Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study

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Summary

Background Data from Kwazulu Natal, South Africa, suggest that almost all patients with extensively drug-resistant (XDR) tuberculosis are HIV-positive, with a fatal outcome. Since, there are few data for the treatment-related outcomes of XDR tuberculosis in settings with a high HIV prevalence, we investigated the associations of these diseases in such settings to formulate recommendations for control programmes.

Methods In a retrospective cohort study, we analysed the case records of patients (>16 years old) with XDR tuberculosis (culture-proven at diagnosis) between August, 2002, and February, 2008, at four designated provincial treatment facilities in South Africa. We used Cox proportional hazards regression models to assess risk factors associated with the outcomes—mortality and culture conversion.

Findings 195 of 227 patients were analysed. 21 died before initiation of any treatment, and 174 patients (82 with HIV infection) were treated. 62 (36%) of these patients died during follow-up. The number of deaths was not significantly different in patients with or without HIV infection: 34 (41%) of 82 versus 28 (30%) of 92 (p=0·13). Treatment with moxifloxacin (hazard ratio 0·11, 95% CI 0·01–0·82; p=0·03), previous culture-proven multidrug-resistant tuberculosis (5·21, 1·93–14·1; p=0·001), and number of drugs used in a regimen (0·59, 0·45–0·78, p<0·0001) were independent predictors of death. Fewer deaths occurred in patients with HIV infection given highly active antiretroviral therapy than in those who were not (0·38, 0·18–0·80; p=0·01). 33 (19%) of 174 patients showed culture conversion, of which 23 (70%) converted within 6 months of initiation of treatment.

Interpretation In South Africa, patients with XDR tuberculosis, a substantial proportion of whom are not infected with HIV, have poor management outcomes. Nevertheless, survival in patients with HIV infection is better than previously reported. The priorities for the country are still prevention of XDR tuberculosis, and early detection and management of multidrug-resistant and XDR tuberculosis through strengthened programmes and laboratory capacity.

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Introduction

Tuberculosis kills 1·8 million people per year worldwide. Unsuccessful treatment programmes for disease control,[1,2] and continued transmission[3] are contributing to the emergence and spread of multidrug-resistant (MDR) tuberculosis (ie, bacillary resistance to at least rifampicin and isoniazid). The diagnosis and treatment of MDR tuberculosis in such settings diverts much needed resources away from case finding and the treatment of drug-susceptible tuberculosis. Extensively drug-resistant (XDR) tuberculosis (ie, bacillary resistance to at least rifampicin, isoniazid, a fluoroquinolone, and at least one second-line injectable drug—amikacin, kanamycin, or capreomycin) has been identified in more than 59 countries and, because of the changes in international travel and migration patterns, the threat is global.[4] Treatment of XDR tuberculosis shows varying results.[5–8] Data from Estonia suggested that the outcome associated with resistance to capreomycin was poor.[8,9] However, data from Peru, although few, are encouraging because 60% of patients with XDR tuberculosis completed treatment or were cured with intensive multidrug treatment.[10]

However, despite having the highest caseload of tuberculosis, there have been few reports of XDR tuberculosis from high-burden settings, including those with a high prevalence of HIV/AIDS, possibly because of the absence of comprehensive drug-susceptibility testing. Reports from KwaZulu Natal, South Africa, showed that almost all patients with XDR tuberculosis acquired in a hospital or community outbreak were infected with HIV and had a short-term mortality rate of more than 80%.[11,12] Because of the initial global media scare, XDR tuberculosis in Africa is generally perceived to arise predominantly in HIV-infected patients, who have increased susceptibility to infection and poor survival. Subsequently, 4% of cases

of MDR tuberculosis were in fact shown to be cases of XDR disease, and were present in most provinces in South Africa.[13,14]

Thus, the high rate of HIV infection and poorly functioning tuberculosis control programmes in many parts of Africa are likely to contribute to the emergence of XDR tuberculosis, which could destabilise the control of tuberculosis in these areas.[15] Urgent implementation of rational control policies is required to counter this threat. However, there are few data from Africa on which to base such recommendations, including those relevant to advocacy, diagnosis, case finding, and treatment. Furthermore, several uncertainties have implications for the design and implementation of tuberculosis control programmes, including the prognosis for individuals with XDR tuberculosis with or without HIV infection in a non-outbreak setting, the effect of highly active antiretroviral therapy on outcome in patients with HIV infection, the best treatment regimens for XDR tuberculosis, and predictors of poor outcome in a resource-poor setting. We therefore attempted to address these issues on the basis of our experience of treating patients with XDR tuberculosis at several centres in South Africa.

Methods

Study setting and participants

We retrospectively reviewed the case records of 227 patients (>16 years) with XDR tuberculosis, diagnosed (culture proven) between August, 2002, and February, 2008, at four of nine dedicated provincial facilities for the treatment of XDR tuberculosis in South Africa. This country has a population of nearly 48 million people, HIV prevalence of 11% in 2008, tuberculosis notification of 948 per 100 000 population in 2007, and notification of more than 7000 cases of MDR disease in 2007. National policy recommends that drug-susceptibility testing should be done for rifampicin, isoniazid, and ethambutol in all new cases of tuberculosis who have not culture-converted by 3 months, all retreatment cases at presentation, and high-risk patients. Second-line testing was done by use of the indirect method on solid media (Middlebrook 7H11, Becton Dickinson, Le Pont de Claix, France) at dedicated provincial tuberculosis laboratories (Cape Town, Port Elizabeth, Kimberley, and Johannesburg) with standardised reagents and methods, which did not change during the study. Ethical approval was obtained from the University of Cape Town, South African Medical Research Council, and the University of Witwatersrand's human research ethics committees.

