Drug susceptibility testing and mortality in patients treated for tuberculosis in high-burden countries: a multicentre cohort study

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Lancet Infect Dis 2019; 19: 298–307

Published Online February 7, 2019 http://dx.doi.org/10.1016/S1473-3099(18)30673-X

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Summary

Background Drug resistance is a challenge for the global control of tuberculosis. We examined mortality in patients with tuberculosis from high-burden countries, according to concordance or discordance of results from drug susceptibility testing done locally and in a reference laboratory.

Methods This multicentre cohort study was done in Côte d'Ivoire, Democratic Republic of the Congo, Kenya, Nigeria, South Africa, Peru, and Thailand. We collected *Mycobacterium tuberculosis* isolates and clinical data from adult patients aged 16 years or older. Patients were stratified by HIV status and tuberculosis drug resistance. Molecular or phenotypic drug susceptibility testing was done locally and at the Swiss National Center for Mycobacteria, Zurich, Switzerland. We examined mortality during treatment according to drug susceptibility test results and treatment adequacy in multivariable logistic regression models adjusting for sex, age, sputum microscopy, and HIV status.

Findings We obtained *M tuberculosis* isolates from 871 patients diagnosed between 2013 and 2016. After exclusion of 237 patients, 634 patients with tuberculosis were included in this analysis; the median age was 33·2 years (IQR 26·9–42·5), 239 (38%) were women, 272 (43%) were HIV-positive, and 69 (11%) patients died. Based on the reference laboratory drug susceptibility test, 394 (62%) strains were pan-susceptible, 45 (7%) monoresistant, 163 (26%) multidrug-resistant (MDR), and 30 (5%) had pre-extensively or extensively drug resistant (pre-XDR or XDR) tuberculosis. Results of reference and local laboratories were concordant for 513 (81%) of 634 patients and discordant for 121 (19%) of 634. Overall, sensitivity to detect any resistance was 90·8% (95% CI 86·5–94·2) and specificity 84·3% (80·3–87·7). Mortality ranged from 6% (20 of 336) in patients with pan-susceptible tuberculosis treated according to WHO guidelines to 57% (eight of 14) in patients with resistant strains who were under-treated. In logistic regression models, compared with concordant drug susceptibility test results, the adjusted odds ratio of death was 7·33 (95% CI 2·70–19·95) for patients with discordant results potentially leading to under-treatment.

Interpretation Inaccurate drug susceptibility testing by comparison with a reference standard leads to under-treatment of drug-resistant tuberculosis and increased mortality. Rapid molecular drug susceptibility test of first-line and second-line drugs at diagnosis is required to improve outcomes in patients with MDR tuberculosis and pre-XDR or XDR tuberculosis.

Funding National Institutes of Allergy and Infectious Diseases, Swiss National Science Foundation, Swiss National Center for Mycobacteria.

Introduction

Tuberculosis is a global public health concern. In 2017, an estimated 10·0 million individuals developed active tuberculosis worldwide, of whom an estimated 0·9 million (920 000; 9%) were HIV positive.[1] Scale-up of combination antiretroviral therapy (ART) has substantially improved the prognosis of HIV-positive patients[2,3] and reduced the incidence of tuberculosis in this population.[4,5] However, the risk of tuberculosis among HIV-positive patients on ART remains four times higher than among HIV-negative patients.[6]

The emergence of multidrug-resistant (MDR) tuberculosis and extensively drug-resistant (XDR) tuberculosis is another threat to the control of this disease.[7–9] In 2017, it was estimated that 3·5% of new patients and 18% (>50% in eastern Europe) of previously treated patients had MDR tuberculosis.[1] Treatment of MDR tuberculosis and XDR tuberculosis is challenging because of the longer treatment duration, adverse effects, and lower efficacy of second-line drugs.[10,11] Strategies to prevent drug-resistant tuberculosis include monitoring of the prevalence of MDR tuberculosis, widespread drug susceptibility testing, and ensuring rapid initiation and completion of full courses of effective treatment regimens.[12,13] Culture-based phenotypic drug susceptibility testing is considered the gold standard, but is time and

Research in context

Evidence before this study

Multidrug-resistant (MDR) tuberculosis and extensively drug-resistant (XDR) tuberculosis are serious threats to WHO's End-TB strategy, because of restricted access to both laboratory tests for rapid identification of drug resistance and appropriate treatment in many countries with a high tuberculosis burden. We searched PubMed for systematic reviews and original research articles published in any language up to March 31, 2018. We combined terms for "tuberculosis", "drug resistance testing", and "mortality". Several individual studies and systematic reviews have documented poor outcomes of MDR tuberculosis and pre-XDR/XDR tuberculosis in high-burden countries. Two Cochrane reviews investigated the accuracy of molecular tests detecting specific mutations associated with resistance, such as the Xpert MTB/RIF, which is recommended by WHO to detect rifampicin resistance directly from sputum.

Added value of this study

To our knowledge, this is the first multicentre cohort study assessing the accuracy of drug susceptibility testing in routine settings in high-burden countries by comparing local drug susceptibility test results with those from a tuberculosis reference laboratory and assessing the impact on mortality. The study shows that the accuracy of local drug susceptibility testing to detect any resistance in high-burden countries was moderate (sensitivity 90·8%, specificity 84·3%). Results from the reference and local laboratories were discordant in about 20% of patients. Mortality during treatment was increased almost two-fold in patients with discordant drug susceptibility test results compared to patients with concordant results. Mortality ranged from 6% in adequately treated patients with pan-susceptible strains to 57% in inadequately treated patients with drug-resistant strains. In multivariable analyses, associations with mortality changed little after adjustment for sex, age, sputum microscopy result, and HIV status. Notably, HIV infection was not associated with mortality during tuberculosis treatment.

