Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study

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Summary

Background The emergence of programmatically incurable tuberculosis threatens to destabilise control efforts. The aim of this study was to collect prospective patient-level data to inform treatment and containment strategies.

Methods In a prospective cohort study, 273 South African patients with extensively drug-resistant tuberculosis, or resistance beyond extensively drug-resistant tuberculosis, were followed up over a period of 6 years. Transmission dynamics, infectiousness, and drug susceptibility were analysed in a subset of patients from the Western Cape using whole-genome sequencing (WGS; n=149), a cough aerosol sampling system (CASS; n=26), and phenotypic testing for 18 drugs (n=179).

Findings Between Oct 1, 2008, and Oct 31, 2012, we enrolled and followed up 273 patients for a median of 20·3 months (IQR 9·6–27·8). 203 (74%) had programmatically incurable tuberculosis and unfavourable outcomes (treatment failure, relapse, default, or death despite treatment with a regimen based on capreomycin, aminosalicylic acid, or both). 172 (63%) patients were discharged home, of whom 104 (60%) had an unfavourable outcome. 54 (31%) home-discharged patients had failed treatment, with a median time to death after discharge of 9·9 months (IQR 4·2–17·4). 35 (20%) home-discharged cases were smear-positive at discharge. Using CASS, six (23%) of 26 home-discharged cases with data available expectorated infectious culture-positive cough aerosols in the respirable range (<5 μm), and most reported inter-person contact with suboptimal protective mask usage. WGS identified 17 (19%) of the 90 patients (with available sequence data) that were discharged home before the diagnosis of 20 downstream cases of extensively drug-resistant tuberculosis with almost identical sequencing profiles suggestive of community-based transmission (five or fewer single nucleotide polymorphisms different and with identical resistance-encoding mutations for 14 drugs). 11 (55%) of these downstream cases had HIV co-infection and ten (50%) had died by the end of the study. 22 (56%) of 39 isolates in patients discharged home after treatment failure were resistant to eight or more drugs. However, five (16%) of 31 isolates were susceptible to rifabutin and more than 90% were likely to be sensitive to linezolid, bedaquiline, and delamanid.

Interpretation More than half of the patients with programmatically incurable tuberculosis were discharged into the community where they remained for an average of 16 months, were at risk of expectorating infectious cough aerosols, and posed a threat of transmission of extensively drug-resistant tuberculosis. Urgent action, including appropriate containment strategies, is needed to address this situation. Access to delamanid, bedaquiline, linezolid, and rifabutin, when appropriate, must be accelerated along with comprehensive drug susceptibility testing.

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Introduction

Resistance to antituberculosis drugs has emerged as a serious and pressing global health concern.[1] During the past decade, widespread resistance to rifampicin and isoniazid (multidrug resistance) has been supplanted by additional resistance to fluoroquinolones and the second-line injectable drugs amikacin, kanamycin, and capreomycin (extensive drug resistance),[2,3] and now resistance beyond extensive drug resistance (known as totally drug-resistant tuberculosis, a non-standardised definition) has emerged.[4] Multidrug-resistant and extensively drug-resistant tuberculosis drive roughly a quarter of global tuberculosis mortality,[1] are unsustainably costly to treat,[5–8] and pose a major threat to health-care workers.[9]

In 2014 there were almost 500 000 cases of multidrug-resistant tuberculosis globally, and 18 734 reported cases of rifampicin-resistant or multidrug-resistant tuberculosis in South Africa (of which roughly 8% were thought to be

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See Comment page 237

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Research in context

Evidence before this study

Reports of programmatically incurable tuberculosis (cases of treatment failure in extensively drug-resistant tuberculosis) have come from countries with a high tuberculosis burden, including China, India, and South Africa. The poor cure rates, high economic cost, and drain on scarce resources of treatment for drug-resistant tuberculosis threatens to destabilise tuberculosis control efforts in affected countries. We searched PubMed for articles published in all languages up to Aug 1, 2016, with the terms "XDR-TB" or "XDR-TB" and "treatment failure" and "outcome". Of 44 articles identified, ten described treatment failure in extensively drug-resistant tuberculosis, two of which concerned the same cohort. Only one study followed up cases of treatment failure to document duration to hospital discharge and post-discharge outcomes including survival. This South African study described a short prospective follow-up of a retrospectively selected cohort and post-discharge outcomes in only 19 cases of treatment failure in extensively drug-resistant tuberculosis. Median survival of patients who had failed treatment from time of discharge was 19·84 months (IQR 4·16–26·04). Infectiousness and transmission dynamics to elicit the magnitude of community-based transmission was not undertaken. No further articles reporting follow-up of treatment failure in extensively drug-resistant tuberculosis were identified. Similarly, a search of PubMed using the terms "XDR" or "MDR" and "TB" and "infectiousness" revealed no studies assessing the infectiousness of individual patients with drug-resistant tuberculosis.

Added value of this study

We describe a large prospective study of clinical outcomes and longevity in home-discharged patients with tuberculosis resistant to first-line and second-line drugs and for whom a curative treatment regimen was not available. More importantly, to our knowledge, our data about respirable aerosols infected with *Mycobacterium tuberculosis* expelled during coughing in this study are the first to describe the infectiousness of individual patients with programmatically incurable tuberculosis. The accompanying whole-genome sequencing data from *M tuberculosis* isolates provides evidence of transmission and post-discharge generation of secondary cases in the community with high mortality. Previous study findings have suggested nosocomial transmission of extensively drug-resistant tuberculosis, but here we provide evidence that highly drug-resistant strains are spreading in the community from home-discharged patients and generating secondary cases with poor outcomes. These data challenge previously held assumptions that highly drug-resistant mycobacteria have reduced fitness and diminished capacity to infect new patients. Our findings also show that, although survival is poor, patients can reside in the community for months or years after discharge home and before death. Evidence presented for regimens likely to be effective include the description of rifabutin-sensitive cases despite resistance to rifampicin.

Implications of all the available evidence

Evidence from this large, prospective study shows that home-discharged patients with infectious, highly drug-resistant tuberculosis survive for considerable periods of time and, more importantly, are a source of transmission, which has serious implications for public health. Previous evidence of nosocomial transmission of extensively drug-resistant tuberculosis, such as the outbreak associated with an HIV clinic in Tugela Ferry, demonstrate the crucial importance of infection control. Our findings highlight an urgent need to interrupt transmission in communities. The increasing incidence of extensively drug-resistant tuberculosis reported by WHO and reports of programmatically incurable tuberculosis from countries such as China and India indicate that such patients are not unique to South Africa. Investment is urgently needed to halt the rise of programmatically incurable tuberculosis that risks taking us back to the pre-antibiotic era. Cure rates are enhanced by use of the new and repurposed drugs (bedaquiline, delamanid, and linezolid) but are hampered by their lack of availability in many countries. Poor access to drug susceptibility tests and empirical treatment of drug-resistant disease risks amplification of resistance when a curative regimen might no longer be possible. Thus, the available evidence indicates an urgent need to rollout new drugs and drug resistance testing, reduce opportunities for transmission, and facilitate care for individuals with highly resistant and incurable tuberculosis when discharged from hospital.