Diagnosis of XDR tuberculosis

According to national policy, isolates resistant to isoniazid and rifampicin should be sent for second-line testing (ethionamide, ofloxacin, amikacin, and streptomycin in selected centres). Patients with MDR tuberculosis who were still culture-positive at the end of the intensive phase of treatment were also sent for second-line testing. In this study, patients with isolates of *Mycobacterium tuberculosis* that were resistant at diagnosis (time of sputum collection) to at least isoniazid, rifampicin, a fluoroquinolone, and at least one of the second-line injectable drugs (amikacin, kanamycin, or capreomycin) were judged to have XDR tuberculosis. In early 2007, with the newly adopted definition of XDR tuberculosis and revised guidelines, second-line drug-susceptibility testing again became widely available. Thus, XDR tuberculosis was only widely diagnosed and treated from late 2006 and early 2007, with a few cases identified retrospectively from before this

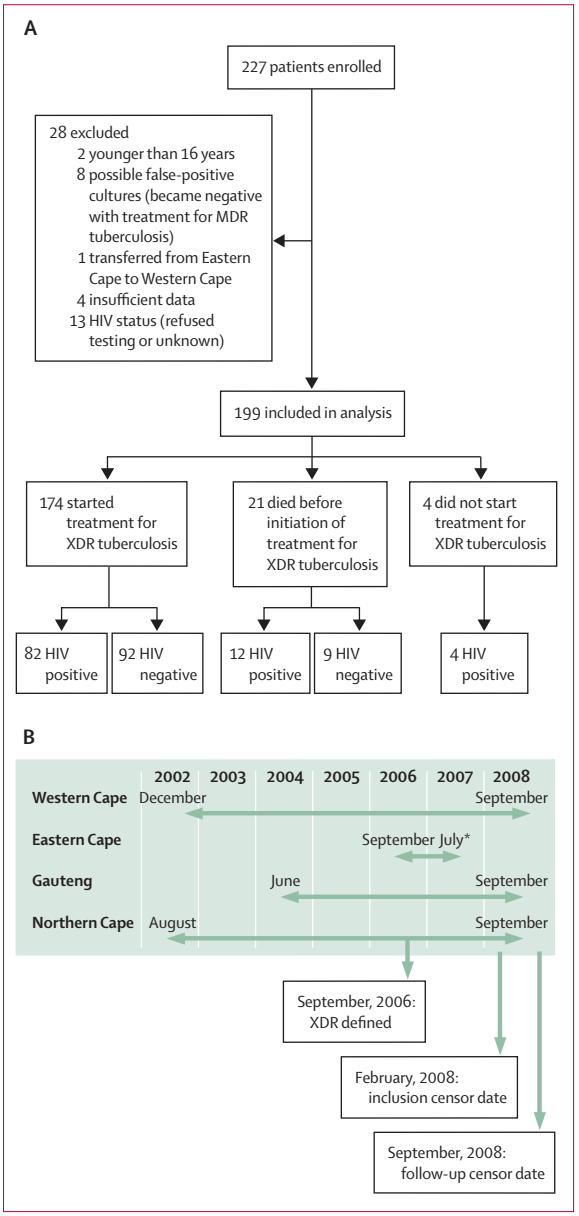

Figure 1: **Study profile (A), and timeline for data gathering stratified by recruitment institution (B)**
MDR=multidrug-resistant. XDR=extensively drug-resistant. *Local approval was only obtained for this period.

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	Mortality status			Sputum-culture conversion		
	Alive	Died	p value	Yes	No	p value
Patients	112 (64%)	62 (36%)	..	33 (19%)	141 (81%)	..
Age (years; median, IQR)	32·1 (25·8–42·8)	37·2 (28·5–47·1)	0·07	33·2 (26·3–044·7)	33·4 (26·3–44·4)	0·99
HIV status	..	..	0·13	..	..	0·83
Positive	48 (43%)	34 (55%)	..	15 (45%)	67 (48%)	..
Negative	64 (57%)	28 (45%)	..	18 (55%)	74 (52%)	..
Sex	..	..	0·59	..	..	0·47
Female	59 (53%)	30 (48%)	..	15 (45%)	67 (48%)	..
Male	53 (47%)	32 (52%)	..	18 (55%)	74 (52%)	..
Ethnic origin	..	..	0·37	..	..	0·11
Black	63 (56%)	41 (66%)	..	20 (61%)	84 (60%)	..
Mixed	48 (43%)	21 (34%)	..	12 (36%)	57 (40%)	..
White	1 (0·9%)	0	..	1 (3%)	0	..
Smoking history	..	..	0·004	..	..	0·10
Yes	39 (35%)	10 (16%)	..	9 (27%)	40 (28%)	..
No	34 (30%)	15 (24%)	..	14 (42%)	35 (25%)	..
Unknown	39 (35%)	37 (60%)	..	10 (30%)	66 (47%)	..
Previous multidrug-resistant tuberculosis	..	..	<0·0001	..	..	0·11
Yes	70 (63%)	55 (89%)	..	20 (61%)	105 (74%)	..
No	42 (38%)	7 (11%)	..	13 (39%)	36 (26%)	..
Weight (kg; median, IQR; n=65)	50·0 (44·0–60·0)	44·0 (39·0–49·0)	0·004	54·5 (49·8–61·3)	48·0 (39·0–52·5)	0·007
Drugs used	..	..	..	..	..	..
Ofloxacin	..	..	0·02	..	..	0·50
Yes	25 (22%)	5 (8%)	..	7 (21%)	23 (16%)	..
No	87 (78%)	57 (92%)	..	26 (79%)	118 (84%)	..
Capreomycin	..	..	0·43	..	..	0·33
Yes	103 (92%)	58 (94%)	..	32 (97%)	130 (92%)	..
No	9 (8%)	3 (5%)	..	1 (3%)	11 (8%)	..
Ethionamide	..	..	0·61	..	..	0·72
Yes	66 (59%)	39 (63%)	..	19 (58%)	86 (61%)	..
No	46 (41%)	23 (37%)	..	14 (42%)	55 (39%)	..
Ethambutol	..	..	0·01	..	..	0·40
Yes	57 (51%)	44 (71%)	..	14 (42%)	87 (62%)	..
No	55 (49%)	18 (29%)	..	19 (58%)	54 (38%)	..
Para-aminosalicylic acid	..	..	0·41	..	..	0·13
Yes	102 (91%)	54 (87%)	..	32 (97%)	124 (88%)	..
No	10 (9%)	8 (13%)	..	1 (3%)	17 (12%)	..
Moxifloxacin	..	..	0·02	..	..	0·24
Yes	13 (12%)	1 (2%)	..	1 (3%)	13 (9%)	..
No	99 (88%)	61 (98%)	..	32 (97%)	128 (91%)	..

Data are number (%), unless otherwise indicated.

