24)
Died whilst on treatment	12 (7)	14 (8)	14 (8)	14 (8)	14 (8)
Treatment failed/ default/ relapsed	37 (22)	64 (37)	78 (45)	87 (51)	90 (52)
Transferred out or lost to follow-up	6 (3)	10 (6)	16 (9)	20 (12)	24 (14)
On-going treatment	116 (67)	74 (43)	44 [B] (26)	15 (9)	2 (1)
Death irrespective of initial outcome [C]	53 (31)	78 (45)	85 (49)	89 (52)	89 (52)

[A]Two patients were followed for 60·3 and 64·9 months.
[B]Two patients had a treatment outcome of on-going treatment after three years of follow-up.
[C]Number (%) of individuals who died in the given year of follow-up irrespective of the treatment outcome

Table 9: Clinical characteristics, stratified by HIV status, in (A) all home-discharged XDR-TB patients and (B) home-discharged patients with unfavourable treatment-related outcomes

(A)

	Home-discharged XDR-TB patients			
	HIV-uninfected 102/172 (59)	**HIV-infected** 70/172 (41)	**Total** 172/272 (63)	**p value** 0.23
Female	38 (37.3)	30 (42.9)	68 (39.5)	0.56
Mixed ancestry≠	75 (73.5)	22 (31.4)	97 (56.4)	< 0.0001
Weight <50kg	47 (47)$	23 (33)	70 (41)	0.09
Died in community	52 (51)	35 (50)	87 (50.6)	1
Clinical defined primary XDR-TB*	31 (30.1)	25 (35.7)	56 (32.6)	0.57
Positive smear result at discharge from hospital (n=170)*	24 (24)	18 (25.7)	42 (24.7)	0.94

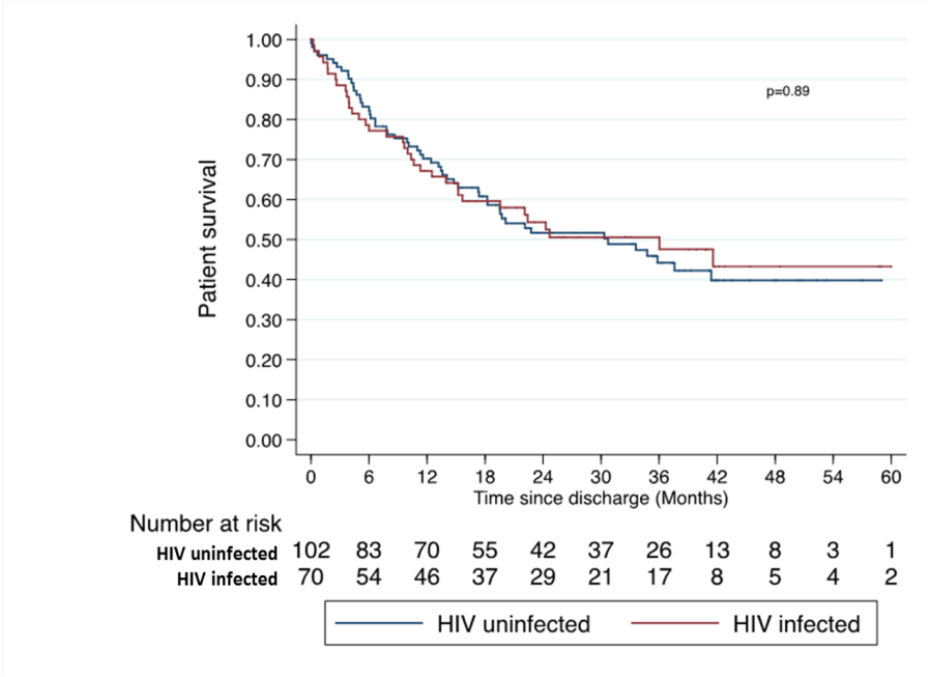
* Two HIV-uninfected patients had no smear results when discharged to community

(B)

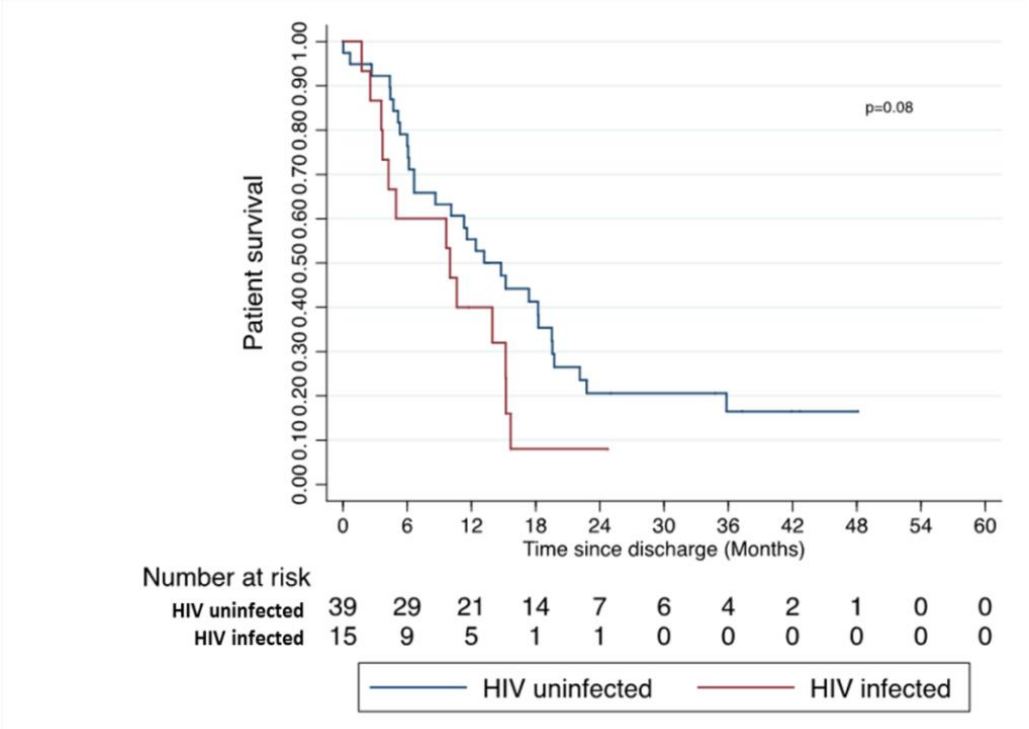
	Home-discharged XDR-TB patients with unfavourable treatment outcomes			
	HIV-uninfected 66/104 (63.5)	**HIV-infected** 38/104 (36.5)	**Total** 104/172 (60.5)	**p value** 0.22
Female	25 (18.2)	12 (31.6)	37 (35.6)	0.67
Mixed ancestry≠	52 (78.8)	16 (42)	68 (65.4)	< 0.0004
Weight <50kg	32 (48.5)	15 (39.5)	47 (45.2)	0.49
Died in community	42 (64)	30 (79)	87 (83.7)	0.16
Clinical defined primary XDR-TB*	20 (30.3)	11 (29)	31 (30)	1
Positive smear result at discharge from hospital (n=104)	21 (31.8)	14 (36.8)	35 (33.7)	0.76

Figure 5: Probability of survival, stratified by HIV status, in (A) all home-discharged XDR-TB patients and (B) home-discharged XDR-TB patients who failed treatment

(A)



(B)



5.4 DISCUSSION

There are limited data on treatment-related outcomes in XDR-TB HIV co-infected patients. Key findings of this study were (i) HIV-infected patients had poorer survival despite receiving ART, however, (ii) survival of home-discharged HIV-infected patients was similar to that of uninfected patients irrespective of an unfavourable treatment outcome; (iii) predictors of survival, ie weight at XDR-TB diagnosis, time to culture positivity, and number of drugs in an XDR-TB regimen were similar in HIV-infected and uninfected patients; (iv) rates of adverse events, smear positivity at discharge, and levels of strain phenotype were similar in HIV-infected and uninfected patients despite HIV-infected patients more commonly having clinically defined primary XDR-TB.

Overall our analysis showed poorer survival in HIV-infected persons who started XDR-TB treatment. This might be due to patients being more immune-compromised and thus having a higher event rates (opportunistic infections and death). Another reason for the overall poorer survival in HIV-infected patients could be related to malnutrition, which might have resulted in attenuated immunity and reduced the effectiveness of the XDR-TB regimen. However, surprisingly HIV-infected patients weighed significantly more at time of XDR-TB diagnosis compared to uninfected patients. The fact that almost all HIV-infected patients received ART might explain this finding. Yet, the nutritional status of HIV-infected patients could have changed considerably during the duration of XDR-TB treatment. Furthermore, given the large percentage (83%) mild-moderate adverse events, including 38% gastro-intestinal related, experienced by HIV-infected patients malnutrition could have occurred during XDR-TB

treatment. HIV-infected patients defaulted, and thus interrupted their treatment more compared to uninfected patients. Thus interruption of treatment could have led to poorer overall survival given the pill burden. Another possible explanation for poorer survival could be drug-drug interactions. We, however, could not discern the difference between drug-drug interactions versus adverse events [139, 140]. Adverse events may have contributed to the disproportionally lower survival in HIV-infected persons. However, we found no differences in adverse event rates comparing HIV-infected and uninfected persons. Finally, overall predictors of survival ie: weight; number of drugs in the XDR-TB regimen, and time to culture positivity was similar in HIV-infected and uninfected patients.

A large proportion of XDR-TB patients who mostly had unfavourable treatment-related outcomes, regardless of HIV status, were discharged from hospital back to the community. More than a third of these patients were smear positive at discharge. Surprisingly, survival in home-discharged HIV-infected patients, irrespective of an unfavourable outcome, was similar to HIV-uninfected patients. In fact longevity in more than halve of programmatically incurable home-discharged HIV-infected patients was at least 12 months. This could be as a result of the more intensive HIV care and support patients received compared to uninfected patients [154]. HIV-infected persons were all on ART and thus self-selected for survival. O'Donnell et al. reported an association between ART and survival in XDR-TB HIV co-infected patients [122]. Longevity in home-discharged HIV-infected patients in our cohort, however, was unlike survival in XDR-TB patients from KwaZulu-Natal where all patients, irrespective of HIV status, died within 12 months from start of treatment [122].

While we found the proportion of primary XDR-TB in HIV-infected persons as higher compared to uninfected patients, primary XDR-TB was not a predictor of survival. This finding is unsurprising as HIV-infected are more prone to infections and reflects immune suppression in this vulnerable group. Furthermore, HIV health care service delivery and access to support groups might have resulted in early detection of XDR-TB in these patients. Our finding thus underscores the need for rapid diagnosis to be offered to HIV-infected persons including sputum induction to obtain a suitable specimen. It also points to the fact that HIV-infected contacts must be screened. This is currently not happening in the national TB programme.

Although AEs were frequent (71%) there were no differences in HIV-infected and uninfected patients. This is unexpected as HIV-infected patients are known to generally have higher advert event rates, especially with TB drugs [139]. However, predictors of adverse events include CD4 count but the median CD4 count of our cohort was close to 200 cells/mm^3. Furthermore, higher default and death rates in HIV-infected persons in our cohort could have masked higher adverse rates in these patients.

Treatment failure was surprisingly higher in HIV-uninfected persons. This could be due to selection bias but more likely because most HIV-infected patients were on ART. Thus we self-selected for survivors. Furthermore, HIV-infected patients defaulted more. The difference between HIV-infected and uninfected patients who failed treatment disappeared when

treatment failure and default were categorized together and deemed an unfavourable outcome.

This study has several limitations. Patients were treated at two specialised TB units in South Africa. Findings from this research might therefore not be applicable to other global or African setting with localised or extended outbreaks of XDR-TB, a low and intermediate TB burden or a dissimilar HIV prevalence. In this study treatment-related outcomes might thus have been underestimated due to selection bias as we did not account for patients who did not reach hospitalisation because of non-diagnosis, delays in diagnostic results or death prior to admission. Furthermore, we did not distinguish between deaths related to XDR-TB versus HIV or other medical conditions. We did not perform molecular epidemiology to confirm primary XDR-TB in patients. Drug susceptibility test and strain type results were based on a proportion of isolates from the Western Cape Province only. The results reported might thus misrepresent the true proportion of Beijing strain phenotype, and levels of phenotypic drug resistance in XDR-TB patients.

5.5 CONCLUSION

Overall HIV-infected persons had marginally poorer survival. However, post-discharge survival was similar for HIV-infected and uninfected patients. HIV-infected persons had more clinically defined primary XDR-TB, however, adverse event rates, smear positivity at discharge, and percentage Beijing strain were similar in HIV-infected and uninfected patients. XDR-TB treatment failure, and hence programmatically incurable TB, is a growing phenomenon

especially in home-discharged patients. This is also a problem in HIV-infected persons on ART who have surprisingly substantial longevity post discharge.

These data inform the need for resource allocation and interventional strategies to contain the TB epidemic. Better access to phenotypic and genotypic diagnostic tools, including whole genome sequencing, will facilitate individualised XDR-TB treatment. The use of effective regimens containing new and repurposed drugs, and prevention of amplification of resistance through optimal dosing and adherence promotion are vital. Strengthening the national TB programme and the identification of the most infectious community-based patients is urgently required. Although such approaches will reduce the overall burden of both infectious diseases prevention strategies should also incorporate alleviation of poverty and management of overcrowding.

CHAPTER 6: NEEDS AND WANTS OF TREATMENT DESTITUTE COMMUNITY-BASED EXTENSIVELY DRUG-RESISTANT PATIENTS

MANUSCRIPT 3: **Lifestyle, attitudes and needs of uncured XDR-TB patients living in the communities of South Africa: a qualitative study**

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PhD context

In this chapter I explore the lifestyle, attitudes and needs of incurable XDR-TB patients living in the community. In chapter 3 I have described the fate of XDR-TB patients and showed that they have substantial longevity in the community [74]. However, I was interested to know how well educated patients were on XDR-TB, whether they understood the implications of their disease, and what their needs and wants were? Although not conclusively proven it was likely that these patients were transmitting disease in the community. However, interventions to interrupt this process would rely on patients who were well informed and educated about infection control, and the nature of the disease. Having insight into these aspects are critical to inform strategies to interrupt transmission and default in patients with XDR-TB. I therefore investigated the experiences, lifestyle, attitudes and needs of patients with XDR-TB who had failed therapy, and were living in the community. I wanted to understand the need for community-based palliative care, the need for economic support, the need for psychosocial support, and the need for home-based infection control, not to mention implications reduction in transmission.

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Lifestyle, attitudes and needs of uncured XDR-TB patients living in the communities of South Africa: a qualitative study

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Summary

OBJECTIVE Patient-level data are required to inform strategies interrupting transmission and default in patients with extensively drug-resistant TB (XDR-TB) to improve models of care and identify potential routes of transmission. We therefore explored the experiences, lifestyle, attitudes and needs of patients with uncured XDR-TB, who failed or interrupted therapy, living without treatment in the community.

METHODS We conducted in-depth interviews with 12 community-based patients from South Africa. Family members were interviewed when patients were unavailable. Interviews were analysed using inductive thematic analysis.

RESULTS The thematic experiences identified from the interviews were as follows: (i) living with but not being cured of XDR-TB, (ii) altered lifestyle in the community, (iii) experiences with community health care, (iv) local community members, and (v) wants and needs. Patients identified mistrust in health care, futility of treatment regimens, a need for a purpose in life and subsistence as major concerns. Restriction of living in the community for patients whose treatment had failed resulted in self-imposed isolation. Defaulters focused more on the never-ending drug regimen and bad experiences with health care contributing to non-adherence. Family members emphasised an under-recognised experience of unforeseen burden, obligation, worry and discomfort. Lack of knowledge and lack of concern about transmission was evident.

CONCLUSION Current models of care are not adequately meeting the needs of patients with uncured XDR-TB and relatives. These data inform the need for community-based palliative care, vocational facilities to improve economic opportunities, home-based infection control and improved psychosocial support to increase patient adherence, reduce transmission, provide income and relieve the burden on family members.

keywords tuberculosis, extensively drug-resistant tuberculosis, South Africa, qualitative, treatment failure, resistance

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis,* remains a public health problem with 8.6 million new cases detected globally in 2012 [1]. Failed TB treatment programmes [2, 3] combined with ongoing transmission of tuberculosis [4] have aided the emergence and spread of drug-resistant tuberculosis (DR-TB). Multi-drug-resistant TB (MDR-TB) is defined as bacterial resistance to at least isoniazid and rifampicin, and extensively drug-resistant TB (XDR-TB) defined as bacterial resistance to at least isoniazid and rifampicin, any fluoroquinolone and at least one of the injectable second-line drugs. XDR-TB has now been reported in 92 countries [1], and DR-TB has led to increased mortality, is a threat to healthcare workers [5] and associated with unfavourable outcomes and unsustainable costs [6]. Indeed, in South Africa, DR-TB consumes ~45% of the total national TB management budget, despite comprising only 2% of the total TB burden [7]. Moreover, resistance beyond XDR-TB, including totally drug-resistant TB (TDR-TB), has now emerged in several countries [8, 9] resulting in serious public health consequences if transmitted.

*These authors contributed equally.

In South Africa, problems with treatment default and community transmission mean that nearly 80% of multi-drug-resistant TB (MDR-TB) [8], and a variable but increasing proportion of XDR-TB [10], is transmitted from person-to-person. Furthermore, incurable cases of XDR-TB are discharged back into the community after failing treatment and not achieving sputum conversion [11]. Thus, these patients remain infectious after discharge into home isolation. Patients also regularly default treatment and may continue to be infectious. Thus, issues associated with treatment default need to be addressed and interrupting transmission is a major goal of disease management.

There are several approaches to curtailing XDR-TB transmission. Most of the literature has evaluated the use of personal protection measures, UV inactivation of bacilli and improved ventilation to impact infection control. However, little attention has been paid to patient-level factors and behaviour impacting transmission, and there are no data in the context of highly drug-resistant TB. Furthermore, qualitative studies on TB have assessed health-seeking behaviour [12, 13], the influence of stigma and perception on seeking health care [14, 15], reasons for delayed diagnosis [15–17] and the meaning of living with a TB diagnosis [18]. However, little or no studies have investigated the lifestyle, attitudes, or adherence of patients with XDR-TB and the experience associated with failing treatment. If effective strategies to curtail transmission and improve patient care are to be introduced, patient-level factors must be considered. We therefore conducted a qualitative study in therapeutically destitute but infectious patients with resistance beyond XDR-TB (or defaulters with XDR-TB) living in the Western Cape community to inform future models of care and transmission control.