extensively or totally drug resistant).[10,11] Further resistance to the drugs used to treat extensively drug-resistant disease has resulted in patients for whom a treatment regimen cannot be constructed with the drugs available in the national programme and who thus have programmatically untreatable tuberculosis.[4,10,12,13] Estimates from Africa, Russia, India, and China suggest that treatment fails to cure 30–75% of patients with extensively or totally drug-resistant tuberculosis.[14–18] New drugs such as bedaquiline,[19,20] and repurposed ones such as linezolid,[21–23] have become available in some settings. However, in many countries access is severely limited and the inability to construct an effective regimen using at least four effective drugs (despite the availability of newer agents) has rendered many patients impossible to treat effectively. Patients in whom treatment has failed are often discharged to home care in the community.[4] The epidemiology and long-term outcomes beyond 2 years for these patients have not been comprehensively and prospectively studied, and potentially effective drug combinations for such cases of treatment failure remains unclear. Furthermore, the capacity of these patients to transmit disease is not known, and although

the transmission of drug-resistant bacteria is thought to be attenuated compared with susceptible bacteria,[24] the detection of drug-resistant tuberculosis has increased in several high-burden countries, and serious epidemics are unfolding in others including South Africa, India, Russia, and several countries in eastern Europe and central Asia.[25]

To interrogate these questions, we did a long-term prospective study in patients with extensively drug-resistant tuberculosis who received treatment regimens based on aminosalicylic acid, capreomycin, or both according to prevailing national guidelines at the time. We postulated that a substantial proportion of home-discharged patients with extensively drug-resistant tuberculosis that had not responded to treatment would remain infectious and generate secondary cases of extensively drug-resistant tuberculosis in the community. We thus undertook whole-genome sequencing of baseline *Mycobacterium tuberculosis* culture isolates and did cough aerosol sampling to quantify the infectiousness of individual patients.

Methods

Study design and participants

Between Oct 1, 2008, and Oct 31, 2012, we recruited all adult patients with microbiologically confirmed, extensively drug-resistant tuberculosis (defined as resistance to at least rifampicin, isoniazid, a fluoroquinolone, and a second-line injectable) who were admitted to two South African treatment facilities (Brooklyn Chest Hospital, Western Cape, and Harry Surtie [previously Gordonia] Hospital, Northern Cape; figure 1A). Both hospitals are designated specialist referral centres for the treatment of all patients diagnosed with extensively drug-resistant tuberculosis within their province. All patients with extensively drug-resistant tuberculosis who initiated treatment were admitted to the designated hospitals during the study period and received a treatment regimen based on aminosalicylic acid, capreomycin, or both according to prevailing national guidelines at the time (linezolid and bedaquiline were not available within the tuberculosis programme). Patients were followed up until Oct 31, 2014 (the study censor date), or death. Patients were reviewed in hospital and data was captured on a quarterly basis by a trained researcher using a standardised case record form. Discharged patients were contacted by telephone and visited at their homes, and treatment in the continuation phase was directly observed. Sputum samples were collected monthly for smear microscopy and culture. Further laboratory and clinical investigations were done only on patients or samples from Brooklyn Chest Hospital in the Western Cape (n=204) and were dependent on the availability of samples. This included extended drug susceptibility testing to 18 drugs (rifampicin, isoniazid, amikacin, kanamycin, streptomycin, cycloserine, ethionomide, ethambutol, moxifloxacin, ofloxacin and aminosalicylic acid, capreomycin, dapsone, clarithromycin, clofazimine, linezolid, pyrazinamide, and rifabutin; n=179), next-generation whole-genome sequencing (n=153), and cough aerosol sampling (n=26; figure 1B). Thus, further investigations were not undertaken on patients or samples from the Harry Surtie Hospital in the Northern Cape.

Ethical approval was obtained from the University of Cape Town Human Research Ethics Committee and participants provided informed written consent.

Procedures

A 5-year study period, including recruitment and follow-up, was selected to enable the recruitment of a sizable cohort and to allow for the assignment of programmatic treatment outcomes. Treatment duration for extensively drug-resistant tuberculosis is 24 months, and therefore at least 2 years of follow-up are required. We routinely recorded death during follow-up (table, figure 2). We additionally recorded programmatic treatment outcomes at 24 months and 60 months, including death, as outlined in the WHO definitions and reporting framework (adapted when appropriate; appendix p 10).[26] We also assessed the proportion of patients who were cured, completed treatment, had treatment failure, died while on treatment, relapsed, defaulted, and had ongoing treatment. In patients who were discharged home, we defined death while on treatment as deaths in patients who were receiving futile treatment (token oral treatment with one or two oral agents [eg, pyrazinamide and ethambutol] was often given for perceived humanitarian reasons by the clinician in charge). Clinical primary disease was defined as any patient with at least one of the following: no previous tuberculosis episode, drug-susceptible tuberculosis cured more than 12 months before the current diagnosis, first empirical treatment for drug-susceptible tuberculosis 9 months or less before the current diagnosis, or pre-extensively drug-resistant tuberculosis (resistance to rifampicin, isoniazid, and either a fluoroquinolone or an aminoglycoside) 6 months or less before the current diagnosis (full definitions are given in the appendix, p 11). Capreomycin was prescribed according to the national guidelines because linezolid and bedaquiline were not available within the national tuberculosis programme at the time. Capreomycin was used even when isolates were phenotypically resistant, due to the paucity of other therapeutic options and the drug's high serum to minimum inhibitory concentration ratio (thus possibly still providing therapeutic benefit). Further details of methodology are presented in the appendix (p 6).

See Online for appendix

Drug susceptibility testing

Phenotypic drug susceptibility testing was done by the National Health Laboratory Service in accordance with the national tuberculosis programme guidelines; included second-line drugs were ofloxacin, amikacin, ethionamide, and (in a subset of patients, at the request of the attending clinician) capreomycin. Additional testing was done on

