Determination of Minimum Inhibitory Concentration (MIC) Breakpoints for second-line drugs associated with clinical outcomes in multidrug-resistant tuberculosis treatment in China

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Running title: MIC testing in MDR-TB treatment

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Abstract

Background Our study aims to identify the clinical breakpoints (CBPs) of second-line drugs (SLDs) above which standard therapy fails, in order to improve multidrug-resistant tuberculosis (MDR-TB) treatment.

Methods MICs of SLDs were determined for *M. tuberculosis* isolates, cultured from 207 MDR-TB patients in a prospective cohort study in China between January 2010 and December 2012. Classification and Regression Tree (CART) analysis was used to identify the CBPs predictive of treatment outcome.

Results Of the 207 MDR-TB isolates included in the present study, the proportion of isolates above the critical concentration recommended by WHO, ranged from 5.3% in pyrazinamide to 62.8% in amikacin. By selecting pyrazinamide as primary node (CBP: 18.75mg/L), 72.1% of sputum culture conversions at month four could be predicted. As for treatment outcome, pyrazinamide (CBP: 37.5 mg/L) was selected as primary node to predict 89% of the treatment success, followed by ofloxacin (CBP: 3 mg/L), improving the predictive capacity of the primary node by 10.6%. Adjusted by identified confounders, the CART derived pyrazinamide CBP remained the strongest predictor in the model of treatment outcome.

Conclusions Our findings indicated that the critical breakpoints of some second-line drugs and PZA might need to be reconsidered in order to better indicate MDR-TB treatment outcome.

Keywords: Minimum inhibitory concentration; Multidrug-resistant tuberculosis; China
**Introduction**

*Mycobacterium tuberculosis* (*Mtb*) is a major public health problem worldwide (1). The emergence of multidrug-resistant (MDR) *Mtb* strains have complicated the treatment and are associated with increased treatment failure (2). A reduction in the efficacy of second-line drug (SLD) against MDR-TB strains with resistance to SLDs has been described in observational studies (2, 3). Subtle changes in the drug susceptibility may be predictive of clinical failures, especially when the drug susceptibility testing (DST) result is at the borderline of the susceptibility range.

Susceptibility testing for *Mtb* is increasingly being utilized in diagnostic laboratories to guide TB treatment. However, there has been considerable debate regarding the critical concentrations used to define resistance of anti-tuberculosis drugs (4, 5). By now, the standard approach for identifying antibiotic susceptibility breakpoints has been the epidemiological cut-off method. This method is based on the minimum inhibitory concentration (MIC) distribution of a drug, which identifies the upper 95% cut-off point on the Gaussian curve of wild-type susceptible *Mtb* isolates (6-8). However, Gumbo et al, using Monte Carlo simulations, concluded that current critical concentrations of first-line drugs were over-optimistic and new susceptibility breakpoints should be defined considering microbiologic and clinical outcomes (4). Therefore, clinical outcome studies including MIC results are needed. Thus, the aim of this study was to identify the clinical breakpoints (CBPs) in a cohort of MDR-TB patients in China and to develop a decision tree to better predict treatment outcomes of MDR-TB patients.
Materials and Methods

Study design

We conducted a prospective cohort study including MDR-TB patients who visited two MDR-TB designated hospitals in China for treatment between January 2010 and December 2012. Patients were included if they had a positive acid-fast bacilli smear and defined as MDR-TB by the DST results before receiving fluoroquinolone-containing regimens and gave informed consent. Patients who were pregnant, below 18 or above 65 years of age, with impaired liver or renal function, receiving treatment with SLDs in the previous six months or infected with extensively drug resistant \textit{M. tuberculosis} strain were excluded. The patients were followed up monthly during the fluoroquinolone-containing treatment, which was given as a directly observed treatment short course (DOT).

Species identification and drug susceptibility testing

Sputum samples were decontaminated and digested with 2\% NaOH. The mixture was concentrated by centrifugation and inoculated on Lowenstein Jensen (LJ) medium. Species identification of mycobacteria was performed by conventional biochemical tests (9).