Implications of all the available evidence

Drug-resistant tuberculosis is difficult to diagnose and treat, particularly in high-burden settings, where resources are scarce. In these settings, inaccurate drug susceptibility testing leading to inappropriate treatment contributes to the high mortality associated with drug-resistant tuberculosis. Local access to accurate and rapid drug susceptibility testing for first-line and second-line drugs is required to improve outcomes in patients with MDR tuberculosis and pre-XDR or XDR tuberculosis. Whole-genome sequencing is the most promising approach to reach this goal, but much work remains to be done to make this approach feasible and affordable in high-burden countries.

resource intensive, and too slow to influence decisions about starting treatment.[14] Molecular-based resistance testing offers an alternative to culture-based drug susceptibility testing.[15] Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) detects resistance to rifampicin directly from sputum and provides results within 1·5 h,[16] whereas line-probe assays (LPAs) from sputum detect resistance to isoniazid, rifampicin, ethambutol, fluoroquinolones, or second-line injectable drugs (amikacin, capreomycin, or kanamycin) and provide results within 1–2 days.[15]

Laboratories in high-burden settings use different tests and strategies to diagnose MDR tuberculosis, but the accuracy of drug susceptibility testing in routine settings in high-burden countries is unknown. We compared results of resistance testing done locally in ART and tuberculosis programmes in countries with a high tuberculosis burden with results from gold standard phenotypic drug susceptibility testing done in a Swiss reference laboratory, and examined mortality in HIV-positive and HIV-negative patients with tuberculosis who had concordant and discordant test results.

Methods

Study design

This multicentre cohort study is part of a larger research project about the development of drug-resistant *Mycobacterium tuberculosis* in the context of HIV co-infection within the International epidemiology Databases to Evaluate AIDS (IeDEA), a global consortium of ART programmes.[17,18] Isolates and clinical data were collected from patients with tuberculosis in seven high-burden countries in sub-Saharan Africa, Asia, and Latin America. The sample size was calculated so that the study had adequate power to detect differences in the prevalence of drug resistance between HIV-positive and HIV-negative patients.

Local institutional review boards or ethics committees approved the study at all participating sites. The study was also approved by the Cantonal Ethics Committee in Bern, Switzerland. Written informed consent was obtained at all sites, except in Nigeria and South Africa, where no informed consent was required for archived samples.

Data collection

Prospective recruitment of participants took place between January, 2013, and December, 2016. We included adult patients aged 16 years or older who were treated for active pulmonary tuberculosis in Côte d'Ivoire, Democratic Republic of the Congo, Kenya, Nigeria, South Africa, Peru, and Thailand. All seven countries are defined by WHO as countries with a high tuberculosis burden; Democratic Republic of the Congo, Kenya, Nigeria, South Africa, and Thailand also have a high MDR tuberculosis burden and high HIV/tuberculosis burden.[19]

HIV-positive patients with tuberculosis were recruited prospectively from ART clinics participating in IeDEA and HIV-negative patients were recruited prospectively

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See Online for appendix

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from tuberculosis clinics serving the same population. In South Africa, recruited patients came from strain collections held at the University of Cape Town. Sites were asked to contribute pulmonary pre-treatment *M tuberculosis* isolates from 25 or more patients within each of the four strata defined by HIV status (positive or negative) and drug resistance (MDR or pan-susceptible), for a total of 100 patients per site. The appendix summarises the characteristics of participating sites. Patient characteristics were entered online in French or English at baseline, with the Research Electronic Data Capture (REDCap) tool,[20] including site, type of tuberculosis patient as defined by WHO, age, sex, HIV status, CD4 cell count at start of tuberculosis treatment (if HIV positive), sputum smear microscopy result, and risk factors for tuberculosis. Treatment regimens were updated and outcomes entered during follow-up visits within routine care.

Outcomes

Treatment outcomes were defined according to WHO as follows: cured, treatment completed, treatment failure, death, lost to follow-up, transferred to other clinics, ongoing treatment at the time of evaluation, or unknown treatment outcome.[21] Treatment success included cured patients and patients who completed treatment.[21] The main outcome for this study was mortality during tuberculosis treatment. Outcome data received up to March 31, 2018, were included in analyses.

Drug susceptibility testing

Drug susceptibility testing was done locally with liquid or solid cultures or molecular methods: Xpert MTB/RIF or LPAs, such as Genotype MTBDR*plus* or MTBDR*sl* tests (Hain Lifesciences, Nehren, Germany). Drug susceptibility testing at participating clinics was dictated by local guidelines and the availability of tests. The reference laboratory of the Swiss National Center for Mycobacteria, Zurich, Switzerland, did drug susceptibility testing with the Mycobacteria Growth Indicator Tube liquid medium system (MGIT, Becton Dickinson, Franklin Lakes, NJ, USA), with the following drug concentrations: 0·1 mg/L for isoniazid, 1·0 mg/L for rifampicin, 100·0 mg/L for pyrazinamide, 5·0 mg/L for ethambutol, 1·0 mg/L for amikacin, and 0·25 mg/L for moxifloxacin, in line with the critical concentrations published by WHO.[22]

