Indian catalogue of *Mycobacterium tuberculosis* mutations and their association with drug resistance - 2022

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NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

Indian Catalogue of *Mycobacterium tuberculosis* Mutations and their Association with Drug Resistance - 2022

Contents

Abbreviations

CC	Critical Concentration
CI	Confidence Interval
CTAB	Cetyl TrimethylAmmonium Bromide
DNA	Deoxyribo Nucleic Acid
DST	Drug Susceptibility Testing
FDR	False Discovery Rate
gDST	Genotypic Drug Susceptibility Testing
indel	Insertion/ Deletion
IRL	Intermediate TB Reference Laboratory
LoF	Loss of Function
lb	Lower Bound
MDR-TB	Multi-Drug Resistant TB
MGIT	Mycobacteria Growth Indicator Tube
MICs	Minimum *Inh*ibitory Concentrations
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium TuBerculosis* Complex
mWRD	Molecular WHO-Recommended Rapid Diagnostic
NA	Not Available
NGS	Next Generation Sequencing
NRL	National Reference Laboratory
NTEP	National Tuberculosis Elimination Programme
OR	Odds Ratio
OR SOLO	Odds Ratio of SOLO Mutation
pDST	phenotypic Drug Susceptibility Testing
PPV	Positive Predictive Value
RRDR	Rifampicin Resistance-Determining Region
STDC	State Tuberculosis Demonstration and training Centre
TAT	Turn Around Time
TB	Tuberculosis
ub	Upper Bound
UT	Union Territory
WGS	Whole Genome Sequencing
WHO	World Health Organization

Drugs

AMK	Amikacin
BDQ	Bedaquiline
CAP	Capreomycin
EMB	Ethambutol
ETO	Ethionamide
FLQ	Fluoroquinolones
INH	Isoniazid
KAN	Kanamycin
LFX	Levofloxacin
LZD	Linezolid
MFX	Moxifloxacin
OFX	Ofloxacin
PAS	Para-aminosalicylic acid
PZA	Pyrazinamide
RIF	Rifampicin
STM	Streptomycin

Study Team

ICMR-NIRT

Principal Investigator	Dr. K.R. Uma Devi, PhD
Site Coordination	Dr. Siva Kumar Shanmugam, PhD Dr. S. Mukesh Kumar, MBBS
Data Analysis and Data Quality Management	Dr. S. Ashok, PhD Mr. Karthick. V
Whole Genome Sequencing	Dr. S. Tamilzhalagan, PhD Mrs. Suganthi Chittibabu Ms. Sudha Solaiyappan
Phenotypic Drug Susceptibility Testing	Dr. Suba Sakthi, PhD Dr. Harresh Adikesavalu, PhD Mrs. C. Dhatchayani Ms. Evanslin Santus Ms. Bhuvaneshwari
Directors	Dr. C. Padmapriyadarsini, MS, PhD (Director) Dr. Soumya Swaminathan, MD (Former Director) Dr. Srikanth Tripathy, MD (Former Director)

US CDC Contributors*

Dr. Patricia Hall-Eidson, PhD, MS
Dr. Heather McLaughlin, PhD
Dr. Jonathan P. Smith, PhD
Dr. Christine Ho, MD
Dr. Patrick K. Moonan, DrPH, MPH

Collaboration

Central TB Division (CTD) and National Tuberculosis Elimination Programme (NTEP)

**The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.*

Introduction

Tuberculosis (TB) is an ancient disease caused by *Mycobacterium tuberculosis* (MTB) and is a major public health concern worldwide. In India, TB remains a national epidemic, infecting over 2.6 million of the nation's population and killing over 493,000 people in 2020 *(1)*. The country's TB burden is the highest in the world. Although numerous advances have rendered tuberculosis more easily diagnosable and curable, the disease is yet advancing by the emergence and continued transmission of drug-resistant MTB. Drug-resistant TB (DR-TB) threatens disease control efforts and is more challenging to both treat and cure. In 2020, resistance to at least isoniazid (INH) and rifampicin (RIF), defined as multidrug-resistant TB (MDR-TB), was observed in an estimated 497,000 people, making India the country with the highest number of MDR-TB cases in the world. The spread of MDR-TB must be arrested by rapid, accurate diagnosis and treatment *(1)*.

The treatment success rate for MDR-TB in India using second-line drugs required to treat the disease is approximately 56% *(1)*. Accurate TB diagnostic and drug susceptibility testing (DST) is required to guide selection of appropriate antituberculosis medicines to include in individualized treatment regimens for patients with DR-TB; improving clinical outcomes by supporting use of regimens that are efficacious. Elucidating genetic markers and mechanisms of MTB resistance to antituberculosis medicines has informed development of molecular assays for resistance detection with substantially reduced testing turn-around-times (TAT) for DST results. The World Health Organisation (WHO) recommends RIF resistance determination, at a minimum, for all TB cases using WHO-recommended rapid molecular diagnostic tests (mWRDs), such as the Xpert MTB/RIF Ultra (Cepheid) and Truenat MTB Plus with Truenat Rif Dx (Molbio) assays. Detection of INH resistance is also recommended, as well as detection of fluoroquinolone resistance for RIF- or INH-resistant cases, using other molecular WHO-recommended diagnostics, such as the MTBDRplus and MTBDR*sl* (Bruker/ Hain) line probe assays. All of these assays target specific regions of the MTB genome known to harbour mutations associated with medicine-specific resistance. For instance, multiple mutations in the Rifampicin Resistance Determining Region (RRDR) of the *rpo*B gene are known to confer resistance to RIF. Similarly, the molecular basis for INH and fluoroquinolone resistance is well understood, while further mutation-based resistance associations for other TB medicines used in drug-sensitive and drug-resistant TB treatment regimens are available or evolving. While use of mWRDs reduces time to DST results, the tests are limited in the number

of drugs that can be analyzed, the number of genes and gene regions that can be targeted, and the sensitivity and specificity with which they can detect resistance-associated variants. Whole genome sequencing (WGS) of MTB offers the most comprehensive solution to genomic-based DST, allowing identification of all mutations that may be associated with resistance (and sensitivity) to first-line, second-line, and new or repurposed antituberculosis drugs within a single platform, while also providing valuable data to establish genetic relatedness between strains of MTB, which is necessary for understanding of potential transmission linkages.

In 2021, the World Health Organisation released the "*Catalogue of Mutations in Mycobacterium tuberculosis-complex and Their Association with Drug Resistance*" (WHO Mutation Catalogue), that summarized and analyzed resistance profiles from a global collection of isolates with complementary WGS and phenotypic DST data *(2, 3)*. The catalogue facilitates standardized interpretation of genotypic data for all currently recommended antituberculosis drugs. However, as the WHO Mutation Catalogue's sources of data and MTB strains are not evenly distributed around the world, catalogued mutations may predominate regionally rather than globally or vary in their strength of association with DR-TB.

To date, there has been no national database or country-specific catalogue summarizing the current DR-TB-associated mutations in circulation in India. Thus, there was an urgent need to collect drug-resistant TB strains from across the country, build a database of MTB genomic and phenotypic drug susceptibility data, and analyze the database to produce a Mutations Catalogue specific to India. To meet this need, we identified and summarized a comprehensive collection of MTB mutations based on extensive analysis of the first and largest nationwide collection of MTB strains presumed to have, or lacking evidence of, drug resistance. A quota sampling method was utilized to collect *M. tuberculosis* isolates from 25 States and four Union Territories for next generation WGS and WHO-approved phenotypic DST. Genotypic and phenotypic drug resistance profiles were then compared and analyzed using a validated bioinformatics pipeline in combination with a setting-adapted classification scheme based on that used in the WHO Mutation Catalogue (see Methodology for a more detailed description of methods used). This document serves as the first nationwide Indian Catalogue of Mutations (Indian Mutation Catalogue) and their association with DR-TB that can be used for surveillance to inform National TB Elimination Programme (NTEP) strategies for combating and treating this high-priority disease.

