Early View

Original research article

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Drug exposure and susceptibility of second-line drugs correlate with treatment response in patients with multidrug-resistant tuberculosis: a multi-centre prospective cohort study in China

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Take-home message

Drug exposure and susceptibility were proved to be associated with treatment responses during multidrug-resistant tuberculosis treatment and identified thresholds may serve as targets for dose adjustment in future clinical studies to improve treatment efficacy.

Abstract

Background. Understanding the impact of drug exposure and susceptibility on treatment response of multidrug-resistant tuberculosis (MDR-TB) will help to optimize treatment. This study aimed to investigate the association between drug exposure, susceptibility and response to MDR-TB treatment.

Methods. Drug exposure and susceptibility for second-line drugs were measured for patients with MDR-TB. Multivariate analysis was applied to investigate the impact of drug exposure and susceptibility on sputum culture conversion and treatment outcome. Probability of target attainment was evaluated. Random Forest and Classification and Regression Tree (CART) analysis were used to identify key predictors and their clinical targets among patients on WHO-recommended regimens.

Results. Drug exposure and corresponding susceptibility were available for 197 patients with MDR-TB. The probability of target attainment was highly variable ranging from 0%

for ethambutol to 97% for linezolid, while patients with fluoroquinolones above targets had higher probability of two-month culture conversion (56.3% vs. 28.6%, adjusted OR 2.91, 95% CI 1.42-5.94) and favourable outcome (88.8% vs. 68.8%, adjusted OR 2.89, 95% CI 1.16-7.17). Higher exposure values of fluoroquinolones, linezolid and pyrazinamide were associated with earlier sputum culture conversion. CART analysis selected moxifloxacin AUC/MIC of 231 and linezolid AUC/MIC of 287 as best predictors for six-month culture conversion in patients receiving identical Group A-based regimen. These associations were confirmed in multivariate analysis.

Conclusions. Our findings indicated that target attainment of second-line drugs was associated with response to MDR-TB treatment. The CART-derived thresholds may serve as targets for early dose adjustment in a future randomized controlled study to improve the treatment outcome.

Background

Multidrug-resistant tuberculosis (MDR-TB) is a global public health crisis and its poor treatment outcome[1] is threatening achieving the END-TB targets by 2035. The MDR-TB treatment success rate was 54% in China in 2019 while it has been reported up to 80-85% in less burdened countries [1]. Adequate drug exposure is key for effective therapy as suboptimal exposures of anti-TB drugs are correlated with delayed sputum culture conversion and poor treatment outcome [2]. Well-designed studies linking drug exposure to treatment outcome are urgently needed to guide dose optimization and implementation of therapeutic drug monitoring (TDM) [3-5].

TDM is a tool considered in the ATS/CDC/ERS/IDSA Clinical Practice Guideline and the WHO guideline to individualize drug doses to maximize the therapeutic effects while minimizing the risk of adverse events, particularly for drugs with narrow therapeutic windows such as linezolid [3, 4]. Although variability in pharmacokinetics and drug susceptibility has been reported for second-line TB drugs [5, 6], clinical targets are predominantly based on pre-clinical models [7-13]. Large clinical studies establishing targets for drug exposure and susceptibility are still lacking due to logistical and financial hurdles, including the need for long-time follow-up, variability in drug regimens and incapability to integrate both drug concentration and susceptibility of *Mycobacterium tuberculosis* [14, 15]. Such barriers prevented implementation of individualised, TDM based therapy [16].

Although new anti-TB drugs and shorter treatment regimens demonstrate improved treatment outcome, there is still a long way to go before all patients will benefit from them. Besides, new drugs are not free of variability in drug exposure [17]. Improving treatment should consider variability in *Mycobacterium tuberculosis* susceptibility and drug exposure[5] in addition to other factors like treatment adherence.

Earlier our international TB research consortium showed that treatment outcomes in patients with drug-susceptible TB could be explained by drug exposure and susceptibility [18]. Thus, we designed a multicentre, prospective, population-based study to determine the association between drug exposure – susceptibility targets and MDR-TB treatment responses.

Materials and Methods

Study design and participants

A multi-centre prospective cohort study was conducted between June 2016 and June 2019 in five hospitals from Guizhou, Henan and Jiangsu Province in China. Eligible patients had an MDR-TB (*Mycobacterium tuberculosis* simultaneously resistant to rifampicin and isoniazid) diagnosis confirmed by bacterial culture and phenotypic drug susceptibility testing (DST), and were aged between 18 and 70 years. Patients were excluded if they were clinically abnormal in liver or kidney function, were pregnant or infected with human immunodeficiency virus, hepatitis B or C virus, having received MDR-TB treatment previously for more than 1 day, or refused to participate. The study was approved by the ethics committee of the School of Public Health, Fudan University (IRB#2015-08-0565) and written informed consent was obtained from all subjects.

MDR-TB treatment and information collection

The patients with MDR-TB were routinely transferred to designated hospitals for two-week inpatient treatment followed by outpatient treatment. A standardized oral regimen of fluoroquinolones, bedaquiline, linezolid, clofazimine and cycloserine for six months, followed by fluoroquinolones, linezolid, clofazimine and cycloserine for 18 months, was used [4, 19]. Treatment modification was made according to phenotypic DST results, clinical characteristics of patients and drug availability. Directly Observed Therapy was implemented daily by study nurses during in-patient treatment and by community healthcare

workers during the outpatient phase [19]. Missing doses and/or treatment interruption and the reasons for these were recorded. Patients were routinely examined once a month during the intensive phase and once every two months during the consolidation phase. A questionnaire was used to collect demographic data, while medical and laboratory data were extracted from hospital records. Sputum samples were collected at each visit and were sent to up-level quality-controlled prefectural TB reference laboratory for analysis [20].

Drug susceptibility testing

Bacterial culture, phenotypic DST and minimum inhibitory concentration (MIC) values for the studied drugs were performed using the BACTEC MGIT 960 system (Becton Dickinson, Franklin Lakes, NJ, USA). Critical concentrations were used for the classification of drug susceptibility of the isolates [21]. The following concentrations were used for MIC testing: levofloxacin 0.06-32 mg/L, moxifloxacin 0.03-16 mg/L, linezolid 0.06-4 mg/L, bedaquiline 0.015-4 mg/L, cycloserine 2-64 mg/L, clofazimine 0.03-4 mg/L, prothionamide 0.3-20 mg/L, pyrazinamide 16-1024 mg/L and ethambutol 0.5-32 mg/L. The MIC was defined as the lowest concentration of a drug that inhibited the bacterial growth. For details see supplement S1.

Drug exposure

After two weeks of inpatient treatment, blood samples were collected via a venous catheter at pre-dose and at 1, 2, 4, 6 and 8 hours after witnessed intake of anti-TB drugs (steady-state) [22]. Additional blood samples at 12 and 18 hours post-dose were collected in patients

receiving bedaquiline. Samples were measured using a validated liquid chromatography tandem mass spectrometry method previously established [23] (see S2 for details). Non-Compartmental Analysis was applied to calculate area under the concentration-time curve (AUC_{0-24h}) for all drugs and percentage of time that concentration persisted above MIC ($\%T_{>MIC}$) for cycloserine. C_{max} was defined as the maximum drug concentration observed.

Response to treatment and main definitions

The response to treatment in this study was evaluated by: a) two-month sputum culture conversion as a marker of early treatment response; b) six-month culture conversion, previously reported to be predictive of treatment outcome [24]; c) time to culture conversion using time-to-event analysis; and d) final treatment outcome. Sputum culture conversion was defined as two consecutive negative cultures of samples taken at least 30 days apart [25]. The treatment outcome was defined according to the WHO guidelines [25]. Cure and treatment completion were considered as a successful treatment outcome, while failure, death and lost to follow-up were considered as unfavourable outcomes. Severe disease was defined as the TB score ≥ 8 [26]. Timika score was used to assess chest X-ray (CXR) severity and a score ≥ 71 was defined as extensive pulmonary disease [27]. Effective drugs were defined as those with confirmed susceptibility by phenotypic DST or no previous exposure history.

Statistical analyses

The statistics for patients' characteristics and treatment response were presented, in line with the STROBE statement (www.strobestatements.org) for observational cohort studies. Between-group differences were evaluated by Chi-square test, Fisher's exact test or Mann-Whitney U test as appropriate. *P* value below 0.05 was considered statistically significant and 95% confidence interval (CI) was calculated. Due to the lack of clinical pharmacokinetic/pharmacodynamic targets, the probability of target attainment for drug exposure - susceptibility ratio (AUC_{0-24h}/MIC) was based on previous *in vitro* studies (the targets for moxifloxacin 56, levofloxacin 160, linezolid 119, cycloserine 25.8, pyrazinamide 11.3, prothionamide 56.2 and ethambutol 119 [7-13]). Patients were grouped based on the quartiles of AUC_{0-24h}/MIC ratio. The association between these groups and time to sputum culture conversion was investigated by Kaplan-Meier survival analysis and then adjusted for potential confounders in Cox proportional hazard regression models with death and lost to follow-up as censored data, while the association with sputum culture conversion at two/six months and treatment outcome was investigated in univariate and multivariate logistic regression models.