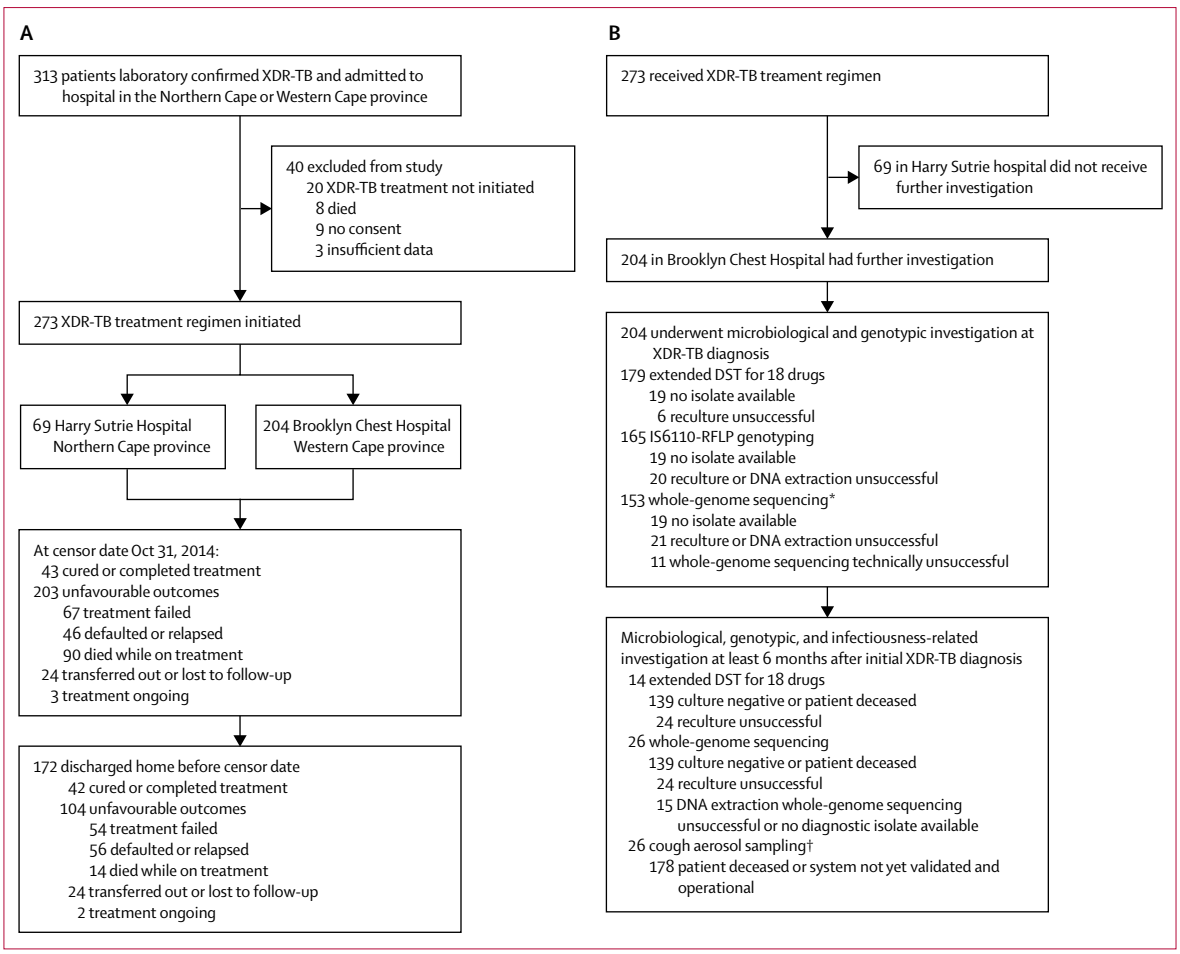


Figure 1: **Study design**
(A) Patient outcomes, assessed in whole cohort. (B) Further investigations, assessed in patients from Brooklyn Chest Hospital only. XDR-TB treatment regimens were based on aminosalicylic acid or capreomycin. XDR-TB=extensively drug-resistant tuberculosis. DST=drug susceptibility testing. RFLP=restriction fragment length polymorphism. SNP=single nucleotide polymorphism. *Four samples failed quality control for genotypic drug resistance analysis; of the remaining 149 readouts, 141 passed quality control for SNP analysis. Of these, 90 were from isolates belonging to home-discharged cases; thus, there were 90 home-discharged cases that had isolates with valid whole-genome sequences. †Cough aerosol sampling was done after diagnosis of extensively drug-resistant tuberculosis, but not necessarily exactly 6 months after diagnosis. Phenotypic drug susceptibility testing was done at the time of cough aerosol sampling in 20 patients. Contact reporting was assessed in 13 patients who underwent cough aerosol sampling.

186 available isolates from Brooklyn Chest Hospital using Sensititre MYCOTB MIC plates (Trek Diagnostic Systems, Cleveland, OH, USA) to establish minimum inhibitory concentrations for rifampicin, isoniazid, amikacin, kanamycin, streptomycin, cycloserine, ethionomide, ethambutol, moxifloxacin, ofloxacin, and aminosalicylic acid. Resistance to capreomycin, dapsone, clarithromycin, clofazimine, linezolid, pyrazinamide, and rifabutin was determined using the BD BACTEC mycobacterial growth indicator tube (MGIT; Becton Dickinson Diagnostic Systems, Sparks, MD, USA; further details are provided in the appendix, p 6). Sensititre or MGIT results were used in analyses if discrepancies were observed compared with programmatic drug susceptibility testing. We did a secondary analysis excluding discrepant patients to rule out possible bias from misclassified samples.

Further investigations were not undertaken in patients from the Northern Cape province because of logistical (inability and lack of approval to transport highly infectious material) and regulatory (provincial and ethical approvals could not be obtained) reasons.

Genome analysis

We used IS*6110* restriction fragment length polymorphism (RFLP) fingerprinting to assess available *M tuberculosis* MGIT cultures from the sputum of patients from Brooklyn Chest Hospital (n=165).[27] We did whole-genome sequencing on extracted DNA (n=153; more details are provided in the appendix, p 7) using Illumina HiSeq2000 technology (available at European Nucleotide Archive with accession PRJEB14199).[28] The data processing pipeline that we used has been described previously.[29] We mapped

raw sequence data uniquely to the corrected H37Rv reference genome (Genbank accession AL123456.3) using bwa-mem (v0.7) software. We used SAMtools (v1.2) and GATK (v3.3-0) to call single nucleotide polymorphisms (SNPs) and indels. Variants with quality scores of Q30 or higher, equating to one error per 1000 base positions, were selected for further analysis. Genotypes were called in positions of ten-fold coverage; otherwise positions were classified as missing. Highly repetitive and variable regions were removed by calculating mappability values along the reference genome using a k-mer length of 50 base pairs and 0·04% of allowed substitutions while mapping. Isolates with a high proportion of missing SNP positions (>10%) were excluded. Large indels and structural variants were identified with Pindel (version 0.2.4w) and Delly2 (version 0.7.3). Phylogenetic analysis was performed with RAxML tree software (v8) with a general time-reversible model and gamma correction for among-site rate variation using 1000 random bootstrap replicates.

To calculate the number of SNPs differing between isolates, we first removed all mutations associated with drug resistance, then clustered isolates by lineage, and filtered out isolates with a proportion of missing calls greater than 10%. Positions where any isolate in a cluster had a missing call were removed and the number of SNPs differing between two isolates were calculated. Finally, we compared mutations associated with drug resistance between isolates. This process was repeated within each cluster until no further separation was observed. We used a threshold of five or fewer SNP differences and identical markers of drug resistance in genes encoding for 14 drugs (amikacin, capreomycin, ethambutol, ethionamide, fluoroquinolones, isoniazid, kanamycin, aminosalicylic acid, pyrazinamide, rifampicin, streptomycin, linezolid, bedaquiline, clofazimine) when assigning transmission events.[30,31] Phylogenetic trees and interaction networks were created using the R packages ape (v 3.3) and qgraph (v1.3.1), respectively.

Genotypic drug resistance

Samples with inadequate sequence coverage of the genes responsible for resistance to rifampicin and isoniazid were removed from the analysis. Variants were analysed against known resistance markers and resistance-conferring genes for each of the major antituberculosis drugs[32] and the newer and repurposed drugs (bedaquiline, clarithromycin, delamanid, linezolid, pretomanid [PA-824; Global Alliance for TB Drug Development, New York, NY, USA], rifabutin, SQ109 [Sequella, Rockville, MD, USA], sutezolid [PNU-100480; Sequella], and TBA-354 [Global Alliance for TB Drug Development]; appendix p 7). Pyrazinamide resistance included all variants in *pncA* except those reported not to cause resistance.[33] Isolates with less than ten-depth coverage or a low confidence structural variant at a resistant marker and no positive resistant marker for the drug under investigation were excluded. For rifabutin, mutations at codon 516* (Asp526Thr/Ser/Val) in *rpoB* (Rv0667) were removed because they do not increase the minimum inhibitory concentration of rifabutin to above the critical threshold of 0·5 µg/mL.[34] Similarly, the naturally occurring variant Arg409Gln in Rv1979c (clofazimine resistance gene) was excluded.