Table 1: **Sociodemographic and clinical characteristics according to outcomes in 174 patients with extensively drug-resistant tuberculosis**

period. Drug-susceptibility testing to capreomycin, cycloserine, terizidone (a derivative containing a double molecule of cycloserine), and fluoroquinolones other than ofloxacin is not available within the provincial laboratories. However, to gain further insight into prevailing patterns, we tested the susceptibility to capreomycin in 57 isolates obtained from patients in Western Cape in accordance with the guidelines of the Centers for Disease Control and Prevention.[16]

Treatment regimens

MDR tuberculosis, was generally treated with a standardised regimen of kanamycin, ofloxacin, ethionamide, ethambutol, and pyrazinamide (terizidone was added for ethambutol-resistant patients) before the diagnosis of XDR tuberculosis. By contrast, treatment of XDR disease was given in hospital, and individualised with the use of capreomycin and para-aminosalicylic acid as the main drugs, with other first-line and second-line drugs used at

the discretion of the attending clinician, or to which the microorganism showed susceptibility. Capreomycin and para-aminosalicylic acid were introduced into the programme in about March, 2007. Linezolid is unavailable through the national tuberculosis programme and moxifloxacin is only accessible at specific centres (in Eastern Cape) or on a restricted basis through non-governmental organisations (eg, in Western Cape). Highly active antiretroviral therapy was offered to all patients infected with HIV at the discretion of the attending physician.

Outcomes

The date of diagnosis of XDR tuberculosis was the same as the date on which the sputum sample was sent to the laboratory, and from which the organism was cultured. There was thus a delay between diagnosis and initiation of treatment. Conversion was judged to have occurred when two consecutively negative cultures were obtained, 1 month apart, and when the first culture was positive at the start of treatment for XDR tuberculosis. Because widespread and targeted screening for XDR tuberculosis was only available in many centres from late 2006 or early 2007, most of the cohort had a short follow-up. Hence, we focused on early outcomes (conversion and mortality).

Strain typing

To establish whether XDR tuberculosis was acquired or transmitted, a subset of isolates (n=52) from patients living in the Western Cape were genotyped by use of IS*6110* DNA fingerprinting,[17] spoligotyping,[18] and DNA sequencing of the *inhA* promoter and the *katG*, *rpoB*, *embB*, *pncA*, *gyrA*, and *rrs* genes. Transmission chains were defined by use of isolates that had identical IS*6110* DNA fingerprints, spoligotypes, and mutations that conferred resistance.

Statistical analysis

A risk-management strategy for data, including double data entry, was used to ensure data integrity. We compared categorical variables by use of the χ^2 test, or Fisher's exact test when appropriate, and we compared continuous variables, because of the non-normal distribution of the analysed variables, using the Mann-Whitney *U* test or Kruskal-Wallis non-parametric test. The Kaplan-Meier method was used to calculate probabilities of events at different timepoints, and the log-rank test was used to compare these probabilities by group. Cox proportional hazards regression models were fitted to determine risk factors associated with outcomes in time-to-event-based analyses. Variables that were significantly associated with the outcome ($p<0·05$) were included in the final model. The proportionality assumption of the Cox models was tested with –ln(–ln [survival]) curves and regression of scaled Schoenfeld residuals on functions of time. The assumption of uninformative censoring (ie, more censored observations at an earlier time in one group than in another group, or a greater proportion of censored survival times in patients with a particular range of values of the explanatory variables) was investigated with observed survival times plotted against the values of the explanatory variables included in the model. Additionally, sensitivity analyses were done to account for patients excluded from the analysis, and for those lost to follow-up.

	Patients given drug	Treatment duration (months; median, IQR)	Resistance pattern (number of patients resistant to treatment/number of susceptibility tests done in treated patients [%])
Ethambutol	103 (59%)	6·8 (4·0–11·4)	62/117 (53%)
Pyrazinamide	140 (80%)	7·2 (3·3–11·5)	ND
Amikacin	3 (2%)	4·2 (2·1–5·8)	124/124 (100%)
Capreomycin	162 (93%)	7·2 (3·1–12·1)	22/42 (52%)
Kanamycin	4 (2%)	13·4 (4·8–15·6)	50/50 (100%)
Moxifloxacin	14 (8%)	12·1 (10·4–18·3)	ND
Ofloxacin	31 (18%)	8·1 (4·2–12·0)	174/174 (100%)
Terizidone	147 (84%)	9·2 (3·5–12·1)	ND
Ethionamide	107 (61%)	8·0 (3·2–11·9)	59/167 (35%)
Para-aminosalicylic acid	156 (90%)	7·3 (3·7–12·2)	ND
Amoxicillin–clavulanate	66 (38%)	7·1 (3·1–12·2)	ND
Clarithromycin	77 (44%)	8·6 (3·7–13·9)	ND
Clofazimine	28 (16%)	8·7 (4·3–14·3)	ND
Dapsone	95 (55%)	6·6 (3·1–11·6)	ND
Azithromycin	11 (6%)	7·9 (2·8–12·1)	ND
Isoniazid+thiacetazone	2 (1%)	7·8 (1·9–13·7)	ND
Rifabutin	1 (<1%)	NA	ND
Streptomycin	1 (<1%)	NA	ND

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

Table 2: **Drugs used in treatment regimens for patients (n=174) with extensively drug-resistant tuberculosis**

Role of the funding source

None of the funding sources had any role in the design or execution of the study. The corresponding author in consultation with the other lead authors had final decision to submit this work for publication. All authors had access to all the data.

Results

Figure 1A shows a summary of the study plan. 199 of 227 enrolled patients were included in the further analysis (figure 1A). All patients diagnosed with XDR tuberculosis were admitted to the facility until conversion of the sputum smear. Figure 1B shows the centre-specific dates of enrolment (Feb 1, 2008, was the enrolment censor date and Sept 1, 2008, was the follow-up censor date).

In the final analyses, the 174 patients started on treatment for XDR tuberculosis were young (median age 33 years, IQR 26–45), mainly of mixed ethnic origin, and 82 (47%) were infected and 92 (53%) were not infected