The Indian Mutation Catalogue provides data on the frequency and interpretation of mutations harbored in MTB strains nationwide, and their association with resistance to 15 drugs used for antituberculosis treatment, including all first-line and second-line medicines. These drugs include: rifampicin (RIF), isoniazid (INH), ethambutol (EMB), pyrazinamide (PZA), levofloxacin (LFX), moxifloxacin (MFX), ofloxacin (OFX), linezolid (LZD), amikacin (AMK), capreomycin (CAP), kanamycin (KAN), streptomycin (STM), ethionamide (ETH), para-aminosalicylic acid (PAS), and bedaquiline (BDQ). Assessment of resistance associated mutations to ofloxacin and para-aminosalicylic acid are specific to this Indian Catalogue, whereas assessments of delamanid and clofazimine resistance are specific to the 2021 WHO Catalogue and, based on scarcity of India-specific data, are not included here. Lastly, this document also serves as a reference point for further genomic DR-TB strain profile comparisons and may be used to guide development of new and improved molecular tests for resistance determination.

The Indian Mutation Catalogue will be updated periodically as more data are generated (or other existing datasets become available to supplement the underlying database), treatment regimens are updated, or NTEP strategic priorities evolve. These future updates will be particularly important to enhance understanding and setting-specific classification of mutation-based resistance and sensitivity associations for the relatively new and repurposed medicines now used to treat DR-TB patients in India.

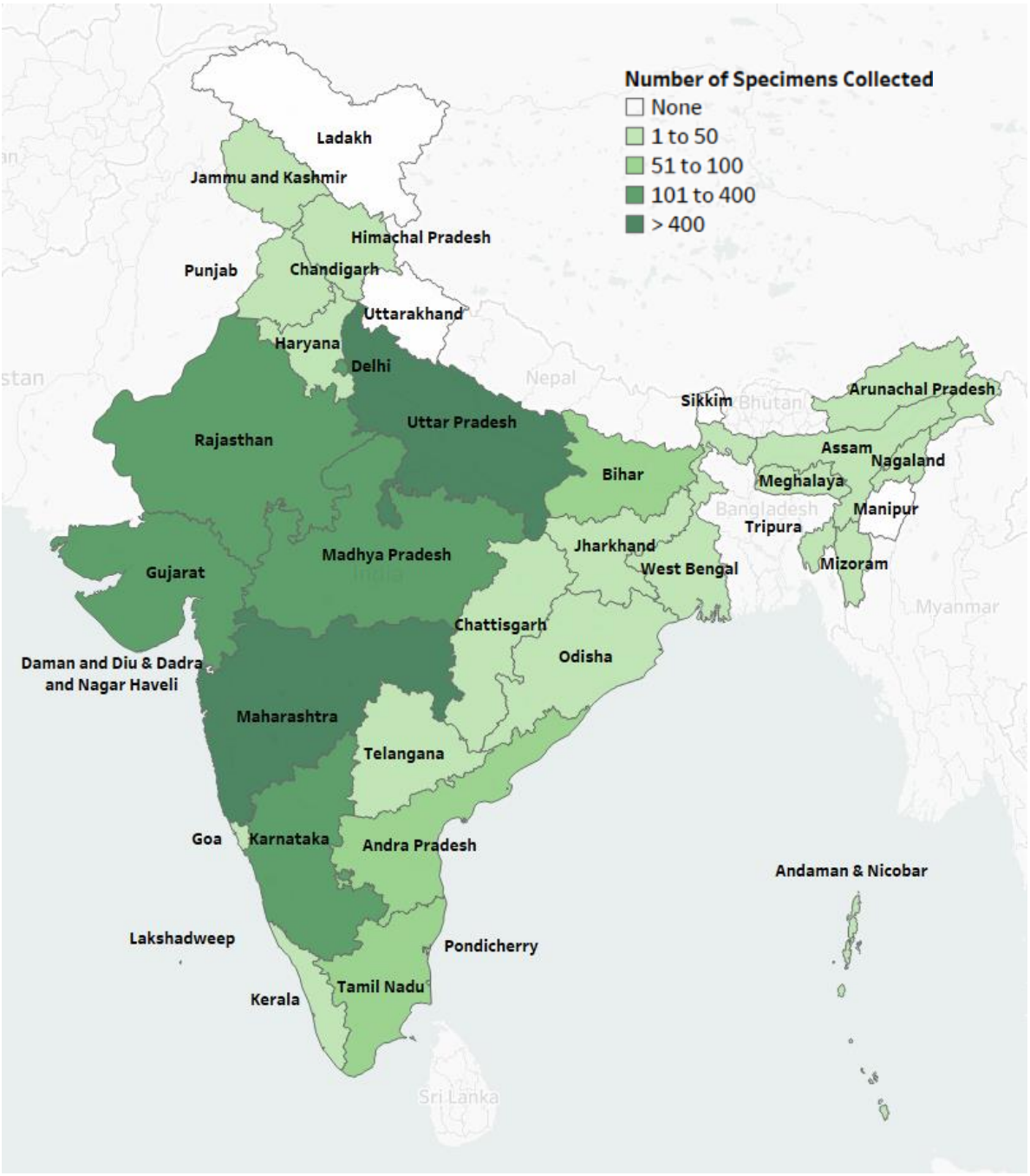
Outline of the Indian Mutation Catalogue

A total of 3,167 *M. tuberculosis-complex* (MTBC) isolates were collected from patients residing in 25 States and four Union Territories using a quota-based sampling frame. Matching WGS data were available for 2,112 isolates on quality filtering which were included for downstream analyses. Among collected samples, 448 were cultures obtained from processed sputum, 1,635 were cultures obtained directly from collection sites, one was of extrapulmonary origin, and 28 did not have processing history available. This catalogue includes characterization of over 8,000 mutations. Refer to the Methodology section of this catalogue for a more detailed sampling description.

Nationwide Geographical Distribution of Data Collection

Out of 28 States and 8 Union Territories, 25 States and 4 Union Territories contributed MTBC isolates and/ or processed sputa for genotypic and phenotypic DST at ICMR-NIRT. The geographic distribution of specimen contribution is presented in Figure 1. MTB samples could not be collected from three states (Manipur, Sikkim and Uttarakhand) and four Union Territories (Ladakh, Lakshadweep, Chandigarh, and Dadra and Nagar Haveli;Daman and Diu).

Figure 1. Geographic distribution of the number MTBC isolates or processed sputum collected by State and Union Territories – India.



Phenotypic DST and Genotypic DST Results

Phenotypic drug susceptibility testing (pDST) and genotypic DST (gDST) were available for 2,112 patient isolates. The number and percent of collected samples with pDST and gDST resistance results for each drug are outlined in Table 1 below. The prevalence of phenotypic resistance to first-line drugs *INH* and RIF among these samples was >60%, whereas resistance to EMB and PZA was detected in 34% and 43% of isolates tested, respectively. The prevalence of phenotypic resistance to AMK, KAN, CPR, LZD, and PAS among these samples was lower (range: 2–11%) (Table 1). In the case of gDST, resistance to the first-line drugs RIF and INH was observed for >70% of isolates tested, whereas resistance to EMB was 67.0% (32.8% higher than reported using pDST) and PZA was 36.5%. Resistance to the fluoroquinolones LFX, MFX and OFX was observed for 59.3% of isolates, while resistance to the second-line drugs AMK, KAN, CPR, LZD, and PAS ranged from 1.7 to 18.0%. Lastly, the percentage of resistance detected to STM was similarly high for pDST and gDST, at 50.4% and 52.6%, respectively. Overall, the percentage of gDST-detected resistance was higher than that detected by pDST for 9 of 15, or 60% of the TB drugs across all isolates tested.

