# Supplemental Material

## Pyrazinamide Resistance Assays and Two-Month Sputum Culture Status in MDR-TB Patients

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**Table S1.** List of *pncA* mutations for MDR-TB isolates, with corresponding DST results and two-month outcomes[a]

| Mutation | Isolates (*N = 121*) | Wayne Method No. Resistant (%) | BACTEC™ MGIT™ 960 No. Resistant (%)[b] | Two-Month Smear No. Positive/Tested (%) | Two-Month Culture No. Positive/Tested (%)[c] |
|---|---|---|---|---|---|
| R2W | 1 | 0 (0.0) | 0 (0.0) | 1/1 (100.0) | 1/1 (100.0) |
| L4S | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| D8A | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| D8E | 2 | 1 (50.0) | 2 (100.0) | 2/2 (100.0) | 2/2 (100.0) |
| Q10R | 19 | 16 (84.2) | 17 (89.5) | 13/19 (68.4) | 17/19 (89.5) |
| A46E | 1 | 1 (100.0) | 0 (0.0) | 1/1 (100.0) | 1/1 (100.0) |
| K48T | 1 | 0 (0.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| indel 48 | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| D49N | 3 | 2 (66.7) | 2 (66.7) | 1/3 (33.3) | 2/3 (66.7) |
| H51R | 13 | 10 (76.9) | 12 (92.3) | 6/13 (46.2) | 9/12 (75.0) |
| H57L | 3 | 3 (100.0) | 2 (66.7) | 2/3 (66.7) | 1/3 (33.3) |
| H57P | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| H57R | 2 | 1 (50.0) | 2 (100.0) | 2/2 (100.0) | 1/2 (50.0) |
| indel 65 | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| L85P | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| indel 98 | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| indel 130 | 1 | 0 (0.0) | 1 (100.0) | 0/1 (0.0) | 0/1 (0.0) |
| V131G | 2 | 1 (50.0) | 2 (100.0) | 1/2 (50.0) | 1/1 (100.0) |
| T135A/P54L | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| T135P | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| D136Y | 1 | 0 (0.0) | 0 (0.0) | 1/1 (100.0) | 1/1 (100.0) |
| V139A | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| R140P | 2 | 2 (100.0) | 2 (100.0) | 1/2 (50.0) | 2/2 (100.0) |
| L159R | 1 | 1 (100.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| L159V | 1 | 0 (0.0) | 0 (0.0) | 0/1 (0.0) | 0/1 (0.0) |
| T160P | 1 | 1 (100.0) | 1 (100.0) | 0/1 (0.0) | 1/1 (100.0) |
| indel 170 | 1 | 0 (0.0) | 1 (100.0) | 0/1 (0.0) | 1/1 (100.0) |

| Mutation | Isolates (N = 121) | Wayne Method No. Resistant (%) | BACTEC™ MGIT™ 960 No. Resistant (%)[b] | Two-Month Smear No. Positive/Tested (%) | Two-Month Culture No. Positive/Tested (%)[c] |
|---|---|---|---|---|---|
| A171T | 1 | 0 (0.0) | 1 (100.0) | 1/1 (100.0) | 1/1 (100.0) |
| V180F | 2 | 1 (50.0) | 2 (100.0) | 2/2 (100.0) | 2/2 (100.0) |
| STOP187W | 1 | 1 (100.0) | 1 (100.0) | 0/1 (0.0) | 0/1 (0.0) |
| Any Mutation | 69 | 51 (73.9) | 60 (87.0) | 46/69 (66.7) | 55/67 (82.1) |
| Wild type | 52 | 9 (17.3) | 11 (21.2) | 25/52 (48.1) | 32/49 (65.3) |
| **Total** | **121** | **60 (49.6)** | **71 (58.7)** | **71/121 (58.7)** | **87/116 (75.0)** |

[a] DST, drug susceptibility testing; MDR-TB, multidrug-resistant tuberculosis; MGIT, mycobacteria growth indicator tube; MIC, minimum inhibitory concentration.

[b] The DST results shown use a BACTEC™ MGIT™ 960 method MIC >100 mg/L to indicate resistance to pyrazinamide.

[c] Two (1.7%) patients had a missing two-month culture result due to a contaminated sample, and three (2.5%) patients for an unknown reason.