Methods

We recruited and interviewed a purposive sample of 12 patients, irrespective of HIV status. Patients meeting our selection criteria were purposely selected from a prospective cohort of 216 patients with XDR-TB who were admitted to Brooklyn Chest Hospital (BCH) in Cape Town between February 2008 and October 2012 (Figure S1). Patients were eligible for inclusion if their treatment had failed (not sputum culture converted) and were living in the community without treatment, or had defaulted treatment and had unknown culture status. Ethics approval was obtained from the human research ethics committee at the University of Cape Town, South Africa.

Data from patient records were analysed to obtain descriptive epidemiological statistics (Table 1) to gain insight into their socio-demographics and clinical histories before interviews were conducted. Informed oral consent was obtained before all interviews. Participants were concurrently informed of the study objectives, and that they could terminate the interview at any time. Participants selected the time and date of their interview. The same trained qualitative interviewer, using a solitary office, conducted the in-depth one-to-one telephone interviews in English over a period of 6 weeks. Interviews ranged from 25 to 65 min, with a median duration of 42 min. Participants incurred no expenses to take part in the study and were not compensated. A one-to-one interview, at their convenience, was conducted with a family member when patients were too ill, had hearing loss or were unreachable. Questions during interviews explored patient's daily lives, life before and after infection with TB, impact of disease, life in hospital and after release and thoughts on their future.

An XDR-TB clinical manager and research nurse at BCH and a community nurse were consulted and interviewed to obtain a picture of healthcare services for TB in the Province. BCH was visited to understand the setting in which participants were treated as well as

Table 1 Patient demographics

Characteristic	Whole study cohort (n = 216)	Qualitative study sample (n = 12)
Median Age at XDR-TB diagnosis (IQR)	33 (26, 41)	36 (30, 46)
Race		
Black	123 (57%)	3 (25%)
Mixed ancestry	92 (42.5%)	9 (75%)
Caucasian	1 (0.5%)	0
Gender (Male)	126 (58%)	10 (83%)
Location		
City Centre and Surroundings	176 (81.5%)	8 (67%)
Peripheral	40 (18.5%)	4 (33%)
HIV Positive	103 (48%)	4 (33%)
Education†		
None	6 (3%)	0
Primary (Grade 0–7)	48 (22%)	4 (33%)
Secondary (Grade 8–12)	118 (55%)	7 (58%)
Tertiary/Further Skills	6 (3%)	1 (8%)
Unknown	38	
Previous established DR-TB History	103 (48%)	8 (67%)
Resistance beyond XDR-TB	–	9‡ (75%)

†Information as in patient records.
‡Extended DST available for 9/12 participants.

community clinics for insight into care in the community. Interviews were recorded, transcribed verbatim and reviewed immediately. Transcribed interviews were read repeatedly for full immersion, and inductive thematic analysis was carried out using Nvivo 9.2. A second researcher was consulted to discuss the themes decided upon.

Results

Seven patients with uncured XDR-TB, not on treatment, and five relatives of uncured patients were interviewed (Table S3). All patients had DR-TB for more than two years, a third (4/12) were HIV-infected and the majority (8/12) lived in Cape Town city centre or immediate surrounding communities of the Western Cape Province (Table 1). In total, 9 of 12 patients have results confirming resistance beyond the definition of XDR-TB (Table S2). The six main themes identified were living with and not being cured of XDR-TB, an altered lifestyle in the community, experiences with healthcare and local community members, wants and needs of patients and experiences of family members caring for patients with XDR-TB (Table S3 and Box S1).

Patients and family members readily reported the experience of receiving 'never-ending' treatment for tuberculosis, gradual disease progression to MDR-TB and XDR-TB as one with long durations of treatment, vast numbers of pills to be taken, daily injections resulting in soreness and side-effects such as vomiting and nausea. Patients experienced futility as treatment continued to fail, leading to mistrust in caregivers and the health advice they provide.

Patients experienced hospitalisation as being lonely, restrictive and feeling like 'jail'. The trauma of seeing other patients die of the same disease was expressed regularly. The desire to not feel restricted, with evidence of unsuccessful treatment, was the reason defaulters discharged themselves from hospital. Patients whose treatment had failed reported the hurt and disappointment of 'being a failure'. This sentiment was associated with a lack of hope and a feeling that they 'lose'.

Blame was evident in different forms. Patients whose treatment had failed reported self-blame and remorse for previously not taking medication consistently and regretted not taking their treatment regimen seriously. Conversely, all patients had an 'it's their fault' outlook regarding healthcare facilities for rendering inadequate care causing them to remain infected for extended periods of time.

Family members blamed patients for not adhering to treatment, either during previous TB infections or during their current XDR-TB infection. Patients described methods of coping, adaptation to any deterioration in their health and hope that they would get better, mainly by a belief in God.

The lifestyle of patients whose treatment failed was described as one of loneliness and isolation as experienced through living alone, living in separate quarters to the rest of the family, not seeing friends, or exclusion from community members.

Patients whose treatment had failed missed companionship and intimate relationships due to the risk of transmission to a partner. Where patients maintained friendships and personal relationships they did so with limitations.

Patients are advised not to use public transport when discharged resulting in many not leaving their homes or communities very often as most can only afford public transport. A key theme that emerged was one of being 'stuck' without a purpose. Furthermore, many felt physically able and wanted to work but were not allowed due to risk of transmission, while others were forced to be housebound due to declines in their health.

Visiting community clinics was met with fear and exclusion for some patients and family members. Participants described traumatic accounts of experiences with staff at community clinics. Some reported discrimination and humiliation by nurses when collecting medication.

Patients no longer on treatment after it failed regimens described primary care clinic staff as being non-supportive and unhelpful when they had sought medical help. Such events reduced faith in health services and led to some patients not attending clinics at all, despite needing care.

Leaving their house and visiting clinics was met with stigma and discrimination for patients. They experienced discrimination living in their community. One patient experienced a petition against them and others felt stared at by local residents. This impacted a patient's likelihood to wear masks around others, an important part of infection control and a requirement to reduce transmission. Mask adherence was linked to how excluded they felt wearing them.

Understanding and awareness of tuberculosis, particularly drug-resistant tuberculosis, were described as being low or non-existent in communities resulting in patients experiencing feelings of being outcast.

HIV and TB co-infected patients (4/12) spontaneously discussed the fact that these infections both carry stigma in local communities. Lack of knowledge among the community means patients appear to be tarnished with a double stigma, or assumed they have HIV, when visiting the clinic. Many patients reported only knowing and understanding TB once experiencing it for themselves either as patients or through infected family members.

All patients reported a desire to be independent, self-sufficient and have a sense of purpose. There was a strong need for subsistence and employment to support themselves and their families and to not have to rely on others or on a disability grant. Patients had a complex mix of wants, needs and desires regarding relationships with family, friends and partners. Family bonds were the strongest relationship most patients had. All patients reported a preference to receive care in their community, in part due to the long duration of treatment. They believed that they would be fine and immediately feel a lot better by not being hospitalised and restricted.

All family members described a sense of responsibility and accountability for their relative's well-being. However, family members uncovered an under-recognised experience of unforeseen burden, obligation, worry and discomfort. This experience was often described by family members who had their own families to look after yet were chosen by the patient, or healthcare services, to be responsible for them. They felt hospitals should be looking after the patient and expressed a need for relief for this responsibility. Extended family members expressed a desire for patients to rather return to their original family home.

Subthemes emerging from family members were a need for psychological and financial support. Family members readily requested this support during interviews, confirming the need. Patients whose treatment had failed discussed the limited life they lived on their disability grant and some sought casual labour as handymen or gardeners to improve their lifestyle. All defaulters engaged in casual labour as they are not eligible for disability grants. Families, and patients, believe that they would not contract tuberculosis from the patient, often due to a belief in God protecting them, which raises a public health concern.

Discussion

This study aimed to identify patient and family member experiences of contracting and living with incurable XDR-TB and to gain insight into such experiences both in hospital and in the community. Our key finding is the need for decentralised treatment and care, palliative care facilities, increased knowledge and awareness, improved support from healthcare workers in multidisciplinary teams, and provision of subsistence, purpose and support by social services.

Quality of care and support for patients needs to be improved. Studies in Peru identified the benefits of psychosocial support to mitigate the difficulty of continuous treatment and confronting stigma and isolation [19]. The need for such psychosocial support was evident from the data in this study. Although such support is mentioned in national TB guidelines for South Africa [20], evidence of such support appears to be lacking and highlights a need to reinforce these guidelines to improve patient adherence and quality of life.

Patients discharged into the community whose treatment had failed have a higher risk of mortality and are likely to be in increased need of palliative care. The need for palliative and end-of-life care for DR-TB patients has been highly recommended [21, 22]. A recent National Government initiative to build capacity regarding care and support to improve patient outcomes (CaSIPO) reflects commitment and vision to render quality palliative care for all, but in particular to HIV- and TB-infected patients. Each patient diagnosed with a life-threatening or life-limiting illness will receive care and support [23].

These findings support this need for patients and families in the form of physical, psychosocial and economical support for patients who have both failed and defaulted from treatment. The need for such care to be community-based rather than centralised is emphasised to enable patients to continue living their lives as normally as possible. The need for day centres or community centres, either stand-alone or through palliative care facilities, for patients to socialise, learn skill sets and be productive was also highlighted by patients who had not responded to treatment reporting feeling 'stuck' and discussing a need for purpose.

Economic stability is essential. Patients whose regimens failed receive financial support through government disability grants but defaulters and hospitalised patients [24] are not eligible for grants. As duration of illness increases, the burden of lost income grows to exacerbate financial constraints on patients and their families [25]. This need for subsistence contributed to the likelihood of hospitalised patients discharging themselves from the hospital. The findings call for a need to enable grants to be available to patients when hospitalised.

For patients living in the community whose treatment had failed, socio-economic intervention and support via palliative care facilities, or day centres, providing vocational activities addressing boredom, their need for purpose, and their need for more income is essential. This could prove more economical and far reaching than increasing access to grants for those living in the community as illustrated by the Innovation Socio-economic Intervention against TB (ISIAT) project [26].

Nearly half of the participants reported experiencing being abused or unsupported by staff at community clinics resulting in them avoiding such services. Similar

concerns were raised with hospital staff causing patients experiencing a desire to leave the hospital. Improved support by training healthcare workers to provide psychosocial support would ensure that health seeking behaviour continues resulting in reduced defaulting. Indeed, healthcare personnel are key providers of emotional support and encouragement for patients to adhere to their treatment [19], calling for a need for more supportive interactions with community and hospital healthcare providers.

In this study, low levels of community awareness and knowledge about tuberculosis were highlighted. Poor knowledge about TB is well reported [27–30] with many family members stating they learned more about the disease once experiencing it themselves. Thus, awareness and understanding of tuberculosis symptoms and transmission are crucial in high-risk populations. Education programmes are needed for families and the wider community to improve community attitudes, hygiene practices and infection control as well as future health-seeking, but also the quality of life of patients in the community. National guidelines [20] state education as part of the management strategy for TB but the degree to which this is enforced appears to be variable.

A theme raised by two family members was one of denial, where family members believed 'we won't get it'. Infection control procedures were ignored due to a belief in God who will protect them. With almost 80% of South Africa's population practicing various denominations of faith [31], the belief of 'we won't get it' may need to be addressed in collaboration with place of worship officials.

A surprising finding in this study was the burden highlighted by family members caring for patients with uncured XDR-TB in the community. This group appears to be neglected by healthcare services despite their need for support in caring for patients with a terminal and infectious disease. Furthermore, families experienced an additional burden being obligated to visit their relatives on a daily basis when patients were unable to live at home due to poor infection control or the presence of young children at home. The family member chosen to be responsible for a patient once they are discharged is patient-led [32]. The emotional and financial struggles associated with such responsibility, as mentioned by family members require a need for improved liaison between health professionals and families before a patient is discharged. This is to ensure that families are equipped, educated and supported for this considerable task.

There were several limitations to this study. Although not ideal, interviews were conducted telephonically due to the potentially fatal and infectious nature of XDR-TB. However, data collection by phone yields comparable data to face-to-face interviews. [33, 34]. Furthermore, due to the highly personal and emotional context discussed during the telephonic interviews, patients came across as being enabled to be more honest and open to talk about personal thoughts and feelings.

Bias could have potentially been introduced in several ways. Patients were required to own or have access to a landline or cell phone. Interviews were conducted in English, limiting the extent to which participants could talk during interviews and in some cases impacting on the rapport established during an interview. The inclusion of patients, after their treatment had failed, defaulters and family members in a small sample limits the generalisability of the themes identified. Furthermore, family members interviewed had varied relationships to patients and were not always immediate family to the patient. The findings do not aim to be generalisable across all patients with XDR-TB and may be unique to patients in the Western Cape of South Africa. Further qualitative research is needed in other parts of South Africa and in other countries.

Conclusion

Current models of care are not meeting the needs of patients with uncured XDR-TB and relatives. Patients are inadequately supported and face stigma in the community increasing feelings of isolation and risks of transmission through non-adherence to infection control. Increased psychosocial support is needed during treatment to reduce chances of defaulting. Quality of life can be improved through community-based palliative care to reduce isolation and vocational facilities to improve economic opportunities and provide income. Increasing home-based infection control and community knowledge and awareness can reduce person-to-person transmission. Psychosocial and economic support for family members is also needed to relieve burden and requires further insight into the challenges they are faced with. Only by addressing these patient-level factors associated with XDR-TB will care be improved, and transmission reduced, in the communities of South Africa.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Study plan indicating how the cohort that participated in the research was selected.
Table S1. Cohort of XDR-TB patients and family members interviewed
Table S2. Extended Drug Sensitivity Tests (DST) for study cohort
Table S3. Themes identified in patients with uncured XDR-TB and their family members living in the communities of the Western Cape
Box S1. Participants' responses to main themes of concerns.

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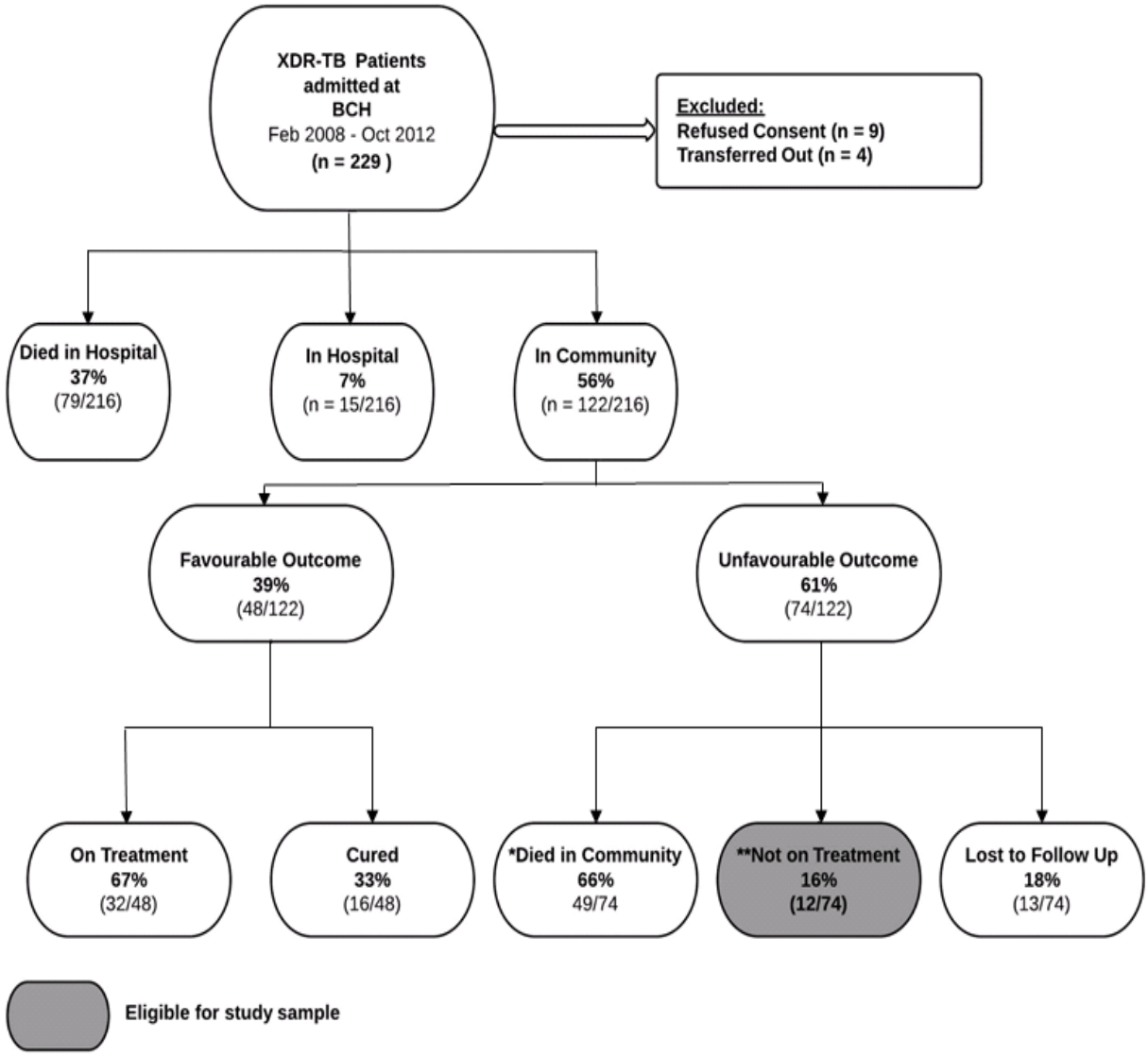
Figure S1. Study plan indicating how the cohort that participated in the research was selected**.**



* Patients who died in the community may have been on treatment, not on treatment, or cured.
** Patients not on treatment include treatment failures and defaulters.