DST for 1\textsuperscript{st}-line anti-TB drugs was performed using the proportion method (10) on LJ medium with the following drug concentrations: isoniazid (INH) (0.2 mg/L), rifampicin (RIF) (40.0 mg/L), streptomycin (STR) (4.0 mg/L) and ethambutol (EMB) (2.0 mg/L). Furthermore, MIC testing for SLDs and pyrazinamide (PZA) was performed on the mycobacterial growth indicator tube (MGIT) 960 platform.
according to standard manufacturer protocols. Briefly, bacterial suspensions were transferred to serial 1:2 diluted MGIT tubes with range 1-32 mg/L for ofloxacin (OFX) and levofloxacin (LVX), range 0.5–256 mg/L for capreomycin (CAP), amikacin (AMK) and kanamycin (KAN) and range 6.2-400 mg/L for PZA. Control plate without any antibiotic were inoculated with 1:100 diluted bacterial suspensions. The MIC was defined as the lowest antibiotic concentration that showed less growth than 100 growth unit when 1:100 diluted control reached 400 growth unit. Duplicates of the pan-susceptible *Mtb* H37Rv reference strain were included in each run as an inter- and intra-replication quality control. The MIC determination was also repeated twice for 10% (*n*=20) of *in vitro* randomly selected isolates (45% resistant to FQs and 40% resistant to second-line injective drugs) to ensure reproducibility. We used the critical concentrations for MGIT-base DST recommended by WHO guideline and the substances mentioned above were bought from Sigma-Aldrich (St. Louis, MO). As there were no published WHO-recommended critical concentrations for KAN DST by MGIT 960 at the time of the study, we used 2.5 mg/L based on the existing literature (11).

**Outcome definition**

Treatment outcome was evaluated by two endpoints: 1. Sputum culture conversion within four months (early sputum culture conversion); 2. Cure or treatment completion by 2 years (treatment success) after commencement of second-line treatment. Sputum samples were cultured on LJ media by the TB lab in the TB designated hospital. Sputum culture conversion was defined as two consecutive negative cultures of samples taken at least 30 days apart with no subsequent recurrence of a positive culture (12). In accordance with WHO guidelines (13), *cure*
was defined as completion of treatment according to the Global Fund program protocol with at least five consecutive negative cultures from sputum samples, collected at least 30 days apart in the last 12 months of treatment. Treatment completion was defined as treatment done with fewer than five consecutive negative cultures in the last 12 months of treatment. Second-line treatment referred to the use of a treatment regimen comprising one or more drugs (except streptomycin) listed in groups 2 to 5 of the WHO classification (14).

Classification and regression tree (CART) analyses

We utilized CART analysis to identify MIC predictive of treatment outcome for each SLDs and to develop the decision tree to predict sputum culture conversion after four months of treatment as well as treatment outcome. CART analysis is a non-parametric method that uses binary recursive partitioning to assign patients to homogenous groups and then present results in the form of intuitive and easy-to-interpret decision trees (15-17). Furthermore, CART analysis has the advantage of handling missing data by identifying and using surrogate variables to minimize ascertainment bias (18). CART analysis searches through potential predictors and all possible cut-off values of the variables to identify the best predictor for classifying between patients with and without the designated outcome (i.e. sputum culture conversion after four months of treatment and treatment outcome). This results in an upside-down ‘tree’ whose root node is the primary predictor. We utilized the Gini criterion function for splitting nodes and attaining the minimum cost tree. The resulting trees were pruned to improve the predictive value of the models by using the receiver operator curve (ROC) score of both the training and test samples. The optimal trees were then chosen, based on relative misclassification costs, complexity and parsimony. We performed 10-fold...
validation of the results as previously described (5). In the cross validation, the dataset was randomly split into learning and test databases, and CART analysis was performed using Salford Predictive Miner System software (San Diego, CA, USA).