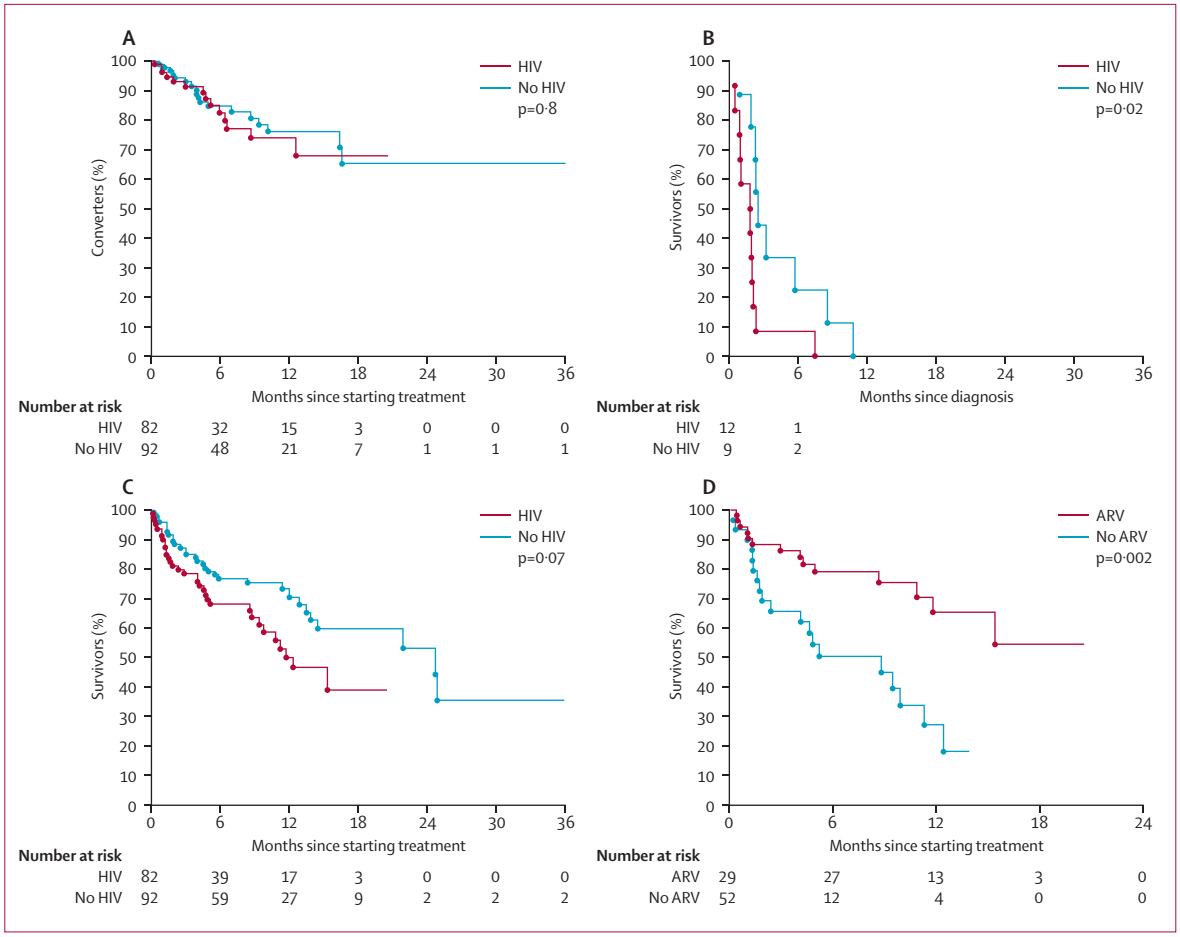

Figure 2: **Kaplan-Meier estimates of culture-conversion and deaths in patients with extensively drug-resistant tuberculosis**
(A) Probabilities of culture conversion in patients with and without HIV infection given treatment; (B) probabilities of death in patients who died before initiation of treatment stratified by HIV status; (C) probabilities of death in patients with and without HIV infection given treatment from date of treatment initiation; and (D) probabilities of death in patients with HIV infection given treatment stratified according to use of highly active antiretroviral therapy.

with HIV. Table 1 shows the demographic variables stratified by mortality and conversion status. There was no difference in the number of deaths or sputum-culture conversions (p>0·05) when these outcomes were stratified by sex, ethnic origin, and the number of previous episodes of MDR tuberculosis (median 1 episode [1–1] each in those who survived and died, 1 episode [1–2] in those who converted, and 1 episode [1–2] in those who did not convert). There was no significant difference in the duration of previous treatment for MDR tuberculosis in patients who survived versus those who died (median 8·0 months [5·0–12·0] *vs* 5·5 months [4–8], respectively; p=0·7), and converters versus non-converters (9 months [6–14] *vs* 6 months [4–10], respectively; p=0·37). Similarly, the median number of previous drug-susceptible episodes of tuberculosis in the same groups was not different (survivors *vs* non-survivors: 1 [1–2] *vs* 1 [1–2], respectively, p=0·1; converters *vs* non-converters: 1 [1–2] *vs* 1 [1–2], respectively, p=0·2). The time from sputum acquisition to start of treatment was significantly longer in those who survived than in those who died (78 days [53–107] *vs* 57 days [36–67]; p=0·001), and in converters than in non-converters (91 days [61–116] *vs* 59 days [43–86], respectively; p=0·001). The median duration of follow-up from the start of treatment to event (death, loss to follow-up, or the date of censor) was 6·9 months (3·1–12·0).

72% of patients with XDR tuberculosis had previous culture-confirmed MDR tuberculosis (table 1), suggesting acquisition of additional resistance during this treatment. 17 patients with XDR tuberculosis had an isolate of *M tuberculosis* with a unique IS*6110* genotype, whereas the remaining 35 patients had an isolate that could be genotypically grouped into one of six clusters. Ten (19%) of these clustered cases showed mutations that caused identical resistance to first-line and second-line drugs, suggesting that resistance to second-line drugs was acquired in 81% of patients from the Western Cape.

174 patients started treatment with a regimen containing capreomycin and para-aminosalicylic acid, and were given a median of seven drugs per regimen (IQR 6–8). The drug-specific resistance and treatment profile are summarised in table 2. Median duration of treatment was

6·9 months (3·1–12·0), and duration of treatment with injectable drugs was 13·4 months (4·7–15·5). 33 (58%) of 57 random isolates that were retrospectively tested for susceptibility to capreomycin were resistant, and 42 (74%) patients were given the drug after the isolate was harvested for susceptibility testing. Only 20 (48%) of these patients had isolates that were capreomycin-susceptible. In the resistant versus susceptible group, there was no significant difference in the number of patients who converted (six [27%] of 22 *vs* three [15%] of 20; p=0·33) or died (seven [32%] of 22 *vs* six [30%] of 20; p=0·9). Overall drug susceptibility profiles did not differ with HIV status or when HIV-infected patients were stratified by CD4-cell count. 52 (63%) of 82 patients with HIV infection given treatment for XDR tuberculosis were also taking highly active antiretroviral therapy; zidovudine, lamivudine, and efavirenz were the most commonly used antiretroviral drugs in 26 (50%) of these patients.