WHO defines monoresistance as resistance to one first-line tuberculosis drug (isoniazid, rifampicin, pyrazinamide, or ethambutol); MDR as resistance to isoniazid and rifampicin; pre-XDR as MDR with additional resistance to any fluoroquinolone or one of the second-line injectable drugs (amikacin, capreomycin, or kanamycin); and XDR as MDR with additional resistance to any fluoroquinolone and at least one of the second-line injectable drugs.[21] The "other drug resistance" category included any other combination. We defined pan-susceptible tuberculosis as no resistance against the six drugs tested at the reference laboratory and any resistance as resistance against at least one of the tested drugs. First-line regimens (standard treatment) included first-line tuberculosis drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) and second-line regimens included a combination of first-line and second-line drugs.[21,23]

Exposure definition and data analysis

We calculated test accuracy statistics for diagnosis of any drug resistance. We further classified comparisons between the phenotypic and molecular drug susceptibility test results obtained in the local laboratories and the reference laboratory as follows: concordant results, discordance potentially leading to under-treatment, discordance potentially leading to over-treatment, and other discordant results. We defined drug regimens received by patients as compatible with WHO guidelines in place during the study period, as under-treatment, or as over-treatment, based on the reference drug susceptibility test results. First-line regimens for pan-susceptible tuberculosis, first-line or second-line regimens prescribed to isoniazid monoresistant patients, and second-line regimens prescribed to rifampicin monoresistant patients, patients with MDR tuberculosis, and patients with pre-XDR or XDR tuberculosis were classified as being in accordance with WHO guidelines. Under-treatment included first-line regimens given to rifampicin monoresistant patients, patients with MDR tuberculosis, and those with pre-XDR or XDR tuberculosis; and over-treatment included second-line regimens given to patients with pan-susceptible tuberculosis. The appendix shows the classification of regimens.

We used descriptive statistics to describe patient characteristics by levels of drug resistance based on drug susceptibility testing done at the reference laboratory and by HIV status. We examined determinants of mortality in multivariable logistic regression models. Patients with unknown or missing treatment outcome, ongoing treatment, missing treatment regimen, missing sputum microscopy, and other drug-resistant tuberculosis were excluded from logistic regression analyses. Logistic regression models were adjusted for age, sex, sputum microscopy result, and HIV status. We stratified models by study site by including an indicator variable for all sites except for South Africa (the reference group). We calculated the population attributable fraction of mortality due to discordant drug susceptibility test results based on the adjusted model as described by Greenland and Drescher.[24]

Other variables, such as smoking history, diabetes, substance abuse, and contact with other patients with tuberculosis worsened the fit of the model. For HIV-positive individuals, models were additionally adjusted for CD4 cell count at the start of tuberculosis treatment. All analyses were done with Stata, version 15.

	Pan-susceptible (n=394)	Any resistance (n=240)	p value	Monoresistance			Polyresistance		
				INH (n=29)	RIF (n=14)	PZA (n=2)	MDR (n=163)	Pre-XDR or XDR (n=30)	Other (n=2)
Sex									
Women	150 (38%)	89 (37%)	0·80	6 (21%)	3 (21%)	0	65 (40%)	14 (47%)	1 (50%)
Men	244 (62%)	151 (63%)	..	23 (79%)	11 (79%)	2 (100%)	98 (60%)	16 (53%)	1 (50%)
Age (years)	34·6 (27·8–44·6)	31·5 (25·3–40·2)	0·003	34·3 (26·5–43·2)	27·1 (24·9–35·5)	26·1 (23·3–28·9)	31·5 (25·4–41·4)	30·3 (24·2–37·5)	27·3 (24·4–30·2)
HIV status									
Negative	200 (51%)	162 (68%)	<0·0001	20 (69%)	8 (57%)	1 (50%)	114 (70%)	18 (60%)	1 (50%)
Positive	194 (49%)	78 (32%)	..	9 (31%)	6 (43%)	1 (50%)	49 (30%)	12 (40%)	1 (50%)
CD4 count at baseline, cells per μL	215 (85–369)	161 (61–369)	0·79	92·5 (55–161)	63·5 (43–81)	43	259 (151–528)	32 (5–105)	213
Number of observations (%)	155 (39%)	45 (19%)	..	6 (21%)	6 (42·9%)	1 (50%)	24 (15%)	7 (23%)	1 (50%)
Treatment regimen									
First line	369 (94%)	46 (19%)	<0·0001	27 (93%)	0	2 (5%)	14 (9%)	2 (7%)	1 (50%)
Second line	25 (6%)	188 (78%)	..	2 (7%)	14 (100%)	0	143 (85%)	28 (93%)	1 (50%)
Unknown	0	6 (3%)	..	0	0	0	6 (6%)	0	0
Treatment outcomes									
Success	287 (73%)	124 (52%)	<0·0001	15 (52%)	7 (50%)	0	88 (54%)	13 (43%)	1 (50%)
Mortality	24 (6%)	45 (19%)	..	7 (24%)	2 (14%)	1 (50%)	24 (15%)	10 (33%)	1 (50%)
Treatment failure	12 (3%)	10 (4%)	..	0	0	1 (50%)	5 (3%)	4 (13%)	0
Lost to follow-up	29 (7%)	30 (13%)	..	1 (3%)	3 (21%)	0	26 (16%)	0	0
Transfer	15 (4%)	14 (6%)	..	0	2 (14%)	0	9 (6%)	3 (10%)	0
Ongoing treatment/ unknown	27 (7%)	17 (7%)	..	6 (21%)	0	0	11 (7%)	0	0
Country									
Côte d'Ivoire	48 (12%)	51 (21%)	<0·0001	3 (10%)	0	0	44 (27%)	4 (13%)	0
Democratic Republic of the Congo	33 (8%)	29 (12%)	..	0	1 (7%)	0	19 (12%)	9 (30%)	0
Kenya	24 (6%)	11 (5%)	..	2 (7%)	1 (7%)	0	8 (5%)	0	0
Nigeria	20 (5%)	36 (15%)	..	1 (3%)	5 (36%)	0	26 (16%)	4 (13%)	0
Peru	66 (17%)	38 (16%)	..	8 (28%)	0	0	27 (17%)	3 (10%)	0
South Africa	130 (33%)	57 (24%)	..	6 (21%)	7 (50%)	1 (50%)	32 (20%)	10 (33%)	1 (50%)
Thailand	73 (19%)	18 (8%)	..	9 (31%)	0	1 (50%)	7 (4%)	0	1 (50%)