Table 1. Phenotypic and genotypic drug susceptibility testing results by drug^

Drug	Number of resistant isolates by pDST (n=2112)	Percentage (95% CI)	Number of resistant isolates by gDST (n=2112)	Percentage (95% CI)
RIF	1394	66.0 (63.9–68.0)	1575	74.6 (72.7–76.4)
INH	1742	82.5 (80.8–84.1)	1668	79.0 (77.2–80.7)
EMB	722	34.2 (32.2–36.3)	1414	67.0 (64.9–69.0)
PZA	900	42.6 (40.5–44.7)	771	36.5 (34.4–38.6)
LFX	1092	51.7 (49.5–53.9)	1253	59.3 (57.2–61.4)
MFX	1013	48.0 (45.9–50.2)	1253	59.3 (57.2–61.4)
OFX	1223	57.9 (55.8–60.0)	1253	59.3 (57.2–61.4)
LZD	58	2.7 (2.1–3.5)	35	1.7 (1.2–2.4)
BDQ				
AMK	164	7.8 (6.7–9.0)	214	10.1 (8.9–11.5)
CAP	163	7.7 (6.6–8.9)	361	17.1 (15.5–18.8)
KAN	223	10.6 (9.3–12.0)	282	13.4 (12.0–14.9)
STM	1064	50.4 (48.2–52.6)	1111	52.6 (50.4–54.8)
ETO	620	29.4 (27.5–31.4)	380	18.0 (16.4–19.7)
PAS	116	5.5 (4.6–6.6)	79	3.7 (2.9–4.6)

^ Bedaquiline not represented, as phenotypic drug susceptibility testing data was only available for the 12 isolates harboring mutations in bedaquiline-resistance associated genes at the time of this report.

Diagnostic Testing of confidence-graded mutations for predicting phenotypic drug susceptibility

The sensitivity, specificity, and Positive Predictive Value (PPV) of final confidence-graded mutations as predictors of phenotypic drug susceptibility are presented in Table 2. The performance metrices are presented by level of association (Group 1, associated with R; Group 2, associated with R – interim), to indicate the contribution of the different confidence-graded mutations. Performance data for all identified mutations are presented for transparency and completeness, irrespective of strain and mutation frequency, which should both be considered when reviewing Table 2 sensitivity, specificity, PPV, and associated confidence interval data.

Table 2. Sensitivity, specificity and PPV of the confidence-graded mutations as predictors of phenotypic drug susceptibility

Drug		1) Associated with Resistance	2) Associated with Interim Resistance	3) Uncertain Significance	4) Not Associated with Interim Resistance	5) Not Associated with Resistance
RIF	Mutations Identified	3	57	767	121	850
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	87.8 (86.0–89.5), 97.9 (96.6–98.8), 98.8 (98.0–99.3)	16.7 (14.8–18.8), 72.0 (68.6–75.3), 53.7 (49.6–57.8)			
INH	Mutations Identified	2	46	1336	26	106
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	98.6 (97.9–99.1), 98.1 (96.1–99.2), 99.6 (99.2–99.8)	91.8 (90.3–93.0), 13.8 (10.4–17.7), 83.4 (82.8–84.0)			
EMB	Mutations Identified	0	7	70	26	893
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	NA	78.4 (75.2–81.3), 77.4 (75.1–79.6), 64.3 (61.9–66.7)			
PZA	Mutations Identified	2	298	47	0	220
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	10.4 (8.5–12.6), 99.3 (98.6–99.7), 91.3 (84.1–95.4)	77.6 (75.5–79.6), 73.5 (70.9–76.0), 79.8 (78.1–81.3)			

Drug		1) Associated with Resistance	2) Associated with Interim Resistance	3) Uncertain Significance	4) Not Associated with Interim Resistance	5) Not Associated with Resistance
LFX	Mutations Identified	5	10	115	9	228
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	93.6 (92.0–95.0), 90.0 (88.0–91.8), 91.0 (89.3–92.3)	15.4 (13.3–17.7), 93.7 (92.1–95.1), 72.4 (66.6–77.6)			
MOX	Mutations Identified	6	9	112	10	230
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	85.3 (83.0–87.4), 92.4 (90.6–93.9), 91.1 (89.3–92.7)	24.7 (22.1–27.5), 85.6 (83.4–87.7), 61.3 (56.9–65.5)			
OFX	Mutations Identified	7	6	118	10	226
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	96.4 (95.3–97.4), 97.1 (95.7–98.1), 98.0 (97.1–98.6)	3.8 (2.8–5.1), 99.0 (98.1–99.5), 83.9 (72.0–91.4)			
LZD	Mutations Identified	1	0	304	10	477
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	51.7 (38.2–65.1), 100.0 (99.8–100), 100.0 (100–100)	NA			
AMK	Mutations Identified	2	0	48	0	383
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	93.3 (88.3–96.6), 99.9 (99.6–100.0), 98.7 (95.0–99.7)	NA			
CAP	Mutations Identified	2	5	5	0	222
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	81.6 (74.8–87.2), 98.9 (98.3–99.3), 85.8 (79.9–90.2)	9.8 (5.7–15.5), 94.2 (93.1–95.2), 12.4 (7.9–18.9)			

Drug		1) Associated with Resistance	2) Associated with Interim Resistance	3) Uncertain Significance	4) Not Associated with Interim Resistance	5) Not Associated with Resistance
KAN	Mutations Identified	5	3	23	0	103
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	82.5 (76.9–87.3), 99.6 (99.2–99.9), 96.3 (92.6–98.2)	4.5 (2.2–8.1), 99.7 (99.3–99.9), 62.5 (38.0–82.0)			
STM	Mutations Identified	4	16	34	4	83
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	83.7 (81.4–85.9), 98.9 (98.0–99.4), 98.7 (97.7–99.2)	18.4 (16.1–20.9), 95.9 (94.5–97.0), 82.0 (76.8–86.2)			
ETH	Mutations Identified	2	177	233	1	312
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	47.4 (43.4–51.4), 97.7 (96.7–98.4), 89.4 (85.7–92.2)	45.3 (41.2–49.3), 86.7 (84.9–88.4), 58.7 (54.8–62.4)			
PAS	Mutations Identified	4	4	72	2	306
	Sensitivity (95% CI), Specificity (95% CI), PPV (95% CI)	43.1 (33.9–52.6), 99.5 (99.0–99.7), 82.0 (70.9–89.5)	7.8 (3.6–14.2), 99.8 (99.5–100), 69.2 (41.3–87.8)			

Catalogue Limitations

There are several limitations to this work. First, the data presented herein are based on a quota-based sampling strategy that, while inclusive of most Indian States and Union Territories, was not statistically representative of the representative location or for the country as whole. Our approach aimed to initiate the systematic collection of MTB strains to assess genetic diversity and characterize mutations associated with drug resistance in India. This approach may not be all-inclusive, and some resistance-association analyses may not be statistically powered to accurately calculate measures of association. Second, the inclusion criteria focused on patient presumed to have drug-resistant TB; thus, the strain collection was largely biased toward collection, testing, and analysis of DR-TB isolates and strains, with drug-sensitive TB isolates accounting for approximately 10% of the final dataset. Focusing on DR-TB allowed for the inclusion of a large volume of DR-TB strains from across the country, but may simultaneously lend a bias in the dataset, where the association of detected mutations among drug-susceptible strains may be incomplete. This bias could contribute to overestimation of mutation-associated resistance for those polymorphisms that are not already known to be resistance-associated. Third, the pDST methods of WHO Catalogue contains both WHO-endorsed methods and methods not endorsed by WHO. In our catalogue, the pDST results were based only from WHO-endorsed methodologies; this may have caused some discordance in phenotypic results when compared to WHO Catalogue. Lastly, the methodology applied was adapted from that of the WHO Mutation Catalogue designed to interrogate a much larger and globally representative dataset. While adaptations to the global algorithms were made to adjust for the smaller sample size of the Indian dataset (see Adapted Methodology/ Confidence Grading below), setting-specific differences in MTB lineage and genotype diversity may not be fully accounted for with the current approach. As with any guide based on an initial dataset, the accuracy and effectiveness of mutation classification in this catalogue should be monitored and evaluated, and the database supplemented with further drug-susceptible and drug-resistant MTB genotypic and phenotypic DST data, particulary for new and repurposed anti-TB medicines for which resistance monitoring will be essential, to inform future versions with updated analytics and outputs.