Cough aerosol sampling

The cough aerosol sampling system is a newly validated technology; its readings positively correlate with tuberculin

	Alive	Deceased	Total	p value for mortality*
All patients with extensively drug-resistant tuberculosis				
Number of patients	87 (32%)	186 (68%)	273	..
Sex				0·90
Men	50 (57%)	104 (56%)	154 (56%)	..
Women	37 (43%)	82 (44%)	119 (44%)	..
Race				0·19
Mixed ancestry	44 (51%)	109 (59%)	153 (56%)	..
Black	43 (49%)	77 (41%)	120 (44%)	..
Age at diagnosis, years	32·62 (26·51–38·29)	35·39 (26·83–44·19)	34·27 (26·52–42·88)	0·10
HIV infected	37 (43%)	82/185 (44%)	119/272 (44%)	0·93
CD4 count, cells per µL	207·5 (70–342·3)	199·5 (89–315)	199·5 (85·8–323)	0·68
Patients with HIV receiving antiretroviral therapy	34 (39%)	74 (40%)	108 (40%)	1·00
Weight at XDR-TB diagnosis ≤50 kg	29/85 (34%)	103/185 (56%)	132/270 (49%)	<0·0001
Number of drugs in XDR-TB tuberculosis regimen	9 (8–10)	9 (8–10)	9 (8–10)	0·11
Follow-up since XDR-TB diagnosis, months	25·5 (18·9–29·4)	15·3 (7·3–26·4)	20·3 (9·6–27·8)	<0·0001
Primary XDR-TB†	32 (37)	59 (32)	91 (33)	0·41
Patients discharged home				
Total	83 (48%)	89 (52%)	172	..
Sex				0·60
Men	48 (58%)	56 (63%)	104 (60%)	..
Women	35 (42%)	33 (37%)	68 (40%)	..
Race				0·10
Mixed ancestry	41 (49%)	56 (63%)	97 (56%)	..
Black	42 (51%)	33 (37%)	75 (44%)	..
HIV-infected	35 (41%)	35 (39%)	70 (41%)	0·82
CD4 count, cells per µL	243 (104·0–330·5)	184 (68·0–326·2)	217 (85·0–335·0)	0·55
Positive smear status at XDR-TB diagnosis	25/69 (36%)	30/81 (37%)	55/150 (37%)	1·00
Weight at XDR-TB diagnosis ≤50 kg	27/81 (34%)	43/89 (48%)	70/170 (41%)	0·068
Number of drugs in XDR-TB regimen	9 (8, 10)	9 (8, 10)	9 (8, 10)	0·89

Table shows data for patients with XDR-TB diagnosed between Oct 1, 2008, and Oct 31, 2012, stratified by mortality status by the end of the study. Data are n (%) or median (IQR). Denominators are given when they do not match the total sample size (as a result of missing data). XDR-TB=extensively drug-resistant tuberculosis. *Difference between alive and deceased groups. †For definition refer to appendix.

Table: **Demographic characteristics**

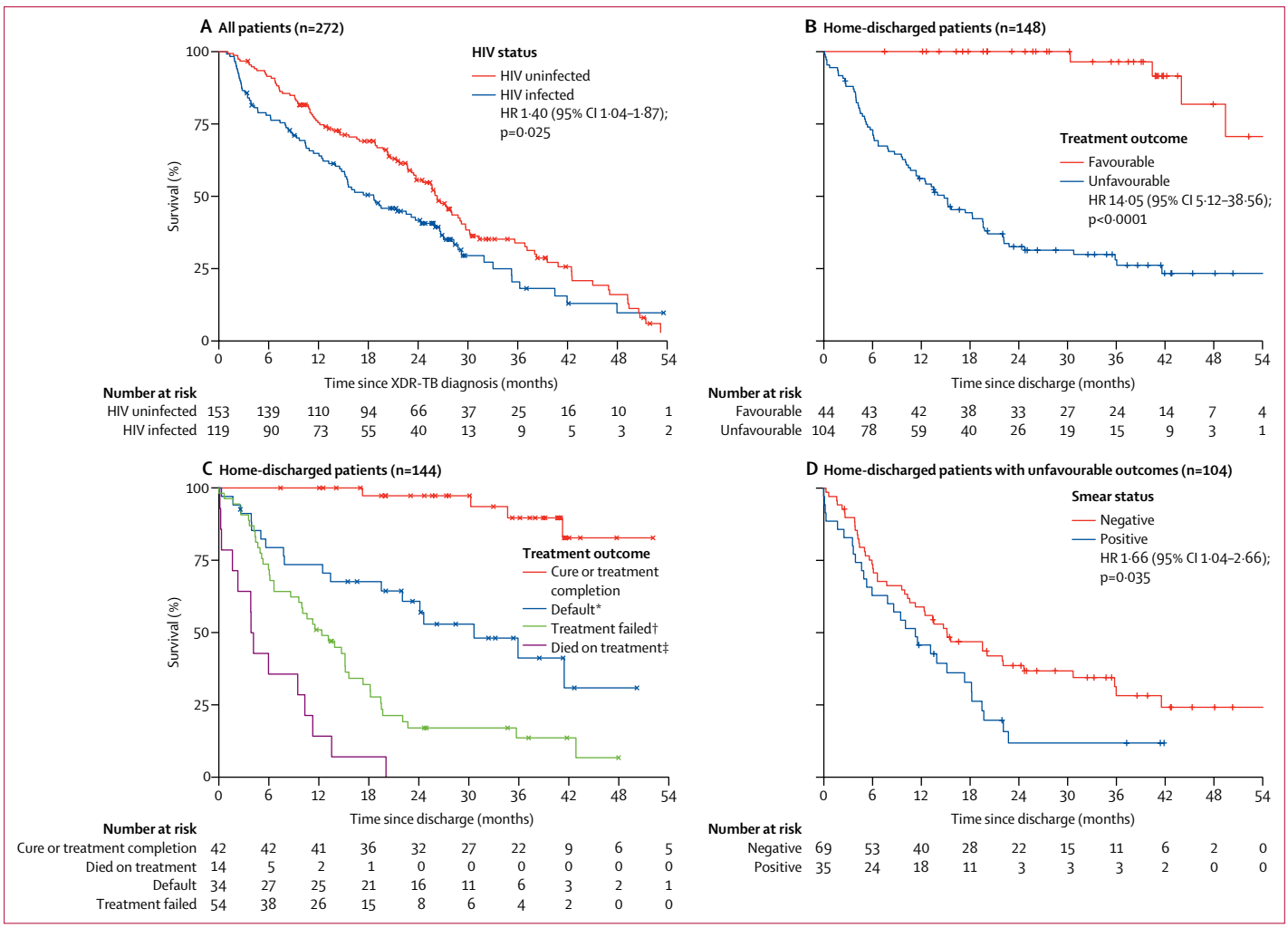


Figure 2: **Kaplan-Meier survival estimates for probability of survival**

(A) Survival since diagnosis in all patients with extensively drug-resistant tuberculosis, stratified by HIV status (n=272; one patient refused testing and was not included in this analysis). (B) Survival since discharge from hospital for home-discharged patients stratified by favourable *vs* unfavourable outcomes (n=148; 172 patients were discharged home but patients who were lost to follow-up and transferred out were not included in this analysis). Unfavourable outcomes are divided into treatment default, treatment failure with no further therapy (death while on no treatment), and treatment failure with death while receiving ineffective treatment after discharge. (C) Survival since discharge from hospital for home-discharged patients by type of unfavourable outcome (treatment failed and not on therapy, defaulted and not on therapy, or death while on treatment; n=144 [two patients relapsed and two patients had ongoing treatment, so were not included in this analysis]). HRs were calculated *vs* a favourable outcome (ie, cure or treatment completion; n=42). (D) Survival since discharge from hospital in home-discharged patients with unfavourable outcomes stratified by smear status at discharge (n=104). HR=hazard ratio. XDR-TB=extensively drug-resistant tuberculosis. *HR 6·82 (95% CI 2·29–20·31); p=0·00056. †HR 18·59 (95% CI 6·61–52·33); p<0·0001. ‡HR 52·54 (95% CI 16·75–164·982); p<0·0001.