Data are n (%) or median (IQR). Analysis based on 634 patients (see appendix). INH=isoniazid. RIF=rifampicin. PZA=pyrazinamide. MDR=multidrug resistant. XDR=extensively drug resistant.

Table 1: **Patient characteristics by phenotypic drug resistance profiles obtained at the Swiss National Center for Mycobacteria**

Role of the funding source

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

Results

We obtained *M tuberculosis* isolates from 871 patients diagnosed between 2013 and 2016. We excluded 237 patients from analyses of the accuracy of drug susceptibility testing, mainly because isolates were contaminated or not viable, and excluded a further 61 patients from analyses of mortality, mainly because treatment was ongoing or outcomes were unknown at the time of closing the database (appendix). Excluded patients were similar in terms of age, sex, HIV status, and site of tuberculosis, but had lower CD4 counts and were more likely to have recurrent tuberculosis and to be on treatment after failure or default (appendix).

634 patients with tuberculosis were included in the analysis; the median age was 33·2 years (IQR 26·9–42·5) and 239 (38%) patients were women (table 1). The reference laboratory identified 394 (62%) pan-susceptible *M tuberculosis* strains, 45 (7%) monoresistant strains, 163 (26%) MDR strains, 30 (5%) pre-XDR or XDR strains, and two (<1%) strains with other drug resistance profiles (appendix). Among the 163 patients with MDR tuberculosis, 85 (52%) had resistance to rifampicin and isoniazid only, whereas the remaining patients were also

	Drug susceptibility test results by laboratory		Test used at local laboratories			
	Reference laboratory (phenotypic)	Local laboratories	Xpert MTB/RIF*	Culture	LPA	Combination of tests
Concordance						
513 (100%)	Total	..	216 (100%)	154 (100%)	11 (100%)	73 (100%)
332 (65%)	Pan-susceptible	Pan-susceptible	167 (77%)	101 (66%)	1 (9%)	5 (7%)
8 (2%)	RIF monoresistance	RIF monoresistance	0	0	0	7 (10%)
8 (2%)	INH monoresistance	INH monoresistance	0	8 (5%)	0	0
153 (30%)	MDR	MDR	49 (23%)	44 (29%)	8 (73%)	52 (71%)
12 (2%)	Pre-XDR or XDR	Pre-XDR or XDR	0	1 (1%)	2 (18%)	9 (12%)
Discordance potentially leading to under-treatment						
23 (100%)	Total	..	8 (100%)	9 (100%)	0	6 (100%)
5 (22%)	MDR	Pan-susceptible	2 (25%)	2 (22%)	0	1 (17%)
18 (78%)	Pre-XDR or XDR	MDR	6 (75%)	7 (78%)	0	5 (83%)
Discordance potentially leading to over-treatment						
67 (100%)	Total	..	5 (100%)	44 (100%)	3 (100%)	14 (100%)
14 (21%)	Pan-susceptible	RIF monoresistance	0	0	3 (100%)	10 (71%)
14 (21%)	Pan-susceptible	MDR	3 (60%)	8 (18%)	0	3 (21%)
33 (49%)	Pan-susceptible	Other monoresistance†	2 (40%)	31 (71%)	0	0
5 (8%)	Other monoresistance‡	MDR	0	5 (11%)	0	0
1 (2%)	MDR	Pre-XDR or XDR	0	0	0	1 (7%)
Other discordance						
31 (100%)	Total	..	16 (100%)	6 (100%)	1 (100%)	7 (100%)
1 (3%)	Pan-susceptible	EMB, SM	0	1 (17%)	0	0
7 (23%)	RIF monoresistance	MDR	2 (13%)	0	0	5 (29%)
17 (55%)	Other monoresistance§	Pan-susceptible	13 (81%)	3 (50%)	0	0
1 (3%)	INH, MOX	INH monoresistance	0	1 (17%)	0	0
1 (3%)	IHN, PZA	MDR	0	1 (17%)	0	0
3 (10%)	MDR	RIF monoresistance	0	0	1 (100%)	2 (71%)
1 (3%)	MDR	EMB, SM	1 (6%)	0	0	0

Data are n (%). Analysis based on 634 patients (see appendix). MDR=multidrug resistance. XDR=extensively drug resistant. RIF=rifampicin. LPA=line probe assay. INH=isoniazid. XDR=extensively drug resistant. EMB=ethambutol. SM=streptomycin. MOX=moxifloxacin. PZA=pyrazinamide. In some patients the test used to diagnose drug-resistant infection at the local laboratories was unknown. Therefore, numbers do not always add up to the row totals. *RIF resistance diagnosed with Xpert MTB/RIF was classified as MDR. †21 strains were resistant to EMB, ten to SM, and two to INH. ‡Five strains were resistant to INH. §15 strains were resistant to INH and two to PZA.