Catalogue Disclaimer Statement

These data were compiled, analyzed, and presented to support DR-TB surveillance and disease control efforts in India. Next generation WGS is not yet endorsed by the WHO or the Indian NTEP as a diagnostic or drug susceptibility testing method for clinical use. Indian Mutation Catalogue data should therefore not be used to guide clinical care of patients in India or elsewhere in the world.

Mutation Catalogue

Reading the tables

The terms and abbreviations used in drug-specific subsections and tables below are listed in Table 3 and mirror those used in the WHO Mutation Catalogue.

Table 3. Terms used in the report and their description

Terms Used in the Mutation Catalogue	Description
Assoc w R	Associated with resistance
Assoc w RI	Associated with resistance – interim
Inf	Infinity
NA	Not Available
Undef	Undefined (0/0)
Not Assoc w R	Not associated with resistance
Not Assoc w RI	Not associated with resistance – interim
Uncert. Sig.	Uncertain significance
WHO-endorsed gDST assay	WHO-endorsed genotypic drug susceptibility testing assay
Drug	Name of drug
Mutations	Mutation, with common name where relevant
Present in R (TP)	Number of resistant isolates with the mutation
Present in S (FP)	Number of susceptible isolates with the mutation
Absent in R (FN)	Number of resistant isolates without the mutation
Absent in S (TN)	Number of susceptible isolates without the mutation
Sensitivity	True positive rate of mutation
Specificity	True negative rate of mutation
PPV	Positive predictive value of mutation
PPV|SOLO	Positive predictive value conditional on being solo
Initial confidence grading	Initial grouping of mutation
Dataset(s)	Dataset(s) used to derive the initial confidence grading
Additional grading criteria	Criterion for changing the initial confidence grading (e.g., previous WHO guidance or WHO-endorsed genotypic DST assays) to determine the final confidence grading
LoF	Loss of function
Final confidence grading	Final grouping of mutation after additional grading criteria were applied

Additional Variables Shown in the Catalogue	Description
Gene Name	Gene Name of Each Mutation
Gene ID	Gene ID for Every Variant
Present SOLO_R	Resistant isolates with the single (solo) mutation
Present SOLO_S	Sensitive isolates with the single (solo) mutation
Present SOLO_SR	Sum of resistant and susceptible isolates with the single (solo) mutation
Sensitivity*	True positive rate of mutation
Specificity*	True negative rate of mutation
PPV*	Positive predictive value of mutation
LR+*	Positive likelihood ratio of mutation
LR-*	Negative likelihood ratio of mutation
OR*	Odds ratio of mutation
OR SOLO*	Odds ratio of solo mutation
OR SOLO_FE-sig	Fisher's exact test for the false discovery rate (FDR)-corrected *P* for the OR SOLO; TRUE = FDR-corrected $P \leq 0.05$, FALSE = FDR-corrected $P > 0.05$
Previous WHO Guidance	2018 WHO Technical Guide on Use of NGS, 2021 WHO Technical Report on the RIF and INH CC, 2021 WHO Mutations Catalogue, 2021 WHO Guidance on DR TB Surveillance, Miotto et al. (PubMed identifier 29284687) *(4 - 9)*
WHO-endorsed Genotypic DST Assays	Cepheid Xpert MTB/RIF, Cepheid Xpert MTB/RIF Ultra, Cepheid Xpert MTB/XDR, Bruker/ Hain Genotype MTBDRplus V2.0, Bruker/ Hain Genotype MTBDR*sl* V2.0, Bruker/ Hain FluoroType MTBDR, Nipro Genoscholar NTM+MDRTB II, Nipro Genoscholar PZA-TB II, Abbott RealT*ime* MTB RIF/INH, BD Max MDR-TB, Roche cobas MTB-RIF/INH, and Molbio Truenat MTB-RIF Dx (without public disclosure of *rpo*B targets *(4)*)

* The lower bound (lb) and upper bound (ub) of the 95% CI are provided as additional columns in the Indian Mutation Catalogue Dataset 2022.

The tables in this report were simplified and abridged to fit the page space (i.e., all Group 3, Group 4 and Group 5 Mutations are not shown; full list available upon request).

A description of methods used to calculate and analyze data for the variables above is presented in the Statistical Analysis section of this document. The thresholds used to define the initial confidence grading are listed below; if they were met, the entry is highlighted in the colour shown in parentheses.

Initial Confidence Grading

Group 1: Associated with resistance

1. Sum of resistant and susceptible isolates with the solo mutation (Present SOLO_SR) ≥ 5 (red)
2. Lower bound of 95% CI of PPV conditional on being solo (PPV | SOLO_lb) ≥ 25% (red)
3. OR > 1, which always applies if criterion 4 is met (red)
4. OR | SOLO > 1 (red)
5. Statistical significance of OR | SOLO (OR SOLO_FE-sig) with Fisher exact FDR-corrected $P \leq 0.05$ (red)

Group 2: Associated with resistance – interim

1. Resistant isolates with the solo mutation (Present SOLO_R) ≥ 2 (Orange)
2. PPV ≥ 50% (Orange)

Group 3: Uncertain significance

Mutations that did not meet the criteria for inclusion in group 1, 2, 4 or 5.

Group 4: Not associated with resistance – Interim

1. PPV conditional on being solo (PPV | SOLO) < 40%
2. Upper bound of 95% CI of PPV conditional on being solo (PPV | SOLO_ub) < 75%

Group 5: Not associated with resistance

Mutations which have PPV less than 50% were considered as Not associated with resistance while WHO filtered those mutations which are less than 10%.

Additional Grading Criteria

After the initial confidence grading, we moved some mutations in Groups 3, 4 and 5 to Group 2 based on expert rules and precedents followed in WHO Catalogue for the same reasons explained in WHO Catalogue. In addition, some mutations with strong association to resistance were missed in group 1 and 2 due to stringent statistical criteria. The reasons for moving the mutations upward are mentioned in the tables (Blue). Lastly, all strain and mutations are displayed in drug-specific tables below, irrespective of frequency, for completeness and accuracy. Presented performance and associated confidence interval data should therefore be interpreted in the context of each mutation's frequency.

The mutations with '**bold'** font in the tables are mutations detected by WGS that are not otherwise targeted for reporting by mWRDs in use in India at the time of this report.

An illustrative example

In the first example below, the drug considered is INH. The variant is in the *kat*G gene, the amino acid change is at codon 315, and the change is from Serine to Threonine. This variant was found in 1421 phenotypically resistant isolates and in 5 susceptible isolates. The mutation was not found in 322 phenotypically resistant isolates and in 363 susceptible isolates. The mutation S315N of *kat*G gene falls into uncertain significance category by initial confidence grading based on its statistical classification. This mutation was moved to Group 2 (Associated with interim resistance) following the expert rule that any non-synonymous mutation in *kat*G, or upstream of *kat*G, can confer INH resistance as described in WHO Mutation Catalogue *(2)*.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
S315T	*kat*G	79	80	1421	5	322	363	99.6	94	89	99.1	81.5	98.6	89.1	Assoc. w R		1) Assoc. w R
C-15T	*fab*G1/ promoter	5	5	296	2	1449	364	99.3	71.4	38	105	17	99.5	Inf	Assoc. w R		1) Assoc. w R
S315N	*kat*G	0	0	25	0	1720	366	100	Inf	Inf	Inf	1.4	100	Inf	Uncert. Sig.		2) Assoc. w RI

The sensitivity, specificity, and PPV represent the performance of this mutation in the dataset. The next four columns indicate the statistical performance of this mutation when it occurs without other mutations (defined as 'SOLO') in the genomic regions selected when assessing *INH* resistance. The values given are the midpoint PPV with the corresponding lower bound (lb) and upper bound (ub), and the odds ratio for the solo mutation (OR SOLO). This template is as per the WHO Mutations Catalogue.