In the subgroup analysis of treatment arms of WHO-recommended Group A-based regimens, Random Forest was used to rank variable importance for all demographic characteristics, clinical features and drug AUC_{0-24h}/MIC ratios. The top ten variables were selected for subsequent analysis. To detect interactions and deal with missing values, Classification and Regression Tree (CART) analysis was used to identify the AUC_{0-24h}/MIC thresholds predictive of treatment response using Salford Predictive Miner System software (San Diego,

CA, USA). The association of derived targets with treatment response was further investigated in modified Poisson regression and Cox proportional hazard regression models. Further statistical analyses details are summarized in supplement S3.

Results

Study patients

In total, 246 patients were newly diagnosed with MDR-TB in the study hospitals during the study period and 201 of them were included, of which data were available for analysis for 197 patients (Figure 1). Of the 197 patients, 71.1% were male, and the mean age and median weight were 42.0 (± 9.9) years and 54 (IQR: 48-66) kg, respectively (Table 1). Baseline DST identified 37 (18.8%) *Mycobacterium tuberculosis* strains with additional resistance to fluoroquinolones.

Treatment regimens and procedures

The number of patients receiving an all-Group A + B drug regimen was 111 (56.3%) and those received a personalized regimen was 86 (43.7%). All patients received a treatment regimen consisting of at least 4 effective drugs.

Of the 197 patients, 1 patient died due to cardiovascular disease after 12-month treatment, while 2 patients were lost to follow-up. During the treatment, 125 patients reported 219 adverse events, including gastrointestinal disorders (33.5%), psychiatric disorders (14.7%) and anaemia (13.2%). Dose reductions were performed for cycloserine (n=5), linezolid (n=4)

and bedaquiline (n=2), while cycloserine was discontinued in 10 patients after a median of 7 (range 6-10) months of treatment. (Table S2)

Treatment responses and risk factors

Sputum culture conversion was achieved in 88 (44.7%) patients after two-month MDR-TB treatment, 128 (65.0%) achieved six-month culture conversion while 156 (79.2%) finally had favourable outcome during follow-up (Table 1). The median time to culture conversion was 4 (IQR: 2-14) months. As shown in Table S1, baseline time to culture positivity (TTP) was found to be significantly associated with two/six-month culture conversion and treatment outcome ($P<0.001$). Patients who had diabetes mellitus type 2 (50.0% vs.68.8%, $P = 0.026$) or currently smoked (57.9% vs.73.3%, $P=0.024$) were less likely to achieve six-month culture conversion. Sex, severe disease and extensive pulmonary disease were not associated with any treatment responses ($P>0.05$). Compared to patients receiving at least two Group A drugs, patients taking only one drug of group A had a lower probability of two-month (23.1% vs. 50.0%, $P=0.002$) and six-month culture conversion (48.7% vs. 69.0%, $P=0.017$) as well as a lower probability of favourable outcome (53.8% vs. 85.4%, $P<0.001$). Patients receiving three Group A drugs had higher treatment success rate compared to others (100.0% vs. 73.9%, $P<0.001$). (Figure S1)

Association between drug exposure – susceptibility ratio and treatment responses

Patients with a higher exposure – susceptibility ratio for fluoroquinolones, linezolid and pyrazinamide had better treatment responses ($P<0.05$), while prothionamide and ethambutol

had little impact (Table 2 and Table S3). A more favourable exposure – susceptibility ratio for bedaquiline (2890.1 vs. 1527.2, $P=0.001$), cycloserine (111.0 vs. 79.2, $P<0.001$) and clofazimine (101.1 vs. 51.7, $P=0.005$) were strongly associated with six-month culture conversion. As shown in Figure 2, time to sputum culture conversion was observed to be significantly shorter in patients with a higher exposure – susceptibility ratio of fluoroquinolones, linezolid, cycloserine and pyrazinamide ($P<0.001$). Similar effects were not observed in bedaquiline, clofazimine and prothionamide ($P>0.05$). These associations were confirmed in a multivariate analysis (Table 3 and 4).

The probabilities of target attainment for moxifloxacin, linezolid and cycloserine were above 85%, while below 45% for levofloxacin, pyrazinamide, prothionamide and ethambutol (Table 5 and Figure S4). Multivariate analysis showed that patients with fluoroquinolone exposure above the previously suggested targets had higher probability of two-month culture conversion (aOR 2.91, 95% CI 1.42-5.94) and treatment success (aOR 2.89, 95% CI 1.16-7.17). Patients with a moxifloxacin exposure above target were more likely to achieve six-month culture conversion (aOR 15.6, 95% CI 1.48-165.0).

CART analysis for clinical drug exposure – susceptibility targets

Random Forest and CART analysis was performed among subgroups of patients receiving “moxifloxacin+linezolid+/-bedaquiline” (n=67) or “levofloxacin+linezolid+/-bedaquiline” (n=61) based regimen. The results showed that the primary node for “moxifloxacin+linezolid+/-bedaquiline” based regimen was moxifloxacin AUC_{0-24h}/MIC of

231, where 97.6% of patients exceeding this target achieved six-month culture conversion compared to 3.8% in those below the target (Figure 3). For “levofloxacin+linezolid+/-bedaquiline” based regimen, linezolid was selected as the primary node with an AUC_{0-24h}/MIC cut-off value of 287 and patients with linezolid above the target had higher probability of sputum culture conversion at six months of treatment (82.4% vs. 10.0%). After adjusting for current smoking, diabetes mellitus type 2, baseline TTP and number of effective drugs, patients with moxifloxacin or linezolid exposure above target had greater probability of six-month culture conversion and showed earlier culture conversion (Table S4).

Discussion

In this large multi-centre study, higher drug exposure in relation to susceptibility for all drugs, except prothionamide and ethambutol, were found to be associated with favourable treatment responses in patients with MDR-TB. This is the first study demonstrating that adequate exposure to fluoroquinolones, bedaquiline and linezolid is strongly associated with sputum culture conversion at various time points during MDR-TB treatment in programmatic regimen.

It is well known that current Group A drugs contribute to improved treatment response. Our study demonstrates that adequate exposure to these drugs translated in higher two-month and six-month culture conversion rates compared to patients below these targets. By showing that targets established in *in vitro* studies are associated with improved treatment

response across the two- and six-month culture conversion, time to culture conversion and overall treatment outcome, our study is the first to bridge the gap between pre-clinical studies and clinical trials evaluating treatment outcome [28-31]. Although the efficacy of levofloxacin and moxifloxacin is believed to be comparable in MDR-TB treatment [4], moxifloxacin was found to play a more important role in driving treatment response in our study. However, the observed difference may well be attributed to under-dosing of levofloxacin [5]. Although a standard levofloxacin dose of 750 mg was recommended [4, 19], physicians still tended to prescribe 500mg, due to the lack of clear dose recommendations for domestically manufactured levofloxacin, as well as concerns of potential adverse events. Under-dosing of fluoroquinolones should be avoided and there is room for treatment optimization using TDM. We urgently request that no country uses 500 mg of levofloxacin as standard dose, as this has been shown to lead to sub-therapeutic drug levels [5, 32, 33].

Having a higher exposure – susceptibility ratio of linezolid and bedaquiline was also associated with a better treatment response. This underpins the critical importance of interpretation of the highly variable exposures of bedaquiline and linezolid in relation to the baseline drug susceptibility [17, 34]. Although the mean bedaquiline exposure after 2 weeks of MDR-TB treatment (AUC_{0-24h} of 41.5 mg*h/L) in our study was higher ($P=0.04$) than a previous study (33.0 mg*h/L) [29], the clinical relevance is unclear. The study by Conradie and colleagues has fuelled the discussion on linezolid dosing as over 80% of the patients in that study experienced toxicity, prompting a dose reduction or interruption of treatment

when receiving a dose of 1200 mg daily [35]. In our study, the linezolid dose was reduced in 4 patients. Meanwhile, most patients (92.9%, 156/168) were eligible for dose reduction while maintaining adequate drug exposure. Clearly there is some room for linezolid dose optimization to balance efficacy and toxicity [6, 30].

Adequate exposure to cycloserine and clofazimine contributed to improved treatment response in our study. A high probability of target attainment for cycloserine supports the use of the agent with currently recommended dose of 10-15 mg/kg [12]. Regarding treatment optimization in the Chinese setting, more efforts are needed to promote the use of Group B drugs since nearly one third of patients received only one of them. The main reason was that these two drugs were not covered by medical insurance in China and needed to be paid by the patient. When susceptibility is proven, our study shows that pyrazinamide is a valuable addition for composing an MDR-TB treatment regimen, as it increased the probability of sputum culture conversion and reduced the time to culture conversion, confirming previous studies [36, 37]. However, as only 11.1% of patients reached the target for pyrazinamide [10], the need of higher dose of pyrazinamide (40mg/kg) should be considered to increase the benefits without compromising its tolerability [38]. The fact that prothionamide and ethambutol had little impact on treatment responses, reflecting their limited bactericidal and/or sterilizing effect compared to other second -line drugs.