Table S1. Cohort of XDR-TB patients and family members interviewed

Patient	Reason Included in Study	Location at time of Interview	Interview conducted with	No. Drugs the isolate was resistant to out of 18 drugs tested
1	Treatment failed	Home	Patient	3***
2	Treatment failed	Home	Patient	6
3	Treatment failed	Home	Patient	12
4	Treatment failed	Home	Patient	8
5	Treatment failed	Home	**Family Member – Aunt** (patient Wanders streets)	Unknown**
6	Treatment failed	Hospital Re-admitted for palliative care only)	**Family Member – Sister**	Unknown**
7	Treatment failed	Home	**Family Member – Mother** (hearing loss in patient)	12
8	Defaulter	Hospital (Re-admitted after self-discharge)	Patient	12
9	Defaulter	Home (discharged themselves)	Patient	10
10	Defaulter	Home (discharged themselves)	Patient	9
11	Defaulter	Residence Unknown	**Family Member - Brother**	7
12	Defaulter	Residence unknown	**Family Member - Cousin**	9

*Died since interview
** Extended DST not performed
*** Extended DST of only 5 drugs

Table S2. Extended Drug Sensitivity Tests (DST) for study cohort

Patient	Capreomycin	PZA	Linezolid	Clofamazine	Clarithromycin	Dapsone	Amikacin	Cycloserine	Ethambutol	Ethionomide	INH	Kanamycin	Moxifloxacin	Ofloxacin	PAS	Rifampicin	Rifambutin	Streptomycin	Total number of drugs patient is resistant to
1	S	S	S	R	-	S	R	R	R	R	R	R	S	R	R	R	R	R	**12/17 (71%)**
2	R	S	S	S	S	S	R	R	R	R	R	R	R	R	S	R	R	R	**12/18 (67%)**
3	R	S	S	R	S	S	R	S	R	R	R	R	R	R	S	R	R	R	**12/18 (67%)**
4	R	S	S	S	-	S	R	S	R	R	R	R	R	R	S	R	R	S	**10/17 (59%)**
5	R	S	S	S	S	S	R	S	R	S	R	R	S	R	S	R	R	R	**9/18 (50%)**
6	R	R	S	S	-	-	R	S	R	S	R	R	S	S	S	R	S	R	**8/17 (47%)**
7	R	S	S	S	R	S	S	S	S	R	R	S	S	R	S	R	R	R	**8/18 (44%)**
8	R	S	S	S	S	S	R	R	S	S	R	R	R	R	S	S	R	S	**8/18 (44%)**
9	R	S	S	R	-	S	R	S	S	S	R	R	S	S	S	R	R	S	**7/17 (41%)**
10*	R	S	S	R	-	S	-	-	-	-	-	-	-	-	-	-	-	-	**3/5 (60%)**
11**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
12**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**

* Extended DST performed for 5 drugs only

** No extended DST performed

Table S3. Themes identified in patients with uncured XDR-TB and their family members living in the communities of the Western Cape

Theme 1: **Living with and not being cured of XDR-TB**	**Treatment failed**	**Defaulters**	**Family members**
"I'm a failure"	X		
"Never-ending" and futile	X	X	
Restriction and fearful premonition of hospitalisation	X	X	
Blame	X	X	X
Bitterness and decline	X		X
"It's out of my control".	X		
Mistrust and disbelief	X	X	
Coping	X		X
Wanting to survive	X	X	
Us and them	X	X	X
Theme 2: **Altered Lifestyle in the community with uncured XDR-TB**			
Isolation, loneliness	X		
Rules, limitations	X		
Altered relationships	X		
Discrimination, stigma	X	X	X
Stuck with no purpose	X		
A 'stopped' life and stunted future	X		
"I feel fine"	X	X	
Fighting disease	X	X	
Theme 3: **Experiences with community healthcare**			
Traumatic experiences	X	X	X
Unsupported	X	X	
Theme 4: **Experiences with the local community**			
Lack of understanding	X	X	X
Exclusion and discrimination	X	X	X
Theme 5: **Wants and Needs of uncured XDR-TB Patients**			
Independence and self-sufficiency	X	X	
'Being Normal'	X	X	

Purpose	X		
Subsistence	X	X	X
Relationships	X		
Support systems	X		
'Outside', not hospitalised	X	X	
Theme 6: **Experience of family members responsible for uncured XDR-TB patients**	**Treatment failed**	**Defaulters**	**Family members**
Worry			X
Burden, Wanting Relief, need for support.	X		X
Blood Obligation			X
Accountability and Responsibility			X
Not my "own family".			X
"We won't get it".			X

Box S1. Participants' responses to main themes of concerns

Living with and not being cured of XDR-TB

"It felt like it was a waste. You kind of put your trust in your caregivers. They tell you to stop smoking, to stop drinking, and live an active life. So you follow all of that and nothing happens". (Failed patient, Male)

"I'm not proud of it sister.I drink my pills and treatment sister but sometimes sister I, I must drink it sister and then I don't drink it sister, it's a lots of pills sister, drinking many pills sister every day sister. I'm human sister, do you understand. Everyone getting, how can I say, frustrated with the time sister, but the time now my TB is 6, 7 years sister I got TB now". (Defaulting patient, Male).

Patient: "My life is different sister. It's not the same.

Interviewer: Could you tell me a bit more? How is it not the same?

Patient: Yes sister..,they told me I'm a failure and they told me I'm….they told me I'm a failure and I lose". (Failed patient, Male)

"They say you have a TB, but all, all are in the wards. In one place all they have got TB, so how can you can be, you can be cured, but …all of us we got TB. We sleep in one…in one hall. (Defaulting Patient, Male)

God is with me, I pray everyday, I'm alive everyday so I believe in God. I not smoke anymore. I'm fine sister. (Failed patient, Male)

Altered Lifestyle in the community

"He was playing pool but all the time before he was ill. And he was a champ. His trophy stands here. But when he get XDR, it's all gone." (Family member of failed patient, Female)

"It changed a lot. Because now, I don't have friends, you understand? Because some of my friends know about me, and then they make excuses when they...you call them and want them to come to visit you they say no we're working, they're busy this weekend. Whether you know these people are lying to you, they're making lots of excuses not to interact with you because they are afraid to get this disease also you understand?" (Failed patient, Male)

Patient: "If, if I can have my own transport...because I've got sore foot, sore foot, it's painful.

Interviewer: If you do go somewhere, what is your transport?

Patient: I say if I can have my own transport, and then someone who understand me I put my own mask, I go, maybe where I want to go. You understand? Like the time we were there in hospital.

Interviewer: But otherwise, is it taxis and things like that?

Patient: They told us we can't. You can't be in public, like, without mask. So instead I'm here in my place". (Failed patient, Female)

"It's not nice to wear a mask in front of all our people. Whereas you are the only one who is doing that. If there are 50 people who are same like me it would be okay, because they won't be able to talk to...once they see someone with something different then people like to talk, you understand? It wasn't very nice". (Failed patient, Male)

"It was very sad for me to leave her alone but I have to for the sake of my children. The sister at the clinic said she must stay on her own. She mustn't stay with me because she can infect my children and she isn't well. So I told her she must stay on her own". (Family member of failed patient, Female,)

"I have a girlfriend who I also limit time with. TB stunts your progress if I could put it that way. There's certain things that she wants out of life and I got TB, she wants to get married and have kids and I always tell her I got TB and I need to sort that out first". (Failed patient, Male)

Experiences with community healthcare

"If the patient is there at the clinic you must help the patient, you can't ask for, for formally, a letter. You must help the patient. And they didn't. After that she didn't go back". (Family member of failed patient, female)

"When mother goes to fetch my medication or something they will talk silly stuff to the sister to my mother. Sisters they say 'look that's the mother of the child who's about to die' you see what the kind of things they say".(Failed patient, Male)

Experiences with the local community

Interviewer: "How do you think people in the community would treat him if they saw him with the mask?

Patient: Oooh, these people here? They will run.

Interviewer: Run away?

Patient: Yeah. This, these people are so full of things here". (Family member of failed patient, female)

"It's not nice to wear a mask in front of all our people. Whereas you are the only one who is doing that. If there are 50 people who are same like me it would be okay, because they won't be able to talk to...once they see someone with something different then people like to talk, you understand? It wasn't very nice". (Failed patient, Male)

"Nobody talk about. And if you are going to clinic you are maybe HIV positive and you have TB, all the people staring at you…people don't want to go for treatment because…people look at them as aliens here". (Family member of failed patient, Female)

Wants and needs of uncured XDR-TB patients

Patient: "Every day sister I do it...I sleep...I sit up I...exercise, I take a...I...I still run to the, to the fire station and come back. Then I wash me and I go to sleep. I keep my fitness… I don't want this...anyone to see... How my body go out of control". (Failed patient, Male)

"I've got a family, when I've got a problem. But now, I don't have problem. I can call my sister of my mother. That time I used to get sick? She's the one who used to come and escort me to the clinic. But now I didn't have problem." (Failed patient, Female)

"It's not right for me sister. Because I want to work for my family sister…I have 2 kids sister. 5, 6, 7 months sister I'm alright, then after that I'm weak sister". (Defaulting patient, Male).

"Outside sister I can heal faster, because why you are roaming outside you can go to the shop and you see different people sister. The people is not like this here". (Defaulting patient, Male).

Experience of family members responsible for uncured XDR-TB patients

"Every day, I set in my mind the news will come now, will come to me that he's gone. Because I don't know where he is exactly. Really really that worries me". (Family member of failed patient, Male)

"If I must get it, that illness, I must get it. If I must, the Lord know. I believe, if you feel ashamed or 'I'm afraid because I'm gonna get that same sick', he's ill and people say don't go over to other

people, then you gonna get sick. But if you believe you won't get sick". (Family member of failed patient, Female)

"They will look after him. I know he will comfortable because he can, can eat.and he can sleep well and by his own family there. So I want him, he must go to his home town". (Family member of defaulting patient, Female)

Patient: "My people are normal sister, they don't say no you can't drink, you can't drink out of one cup or something like that sister man. They don't do that sister, they believe that they, they won't get TB sister.

Interviewer: And do you believe that too?

Patient: Yah sister they don't, they won't get it sister cos we believe in God sister man, we pray every day every morning every night sister". (Defaulting patient, Male).

CHAPTER 7: SUMMARY AND CONCLUSIONS

Recent gains in TB control are being overturned by drug-resistant tuberculosis [3]. Furthermore, drug-resistant TB has high rates of unfavourable outcomes and dismal cure rates, using a non-bedaquiline and linezolid-based regimen, and is a threat to TB control and health care workers in South Africa [155-157]. Even with bedaquiline 30 months sputum culture conversion in XDR-TB is only ~60% [158]. Data on long-term treatment-related outcomes in XDR-TB patients in a period prior to the availability of bedaquiline and linezolid were required to inform clinical management, resource allocation, and policy guidelines. Most studies, hitherto, only reported short-term treatment-related outcomes [122, 125, 126]. Furthermore, the newly adopted WHO End TB Strategy, in line with sustainable developmental goal (SDG) targets and milestones [3] yet again highlights the importance of effective management of XDR-TB for TB control [159].

In this chapter I summarise the work outlined in the thesis and provide relevant conclusions and their implications. Discussion specific for each paper is outlined in the relevant discussion sections of each chapter. This discussion is not duplicated here. The purpose of this final chapter is to provide a summary and final conclusion.

In **Chapter 1** I addressed the context and rationale of the study and in **Chapter 2** provided a background on the burden of TB disease, transmission dynamics, and treatment-related outcomes in XDR-TB globally and in South Africa.

In **Chapter 3** we reported on long-term treatment-related outcomes in a cohort of XDR-TB patients which, to my knowledge, was the first prospective cohort study reporting 60 months treatment-related outcomes in XDR-TB patients. We confirmed that, despite a multidrug capreomycin-based regimen, treatment-related outcomes and prognosis in XDR-TB are poor. Furthermore, patients who failed an XDR-TB regimen were discharged from hospital notwithstanding positive smear microscopy. This data underscored the urgent need for new XDR-TB regimens to become available. Given the advent of community-based incurable XDR-TB we urged for interventions such as community and palliative care facilities to minimise the spread of this deadly disease.

Therapeutic treatment regimen options for cohorts included in this thesis, who received treatment within a resource poor national TB programme, were limited and therapeutic benefits were uncertain. Furthermore, empiric use of capreomycin, the backbone of a presumed effective XDR-TB regimen, was routine during the study period despite little data about the resistance of this second-line anti-TB drug. In **Chapter 4** we investigated factors associated with poor treatment outcomes and proved, in contradistinction to existing practice, a lack of therapeutic benefit related to the use of capreomycin. Furthermore, high levels of capreomycin resistance in XDR-TB isolates, even in capreomycin and aminoglycoside naïve patients, were uncovered. Not only did this data strengthen the urgency for the development of new anti-TB drugs, and an alternative regimen, but also suggested omission of capreomycin in XDR-TB patients with capreomycin resistance. Furthermore, we highlighted the need for

genotypic testing for second-line resistance and the high level of toxicity associated with the use of capreomycin [85], not to mention the prohibitive costs [4].

The WHO highlighted the need for research focusing on special groups like TB HIV co-infected patients [95]. Indeed, in **Chapter 5** we addressed factors associated with treatment-related outcomes in XDR-TB HIV-infected persons. We have shown in TB and HIV endemic setting that long-term treatment-related outcomes are poor, irrespective of HIV status, and despite hospitalisation where fairly good adherence is anticipated. HIV-infected persons who managed to start treatment had marginally poorer survival, though survival in HIV-infected persons on ART was not different to HIV-uninfected patients. Post-discharge survival was, however, similar for HIV-infected and uninfected patients. Although HIV-infected persons had more clinically defined primary XDR-TB, higher rates of adverse events, and higher levels of smear positivity at discharge, strain types were similar in all XDR-TB patients. This data substantiated our previous findings and once more emphasised the need for better approaches to phenotypic and genotypic diagnostic tools, including whole genome sequencing [160] as well as the use of effective regimens containing new and repurposed drugs. We stressed that strengthening of the national TB programme and the identification of the most infectious community-based patients is likely mandatory to reduce the overall burden of HIV and XDR-TB.

In fact in **Chapters 3 and 5** we described high levels of treatment failure in XDR-TB patients. XDR-TB patients in whom treatment failed were home-discharged irrespective of a positive smear microscopy result. Their longevity in the community is likely to contribute to the

persistent burden of TB disease as a result of transmission of community-based XDR-TB disease. Little attention has however been given to patient-level factors and behaviour impacting on community-based care and transmission of XDR-TB. In **Chapter 6** we concluded that current models of care do not meet the needs of these therapeutically destitute community-based XDR-TB patients and their relatives. We highlighted the need for psychosocial support, community-based palliative care and vocational facilities to improve their economic opportunities. However, the value and impact of palliative care on XDR-TB treatment-related outcomes is lacking and worth exploring [161]. A surprise finding from interviews conducted with family members was the level of burden of care they experienced and their particular need for psychological and economic support. It became clear that improved care and reduced TB transmission will likely only be achievable when patient-level factors associated with XDR-TB are addressed.

CONCLUSION

Research completed in this thesis interrogated treatment-related outcomes, molecular epidemiology, and social perspectives related to XDR-TB in an era prior to the use of newer and repurposed anti-TB drugs. Key findings of the study were: (i) treatment-related outcomes in XDR-TB are poor irrespective of HIV status; (ii) community-based incurable XDR-TB is a growing problem and smear positive XDR-TB patients are discharged from hospital; (iii) there is a lack of therapeutic benefit of capreomycin in an XDR-TB regimen and high levels of capreomycin resistance even in aminoglycoside naïve patients; (iv) compared to HIV-uninfected patients survival in HIV-infected patients who reached treatment were marginally poorer, however,

survival in those on ART compared well to HIV-uninfected patients; (v) models of care do not meet the needs of therapeutically destitute community-based XDR-TB patients and their relatives and hence the need for psychosocial support, palliative care and vocational facilities to cater for socio-economic needs of community-based incurable XDR-TB patients.

Poor treatment-related outcomes in XDR-TB are, however, consistent across studies conducted during a similar timeframe as this research [74, 122, 127, 162]. The data presented in this thesis thus underscored the urgent need for testing of new combined regimens for XDR-TB to be made available to National TB programmes. Even with DR-TB treatment success evident since newer and repurposed drugs like bedaquiline and linezolid were introduced [163] programmatic management of DR-TB remains a challenge [164]. Current research efforts to establish a shorter treatment regimen for (X)DR-TB include the STREAM and NeXT studies [6, 165]. Furthermore, improved phenotypic and genotypic diagnostic tools including whole genome sequencing [166] which will further facilitate access to individualised XDR-TB treatment [160] are essential.

During the study decentralised care was initiated due to a limited bed capacity in designated TB hospitals. Consequently, patients who failed treatment were being discharged back to the community where they needed to settle in with their relatives as alternative residential or palliative care facilities were non-existent or limited. Home-based care was reduced to what relatives were able and willing to provide. Thus to minimise transmission from these incurable XDR-TB patients the identification of infectious community-based DR-TB patients is mandatory. Similarly, patient level factors and behaviour impacting XDR-TB transmission require urgent

attention. Furthermore, resistance beyond XDR-TB or totally drug-resistant TB is emerging which is an added threat to public health.

Evidence regarding best practice and thus optimal health care service delivery relevant to the particular socio-economic needs of patients, adherence behaviour, and family needs in high-burden TB HIV countries are, however, lacking. Furthermore, alleviating the burden of DR-TB disease, including innovative design interventions to interrupt the burden of disease, needs an approach that goes beyond medical therapies. Such an approach would need to include addressing social determinants of health [99]. Although the notion of social determinants of health might 'lie outside the health system', [167] it impacts on the health system and more importantly on people using the health system. Thus ignoring social determinants of DR-TB happens at the peril of the patient, the community, health professionals and the disease itself [168, 169]. Furthermore, prevention strategies such as strengthening of the NTP, reduction of the overall burden of TB and HIV alike, and addressing social determinants of health like poverty and overcrowded households are essential to reach the End TB Strategy and ultimately SDGs by 2035 [170].

The goal to end the TB epidemic by 2035 has made a paradigm shift essential. Health care professionals and service providers are required to rapidly reconstruct care and management standards [3] and trans-disciplinary research focusing on reframing problems, and perspective on these problems and challenges might be the only, and even best, place to start [167].

The various chapters in this thesis in totality have some common and overlapping limitations. Cohorts included in the study were limited to four specialised TB units in South Africa. Findings

from this research might therefore not be applicable to other global or African setting with dissimilar XDR-TB and HIV disease burden. Furthermore, considerable changes to treatment regimens, and point-of-care diagnostics occurred since the inception of the study. Treatment-related outcomes, predictors of survival and prognosis reported in this research might thus be out-dated and not appropriate to emerging trends related to drug-resistance and treatment regimens in high burden TB endemic settings.

In conclusion there is an urgent need to roll out new drugs and diagnostic technologies, limit the transmission of XDR-TB, and provide community-based facilities to meet patient needs. However these goals will not be met without addressing socio-economic and political factors. As Nelson Mandela said 'As long as poverty, injustice and gross inequality persist in our world, none of us can truly rest'.

APPENDICES

Appendix 1: PhD declaration

I, Gerbrecht Elizabeth Pietersen, hereby declare that this thesis is in part based on 3 manuscripts (Chapters 3, 4 and 6) published in peer-reviewed journals while I was registered as a PhD candidate. Published manuscripts are presented in the style and formatting of the journal in which manuscripts were published. Manuscripts included in the thesis are listed below, with a description of my contribution to each. All co-authors provided written approval for inclusion of the manuscripts in my thesis.

Chapter 3

Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a prospective cohort study.