The initial confidence grading for *kat*G S315T was group 1 (Assoc w R) because:

- Present_SOLO_SR (see Mutation Catalogue) was 80 and, consequently, ≥ 5.
- PPV|SOLO_lb of 89% was ≥ 25%.
- OR SOLO of 89.1 was > 1 and statistically significant.

Rifampicin

Within this database, all mutations associated with RIF resistance were all harboured within the *rpo*B gene. Group 1 (Associated with resistance) mutations had a sensitivity of 87.8% (95% CI: 86.0–89.5) for RIF resistant phenotype prediction. Three mutations (S450L, D435V and H445D) were classified into Group 1 and four mutations (H445Y, S450W, S441L and H445L) were found in Group 2 (Associated with interim resistance) based on initial confidence grading. Fifty-three mutations initially classified in Groups 3, 4 and 5 were moved to Group 2 based on previous WHO Guidance, WHO-endorsed gDST assays, and additional grading criteria, which included two mutations outside the RRDR (V170F and I491F) and two borderline RIF-resistance mutations (L430P and H445N). The thirty-eight Group 2 mutations were all in the RRDR and they were classified according to the expert rule that any RRDR mutation, except for synonymous mutations, should be assumed to confer RIF resistance. This expert rule was first introduced by WHO in 2018 and reaffirmed in 2021 *(4,10,11).* Of note, two of 60 (3.3%) Group 1 and 2 mutations (*rpo*B mutations V170F and I491F bolded in the table below), detected in 11 of the 1394 rifampicin-resistant strains, are not otherwise detected by the mWRDs used for diagnostic and drug susceptibility testing in India. These findings highlight the added value of sequencing for emergent and existing DR-TB detection and surveillance.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
S450L	*rpo*B	99	99	1122	9	273	708	99.2%	91.67%	86.45%	96.88%	80.43 %	98.74%	Inf	Assoc w R		1) Assoc w R
D435V	*rpo*B	11	11	74	6	1323	709	92.5%	64.71%	41.99%	87.42%	5.3%	99.16%	Inf	Assoc w R		1) Assoc w R
H445D	*rpo*B	7	7	28	0	1369	715	100.0%	100%	100%	100%	2%	100%	Inf	Assoc w R		1) Assoc w R
H445Y	*rpo*B	12	12	26	0	1371	715	100.0%	100%	100%	100%	1.86%	100%	Inf	Assoc w RI		2) Assoc w RI
S450W	*rpo*B	3	3	18	2	1379	713	90.0%	60%	17.06%	100%	1.29%	99.72%	Inf	Assoc w RI		2) Assoc w RI
S441L	*rpo*B	3	3	7	0	1390	715	100.0%	100%	100%	100%	0.5%	100%	Inf	Assoc w RI		2) Assoc w RI
H445L	*rpo*B	2	4	9	2	1388	713	81.8%	50%	1%	99%	0.64%	99.72%	0.5137	Assoc w RI		2) Assoc w RI
L430R	*rpo*B	0	0	10	0	1387	715	100.0%	Undef	Undef	Undef	0.72%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
H445R	*rpo*B	1	1	9	0	1388	715	100.0%	100%	100%	100%	0.64%	100%	Inf	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
H445P	*rpo*B	0	0	5	0	1392	715	100%	Undef	Undef	Undef	0.36%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
V170F	***rpo*B**	**0**	**0**	**8**	**1**	**1389**	**714**	**88.9%**	**0%**	**0%**	**0%**	**0.57%**	**99.86%**	**Undef**	**Not Assoc w RI**	**Outside RRDR**	**2) Assoc w RI**
Q432P	*rpo*B	0	0	7	1	1390	714	87.5%	0%	0%	0%	0.50%	99.86%	Undef	Not Assoc w RI		2) Assoc w RI
Q432K	*rpo*B	0	0	4	0	1393	715	100%	Undef	Undef	Undef	0.29%	100%	Undef	Uncert. Sig.		2) Assoc w RI
Q429H	*rpo*B	0	0	6	1	1391	714	85.7%	0%	0%	0%	0.43%	99.86%	Undef	Not Assoc w RI	RRDR	2) Assoc w RI
T427I	*rpo*B	0	0	2	0	1395	715	100%	Undef	Undef	Undef	0.14%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
Q432L	*rpo*B	0	0	2	0	1395	715	100%	Undef	Undef	Undef	0.14%	100%	Undef	Uncert. Sig.		2) Assoc w RI
S428T	*rpo*B	0	0	1	0	1395	716	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
K446Q	*rpo*B	0	0	2	0	1395	715	100%	Undef	Undef	Undef	0.14%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
S431T	*rpo*B	0	0	2	0	1393	717	100%	Undef	Undef	Undef	0.14%	100%	Undef	Not Assoc w RI	RRDR	2) Assoc w RI
S431R	*rpo*B	1	1	2	0	1393	717	100%	100%	100%	100%	0.14%	100%	Inf	Uncert. Sig.	RRDR	2) Assoc w RI
H445Q	*rpo*B	0	0	3	1	1394	714	75.0%	0%	0%	0%	0.21%	99.86%	Undef	Not Assoc w RI	RRDR	2) Assoc w RI
D435G	*rpo*B	0	1	8	4	1389	711	66.7%	0%	0%	0%	0.57%	99.44%	0	Not Assoc w RI	Previous WHO guidance	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
L452V	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
L452Q	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
D435A	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	Previous WHO guidance	2) Assoc w RI
N437Y	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
T427A	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
N437D	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
H445S	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.		2) Assoc w RI
Q436P	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
R448Q	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
ATTCATG1296ATTCATGTTCATG	*rpo*B	1	1	1	0	1396	715	100%	100%	100%	100%	0.07%	100%	Inf	Uncert. Sig.	RRDR	2) Assoc w RI
ATTC1296ATTCTTC	*rpo*B	1	1	1	0	1396	715	100%	100%	100%	100%	0.07%	100%	Inf	Uncert. Sig.		2) Assoc w RI
S428R	*rpo*B	0	0	3	2	1394	713	60%	0%	0%	0%	0.21%	99.72%	Undef	Not Assoc w RI	RRDR	2) Assoc w RI
H445C	*rpo*B	1	1	4	3	1393	712	57.1%	25.0%	0%	67.4%	0.29%	99.58%	Inf	Not Assoc w RI		2) Assoc w RI
I491F	***rpo*B**	**0**	**3**	**3**	**11**	**1394**	**704**	**21.4%**	**0%**	**0%**	**0%**	**0.21%**	**98.46%**	**0**	**Not Assoc w RI**	**Outside RRDR**	**2) Assoc w RI**