This study has some important implications for future randomized controlled studies on personalized dosing. Though TDM is recommended to optimize MDR-TB treatment in

guidelines, our study is the first to identify clinical targets for moxifloxacin and linezolid. The CART-derived clinical targets are higher compared to the targets reported in *in vitro* studies [11, 13]. However, these differences need to be viewed in a clinical context and methodology of MIC determination, as MIC determination has inherent variability due to laboratory and strain variability [39] and with each two-fold change in the MIC, the target as calculated by AUC_{0-24h}/MIC will double. Moreover, free drug concentrations and tissue penetration to the site of infection (e.g. cavitory disease) need to be considered when applying AUC_{0-24h}/MIC targets in clinical practice. Considering the delay and complexity of phenotypic DST in routine care, we foresee that genotypic testing to determine drug susceptibility in combination with drug exposure assessment would allow for early treatment modifications. Establishing AUC_{0-24h} targets solely based on clinical breakpoints would result in significant overexposure in many patients as most isolates have an MIC lower than the breakpoint.

Our study has some limitations. We excluded patients aged over 70 and patients co-infected with human immunodeficiency virus, hepatitis B or C virus, to reduce heterogeneity of study participants. Therefore, results cannot be extrapolated to these patients. Sputum culture conversion was used to assess treatment response but more sensitive biomarkers should be considered in future studies evaluating interventions on drug dosing in relation to treatment response. We assessed drug exposure after two weeks of treatment (steady-state) and we assumed that intra-patient variability in drug exposure was limited compared to inter-patient variability, as sputum culture conversion at 2 and 6 months were comparable. It's important

to realize that analysing the interaction between drug concentrations, pathogen susceptibility and treatment outcome is complex, and thresholds derived from a population depend on the distribution of different variables in that population [40]. This must be considered when comparing or translating study results.

In conclusion, our findings indicate that targets based on drug exposure – susceptibility ratio are associated with response to treatment for most anti-TB drugs used in MDR-TB treatment, especially for Group A drugs and pyrazinamide. For fluoroquinolones, linezolid and pyrazinamide, there is a clear opportunity for dose optimization in general, in addition to individualisation. We recommend clinical targets for efficacy to be evaluated in a randomized controlled study as a strategy to improve MDR-TB treatment outcome, adjusted for differences in susceptibility testing.

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Competing interests

None to declare.

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Table 1. Demographic characteristics, clinical features and treatment outcome of study participants with multidrug-resistant tuberculosis

Characteristics	No.(%)
Age, years ^a	42.0±9.9
Sex, male	140 (71.1)
Weight, kg ^b	54 (48, 66)
Current smoking	107 (54.3)
Diabetes mellitus type 2	40 (20.3)
Pulmonary cavities	78 (39.6)
Extensive pulmonary disease	38 (19.3)
Severe disease	56 (28.4)
Time to positivity, days ^a	11.9±3.0
Drug susceptibility profile	
MDR-TB alone	160 (81.2)
Pre-XDR-TB	37 (18.8)
Drug intake (mg/kg) ^b	
Levofloxacin (500mg, QD)	7.7 (6.7, 8.4)
Moxifloxacin (400mg, QD)	8.6 (7.2, 10.4)
Linezolid (600mg, QD)	11.3 (9.2, 12.8)
Bedaquiline (400mg, QD)	7.5 (6.2, 8.3)
Clofazimine (100mg, QD)	9.3 (7.6, 10.4)
Cycloserine (500mg, BID)	3.8 (3.2, 4.3)
Prothionamide (600mg, TID)	10.8 (9.3, 12)
Pyrazinamide (1500mg, TID)	26.8 (21.7, 30)
Ethambutol (750mg, QD)	13.9 (11.3, 15.6)
Number of drugs in ^a	
Group A	2.0±0.6
Group B	1.6±0.5
Group C	2.0±1.1
Effective drugs ^a	5.6±0.7
Two-month culture conversion	88 (44.7)
Six-month culture conversion	128 (65.0)
Time to culture conversion, months ^b	4 (2, 14)
Treatment outcome	
Success	156 (79.2)
Failure	38 (19.3)
Death	1(0.5)
Lost to follow-up	2(1.0)

Group A, B and C drugs were referred to the multidrug-resistant tuberculosis treatment guidelines released by the World Health Organization. MDR: multidrug-resistant tuberculosis; XDR-TB: extensively drug-resistant tuberculosis; QD: once daily; BID: twice daily; TID:

three times a day.

^a: mean $\pm$ standard deviation

^b: median (interquartile range)

Table 2. Distribution of drug exposure – susceptibility ratio in patients with multidrug-resistant tuberculosis

Drugs (AUC _{0-24h} /MIC)	Two-month sputum culture result		Six-month sputum culture result		Treatment outcome	
	Positive (n=109)	Negative (n=88)	Positive (n=69)	Negative (n=128)	Unfavourable (n=41) ^c	Successful (n=156)
Levofloxacin (n=78)	93.3 (50.6, 114.3)	108.7 (90.0, 249.1) ^a	63.3 (48.7, 107.4)	108.8 (90.1, 136.8) ^a	52.9 (44.3, 96.6)	108.8 (90.4, 134.0) ^a
Moxifloxacin (n=79)	177.1 (68.5, 268.1)	742.9 (478.7, 887.5) ^a	119.6 (51.6, 192.7)	625.8 (387.4, 834.7) ^a	159.0 (91.5, 194.7)	411.5 (184.3, 763.3) ^a
Linezolid (n=168)	472.2 (372.5, 569.7)	492.4 (432.5, 715.4) ^a	429.6 (273.6, 503.1)	497.7 (439.0, 715.4) ^a	373.4 (129.7, 503.8)	492.4 (433.7, 617.9) ^a
		2901.0 (2103.2, 3765.2)			2382.0	(1441.4,
Bedaquiline (n=70)	2107.5 (914.7, 3275.4) ^a		1527.2 (852.3, 2383.1)	2890.1 (2163.8, 3867.6) ^a	3120.0)	2383.1 (1627.0, 3743.4)
Clofazimine (n=136)	64.4 (30.2, 141.3)	115.7 (45.8, 235.1) ^a	51.7 (26.8, 138.1)	101.1 (45.0, 235.6) ^a	102.7 (34.4, 154.3)	94.8 (36.8, 228.6)
Cycloserine (n=186)	86.2 (46.7, 152.4)	130.0 (65.5, 219.3) ^a	79.2 (33.4, 119.0)	111.0 (65.0, 214.6) ^a	90.7 (33.6, 144.6)	99.4 (59.3, 201.0)
Cycloserine (n=186) ^b	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	100.0 (93.6, 100.0)	100.0 (100.0, 100.0) ^a	100.0 (95.2, 100.0)	100.0 (100.0, 100.0)
Prothionamide (n=86)	38.0 (28.8, 77.4)	51.4 (37.1, 72.4)	39.5 (18.7, 75.9)	51.1 (36.0, 73.4)	37.7 (16.4, 69.5)	51.0 (35.5, 76.0)
Pyrazinamide (n=99)	4.2 (3.1, 5.6)	7.7 (4.6, 11.9) ^a	4.1 (3.4, 5.4)	5.9 (3.5, 9.3) ^a	3.9 (3.4, 5.2)	5.6 (3.5, 9.3) ^a
Ethambutol (n=123)	19.1 (12.7, 23.5)	21.5 (13.9, 31.8) ^a	19.3 (12.9, 23.5)	20.2 (13.5, 27.7)	19.2 (12.7, 23.1)	20.1 (13.5, 27.6)

AUC_{0-24h}: area under drug concentration-time curve; MIC: minimum inhibitory concentration. All data are presented as AUC_{0-24h}/MIC ratio and are shown median with interquartile range otherwise specified. Mann-Whitney U test was applied for comparison.

^a $P < 0.05$

^b Percentage of time that concentration persisted above MIC (%T_{>MIC}), was presented as a median with interquartile range.

^c Unfavourable outcome was defined as the sum of failure, death and lost to follow-up.

