

SUPPL TABLE 1: Phenotypic and Genotypic Characteristics of Clinical PZA-R Isolates

| ISOLATE | pncA ^a | rpsA ^b | PHENOTYPE ^c | | GENOTYPE ^d | | | PYRAZINAMIDASE/pncA MUTATIONS ^e | | DRUG RESISTANCE ^f | | | | | | | | |
|---------|-------------------|-------------------|------------------------|---------------------|-----------------------|---------------|-----------------|--|---------------------------------------|------------------------------|-----|----|-----|----|----|-----|-----|-----|
| | | | 460TB | BT960 | MTBC LINEAGE | MIRU-VNTR | SPOLOGOTYPE | NUCLEOTIDE CHANGE | AMINO ACID CHANGE | PZA | INH | RM | EMB | SM | AK | CAP | ETA | OFL |
| 1 | WT | WT | MDR | No Growth | EAST ASIAN | 222325173533 | 00000000003771 | WT | WT | R | R | R | S | S | S | S | R | - |
| 2 | WT | WT ⁴ | MDR | No Growth | EAST ASIAN | 223325153533 | 00000000003771 | WT | WT | R | R | R | S | S | S | S | R | - |
| 3 | WT | WT ⁴ | MDR | No Growth | INDO OCEANIC | 228225133433 | 77777777777771 | WT | WT | R | R | R | R | S | S | S | S | R |
| 4 | WT | WT | PZA Only | PZA ^{SENS} | EURO AMERICAN | 226325153323 | 77770007720771 | WT | WT | S | S | S | S | S | - | - | - | - |
| 5 | WT | WT | PZA Only | PZA ^{SENS} | EAST AFRICAN INDIAN | 224325153324 | 700076700000071 | WT | WT | S | S | S | S | S | - | - | - | - |
| 6 | WT | WT ⁴ | PZA Only | PZA ^{SENS} | EAST ASIAN | 223325173533 | 00000000003771 | WT | WT | S | S | S | S | S | - | - | - | - |
| 7 | WT ² | WT | PZA Only | PZA ^{SENS} | EAST AFRICAN INDIAN | 236425163335 | 703777440003771 | WT; [C195T] | WT [Ser65Ser] | S | S | S | S | S | - | - | - | - |
| 8 | WT | WT ⁴ | POLY | PZA ^{SENS} | INDO OCEANIC | 238225173333 | 77757777777771 | WT | WT | S | R | S | S | R | S | S | S | S |
| 9 | WT ¹ | WT | PZA Only | PZA Only | EURO AMERICAN | 223125143322 | 61777777760771 | WT | WT | R | S | S | S | - | - | - | - | - |
| 10 | NS ¹ | WT | MDR | MDR | EURO AMERICAN | 224325152325 | 77773677760771 | G538T | Val180Phe | R | R | R | S | S | S | S | S | S |
| 11 | DEL ¹ | WT | MDR | MDR | EURO AMERICAN | 225125113324 | 770000017760771 | 1 bp deletion @ 260 | Frameshift | R | R | R | R | R | S | S | S | S |
| 12 | PRO | - | PZA Only | - | EAST AFRICAN INDIAN | 227723173533 | 703760000000331 | A(-11)G | Promoter | R | S | S | S | S | - | - | - | - |
| 13 | PRO | - | PZA Only | - | INDO OCEANIC | 254325223433 | 777637777413771 | G(-13)T | Promoter | R | S | S | S | - | - | - | - | - |
| 14 | PRO | - | POLY | - | EAST ASIAN | 223315173634 | 00000000003771 | A(-11)G | Promoter | R | R | S | R | R | S | S | R | S |
| 15 | NS | - | PZA Only | - | EURO AMERICAN | 22426143321 | 77777760740771 | A573C | Ser179Arg | R | S | S | S | S | - | - | - | - |
| 16 | NS | - | PZA Only | - | EURO AMERICAN | 222325143323 | 57777777400731 | C161G | Pro54Arg | R | S | S | S | S | - | - | - | - |
| 17 | NS | - | PZA Only | - | INDO OCEANIC | 294326223512 | 763000000000031 | C174G | Phe58Leu | R | S | S | S | S | - | - | - | - |
| 18 | NS | - | PZA Only | - | INDO OCEANIC | 228225173433 | 77777777777771 | T269G | Ile90Ser | R | S | S | S | S | - | - | - | - |
| 19 | NS | - | PZA Only | - | EAST ASIAN | 223325173433 | 00000000003771 | G395A | Gly132Asp | R | S | S | S | S | - | - | - | - |
| 20 | NS | - | PZA Only | - | INDO OCEANIC | 274326223422 | 677777477413771 | A35C | Asp12Ala | R | S | S | S | S | - | - | - | - |
| 21 | NS | - | PZA Only | - | EURO AMERICAN | 224325143324 | 70007677760771 | A23G | Asp8Gly | R | S | S | S | - | - | - | - | - |
| 22 | NS | - | PZA Only | - | INDO OCEANIC | 254326223713 | 777777777413731 | G463A | Val155Met | R | S | S | S | - | - | - | - | - |
| 23 | NS | - | PZA Only | - | EAST ASIAN | 325525173533 | 00000000003771 | C217G | His51Asp | R | S | S | S | - | - | - | - | - |
| 24 | NS | - | PZA Only | - | M. bovis | 232324250222 | 67660177767600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 25 | NS | - | PZA Only | - | M. bovis BCG | 2252324253322 | 67673777777600 | C169G; [C222T] | His57Asp; [Ser74Ser] | R | S | S | S | - | - | - | - | - |
| 26 | NS | - | PZA Only | - | M. bovis BCG | 2252324253322 | 67673777777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 27 | NS | - | PZA Only | - | M. bovis BCG | 2252324223322 | 67673777777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 28 | NS | - | PZA Only | - | M. bovis BCG | 2252324253322 | 67673777777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 29 | NS | - | PZA Only | - | M. bovis BCG | 2252324253322 | 67673777777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 30 | NS | - | PZA Only | - | M. bovis | 232324253322 | 67673677777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 31 | NS | - | PZA Only | - | M. bovis | 232324253322 | 66673677777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 32 | NS | - | PZA Only | - | M. bovis BCG | 2152324253322 | 67673777777600 | C169G | His57Asp | R | S | S | S | - | - | - | - | - |