Data collection and analysis

Age, sex, history of prior TB, year of diagnosis and site of TB disease were obtained from the provincial reportable diseases registry. Information on deaths and the dispensing of TB drugs was obtained from the death registration system. Furthermore, hospital medical records were reviewed to obtain additional clinical, epidemiological, treatment and outcome data. Absent clinical outcomes were due to lack of patient examination or loss to follow-up during the treatment phase. Duration of treatment was defined as months from the first to the last anti-tuberculosis drug dispensed. Multivariate models were reviewed for appropriateness using the Hosmer–Lemeshow goodness-of-fit test. IBM SPSS 20.0 (IBM Corp., Armonk, NY) was used to perform univariate analysis and logistic regression.

Results

Demographics and clinical characteristics

All pulmonary TB cases were reviewed during the study period. In total, 226 patients with MDR-TB were identified during the period. Of these, six patients were immediately transferred to other hospitals and 13 had received SLDs previously. As a result, 207 diagnosed MDR-TB patients were included in the study. The mean [standard deviation presented] age was 50.1 years (±16.9), and 66.7% of the patients were male. Of the 207 studied MDR patients, 72 (34.8%) were previously treated with
first line anti-TB drugs. The most common comorbidity was cardiovascular disease (15.9%), followed by diabetes (11.1%).

**Treatment outcome**

With regard to sputum culture conversion, 121 patients (58.5%) still had a positive culture or smear after the first four months of treatment. As for treatment outcome of the 185 patients, 22 patients were lost to follow up, 68 patients (36.8%) had treatment failure (43 patients were persistently sputum smear positive, two patients died and 23 patients relapsed). During the follow-up period, another four patients died, three from cardiovascular disease and one patient died of lung cancer.

**MIC distribution of the *Mtb* isolates for SLDs and PZA among the MDR-TB patients**

The MIC distributions for SLDs and PZA are presented in Figure 1. The median ofloxacin (OFX) and levofloxacin (LVX) MICs were 2 mg/L (range 1-32 mg/L) and 1 mg/L (range 0.25-16 mg/L) respectively, with 49.0% and 13.0% of the *Mtb* isolates above the respective DST critical concentrations of 2 mg/L for OFX and 2 mg/L for LVX. As for the injectable drugs, the median MICs were 2 mg/L (0.5-128 mg/L) for KAN, 4 mg/L (0.5-128 mg/L) for AMK and 1 mg/L (0.5-128 mg/L) for CAP, while 48.3%, 62.8% and 15.5% of the *Mtb* isolates were above the DST critical concentrations for KAN (2.5 mg/L), AMK (1 mg/L) and CAP (2.5 mg/L) respectively. For PZA, the median MIC was 25 mg/L (6.2-400 mg/L) and 5.3% of *Mtb* isolates were above the DST critical concentrations of 100 mg/L.

Our tentative CBPs with respect to sputum culture conversion by four months and
treatment outcome are summarized in Table 2. The CBPs for OFX (3 mg/L), LVX (1.5 mg/L) and CAP (5 mg/L) were close to WHO-recommended DST critical concentrations, while the CBP for PZA (37.5 mg/L) in our study was lower than the WHO-recommended DST critical concentration.

**Identification of MIC decision tree to predict treatment outcome**

Since combination therapy is used for MDR-TB treatment, we included all the MIC values of SLDs to illustrate a decision tree to predict treatment outcome. By selecting PZA as primary node (CBP: 18.75 mg/L), 72.1% of the sputum culture conversion at month four could be correctly predicted. As for the treatment outcome, pyrazinamide was selected as primary node (CBP: 37.5 mg/L) to predict 89% of patients with treatment success, followed by OFX (CBP: 3 mg/L) improving the predictive capacity of primary node by 10.6%. The strongest predictor in treatment outcome of MDR-TB was an MIC level of 37.5 mg/L for PZA, with the variable importance of 100.0%, while the variable importance of the second node of OFX was just 9.1%. Overall, the decision tree was capable of predicting 83.5% of the failure to sputum culture convert by month four and predicting 85.3% of the treatment failure, with a specificity of 72.1% and 94.9% respectively. Based on the test samples, the ROC score of short-term and long-term treatment efficacy CART model was 0.777 and 0.946, respectively.