Elize Pietersen, Elisa Ignatius, Elizabeth M Streicher, Barbara Mastrapa, Xavier Padanilam, Anil Pooran, Motasim Badri, Maia Lesosky, Paul van Helden, Frederick A Sirgel, Robin Warren, Keertan Dheda.

Lancet. 2014;383(9924):1230-9. doi: 10.1016/S0140-6736(13)62675-6. PubMed PMID: 24439237

In Chapter 3 I included a manuscript focusing on the 10 year follow-up of XDR-TB patients diagnosed shortly after the outbreak of XDR-TB in South Africa. Professor Keertan Dheda requested that I lead and manage the follow-up of these XDR-TB patients from 3 provinces in

South Africa (cohort A). I reviewed all patient records at the three specialist hospitals where patients were treated. I summarised and edited the data in collaboration with the joint first author. Co-authors provided intellectual contributions to the structure and content of the manuscript, including editorial input to the final draft.

Chapter 4

High Frequency of Resistance, Lack of Clinical Benefit, and Poor Outcomes in Capreomycin Treated South African Patients with Extensively Drug-Resistant Tuberculosis.

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We noticed that long-term treatment-related outcomes (as reported in Chapter 3) in XDR-TB patients are poor and questioned the effectiveness of capreomycin, the injectable that forms the backbone an XDR-TB regimen. In Chapter 4 I included a manuscript that focused on the therapeutic benefit of a capreomycin-based XDR-TB regimen, the frequency of capreomycin resistance and associated treatment-related outcomes in XDR-TB. I collated clinical data from all patient records (cohort A+B), liaised with the molecular epidemiologist in terms of available biological material and conducted the data analysis. I summarised and edited the data after discussions with Professors Dheda and Warren and Dr Peter. Co-authors provided intellectual

contributions to the structure and content of the manuscript, including editorial input to the final draft.

Chapter 6

Lifestyle, attitudes and needs of uncured XDR-TB patients living in the communities of South Africa: a qualitative study.

Meera Senthilingam*, Elize Pietersen*, Ruth McNerney, Julian te Riele, Pat Sedres, Ruth Wilson and Keertan Dheda. *The first two authors contributed equally

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During the course of the study National TB programme policy changed to decentralised XDR-TB patient care. This resulted in the discharge, back to the community, of XDR-TB patients who failed their XDR-TB treatment. Through telephonic follow-up of these patients I became aware of constraints and numerous predicaments these home-discharged patients, and their family members, deal with. Professor Dheda agreed that we interview community-based XDR-TB patients who were living with an incurable life-threatening disease. In Chapter 6 I included a manuscript that reported on the wants, needs and experiences of incurable community-based XDR-TB patients (cohort B). The joint first author and I discussed interview transcripts and debated probable thematic themes from data extracted. We reflected on the thematic themes with Professors Dheda and McNerny to facilitate concluding of themes. Co-authors provided

intellectual contributions to the structure and content of the manuscript, including editorial input to the final draft.

This thesis is presented for examination in fulfilment of the requirements for the degree of Doctor of Philosophy in Medicine.

I confirm that no part of this thesis has been submitted in the past, or is being, or is to be submitted for additional degree purposes at the University of Cape Town or any other university. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or in part for the purposes of research or teaching.

Gerbrecht Elizabeth Pietersen

October 2016

As the senior author and supervisor of all the above original manuscripts, and on behalf of all co-authors, I confirm the above authors' contributions to be accurate.

Signed by candidate

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Professor Keertan Dheda

October 2016

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Appendix 2: Chapter 4: Co-authored manuscript: *Drug-associated adverse event and their relationship with outcomes in patients receiving treatment for extensively drug-resistant tuberculosis in South Africa*

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Drug-Associated Adverse Events and Their Relationship with Outcomes in Patients Receiving Treatment for Extensively Drug-Resistant Tuberculosis in South Africa

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Abstract

Background: Treatment-related outcomes in patients with extensively drug-resistant tuberculosis (XDR-TB) are poor. However, data about the type, frequency and severity of presumed drug-associated adverse events (AEs) and their association with treatment-related outcomes in patients with XDR-TB are scarce.

Methods: Case records of 115 South-African XDR-TB patients were retrospectively reviewed by a trained researcher. AEs were estimated and graded according to severity [grade 0 = none; grade 1–2 = mild to moderate; and grade 3–5 = severe (drug stopped, life-threatening or death)].

Findings: 161 AEs were experienced by 67/115(58%) patients: 23/67(34%) required modification of treatment, the offending drug was discontinued in 19/67(28%), reactions were life-threatening in 2/67(3.0%), and 6/67(9.0%) died. ~50% of the patients were still on treatment at the time of data capture. Sputum culture-conversion was less likely in those with severe (grade 3–5) vs. grade 0–2 AEs [2/27(7%) vs. 24/88(27%); p = 0.02]. The type, frequency and severity of AEs was similar in HIV-infected and uninfected patients. Capreomycin, which was empirically administered in most cases, was withdrawn in 14/104(14%) patients, implicated in (14/34) 41% of the total drug withdrawals, and was associated with all 6 deaths in the severe AE group (renal failure in five patients and hypokalemia in one patient).

Conclusion: Drug-associated AEs occur commonly with XDR-TB treatment, are often severe, frequently interrupt therapy, and negatively impact on culture conversion outcomes. These preliminary data inform on the need for standardised strategies (including pre-treatment counselling, early detection, monitoring, and follow-up) and less toxic drugs to optimally manage patients with XDR-TB.

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Introduction

Over the last two decades the entity of multidrug resistant tuberculosis (MDR-TB i.e. resistance to at least isoniazid and rifampicin) has emerged. In 2008 there were approximately 440 000 cases of MDR-TB globally[1]. 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, any fluoroquinolone and one of the 2nd 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[2].

In high burden settings treatment outcomes of MDR-TB are disappointing with only ~50% of patients successfully completing treatment[3]. Outcomes in XDR-TB are poorer. We and others have recently shown that, in contrast to low and intermediate burden settings[4], less that 20% of patients with XDR-TB

culture-convert in high burden settings[5,6]. 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[5]. Thus, treatment interruption due to any cause may potentially subvert successful outcome in patients with XDR-TB. Failure to identify and manage presumed drug-associated adverse events (AEs) may also have serious implications for patient perceptions about toxicity versus benefit, and thus may impact on compliance. Even in adherent inpatients, we and others have recently shown that AEs are common in patients with XDR-TB[5], [7].

However, data about the relationship between AEs and 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 AEs, and associated clinical outcomes. Given that capreomycin modulates outcomes and is a vital backbone of most XDR-TB treatment regimens[6], the frequency of AEs 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 association of AEs with outcomes we reviewed the case records of 115 patients treated for XDR-TB at three treatment centres in South Africa.

Methods

Study setting and participants

We retrospectively reviewed the case records of 115 consecutive laboratory-confirmed XDR-TB patients diagnosed between August 2002 and February 2008 at three designated XDR-TB treatment centres in South Africa (see Figure 1 for the study outline). Patients were admitted to the facilities for the duration of their treatment and thus adherence was assumed to be excellent unless the patients self discharged (designated as default from treatment). Case records were comprehensively reviewed by a trained researcher for AEs listed in Table 1 (including duration, type and severity), drug regimen used (dose, indication, route of administration), culture conversion and mortality outcomes, and HIV status. Associated demographic and clinical information were also transcribed into a case record form, and the information captured by double data entry.

Ethics

Ethical approval was provided by the UCT Research Ethics Committee. As per the regulations at UCT patient-provided written informed consent was not required as retrospective annonymised data was used in this study.

Diagnosis and treatment regimens

The standard definition of XDR-TB was used to define patient inclusion[8]. Drug-susceptibility testing to capreomycin, terizidone/cycloserine and fluoroquinolones other than ofloxacin was unavailable as these tests are not undertaken by the National Treatment Program (NTP). The drugs used in the treatment regimens are shown in Table 2. XDR-TB treatment was only initiated and administered in hospital with the use of capreomycin and para-aminosalycilic acid (PAS) as the anchor drugs since late 2006/early 2007. Treatment with capreomycin was empiric and in almost all cases was given in the absence of prior susceptibility testing. Third-line drugs (clarithromycin, dapsone, amoxicillin/clavulanate and azithromycin) were used at the discretion of the attending clinician. High-dose INH was administered at a dose of 10 mg/kg. Clofazimine and moxifloxacin was used in selected centres on a limited basis. ART was offered to all HIV co-infected patients irrespective of the patients' CD4 count.

Definition of adverse drug reactions

For the purposes of analysis grades 1 and 2 AEs were considered mild to moderate, and grade 3–5 severe (see definitions in Table 1). Events where the drug was discontinued was designated grade 3. Multiple events of the same AE were counted separately.

Outcomes

All cause mortality and culture conversion were the primary outcomes of interest. Conversion was judged to have occurred when two consecutively negative cultures were obtained, 1 month apart, and providing that a culture taken at initiation of XDR-TB treatment was positive. Time to conversion was measured in days from treatment start date to the take date of the first of the two negative cultures.

Mycobacterium tuberculosis strain typing

A subset of 53 XDR-TB isolates from patients from the Western Cape were genotyped by IS*6110* DNA fingerprinting [9] and spoligotyping [10]. Strains were categorised as Beijing or non-Beijing according the their spoligotype signature [11].

Data handling and statistical analysis

A data risk management tool, including double data entry, was used to ensure data integrity. Categorical data were compared using χ^2 test and continuous data were compared using Mann-Whitney test or Kruskal-Wallis test (SPSS, Version 17). Cox proportional hazards regression models were fitted to determine risk factors associated with outcomes in a time-to-event (conversion and mortality) based analysis. These factors included AEs, previous MDR-TB, 6 month culture conversion (when assessing risk factors for death), HIV status, usage of ART, weight, age, sex, ethnicity, number of previous TB episodes, number of drugs used in a regimen. Factors found to be significant in univariate were included in the final multivariate analysis. Kaplan-Meier's method was used to calculate probabilities of events, and the Log-rank test was used to compare these probabilities by group. All tests were two-sided, and a p-value <0.05 was considered significant. The proportionality assumption of the Cox models was tested using –ln[–ln (survival)] curves and regression of scaled Schoenfeld residuals on functions of time.

Results

Demographic and clinical characteristics

AEs were reported in 58.3% (67/115) of patients. The breakdown by severity of AE and HIV status, stratified by number of patients and total number of AEs, is shown in Figure 1. The median CD4 count in HIV-infected persons was 204 (range 13–893) cells/mm^3. We could not identify any demographic and clinical variables that were specifically associated with the development of AEs (grade 1 to 5) compared to those who did not develop an AE (grade 0). However, patients with severe AEs (grade 3 to 5), when compared to those with mild, moderate or no AEs (grade 0, 1 and 2), were more likely to be female, have had previous MDR-TB or drug sensitive TB, and had fewer drugs in their treatment regimens (Table 3). Furthermore, in the multivariate analysis only a history of previous MDR-TB was indepen-

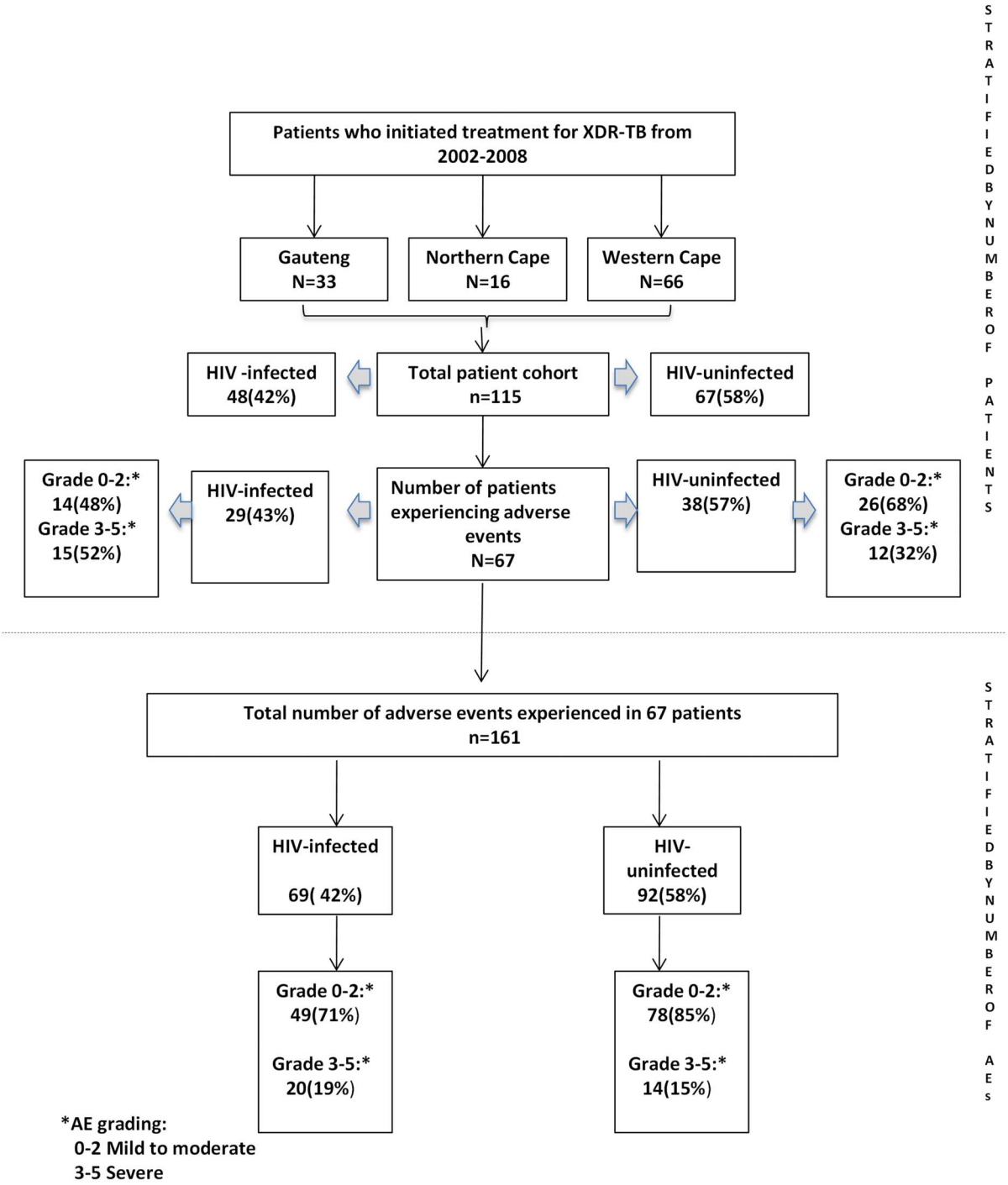

Figure 1. Study plan stratified according to treatment site, HIV status and severity of adverse drug reactions.
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dently associated with the likelihood of developing severe AEs (grade 3 to 5); p = 0.009. The overall median (IQR) duration of follow-up (months) within the cohort was 7.3 (3.1–12.6). As at the study censure date 21% of patients had died, 22% had defaulted treatment, 7% were cured or had completed treatment, and the remaining of 50% were on on-going treatment.

Frequency and severity of AEs

161 AEs were experienced by 67/115 (58%) patients (Figure 1; upper panel). When the results were stratified by the number of patients 17/67 (25.4%) patients required no intervention (grade 1); 23/67 (34.3%) required modification of treatment (grade 2), the offending drug was discontinued in 19/67 (28.3%) of patients (grade 3); reactions were life-threatening in 2/67(3.0%; grade 4), and 6/67(9.0%) died (grade 5). When the results were analysed by the number of AEs (Figure 1; lower panel): in 58/161 (36%)

Table 1. Definitions used to grade, identify and classify adverse events.

A. Grading of adverse events[1]	
grade 0	no AE
grade 1	mild AE i.e. described in the patient's management records but no action was taken
grade 2	moderate AE resulting in either changing the dose or frequency of the offending drug or another drug(s) was added to manage the AE
grade 3	the side effect was severe enough for the offending drug to be stopped
grade 4	the AE was life threatening or disabling
grade 5	the AE caused the death of the patient
B. Definitions used to identify and classify adverse events.	
Nausea, vomiting, diarrhoea. Other GI symptoms: abdominal pain, dyspepsia, and epigastric discomfort	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
Hypokalaemia	<3.5 meq/L (normal range: 3.5–5.5 meq/L)
Hypothyroidism	At least one thyroid stimulating hormone (TSH) result >4.94 IU/ml (normal 0.35–4.94) that was thought to be unrelated to the sick euthyroid syndrome
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].
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instances an AE 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 AE; the offending drug was withdrawn in 34/161 (21%); the AE was life-threatening in 2/161 (1.2%) instances (both AEs were due to renal failure), and death was associated with 6/161 (4%) of AEs. All 6 deaths were associated with 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 therapy including Capreomycin. The severity of AEs was not associated with the frequency and duration with which the drug was used, or the resistance pattern of the drug.

Culture conversion and mortality outcomes stratified by HIV status

Culture conversion occurred in 26/115 (22.6%) of patients. Patients with grade 3–5 AEs had a lower sputum culture conversion rate compared with those with grade 0–2 AEs [2/27 (7.4%) vs. 24/88 (27.3%); p = 0.02; Figure 2A]. In a Cox regression of the whole cohort the hazard ratio for AE (grade 3–5 AEs compared with grade 0–2 AEs) 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 AEs (grade 3–5) had a significantly lower sputum culture conversion rate than those with grade 0–2 AEs [0/15 (0%) vs. 10/33 (30.3%), p = 0.02].

Of the 115 patients in the cohort, 30 (26.1%) died. Patients with grade 3–5 AEs had a higher death rate compared with those with grade 0–2 AEs [13/27 (48.1%) vs. 17/88 (19.3%); p = 0.003; Figure 2D]. However, in a multivariate Cox regression model for risk factors for death in the whole cohort, only culture non-conversion and previous MDR-TB, but not adverse events, were independently associated with death (Table 4).

In HIV-infected patients mortality rates were higher in those with grade 3–5 (severe) AEs compared to those with grade 0–2 AEs [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 AEs had a higher death rate compared to those without severe AEs [6/12 (50.0%) vs. 9/55 (16.4%); p = 0.02; Figure 2F]. Of the 13 all-cause deaths occurring in the severe AE group, 6 were due to an AE itself (5 due to renal failure and 1 due to hypokalaemia-all likely ascribable to capreomycin). These patients were not

Table 2. Specific drugs, the dosages used in XDR-TB treatment regimens, and the frequency of drug withdrawal due to adverse events relative to the number of patients prescribed the drug.