Table 3 Univariate and multivariate analysis for drug exposure - susceptibility ratio with two-month/six-month culture conversion

	Two-month culture results			Six-month culture results		
	Negative (%)	OR (95%CI)	aOR (95%CI) ^a	Negative (%)	OR (95%CI)	aOR (95%CI) ^a
Fluoroquinolones (n=157)						
The first quartile	3 (7.5)	1	1	7 (17.5)	1	1
The second quartile	9 (23.1)	3.70 (0.92, 14.9)	4.02 (0.91, 17.7)	22 (56.4)	6.10 (2.17, 17.1)	5.92 (1.93, 18.2)
The third quartile	26 (65.0)	22.9 (5.97, 87.8)	31.3 (6.61, 148.0)	33 (82.5)	22.2 (7.01, 70.4)	23.4 (6.38, 85.6)
The fourth quartile	29 (76.3)	39.7 (9.86, 160.2)	53.3 (10.6, 268.8)	34 (89.5)	40.1 (10.7, 149.8)	36.4 (8.31, 159.3)
Linezolid (n=168)						
The first quartile	13 (31.0)	1	1	17 (40.5)	1	1
The second quartile	25 (59.5)	3.28 (1.34, 8.06)	2.91 (1.11, 7.64)	33 (78.6)	5.39 (2.06, 14.1)	4.92 (1.74, 13.9)
The third quartile	18 (42.9)	1.67 (0.68, 4.10)	1.36 (0.52, 3.57)	30 (71.4)	3.68 (1.48, 9.13)	2.85 (1.06, 7.64)
The fourth quartile	24 (57.1)	2.97 (1.22, 7.28)	2.59 (0.99, 6.78)	36 (85.7)	8.82 (3.05, 25.5)	8.52 (2.74, 26.4)
Bedaquiline (n=70)						
The first quartile	6 (35.3)	1	1	7 (41.2)	1	1
The second quartile	10 (55.6)	2.29 (0.59, 8.94)	2.70 (0.62, 11.8)	14 (77.8)	5.00 (1.15, 21.8)	7.71 (1.43, 41.6)
The third quartile	15 (83.3)	9.17 (1.87, 44.9)	9.74 (1.64, 57.9)	15 (83.3)	7.14 (1.48, 34.4)	6.38 (1.04, 39.1)
The fourth quartile	11 (64.7)	3.36 (0.82, 13.7)	2.69 (0.52, 14.0)	15 (88.2)	10.7 (1.84, 62.5)	8.35 (1.11, 63.0)
Cycloserine (n=186)						
The first quartile	17 (36.2)	1	1	23 (48.9)	1	1
The second quartile	16 (34.8)	0.94 (0.40, 2.20)	0.93 (0.37, 2.30)	28 (60.9)	1.62 (0.71, 3.70)	1.58 (0.65, 3.83)
The third quartile	17 (36.2)	1.00 (0.43, 2.32)	1.07 (0.44, 2.64)	30 (63.8)	1.84 (0.81, 4.20)	2.05 (0.84, 5.01)
The fourth quartile	30 (65.2)	3.31 (1.41, 7.74)	3.45 (1.38, 8.61)	38 (82.6)	4.96 (1.91, 12.9)	5.26 (1.88, 14.7)
Clofazimine (n=136)						
The first quartile	10 (29.4)	1	1	18 (52.9)	1	1
The second quartile	16 (47.1)	2.13 (0.79, 5.79)	2.23 (0.79, 6.27)	25 (73.5)	2.47 (0.89, 6.83)	2.66 (0.91, 7.76)

The third quartile	17 (50.0)	2.40 (0.89, 6.51)	2.34 (0.83, 6.59)	20 (58.8)	1.27 (0.49, 3.31)	1.13 (0.41, 3.13)
The fourth quartile	21 (61.8)	3.88 (1.41, 10.7)	3.81 (1.34, 10.9)	30 (88.2)	6.67 (1.93, 23.1)	6.57 (1.81, 23.8)
Pyrazinamide (n=99)						
The first quartile	5 (20.0)	1	1	14 (56.0)	1	1
The second quartile	5 (20.0)	1.00 (0.25, 4.00)	1.21 (0.27, 5.47)	9 (36.0)	0.44 (0.14, 1.38)	0.39 (0.11, 1.35)
The third quartile	8 (32.0)	1.88 (0.52, 6.84)	2.05 (0.51, 8.15)	14 (56.0)	1.00 (0.33, 3.06)	0.96 (0.30, 3.12)
The fourth quartile	17 (70.8)	9.71 (2.60, 36.3)	12.5 (2.81, 55.3)	20 (83.3)	3.93 (1.04, 14.9)	3.30 (0.81, 13.5)
Prothionamide (n=86)						
The first quartile	8 (36.4)	1	1	11 (50.0)	1	1
The second quartile	9 (42.9)	1.31 (0.39, 4.47)	1.08 (0.25, 4.64)	15 (71.4)	2.50 (0.71, 8.84)	3.16 (0.78, 12.8)
The third quartile	15 (68.2)	3.75 (1.08, 13.1)	5.88 (1.33, 25.9)	17 (77.3)	3.40 (0.93, 12.5)	4.08 (1.00, 16.7)
The fourth quartile	8 (38.1)	1.08 (0.31, 3.71)	1.11 (0.27, 4.53)	13 (61.9)	1.63 (0.48, 5.47)	1.77 (0.47, 6.60)
Ethambutol (n=123)						
The first quartile	11 (35.5)	1	1	19 (61.3)	1	1
The second quartile	11 (35.5)	1.00 (0.35, 2.83)	0.77 (0.25, 2.42)	19 (61.3)	1.00 (0.36, 2.78)	0.70 (0.23, 2.17)
The third quartile	11 (35.5)	1.00 (0.35, 2.83)	0.83 (0.27, 2.53)	17 (54.8)	0.77 (0.28, 2.11)	0.52 (0.17, 1.60)
The fourth quartile	19 (63.3)	3.14 (1.10, 8.93)	2.38 (0.76, 7.40)	24 (80.0)	2.53 (0.80, 7.98)	1.47 (0.42, 5.17)

OR: odds ratio; CI: confidence interval

^a adjusted according to current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment

Table 4 Univariate and multivariate analysis for drug exposure - susceptibility ratio with treatment outcome and time to culture conversion

	Treatment outcome			Time to culture conversion		
	Successful (%)	OR (95%CI)	aOR (95%CI) ^a	Time to conversion (IQR)	HR (95%CI)	aHR (95%CI) ^a
Fluoroquinolones (n=157)						
The first quartile	22 (55.0)	1	1	20 (12.5, 24)	1	1
The second quartile	30 (76.9)	2.73 (1.03, 7.2)	2.09 (0.73, 6.04)	6 (4, 10)	2.14 (1.22, 3.74)	1.97 (1.10, 3.52)
The third quartile	35 (87.5)	5.73 (1.86, 17.64)	4.16 (1.19, 14.49)	2 (1, 6)	3.62 (2.10, 6.26)	3.82 (2.14, 6.83)
The fourth quartile	37 (97.4)	30.27 (3.78, 242.72)	24.81 (2.68, 229.61)	1 (1, 2.5)	6.26 (3.61, 10.9)	6.49 (3.53, 12.0)
Linezolid (n=168)						
The first quartile	25 (59.5)	1	1	14 (1.8, 24)	1	1
The second quartile	41 (97.6)	27.88 (3.49, 222.54)	30.29 (3.51, 261.57)	2 (1, 6)	2.71 (1.63, 4.49)	2.45 (1.45, 4.14)
The third quartile	37 (88.1)	5.03 (1.64, 15.4)	4.93 (1.45, 16.72)	4 (1.8, 10.5)	1.98 (1.19, 3.30)	1.79 (1.06, 3.03)
The fourth quartile	39 (92.9)	8.84 (2.35, 33.3)	8.94 (2.25, 35.56)	2 (1.8, 6)	2.58 (1.54, 4.31)	2.30 (1.35, 3.90)
Bedaquiline (n=70)						
The first quartile	16 (94.1)	1	1	10 (1.5, 14)	1	1
The second quartile	16 (88.9)	0.5 (0.04, 6.08)	0.92 (0.06, 15.18)	2 (1, 6.5)	1.31 (0.65, 2.64)	1.57 (0.76, 3.26)
The third quartile	16 (88.9)	0.5 (0.04, 6.08)	0.85 (0.06, 12.23)	1 (0.9, 2)	1.67 (0.83, 3.37)	1.96 (0.89, 4.33)
The fourth quartile	17 (100)	/	/	2 (1, 6)	1.98 (0.98, 4.02)	1.76 (0.76, 4.11)
Cycloserine (n=186)						
The first quartile	34 (72.3)	1	1	8 (2, 24)	1	1
The second quartile	38 (82.6)	1.82 (0.67, 4.91)	1.84 (0.62, 5.4)	6 (2, 20)	1.22 (0.77, 1.94)	1.17 (0.73, 1.87)
The third quartile	32 (68.1)	0.82 (0.34, 1.98)	0.9 (0.34, 2.38)	6 (2, 24)	0.98 (0.61, 1.60)	0.99 (0.61, 1.61)
The fourth quartile	42 (91.3)	4.01 (1.2, 13.44)	4.87 (1.28, 18.49)	2 (1, 4)	2.18 (1.38, 3.45)	2.06 (1.30, 3.27)
Clofazimine (n=136)						
The first quartile	28 (82.4)	1	1	6 (2, 15.5)	1	1
The second quartile	29 (85.3)	1.24 (0.34, 4.54)	0.83 (0.2, 3.45)	4 (1, 8.5)	1.29 (0.76, 2.17)	1.22 (0.72, 2.06)