Table 2: **Concordance and discordance of drug susceptibility results obtained from reference and local laboratories (n=634)**

resistant to pyrazinamide or ethambutol, or both. Among the 24 patients with pre-XDR tuberculosis, resistance to moxifloxacin (n=15) was more frequent than resistance to amikacin (n=9; appendix). Patients with resistant strains were more likely to receive second-line tuberculosis treatment and to experience unfavourable treatment outcomes than were patients with pan-susceptible strains (table 1).

272 (43%) patients with tuberculosis were HIV positive, with a median CD4 cell count at the start of tuberculosis treatment of 192 cells per µL (IQR 77·5–369). Among them, 175 (64%) were either on ART at the start of tuberculosis treatment or initiated ART within 3 months; the ART status of the remaining patients was unknown. Compared to HIV-negative individuals, HIV-positive patients were more likely to be women, more likely to have both pulmonary and extrapulmonary disease, and more likely to be patients with recurrent tuberculosis (appendix). HIV-positive patients were also more likely to have a negative sputum smear microscopy result and more likely to have a pan-susceptible *M tuberculosis* infection than HIV-negative patients.

Local laboratories used the Xpert MTB/RIF system, culture, LPAs, or a combination of these methods to diagnose drug-resistant infections and inform treatment regimens (table 2; appendix). Among the 27 isolates assessed by a combination of tests, Xpert MTB/RIF and LPA were used in 17 (63%), Xpert MTB/RIF and culture in eight (30%), culture and LPA in one, and Xpert MTB/RIF, culture and LPA in another isolate.

Comparing local results with reference laboratory results for any resistance, there were 218 true and 62 false positives and 332 true and 22 false negatives, for an overall sensitivity of 90·8% (95% CI 86·5–94·2) and specificity of 84·3% (80·3–87·7). For Xpert MTB/RIF, sensitivity was 79·5% (95% CI 68·4–88·0) and specificity 97·1% (93·3–99·0); for culture, sensitivity was 93·1% (84·5–97·7) and specificity

	Concordant results	Discordant results	Total
Pan-susceptible	17/302 (6%)	6/57 (11%)	23/359 (6%)
Any resistance	29/164 (18%)	15/50 (30%)	44/214 (21%)
Monoresistance			
INH	5/8 (63%)	2/15 (13%)	7/23 (30%)
RIF	0/7 (0%)	2/7(29%)	2/14 (14%)
PZA	..	1/2 (50%)	1/2 (50%)
Polyresistance			
MDR	22/138 (16%)	2/8 (25%)	24/146 (16%)
Pre-XDR or XDR	2/11 (18%)	8/18 (44%)	10/29 (34%)
Total	46/466 (10%)	21/107 (20%)	67/573 (12%)

Analysis based on 573 patients with complete data (see appendix). INH=isoniazid. RIF=rifampicin. PZA=pyrazinamide. MDR=multidrug resistance. XDR=extensively drug resistant.

***Table* 3: Mortality by phenotypic drug resistance profiles obtained at the Swiss National Centre for Mycobacteria and by concordance with local results**

71·6% (63·4–78·9); for LPA, sensitivity was 100% (71·5–100·0) and specificity 25·0% (0·63–80·6); and for combinations of tests, sensitivity was 98·8% (93·4–100·0) and specificity 27·8% (9·7–53·5). For all four categories of drug resistance considered together (rifampicin monoresistance, isoniazid monoresistance, MDR, and pre-XDR or XDR), results from the reference laboratory and local laboratories were concordant for 513 (81%) of 634 patients and discordant for 121 (19%). Results were concordant in 216 (88%) of 245 patients for Xpert MTB/RIF, in 154 (72%) of 213 for culture, in 11 (73%) of 15 for LPA, and in 73 (73%) of 100 for a combination of tests (p<0·0001).

23 (4%) of 634 patients had discrepancies potentially leading to under-treatment, 67 (11%) had discordant results potentially leading to over-treatment, and 31 (5%) had other discordances (table 2; appendix). Treatments received were compatible with WHO guidelines in 491 (97%) of 507 patients with concordant drug susceptibility test results compared with 94 (78%) of 121 patients with discordant results (p<0·0001).

After excluding 61 (10%) of 634 patients with unknown treatment outcomes, missing data, or other drug resistance (appendix), mortality ranged from 6% (17 of 302) among patients with pan-susceptible strains and concordant drug susceptibility test results to 44% (eight of 18) among patients with pre-XDR or XDR tuberculosis and discordant drug susceptibility test results (table 3). In patients with discordant results potentially leading to over-treatment, mortality was 10% (six of 61) whereas in patients with discordant results potentially leading to under-treatment it was 41% (nine of 22; figure, table 4). Mortality ranged from 6% (23 of 359) in patients with pan-susceptible strains to 35% (ten of 29) in patients with pre-XDR or XDR tuberculosis (table 4). Mortality was higher in patients with isoniazid monoresistant strains (seven [30%] of 23) than in patients with rifampicin monoresistant strains (two [14%] of 14) but the difference