**Evaluation of decision tree in a multivariate model of MDR-TB treatment outcome**

Since several factors affect treatment outcome, we compared the distribution of these factors between patients with *Mtb* isolates with an MIC above our CART
analysis-derived breakpoints and those below (Supplemented Table 1). The two groups of patients had very similar risk factors, except for pulmonary cavities and severe pulmonary disease on chest X-ray (CXR), which were more frequent in the group of patients with *Mtb* isolates with higher MICs. After adjusting for these variables, any differences in the sputum culture conversion by four month and treatment outcome can be attributed to the CART analysis-derived susceptibility breakpoints.

Adjusted for cavity and severe pulmonary disease on CXR in the Binary Logistical Regression model comparing the group of patients with *Mtb* isolates with higher and lower MICs than the CART-derived breakpoints, the CBPs value of PZA (OR=0.07, 95% CI 0.04-0.15), OFX (OR=0.48, 95% CI 0.27-0.84) and AMK (OR=0.46; 95% CI: 0.26-0.81) were the most statistically significant indicators for sputum culture conversion after four months of treatment (Table 3). The association between the CBPs of PZA, OFX, LVX, KAN and MDR-TB treatment success was strong and the odds ratios (95% CI) for these drugs were 0.01 (0.003-0.03), 0.30 (0.15-0.57), 0.30 (0.13-0.69), 0.29 (0.15-0.55), respectively. Furthermore, the CART decision tree remained to be the strongest indicator for the four month treatment sputum conversion (OR: 0.07; 95% CI: 0.04-0.15) as well as treatment success (OR: 0.01; 95% CI: 0.002-0.02). Additionally, *Mtb* isolates with MICs below the DST critical concentrations for PZA, OFX and AMK were associated with a higher rate of sputum culture conversion after four months of treatment and treatment success, although the association was weaker than our suggested CBPs. Furthermore, the DST critical concentrations of CAP and KAN were more likely to predict sputum culture conversion after four months of treatment rather than the treatment outcome.
Other factors associated with the treatment outcome

The frequency of elderly patients (≥65 years) in the treatment failure group and the treatment success group was not significantly different (22.1% vs. 21.4%; P = 0.912). Patients with previous treatment history were more frequent in the treatment failure group than in the treatment success group, and this was statistically significant (61.8%) vs. 29 (24.8%); P = 0.000]. Furthermore, the severity of the disease was also significantly associated with the treatment failure.

Discussion

Susceptibility testing is an important guide for clinician to do the evaluation of likely patient response to a particular drug (3). The critical concentration of SLDs recommended by WHO are based on wild-type susceptible *Mtb* isolates. However, our research indicates that the MIC distribution of MDR-TB isolates show disparity with the MIC distribution summarized by EUCAST (Fig. 1). These differences may be caused by phenotypic characteristics or genetic mutations in MDR-TB isolates. Considering the low cure rates of MDR-TB patients, guiding treatment merely based on efficacy of bacterial killing may not be sufficient. Therefore, we performed CART analyses to identify tentative CBPs of SLDs and develop an MIC decision tree to better predict sputum culture conversion after four months of treatment and the treatment outcome.

We propose that the CBPs of some of the SLDs, as well as PZA, should be considered to be lower than the current standard. MDR-TB treatment is guided by DST result in China. However, the current DST critical concentration is selected based on epidemiological cut-off value (ECOFF) (5), which is used to define microbiological
resistance. However as indicated in our study, the MIC above which therapeutic failure occurs is not necessarily linked to the ECOFF derived from MIC distribution. As previous authors suggest (19), the DST critical concentration of PZA (100 mg/L) need to be lowered and we also propose the inclusion of an intermediate category showing MICs at 64-128 mg/L. In our study, the CBPs for LVX and PZA were close to the mean MIC; thus, half of the patients in our current study would be considered to have isolates with LVX or PZA clinical resistance. Moreover, our proposed breakpoints are based on the failure of patients to respond to therapy and is therefore not defined by chromosomal mutations in the classic resistance genes, as is the case with Gene-Xpert or or line probe assays, such as GenoType(R) MTBDRplus and sl (Hain Life science, Nehren, Germany). Not all drug resistance is due to mutations; the MICs for some Mtb isolates are naturally high, while other mechanisms of drug resistance, such as efflux pump induction, could also lead to drug resistance (20, 21). Our results suggest that adjustment of critical concentrations of some SLDs should be considered to better guide MDR-TB treatment.