The third quartile	25 (73.5)	0.6 (0.19, 1.91)	0.48 (0.13, 1.76)	3 (1, 24)	1.03 (0.6, 1.77)	0.98 (0.57, 1.69)
The fourth quartile	31 (91.2)	2.21 (0.51, 9.7)	2.14 (0.43, 10.74)	2 (1, 6)	1.7 (1.01, 2.84)	1.65 (0.98, 2.78)
Pyrazinamide (n=99)						
The first quartile	16 (64.0)	1	1	4 (4, 24)	1	1
The second quartile	12 (48.0)	0.52 (0.17, 1.61)	0.44 (0.13, 1.51)	24 (4, 24)	0.65 (0.31, 1.38)	0.64 (0.30, 1.37)
The third quartile	17 (68.0)	1.2 (0.37, 3.86)	1.15 (0.34, 3.94)	6 (2, 24)	1.17 (0.59, 2.32)	1.14 (0.57, 2.26)
The fourth quartile	23 (95.8)	12.94 (1.49, 112.4)	9.51 (1.04, 87.14)	2 (2, 5.5)	2.47 (1.30, 4.72)	2.24 (1.14, 4.39)
Prothionamide (n=86)						
The first quartile	14 (63.6)	1	1	7 (2, 24)	1	1
The second quartile	19 (90.5)	5.43 (1, 29.61)	8.16 (1.2, 55.34)	4 (2, 20)	1.69 (0.85, 3.37)	1.48 (0.73, 3.00)
The third quartile	18 (81.8)	2.57 (0.64, 10.31)	3.36 (0.7, 16.09)	2 (0.5, 9)	2.12 (1.05, 4.27)	2.46 (1.18, 5.09)
The fourth quartile	17 (81.0)	2.43 (0.6, 9.78)	2.72 (0.58, 12.75)	4 (2, 11)	1.57 (0.77, 3.19)	1.49 (0.72, 3.08)
Ethambutol (n=123)						
The first quartile	23 (74.2)	1	1	4 (2, 24)	1	1
The second quartile	22 (71.0)	0.85 (0.28, 2.6)	0.64 (0.19, 2.19)	4 (2, 24)	0.92 (0.51, 1.64)	0.78 (0.43, 1.43)
The third quartile	20 (64.5)	0.63 (0.21, 1.88)	0.48 (0.15, 1.58)	6 (2, 24)	0.84 (0.46, 1.52)	0.75 (0.41, 1.39)
The fourth quartile	27 (90.0)	3.13 (0.74, 13.2)	1.7 (0.36, 8.05)	2 (1, 6)	1.92 (1.10, 3.36)	1.58 (0.88, 2.84)

OR: odds ratio; IQR: interquartile range; HR: hazard ratio; CI: confidence interval

^a adjusted according to current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment

Table 5 Association between drug exposure – susceptibility targets and treatment response in patients with multidrug-resistant tuberculosis ^a

Drugs (AUC _{0-24h} /MIC)	Overall	Two-month sputum culture result		Six-month sputum culture result		Treatment outcome	
		Negative (%)	aOR (95%CI) ^b	Negative (%)	aOR (95%CI) ^b	Successful (%)	aOR (95%CI) ^b
Moxifloxacin (n=79)							
≤56	10 (12.7)	0 (0.0)	/	1 (10.0)	1	8 (80.0)	1
>56	69 (87.3)	34 (49.3)	/	45 (65.2)	15.6 (1.48, 165.0) ^c	60 (87.0)	1.15 (0.08, 6.95)
Levofloxacin (n=78)							
≤160	67 (85.9)	22 (32.8)	/	39 (58.2)	/	45 (67.2)	/
>160	11 (14.1)	11 (100.0)	/	11 (100.0)	/	11 (100.0)	/
Fluoroquinolones (n=157)							
≤target	77 (49.0)	22 (28.6)	1	40 (51.9)	1	53 (68.8)	1
>target	80 (51.0)	45 (56.3)	2.91 (1.42, 5.94) ^c	56 (70.0)	1.63 (0.79, 3.36)	71 (88.8)	2.89 (1.16, 7.17) ^c
Linezolid (n=168)							
≤119	5 (3.0)	0 (0.0)	/	0 (0.0)	/	0 (0.0)	/
>119	163 (97.0)	80 (49.1)	/	116 (71.2)	/	142 (87.1)	/
Cycloserine (n=186)							
≤25.8	18 (9.7)	7 (38.9)	1	8 (44.4)	1	11 (61.1)	1
>25.8	168 (90.3)	73 (43.5)	1.03 (0.36, 2.96)	111 (66.1)	2.21 (0.78, 6.32)	135 (80.4)	2.37 (0.78, 7.21)
Pyrazinamide (n=99)							
≤11.3	88 (88.9)	25 (28.4)	1	46 (52.3)	/	57 (64.8)	/
>11.3	11 (11.1)	10 (90.9)	31.4 (3.50, 281.1) ^c	11 (100.0)	/	11 (100.0)	/
Prothionamide (n=86)							
≤56.2	48 (55.8)	21 (43.8)	1	30 (62.5)	1	37 (77.1)	1
>56.2	38 (44.2)	19 (50.0)	1.63 (0.60, 4.45)	26 (68.4)	1.36 (0.51, 3.61)	31 (81.6)	1.49 (0.46, 4.79)
Ethambutol (n=123)							

Ethambutol (n=123)

≤119	123 (100.0)	52 (42.3)	/	79 (64.2)	/	92 (74.8)	/
>119	0 (0.0)	/	/	/	/	/	/

AUC_{0-24h}: area under drug concentration-time curve; MIC: minimum inhibitory concentration. “/” indicates the 95%CI was infinite due to the small number of patients in subgroup.

^a No AUC_{0-24h}/MIC targets were found for prothionamide, bedaquiline and clofazimine in published studies. As an alternative, the AUC_{0-24h}/MIC target of ethionamide was applied for prothionamide in this study.

^b adjusted according to current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment

^c $P < 0.05$ and 95%CI of OR did not include 1

Figure 1. Enrolment of patients with multidrug-resistant tuberculosis in this study

MDR-TB, multidrug-resistant tuberculosis

Group A drugs

Group B drugs

Group C drugs

Figure 2. Time to culture conversion among patients with multidrug-resistant tuberculosis grouped by quartiles of drug exposure – susceptibility ratio

Figure 3. Random Forest and Classification and Regression Tree analysis for six-month sputum culture conversion among patients receiving “Moxifloxacin+Linezolid+/-Bedaquiline” (A) and “Levofloxacin+Linezolid+/-Bedaquiline” (B) based regimen. AUC_{0-24h}: area under drug concentration-time curve; MIC: minimum inhibitory concentration; TTP: time to culture positivity.

S1 Microbiological laboratory work

Cultures

For the diagnosis of tuberculosis, primary isolation and culture were performed using the BD MGIT960 system according to the manufacturer (BACTEC MGIT 960, Becton Dickinson, Franklin Lakes, NJ, USA). After turning positive, MGIT 960 inoculum was directly used for phenotypic drug susceptibility testing and also inoculated onto Lowenstein-Jensen medium for determination of minimum inhibitory concentration.

Chemicals

All studied drugs were ordered in chemical pure form including: prothionamide (Riemser, Germany), bedaquiline (Janssen, USA), clofazimine (Sigma-Aldrich, Germany), linezolid (Sigma-Aldrich, Germany), moxifloxacin (Bayer Health Care AG, Germany), levofloxacin (Spring House, PA), cycloserine (Sigma-Aldrich, Germany), pyrazinamide (Sigma-Aldrich, Germany) and ethambutol (Sigma-Aldrich, Germany). As manufacturer recommended, all the chemicals were stored at -20°C. Levofloxacin was dissolved in deionized water, while moxifloxacin was in 0.1 M NaOH with subsequent dilutions in deionized water. All stock solutions were sterilized using 0.22 µm-pore sized membrane filtration (Millipore, Bedford, MA) and then stored at -80°C until use. The frozen solution was used immediately after thawing. The working solution was prepared freshly from the stock solution and the serial dilutions were carried out to achieve the desired concentrations.

Phenotypic drug susceptibility testing (DST)

The inoculum prepared from MGIT tubes was standardized to a 0.5 McFarland standard and 0.5ml of bacterial suspension was inoculated to each drug containing tube. For the drug-free growth control preparation, the *M. tuberculosis* suspension was diluted 1:100 in sterile saline and 0.5ml of dilution was inoculated (proportion testing). Due to specific pH requirements, pyrazinamide (PZA) susceptibility was determined using the BACTEC MGIT 960 PZA susceptibility test, with a pH of 5.9 as previously described (1). Referred to the WHO technical guideline, critical concentrations were used for the classification of strains into resistant or susceptible.