Initiation of treatment resulted in culture conversion in 33 (19%) of 174 patients (table 1), and the probability of culture conversion did not differ by HIV status (figure 2A). 23 (70%) of these patients converted by 6 months, 28 (85%) by 9 months, and 30 (91%) by 12 months. Before the date for censor follow-up (Sept 30, 2008), two (6%) of 33 patients with XDR tuberculosis who had converted had reverted back to culture-positive status. In a univariate Cox regression analysis, low weight (<50 kg) before treatment was associated with conversion failure (hazard ratio 3·31, 95% CI 1·08–10·1; p=0·04).

The cause of death could not be ascertained for the 21 patients who died before the start of treatment for XDR tuberculosis, but the proportion of deaths in patients with HIV infection was significantly higher than in the uninfected patients in the time-to-event analysis (figure 2B). 195 of 227 enrolled patients with XDR tuberculosis were analysed for the mortality outcome. 62 (36%) of 174 died during follow-up. Numbers of deaths in patients with or without HIV infection were not significantly different: 34 (41%) of 82 versus 28 (30%) of 92 (p=0·13; table 1). Additionally, the probability of survival did not differ in the two groups (figure 2C).

In the Cox multivariate model, treatment with moxifloxacin and increasing number of drugs used in a regimen were independent predictors of a reduction in the number of deaths, whereas previous culture-proven MDR tuberculosis was an independent predictor of death (table 3). Survival did not differ with CD4-cell count (data not shown), but the analysis was based on a small number of participants with verifiable CD4-cell counts at the time of initiation of treatment (35 of 82 patients; median CD4-cell count 273 cells per mm³ [IQR 169–396]). Results for survival were similar when the date of diagnosis rather than the date of treatment initiation was used, and outcomes (mortality and culture conversion) were similar before and after March, 2007, when capreomycin and para-aminosalicylic acid were introduced into the programme (data not shown). In a sub-analysis of patients with HIV infection, only highly antiretroviral therapy and moxifloxacin were independent predictors of survival (table 3). Significantly fewer individuals with HIV infection given highly antiretroviral therapy died than did those not given this therapy (table 3), and number of deaths at 12 months was 13 (25%) of 52 versus 19 (66%) of 29 (figure 2D), respectively. The median CD4-cell counts in patients taking highly antiretroviral therapy were 267 per mm³ (160–365) versus 440 per mm³ (170–477) in those not taking this therapy. A formal test of the assumptions of the proportional hazards, and of uninformative censoring, indicated that these assumptions were not violated.

Case records from three treatment centres (Western Cape, Northern Cape, and Gauteng) were comprehensively reviewed for adverse drug reactions, which were reported in 67 (58%) of 115 patients. Table 4 shows the drug-specific adverse effects. 58 (36%) of 161 adverse drug reactions required no intervention; 69 (43%) needed modification in the duration of treatment or frequency of administration of the drug, or prescription of an additional drug to treat the adverse event; the drug causing the adverse reaction was stopped in 26 (16%) reactions; and six (4%) patients died (five with rapidly deteriorating renal failure and one with hypokalaemia) at a median of 14 days (IQR 9–73) after starting capreomycin. Highly active antiretroviral therapy was generally well tolerated.

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	p value*	Hazard ratio (95% CI)	p value
All patients (n=174)				
Isoniazid	0·20 (0·08–0·49)	<0·0001	0·76 (0·24–2·51)	0·68
Moxifloxacin	0·12 (0·02–0·89)	0·03	0·11 (0·01–0·82)	0·03
Ethambutol	1·87 (1·08–3·26)	0·03	1·49 (0·69–3·23)	0·31
Dapsone	2·19 (1·25–3·84)	0·006	1·79 (0·84–3·85)	0·13
Clofazamine	0·30 (0·11–0·82)	0·02	0·54 (0·14–2·08)	0·37
Clarithromycin	0·55 (0·32–0·94)	0·03	1·46 (0·61–3·52)	0·40
Terizidone	0·48 (0·27–0·84)	0·01	1·35 (0·71–2·55)	0·37
Number of drugs used	0·74 (0·61–0·88)	0·001	0·59 (0·45–0·78)	<0·0001
Previous treatment for multidrug-resistant tuberculosis	3·73 (1·69–8·22)	0·001	5·21 (1·93–14·1)	0·001
HIV-infected patients (n=82)				
Highly active antiretroviral therapy	0·31 (0·15–0·61)	0·001	0·38 (0·18–0·80)	0·01
Isoniazid	0·17 (0·05–0·56)	0·005	0·41 (0·06–2·96)	0·39
Moxifloxacin	13·00 (0·02–0·92)	0·006	0·08 (0·01–0·61)	0·02
Pyrazinamide	4·10 (1·00–17·50)	0·04	3·48 (0·74–16·50)	0·12
Clofazamine	0·21 (0·05–0·86)	0·03	1·37 (0·14–13·82)	0·79
Number of drugs used	0·74 (0·56–0·99)	0·04	0·87 (0·58–1·26)	0·43
Previous treatment for multidrug-resistant tuberculosis	7·46 (1·79–31·20)	0·006	4·50 (0·83–24·40)	0·08

*Wald test. Ofloxacin not included because it was significant in the χ^2 test (p=0·02), but not in the time-to-event analysis with Cox regression model (p>0·05).

Table 3: **Cox proportional hazards regression model of factors associated with risk of death in all patients given treatment for extensively drug-resistant tuberculosis, and in HIV-infected patients only**

	Reactions	No action taken*	Change in dose or frequency*	Implicated drug stopped*	Life threatening*	Death*
Nausea or vomiting	35 (22%)	9 (26%)	16 (46%)	10 (29%)	0	0
Diarrhoea	22 (14%)	3 (14%)	17 (77%)	2 (9%)	0	0
Other gastrointestinal symptoms (abdominal pain, dyspepsia, epigastric discomfort, cramps)	22 (14%)	11 (50%)	8 (36%)	3 (14%)	0	0
Dizziness, disorientation, or confusion	13 (8%)	13 (100%)	0	0	0	0
Loss of hearing	10 (6%)	5 (50%)	2 (20%)	3 (30%)	0	0
Renal failure†	7 (4%)	0	0	0	2 (28%)	5 (71%)
Body aches or pains	10 (6%)	6 (60%)	3 (30%)	1 (10%)	0	0
Headache	8 (5%)	4 (50%)	4 (50%)	0	0	0
Skin reaction	7 (4%)	2 (29%)	5 (71%)	0	0	0
Hypokalaemia	7 (4%)	1 (14%)	4 (57%)	1 (14%)‡	0	1 (14%)§
Hypothyroidism	6 (4%)	0	5 (83%)	1 (17%)	0	0
Depression	2 (1%)	1 (50%)	0	1 (50%)	0	0
Sore tongue or throat	2 (1%)	0	2 (100%)	0	0	0
Numbness of extremities	2 (1%)	0	0	2 (100%)	0	0
Generalised itchiness	2 (1%)	1 (50%)	1 (50%)	0	0	0
Psychosis	1 (<1%)	0	0	1 (100%)	0	0
Renal impairment	1 (<1%)	0	0	1 (100%)	0	0
Fatigue	1 (<1%)	1 (100%)	0	0	0	0
Visual disturbance	1 (<1%)	1 (100%)	0	0	0	0
Thrombophlebitis	1 (<1%)	0	1 (100%)	0	0	0
Arthralgia	1 (<1%)	0	1 (100%)	0	0	0
Total	161 (100%)	58 (36%)	69 (43%)	26 (16%)	2 (1%)	6 (4%)