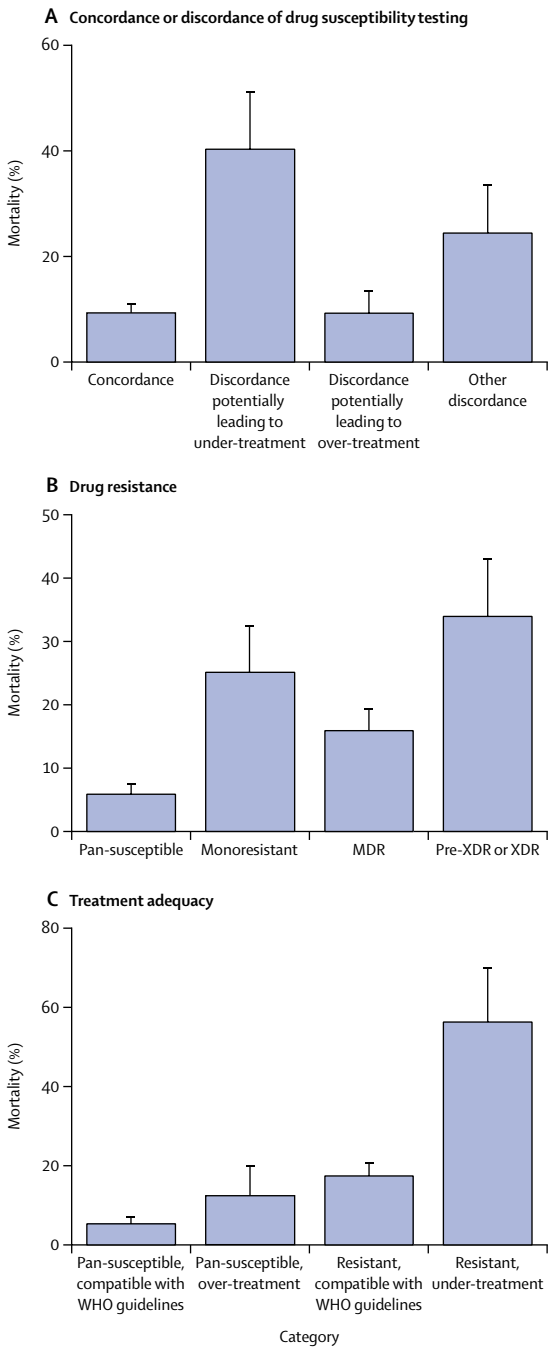

***Figure*: Mortality according to drug resistance, concordance or discordance of drug susceptibility test results, and treatment adequacy**
Error bars are standard errors. All p values are less than 0·001 for difference in mortality across categories. Analysis based on 573 patients with complete data.

was not significant (p=0·38, table 3) and the two categories were combined in further analyses. Finally, mortality ranged from 6% (20 of 336) in patients with pan-susceptible tuberculosis treated according to WHO guidelines to 57% (eight of 14) in patients with resistant strains who were under-treated (figure, table 4).

	Number of patients	Number of deaths (%)	Model 1, aOR (95% CI)	Model 2, aOR (95% CI)	Model 3, aOR (95% CI)
Concordance or discordance of drug susceptibility test results					
Concordance	466	46 (10%)	1	..	..
Discordance potentially leading to under-treatment	22	9 (41%)	7·33 (2·70–19·95)	..	..
Discordance potentially leading to over-treatment	61	6 (10%)	0·81 (0·31–2·11)	..	..
Other discordance	24	6 (25%)	4·92 (1·69–14·33)	..	..
Drug resistance*					
Pan-susceptible	359	23 (6%)	..	1	..
Monoresistance	39	10 (26%)	..	6·05 (2·36–15·56)	..
MDR	146	24 (16%)	..	3·83 (1·88–7·81)	..
Pre-XDR or XDR	29	10 (35%)	..	15·19 (5·45–42·36)	..
Treatment adequacy by drug resistance					
Pan-susceptible, compatible with WHO guidelines	336	20 (6%)	..	..	1
Pan-susceptible, over-treatment	23	3 (13%)	..	..	3·31 (0·82–13·45)
Any resistance, compatible with WHO guidelines	200	36 (18%)	..	..	4·66 (2·38–9·14)
Any resistance, under-treatment	14	8 (57%)	..	..	19·32 (5·59–66·73)
Sex					
Women	219	20 (10%)	1	1	1
Men	354	47 (13%)	1·47 (0·81–2·67)	1·42 (0·78–2·60)	1·46 (0·80–2·70)
Age (per 1-year increase)	573	67 (12%)	1·04 (1·01–1·06)	1·04 (1·01–1·06)	1·04 (1·01–1·06)
Sputum microscopy					
Negative	111	10 (9%)	1	1	1
Positive	462	57 (12%)	1·14 (0·51–2·56)	1·03 (0·45 -2·37)	0·90 (0·40–2·07)
HIV status					
Negative	337	43 (13%)	1	1	1
Positive	236	24 (10%)	0·90 (0·50–1·61)	1·19 (0·65–2·20)	1·19 (0·65–2·20)

Models based on 573 patients with complete data for all variables shown (see appendix). Model 1 was adjusted for concordance or discordance of drug susceptibility test results, sex, age, sputum microscopy, and HIV status; model 2 was adjusted for drug resistance, sex, age, sputum microscopy, and HIV status; model 3 was adjusted for treatment adequacy, sex, age, sputum microscopy, and HIV status. aOR=adjusted odds ratio. MDR=multidrug resistant. XDR=extensively drug-resistant. *Results from the Swiss National Reference Center for Mycobacteria.

Table 4: **Results from logistic regression models showing the probability of death during tuberculosis treatment**

In multivariable logistic regression models adjusted for sex, age, sputum microscopy result, and HIV status, discordant drug susceptibility test results continued to be associated with increased mortality compared with concordant drug susceptibility test results (table 4). Compared with concordant results, the adjusted odds ratio (aOR) of death was 7·33 (95% CI 2·70–19·95) for patients with discordant results potentially leading to under-treatment. The population attributable fraction for mortality associated with any type of discordance obtained from the logistic model was 15·15% (95% CI 2·08–26·47).