Minimum inhibitory concentration (MIC) determination

MIC determination was performed with MGIT 960 growth supplement in the BACTEC MGIT 960 system (Becton Dickinson) as well. Briefly, the bacterial suspension was prepared from 2- to 3-week fresh subcultures performed on Lowenstein-Jensen medium. A suspension of 1.0 McFarland Standard was prepared and allowed to rest for around 20 min in order for large bacterial clumps to settle. The supernatant was transferred to another sterile tube and allowed to rest for another 15 min before transferring the supernatant to another tube and adjusted to 0.5 mg/ml using M7H9. This suspension was diluted 1:5 in sterile physiological water to be used as MIC inoculum. The drug-free control was inoculated with 0.5 ml of a 1:100 dilution of inoculum representing 1% of the bacterial population. The MIC was determined to be the lowest drug concentration that tested susceptible.

Quality assurance

All these tests were done in duplicates of the studied isolates. In addition, H37Rv reference strains (ATCC 27294) were included in each batch for inter- and intra-run quality control. For internal quality assurance, DST was repeated for 10% of the isolates by the collaborating Jiangsu Province-level reference laboratory who had passed the WHO's external quality control with a consistency of over 98%. For external quality assessment, 5% of isolates were sent to Shanghai Public Health Clinical Centre, serving as WHO collaborating centre for clinical management, training and research on emerging and re-emerging infectious diseases. The consistency between the two rounds was over 95%.

Reference

1. WHO. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. 2018. [Available from: https://www.who.int/tb/publications/2018/WHO_technical_drug_susceptibility_testing/en/]

S2 LC-MS/MS methods for measurement of linezolid, bedaquiline, cycloserine and clofazimine

Plasma concentrations of linezolid, bedaquiline, cycloserine and clofazimine were determined using a validated liquid chromatography with tandem mass spectrometry method (LC-MS/MS) in Agilent co-constructed laboratory, department of environment science, Fudan University. The linezolid-d3, bedaquiline-d6, carbamazepine and clofazimine-d7 were used as the internal standards. Chromatographic separations were performed on a 30 mm Polaris C18 column (Agilent Technology) with a gradient elution. Mobile phases consisted of 25% 0.01 M ammonium formate (pH 4) and 75% methanol, with a flow rate of 1.5 ml/min. Positive ionization mode and multiple reaction monitoring were applied for detection of linezolid, bedaquiline, cycloserine and clofazimine with m/z of 338.01 $\rightarrow$ 296.03, 555.2 $\rightarrow$ 58.0, 335.9 $\rightarrow$ 157.2 and 473.8 $\rightarrow$ 431.4, respectively. The analytical ranges for linezolid, bedaquiline, cycloserine and clofazimine were 0.001~30, 0.001~40.0, 0.0033~40.0 and 0.004~40.0 mg/L, respectively, with good linearity of $r^2 > 99.83\%$ for all analyses. The inter- and intra-day variation ranged from 2.3% to 8.5% for all drugs.

S3 Random Forest, Classification and Regression Tree and modified Poisson regression analysis

To control heterogeneity within and between study populations, Random Forest and Classification and Regression Tree (CART) analysis were performed among patients receiving identical Group A-based regimen. A two-step approach was applied for data analysis. Firstly, Random Forest was used to generate relative variable importance ranking scores for each variable in prediction of six-month culture conversion. One thousand trees were conducted with out of bag data for testing. Variable importance was calculated on the basis of the number of times each variable was used for splitting the data. The most important variable was applied as the reference, i.e. a score of 100%, while subsequent variables were scored relative to the first one. Overall, 22 potential predictors were assessed in the model, including AUC_{0-24h}/MIC of levofloxacin, moxifloxacin, linezolid, bedaquiline, cycloserine, clofazimine, prothionamide, pyrazinamide and ethambutol, number of group A, group B and group C drugs, sex, age, weight, current smoking, diabetes mellitus type 2, baseline time to culture positivity, severe disease, extensive pulmonary disease, pulmonary cavity and baseline drug susceptibility profile.

Then, CART analysis was used to identify the key predictors predictive of six-month sputum culture conversion and to determine their cut-off values from the top ten variables identified in Random Forest. As a nonparametric method, CART analysis

uses binary recursive partitioning to assign patients to homogenous groups on the basis of Gini criterion function. The results were presented in the form of intuitive and easy-to-interpret decision trees, which had the advantage of handling missing data by identifying and using surrogate variables to minimize ascertainment bias. The root node, considered as the primary predictor, was applied for splitting nodes. The fully growing trees were pruned to avoid overmatching. The tree within one standard error of minimum was chosen as the optimal one, which is comparable to the minimum cost tree but less complex. We applied receiver operating characteristic (ROC) analysis and 10-fold cross-validation to evaluate the goodness-of-fit for each model.

Finally, given the advantages of numerically stable procedure and no convergence problem, modified Poisson regression model was applied to estimate risk ratio. Subsequently, the Cox proportional hazard regression model was used to calculate hazard ratio for the cut-off values derived from CART analysis. Both these two analyses were adjusted for current smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment.

Table S1. Demographic and clinical characteristics of patients with multidrug-resistant tuberculosis in different treatment response groups

Characteristics	No. (%) of patients with two-month sputum culture		No. (%) of patients with six-month sputum culture		No. (%) of patients with treatment outcome	
	Positive (n=109)	Negative (n=88)	Positive (n=69)	Negative (n=128)	Unfavourable (n=41)	Successful (n=156)
Age, years ^a	42.2±10.0	41.9±9.9	43.2±10.7	41.4±9.4	44.6±11.8	41.4±9.3
Sex						
Male	75 (53.6)	65 (46.4)	45 (32.1)	95 (67.9)	27 (19.3)	113 (80.7)
Female	34 (59.6)	23 (40.4)	24 (42.1)	33 (57.9)	14 (24.6)	43 (75.4)
Body mass index ^a	21.1±4.8	20.4±4.2	21.5±5.0	20.4±4.3	21.8±5.2	20.5±4.4
Current smoking						
No	43 (47.8)	47 (52.2)	24 (26.7)	66 (73.3) ^b	15 (16.7)	75 (83.3)
Yes	66 (61.7)	41 (38.3)	45 (42.1)	62 (57.9)	26 (24.3)	81 (75.7)
Diabetes type 2						
No	83 (52.9)	74 (47.1)	49 (31.2)	108 (68.8) ^b	29 (18.5)	128 (81.5)
Yes	26 (65.0)	14 (35.0)	20 (50.0)	20 (50.0)	12 (30.0)	28 (70.0)
Severe disease						
No	77 (54.6)	64 (45.4)	48 (34.0)	93 (66.0)	26 (18.4)	115 (81.6)
Yes	32 (57.1)	24 (42.9)	21 (37.5)	35 (62.5)	15 (26.8)	41 (73.2)
Extensive pulmonary disease						
No	85 (53.5)	74 (46.5)	53 (33.3)	106 (66.7)	29 (18.2)	130 (81.8)
Yes	24 (63.2)	14 (36.8)	16 (42.1)	22 (57.9)	12 (31.6)	26 (68.4)
TTP, days ^a	11.2±3.0	12.8±2.7 ^b	10.9±2.8	12.5±2.9 ^b	10.2±2.7	12.4±2.9 ^b
DST profile						
MDR-TB alone	91 (56.9)	69 (43.1)	62 (38.8)	98 (61.2) ^b	34 (21.3)	126 (78.7)
Pre-XDR-TB	18 (48.6)	19 (51.4)	7 (18.9)	30 (81.1)	7 (18.9)	30 (81.1)
Drug intake						
Levofloxacin	45 (57.7)	33 (42.3)	28 (35.9)	50 (64.1)	22 (28.2)	56 (71.8) ^b
Moxifloxacin	45 (57.0)	34 (43.0)	33 (41.8)	46 (58.2)	11 (13.9)	68 (86.1)
Linezolid	88 (52.4)	80 (47.6)	52 (31.0)	116 (69.0) ^b	26 (15.5)	142 (84.5) ^b
Bedaquiline	28 (40.0)	42 (60.0)	19 (27.1)	51 (72.9)	5 (7.1)	65 (92.9) ^b
Clofazimine	72 (52.9)	64 (47.1)	43 (31.6)	93 (68.4)	23 (16.9)	113 (83.1) ^b
Cycloserine	106 (57.0)	80 (43.0)	67 (36.0)	119 (64.0)	40 (21.5)	146 (78.5)
Prothionamide	46 (53.5)	40 (46.5)	30 (34.9)	56 (65.1) ^b	18 (20.9)	68 (79.1)
Pyrazinamide	64 (64.6)	35 (35.4)	42 (42.4)	57 (57.6)	31 (31.3)	68 (68.7) ^b
Ethambutol	71 (57.7)	52 (42.3)	44 (35.8)	79 (64.2)	31 (25.2)	92 (74.8)
Number of drugs in ^a						
Group A	1.9±0.7	2.2±0.6 ^b	1.9±0.7	2.1±0.6	1.6±0.5	2.1±0.6 ^b
Group B	1.6±0.5	1.6±0.5	1.7±0.5	1.6±0.5	1.7±0.5	1.6±0.5
Group C	2.0±1.1	2.1±1.1	2.0±1.0	2.0±1.1	2.0±0.9	2.0±1.1
Effective drugs ^a	5.6±0.6	5.7±0.7	5.7±0.6	5.6±0.7	5.6±0.6	5.7±0.7

DST: drug susceptibility testing; TTP: time to culture positivity recorded by the MGIT 960 system. Group A, B and C drugs were referred to the multidrug-resistant tuberculosis treatment guidelines released by the World Health Organization.