| 33 | NS | - | POLY | - | M. bovis | 232324251222 | 67673777777600 | C169G | His57Asp | R | S | S | S | S | S | S | S | S |
| 34 | NS | - | POLY | - | M. bovis | 23224123422 | 66403777777600 | C169G | His57Asp | R | S | S | S | S | S | S | S | S |
| 35 | NS | - | POLY | - | EAST ASIAN | 223325173533 | 00000000003771 | A226C | Thr76Pro | R | R | S | R | R | S | S | - | S |
| 36 | NS | - | POLY | - | EAST ASIAN | 223325153533 | 00000000003371 | A152G | His51Arg | R | R | S | R | S | S | R | S | R |
| 37 | NS | - | POLY | - | EAST ASIAN | 223325153533 | 00000000003371 | A152G | His51Arg | R | R | S | R | S | S | R | S | R |
| 38 | NS | - | MDR | - | EAST ASIAN | 203325183533 | 00000000003371 | A226C | Thr76Pro | R | R | S | R | S | S | R | S | R |
| 39 | NS | - | MDR | - | INDO OCEANIC | 254326223432 | 677777477413471 | C211T | His71Tyr | R | R | R | S | S | S | R | S | R |
| 40 | NS | - | MDR | - | INDO OCEANIC | 254326223432 | 677777477413471 | T307T | Tyr103Asp | R | R | R | S | S | S | R | S | R |
| 41 | NS | - | MDR | - | EURO AMERICAN | 223125153322 | 77773777760771 | G463A | Val155Met | R | R | R | R | S | S | R | S | R |
| 42 | NS | - | MDR | - | EAST ASIAN | 222325183523 | 00000000002771 | T199C | Ser67Pro | R | R | R | R | S | S | R | S | R |
| 43 | NS | - | MDR | - | EURO AMERICAN | 223226153321 | 67777760760771 | T254A | Leu85Gln | R | R | R | R | - | - | - | - | - |
| 44 | NS | - | MDR | - | EURO AMERICAN | 223226153321 | 67777760760771 | T254A | Leu85Gln | R | R | R | R | S | S | S | S | R |
| 45 | NS | - | MDR | - | EAST ASIAN | 223315173533 | 00000000003771 | A170G | His57Arg | R | R | R | R | S | S | R | S | R |
| 46 | NS | - | MDR | - | EAST ASIAN | 223315173534 | 00000000003771 | A226C | Thr76Pro | R | R | R | R | S | S | S | S | R |
| 47 | NS | - | MDR | - | EURO AMERICAN | 223325143323 | 77773777760401 | C137T | Ala46Val | R | R | R | R | S | S | R | S | R |
| 48 | NS ² | - | MDR | - | EAST AFRICAN INDIAN | 226425173523 | 703777440003771 | T11G; [C195T] | Leu4Trp; [Ser65Ser] | R | R | R | R | S | S | S | S | - |
| 49 | NS | - | MDR | - | INDO OCEANIC | 254326223432 | 677777477413771 | C263A | Nonsense | R | R | R | R | R | R | R | S | S |
| 50 | NS | - | MDR | - | EAST ASIAN | 223325163533 | 00000000003771 | A188G | Asp63Gly | R | R | R | R | S | S | S | S | S |
| 51 | NS | - | MDR | - | EAST ASIAN | 223325173533 | 00000000003771 | C512T | Ala171Val | R | R | R | R | S | S | R | S | R |
| 52 | NS ² | - | MDR | - | EAST AFRICAN INDIAN | 225425133533 | 303777440003671 | A422C; [C195T] | Gln141Pro; [Ser65Ser] | R | R | R | R | S | S | S | S | S |
| 53 | NS | - | MDR | - | EAST ASIAN | 2233-5153532 | 00000000003771 | T452C | Leu151Ser | R | R | R | R | S | S | R | S | R |
| 54 | NS | - | XDR | - | EURO AMERICAN | 124226154222 | 77774077560771 | A152G | His51Arg | R | R | R | R | S | S | R | R | R |
| 55 | INS | - | POLY | - | EAST ASIAN | 322325173543 | 00000000003771 | 1 bp insert G @ 339 | Frameshift (after Gly113) | R | R | S | R | S | S | S | S | R |
| 56 | INS | - | POLY | - | EAST ASIAN | 223325173433 | 00000000003771 | 9 bp insertion: ATGTGGTCC @ 395 | In Frame Insertion: Asp-Val-Val @ 132 | R | R | S | R | S | S | S | S | R |
| 57 | INS | - | MDR | - | EAST AFRICAN INDIAN | 223425173533 | 70377770003771 | 1 bp insertion G @ 408; [C195T] | Frameshift; [Ser65Ser] | R | R | S | S | S | S | S | S | S |
| 58 | INS ² | - | MDR | - | EAST AFRICAN INDIAN | 226425163532 | 703777440003171 | 1 bp insertion G @ 391; [C195T] | Frameshift; [Ser65Ser] | R | R | R | R | S | S | R | S | R |
| 59 | INS | - | MDR | - | EURO AMERICAN | 224226163321 | 67773760760771 | 2 bp insertion GG @ 392 | Frameshift | R | R | R | R | S | S | R | S | R |
| 60 | INS | - | MDR | - | EURO AMERICAN | 223226153323 | 777737777000371 | 1 bp insertion A @ 408 | Frameshift | R | R | R | S | S | S | S | S | R |
| 61 | DEL ¹ | - | PZA Only | - | EURO AMERICAN | 253325153324 | 77637777760731 | C418A; 8 bp deletion @ 446 | Arg140Ser; Frameshift | R | S | S | S | S | - | - | - | - |
| 62 | DEL ¹ | - | PZA Only | - | EURO AMERICAN | 233325163324 | 77637777760771 | C418A; 8 bp deletion @ 446 | Arg140Ser; Frameshift | R | S | S | S | - | - | - | - | - |
| 63 | DEL ¹ | - | PZA Only | - | EURO AMERICAN | 253325153324 | 77637777760731 | C418A; 8 bp deletion @ 446 | Arg140Ser; Frameshift | R | S | S | S | - | - | - | - | - |
| 64 | DEL ⁴ | - | MDR | - | EAST AFRICAN INDIAN | 124226154222 | 700377740003771 | 2 bp deletion @ 282; [C195T] | Frameshift; [Ser65Ser] | R | R | R | R | S | S | S | S | S |