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
L452P	*rpo*B	1	16	7	23	1390	692	23.3%	4.2%	0%	12.2%	0.50%	96.78%	0.0332	Not Assoc w RI		2) Assoc w RI
D435Y	*rpo*B	2	10	27	44	1370	671	38.0%	4.4%	0%	10.2%	1.93%	93.85%	0.1224	Not Assoc w RI		2) Assoc w RI
L430P	*rpo*B	3	21	23	64	1374	651	26.4%	4.5%	0%	9.4%	1.65%	91.05%	0.079	Not Assoc w RI	Border line	2) Assoc w RI
H445N	*rpo*B	1	1	7	20	1390	695	25.9%	4.8%	0%	13.9%	0.50%	97.20%	Inf	Not Assoc w R	Border line	2) Assoc w RI
S431G	*rpo*B	0	0	1	1	1396	714	50%	0%	0%	0%	0.07%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
S428I	*rpo*B	0	0	1	1	1396	714	50%	0%	0%	0%	0.07%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
GAACAAC1308GAAC	*rpo*B	0	0	1	2	1396	713	33.3%	0%	0%	0%	0.07%	99.72%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
GGCACCAGC1276GGC	*rpo*B	0	0	1	3	1396	712	25.0%	0%	0%	0%	0.07%	99.58%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
M434I	*rpo*B	0	0	1	4	1396	711	20.0%	0%	0%	0%	0.07%	99.44%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
CAATTCATGGA1294CA	*rpo*B	0	0	0	1	1397	714	0%	0%	0%	0%	0%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
M434L	*rpo*B	0	0	0	1	1397	714	0%	0%	0%	0%	0%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
A451V	*rpo*B	0	0	0	1	1397	714	0%	0%	0%	0%	0%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
M434V	*rpo*B	0	0	0	1	1397	714	0%	0%	0%	0%	0%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
CACCAGCCAGC TGA1278CA	*rpo*B	0	0	0	1	1397	714	0%	0%	0%	0%	0%	99.86%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
S428C	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
S431C	*rpo*B	0	0	2	1	1395	714	66.7%	0%	0%	0%	0.14%	99.86%	Undef	Not Assoc w RI	RRDR	2) Assoc w RI
Q432R	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
F433L	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
N438T	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
S441!	*rpo*B	0	0	0	2	1397	713	0%	0%	0%	0%	0%	99.72%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
S441P	*rpo*B	0	0	0	3	1397	712	0%	0%	0%	0%	0%	99.58%	Undef	Not Assoc w R	RRDR	2) Assoc w RI
S450P	*rpo*B	0	0	3	0	1394	715	100%	Undef	Undef	Undef	0.21%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
S450!	*rpo*B	0	0	2	0	1395	715	100%	Undef	Undef	Undef	0.14%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI
L452R	*rpo*B	0	0	1	0	1396	715	100%	Undef	Undef	Undef	0.07%	100%	Undef	Uncert. Sig.	RRDR	2) Assoc w RI

Isoniazid

For Isoniazid Group 1 mutations, we had a sensitivity of 98.6% (95% CI: 97.9–99.1) for INH resistant phenotypic prediction. Two promoter mutations were detected, namely C-15T of *fab*G1 (Group 1) and C-81T of *ahp*C (Group 2). Only one *kat*G mutation (S315T) was found in Group 1, while two (S315T2 and R463L) were found in Group 2. Thirty-seven *kat*G mutations classified into Group 3 (Uncertain Significance) and six classified into Group 5 (Not associated with resistance) were moved to Group 2 based on Additional Grading Criteria (Indel or premature stop codon loss of function [LoF]) *(2– 4, 9).* Of note, two INH Group 2 resistance-associated single nucleotide changes (*kat*G R463L and *ahp*C C-81T) and 42 unique instances of inserts, deletions, or stop codons detected by WGS are not specifically targeted for detection by available mWRDs used for clinical detection of INH resistance in India. As discussed for Rifampicin above, these findings highlight the value of sequencing-based surveillance for enhanced control of INH-resistant transmission.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
S315T	*kat*G	79	80	1421	5	322	363	99.7%	94.1%	89.0%	99.1%	81.53%	98.64%	89.059	Assoc w R		1) Assoc w R
C-15T	*fab*G1/ promoter	5	5	296	2	1449	364	99.3%	71.4%	38.0%	100%	16.96%	99.45%	Inf	Assoc w R		1) Assoc w R
S315T2	*kat*G	2	2	15	0	1730	366	100%	100%	100%	100%	0.86%	100%	Inf	Assoc w RI		2) Assoc w RI
R463L	***kat*G**	**8**	**185**	**1511**	**313**	**234**	**53**	**82.8%**	**2.3%**	**0.8%**	**4.2%**	**86.59%**	**14.48%**	**0.0102**	**Assoc w RI**		**2) Assoc w RI**
C-81T	***ahp*C/ promoter**	**2**	**2**	**5**	**0**	**1740**	**366**	**100%**	**100%**	**100%**	**100%**	**0.29%**	**100%**	**Inf**	**Assoc w RI**		**2) Assoc w RI**
S315N	*kat*G	0	0	25	0	1720	366	100%	Undef	Undef	Undef	1.43%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GCCCCC372GCCCC	***kat*G**	**0**	**0**	**3**	**0**	**1742**	**366**	**100%**	**Undef**	**Undef**	**Undef**	**0.17%**	**100%**	**Undef**	**Uncert. Sig.**	**Indel or premature stop codon (LoF)**	**2) Assoc w RI**

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
TC1003TCC	*katG*	0	0	2	0	1743	366	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCC1177TCCC	*katG*	0	0	2	0	1743	366	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGG1145AGGG	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACC1334ACCCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CTCGGGTTCGGG867CTCGGGTTCGGGTTCGGG	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
W668!	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CG486CGG	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCCCC372GCCCCCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GC1920GCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCC1433GCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCC1285GCC	*katG*	0	0	1	1	1744	365	50%	0%	0%	0%	0.06%	99.73%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GC1367GCC	*katG*	0	0	2	0	1743	366	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
W728!	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CTTACCGCTGTAACG639C	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACC1550ACCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
TTGT1734TTGTGT	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CTT474CT	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACCTTGCCACTGCCATCCTTGCC2060ACCTTGCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGCGC1921AGCGCGC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TC1868TCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACC1485ACCCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGG18TGGGG	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGCTTGGGGACCAGC1301TGC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GC1855GCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TC29TCC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CCCGGCGCC G369CCCG	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CACGACGGG AC69CAC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCCC644TCCC CC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CA1614CATA	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
W39!	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
W341!	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GACAC1616G ACACAC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
C2106CA	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CGGG717CGG GG	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AC2081ACC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CCGGTGGTGGTTTC TGTAATGGGTGGGT GTTGC41CCGGTGGT GGTTTCTGTAATGG GTGGGTGTTGCGGT GGTGGTTTCTGTAAT GGGTGGGTGTTGC	*katG*	0	0	1	0	1744	366	100%	Undef	Undef	Undef	0.06%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Q190!	*katG*	0	0	0	1	1745	365	0%	0%	0%	0%	0%	99.73%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
!741C	*katG*	0	0	0	1	1745	365	0%	0%	0%	0%	0%	99.73%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GACA61GA	*katG*	0	0	0	1	1745	365	0%	0%	0%	0%	0%	99.73%	Undef	Not Assoc w R	Indel or premature	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
																stop codon (LoF)	
K554!	*katG*	0	0	0	1	1745	365	0%	0%	0%	0%	0%	99.73%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCCC703GCCCCC	*katG*	0	0	0	1	1745	365	0%	0%	0%	0%	0%	99.73%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Ethambutol