Drug resistance of any type was associated with higher mortality than pan-susceptible tuberculosis (aOR 5·18; 95% Cl 2·78–9·66) and mortality was highest for pre-XDR or XDR tuberculosis (15·19; 5·45–42·36; table 4). Finally, when compared with patients treated according to WHO guidelines with pan-susceptible strains, adequately treated patients with resistant strains had higher mortality (aOR 4·66; 95% CI 2·38–9·14), as did patients with resistant strains receiving inadequate regimens (19·32; 5·59–66·73; table 4). Patients with pan-susceptible tuberculosis who were over-treated also had an increased risk of death compared with patients who had pan-susceptible tuberculosis treated according to WHO guidelines, although the difference was not significant (aOR 3·31; 95% CI 0·82–13·45; p=0·10). Sex, positive sputum smear microscopy, and HIV status were not associated with the odds of death (table 4). Results from univariable models were similar to those from multivariable models (appendix). When restricting the analysis to HIV-positive patients, mortality was higher among patients with CD4 cell counts less than 50 cells per µL than in patients with higher CD4 counts at the start of tuberculosis treatment (aOR 6·89; 95% CI 1·57–30·26).

Discussion

The results of this multicentre cohort study of patients treated for drug-resistant or drug-susceptible tuberculosis

in seven high tuberculosis burden countries show that the accuracy of drug susceptibility testing in routine care was moderate, with discordant results from local drug susceptibility testing compared with phenotypic drug susceptibility testing in a reference laboratory in about 20% of patients. Discordant results led to inadequate treatment and contributed to the excess mortality associated with drug-resistant tuberculosis. As expected, mortality was highest in patients with pre-XDR or XDR tuberculosis and higher in patients who were under-treated than in those who were adequately treated. Patients with pan-susceptible tuberculosis who were over-treated also had higher mortality than did those who were adequately treated, although the difference was not significant. It is possible that over-treated patients had worse adherence and were at higher risk of adverse drug effects than were adequately treated patients. To our knowledge, this is the first study to assess the accuracy of drug susceptibility testing in real-world, routine settings and to examine the impact of inaccurate results on mortality. Our findings support the recent call for a precision medicine approach to the treatment of drug-resistant tuberculosis, guided by detailed molecular drug susceptibility testing done locally, to replace the standardised, empirical combination regimens used in many low-income and middle-income countries with a high tuberculosis burden.[25]

At present, WHO recommends that "Xpert MTB/RIF be used as an initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB",[26] based on a Cochrane review of test accuracy studies in adults with suspected rifampicin resistance or MDR tuberculosis.[27] In line with this recommendation, Xpert MTB/RIF was the most commonly used test in our study sites. The Cochrane review reported a pooled sensitivity of 95%, based on 17 studies and 555 patients with rifampicin-resistant strains.[27] The pooled specificity was 98%. We examined the accuracy of drug susceptibility testing strategies at the level of the local laboratories in high-burden countries, in routine care settings, rather than by examining a single test. Our estimates of sensitivity and specificity, for detection of any drug resistance, were lower overall (90·8% and 84·3%), and lower for Xpert MTB/RIF (79·5% and 97·1%) and for culture (93·1% and 71·6%), indicating that drug susceptibility testing is less accurate in routine settings than in test accuracy studies.[27]

There are concerns about both false-negative and false-positive Xpert MTB/RIF test results, and a policy of confirmatory testing has been introduced in South Africa and Brazil.[28,29] The discordant drug susceptibility test results that potentially led to under-treatment of drug-resistant tuberculosis (false negative for resistance) were mainly based on locally done cultures, Xpert MTB/RIF tests, or a combination of the two. Notably, the recently developed Xpert MTB/RIF Ultra assay has been shown to improve detection of rifampicin resistance.[30] Culture-based tests dominated discordance that potentially led to over-treatment, whereas Xpert MTB/RIF dominated in the category of discordance with unclear clinical significance. Some discordance could be explained by mixed infections, heteroresistance, or minority resistant populations.[31,32]

LPAs were rarely used in our study, possibly because they have been widely replaced by Xpert MTB/RIF, which is easier to use and provides results in a shorter time. Additionally, LPAs have suboptimal accuracy for isoniazid resistance, and WHO recommends that culture-based drug susceptibility testing for isoniazid should still be used, particularly in patients with suspected MDR tuberculosis in whom the LPA result does not detect isoniazid resistance.[33] In one case, the local laboratory detected resistance to ethambutol but this could not be confirmed in the reference laboratory: drug susceptibility testing is challenging for ethambutol and less reproducible.[34]

Data about treatment outcomes in drug-resistant tuberculosis are scarce, particularly for sub-Saharan Africa. A systematic review of treatment outcomes in patients with MDR tuberculosis included data on mortality among adults from seven studies done in sub-Saharan Africa, six in South Africa and one in Lesotho.[35] In these studies, mortality during tuberculosis treatment ranged from 12·4% in patients with MDR tuberculosis treated in a referral hospital in the Western Cape, South Africa,[36] to 45·8% in a study of patients with XDR tuberculosis from three South African provinces.[37] Our results extend these data to other countries in the region and also provide data for Peru and Thailand.