	Drug dosages used	No. of patients who received a drug as part of the XDR-TB regimen n = 115(%)	Number of patients in whom the drug was withdrawn relative to the total number receiving the drug (%)	Proportion of severe AE [total = 34] due to a specific drug (%)
Isoniazid	4–6 mg/kg/daily	39/115(34)		-
Ethambutol	25 mg/kg/daily	46/115 (40)	1/46(2.2)	1/34(2.9)
Pyrazinamide	30–40 mg/kg/daily	80/115(69.6)	-	-
Amikacin	15–20 mg/kg/daily*	3/115(2.6)	1/3 (33.3)	1/34(2.9)
Kanamycin	15–20 mg/kg/daily*	4/115(3.5)	-	-
Ofloxacin	600–800 mg daily	29/115(25.2)	-	-
Moxifloxacin	400 mg daily	2/115(1.7)	-	-
Ethionamide	15–20 mg/kg/daily	66/115(57.3)	7/66(10.6)	7/34(20.6)
Capreomycin	15–20 mg/kg/daily*	104/115(90.4)	14/104 (13.5)	14/34(41.2)
Para-aminosalicylic acid	8 g (400 mg BD)	101/115(87.8)	7/101(6.9)	7/34(20.5)
Terizidone/Cycloserine	500–750 mg daily	104/115(90.4)	2/104(1.9)	2/34(5.9)
Clarithromycin	1 g (500 mg BD)	77/115(66.9)	-	-
Amoxicillin-clavulanate	375 mg	65/115(56.5)	2/65(3.1)	2/34(5.9)
Clofazimine	200 mg (100 mg BD)	28/115(24.3)	-	-
Dapsone	100–200 mg daily	36/115(31.3)	-	-
Azithromycin	500 mg 3xweekly	11/115(9.6)	-	-
INAT (INH+thiacetazone)	3 tabs daily	2/115(1.7)	-	-
Rifabutin	300 mg daily	1/115(0.87)	-	-
Type of ART	**Dosage used**	**Number of XDR-TB patients receiving drug**	**Number of HIV-infected persons receiving ART**	
		n = 115 (%)	**n = 34 (%)**	
3TC (Lamivudine)	80 mg (40 mg BD)	29/115 (25)	29/34 (85.3)	-
D4T (Stavudine)	300 mg (150 mg BD)	25/115 (22)	25/34 (73.5)	-
EFV (Efavirenz)	600 mg nocte	25/115 (22)	25/34 (73.5)	-
NVP (Nevirapine)	200 mg BD	4/115 (3)	4/34 (11.8)	-
AZT (Zidovudine)	600 mg (300 mg BD)	5/115 (4)	5/34 (14.7)	-
Lopinavir/Ritonavir	800 mg (400 mg BD)	1/115 (1)	1/34 (2.9)	-

*(Maximum dose, 1 g) 5 days/week.

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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 from AEs were HIV-infected.

25/115 (21.7%) of patients defaulted (self discharged) from the inpatient facilities. There was no difference between the proportion of patients with severe AEs among defaulters and the proportion with severe AEs among non-defaulters [7/25 (28%) vs. 27/90 (30%); p = 0.96].

AEs by HIV status

In HIV-infected versus uninfected persons there was no significant difference between the proportion of persons with AEs [29/48 (60.4%) vs. 38/67 (56.7%); p = 0.26)], the number of total AEs per person [2.37 vs. 2.42 AEs per person; p = 0.15], and the number of severe AEs [20/69(29.0%) vs. 14/92(15.2%); p = 0.31]. Thus, the type, frequency and severity of the number of AEs was similar in HIV-infected and uninfected patients. However, those who died of an AE 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 ART (active anti-retroviral therapy). 23/34 (68%) of patients were on a combination of lamivudine (3TC), stavudine (D4T) and efavirenz (EFV). In HIV-infected patients the number of patients experiencing an AE was not significantly different in those taking ART vs. those not taking ART [29/34 (85.3%) vs. 8/14 (57.1%); p = 0.71]. Similarly, the frequency of severe AEs was not significantly different in the same groups [11/34 (32.3%) vs.6/14 (43.0%)]. Thus, ART did not impact on the frequency of AEs and was generally well tolerated. The role of overlapping toxicities between ART and anti-TB drugs could not specifically be evaluated but the number of patients experiencing an AE was significantly higher in those taking ART compared to HIV un-infected patients [29/34 (85.3%) vs. 38/67 (56.7%); p = 0.008]. Nevertheless, the proportion of patients experiencing a severe AE was not significantly different in those taking ART compared to HIV un-infected patients [11/34 (32.3%) vs. 12/67 (17.9%); p = 0.17].

Table 3. Socio-demographic and treatment related clinical characteristics of 115 patients who initiated treatment for extensively drug-resistant tuberculosis (XDR-TB).

	Grade: 0–2 (none/mild/moderate) adverse events	Grade 3–5 (severe/life threatening/ death) adverse events	P value
	n = 88 (% unless otherwise stated)	n = 27 (% unless otherwise stated)	
Sex			
male	53(60.2)	9(33.3)	0.014
Ethnicity			
mixed origin	46(52.3)	14(51.9)	0.969
HIV status			
infected	33(37.5)	15(55.6)	0.096
ART			
yes	23(71.8)	11(73.3)	0.917
Number of previous sensitive TB episodes (IQR)	1(1–2)	1(1–2)	0.033
Previous MDR-TB episodes			
yes	47(53.4)	22(81.5)	0.009
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			
current	37(42)	12(44.4)	0.454
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
Outcome-related variables			
Died			
yes	17(19.3%)	13(48.1%)	0.003
Conversion			
yes	24(27.3%)	2(7.4%)	0.035

*The only other ethnic group in the cohort was Black. Grade 0–2 AE 42(47.7%). Grade 3–5 13(48.1%).
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Type of adverse events and drug withdrawal

Of all drug discontinuations, (n = 34), capreomycin (Capstat; Pharmacare Johannesburg) was the drug withdrawn most often in 14/34 (44.1%) of cases, followed by PAS in 7/34 (20.5%), and ethionamide in 7/34 (20.6%) (Table 2). The withdrawal of capreomycin due to an AE occurred at a median of 73 days (range 9–485) days after initiation of therapy. Persons who took capreomycin and had AEs, compared to those that took capreomycin but had no AEs, were more likely to be taking concurrent ethambutol, augmentin, ethionamide and PZA ($p<0.05$).

The breakdown of AEs by cause is shown in Table 5. Overall, nausea and vomiting (22%), diarrhoea (14%), and other GI symptoms (14%) were the commonest causes of AEs and their frequency did not differ significantly by HIV status. The most common cause of grade 0–2 AEs (79% of the total number of AEs) were GI symptoms overall (nausea, vomiting, diarrhoea and others), which caused ~50% of AEs in this severity category. The most common causes of grade 3–5 AEs (21% of the total number of AEs) were specifically vomiting (29% of severe AEs) and renal failure (21%).

Some patients experienced multiple AEs. 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. AE clustering was also evident in the neurological category (overlapping symptoms of headaches, dizziness, generalised aches and pains etc.).

For the 18 drugs used in the XDR-TB treatment regimens, the severity of AEs was not related to the number of patients who received each drug, total duration of treatment (months), or the proportion of resistant isolates.

AEs 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 [43(81%) vs. 10 (1%); p = 0.0001]. The severity of AEs was not significantly different in the Beijing and non-Beijing families (Table 6).

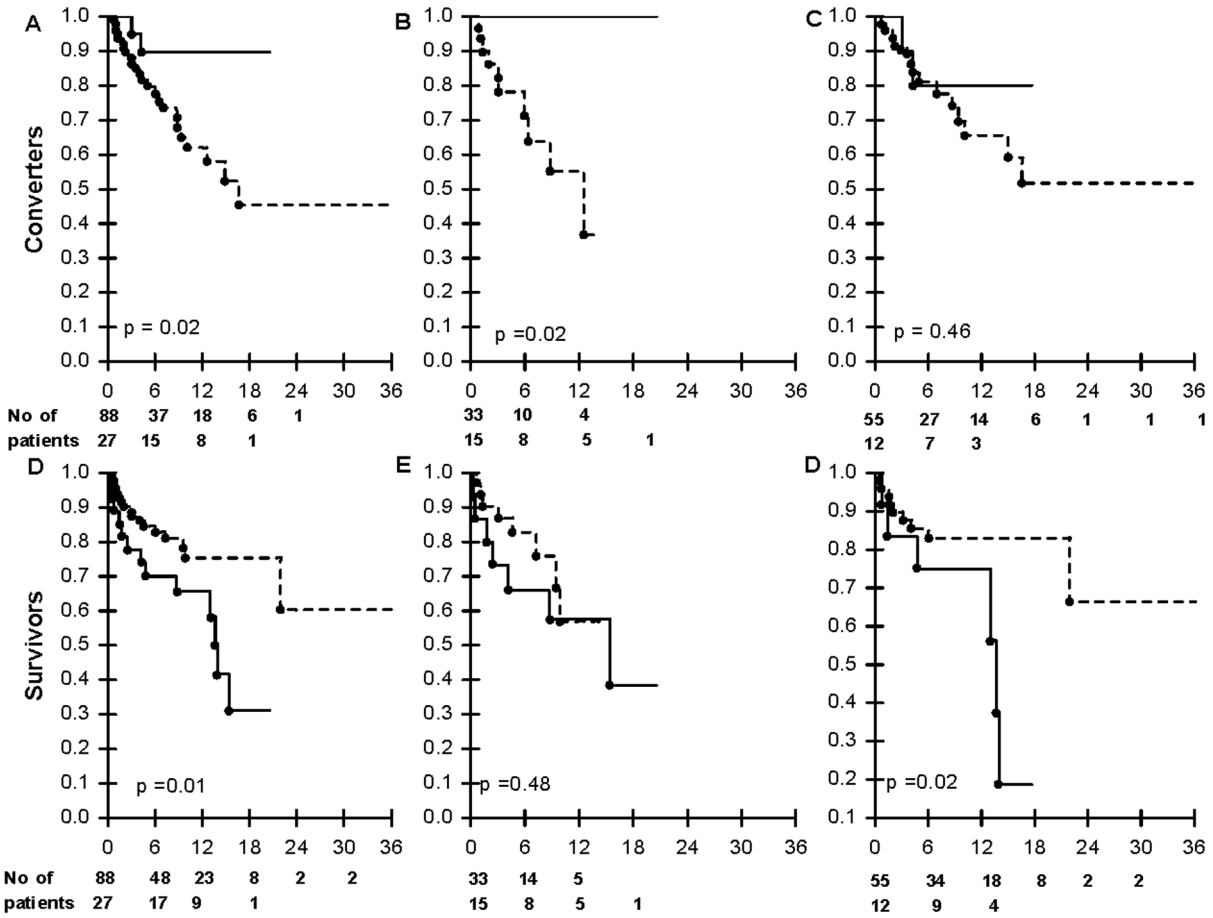

Figure 2. Kaplan-Meier probabilities of XDR-TB culture-conversion in: (A) The whole cohort of patients who experienced AEs stratified by severity score i.e. none or mild to moderate (grade 0, 1 and 2; dashed line) versus severe (grade 3 to 5; solid line); (B) HIV-infected patients whom experienced AEs stratified by stratified by severity score; (C) HIV-uninfected patients who experienced AEs 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 AEs stratified by severity score, and (F) HIV-uninfected patients who experienced AEs stratified by severity categories.
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Discussion

This is the first comprehensive report of AEs and their association with outcomes in a large cohort of patients with XDR-TB. Our key findings were that: (i) the frequency of AEs with XDR-TB treatment regimens is high (~60%), and in ~40% of patients the AE was associated with interruption of therapy, life-

Table 4. Univariate and multivariate analysis of factors associated with mortality in 115 patients with XDR-TB.

Factor	Univariate analysis		Multivariate analysis	
	Hazard Ratio (95%CI)	P-value	Hazard Ratio (95%CI)	P-value
Adverse event				
Grade 3–5	2.39(1.14–4.97)	0.02	1.43(0.67–3.05)	0.35
Grade 0–2	1		1	
Previous MDR TB				
Yes	3.27(1.32–8.03)	0.01	2.91(1.16–7.35)	0.02
No	1		1	
6 month Culture conversion				
Yes	0.09(0.01–0.63)	0.02	0.10(0.01–0.747)	0.03
No	1		1	

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Table 5. Type of adverse event that occurred (n = 161) and total number of patients experiencing these adverse events (n = 115) in patients from the Western Cape, Northern Cape and Gauteng provinces who initiated treatment for XDR-TB.

Presumed drug-associated adverse event	Number of AEs stratified by HIV status (n = 161)			Number of patients with AEs stratified by HIV status (n = 115)		
	n (%)			n (%)		
	HIV+(69)	HIV- (92)	Total AE (161)	HIV+(48)	HIV- (67)	Total patients (115)
Nausea and/or vomiting	15(9)	20(12)	35 (22)	15(31)	20(30)	35(30)
Diarrhoea	8(5)	14(9)	22 (14)	8(17)	14(21)	22(19)
Other GI symptoms: abdominal pain, dyspepsia, epigastric discomfort, cramps	6 (4)	16(10)	22 (14)	6(13)	16(24)	22(19)
Dizziness/disorientation	4(2)	9(6)	13 (8)	4(8)	9(13)	13(11)
Hearing loss	2(1)	8(5)	10 (6)	2(4)	8(12)	10(9)
Renal failure	4(2)	3(2)	7 (4)	4(8)	3(4)	7(6)
Body aches/cramps	5(3)	5(3)	10 (6)	5(10)	5(7)	10(9)
Headache	6(4)	2(1)	8 (5)	6(13)	2(3)	8(7)
Skin reaction	3(2)	4(2)	7 (4)	3(6)	4(6)	7(6)
Hypokalaemia	5(3)	2(1)	7 (4)	5(10)	2(3)	7(6)
Hypothyroidism	3(2)	3(2)	6 (4)	3(6)	3(4)	6(5)
Depression	1(1)	1(1)	2 (1)	1(2)	1(1)	2(2)
Sore tongue/throat	1(1)	1(1)	2 (1)	1(2)	1(1)	2(2)
Numbness of extremities	2(1)	0	2 (1)	2(4)	0	2(2)
Generalised itchiness	1(1)	1(1)	2 (1)	1(2)	1(1)	2(2)
Psychosis	0	1(1)	1(1)	0	1(1)	1(1)
Renal impairment	1(1)	0	1(1)	1(2)	0	1(1)
Fatigue	0	1(1)	1(1)	0	1(1)	1(1)
Visual disturbance	1(1)	0	1(1)	1(2)	0	1(1)
Thrombophlebitis	0	1 (1)	1(1)	0	1(1)	1(1)
Arthralgia	1(1)	0	1(1)	1(2)	0	1(1)
Total # AE stratified by HIV Status	69(43	92(57)	161(100)			

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threatening reactions, or fatal consequences; (ii) those who died of an AE were more likely to be HIV-infected and thus greater vigilance is required in this group; (iii) those with severe AEs have poorer culture conversion but not higher mortality underscoring the need for careful treatment monitoring for early detection of AEs, and (iv) capreomycin was likely the most common cause of drug withdrawal (44% of all withdrawals), was likely responsible for over 40% of severe AEs and all AE-related deaths, and thus careful monitoring of this drug is mandatory.

Table 6. Effect of TB strain type on AEs stratified by Beijing and non-Beijing strain type.

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

*p = 0.03 (severe vs. mild to moderate AEs).
**p = 0.0001 (total Beijing versus non-Beijing).
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A fundamental finding of this study is that XDR-TB patients with severe AEs had poorer culture-conversion outcomes. By contrast, in patients with MDR-TB from Turkey[12] and Russia where AEs were common (~70% of patients) AEs were not associated with unfavourable outcomes[13]. Thus, in contrast to MDR-TB, in XDR-TB patients the consequences of AE-associated interruption of individual drugs impacts on culture-conversion outcomes. This most likely reflects discontinuation of crucial drugs like capreomycin. Thus, interruption of drug therapy has deleterious consequences. In keeping with the findings of O'Donnell *et al* [7] we found no association between AEs and mortality in the multivariate analysis.

In our study persons who took capreomycin and had AEs, compared to those that took capreomycin but had no AEs, were more likely to be taking concurrent ethambutol, augmentin, ethionamide and PZA, raising the possibility that capreomycin withdrawal in some cases may have been unwarranted. However, these confounding drugs are rarely a cause of renal failure and are

not associated with hypokalemia. The high capreomycin toxicity seen in our study (almost half of all drug withdrawals due to an AE) 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[14]. 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. Our data also raises the question of routine supplementation of electrolytes in patients on capreomycin treatment, and capreomycin drug susceptibility testing in all patients with suspected or proven XDR-TB. We suggest active monitoring for AEs, correct dosing by body weight, correction of dehydration, and regular monitoring of renal function and electrolytes, 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). 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[4]. 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 AEs, health care workers should be educated about the recognition, management, as well as appropriate referral pathways of those experiencing AEs, and patients should be followed up more closely and offered appropriate counselling to ensure drug adherence. 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.[13], was the most common reason for discontinuation 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. AEs although frequent were not more common in HIV-infected patients unlike observations that we[15] and others[16] have documented in patients with drug-sensitive TB. The reasons for this are unclear but could reflect poorer absorption of second line drugs and hence lower serum levels, or, be an ascertainment bias as HIV-infected patients may have died prior to diagnosis. Nevertheless, HIV-infected patients were more likely to die from severe AEs and increased vigilance and correct dosing by body weight is required in this group.

The frequency of AEs in this study (~60%) are similar to those that evaluated AEs to second line drugs in the context of XDR-TB (58%)[7] and MDR-TB (73.3% in Tomsk, Russia[13] and 69,2% in Istanbul Turkey[12]) but twice that of AEs to first line drugs in those with drug-sensitive TB[17]. Suspension of any agent (28% in our study) occurred at a similar frequently compared to a large multi-centric study in patients with MDR-TB (30%)[18] and in patients with XDR-TB[7], more frequently in a Peruvian study in patients with MDR-TB (14%) [19], but less frequently than in Turkish patients with MDR-TB (55%)[12]. This may reflect the heterogeneity of several factors including HIV rates, previous history of TB of any type, resistance profiles, drug regimens, physician management and ascertainment bias.