skin test conversion and incident tuberculosis in household contacts of index patients with active tuberculosis.[35] A cough aerosol sampling system was installed at Brooklyn Chest Hospital towards the end of the study (February, 2013), and was thus only done on a subset of 26 patients admitted to Brooklyn Chest Hospital. The system enumerates culturable, respirable cough aerosol particles (<5 µm; able to reach the alveolar compartment) to assess the infectiousness of individual patients.[36–38] Briefly, patients were asked to cough into a sterile chamber containing a six-stage Andersen cascade impactor (Thermo Scientific, Rockford, IL, USA) that collects airborne particles of known size distribution (appendix p 8). Patients coughed as frequently as was comfortable for two 5 min sessions, separated by a rest of approximately 5 min. Cough aerosol particles were deposited on 7H11 media (BD Biosciences, San Jose, CA, USA), supplemented with Mycobacteria Selectatab (Kirchner Mast Group, Merseyside, UK). Colony-forming units of *M tuberculosis* from droplets in the respirable range were recorded after incubation at 37°C. Sputum specimens collected during the cough aerosol sampling system visit were processed for smear microscopy, culture, and phenotypic drug susceptibility testing. After sampling, patients were provided with surgical facemasks and requested to report mask usage and contact with other individuals.

Statistical analysis

We calculated medians and IQRs for continuous variables and frequency (percent) for categorical variables. We compared continuous variables with Wilcoxon rank sum tests and categorical variables with χ^2 tests. Kaplan-Meier curves were estimated for the probability of survival either from date of diagnosis or date of discharge. End of follow-up was date of death, date of loss to follow-up, or censor date. Comparisons between strata (eg, HIV-infected *vs* HIV-uninfected individuals) were made by the log-rank test. Univariate Cox proportional hazards models were used to estimate the relation between explanatory variables and time-to-event outcomes. We included the variables of HIV status, antiretroviral therapy status, sex, race, treatment outcome (favourable or unfavourable), *M tuberculosis* lineage, smoking, education, weight at diagnosis, age at diagnosis, number of drugs prescribed, history of tuberculosis, and history of multidrug-resistant tuberculosis, and a binary variable for each of the drugs prescribed. Multivariate Cox proportional hazards models for mortality included variables that were significantly associated with outcome ($p<0·1$) and the prespecified variables (sex, race, education, HIV status). We used a 90% threshold so that we would not exclude variables that, although not significant at an alpha of 0·05 in univariate analysis, might be significant in a multivariate analysis. The underlying proportional hazards assumption was assessed using the (weighted) scaled Schoenfeld residuals test. We regarded p values as statistically significant at a nominal value of $p<0·05$, but in view of the large number of exploratory outcomes significance should not be taken as confirmatory. Thus, patients were included in the analysis that were still in the study at the time the measurement under investigation was taken, and for whom a result was available, even if they were later lost to follow-up. Statistical analysis was done in in R (version 3·0) and graphics generated with the package ggplot2.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. KD, JDL, EP, and RM had full access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

273 patients, 204 (75%) from Brooklyn Chest Hospital and 69 (25%) from Harry Surtie Hospital, were recruited into the study. Patient demographics are summarised in the table and the appendix (p 15). 119 (44%) patients had HIV, with a median CD4 cell count of 199·5 cells per µL (IQR 85·8–323·0). All but five patients with HIV were on antiretroviral therapy. We noted clinically defined, primary tuberculosis with extensive drug-resistance in 91 (33%) patients.

The median duration of follow-up was 20·3 months (IQR 9·6–27·8). At censor date, 186 (68%) patients had died (table). 203 (74%) patients had unfavourable treatment outcomes (treatment failure, treatment default, relapse, or death while on treatment; appendix p 16), and thus programmatically incurable tuberculosis. Multivariate analysis showed that bodyweight of 50 kg or more ($p<0·0001$), increased time to culture positivity ($p<0·0001$), increased age at diagnosis ($p=0·004$), and treatment with aminosalicylic acid ($p=0·001$) were associated with survival (appendix p 19).

172 (63%) patients were home discharged from hospital into the community; patient outcomes are summarised in the appendix (p 25). 53 (31%) of these patients had died in the first 12 months after discharge and 89 (52%) had died by the end of study. Unfavourable outcomes occurred in 104 (60%) of home-discharged patients (appendix p 25) with treatment failing in 54 (31%); 43 (80%) of these patients later died. Their median time to death after home-discharge was 9·9 months (IQR 4·2–17·4). Positive sputum smear results were recorded for 35 (20%) of home-discharged patients a median of 21·0 days (IQR 10·5–36·0) from discharge, and were associated with reduced longevity (figure 2).

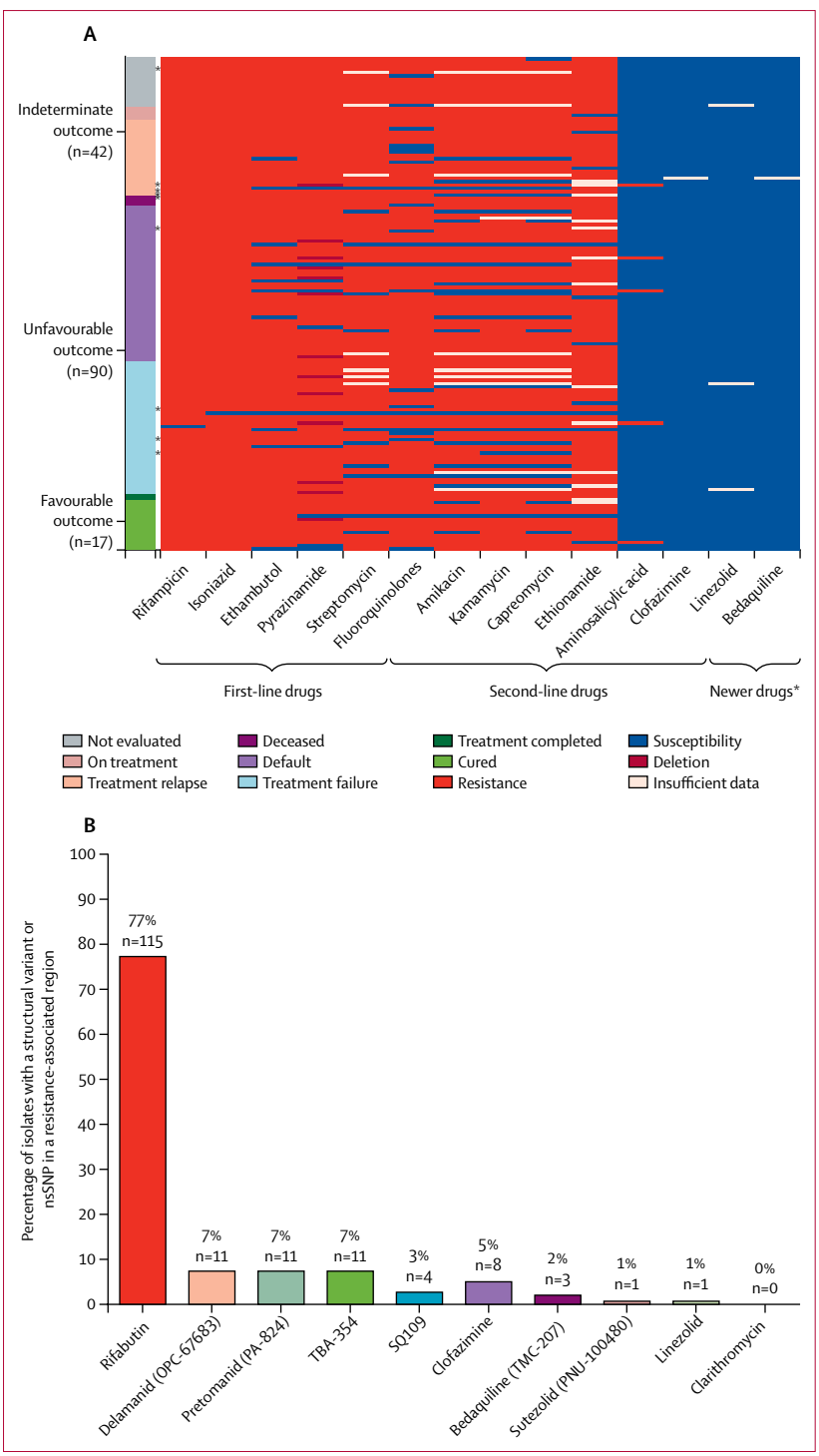
We noted high levels of drug resistance for first-line and second-line drugs (figure 3, appendix p 13). For logistical and regulatory reasons, phenotypic drug susceptibility testing to 18 antituberculosis drugs was done only on patient isolates from Brooklyn Chest Hospital, accounting for 179 (66%) of the total cohort (figure 1B). 135 (75%) patients were resistant to eight or more drugs, 77 (43%) were resistant to ten or more drugs, and four (2%) were resistant to a maximum of 13 drugs.