Our study confirms the poor outcome in patients with isoniazid monoresistant tuberculosis who are treated with first-line regimens (as recommended by WHO during the study period[38]), in line with a study from Durban, South Africa,[39] and a systemic review and meta-analysis.[40] Mortality in patients with monoresistant tuberculosis, especially isoniazid-resistant tuberculosis, was higher than in patients with MDR tuberculosis. This might be due to the treatment of almost all patients with isoniazid monoresistant tuberculosis with first-line regimens, whereas most patients with MDR tuberculosis received second-line treatment. WHO has updated its guidelines recommending the inclusion of fluoroquinolones in the treatment of isoniazid monoresistant tuberculosis.[41] Chance is another explanation: few patients had monoresistant tuberculosis and in the analysis of mortality the confidence intervals of the odds ratios for monoresistant and MDR tuberculosis overlapped widely.

In patients with HIV co-infection, treatment of drug-resistant tuberculosis is challenging for several reasons, including poorer absorption of drugs,[42] the risk of immune reconstitution inflammatory syndrome,[43] and interactions between antiretroviral and second-line tuberculosis drugs.[44–46] In contrast to previous studies from South Africa, which reported higher mortality at the end of treatment in HIV-positive patients with MDR tuberculosis compared with HIV-negative patients with

MDR tuberculosis,[36,47] we found no association with HIV infection, although the confidence intervals were wide. The median CD4 cell count of HIV-positive patients was considerably higher in our study (192 cells per µL) than in the South African studies,[36,47] which might explain the discrepant results. A study from Lesotho[48] also found little evidence for a difference in mortality between HIV-positive patients (median CD4 cell count 185 cells per µL) and HIV-negative patients. Finally, for patients with XDR tuberculosis, treatment outcomes have been uniformly poor in previous studies, irrespective of HIV status.[37]

Our study has several limitations. We sampled eligible patients within strata defined by drug resistance and HIV infection, and therefore could not estimate the incidence or prevalence of drug-resistant tuberculosis in HIV-positive or HIV-negative patients. In previous studies, HIV infection has not been consistently associated with drug resistance,[28] but it is clear that in regions with a high burden of HIV, the majority of patients with MDR tuberculosis will have HIV co-infection.[28] Although we initially exceeded the planned sample size, about a quarter of patients had to be excluded from analyses of drug susceptibility, mainly because of contamination or insufficient growth of cultures, and about a third were excluded from the analysis of mortality outcomes, mainly because vital status was unknown at database closure. The reference laboratory tested resistance against six drugs, and we would have missed resistance against other drugs used, such as kanamycin, ethionamide, or levofloxacin. Furthermore, the presence of different subpopulations of *M tuberculosis* in isolates tested at the local sites versus the reference laboratory might have introduced variability in phenotypic or molecular drug susceptibility testing.[49]

In conclusion, our study shows that the accuracy of drug susceptibility testing in routine care in high-burden countries was inadequate and that inaccurate results led to inadequate treatment and contributed to the excess mortality associated with drug-resistant tuberculosis. Our results support the notion that access to rapid molecular drug susceptibility testing of first-line and second-line drugs at treatment initiation is required to improve outcomes in patients with MDR tuberculosis and pre-XDR/XDR tuberculosis.[28] Whole-genome sequencing is the most promising approach to reach this goal, but much work remains to be done to make this approach feasible and affordable in low-income and middle-income countries.[28] In particular, direct testing of sputum samples should become routine to circumvent lengthy mycobacterial cultures.[40] A standardised approach for the interpretation of mutations conferring drug resistance has been developed.[50] In the meantime, the capacity for the phenotypic and molecular drug susceptibility testing recommended by WHO should be increased to ensure the most adequate treatment of drug-resistant tuberculosis in these settings.

Contributors

KZ, MB, and ME wrote the first draft of the paper, which was reviewed by all authors and revised on the basis of the comments received by co-authors. MB coordinated data and strain collection across study sites. ECB and PMK supervised drug susceptibility testing at the Swiss National Center for Mycobacteria, which were done by RHö. HC, JG, OM, MY, LD, EJC, NR, RJW, NE, AGA, JC, AA, and KK supervised drug susceptibility testing at the local laboratory and the collection of clinical data. ME and KZ did statistical analyses. All authors approved the final version of the manuscript.

Declaration of interests

AA has received honoraria fees from Jensen-Cilag, Gilead, and Bristol-Myers Squibb. All other authors declare no competing interests.

Acknowledgments

We thank all sites that participated in this survey and the patients whose data were used in this study. We are grateful to the Tuberculosis Working Group of IeDEA for helpful discussions, and to the reviewers for their thoughtful comments. We also thank all regional data centres that contributed to the coordination of the study. This research was supported by the Swiss National Science Foundation (grant numbers 153442, 310030_166687 and 174281), the National Institutes of Allergy and Infectious Diseases (NIAID) under award numbers U01 AI096299, U01 AI069919, U01 AI069924, U01 AI069911, U01 AI069907, U01 AI096186, and U01 AI069923, and the Swiss National Center for Mycobacteria, University of Zurich, Switzerland. RJW is supported by the Francis Crick Institute (grant number 10218), which is funded by the Wellcome Trust, Cancer Research UK, and Research Councils UK. RJW also receives support from the Wellcome Trust (104803 and 203135). HC is supported by a Wellcome Trust fellowship and reports grants from UK Medical Research Council and the National Research Foundation of South Africa. SG is supported by the Swiss National Science Foundation (310030_166687, IZRJZ3_164171, IZLSZ3_170834, and CRSII5_177163), the European Research Council (309540-EVODRTB) and SystemsX.ch. KK was supported by the National Research University Program, Office of the Higher Education Commission, Bangkok, Thailand.

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