No EMB resistance-associated mutations were classified into Group 1 and only seven were classified into Group 2. All seven mutations associated with interim resistance were within the well-characterized *emb*B gene, including one within its promoter region. The resulting sensitivity of Group 2 was 78.4% (95% CI: 75.2–81.3), specificity was 77.4% (95% CI: 75.1–79.6), and PPV was 64.3% (95% CI: 61.9–66.7%), all of which were relatively low. These low performance values may be explained by the known association of *emb*B resistance-associated mutations to confer minimum inhibitory concentrations (MICs) close to the phenotypic DST critical concentration (CC), resulting in poor categorical agreement with phenotypic DST *(12–15)*. This rational is similarly explained in the WHO Mutation Catalogue. Of note, none of the mWRDs used for diagnostic and drug susceptibility testing in India currently target ethambutol resistance-associated genes or targets, making this list below an important resource for genotypic drug susceptibility testing data that may be compared with phenotypic DST findings.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
M306V	*emb*B	62	131	364	176	358	1213	67.4%	26.1%	20.5%	31.6%	50.42%	87.33%	3.0445	Assoc w RI		2) Assoc w RI
C-16T	*emb*A/promoter	2	9	48	15	672	1376	76.2%	11.8%	0%	27.1%	6.67%	98.92%	0.585	Assoc w RI		2) Assoc w RI
Q497R	*emb*B	27	55	103	92	617	1299	52.8%	22.7%	15.2%	30.2%	14.31%	93.39%	2.0302	Assoc w RI		2) Assoc w RI
D354A	*emb*B	5	9	13	5	707	1386	72.2%	50.0%	19.0%	81.0%	1.81%	99.64%	2.4505	Assoc w RI		2) Assoc w RI
Q497K	*emb*B	3	5	9	2	711	1389	81.8%	60.0%	17.1%	100%	1.25%	99.86%	2.9304	Assoc w RI		2) Assoc w RI
D1024N	*emb*B	4	4	22	21	698	1370	51.2%	16.0%	1.6%	30.4%	3.06%	98.49%	Inf	Assoc w RI		2) Assoc w RI
Y319S	*emb*B	4	7	7	3	713	1388	70.0%	57.1%	20.5%	93.8%	0.97%	99.78%	2.5956	Assoc w RI		2) Assoc w RI

Pyrazinamide

Mutations associated with PZA resistance (Group 1) had a sensitivity, specificity, and PPV of 10.4% (95% CI: 8.5–12.6), 99.3% (95% CI: 98.6–99.7), and 91.3% (95% CI: 84.1–95.4), respectively. Those classified as Group 2 had a sensitivity, specificity, and PPV of 77.6% (95% CI: 75.5–79.6),73.5 (95% CI: 70.9–76.0), and 79.8% (95% CI: 78.1–81.3), respectively. In total, 300 mutations were found to be associated with PZA resistance. Two differing mutations were found in Group 1, while 46 were found in Group 2, based on initial confidence grading including eight indel mutations and one promoter mutation. The expert rule that any nonsense mutation and indel in the coding region of *pnc*A, as well as all non-synonymous mutations, are presumed to cause loss of function resistance phenotypes (unless disproven) in rifampicin-resistant isolates was applied. By this rule, an additional 143 *pnc*A mutations (Green) were moved to Group 2 and ultimately classified as associated with interim resistance to PZA. An additional 109 mutations (Blue) were moved to Group 2 based on previous WHO guidance and WHO-endorsed gDST assays *(16–19).*