Whole-genome sequencing of the 149 isolates that passed quality control for genotypic drug resistance analysis showed few mutations associated with resistance to new, novel, and repurposed drugs (figure 3, appendix p 20). One isolate had an Ser68Asn mutation in the *Rv0678* gene, thought to be likely predictive of bedaquiline resistance.[39]

SNP analysis suggested susceptibility to rifabutin in 34 (23%) of the 149 extensively drug-resistant tuberculosis isolates sequenced; confirmatory phenotypic drug susceptibility testing was done with the MGIT 960 system and 32 (97%) of 34 isolates were rifabutin sensitive at a critical concentration of 0·5 µg/ml. Of the 31 home-discharge cases with whole-genome sequencing data, five (16%) were confirmed as rifabutin-sensitive. Importantly, 19 of the confirmed rifabutin-sensitive cases were in patients with unfavourable treatment outcomes (11 were home discharged and living in the community for a mean of 21·0 months [SD 14·2]).

Phenotypic analysis of sequential isolates collected at least 6 months after the initial diagnostic isolate showed that one (3%) of 34 isolates gained resistance to clofazimine (the patient did not receive clofazimine), six (21%) of 29 isolates gained resistance to dapsone (two

of the six patients had received dapsone), and four (67%) of six isolates gained resistance to capreomycin (all four patients received capreomycin; appendix p 21). Of the 26 sequential isolate pairs examined by whole-genome sequencing, three (50%) of six patients who received capreomycin gained drug-specific resistance mutations (appendix p 22).



Whole-genome sequencing was performed on 153 diagnostic patient isolates from Brooklyn Chest Hospital in the Western Cape (figure 1). Genotyping these isolates revealed lineage 2 (Beijing) strains to be dominant (appendix p 23). Genome sequences from 141 isolates passed quality control measures for SNP analysis. 16 (11%) of these isolates had evidence suggestive of hetero-resistance (both wildtype and drug-resistance-conferring mutations). 11 had evidence of mixed infection (ie, more than one strain of *M tuberculosis*), two of which had evidence of heteroresistance.

Using longitudinal samples from our cohort, we calculated the median number of SNPs accumulated per isolate genome per year as 0·99 (95% CI 0·79–1·67), which translates into a five-SNP change (using the lower confidence limit) occurring over 38 months, compared with a median diagnostic time difference between any two patients with extensively drug-resistant tuberculosis of 14·1 months (IQR 6·4–23·3).

Interpatient comparisons showed substantial similarity between isolates. The proportion of isolates differing by one or fewer, two or fewer, or five or fewer SNPs from at least one other patient isolate while having identical genotypic drug resistance profiles were 25 (18%) of 141, 41 (29%) of 141, and 65 (46%) of 141, respectively (appendix p 24). 46 (18%) of 273 patients self-reported exposure to tuberculosis before their diagnosis and 12 (4%) self-reported exposure to individuals with known extensively drug-resistant tuberculosis. Whole-genome sequencing or IS*6110*-RFLP data were available for six patient–contact pairs (appendix p 25). Whole-genome sequencing and RFLP data suggested transmission (five or fewer SNPs different, identical IS*6110*-RFLP patterns) in four of five cases with data available; in one case for which whole-genome sequencing data were unavailable, we noted identical IS*6110*-RFLP patterns (appendix p 25).

Isolates from 37 (41%) of the 90 home-discharged patients from Brooklyn Chest Hospital with whole-genome sequencing data available were highly similar and clustered (five or fewer SNPs different and with identical resistance-encoding mutations for 14 drugs; figure 4). The median time spent by these patients in the community after

Figure 3: **Genome-based analysis of polymorphisms predictive of resistance to antituberculosis drugs**

(A) Heat map depicting the genotypic resistance patterns of 149 *Mycobacterium tuberculosis* isolates obtained at diagnosis of extensively drug-resistant tuberculosis. Each row represents a single patient. The isolates are stratified according to treatment outcome. Newer drugs were not used in the treatment of these patients. (B) Proportion of the 149 isolates with resistance-associated mutations for a specific newer or repurposed antituberculosis drug (at least one nsSNP or structural variant in any gene related to the mechanism of action of the drug). Genes analysed are listed in the appendix (p 12). 60 (58%) of 104 isolates were from patients discharged home; of those, 3/60 (5%) were resistant to bedaquiline, 3/60 (5%) to delamanid, 7/60 (12%) to clofazimine, and 47/60 (78%) to rifabutin. ns=non-synonymous. SNP=single nucleotide polymorphism. *Pre-extensively drug-resistant tuberculosis isolates from patient taken within 3 months of a subsequent extensively drug-resistant tuberculosis diagnosis.

discharge was 15·2 months (IQR 8·7–33·3), and 20 remained living in the community at the censor date. 17 (19%) patients were discharged home and living in the community before the diagnosis of 20 patients with extensively drug-resistant tuberculosis and highly similar genomic sequences (ie, ≤5 SNPs different, and identical resistance-encoding mutations for 14 drugs), suggesting community-based transmission. The median time between home discharge and diagnosis of a secondary case was 9·2 months (IQR 5·4–9·7), and 11 (55%) of the 20 possible secondary cases were HIV-infected. 12 (60%) of these secondary cases had unfavourable outcomes and ten (50%) had died by the end of the study.