a WT = wild type pncA; PRO = promoter mutation; NS = nonsynonymous mutation; INS = mutation due to insertion, DEL = mutation due to deletion; 1 = target resequenced; 2 = sequence includes the synonymous mutation C195T; 3 = 'Quebec' mutation

b WT = wild type rpsA; 4 = sequence includes the synonymous mutation A636C

c Resistance phenotype as determined with the BACTEC 460 system (all isolates) and MGIT 960 platform (11 discordant isolates)

PZA Only = pyrazinamide mono-resistant; POLY = resistant to at least pyrazinamide and isoniazid; MDR = resistant to at least pyrazinamide, isoniazid and rifampin; XDR = extremely drug resistant

d Lineage designations were assigned using a combination of 12 loci MIRU-VNTR and Spoligotyping Data and using the Gagneux Nomenclature. MIRU-VNTRplus [1] and SPOL DB4 [2] were used to assist lineage assignments.

[1] Allix-Béguec C., D. Harmsen, T. Weniger, P. Supply, S. Niemann. 2008. Evaluation and user-strategy of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of *M. tuberculosis* complex isolates. *J Clin Microbiol.* 46:2692-2699.

[2] Brudley K., J.R. Driscoll, L Rigouts et al.2006. Mycobacterium tuberculosis complex genetic diversity : mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.*6:23

e WT = wild type; square brackets designate synonymous mutations

f R = resistant; S = sensitive; - = not tested

First line drugs are: PZA = pyrazinamide; INH = isoniazid; RIF = rifampin; EMB = ethambutol

Second Line drugs are: SM = streptomycin; AK = amikacin; CAP = capreomycin; ETA = ethionamide; OFL = ofloxacin; PAS = p-aminosalicylic acid