We found no significant association between strain genotype and the frequency or severity of AEs. This may reflect a true lack of association or type 2 error given the small numbers of isolates that were accessible for genotyping. Association with strain type is of interest because Beijing strains are thought to be more virulent (more cavitation and greater disease extent) and such patients may require a prolonged injectable phase and an increased number of drugs in a regimen. Moreover, 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[20]. 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-sensitive TB[16,17,21], a higher number of women experienced AEs. The reason for this remains unclear. Similar to the findings in the context of drug-sensitive TB[21], the higher rate of AEs 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, lack of complete adherence data, ascertainment bias due to retrospective data capture from medical notes, physician bias, use of a single researcher to capture data on a standardised template, inability to calculate drug-specific AE rates and AE rates per person months of exposure, or to definitively delineate AEs 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 AE to a specific drug in a multidrug regimen. Thus, we chose the term adverse event (rather than adverse drug reaction) as in some cases it was impossible to ascribe the event to a drug rather than HIV, and in other instances it was impossible to determine whether it was TB drug or ARV-related, and in each of these cases which specific drug was implicated. 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., and we could only captured events ascribed by a clinician to be significant. Thus, our analysis may reflect this clinical bias. Given that DST for capreomycin was unavailable, we may have in many, or possibly the majority of cases, inappropriately treated with capreomycin and hence over-estimated the magnitude of AE. However, DST for capreomycin is unreliable and clinical benefit may still occur, even in the face *in-vitro* resistance, and cross-resistance between capreomycin and amikacin is greater than for kanamycin, which is used in our treatment programme. A further limitation is that although all the patients were hospitalised and drugs administered strictly on a DOT basis, it remains unclear to what extent patients may have circumvented this process, and this could have confounded our findings. Furthermore, we did not capture the pill burden or its relationship to the frequency and severity of adverse events. Survivor selection bias may have led to an underestimate of the true mortality in the HIV-infected subgroup whilst late detection and delayed management of AEs could have contributed to mortality given that severe AEs occurred on average about 2 months after mild to moderate AEs. Only a prospective study will be able to address this hypothesis. There were few events in the group with severe AEs and thus CIs were wide, and larger studies are needed to confirm our findings. Finally, 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.

In conclusion, the frequency of AEs with XDR-TB treatment regimens is high and often severe. Those with severe AEs have poorer treatment-related outcomes. Early detection and monitoring of AEs is thus crucial, and XDR-TB patients with AEs 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.

Author Contributions

Conceived and designed the experiments: KS KD MB. Performed the experiments: KS KD MB. Analyzed the data: KS ES EP GS RVZS GT RL XP PW TCV PVH MG RW MB KD. Contributed reagents/materials/analysis tools: KD RW. Wrote the paper: KS ES EP GS RVZS GT RL XP PW TCV PVH MG RW MB KD.

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Appendix 3: Chapter 4: Co-authored manuscript*: What is the cost of diagnosis and management of drug-resistant tuberculosis in South Africa?*

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What is the Cost of Diagnosis and Management of Drug Resistant Tuberculosis in South Africa?

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Abstract

Background: Drug-resistant tuberculosis (DR-TB) is undermining TB control in South Africa. However, there are hardly any data about the cost of treating DR-TB in high burden settings despite such information being quintessential for the rational planning and allocation of resources by policy-makers, and to inform future cost-effectiveness analyses.

Methodology: We analysed the comparative 2011 United States dollar ($) cost of diagnosis and treatment of drug sensitive TB (DS-TB), MDR-TB and XDR-TB, based on National South African TB guidelines, from the perspective of the National TB Program using published clinical outcome data.

Principal Findings: Assuming adherence to national DR-TB management guidelines, the per patient cost of XDR-TB was $26,392, four times greater than MDR-TB ($6772), and 103 times greater than drug-sensitive TB ($257). Despite DR-TB comprising only 2.2% of the case burden, it consumed ~32% of the total estimated 2011 national TB budget of US $218 million. 45% and 25% of the DR-TB costs were attributed to anti-TB drugs and hospitalization, respectively. XDR-TB consumed 28% of the total DR-TB diagnosis and treatment costs. Laboratory testing and anti-TB drugs comprised the majority (71%) of MDR-TB costs while hospitalization and anti-TB drug costs comprised the majority (92%) of XDR-TB costs. A decentralized XDR-TB treatment programme could potentially reduce costs by $6930 (26%) per case and reduce the total amount spent on DR-TB by ~7%.

Conclusion/Significance: Although DR-TB forms a very small proportion of the total case burden it consumes a disproportionate and substantial amount of South Africa's total annual TB budget. These data inform rational resource allocation and selection of management strategies for DR-TB in high burden settings.

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Introduction

Tuberculosis (TB) remains a major public health crisis in sub-Saharan Africa despite declining global TB incidence rates [1]. Achieving the United Nations Millennium Development goal to reduce the burden of TB by 50% in 2015 seems unlikely in this region [2]. This is due to several reasons including unsuccessful treatment programmes, the HIV epidemic, increasing economic deprivation and the emergence of drug resistant TB (DR-TB) [3,4]. Multidrug-resistant TB (MDR-TB), defined as culture-confirmed resistance to rifampicin and isoniazid, comprises ~3% of new and retreatment TB cases in Africa [1]. Approximately 5 to 10% of all MDR-TB cases are extensively-drug-resistant TB (XDR-TB), defined as MDR-TB plus additional culture-confirmed resistance to a fluoroquinolone and an injectable agent (2^{nd} line aminoglycoside or capreomycin) [3]. The situation, fuelled by high transmission rates and HIV co-infection, is particularly dire in South Africa which has the one of the highest TB incidence rates and the 5^{th} highest DR-TB burden globally [1,5].

Compared to drug-susceptible TB (DS-TB), MDR-TB and XDR-TB requires longer, more toxic treatment, and is associated with poorer outcomes (less than 20% of XDR-TB cases culture-convert in South Africa [6–8] compared to other high burden settings where culture conversion rates were higher [9]). Drug costs for treatment of DR-TB are considerably higher and divert resources away from managing a national TB program (NTP). In 2011, the NTP budget in South Africa was approximately US$218 million and a crude preliminary estimate suggests that almost half was allocated to managing MDR-TB [1,10]. More accurate per case and total estimates are required by NTPs and policy makers for rational planning and allocation of resources, to determine optimal preventative and management strategies, to prioritise competing health care issues, and to inform future cost-effectiveness analyses. These data are also relevant to the proposed scaling up of TB diagnostic capacity using nucleic acid amplification platforms such as Xpert MTB-RIF (Cepheid, USA), and Genotype MTBDR*plus* and MTBDR*sl* assays (Hain Lifesciences,

Germany) as such tests are likely to sharply increase the number of newly diagnosed cases of DR-TB [11]. However, there are limited data globally about the cost of treating multi-drug resistant TB [12–17] and none about management-related costs in South Africa. Furthermore, there are no studies that have directly assessed the cost of XDR-TB in South Africa or elsewhere.

To address these gaps in our knowledge we performed a comprehensive cost analysis of MDR-TB and XDR-TB in the Western Cape province of South Africa, based on the current national DR-TB guidelines. As TB treatment costs in different provinces are similar, our analysis reflects costs of DR-TB treatment in South Africa in general. Additionally, we evaluated the costs of a hypothetical decentralized treatment programme for XDR-TB that could potentially reduce the financial burden on South Africa's healthcare system.

Methods

We performed a cost analysis to determine the economic impact of DR-TB on the National TB Programme in South Africa. The analysis was performed from the perspective of the South African National TB Program which incurs all TB related management costs, including ADR management, surgery, drugs, hospitalization and diagnostic/monitoring tests. Strict adherence to National South African DR-TB management guidelines was assumed in the analysis. All direct and indirect medical and non-medical costs were included for the year 2011. The time horizon for the analysis was 6 months for DS-TB and 24 months for DR-TB, which is the length of a full course of anti-DS-TB and anti-DR-TB treatment, respectively. Future costs were adjusted for inflation using the South African Consumer Price Index where appropriate [18]. All costs were expressed in 2011 $US at an exchange rate of $1USD = ZAR7.05 [19]. Estimates of DS-TB, MDR-TB and XDR-TB disease outcomes were taken from published cohort studies specifically conducted in South Africa [1,6–8,20–30]. Component costs are shown in table 1.

Unit Costs

Inpatient, outpatient and clinic costs. The Western Cape Province has the third highest incidence of DR-TB in South Africa and accounts for 15% of DR-TB cases [5]. Brooklyn Chest Hospital (BCH) is a designated DR-TB hospital which provides inpatient and outpatient services for the majority of MDR-TB and XDR-TB patients in the Western Cape. Hospital-associated costs were provided by BCH and are assumed to be representative of this type of facility in South Africa. Inpatient and outpatient costs were calculated using a WHO standardised tool for economic analyses [31]. Personnel costs, including nursing, medical, patient support and other general staff, were obtained from provincial government salary scales and included basic salaries, medical aid contributions and housing allowances. Capital costs included all buildings, equipment, vehicles and furniture. Building and vehicle costs were calculated based on the current replacement costs at BCH, according to standard protocols [31]. Capital costs were annualized at a discount rate of 3%. The useful lifetime was assumed to be 50 years for buildings and 10 years for vehicles and equipment. Outpatient services were assumed to use a proportion of hospital overhead costs determined by standard methods [31]. Medical consumables and other recurrent overhead costs, including utilities and maintenance costs were obtained from 2011 BCH expenditure reports. Ancillary costs, including kitchen and laundry services, were attributable to inpatient costs only and were also obtained from BCH expenditure reports. Outpatient services were assumed to use a proportion of hospital overhead costs [31]. Total inpatient and outpatient costs were divided by the total number of BCH inpatient days and outpatient visits to calculate the cost per inpatient day and outpatient visit, respectively. In South Africa, the majority of DS-TB cases are diagnosed and treated at primary care clinics under the National Directly Observed Treatment Short-Course (DOTS) Programme. Primary care clinic visit costs were calculated, in a similar manner to inpatient and outpatient costs, using data from two TB clinics located in the Cape Town Metro region, Langa Clinic and Chapel Street Clinic. An inpatient day at BCH costs $56.07 whereas an outpatient visit costs $21.16. The cost of a primary care clinic visit (average of two clinics) was $6.64. The cost of a DOTS clinic visit was $1.10 (DS-TB). M/XDR-TB and DS-TB retreatment patients incur a higher DOTS clinic visit cost as they require a nurse for administering injectable drugs ($2.01 per visit).

Drug costs. Drug costs were based on current government tender prices and include those prescribed in standard treatment regimens for DS-TB, MDR-TB and XDR-TB. DR-TB regimens are based on country specific drug resistance profiles and use of second line drugs [5]. DS-TB drugs include four first line agents isoniazid, rifampicin, ethambutol and pyrazinamide as well as streptomycin for retreatment cases. MDR-TB and XDR-TB require much longer treatment duration using second and third line anti-tuberculosis agents. MDR-TB patients use kanamycin in the intensive treatment phase and ethionamide, pyrazinimide, moxifloxacin and terizidone in both the intensive and continuation phase. Primary XDR –TB patients are treated with capreomycin in the intensive phase and ethionamide, pyrazinimide, moxifloxacin, clofazimine, terizidone and para-aminosalycilic acid (PAS) in both intensive and continuation phases. Acquired XDR-TB patients substitute ethionamide and terizidone for augmentin, clarithromycin and high dose isoniazid in their treatment regimen. HIV ARV drug costs have been excluded from the analysis as the incremental costs would be zero for MDR-TB, XDR-TB and DS-TB if HIV ARV therapy was assessed for an equivalent period. However, the inclusion of HIV ARV costs was assessed in the sensitivity analysis. Total treatment regimen costs were calculated based on these standardised regimens for a 50–70 kg patient given treatment 6 days a week.

Diagnostic and monitoring test costs. The costs of all laboratory based tests, for bacteriological assessment, drug susceptibility testing (DST), HIV and adverse drug reaction (ADR) monitoring were provided by the National Health Laboratory Service (NHLS). The NHLS is a reference lab which provides services for the public healthcare system so these costs represent the actual costs incurred by the NTP. HIV monitoring test costs were included in the analysis according to current DR-TB management guidelines [5]. Test costs obtained directly from the NHLS have been used in previous health economic analyses [11,32]. The cost of a chest X-ray (CXR) was obtained from Groote Schuur Hospital (a CXR referral centre for primary care clinics in Cape Town). The cost of an audiogram was calculated from BCH data using an ingredient's approach. The cost of an Xpert MTB-RIF was calculated from WHO estimates [33] and South African specific data using an ingredient's approach. Specimen transport costs were calculated separately using data from BCH according to standard protocols [31] and incorporated in the total test costs. Total test costs were determined by multiplying unit costs provided by the NHLS by the frequency of tests performed for the period of treatment as recommended in the national TB and Drug Resistant TB policy guidelines [5,34] (Table S1).

Adverse drug reaction (ADR) costs. An ADR, due to either first-line or second-line anti-TB drugs, was assumed to be 3 weeks

Table 1. Costs of components associated with diagnosis and treatment of drug sensitive, multi-drug resistant and extensively drug resistant tuberculosis.

Cost Component	Cost ($US)	Source
Inpatient stay (per day)		BCH expenditure reports, Department of Health, BCH statistical reports, staff interviews
Capital costs (Buildings and Equipment)	$4.70	
Nursing & Medical staff	$18.42	
Support staff (OT, PT, Dietician, Psychologist, Data Capturer, etc)	$4.77	
Administrative staff	$2.93	
Staff overhead (excluding administrative staff)	$7.20	
Ancillary (Kitchen & Laundry)	$9.23	
Recurrent medical consumables	$3.91	
Non personnel recurrent overheads (utilities and other general supplies)	$4.93	
Total	**$56.07**	
Outpatient visit (per visit)		BCH expenditure reports, Department of Health, BCH statistical reports, staff interviews
Capital costs (Buildings and Equipment)	$1.59	
Nursing & Medical staff	$6.13	
Support staff (OT, PT, Dietician, Psychologist, Data Capturer, etc.)	$7.15	
Administrative staff	$0.97	
Staff overhead (excluding administrative staff)	$2.39	
Recurrent medical consumables	$1.30	
Non personnel recurrent overheads (utilities and other general supplies)	$1.64	
Total	**$21.16**	
Clinic visit (per visit)		Langa and Chapel St Clinic expenditure reports, Cape Town City Health, clinic staff interviews
Capital costs (Buildings and Equipment)	$0.61	
Staff (Nursing, Medical and General administrative staff)	$4.44	
Recurrent medical consumables	$0.90	
non personnel recurrent overheads (utilities and other general supplies)	$0.69	
Total	**$6.64**	
10 minute DOTS worker visit (per visit)	$1.10	Provincial government salary scales
Drugs		BCH pharmacy, Cape Town City Health
DS-TB		
2 mth Intensive (RHZE)	$16.77	
4 mth Continuation (RH)	$20.96	
DS-TB Retreatment		
3 mth Intensive (RHZES)	$135.45	
5 mth Continuation (RHE)	$53.03	
MDR-TB		
6 mth Intensive (Km-Z-Mxf-Eto-Trd)	$1,438.03	
18 mth Continuation (Z-Mxf-Eto-Trd)	$3,671.48	
XDR-TB Primary		
6 mth Intensive (Cm-Z-Mxf-Eto-Trd-PAS-Cfz)	$5,272.91	
18 mth Continuation (Cm-Z-Mxf-Eto-Trd-PAS-Cfz)	$15,015.62	
XDR-TB Acquired		
6 mth Intensive (Cm-Z-Mxf-Clm-Aug-hdH-PAS-Cfz)	$4,884.80	
18 mth Continuation (Z-Mxf-Clm-Aug-hdH-PAS-Cfz)	$13,406.14	
Tests		National Health Laboratory Services, Groote Schuur Hospital, WHO
Auramine Fluorescent Smear Microscopy	$3.71	
MGIT 960 liquid Culture	$14.02	
Xpert MTB-RIF	$21.39	

Table 1. Cont.

Cost Component	Cost ($US)	Source
1st line DST (Line Probe Assay)	$26.74	
2nd line DST for 4 drugs (MGIT 960 liquid culture)	$37.83	
Chest X-ray	$31.91	
Audiogram	$25.60	
Liver function (Potassium, Urea, Creatinine)	$15.05	
Kidney function (ALT, AST, Bilirubin)	$10.86	
Thyroid function	$20.89	
HIV Rapid screening test	$5.26	
Other HIV associated tests (CD4 count, viral load)	$51.75	
Specimen transport		Department of Transport, BCH expenditure reports, staff interviews
Capital	$0.28	
Recurrent cost	$1.03	
Personnel	$1.40	
Total	**$2.72**	
Surgery		Groote Schuur Hospital
Pneumonectomy	$5,549.36	
Death i.e. Removal of body from premises	$70.92	BCH financial department

DS-TB - Drug sensitive tuberculosis, MDR-TB – Multi-drug resistant tuberculosis, XDR-TB – Extensively Drug-resistant tuberculosis, ADR - Adverse Drug reaction, OT - Occupational Therapist, PT - Physiotherapist, BCH – Brooklyn Chest Hospital, DOTS – Directly Observed Treatment Short Course, MGIT – Mycobacterial Growth In-tube, DST – Drug susceptibility test, AST - aspartate aminotransferase, ALT - alanine aminotransferase, R - Rifampicin, H - Isoniazid, Z - Pyrazinamide, E - Ethambutol, S - Streptomycin, Km – Kanamycin, Mxf - Moxifloxacin, Eto - Ethionamide, Trd – Terizidone, Cm - Capreomycin, PAS – para-amionsalicylic acid, Cfz – Clofazimine, Clm-Clarithromycin, Aug - Augmentin, hdH - high dose Isoniazid.
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in duration and the total cost included that of ancillary drugs, extra weekly monitoring tests and hospital outpatient or clinic visits during this timeframe, in accordance with national guidelines [5,34,35]. The incidence of ADRs associated with DS-TB, MDR-TB and XDR-TB treatment was taken from the literature [6,25,26]. Additionally, we assumed that some patients who develop severe ADRs [30% for MDR-TB and XDR-TB [6,29]; 10% for DS-TB [25]] will require hospitalization for 2 weeks [36]. Given that first line drugs are associated with less severe ADRs [37] and DS-TB patients are generally not as sick as DR-TB patients, we assume that DS-TB associated ADRs only require 1 week hospitalization [38]. We did not account for the reduction in costs associated with lowering the dosage or withdrawing TB drugs and we assumed that substitution of drugs will occur at no additional cost [39]. Additionally, we assumed the same ADRs occur with the same frequency in HIV infected and HIV uninfected individuals.

Surgery costs. The cost of a pneumonectomy procedure was used to represent the cost of surgical resection in DR-TB cases. This cost was provided by the Groote Schuur Surgical Department and included the costs of facilities, medication, personnel and tests associated with surgery.

Death-related costs. Death-related costs were only incurred by inpatients that died in hospital and included the cost for removal of the body from the hospital premises. We assumed all other death related costs to be borne by the patient.

Diagnostic and Treatment Algorithms

Protocols for the diagnosis and treatment of DS-TB, MDR-TB and XDR-TB were followed according to South African National Department of Health Guidelines [5,34]. Treatment associated outcomes have been defined in line with these guidelines. Only new and retreatment cases of pulmonary DS-TB and new cases of MDR-TB and XDR-TB were considered in the analysis. All outcome probabilities are shown in Table 2.