The cough aerosol sampling technology became available only towards the end of the study period. 26 smear-positive individuals underwent cough aerosol sampling system to assess their degree of infectiousness. One (4%) patient of 26 was cured, with the remaining 25 (96%) patients having unfavourable outcomes. 22 (85%) patients were home discharged with 11 (42%) living in the community at the time of testing. Six (23%) had positive cough aerosol cultures in the less than 5 µm respirable range and thus were highly infectious (appendix p 27). All six had an unfavourable outcome (four with treatment failure) and five of these six (19%) were discharged home. One secondary case thought to have arisen from a home-discharged patient was cough aerosol sampling system-positive. Phenotypic drug susceptibility testing showed resistance to an average of six of 11 drugs in 20 (77%) sputum culture isolates acquired at the time of cough aerosol sampling system (six isolates were culture negative or contaminated). 13 patients who underwent cough aerosol sampling self-reported contact with other individuals, with a median of five contacts per day

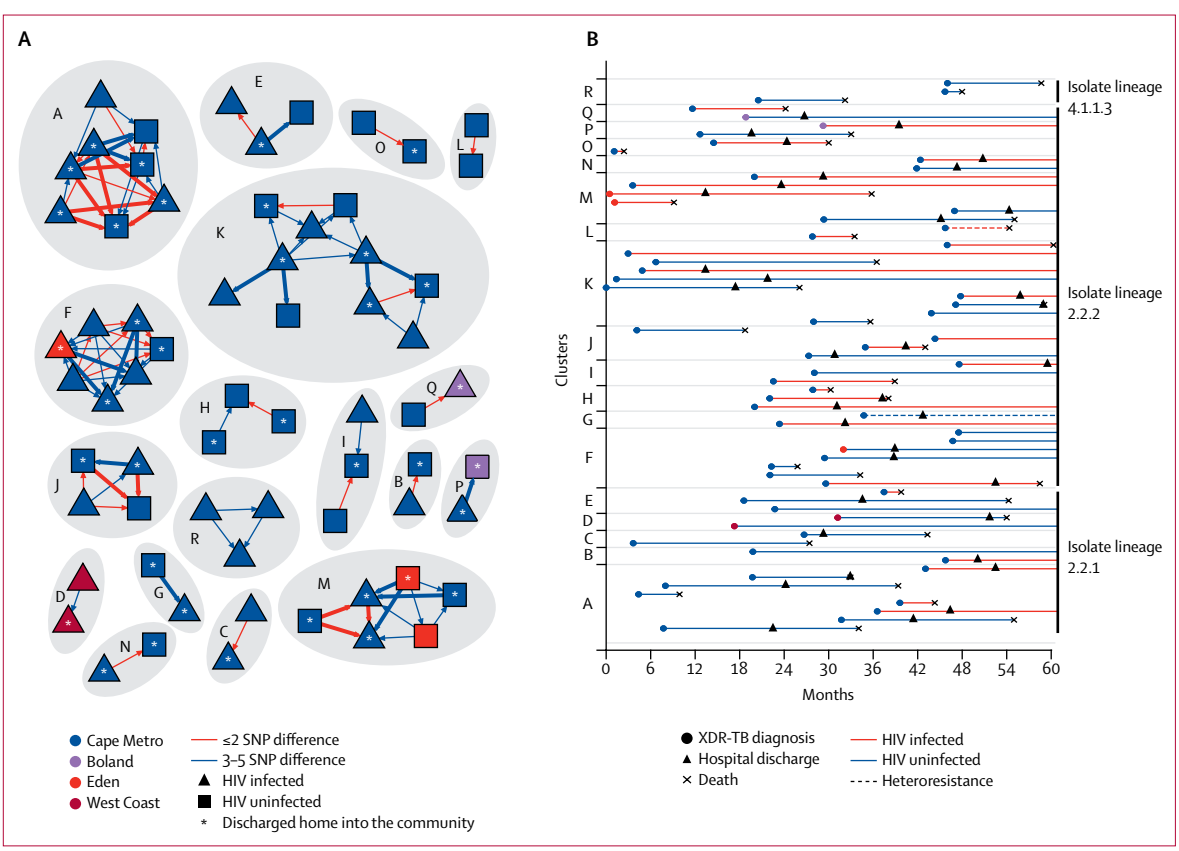


Figure 4: **Molecular epidemiology and transmission dynamics of extensively drug-resistant tuberculosis**
(A) An interaction network showing the linkage of isolates with two or fewer SNPs difference, and three to five SNPs difference, and matching drug resistance markers. Asterisks indicate patients who were discharged home into the community. Arrows originate on the patient with the earliest XDR-TB diagnosis date; geographical origin of the patient refers to the district of residence at the time of diagnosis. 65 (46%) of 141 isolates are represented in 18 clusters, suggestive of transmission. The thick lines represent a patient discharged home before the diagnosis of subsequent extensively drug-resistant tuberculosis case with an almost identical genetic profile (the head of the arrow identifies the downstream case). Thus, 17 (19%) of 90 home-discharged patients probably caused a secondary case of extensively drug-resistant tuberculosis. (B) Timelines for patients with clustered isolates of five or fewer SNPs difference and matching drug resistance markers. The median duration from diagnosis of extensively drug-resistant tuberculosis to end of follow-up was 20·3 months (IQR 9·6–27·8). The y-axis indicates clustered isolates with five or fewer SNP differences as in part (A). IS*6110* restriction fragment length polymorphism patterns were within one band for all isolates in the respective clusters. 72% (47/65) of isolates remained when we assumed a closed network for interaction ≤5 and remove one isolate per node, indicating likely primary transmission in 33% (47/141) of patients. A map of districts is provided in the appendix (p 28). XDR-TB=extensively drug-resistant tuberculosis. SNP=single nucleotide polymorphism.

(IQR 3·2–7·3) and a face mask not being worn for 458/610 (75%) of these interactions (appendix p 27). RFLP data were available for three contact pairs (appendix p 27) and were suggestive of transmission in each case.

Discussion

To our knowledge, this study is the first prospective long-term report on the outcomes and infectiousness of patients with extensively drug-resistant tuberculosis in whom treatment had failed, and hence in patients with programmatically incurable tuberculosis. Overall, unfavourable outcomes occurred in 203 (74%) patients, with 67 (33%) of these individuals having failed treatment. 172 (63%) of all 273 patients in our study were discharged back into the community and 104 (60%) of these 172 patients had an unfavourable outcome. Although such patients are perceived to be terminally ill, in reality the proportion of patients who had survived at 12 months after discharge was 21% (57 of 273). Continued treatment was a post-discharge predictor of mortality, although we believe this finding to be an analytical artifact due to selection bias (with those who were the sickest and with most extensive disease being more likely to receive treatment).

Our data demonstrate that uncured survivors with extensively drug-resistant tuberculosis have considerable longevity in the community, not dissimilar to the pre-chemotherapeutic era when time to death occurred over years and roughly 20–30% of patients had chronic active disease.[40] By contrast, we previously reported an overall survival rate of about 70% in home-discharged patients in whom treatment had failed in a retrospectively selected follow-up cohort, possibly reflecting selection and survival bias in that study.[14] High rates of treatment failure in extensively drug-resistant tuberculosis have been described in several tuberculosis-endemic countries,[14–16,18] and substantial patient longevity and transmission from home-discharged patients within the community is likely to be occurring in such settings (although this has not yet been formally reported). In this study, both individuals with HIV infection and those without HIV infection were affected; multicentre studies are required to assess the global scale of this problem.