Data are number (%). *Proportion of specific adverse drug reactions. †Creatinine concentration greater than six times the upper limits of normal. ‡Potassium concentration 2·0–2·4 mmol/L. §Potassium concentration less than 2·0 mmol/L.

Table 4: **Adverse drug reactions (n=161) in 67 of 115 patients from the Western Cape, Northern Cape, and Gauteng provinces started on treatment for extensively drug-resistant tuberculosis**

Ten (7%) of 135 patients with XDR tuberculosis were assessed for surgery at a centre with facilities for thoracic surgery. Four of these patients refused surgery, three were judged to be unsuitable for surgery, and three underwent the procedure (two pneumonectomies and one lobectomy). One of the patients who had surgery converted and continued treatment but later relapsed, the other two died 12 days and 24 days after surgery. Four patients with XDR tuberculosis had previously undergone surgery for MDR tuberculosis.

Discussion

We have shown several associations of XDR tuberculosis in settings with high HIV prevalence. First, XDR tuberculosis in South Africa is not predominantly associated with HIV infection as perceived from studies done in KwaZulu Natal in 2006 and 2009.[11,12] Although the numbers of deaths were high, we noted no difference in treatment outcomes (mortality and culture-conversion status) when comparing patients who were HIV-positive with those who were HIV-negative. This finding is important for the design of policy guidelines and programmes for management, reduction of stigma associated with a fatal prognosis, and advocacy purposes. Second, fewer deaths occurred in patients with concomitant HIV infection and XDR tuberculosis given highly antiretroviral therapy than in untreated patients. These data also have implications for the design of programmes for the treatment of XDR tuberculosis since the data from the studies in KwaZulu Natal indicated an invariably fatal outcome in individuals with concomitant HIV infection, showing the importance of WHO's recommended strategy for treatment of HIV/AIDS and tuberculosis.[19] Third, that moxifloxacin was an independent predictor of survival is another useful finding because there are no new licensed drug options for XDR tuberculosis in high-burden settings. Fourth, previous culture-proven MDR tuberculosis, and high number of drugs used in a regimen were predictors of survival and culture conversion. Fifth, the outcome of management of patients with XDR tuberculosis in South Africa is poor, despite good adherence and availability of drugs.

Our results are relevant to African governments and the WHO Stop TB Partnership to improve management guidelines for XDR tuberculosis programmes, case finding, diagnostic algorithms, and outcomes of treatment in patients with and without HIV infection in resource-limited settings.

Another important finding was that overall prognosis for the patient was poor irrespective of HIV status, and despite supervised inpatient multidrug treatment and access, when available, to surgical resection of diseased lung. Indeed, conversion rates were low (19%) and culture reversion occurred in some patients (6% by end of study and, at the time of writing, 54% of 13 converters in Western Cape for whom we have follow-up data after the study). Moreover, the overall 12-month mortality rate was higher than the rates associated with most aggressive malignant diseases. By contrast, results from four studies in intermediate or high-burden settings, with smaller cohorts, were better than our results.[7,10,20,21] Mitnick and colleagues[10] in Peru showed that 32 (67%) of 48 patients with XDR tuberculosis in the cohort had converted by 4 months (median time to conversion 90 days), and 11 (23%) died. Possible reasons for the poorer survival in our cohort of patients, independent of HIV status, include the long duration of drug-resistant and drug-susceptible tuberculosis before initiation of treatment for XDR tuberculosis; delays in initiation of such treatment; malnutrition; co-exposures such as smoking, alcohol, and illicit drug use; different *M tuberculosis* strains (their pathogenic characteristics and resistance patterns); lack of treatment with moxifloxacin; and exposure to helminths and other environmental mycobacteria, which might alter the underlying immunological phenotype,[22] thus reducing effectiveness of treatment. Other causes such as malabsorption of drugs, and interactions between antituberculosis drugs and highly active antiretroviral therapy seem unlikely as putative causes because of similar outcomes in patients with or without HIV infection, and because highly active antiretroviral therapy was well tolerated. The poor outcomes draw attention to the need to urgently develop and prospectively validate new interventions for XDR tuberculosis.[23] The outcome data and the high proportion of acquired resistance to second-line drugs in this study and nationally[13,24] suggest that the national tuberculosis control programmes need to aggressively ensure treatment adherence with effective regimens to restrict the spread of resistance. This prevention of drug resistance can be achieved by programme strengthening to prevent system failures,[25,26] integration of treatment for drug-resistant tuberculosis and HIV/AIDS,[27] intensive counselling and follow-up, and surgery when necessary and available.

Available data for XDR tuberculosis from Africa indicate that this disease was almost exclusively associated with HIV infection, and that prognosis in patients with both diseases is poor with a high 30-day mortality rate.[11,12] Hence, since the data were first reported in 2006, and again in 2009 from Kwazulu Natal, the general opinion is that XDR tuberculosis in Africa arises predominantly in HIV-infected individuals who have increased susceptibility to tuberculosis.[28] By contrast, our data show that a substantial proportion (53%) of patients with XDR tuberculosis outside the KwaZulu Natal province are HIV-negative. Moreover, survival in patients with HIV infection in our study cohort, from a wider South African setting, is substantially longer (50% died at 12 months *vs* 83–100% in KwaZulu Natal[11,12]). Although not small, this proportion is substantially better than in KwaZulu Natal.[11,12] The reasons are unclear but might include patients presenting at different stages of their illness, degree of immunosuppression, different drug-susceptibility profiles of isolates, lack of availability of capreomycin before 2006, nutritional status, and local differences in strain virulence. Furthermore, the KwaZulu Natal strain showed different pathogenic properties due to its high capacity to cause recrudescence through reinfection, and clonal expansion through transmission compared with the findings in the Western Cape, where resistance was mostly acquired.[29,30] We also show that highly active antiretroviral therapy, despite its overlapping toxicity and adverse effects with antituberculosis drugs, and the high pill burden, substantially improves survival in patients with concomitant HIV/AIDS and XDR tuberculosis, and was generally well tolerated. Our data suggest that highly active antiretroviral therapy should be used at an early stage in patients with HIV infection and XDR tuberculosis. These findings are important for advocacy purposes because they support the notion that treatment for XDR tuberculosis in patients with HIV infection is not without hope, and thus counteract the stigmatisation of this group of patients.