^a: mean $\pm$ standard deviation

^b: $P < 0.05$

Figure S1. Treatment responses in patients with multidrug-resistant tuberculosis receiving different type and number of Group A drugs

Table S2. Drug change and adverse events during the treatment in correspondence to the treatment categories

	No. (%) of Patients treated with			
	FQs+LZD+BDQ (n=40)	FQs+LZD (n=88)	LZD+BDQ (n=30)	Others (n=39)
Drug change during the treatment				
Group A regimen	3(7.5)	1(1.1)	2(6.7)	0(0.0)
Group B regimen	6(15.0)	4(4.5)	3(10.0)	3(7.7)
Group C regimen	0(0)	1(1.1)	0(0)	1(2.6)
Adverse events ^a				
Gastrointestinal disorders	13(32.5)	31(35.2)	10(33.3)	12(30.8)
Psychiatric disorders	5(12.5)	12(13.6)	6(20.0)	6(15.4)
Central nerve system disorders	1(2.5)	3(3.4)	3(10.0)	3(7.7)
Peripheral neuropathy	3(7.5)	6(6.8)	2(6.7)	1(2.6)
Liver injury	2(5.0)	7(8.0)	2(6.7)	5(12.8)
Kidney injury	1(2.5)	4(4.5)	2(6.7)	0(0.0)
Anaemia	5(12.5)	14(15.9)	5(16.7)	2(5.1)
QTc prolongation	12(30)	3(3.4)	8(26.7)	2(5.1)
Others (pain, skin hyperpigmentation, et al)	5(12.5)	14(15.9)	4(13.3)	6(15.4)

FQs: fluoroquinolones; LZD: linezolid; BDQ: bedaquiline

^a Adverse events were defined as follows:

Gastrointestinal disorders: presence of nausea, vomiting, abdominal pain, diarrhea, epigastric discomfort, hematemesis, melena, and positive endoscopic findings;

Psychiatric disorders: presence of depression, anxiety, psychosis, suicide, nightmares, and convulsion;

Central nervous system disorders: headache and seizure activity as reported by patient or witness;

Peripheral neuropathy: numbness, weakness, tingling, dizziness, burning/pain in the extremities, diagnosed by physician or electromyography;

Liver injury: alanine transaminase elevations above five times the upper limit of normal (ULN) or above three times the ULN with total bilirubin above 2 times the ULN;

Kidney injury: an abnormal estimated glomerular filtration rate based on serum creatinine using the Modification of Diet in Renal Disease formula;

Anaemia: haemoglobin level below 120 g/L for women and 130 g/L for men;

QTc prolongation: a corrected QT interval with the use of Fridericia's formula of more than 450 msec.

Table S3. Distribution for drug exposure - susceptibility ratio in patients with multidrug-resistant tuberculosis

	Two-month culture conversion			Six-month culture conversion			Treatment outcome		
	Positive (n=109)	Negative (n=88)	<i>P</i>	Positive (n=69)	Negative (n=128)	<i>P</i>	Unfavourable (n=41)	Successful (n=156)	<i>P</i>
Levofloxacin (n=78)									
C _{max} , mg/L	4.8 (4.4, 5.3)	4.6 (4.1, 5.1)	0.36	4.9 (4.4, 5.3)	4.7 (4.1, 5.2)	0.624	4.9 (4.2, 5.1)	4.7 (4.3, 5.3)	0.85
AUC _{0-24h} , mg·h/L	53.2 (44.9, 60.0)	51.6 (39.5, 62.8)	0.915	52.5 (45.1, 56.6)	52.2 (39.6, 62.1)	0.95	51.4 (44.3, 55.8)	53.2 (42.7, 62.4)	0.556
C _{max} /MIC	9.0 (4.9, 10.4)	9.8 (8.6, 22.0)	0.002	5.3 (4.5, 10.1)	9.6 (8.6, 11.8)	<0.001	5.0 (4.4, 8.1)	9.6 (8.9, 11.7)	<0.001
AUC _{0-24h} /MIC	93.3 (50.6, 114.3)	108.7 (90.0, 249.1)	0.002	63.3 (48.7, 107.4)	108.8 (90.1, 136.8)	<0.001	52.9 (44.3, 96.6)	108.8 (90.4, 134.0)	<0.001
Moxifloxacin (n=79)									
C _{max} , mg/L	3.0 (2.2, 4.0)	3.7 (3.1, 5.0)	0.01	2.8 (2.1, 3.9)	3.6 (3.0, 5.3)	0.007	3.0 (2.8, 6.5)	3.4 (2.7, 4.8)	0.91
AUC _{0-24h} , mg·h/L	45.8 (24.2, 60.6)	54.8 (47.2, 64.9)	0.02	46.4 (23.8, 60.3)	52.3 (44.5, 64.9)	0.054	49.1 (45.8, 88.6)	49.4 (40.0, 60.2)	0.346
C _{max} /MIC	10.8 (4.9, 19.5)	49.1 (38.3, 60.1)	<0.001	7.2 (4.6, 13.3)	43.0 (25.0, 57.0)	<0.001	9.5 (5.5, 13.5)	28.1 (10.1, 52.0)	<0.001
AUC _{0-24h} /MIC	177.1 (68.5, 268.1)	742.4 (476.9, 871.0)	<0.001	119.6 (51.6, 192.7)	624.8 (387.2, 823.9)	<0.001	159.0 (91.5, 194.7)	410.6 (155.0, 758.8)	0.016
Linezolid (n=168)									
C _{max} , mg/L	7.5 (7.1, 8.7)	7.6 (7.2, 8.6)	0.904	7.4 (6.1, 8.3)	8.0 (7.3, 8.8)	0.006	7.3 (5.4, 8.3)	7.6 (7.2, 8.8)	0.016
AUC _{0-24h} , mg·h/L	113.0 (92.4, 124.7)	114.5 (101.9, 125.4)	0.542	104.9 (73.9, 122.0)	117.0 (105.2, 126.5)	0.002	93.9 (64.9, 122.6)	114.6 (102.2, 125.5)	0.012
C _{max} /MIC	32.1 (28.5, 38.7)	33.7 (29.5, 51.9)	0.038	29.6 (18.4, 35.0)	34.4 (29.8, 51.9)	<0.001	27.3 (10.8, 33.6)	33.1 (29.5, 46.0)	<0.001
AUC _{0-24h} /MIC	472.2 (372.5, 569.7)	492.4 (432.5, 715.4)	0.039	429.6 (273.6, 503.1)	497.7 (439.0, 715.4)	<0.001	373.4 (129.7, 503.8)	492.4 (433.7, 617.9)	<0.001
Bedaquiline (n=70)									
C _{max} , mg/L	2.2 (1.2, 3.9)	2.7 (2.2, 3.9)	0.172	2.0 (1.1, 3.2)	2.7 (2.2, 4.1)	0.039	2.4 (1.6, 3.0)	2.6 (1.9, 4)	0.445
AUC _{0-24h} , mg·h/L	34.9 (15.3, 55.1)	44.1 (32.3, 56.5)	0.208	30.5 (13.0, 51.3)	43.7 (32.8, 58.0)	0.036	35.7 (21.6, 46.8)	42.1 (28.9, 57)	0.418
C _{max} /MIC	134.1 (75.5, 209.1)	180.7 (134.6, 254.9)	0.045	98.4 (64.4, 158.3)	180.2 (135.1, 268.7)	0.001	158.3 (104.6, 196.7)	167.8 (110.5, 256.2)	0.707
AUC _{0-24h} /MIC	2107.5 (914.7, 3275.4)	2901.0 (2103.2, 3765.2)	0.028	1527.2 (852.3, 2383.1)	2890.1 (2163.8, 3867.6)	0.001	2382 (1441.4, 3120.0)	2383.1 (1627, 3743.4)	0.657
Clofazimine (n=136)									
C _{max} , mg/L	1.0 (0.9, 1.6)	1.0 (0.9, 1.4)	0.711	1.0 (0.7, 1.5)	1.0 (0.9, 1.4)	0.473	1.1 (0.9, 1.6)	1.0 (0.9, 1.4)	0.445
AUC _{0-24h} , mg·h/L	16.3 (13.2, 23.8)	16.3 (12.1, 20.9)	0.456	16.5 (9.4, 22.7)	16.3 (13.3, 22.3)	0.554	18.5 (12.6, 23.9)	15.6 (12.7, 21.6)	0.364