DS-TB. DS-TB patients are initially assessed and diagnosed at a primary health care clinic and are followed up at subsequent visits. Bacteriological monitoring tests are performed at diagnosis and periodically during treatment, according to National TB guidelines (Table S1). Xpert MTB-RIF is also performed on all TB and DR-TB suspects at initial diagnosis only, in line with current recommendations [5]. Treatment is initiated under the DOTS program for the first two weeks at the clinic followed by community-based DOTS treatment. The standard DS-TB treatment regimen includes a 2-month intensive phase using 4 drugs (isoniazid/rifampicin/ethambutol/pyrazinamide) followed by a 4-month continuation phase (isoniazid/rifampicin). Retreatment cases require 3 months of intensive phase treatment with streptomycin included in the regimen, followed by a 5-month continuation phase (isoniazid/rifampicin/ethambutol). Patients who do not sputum convert (i.e. become smear negative) after the initial intensive phase of treatment receive an extra month of intensive phase treatment. We assume that DS-TB patients who die or default from treatment only incur half the cost of a treatment regimen [13]. Furthermore, as the vast majority of DS-TB cases are treated in the community, we assume that no DS-TB patients are hospitalized.

MDR-TB. Decentralized MDR-TB treatment in South Africa was initiated in 2002, under the WHO DOTS-Plus program. The national DR-TB guidelines recommend that the majority of cases be treated as outpatients and only severely ill MDR-TB patients should be admitted for hospitalization (10% of cases). Diagnostic and monitoring tests are performed (Table S1) as recommended in

Table 2. Per case probability estimates of different diagnosis- and treatment-related outcomes for drug sensitive, multi-drug resistant and extensively-drug-resistant tuberculosis.

Outcome	DS-TB		MDR-TB		XDR-TB	
	estimate	source	estimate	source	estimate	source
Diagnostic outcomes and assumptions						
HIV prevalence	0.50	[1]	0.60	[43]	0.60	[6–8]
Proportion of smear positives	0.6	[44]	Not included*			
Proportion of smear negatives	0.21	[44]	Not included*			
Proportion of retreatment cases	0.19	[44]	Not included*			
Treatment outcomes and assumptions						
Proportion treated as hospital inpatients	0	Assumed†	0.10	Assumed‡	1.00 0.5	[5] Assumed§
Proportion treated as hospital outpatients/PCC	1.00	Assumed†	0.90	Assumed‡	0 0.5	[5] Assumed§
Duration of treatment (assuming treatment completion)	6 months	[34]	2 years	[5]	2 years	[5]
Proportion who culture convert and complete treatment	Not included¶		0.50	[22,23,29]	0.20	[6,8]
Average time from diagnosis to culture conversion	Not included¶		4 months	[23,29]	6 months	[6,7]
Death during Treatment	0.05	[44]	0.20	[21,22,29]	0.40	[6–8]
Average time from diagnosis to death	3 months	[13]	5 months	[20,21]	7 months	[6]
Treatment default	0.1	[44]	0.2	[20]	Assumed same as MDR-TB	
Time to treatment default	3 months	[13]	5 months	[13]	Assumed same as MDR-TB	
Treatment failure	Not included¶		0.10	[22,29]	0.40	[6]
Average time from diagnosis to treatment failure & discharge from hospital	Not included¶		12 months	Assumed #	12 months	[6]
Proportion developing ADRs	0.05	[25]	0.30	[26]	0.60	[6]
Proportion of patients developing ADRs that are hospitalized	0.10	Assumed, [25]	0.30	[29]	0.60	[6]
Proportion undergoing surgery	0.00	Assumed	0.02	Assumed #	0.02	[6,24]

*These proportions were not included in the model as all MDR-TB and XDR-TB cases follow the same diagnosis and treatment protocols regardless of smear status in accordance with national guidelines. Additionally, we only modelled new cases of MDR-TB and XDR-TB and did not include retreatment cases.
†Assumed that all DS-TB patients are treated at a primary care clinic and none are hospitalized.
‡This estimate was provided by Brooklyn Chest Hospital.
§A figure of 50% is assumed for the decentralized XDR-TB model based on a proportion of patients from an XDR-TB cohort who weigh >50 kg,
¶Not incorporated into our model as we assume all DS-TB patients complete a full course of treatment, whether they are cured or they fail treatment.
#Assumed to be the same as XDR-TB.
DS-TB - Drug sensitive tuberculosis, MDR-TB – Multi-drug resistant tuberculosis, XDR-TB – Extensively Drug-resistant tuberculosis, PCC - primary care clinic, ADR - Adverse Drug reaction.
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the DR-TB management guidelines. We assume HIV infected patients undergo six-monthly assessments of CD4 counts and viral loads for the duration of TB treatment. A standardized MDR-TB regimen consists of a 6-month intensive phase of 5 drugs (Ethionamide/Pyrazinimide/Kanamycin/Moxifloxacin/Terizidone) followed by an 18-month continuation phase with 4 drugs (Ethionamide/Pyrazinimide/Moxifloxacin/Terizidone). In addition to monthly hospital outpatient facility visits for scheduled medical checkups, MDR-TB outpatients attend local TB clinics daily to receive their drugs and monitoring for development of ADRs. Inpatients remain hospitalized until they culture convert (i.e. have two successive months of negative sputum cultures), then continue treatment as outpatients, including daily attendance at TB clinics for drugs and ADR monitoring during the continuation phase of treatment. If patients do not culture convert after 12 months of treatment, they are considered treatment failures. We assume these 'non converters' will be referred for XDR-TB treatment and no longer incur MDR-TB associated costs.

XDR-TB. In South Africa, national DR-TB management guidelines recommend hospitalization of all confirmed XDR-TB patients. A standardized regimen for primary XDR-TB (25% of XDR-TB cases [6,7]) consists of a 6–month intensive phase of 7 drugs (Ethionamide/Pyrazinimide/Capreomycin/Moxifloxacin/Clofazimine/Terizidone/Para-aminosalycilic acid (PAS)) followed by an 18-month continuation phase of 6 drugs (Ethionamide/Pyrazinimide/Moxifloxacin/Clofazimine/Terizidone/PAS). An acquired XDR-TB (75% of XDR-TB cases) treatment regimen is the same except that ethionamide and terizidone are substituted for augmentin, clarithromycin and high dose isoniazid. Clinic visits, bacteriological, radiological and HIV monitoring tests are performed with the same frequency as during MDR-TB management. However, due to the increased toxicity of XDR-TB drugs, such as capreomycin and PAS, we assume certain ADR monitoring tests (kidney function tests, thyroid function test) are performed more frequently (Table S1). In South Africa, provincial review boards decide on whether or not to stop treatment in patients who fail to culture convert after 12 months of XDR-TB treatment. Based on clinical guidance, we assume that these 'treatment failures' are discharged from hospital after 12 months but receive an extra 3 months of continuation phase treatment before a review board decides to stop treatment completely.

Alternative XDR-TB decentralization programme. Due to resource constraints on centralized treatment facilities, we propose a decentralized programme where XDR-TB treatment is

Table 3. Total costs and breakdown per patient for drug sensitive tuberculosis, multi-drug resistant tuberculosis and extensively drug-resistant tuberculosis. Costs are expressed in $US.

Cost Components	Drug Sensitive TB			MDR-TB		XDR-TB	
	Smear Positive	Smear negative	Re-treatment	Outpatient Treatment	Inpatient treatment	Outpatient Treatment	Inpatient treatment
Hospital inpatient stay	$0.00	$0.00	$0.00	$0.00	$8,746.99	$0.00	$14,802.60
Hospital outpatient visit	$0.00	$0.00	$0.00	$240.22	$130.16	$524.46	$105.40
PCC visit	$32.85	$37.49	$76.98	$749.54	$498.08	$724.39	$240.82
Anti-TB drugs	$40.24	$37.73	$188.48	$3,321.70	$3,321.70	$9,501.57	$9,501.57
Diagnostic/Monitoring Tests	$112.30	$171.05	$183.77	$1,408.21	$1,413.28	$1,477.66	$1,409.97
ADRs	$6.27	$6.27	$6.27	$99.37	$99.37	$192.29	$192.29
Surgery	$0.00	$0.00	$0.00	$110.99	$110.99	$110.99	$110.99
Death	$0.00	$0.00	$0.00	$0.00	$28.37	$0.00	$28.37
TOTAL	**$191.66**	**$252.54**	**$455.50**	**$5,930.02**	**$14,348.94**	**$12,531.36**	**$26,392.01**
Prevalence in each group	**0.60**	**0.20**	**0.20**	**0.90**	**0.10**	**0.00* 0.50**	**1.00* 0.50**
Overall cost (group prevalence x cost per patient)†	**$256.61**			**$6771.92**		**Current strategy* $26,392.01 Decentralized strategy $19,461.68**	

*In the current model 100% of XDR-TB patients are hospitalized whereas in the proposed decentralized model 50% are treated as outpatients and the remaining 50% are hospitalized.

†For example, the total costs of illness arising from DS TB per patient were calculated as (0.6*191.66)+(0.2*252.54)+(0.2*455.50) = $256.61.

DS-TB - Drug sensitive tuberculosis, MDR-TB – Multi-drug resistant tuberculosis, XDR-TB – Extensively Drug-resistant tuberculosis, PCC- primary care clinic, ADR- Adverse Drug reaction.

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initiated in an outpatient facility and follow similar protocols to the decentralized MDR-TB strategy. We estimate that approximately 50% of XDR-TB cases will be suitable for ambulatory care (based on clinical experience and estimated by the proportion of XDR-TB patients who weigh <50 kg at diagnosis, which has shown to be associated with worse treatment outcomes [6]). However, in the absence of regulatory guidelines regarding the monitoring frequency of XDR-TB outpatients and due to the poorer XDR-TB outcomes, we assume these patients will be monitored more closely than in MDR-TB. This includes hospital outpatient visits twice a month during the intensive phase and monthly for the continuation phase. We assume that monitoring tests will be conducted with the same frequency as hospitalized patients and XDR-TB drugs will continue to be dispensed at local TB clinics for the entire course of treatment.

Sensitivity Analysis

A univariate sensitivity analysis was performed to measure uncertainties in the component costs and outcome data used in the analysis. Parameters that were varied include cost and length of hospitalization, length of treatment, discount rate for capital items, ADR prevalence, the incidence of surgery and the proportion of MDR-TB and XDR-TB patients hospitalized. Additionally, the inclusion of HIV ARV drug costs and HIV prevalence were varied in the sensitivity analysis. HIV ARVs costs were determined for the period of TB treatment and included 5 standard first-line drugs (Efavirenz/Stavudine/Lamuvidine/Co-Trimoxizole/Pyridoxine) given seven days a week.

Despite decentralized MDR-TB treatment being the current policy in South Africa, many provinces still hospitalize the majority of MDR-TB patients until culture conversion. We therefore perform a sensitivity analysis to determine the per patient and total national costs of MDR-TB assuming a higher rate of hospitalization among MDR-TB patients (70% in South Africa according to the WHO [5]).

Results

TB treatment costs and cost breakdown are shown in table 3. The cost per case of smear-positive DS-TB was $191.66 whereas the cost of a smear-negative and retreatment case was more expensive, at $252.54 and $455.50 respectively, mainly due to the increased number of diagnostic tests and the longer and more expensive retreatment regimen. When the proportion of cases in each category (smear positive, smear negative, retreatment), based on 2010 TB case findings [1], was multiplied by the total costs of each category, the overall cost of DS-TB was $256.61. The cost of MDR-TB was much more expensive than DS-TB, at $5,930.02 for an MDR-TB outpatient and $14,348.94 for an MDR-TB inpatient. We assumed that approximately 90% of MDR-TB cases are treated as outpatients (according to national DR-TB guidelines) while the remainder are hospitalized due to severe illness. Based on these proportions, the overall cost per case of MDR-TB was $6,771.92. This cost was twenty-six times greater than DS-TB with 49% of these costs being attributable to anti-MDR-TB drugs (Figure 1). Conversely, all confirmed XDR-TB cases require hospitalization. Management of an XDR-TB case costs $26,392.01, four times greater than the cost of an MDR-TB case and 103 times greater than that of a DS-TB case. While XDR-TB drugs do make up a significant proportion of these costs (36%), hospitalization contributes to 56% of the total XDR-TB costs (Figure 1).

These costs were applied to a national level, where the total number of notified pulmonary TB cases in 2010 in South Africa [1,5] was used to obtain a generalized total cost spent on diagnosis and treatment of confirmed TB (Figure 2). In terms of total cases, only a small proportion were MDR-TB and XDR-TB (2% and

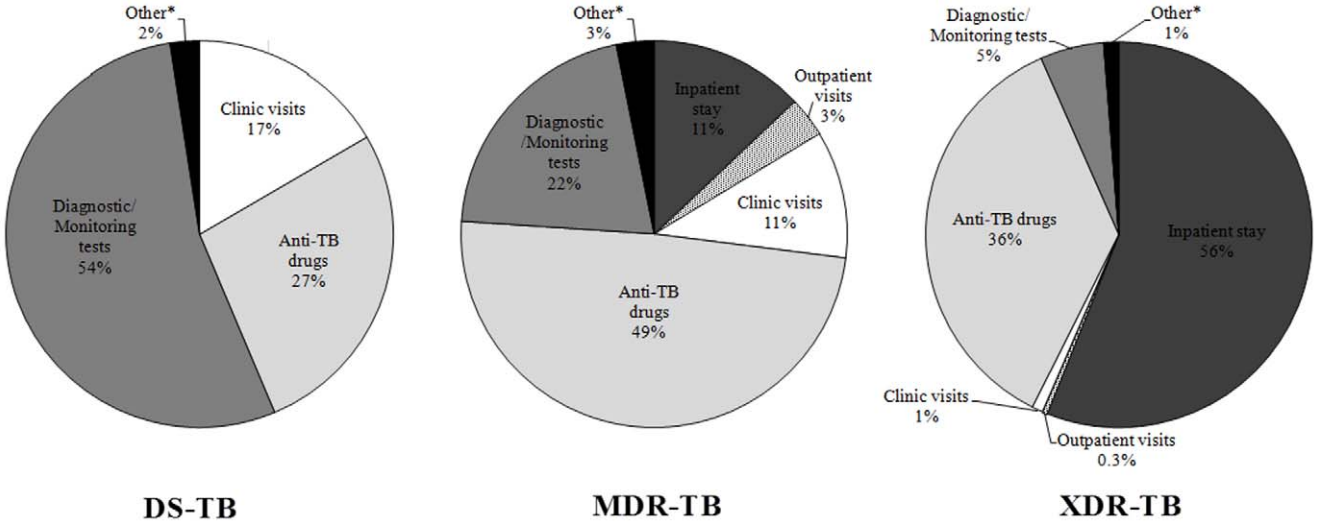

Figure 1. The cost breakdown of the total cost per patient for drug sensitive (DS-TB), multi-drug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) tuberculosis. *Other indicates surgery, ADRs and death related costs.
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0.2%, respectively) but these cases contributed to a significant proportion of the total TB diagnosis and treatment costs (32% and 13%, respectively). Anti-TB drugs is a major contributor to the total cost of TB diagnosis and treatment ($55 million) and DR-TB drugs made up 58% of these costs (Figure 3).

We also proposed an alternative XDR-TB management programme where healthier XDR-TB patients are managed through hospital outpatient facilities and primary care clinics with vigorous follow-up procedures. In this model, hospital costs were eliminated which reduced the cost of diagnosing and treating an XDR-TB case to $12,531.36, a cost saving of 53%. Furthermore, we assume 50% of XDR-TB patients are suitable for ambulatory care. If these patients are treated in the community while the remainder are still treated as inpatients, then XDR-TB would cost $19,461.68 per case and reduce the cost by $6930 compared to current XDR-TB management practice.

We conducted a sensitivity analysis where model parameters (costs and outcomes) were varied to assess any uncertainty in these variables (Table 4). Inpatient day costs had the most significant influence on XDR-TB costs followed by the proportion of patients

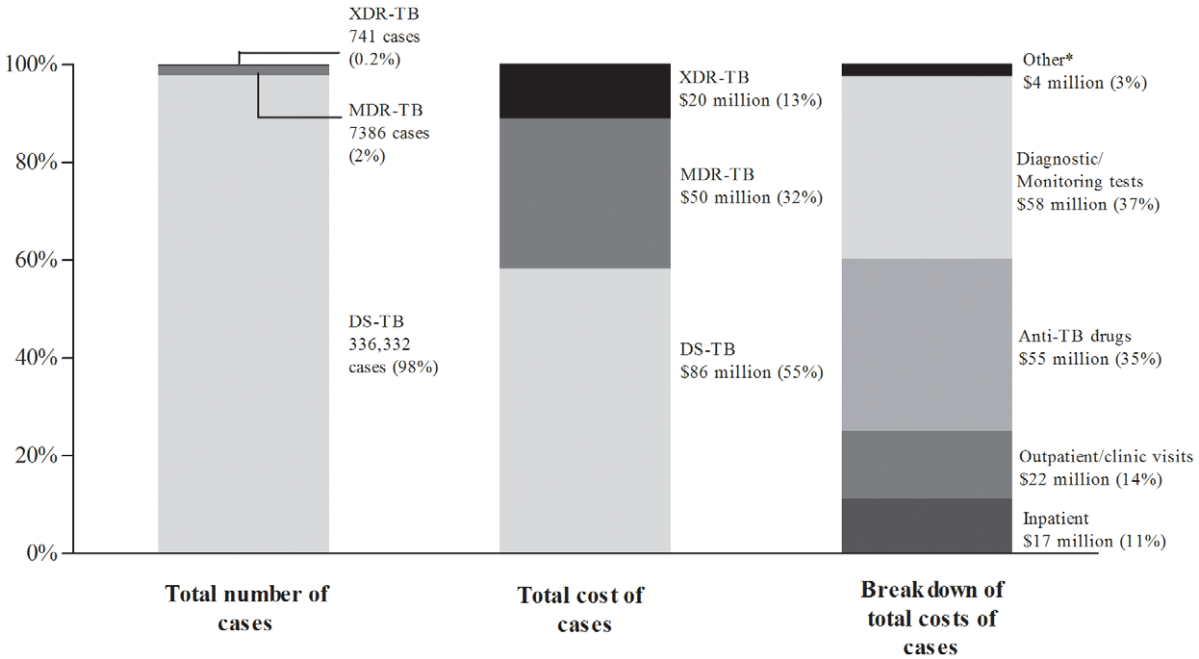

Figure 2. The total number, national costs and cost breakdown of notified cases of drug sensitive (DS-TB), multi-drug resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) reported in 2010. Costs are expressed in $US and refer to the cost of diagnosis and treatment of confirmed cases. *Other indicates surgery, ADRs and death related costs.
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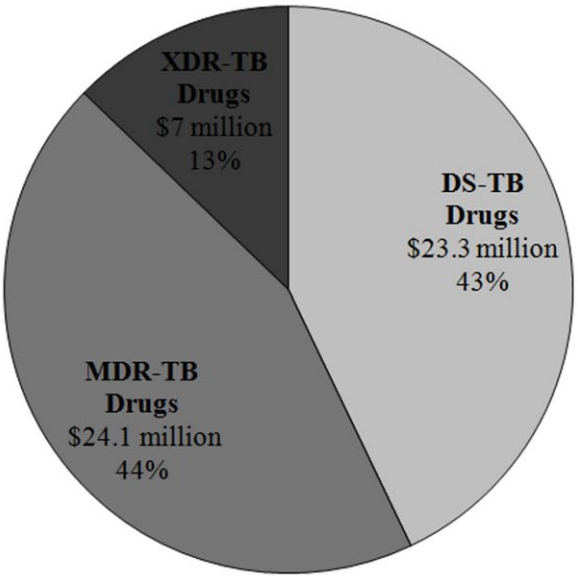

Figure 3. The total drugs costs of notified cases of drug sensitive (DS-TB), multi-drug resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) reported in 2010. Costs are expressed in $US.
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treated as inpatients, which is not surprising as hospitalization comprises a substantial proportion of per patient XDR-TB costs. Similarly, the MDR-TB analysis was most sensitive to changes in the proportion hospitalised and duration of treatment. Variation in the proportion of non converters (treatment failures) also caused noticeable changes in overall DR-TB costs, mainly due to the increased length of hospitalization required in these individuals. Overall costs of DS-TB, MDR-TB and XDR-TB changed minimally when other parameters, such as inclusion of HIV ARV drug costs, were varied.