Treatment with moxifloxacin was an independent predictor of survival in ofloxacin-resistant patients with XDR tuberculosis and has hitherto not been described. Evidence suggests that the incomplete cross-resistance within the quinolone class can be explained by differential drug-specific binding to DNA gyrase.[31] Unlike the cohort in Mitnick and colleagues' study,[10] with 34 (72%) of 47 patients ever treated with moxifloxacin, our programme does not provide this drug nationally. Prospective clinical and in-vitro studies are urgently needed to investigate how best to use fluoroquinolones for treatment of XDR tuberculosis. Meanwhile, we recommend that, in the absence of specific results of drug-susceptibility testing, moxifloxacin should be used in treatment regimens for XDR tuberculosis unless contraindicated. With our findings and those from studies of MDR tuberculosis,[32,33] we also suggest that patients with a weight of less than 50 kg should be given nutritional supplementation, intensive follow-up, and a bolstered regimen. These recommendations, which also have importance for the design of treatment programmes, need to be prospectively validated. The high capreomycin resistance is cause for concern since this antibiotic is the mainstay of regimens for the treatment of XDR tuberculosis that are used globally, and it has not previously been widely used in South Africa. The reasons are not known, but might partly indicate the unreliability of capreomycin-susceptibility testing, or the potentially high cross-resistance with aminoglycosides like kanamycin.[34,35]

An additional key finding was that 21 of 195 patients died before starting treatment, indicating that they had late access to treatment or inappropriate delivery of health services. Our data therefore emphasise the need to reduce delays in diagnosis and initiation of treatment through intensified case finding,[36] improve patient access and diagnostic reporting systems,[25,37] and improve the rollout of rapid diagnostic tests for MDR and XDR tuberculosis.[38] Because existing nucleic acid amplification tests are not efficient enough in smear-negative cases,[38] application of rapid drug-sensitivity testing to all smear-positive patients would be rational at first presentation. A favourable cost-benefit analysis of such an approach is supported by mathematical models.[39] Development and validation of alternative methods that could be useful for detection of sputum-smear-negative tuberculosis are also urgently needed.[40,41]

Our data showed a high incidence of adverse events associated with drugs used for the treatment of XDR tuberculosis. The most serious adverse effects were caused by capreomycin-associated renal dysfunction, which can occur at any time during the course of treatment; thus monitoring of capreomycin-based regimens, even in resource-poor settings is mandatory.

Limitations of our findings are due to the retrospective study design. We have tried to reduce bias by excluding patients with incomplete treatment and microbiological data, by excluding individuals who had culture converted before initiation of treatment for XDR tuberculosis, by crosschecking patients against clinical and laboratory databases, and by using methods to ensure data integrity. Selection bias, particularly in the HIV-infected subgroup, might have led to an underestimate of the true number of deaths since survivors would have had an increased likelihood of being included in our study. Since all cases of XDR tuberculosis were detected passively, we are likely to have missed many patients who had remained undiagnosed or died before susceptibility testing, and patients who did not have access to health care, or in whom appropriate susceptibility testing was not undertaken because of capacity limitations. Nevertheless, the proportion of deaths did not differ in centres with a high urbanised population and easy medical access (Gauteng and Western Cape) compared with those that had high rural populations with restricted access (Eastern Cape and Northern Cape). Although we noted a survival benefit with moxifloxacin, the number of patients treated with this drug was small, and we could not correlate outcomes with quinolone-specific resistance profiles because of the lack of specific data for drug-susceptibility testing. Our findings are only generalisable to a resource-poor high HIV prevalence setting like South Africa. Outcomes in settings with low HIV prevalence and in those that are even more severely resource-constrained might be different.

Since patients with XDR tuberculosis have poor management outcomes in South Africa, prevention of this type of tuberculosis, and early detection and management of MDR and XDR tuberculosis, through strengthened programmes and laboratory capacity remain the priorities for areas that have high endemicity of tuberculosis and HIV/AIDS.

Contributors

KD, AZ, and MB designed the study. EMS, PDH, TV, and RW did the strain-typing analysis. MB and KD did the statistical analysis. KD, MB, and AZ wrote the draft of the report. All authors participated in data gathering, and critical appraisal of the report.

Conflicts of interest

We declare that we have no conflicts of interest.

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References

1 Corbett EL, Marston B, Churchyard GJ, De Cock KM. Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. *Lancet* 2006; **367:** 926–37.
2 Maartens G, Wilkinson RJ. Tuberculosis. *Lancet* 2007; **370:** 2030–43.
3 van Rie A, Warren RM, Beyers N, et al. Transmission of a multidrug-resistant Mycobacterium tuberculosis strain resembling "strain W" among noninstitutionalized, human immunodeficiency virus-seronegative patients. *J Infect Dis* 1999; **180:** 1608–15.
4 Sotgiu G, Ferrara G, Matteelli A, et al. Epidemiology and clinical management of XDR-TB: a systematic review by TBNET. *Eur Respir J* 2009; **33:** 871–81.
5 Chan ED, Strand MJ, Iseman MD. Treatment outcomes in extensively resistant tuberculosis. *N Engl J Med* 2008; **359:** 657–59.
6 Migliori GB, Besozzi G, Girardi E, et al; SMIRA/TBNET Study Group. Clinical and operational value of the extensively drug-resistant tuberculosis definition. *Eur Respir J* 2007; **30:** 623–26.
7 Kwon YS, Kim YH, Suh GY, et al. Treatment outcomes for HIV-uninfected patients with multidrug-resistant and extensively drug-resistant tuberculosis. *Clin Infect Dis* 2008; **47:** 496–502.
8 Migliori GB, Lange C, Centis R, et al. Resistance to second-line injectables and treatment outcomes in multidrug-resistant and extensively drug-resistant tuberculosis cases. *Eur Respir J* 2008; **31:** 1155–59.
9 Migliori GB, Lange C, Girardi E, et al; SMIRA/TBNET Study Group. Fluoroquinolones: are they essential to treat multidrug-resistant tuberculosis? *Eur Respir J* 2008; **31:** 904–05.
10 Mitnick CD, Shin SS, Seung KJ, et al. Comprehensive treatment of extensively drug-resistant tuberculosis. *N Engl J Med* 2008; **359:** 563–74.
11 Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006; **368:** 1575–80.
12 Gandhi NR, Shah NS, Andrews JR, et al. HIV Co-infection in Multidrug- and Extensively Drug-resistant Tuberculosis Results in High Early Mortality. *Am J Respir Crit Care Med* 2010; **181:** 80–86.
13 Mlambo CK, Warren RM, Poswa X, Victor TC, Duse AG, Marais E. Genotypic diversity of extensively drug-resistant tuberculosis (XDR-TB) in South Africa. *Int J Tuberc Lung Dis* 2008; **12:** 99–104.