As demonstrated by cough aerosol sampling, six (23%) home-discharged patients assessed were highly infectious and a substantial proportion were smear positive. Culture-positive cough aerosols in the respirable range are the best predictor of recent tuberculosis infection (tuberculin skin test conversion), and subsequent development of active tuberculosis in household contacts,[35,37] although this method has limited scalability and potential as a public health intervention in view of its technical complexity, cost, time to result (6–8 weeks), and lack of portability (large, non-mobile equipment). We also noted high interperson contact scores and suboptimal mask usage among home-discharged patients.

We previously documented possible transmission from a single patient discharged home after treatment failure, causing a secondary case of extensively drug-resistant tuberculosis, in a retrospectively selected follow-up cohort.[14] Here, we demonstrate that 17 (19%) home-discharged patients with extensively drug-resistant tuberculosis might have caused a secondary case. One of the prerequisites for inferring such transmission was the absence of concurrent treatment at the same health-care facility (ie, the index cases of extensively drug-resistant tuberculosis were in the community before diagnosis of the secondary cases). This figure could be an underestimate, because it includes only secondary cases with development of active disease during the study period. Extended follow-up would be required to assess latently infected individuals who might progress to disease at a later date. Thus, transmission of extensively drug-resistant tuberculosis from programmatically incurable patients discharged home is not uncommon. The mortality rate in secondary cases was substantial (50%), and we reiterate the urgent need to establish community-based containment strategies. These strategies should include voluntary long-term community stay facilities and palliative care;[41] few such amenities exist in tuberculosis-endemic settings.

We did whole-genome sequencing (using a threshold of five SNPs and matching drug-resistance markers) to study the molecular epidemiology of tuberculosis transmission. Although there are some drawbacks with the use of whole-genome sequencing to infer person-to-person transmission, it is a well accepted approximation.[30,31] Investigators of previous studies have justified the use of a five-SNP threshold, showing its high predictive value to infer transmission.[42,43] However, any threshold needs to be context-specific and related to mutation rate. Previously reported *M tuberculosis* mutation rates have varied between roughly 0·25 and 0·75 SNPs per genome per year in longitudinally sampled isolates from patients who were on antituberculosis treatment.[30] The median number of SNPs accumulated per isolate genome per year, 0·99 (95 CI 0·79–1·67), translates into a five-SNP change (using the lower confidence limit) occurring over 38 months; this figure is considerably more than the median duration of the diagnostic time difference between any two patients with extensively drug-resistant tuberculosis in our study of 14·1 months (IQR 6·4–23·3). Thus, although the use of SNP thresholds to infer resistance is contentious, we chose a conservative threshold of five SNPs, and additionally required that isolates had identical mutations in all resistance-encoding genes. When we omitted the requirement for identical resistance mutations, 100 (71%) of 141 isolates shared five or fewer SNP differences with at least one other isolate, compared with 65 (46%) of 141 isolates when this requirement was included in the analysis. Our estimates are therefore likely to be robust and have predictive value for inferring transmission.

We present evidence of transmission of extensively drug-resistant tuberculosis in that 65 (46%) isolates had

negligible genetic differences compared with at least one other patient isolate. This finding suggests that transmission is not uncommon in this setting. This situation is not unique to South Africa; reports from China suggest that almost half of extensively drug-resistant tuberculosis is due to primary transmission.[42] Access to rapid molecular technologies, and newer and repurposed drugs such as bedaquiline, delamanid, and linezolid, are urgently needed in tuberculosis-endemic countries. South Africa has a programme that offers these drugs to a small number of patients, but they are not available to most patients in tuberculosis-endemic countries. Moreover, some individuals are denied access to these agents because of high-grade resistance that precludes the construction of an effective regimen. Although bedaquiline and linezolid are probably good choices to include in regimens against extensively drug-resistant tuberculosis in this population, resistance to the nitroimidazoles is currently being reported, and treatment failures are already emerging. Findings from a 2016 multicentre study of a bedaquiline-based regimen showed that 38% of patients with extensively drug-resistant tuberculosis had not culture converted by 30 months after treatment.[44] Thus, there is and will continue to be a growing population of incurable tuberculosis cases in the community, underscoring the need for appropriate containment facilities to curtail transmission. Strategies to preserve new drugs should include prevention of pharmacokinetic mismatch and measures to ensure adherence.

In this context, a finding worthy of further investigation is the susceptibility to rifabutin of some rifampicin-resistant strains. Although tentative, some data[45] suggest that treatment of multidrug-resistant tuberculosis with rifabutin in appropriate patients improves outcomes compared with the use of conventional regimens. Additionally, more than 90% of isolates did not have mutations associated with resistance to linezolid, bedaquiline, or delamanid. A regimen with these agents could reduce mortality and infectiousness but, although approved for clinical use, these drugs are often inaccessible in tuberculosis-endemic countries; there is urgent need to accelerate rollout to where they are most needed.

There were several limitations to this study. We followed up outcomes in patients with extensively drug-resistant tuberculosis who were admitted to hospital and receiving treatment, thus selection bias and survival bias might have affected the findings. However, during the period of this study local policy was to admit all patients diagnosed with extensively drug-resistant tuberculosis, which would have (if anything) underestimated poor outcomes because patients not diagnosed and thus not placed on treatment were not captured. However, these findings may be less generalisable to settings with low prevalence of HIV co-infection. Indeed, we recruited all the patients in only two provinces of South Africa; in other provinces such as KwaZulu-Natal HIV co-infection rates are considerably higher. Other limitations include missing data due to default and loss to follow-up, and the fact that cause of death was not ascertained. Few patient isolates underwent extended phenotypic drug susceptibility testing and whole-genome sequencing; however, these isolates were only accessible from the Western Cape province, where 91% (186/204) of isolates underwent extended drug susceptibility testing and 75% (153/204) underwent whole-genome sequencing. We were unable to present conclusive geospatial linkages between patients due to logistical constraints given the sheer burden of disease in this setting. Although we did identify clusters of active disease within friend and family groups, we did not comprehensively and actively screen all contacts for disease (which is currently not done within the national tuberculosis programme). However, in patients with demonstrated geospatial linkages, isolate genomes were very similar. Inadequate access to compounds for phenotypic drug susceptibility testing, lack of consensus about cutpoints, and incomplete knowledge of genotypic markers for resistance to the new and repurposed drugs precluded a full assessment of susceptibility to these drugs. Almost half of the patients tested with cough aerosol sampling who were discharged home after treatment failure were highly infectious.

In conclusion, treatment failure of extensively drug-resistant tuberculosis, and hence programmatically incurable tuberculosis, is a nascent but growing phenomenon. Using innovative tools we have demonstrated that substantial numbers of patients with highly drug-resistant and infectious tuberculosis are being discharged into the community after treatment failure and could be generating secondary cases of incurable tuberculosis. Data about these patients are vital to inform resource allocation and interventional strategies to contain this epidemic. Better approaches, including individualised treatment directed by whole-genome sequencing,[46] use of effective regimens containing new and repurposed drugs, prevention of the amplification of resistance through optimal dosing and adherence promotion, and identification of the most infectious patients for transmission interruption strategies are urgently needed.

Contributors

KD conceived, initiated, and obtained funding for the study. KD, RMW, EP, BM, EMS, TD, AMA, FB-R, JS, FAS, GT, and AP facilitated data collection. BM, EMS, TD, AMA, FB-R, JS, and FAS did the laboratory experiments. KD, JDL, EP, JP, ML, GT, TGC, and RMW analysed the data. KD, JDL, EP, ML, RMcN, GT, TGC, and RMW wrote the manuscript. All authors intellectually contributed to the manuscript.

Declaration of interests

We declare no competing interests.

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