C _{max} /MIC	4.2 (1.9, 9.0)	7.8 (3.4, 15.4)	0.009	3.8 (1.9, 8.1)	7.0 (3.2, 15.5)	0.005	6.3 (2.0, 12.6)	5.9 (2.5, 15.0)	0.495
AUC _{0-24h} /MIC	64.4 (30.2, 141.3)	115.7 (45.8, 235.1)	0.013	51.7 (26.8, 138.1)	101.1 (45.0, 235.6)	0.005	102.7 (34.4, 154.3)	94.8 (36.8, 228.6)	0.548
Cycloserine (n=186)									
C _{max} , mg/L	22.7 (15.4, 42)	27.9 (20.6, 43.0)	0.216	22.8 (11.7, 41.8)	24.1 (18.6, 43.0)	0.189	23.6 (15.8, 42.1)	23.0 (18.3, 42.8)	0.73
AUC _{0-24h} , mg· h/L	465.4 (257.3, 688.9)	563.3 (349.2, 718)	0.274	478 (187.9, 679.7)	478.3 (318.4, 718.3)	0.24	479.3 (263.9, 678.6)	478.2 (313.9, 716.4)	0.683
C _{max} /MIC	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	0.269	100.0 (93.6, 100.0)	100.0 (100.0, 100.0)	0.011	100.0 (95.2, 100.0)	100.0 (100.0, 100.0)	0.073
AUC _{0-24h} /MIC	86.2 (46.7, 152.4)	130.0 (65.5, 219.3)	0.004	79.2 (33.4, 119)	111.0 (65.0, 214.6)	0.001	90.7 (33.6, 144.6)	99.4 (59.3, 201.0)	0.048
Prothionamide (n=86)									
C _{max} , mg/L	1.3 (1.1, 1.5)	1.3 (1.2, 1.7)	0.225	1.3 (1.1, 1.5)	1.3 (1.2, 1.6)	0.514	1.2 (1.0, 1.4)	1.3 (1.2, 1.6)	0.08
AUC _{0-24h} , mg· h/L	21.6 (18.8, 25.2)	23.1 (21.0, 28.6)	0.158	20.9 (18.8, 25.5)	22.3 (20.5, 28.3)	0.306	20.2 (18.1, 24.4)	22.6 (20.7, 28.3)	0.06
C _{max} /MIC	2.2 (1.5, 4.5)	3.0 (2.1, 4.4)	0.239	2.2 (1.1, 4.4)	3.0 (2.0, 4.4)	0.186	2.0 (1.0, 4.2)	2.9 (2.0, 4.5)	0.14
AUC _{0-24h} /MIC	38.0 (28.8, 77.4)	51.4 (37.1, 72.4)	0.25	39.5 (18.7, 75.9)	51.1 (36.0, 73.4)	0.195	37.7 (16.4, 69.5)	51.0 (35.5, 76.0)	0.161
Pyrazinamide (n=99)									
C _{max} , mg/L	15.6 (12.4, 20.2)	16.5 (9.1, 19.2)	0.473	15.9 (12.6, 19.6)	16.3 (10.7, 20.4)	0.821	16.0 (13.2, 19.5)	16.0 (10.9, 20.4)	0.587
AUC _{0-24h} , mg· h/L	253.5 (191.7, 332.4)	253.5 (150.5, 310.5)	0.409	248.3 (195.4, 332.3)	255.1 (169.5, 316.2)	0.605	250.2 (215.7, 332.3)	254.3 (169.6, 323.0)	0.449
C _{max} /MIC	0.3 (0.2, 0.4)	0.5 (0.3, 0.7)	<0.001	0.3 (0.2, 0.3)	0.4 (0.2, 0.6)	0.004	0.2 (0.2, 0.3)	0.4 (0.2, 0.5)	0.001
AUC _{0-24h} /MIC	4.2 (3.1, 5.6)	7.7 (4.6, 11.9)	<0.001	4.1 (3.4, 5.4)	5.9 (3.5, 9.3)	0.014	3.9 (3.4, 5.2)	5.6 (3.5, 9.3)	0.005
Ethambutol (n=123)									
C _{max} , mg/L	2.1 (1.5, 3.1)	2.7 (1.8, 3.3)	0.097	2.1 (1.5, 3.5)	2.4 (1.8, 3.2)	0.507	2.1 (1.5, 3.8)	2.3 (1.6, 3.2)	0.998
AUC _{0-24h} , mg· h/L	19.4 (13.8, 26.4)	24.3 (15.5, 30.2)	0.132	19.5 (13.8, 37.9)	22.3 (15.4, 28.0)	0.839	19.4 (13.8, 40.3)	21.9 (14.4, 27.9)	0.749
C _{max} /MIC	2.1 (1.4, 2.6)	2.3 (1.6, 3.6)	0.024	2.0 (1.4, 2.3)	2.2 (1.5, 3.2)	0.061	2.0 (1.5, 2.3)	2.2 (1.5, 3.2)	0.192
AUC _{0-24h} /MIC	19.1 (12.7, 23.5)	21.5 (13.9, 31.8)	0.035	19.3 (12.9, 23.5)	20.2 (13.5, 27.7)	0.213	19.2 (12.7, 23.1)	20.1 (13.5, 27.6)	0.053

C_{max}: maximum drug concentration; AUC_{0-24h}: area under drug concentration-time curve; MIC: minimum inhibitory concentration. Results are shown median with interquartile range. Mann-Whitney U test was applied for comparison.

Figure S2. The distribution of minimum inhibitory concentrations for second-line anti-tuberculosis drugs in study patients with multidrug-resistant tuberculosis (n=197). Critical concentrations, recommended by the technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis 2018, were presented as the dotted lines.

Figure S3. The distribution of area under drug concentration-time curve (AUC_{0-24h}) for second-line anti-tuberculosis drugs in patients with multidrug-resistant tuberculosis

Table S4. Association between CART-derived drug exposure – susceptibility targets and six-month sputum culture conversion

	Modified Poisson regression model			Cox proportional hazards regression model		
	No. of patients with Sputum culture conversion in Month 6 (%)	RR (95%CI)	aRR (95%CI) ^a	Median Time to culture conversion (IQR)	HR (95%CI)	aHR (95%CI) ^a
“Moxifloxacin+Linezolid+/-Bedaquiline” (n=67)						
Moxifloxacin AUC _{0-24h} /MIC≤231	1 (3.8)	1	1	6 (6, 6)	1	1
Moxifloxacin AUC _{0-24h} /MIC>231	40 (97.6)	25.4 (3.7, 173.5)	24.5 (3.4, 176.2)	1 (1, 1)	65.9 (8.8, 493.0)	71.7 (9.3, 550.9)
“Levofloxacin+Linezolid+/-Bedaquiline” (n=61)						
Linezolid AUC _{0-24h} /MIC≤287	1 (10.0)	1	1	6 (6, 6)	1	1
Linezolid AUC _{0-24h} /MIC>287	42 (82.4)	8.2 (1.3, 53.1)	7.8 (1.2, 50.1)	2 (2, 2)	12.9 (1.8, 93.9)	13.4 (1.8, 100.3)

CART: classification and regression tree; IQR: interquartile range; RR: rate ratio; HR: hazard ratio.

^a adjusted according to smoking, diabetes mellitus type 2, time to culture positivity at baseline and effective drug numbers at the onset of treatment

Figure S4. The distribution of minimum inhibitory concentration (MIC) and probability of target attainment for anti-tuberculosis drugs in patients with multidrug-resistant tuberculosis. Patients were divided into four groups according to the quartiles of respective area under drug

concentration-time curve (AUC_{0-24h}). The critical concentrations for moxifloxacin, levofloxacin, linezolid, prothionamide, pyrazinamide and ethambutol were referred as 0.25, 1, 1, 2.5, 100 and 5 mg/L, respectively. No critical concentration was reported for cycloserine. (A) AUC_{0-24h}/MIC ratios of 56, 160, 119, 25.8, 11.3, 56.2 and 119 from *in vitro* studies were applied as the target for moxifloxacin, levofloxacin, linezolid, cycloserine, prothionamide, pyrazinamide and ethambutol, respectively. (B) AUC_{0-24h}/MIC ratios of 231 and 287 from this study were applied as the clinical target for moxifloxacin and linezolid, respectively.