14 O'Donnell MR, Padayatchi N, Master I, Osburn G, Horsburgh CR. Improved early results for patients with extensively drug-resistant tuberculosis and HIV in South Africa. *Int J Tuberc Lung Dis* 2009; **13:** 855–61.

15 Jones KD, Hesketh T, Yudkin J. Extensively drug-resistant tuberculosis in sub-Saharan Africa: an emerging public-health concern. *Trans R Soc Trop Med Hyg* 2008; **102:** 219–24.

16 Isenberg HD, American Society for Microbiology. Clinical microbiology procedures handbook. Washington, DC: ASM Press, 2004.

17 Warren R, de Kock M, Engelke E, et al. Safe Mycobacterium tuberculosis DNA extraction method that does not compromise integrity. *J Clin Microbiol* 2006; **44:** 254–56.

18 Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. *J Clin Microbiol* 1997; **35:** 907–14.

19 WHO. Interim policies on collaborative TB/HIV activities. Geneva: World Health Organization, 2004.

20 Bonilla CA, Crossa A, Jave HO, et al. Management of extensively drug-resistant tuberculosis in Peru: cure is possible. *PLoS ONE* 2008; **3:** e2957.

21 Keshavjee S, Gelmanova IY, Farmer PE, et al. Treatment of extensively drug-resistant tuberculosis in Tomsk, Russia: a retrospective cohort study. *Lancet* 2008; **372:** 1403–09.

22 Rook GA, Dheda K, Zumla A. Immune responses to tuberculosis in developing countries: implications for new vaccines. *Nat Rev Immunol* 2005; **5:** 661–67.

23 Rook GA, Lowrie DB, Hernandez-Pando R. Immunotherapeutics for tuberculosis in experimental animals: is there a common pathway activated by effective protocols? *J Infect Dis* 2007; **196:** 191–98.

24 Pillay M, Sturm AW. Evolution of the extensively drug-resistant F15/LAM4/KZN strain of Mycobacterium tuberculosis in KwaZulu-Natal, South Africa. *Clin Infect Dis* 2007; **45:** 1409–14.

25 Loveday M, Thomson L, Chopra M, Ndlela Z. A health systems assessment of the KwaZulu-Natal tuberculosis programme in the context of increasing drug resistance. *Int J Tuberc Lung Dis* 2008; **12:** 1042–47.

26 Padayatchi N, Friedland G. Decentralised management of drug-resistant tuberculosis (MDR- and XDR-TB) in South Africa: an alternative model of care. *Int J Tuberc Lung Dis* 2008; **12:** 978–80.

27 Gandhi NR, Moll AP, Lalloo U, et al. Successful integration of tuberculosis and HIV treatment in rural South Africa: the Sizonq'oba study. *J Acquir Immune Defic Syndr* 2009; **50:** 37–43.

28 LoBue P. Extensively drug-resistant tuberculosis. *Curr Opin Infect Dis* 2009; **22:** 167–73.

29 Andrews JR, Gandhi NR, Moodley P, et al. Exogenous reinfection as a cause of multidrug-resistant and extensively drug-resistant tuberculosis in rural South Africa. *J Infect Dis* 2008; **198:** 1582–89.

30 Ioerger TR, Koo S, No EG, et al. Genome analysis of multi- and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa. *PLoS ONE* 2009; **4:** e7778.

31 Kam KM, Yip CW, Cheung TL, Tang HS, Leung OC, Chan MY. Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant Mycobacterium tuberculosis: correlation with ofloxacin susceptibility. *Microb Drug Resist* 2006; **12:** 7–11.

32 Leimane V, Riekstina V, Holtz TH, et al. Clinical outcome of individualised treatment of multidrug-resistant tuberculosis in Latvia: a retrospective cohort study. *Lancet* 2005; **365:** 318–26.

33 Mitnick C, Bayona J, Palacios E, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. *N Engl J Med* 2003; **348:** 119–28.

34 Jugheli L, Bzekalava N, de Rijk P, Fissette K, Portaels F, Rigouts L. High level of cross-resistance between kanamycin, amikacin, and capreomycin among Mycobacterium tuberculosis isolates from Georgia and a close relation with mutations in the *rrs* gene. *Antimicrob Agents Chemother* 2009; **53:** 5064–68.

35 Via LE, Cho SN, Hwang S, et al. Polymorphisms associated with resistance and cross-resistance to aminoglycosides and capreomycin in Mycobacterium tuberculosis isolates from South Korean Patients with drug-resistant tuberculosis. *J Clin Microbiol* 2010; **48:** 402–11.

36 Basu S, Friedland GH, Medlock J, et al. Averting epidemics of extensively drug-resistant tuberculosis. *Proc Natl Acad Sci USA* 2009; **106:** 7672–77.

37 Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC Public Health* 2008; **8:** 15

38 Ling DI, Zwerling AA, Pai M. Genotype MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *Eur Respir J* 2008; **32:** 1165–74.

39 Dowdy DW, Chaisson RE, Maartens G, Corbett EL, Dorman SE. Impact of enhanced tuberculosis diagnosis in South Africa: a mathematical model of expanded culture and drug susceptibility testing. *Proc Natl Acad Sci USA* 2008; **105:** 11293–98.

40 Migliori GB, Matteelli A, Cirillo D, Pai M. Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Current standards and challenges. *Can J Infect Dis Med Microbiol* 2008; **19:** 169–72.

41 Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part II. Active tuberculosis and drug resistance. *Expert Rev Mol Diagn* 2006; **6:** 423–32.