We assessed whether CBPs derived by CART analysis can improve predictive accuracy to better guide MDR-TB treatment. In Figure 1, the CBP of AMK derived by CART analysis was 96 mg/L, while the ECOFF is much lower, 1.0 mg/L. The predictive sensitivity and specificity of the CBP for AMK was 98.3% and 11.8%, respectively. In other words, 98.3% of the treatment success, and only 11.8% of the treatment failure, can be predicted correctly. Therefore, the clinical significance of the CBP of AMK deserves further demonstration especially in local settings. The CBP of PZA showed excellent accuracy (sensitivity: 88.9%; specificity: 92.6%) with more potential clinical significance compared to other SLDs, including LVX, CAP, AMK
and KAN. However, MDR-TB treatment consists of four to five effective drugs, so
the decision tree based on all SLDs may be more useful than those based on a single
drug.

In our study, the decision tree based on PZA and/or MICs of SLDs had excellent
predictive accuracy of clinical outcomes, both after four months treatment and at the
end of MDR-TB treatment. This relationship has been shown in previous studies (2)
(22), as well as a large meta-analysis. In addition, there were no differences in
important variables related to treatment outcome, except for the higher frequency of
pulmonary cavities and severe pulmonary disease on CXR in the group of patients
with MICs above the CART derived breakpoints. After adjustment for these variables,
the treatment outcome of MDR-TB is mainly influenced by the drug susceptibilities
of the \textit{Mtb} isolate. \textit{In vitro} data have suggested that PZA or FQs may be less active
against \textit{Mtb} strains with higher MIC (23, 24). Strains with high MIC of a drug might
have a thicker cell wall, which could cause a suboptimal response to the drug.(25).
Although high drug MIC in \textit{Mtb} isolates has been related to previous exposure to
these drugs (26), none of the patients with high MICs in our study had been exposed
to SLDs in the previous six months. This emphasizes the necessity of adequate
management of these patients. As expected, clinical manifestations, like the presence
of pulmonary cavity and severe pulmonary disease on CXR, were associated with
treatment outcome. Despite the importance of these variables, the relationship
between high MIC for PZA and negative treatment outcome remained in the
multivariate model. Therefore, as with many other pathogens such as standard
Gram-negative and Gram-positive bacteria, the MICs of anti-tuberculosis drugs and
their interaction could affect clinical outcome of MDR-TB.
Causal inferences between the MICs of the SLDs and worse treatment outcomes should be made with caution. Although drug resistance requires modification of drug regimen and is known to be associated with worse treatment outcomes, other factors, such as non-adherence, medical comorbidities and pharmacokinetic variability, also contribute to poor outcome of tuberculosis treatment (2) (27) (28) (29). Several factors showed at least a weak association with early sputum culture conversion or treatment outcome. In a univariate analysis, we found that prior TB treatment was significantly associated with both the failure to sputum culture convert by four months of treatment and poor treatment outcome, highlighting the importance of appropriate treatment for MDR-TB. The relationship between the previous TB treatment and poor treatment outcome had been confirmed already in a meta-analysis (2). Additionally, indicators of disease severity, such as cavity and severe pulmonary disease on CXR, increase the risk of poor treatment outcome by inadequate penetration of the drug into the most diseased tissue, due to the damaged lung parenchyma (31-33). These factors might also need to be taken into account when predicting treatment outcome of MDR-TB.