**Table S2.** PZA resistance assay test characteristics among 55 EMB-resistant MDR-TB patients, using a consensus gold standard derived from a LCA model as the comparator[a]

| PZA resistance assay | Sensitivity % (95% CI) | Specificity % (95% CI) | AUC of ROC (95% CI) |
|---|---|---|---|
| Wayne method | 79.5 (63.5-90.7) | 100.0 (79.4-100.0) | 0.90 (0.83-0.96) |
| BACTEC™ MGIT™ 960 | 94.9 (82.7-99.4) | 87.5 (61.7-98.4) | 0.91 (0.82-1.00) |
| *pncA* sequencing | 97.4 (86.5-99.9) | 75.0 (47.6-92.7) | 0.86 (0.75-0.97) |

[a] AUC, area under the curve; CI, confidence interval; EMB, ethambutol; LCA, latent class analysis; MDR-TB, multidrug-resistant tuberculosis; MGIT, mycobacteria growth indicator tube; PZA, pyrazinamide; ROC, receiver operating characteristic.

**Table S3.** Univariate predictors of two-month culture positivity among unsuspected EMB-resistant MDR-TB patients receiving standard first-line antituberculous therapy[a]

| Variable | Two-month culture (*n = 54*)[b] | |
| --- | --- | --- |
| | No. (%)[c] | Crude OR (95% CI)[d] |
| Age, yr | 54 (100) | 1.01 (0.96-1.07) |
| Sex | | |
|    Female | 21 (38.9) | 1.70 (0.30-9.67) |
|    Male | 33 (61.1) | Reference |
| Smoking | | |
|    Yes | 0 (0) | N/A |
|    No | 53 (98.1) | Reference |
|    Unknown | 1 (1.9) | N/A |
| Alcohol use | | |
|    Yes | 21 (38.9) | 0.89 (0.18-4.45) |
|    No | 31 (57.4) | Reference |
|    Unknown | 2 (3.7) | N/A |
| Previous TB treatment | | |
|    Yes | 14 (25.9) | 2.29 (0.25-20.9) |
|    No | 40 (74.1) | Reference |
| Baseline smear status | | |
|    Positive | 48 (88.9) | 1.40 (0.14-14.1) |
|    Negative | 6 (11.1) | Reference |
| Cavitary disease | | |
|    Yes | 12 (22.2) | 0.32 (0.06-1.71) |
|    No | 41 (75.9) | Reference |
|    Unknown | 1 (1.9) | N/A |
| Baseline PZA resistance (Wayne) | | |
|    Yes | 31 (57.4) | **10.6 (1.17-95.5)** |
|    No | 23 (42.6) | Reference |
| Baseline PZA resistance (MGIT™) | | |
|    Yes | 39 (72.2) | *4.36 (0.84-22.5)* |
|    No | 15 (27.8) | Reference |
| Baseline PZA resistance (*pncA*) | | |
|    Yes | 42 (77.8) | **6.50 (1.21-34.8)** |
|    No | 12 (22.2) | Reference |
| Baseline PZA resistance (Consensus) | | |
|    Yes | 39 (72.2) | *4.36 (0.84-22.5)* |
|    No | 15 (27.8) | Reference |

[a] CI, confidence interval; EMB, ethambutol; MDR, multidrug resistant; MGIT, mycobacteria growth indicator tube; OR, odds ratio; PZA, pyrazinamide; TB, tuberculosis.
[b] One (1.8%) patient had a missing two-month culture result due to a contaminated sample.
[c] The sample size varies across covariates due to missing data. Because of rounding, the sum of percentages may not equal 100%.
[d] *P* values < 0.05 are shown in bold, and *P* values ≥ 0.05 and < 0.1 are shown in italics.

**Table S4.** Multivariate comparison of two-month culture positivity among unsuspected EMB-resistant MDR-TB patients receiving standard first-line antituberculous therapy, by PZA resistance assay and the consensus gold standard[a]

| Variable | Two-month culture ($n = 54$)[b] Adjusted OR (95% CI)[c] |
|---|---|
| Baseline PZA resistance (Wayne) | |
|    Yes | **12.4 (1.31-118.5)** |
|    No | Reference |
| Baseline PZA resistance (MGIT™) | |
|    Yes | *5.10 (0.89-29.2)* |
|    No | Reference |
| Baseline PZA resistance (*pncA*) | |
|    Yes | **8.57 (1.42-51.9)** |
|    No | Reference |
| Baseline PZA resistance (Consensus) | |
|    Yes | *4.93 (0.89-27.3)* |
|    No | Reference |

[a] CI, confidence interval; EMB, ethambutol; MDR-TB, multidrug-tuberculosis; MGIT, mycobacteria growth indicator tube; OR, odds ratio; PZA, pyrazinamide.
[b] One (1.8%) patient had a missing two-month culture result due to a contaminated sample.
[c] *P* values $< 0.05$ are shown in bold, and *P* values $\geq 0.05$ and $< 0.1$ are shown in italics. All multivariate models are adjusted for age and sex.