Under the assumption of 70% hospitalization among MDR-TB patients, the per patient cost of MDR-TB was $11,823, a difference of $5,051 compared to MDR-TB costs in our primary analysis ($6772 per case). Not surprisingly, inpatient costs comprise the majority of these per patient costs (52%). Furthermore, in this scenario, MDR-TB costs constitute 49% of the total national TB diagnosis and treatment costs (compared to 32% in the primary analysis). Consequently, the national DR-TB diagnosis and treatment costs consume ~50% of the 2011 NTP budget. Hospitalisation costs comprise the majority of the DR-TB national costs (58%) under this assumption.

Discussion

We performed a cost analysis to determine the specific costs associated with the diagnosis and treatment of MDR-TB and XDR-TB in South Africa, including hospital inpatient and outpatient, and primary care clinic costs, diagnostic and treatment monitoring costs, drug costs, surgical costs, and costs associated with HIV and ADR management. This is, to our knowledge, the first study to accurately quantify the cost of treating XDR-TB and the first to assess the costs of DR-TB management in South Africa according to the current South African DR-TB guidelines. It will facilitate planning and resource allocation, and will inform future cost-effectiveness studies. Our main findings were (i) despite DR-TB comprising only 2.2% of the total case burden it consumed 32% of the estimated 2011, ~US$218 million, total national TB budget. (ii) XDR-TB consumed 28% of total DR-TB management costs and 9% of the total national TB budget; (iii) overall, 45% and 25% of DR-TB costs were attributed to anti-TB drugs and hospitalization, respectively (49% and 13% of MDR-TB costs; 36% and 56% of XDR costs, respectively); (iv) a decentralised XDR-TB treatment model could reduce overall DR-TB costs by ~7% and the XDR-TB-specific costs by ~26%.

Despite DR-TB cases comprising a trivial proportion of the total case burden they consumed a disproportionately large amount of total TB costs. This was due to the high cost of managing DR-TB. Indeed, despite the small number of total cases of XDR-TB (0.2% of total notified cases in 2010), the cost was disproportionately expensive ($26,392.01 per case and representing 9% of the estimated total national TB costs). High drug prices [40] and the need for extensive supervised patient care contribute to the high cost of diagnosing and treating DR-TB. These costs (as a % of total costs) are likely to increase substantially as the South African Department of Health has recently recommended implementation of the new Xpert MTB/RIF assay as the primary TB diagnostic test in all persons with suspected TB [41]. This test, which also detects rifampicin resistance, will likely increase the detection rate and, consequently, the total costs of DR-TB [11]. Thus, effective and sustainable financial and management strategies will be required to deal with this expected rise in notified MDR-TB (and XDR-TB) cases.

What are the major components driving the high DR-TB costs? XDR-TB despite forming only 0.2% of the total case load consumed ~26% of DR-TB diagnosis and treatment costs (and ~9% of total diagnosis and treatment costs). The major drivers here were hospitalisation (56% of total XDR-TB costs) and drug costs (36% of total XDR-TB costs). Drugs such as clarithromycin and augmentin contributed to these costs (4% of total XDR-TB drug costs) despite being of uncertain value in the effective treatment of cases. Such costs could be diverted to other aspects of the programme. By contrast, for MDR-TB laboratory testing and anti-TB drugs comprised the majority (71%) of MDR-TB costs. The MDR-TB per case costs in our study ($6,772) was different compared to Russia (US$14,600) and Peru (US$2400) [17] mainly reflecting differences in hospitalization and drug prices, but also inclusion or exclusion of specific cost components.

A substantial proportion of DR-TB costs were attributed to XDR-TB. Thus, targeting this component of DR-TB could potentially reduce costs substantially. If, in contrast to the existing model where all cases of XDR-TB are admitted to hospital, a decentralized XDR-TB treatment strategy was adopted (where patients receive their drugs and undergo routine clinical assessments at their local TB clinics), costs savings of $6930 per case of XDR-TB could potentially be made depending on the rate of hospitalisation of these patients. These savings would primarily be made through reduced hospitalization costs, which contribute to over half of all XDR-TB costs. This is an attractive strategy because DR-TB treatment facilities in South Africa are severely overburdened [3] and there are often long waiting times for limited bed space thus facilitating community transmission. Indeed, in some areas like the Northern Cape out-patient treatment of XDR-TB has already begun. Furthermore, lack of proper infection control facilities in provinces like Kwa-Zulu Natal results in nosocomial transmission, which may be responsible for almost half of all XDR-TB cases in that province [42]. Given that approximately 50% of patients meet these criteria XDR-TB-related costs can be reduced by ~$6930 per case. Based on notified XDR-TB cases in 2010, this represents a total savings of US$5.1 million per annum (~2% of the National TB budget and ~7% of the estimated DR-TB budget). In addition to the potential

Table 4. Sensitivity analysis. Costs represent the cost per case and are expressed in $US.

Variables		DS-TB	MDR-TB	XDR-TB current practice	XDR decentralized strategy
Baseline		**$256.61**	**$6,771.92**	**$26,392.01**	**$19,461.68**
Discount rate					
	10%	**$261.33**	**$6,973.50**	**$28,514.62**	**$20,589.74**
	1%	**$255.55**	**$6,728.00**	**$25,934.11**	**$19,218.09**
Treatment duration					
	30 months	**$256.61**	**$7,787.05**	**$27,111.95**	**$20,181.62**
	18 months	**$256.61**	**$5,813.29**	**$25,706.93**	**$18,776.61**
Cost of Hospitalization/Outpatient visit					
	doubled	**$260.53**	**$7,965.52**	**$41,479.40**	**$27,357.31**
	halved	**$254.65**	**$6,175.11**	**$18,848.31**	**$15,513.87**
HIV prevalence					
	90%	**$279.48**	**$6,825.74**	**$26,430.66**	**$19,500.34**
	30%	**$245.17**	**$6,718.09**	**$26,353.35**	**$19,423.03**
HIV costs					
Inclusion of ARVs	(period of TB Treatment)	**$329.84**	**$7,012.12**	**$26,632.21**	**$19,701.88**
ADR frequency					
MDR					
	50%	**$256.61**	**$6,838.16**	**$26,392.01**	**$19,461.68**
	10%	**$256.61**	**$6,705.67**	**$26,392.01**	**$19,461.68**
XDR					
	80%	**$256.61**	**$6,771.92**	**$26,456.10**	**$19,525.78**
	30%	**$256.61**	**$6,771.92**	**$26,295.86**	**$19,365.54**
Surgery					
	10%	**$256.61**	**$7,215.86**	**$26,835.95**	**$19,905.63**
	1%	**$256.61**	**$6,716.42**	**$26,336.51**	**$19,406.19**
Time to culture conversion (Length of hospitalization)					
MDR-TB					
	8 months	**$256.61**	**$7,095.49**	**$26,392.01**	**$19,461.68**
	2 months	**$256.61**	**$6,610.00**	**$26,392.01**	**$19,461.68**
XDR-TB					
	12 months	**$256.61**	**$6,771.92**	**$28,327.12**	**$20,429.24**
	2 months	**$256.61**	**$6,771.92**	**$25,118.86**	**$18,825.11**
% non converters					
MDR-TB					
	50%	**$256.61**	**$5,828.11**	**$26,392.01**	**$19,461.68**
	30%	**$256.61**	**$6,300.01**	**$26,392.01**	**$19,461.68**
XDR-TB					
	55%	**$256.61**	**$6,771.92**	**$27,362.82**	**$19,744.69**
	10%	**$256.61**	**$6,771.92**	**$24,450.37**	**$18,895.66**
% hospitalized					
MDR-TB					
	50%	**$256.61**	**$10,139.48**	**$26,392.01**	**$19,461.68**
	80%	**$256.61**	**$12,665.16**	**$26,392.01**	**$19,461.68**
XDR-TB					
	90%	**$256.61**	**$6,771.92**	**$26,392.01**	**$25,005.94**
	10%	**$256.61**	**$6,771.92**	**$26,392.01**	**$13,917.42**

DS-TB - Drug sensitive tuberculosis, MDR-TB – Multi-drug resistant tuberculosis, XDR-TB – Extensively Drug-resistant tuberculosis, ADR – Adverse Drug reaction, ARVs – Anti-Retroviral drugs.

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direct cost savings, a decentralized strategy would free up bed space for patients with more extensive disease and encourage treatment adherence among patients with limited access to these centralized facilities. Decentralized MDR-TB management, currently performed in some provinces in South Africa, is a cost effective option in other resource-poor settings as shown in our analysis and elsewhere [17]. However, implementation of such a strategy remains contentious as these patients need to be closely monitored, (particularly for capreomycin-related electrolyte abnormalities) and educated in proper infection control practices. Treatment default is also an issue which, in MDR-TB outpatients, can be up to 20% [20].

There are a number of limitations to our analysis. Our study is a simple cost analysis rather than a cost-effectiveness study, which also evaluates effectiveness of a management strategy. However, the purpose of our study was to report the costs of DR-TB in South Africa rather than compare the cost-effectiveness of different management strategies, which should be the focus of future studies. The use of cost data from Brooklyn Chest Hospital BCH is representative of the Western Cape but may not be reflect the rest of South Africa as hospitalization and treatment facility costs, as well as treatment regimens and some treatment policies for DR-TB, can vary slightly across provinces. However, we used standardized diagnosis and treatment protocols in our analysis, according to South African national TB guidelines, and varied the parameters that tend to be different across the provinces in the sensitivity analysis. We used treatment outcomes from published retrospective cohort studies due to lack of accurate surveillance data and to generalize our results for South Africa. However, we realize these results may not accurately reflect current disease outcomes. While decentralized MDR-TB treatment is now the current policy in South Africa, it is not fully implemented across all provinces and our assumption of predominant outpatient care for these patients may underestimate the cost of MDR-TB. However, we conducted a sensitivity analysis using a higher estimate of MDR-TB patients hospitalised (70%) and reported the per patient and total national costs using this estimate. Indeed, an increased incidence of inpatient care among MDR-TB patients does increase the DR-TB costs. However, our analysis calculates DR-TB costs based on adherence to current national DR-TB management guidelines (which recommends outpatient care for most MDR-TB cases) rather than the specific treatment practices in other provinces. Strict implementation of decentralized MDR-TB care is likely to become widespread across South Africa in the near future. ADRs are difficult to cost as the frequency and types of ADR varies widely among different cohort studies and one patient may experience more than one ADR several times during the course of treatment. Additionally, HIV infected individuals are likely to have a higher frequency of ADRs due to additional drug interaction with ARVs. As such, ADR costs may be underestimated. Total national TB costs refers to costs associated with diagnosis and treatment of confirmed TB cases and excludes certain costs, such as those associated with diagnosis of non TB cases. As such these costs may also be underestimated. Our decentralized model for XDR-TB did not include detailed costs associated with increased transmission or infection control. As such, the cost-saving of this model is likely to be overestimated. Future setting-specific detailed cost-feasibility studies are now needed to determine the practicality of implementing this model. Societal costs such as patient-related costs were not included as it is difficult to accurately capture patients' out of pocket expenses and their socio-economic status during the time of illness. It is likely that such costs will be substantial given the long hospitalization periods, and extensive morbidity and mortality associated with DR-TB. Assessment of these costs should be the focus of future cost-analysis studies.

In conclusion, DR-TB in South Africa is extremely expensive with a small number of DR-TB cases disproportionately consuming a large chunk of the total NTP budget. Based on South African National DR-TB guidelines, the current cost of XDR-TB management is 103 times greater than DS-TB, the majority of which is attributable to the cost of hospitalization. Implementation of decentralized XDR-TB care is a viable option and can reduce these costs but will require intensive follow up and appropriate infection control measures to be put into place to minimise disease transmission.

Supporting Information

Table S1 Frequency and duration of hospitalization, outpatient/clinic visits, treatment and diagnostic/monitoring tests during the period of treatment for drug sensitive tuberculosis, multi-drug resistant tuberculosis and extensively drug-resistant tuberculosis according to the South African Drug Resistant TB guidelines. The reported frequencies refer to the period from diagnosis till the end of treatment.
(DOCX)

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Author Contributions

Conceived and designed the experiments: AP EP KD. Performed the experiments: AP EP. Analyzed the data: AP MD GT. Wrote the paper: AP MD GT KD.

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Supporting Information:

What is the cost of diagnosis and management of drug resistant tuberculosis in South Africa?

Table S1: Frequency and duration of hospitalization, outpatient/clinic visits, treatment and diagnostic/monitoring tests during the period of treatment for drug sensitive tuberculosis, multi-drug resistant tuberculosis and extensively drug-resistant tuberculosis according to the South African Drug Resistant TB guidelines. The reported frequencies refer to the period from diagnosis till the end of treatment.

Component		DS-TB				MDR-TB		XDR-TB	
		Smear positive converters	**Smear positive non converters**	**Smear negative**	**Retreatment**	**Culture converters**	**Culture non converters**	**Culture converters**	**Culture non converters**
Hospital, outpatient and clinic visit frequency	Hospital inpatient months (hospitalized patients only)	ND	ND	ND	ND	4 months	12 months	6 months	12 months
	Hospital outpatient visits	ND	ND	ND	ND	Once per month during intensive phase, every 2 months in continuation phase		Twice per month during intensive phase, every month in continuation phase	
	Clinic visits (check-up)	1 at diagnosis, 2 during Rx	1 at diagnosis, 3 during Rx	1 at diagnosis, 3 during Rx	1 at diagnosis, 4 during Rx	Once a month for monitoring ADRs (outpatients only)		Once a month for monitoring ADRs (outpatients only)	
	DOTS clinic visits (administering drugs)	1st 2 weeks of Rx	1st 2 weeks of Rx	1st 2 weeks of Rx	daily during intensive phase	Daily for duration of Rx (outpatients only)		Daily for duration of Rx (outpatients only)	
Treatment regimen and duration	Length of intensive phase	2 months	3 months	2 months	3 months	6 months	12 months	6 months	12 months
	Intensive phase regimen	R-H-Z-E	R-H-Z-E	R-H-Z-E	R-H-Z-E-S	Km-Z-Mxf-Eto-Trd		(Primary) Cm-Z-Mxf-Eto-Trd-PAS-Cfz (Acquired) Cm-Z-Mxf-Clm-Aug-hdH -PAS-Cfz	
	Length of	4 months	4 months	4 months	5 months	18 months	-	18 months	3 months

	continuation phase								
	Continuation phase regimen	R-H	R-H	R-H	R-H-E	Z-Mxf-Eto-Trd		(Primary) Z-Mxf-Eto-Trd-PAS-Cfz (Acquired) Z-Mxf-Clm-Aug-hdH -PAS-Cfz	
Diagnostic and monitoring test frequency	Sputum smear microscopy	2 at diagnosis, 4 during Rx	2 at diagnosis, 5 during Rx	2 at diagnosis, 5 during Rx	2 at diagnosis, 5 during Rx	At diagnosis and monthly		At diagnosis and monthly	
	Sputum liquid culture	ND	After 2 months of Rx	At diagnosis	At diagnosis	At diagnosis and monthly		At diagnosis and monthly	
	1st line DST	ND	ND	ND	At diagnosis	At diagnosis		At diagnosis	
	2nd line DST	ND	ND	ND	ND	At diagnosis	At diagnosis and after 6 months of Rx	At diagnosis	At diagnosis and after 6 months of Rx
	Chest X-ray	ND	ND	at diagnosis	at diagnosis	At diagnosis and every 6 months		At diagnosis and every 6 months	
	Full blood count	ND	ND	ND	ND	Baseline (every 6 months in HIV-infected)		Baseline (every 6 months in HIV-infected)	
	Urea	ND	ND	ND	ND	Baseline		Baseline	
	Kidney (creatinine, potassium)	ND	ND	ND	ND	Monthly during intensive phase		1.5 per month during intensive phase	
	Liver (ALT, AST, Bilirubin)	ND	ND	ND	ND	Every 3 months		Every 3 months	
	TSH	ND	ND	ND	ND	Every 6 months		Every 4 months	

	Audiogram	ND	ND	ND	ND	Monthly during injectable phase and every 3 months in continuation phase	Monthly during injectable phase and every 3 months in continuation phase
	CD4 count (HIV only)	Once during Rx period				Every 6 months	Every 6 months
	Viral load (HIV only)	Once during Rx period				Every 6 months	Every 6 months

ND – not routinely done, R - Rifampicin, H - Isoniazid, Z - Pyrazinamide, E - Ethambutol, S - Streptomycin, Km – Kanamycin, Mxf - Moxifloxacin, Eto - Ethionamide, Trd – Terizidone, Cm - Capreomycin, PAS – para-amionsalicylic acid, Cfz – Clofazimine, Clm - Clarithromycin, Aug - Augmentin, hdH - high dose Isoniazid

DST – Drug susceptibility test, AST - aspartate aminotransferase, ALT - alanine aminotransferase, TSH – Thyroid stimulating hormone, Rx – Treatment, DOTS – Directly Observed Treatment Short Course, ADR - Adverse drug reaction

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