Our study has some limitations. Firstly, the relatively small sample size could limit the generalizability of the findings. However, CART has been able to correctly identify thresholds with similarly small populations in the past (19). Secondly, several other clinical factors also determine clinical outcomes, such as the presence of pulmonary cavities. However, these factors do not exclude a role for MICs in outcome prediction. Indeed, our CART analysis also examined some other possible predictors but they were outranked by MICs. Thirdly, one potential limitation of CART analysis is fitting and biasing toward covariates with many possible splits. Thus, our findings
should be taken with these factors in mind. Nevertheless, cross-validation identified the same MIC thresholds, which were virtually identical to Monte Carlo simulation results published in recent years (4). In other words, the same breakpoints have now been identified by two different methods. Another limitation is the absence of pharmacokinetics-pharmacodynamics (PK/PD) data in our study. Measuring drug concentrations is not standard of care in most medical facilities in China. However, population-based study design reduces the possible bias from the PK/PD variables to some degree. We have an ongoing prospective clinical study where PK/PD data as well as MIC distributions will be available and the clinical significance of these variables in MDR-TB treatment can be determined.

Conclusions

This study revealed that MDR-TB patients infected by an Mtb isolate with higher MIC (especially for PZA and FQs) compared to patients whose isolates had lower MICs, had increased risk of negative treatment outcome. These results suggest that the critical concentration of PZA could be reconsidered. In addition, the use of an MIC decision tree might have significance in guiding MDR-TB treatment.

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Conflict of interest

We declare no conflict of interest.
Reference


Table 1 Clinical and demographic factors of 207 patients treated for tuberculosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (N=207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>69 (33.3)</td>
</tr>
<tr>
<td>Age, years(^a)</td>
<td>50.1 ± 16.9</td>
</tr>
<tr>
<td>Prior tuberculosis treatment</td>
<td>72 (34.8)</td>
</tr>
<tr>
<td>Pulmonary cavity</td>
<td>56 (27.1)</td>
</tr>
<tr>
<td>Severe pulmonary disease on CXR</td>
<td>35 (16.9)</td>
</tr>
<tr>
<td>Extra pulmonary tuberculosis</td>
<td>36 (17.4)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>58 (28.0)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>33 (15.9)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>23 (11.1)</td>
</tr>
</tbody>
</table>

\(^a\) Continuous variable, mean ± standard deviation presented.

Note: CXR: chest X-ray

Data are presented as No. (%) unless otherwise specified.
Table 2, CART analysis-derived MIC clinical breakpoints for *Mtb* isolates from 207 MDR-TB patients.

<table>
<thead>
<tr>
<th>2&lt;sup&gt;nd&lt;/sup&gt; drug</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; month culture conversion</th>
<th>Treatment success</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC CBP (mg/L)</td>
<td>Se</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>18.75</td>
<td>0.721</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>3</td>
<td>0.616</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>5</td>
<td>0.930</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>48</td>
<td>0.977</td>
</tr>
<tr>
<td>Amikacin</td>
<td>3</td>
<td>0.523</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>96</td>
<td>0.977</td>
</tr>
</tbody>
</table>

Note: CBPs: clinical breakpoints
Table 3. Univariate and multivariable analysis of factors associated with MDR-TB treatment outcome

<table>
<thead>
<tr>
<th></th>
<th>Univariate logistic regression analysis</th>
<th>Multivariate logistic regression analysisa</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>4th month sputum culture conversion</td>
<td>Treatment success</td>
</tr>
<tr>
<td></td>
<td>P OR 95%CI</td>
<td>P OR 95%CI</td>
</tr>
<tr>
<td>CART Model</td>
<td>0.000 0.08 0.04-0.15</td>
<td>0.000 0.01 0.002-0.02</td>
</tr>
<tr>
<td></td>
<td>0.000 0.07 0.04-0.15</td>
<td>0.000 0.01 0.002-0.02</td>
</tr>
<tr>
<td>PZA CBP</td>
<td>0.000 0.08 0.04-0.15</td>
<td>0.000 0.01 0.003-0.03</td>
</tr>
<tr>
<td></td>
<td>0.000 0.07 0.04-0.15</td>
<td>0.000 0.01 0.003-0.03</td>
</tr>
<tr>
<td>PZA DST CC</td>
<td>0.000 0.32 0.18-0.58</td>
<td>0.000 0.24 0.13-0.45</td>
</tr>
<tr>
<td></td>
<td>0.000 0.32 0.18-0.59</td>
<td>0.000 0.23 0.12-0.45</td>
</tr>
<tr>
<td>OFX CBP</td>
<td>0.010 0.48 0.27-0.84</td>
<td>0.000 0.32 0.17-0.60</td>
</tr>
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<td></td>
<td>0.010 0.48 0.27-0.84</td>
<td>0.000 0.30 0.15-0.57</td>
</tr>
<tr>
<td>OFX DST CC</td>
<td>0.003 0.27 0.11-0.65</td>
<td>0.001 0.24 0.10-0.56</td>
</tr>
<tr>
<td></td>
<td>0.003 0.27 0.11-0.65</td>
<td>0.001 0.23 0.10-0.55</td>
</tr>
<tr>
<td>LVX CBP</td>
<td>0.034 0.36 0.14-0.93</td>
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<td>0.032 0.35 0.13-0.91</td>
<td>0.005 0.30 0.13-0.69</td>
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<tr>
<td>LVX DST CC</td>
<td>0.275 0.57 0.21-1.56</td>
<td>0.388 1.81 0.47-6.91</td>
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<tr>
<td></td>
<td>0.262 0.56 0.21-1.54</td>
<td>0.554 1.51 0.38-5.98</td>
</tr>
<tr>
<td>CAP CBP</td>
<td>0.036 0.20 0.04-0.90</td>
<td>0.025 0.41 0.19-0.89</td>
</tr>
<tr>
<td></td>
<td>0.037 0.20 0.04-0.90</td>
<td>0.015 0.36 0.16-0.82</td>
</tr>
<tr>
<td>CAP DST CC</td>
<td>0.004 0.11 0.03-0.50</td>
<td>0.949 0.97 0.34-2.79</td>
</tr>
<tr>
<td></td>
<td>0.003 0.11 0.03-0.48</td>
<td>0.657 0.78 0.26-2.32</td>
</tr>
<tr>
<td></td>
<td>KAN CBP</td>
<td>KAN DST CC</td>
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<tr>
<td></td>
<td>0.015</td>
<td>0.16</td>
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<tr>
<td></td>
<td>0.04-0.70</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>0.17-0.60</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>0.173</td>
</tr>
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<td></td>
<td>0.16</td>
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<tr>
<td></td>
<td>0.04-0.69</td>
<td>0.30-1.24</td>
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<tr>
<td></td>
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<td></td>
<td>0.29</td>
<td>0.11-0.59</td>
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<td></td>
<td>0.15</td>
<td>0.50</td>
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<tr>
<td></td>
<td>0.055</td>
<td>0.24-1.06</td>
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* Adjusted for the presence of pulmonary cavity and severe pulmonary disease on CXR.

Note: CBP: clinical breakpoints; CC: critical concentration.
Figure 1. MIC distribution of second-line drugs for Mtb isolates from 207 MDR-TB patients. The Gaussian distribution is skewed towards the right and different from the MIC distribution summarized by EUCAST.

\(^{a}\) The critical concentration of DST recommended by WHO except kanamycin, which...
was derived from existing literature (11)

b The suggested clinical breakpoints (CBPs) derived by CART analysis

Note: CBP: clinical breakpoint; CC: critical concentration
Figure 2. Variables predictive of sputum culture conversion after four months of treatment (A) and long-term treatment outcome (B) in 207 and 185 MDR-TB patients, respectively. MICs of second-line anti-TB drugs and confounders were examined in the classification and regression trees. In tree A, MIC of pyrazinamide (18.75 mg/L) was the best predictors of sputum culture conversion after four months of treatment. Furthermore, 75.6% of patients with a pyrazinamide MIC below threshold were sputum negative after four months of treatment. In tree B, the decision nodes demonstrate that the primary node was the MIC of pyrazinamide (37.5 mg/L), followed by ofloxacin MIC. The MIC cutoff values that were identified as important predictive factors are shown. In those who had an MIC of pyrazinamide below the threshold (37.5 mg/L), only 4.6% failed the treatment.