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
V139A	*pnc*A	6	8	42	2	850	1206	95.5%	75.0%	45.0%	100%	4.71%	99.83%	4.2565	Assoc w R		1) Assoc w R
A-11G	*pnc*A/promoter	8	12	52	7	839	1202	88.1%	53.3%	28.1%	78.6%	5.84%	99.42%	2.8653	Assoc w R		1) Assoc w R
R212R	*rps*A	19	182	500	191	392	1017	72.4%	9.1%	5.2%	12.9%	56.05%	84.19%	0.3024	Assoc w RI		2) Assoc w RI
I5S	*pnc*A	2	3	36	2	856	1206	94.7%	50.0%	1.0%	99.0%	4.04%	99.83%	2.8178	Assoc w RI		2) Assoc w RI
ACC392ACCCC	*pnc*A	3	4	26	4	866	1204	86.7%	42.9%	6.2%	79.5%	2.91%	99.67%	4.1709	Assoc w RI		2) Assoc w RI
V139G	*pnc*A	5	5	11	0	881	1208	100%	100%	100%	100%	1.23%	100%	Inf	Assoc w RI		2) Assoc w RI
T76P	*pnc*A	3	4	12	1	880	1207	92.3%	75.0%	32.6%	100%	1.35%	99.92%	4.1148	Assoc w RI		2) Assoc w RI
V128G	*pnc*A	3	3	9	0	883	1208	100%	100%	100%	100%	1.01%	100%	Inf	Assoc w RI		2) Assoc w RI
W68G	*pnc*A	3	3	8	0	884	1208	100%	100%	100%	100%	0.9%	100%	Inf	Assoc w RI		2) Assoc w RI
W68R	*pnc*A	5	5	7	0	885	1208	100%	100%	100%	100%	0.78%	100%	Inf	Assoc w RI		2) Assoc w RI
F94L	*pnc*A	5	5	5	0	887	1208	100%	100%	100%	100%	0.56%	100%	Inf	Assoc w RI		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
L4W	*pncA*	2	2	5	0	887	1208	100%	100%	100%	100%	0.56%	100%	Inf	Assoc w RI		2) Assoc w RI
G97D	*pncA*	3	3	4	0	888	1208	100%	100%	100%	100%	0.45%	100%	Inf	Assoc w RI		2) Assoc w RI
P62L	*pncA*	3	3	4	0	888	1208	100%	100%	100%	100%	0.45%	100%	Inf	Assoc w RI		2) Assoc w RI
V7G	*pncA*	4	4	4	0	888	1208	100%	100%	100%	100%	0.45%	100%	Inf	Assoc w RI		2) Assoc w RI
A-11C	*pncA*/promoter	2	2	4	0	888	1208	100%	100%	100%	100%	0.45%	100%	Inf	Assoc w RI		2) Assoc w RI
Q141P	*pncA*	2	2	4	0	888	1208	100%	100%	100%	100%	0.45%	100%	Inf	Assoc w RI		2) Assoc w RI
V180F	*pncA*	5	5	5	1	887	1207	83.3%	83.3%	53.5%	100%	0.56%	99.92%	Inf	Assoc w RI		2) Assoc w RI
K96R	*pncA*	5	6	5	1	887	1207	83.3%	83.3%	53.5%	100%	0.56%	99.92%	6.8038	Assoc w RI		2) Assoc w RI
D12A	*pncA*	2	3	6	2	886	1206	75.0%	50.0%	1.0%	99.0%	0.67%	99.83%	2.7223	Assoc w RI		2) Assoc w RI
ACC392ACCC	*pncA*	2	3	6	2	886	1206	75.0%	50.0%	1.0%	99.0%	0.67%	99.83%	2.7223	Assoc w RI		2) Assoc w RI
F58L	*pncA*	2	2	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
G162R	*pncA*	2	2	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
P62T	*pncA*	3	3	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
Q10K	*pncA*	2	2	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
T135P	*pncA*	3	3	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
P54R	*pncA*	3	3	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
P54L	*pncA*	2	2	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
A3P	*pncA*	2	2	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
S67P	*pncA*	2	2	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Assoc w RI		2) Assoc w RI
L35P	*pncA*	2	3	4	1	888	1207	80%	66.7%	13.3%	100%	0.45%	99.92%	2.7185	Assoc w RI		2) Assoc w RI
TCC473TCCC	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
TGGTATCGG502TGGTATCGGTATCGG	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
V180L	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
K96T	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
CCGACCACATCGACC395CCGACC	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
T47I	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
A46T	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
AT408ATT	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
TGG91TG	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
A171E	*pncA*	2	2	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Assoc w RI		2) Assoc w RI
A146T	*pncA*	2	2	3	1	889	1207	75.0%	66.7%	13.3%	100%	0.34%	99.92%	Inf	Assoc w RI		2) Assoc w RI
T47A	*pncA*	2	2	5	3	887	1205	62.5%	40.0%	0%	82.9%	0.56%	99.75%	Inf	Assoc w RI		2) Assoc w RI
Q10P	*pncA*	2	3	4	2	888	1206	66.7%	50.0%	1.0%	99.0%	0.45%	99.83%	2.7162	Assoc w RI		2) Assoc w RI
G132D	*pncA*	2	3	3	2	889	1206	60.0%	50.0%	1.0%	99.0%	0.34%	99.83%	2.7132	Assoc w RI		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
T76I	*pncA*	2	3	2	1	890	1207	66.7%	66.7%	13.3%	100%	0.22%	99.92%	2.7124	Assoc w RI		2) Assoc w RI
GC418GCC	*pncA*	2	2	2	1	890	1207	66.7%	66.7%	13.3%	100%	0.22%	99.92%	Inf	Assoc w RI		2) Assoc w RI
Q10!	*pncA*	2	2	2	2	890	1206	50.0%	50.0%	1.0%	99.0%	0.22%	99.83%	Inf	Assoc w RI		2) Assoc w RI
L27P	*pncA*	1	1	91	1	801	1207	98.9%	50.0%	0%	100%	10.2%	99.92%	Inf	Uncert. Sig.		2) Assoc w RI
G132A	*pncA*	1	2	91	4	801	1204	95.8%	20.0%	0%	55.1%	10.2%	99.67%	1.5031	Not Assoc w RI		2) Assoc w RI
L182S	*pncA*	0	0	17	0	875	1208	100%	Undef	Undef	Undef	1.91%	100%	Undef	Uncert. Sig.		2) Assoc w RI
T177P	*pncA*	0	0	15	2	877	1206	88.2%	0%	0%	0%	1.68%	99.83%	Undef	Not Assoc w RI		2) Assoc w RI
D136G	*pncA*	1	1	9	0	883	1208	100%	100%	100%	100%	1.01%	100%	Inf	Uncert. Sig.		2) Assoc w RI
A3E	*pncA*	0	0	8	0	884	1208	100%	Undef	Undef	Undef	0.9%	100%	Undef	Uncert. Sig.		2) Assoc w RI
L172P	*pncA*	1	1	7	0	885	1208	100%	100%	100%	100%	0.78%	100%	Inf	Uncert. Sig.		2) Assoc w RI
C138R	*pncA*	1	1	7	0	885	1208	100%	100%	100%	100%	0.78%	100%	Inf	Uncert. Sig.		2) Assoc w RI
G108R	*pncA*	1	1	10	2	882	1206	83.3%	33.3%	0%	86.7%	1.12%	99.83%	Inf	Uncert. Sig.		2) Assoc w RI
D49G	*pncA*	1	1	5	0	887	1208	100%	100%	100%	100%	0.56%	100%	Inf	Uncert. Sig.		2) Assoc w RI
A134V	*pncA*	1	1	5	0	887	1208	100%	100%	100%	100%	0.56%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H71Y	*pncA*	0	1	8	2	884	1206	80.0%	0%	0%	0%	0.9%	99.83%	0	Not Assoc w RI		2) Assoc w RI
G78V	*pncA*	0	0	4	0	888	1208	100%	Undef	Undef	Undef	0.45%	100%	Undef	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
D49A	*pncA*	1	1	4	0	888	1208	100%	100%	100%	100%	0.45%	100%	Inf	Uncert. Sig.		2) Assoc w RI
CG394CGG	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GA18GAA	*pncA*	0	0	3	0	889	1208	100%	Undef	Undef	Undef	0.34%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
H57Y	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
L4S	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
T160A	*pncA*	0	0	3	0	889	1208	100%	Undef	Undef	Undef	0.34%	100%	Undef	Uncert. Sig.		2) Assoc w RI
A146V	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
M1I	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
M175V	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
A46V	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
S104R	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H51P	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H51R	*pncA*	1	1	3	0	889	1208	100%	100%	100%	100%	0.34%	100%	Inf	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
C14!	*pncA*	0	0	4	1	888	1207	80.0%	0%	0%	0%	0.45%	99.92%	Undef	Not Assoc w RI	Indel or premature stop codon (LoF)	2) Assoc w RI
T142A	*pncA*	0	1	4	1	888	1207	80.0%	0%	0%	0%	0.45%	99.92%	0	Not Assoc w RI		2) Assoc w RI
D12G	*pncA*	1	2	4	1	888	1207	80.0%	50.0%	0%	100%	0.45%	99.92%	1.3592	Uncert. Sig.		2) Assoc w RI
E91K	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
AG451AGG	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
G97S	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H51Y	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
F106L	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
T47P	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
R121P	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
W119!	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
L120R	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
L159R	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H57R	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
L120P	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
H51Q	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
A146E	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
G17D	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
Y41!	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
!187R	*pncA*	0	0	2	0	890	1208	100%	Undef	Undef	Undef	0.22%	100%	Undef	Uncert. Sig.		2) Assoc w RI
L85R	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H57D	*pncA*	1	1	2	0	890	1208	100%	100%	100%	100%	0.22%	100%	Inf	Uncert. Sig.		2) Assoc w RI
T167I	*pncA*	1	2	2	1	890	1207	66.7%	50.0%	0%	100%	0.22%	99.92%	1.3562	Uncert. Sig.		2) Assoc w RI
V125F	*pncA*	1	2	2	1	890	1207	66.7%	50.0%	0%	100%	0.22%	99.92%	1.3562	Uncert. Sig.		2) Assoc w RI
T100P	*pncA*	1	2	2	1	890	1207	66.7%	50.0%	0%	100%	0.22%	99.92%	1.3562	Uncert. Sig.		2) Assoc w RI
D49E	*pncA*	1	2	2	1	890	1207	66.7%	50.0%	0%	100%	0.22%	99.92%	1.3562	Uncert. Sig.		2) Assoc w RI
V7A	*pncA*	1	2	2	1	890	1207	66.7%	50.0%	0%	100%	0.22%	99.92%	1.3562	Uncert. Sig.		2) Assoc w RI
F106C	*pncA*	0	0	2	1	890	1207	66.7%	0%	0%	0%	0.22%	99.92%	Undef	Not Assoc w RI		2) Assoc w RI
CG54CGG	*pncA*	0	1	2	1	890	1207	66.7%	0%	0%	0%	0.22%	99.92%	0	Not Assoc w RI		2) Assoc w RI
H82R	*pncA*	1	2	2	1	890	1207	66.7%	50.0%	0%	100%	0.22%	99.92%	1.3562	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
Q10R	*pncA*	0	1	2	1	890	1207	66.7%	0%	0%	0%	0.22%	99.92%	0	Not Assoc w RI		2) Assoc w RI
ACATCGACCTCATCGAC389ACATCGAC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
TG183T	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
CGTGT261CGTGTGT	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
P69R	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
CCGCTGTCAGG484CCGCTGTCAGGCGCTGTCAGG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CG419CGG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
TG16TGG	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GCC455GCCCC	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GCC315GCCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GGT475GGTGT	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
ATGTGGAAGTCCTTG155ATG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
ACC396ACCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
GCC339GCCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
I6L	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
GCATACGTCCACCATACGT4GCATACGT	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
TA296T	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
C522CA	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
AAACCAACTCGA550AA	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
TGCGC529TGCGCGC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CA292CAA	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
V169I	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
G162S	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GGCACCCTTGT294G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GCC72GCCCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGCCA457TGGCCAGGCCA	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCC257TCCC	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GCC315GCCCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
L4!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
AGG295AGGG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
TG259TGG	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
CG97C	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
G97V	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
TGG457TGGGG	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CGGG232CGG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACCC486ACC	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GCCGCTGTAC GCTCCG315GC CG	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCC119TCCCC	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GC420G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
TCCAGACTGGGATGGAAG257TCCAGACTGGGATGGAAGCCAGACTGGGATGGAAG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
TCACC167TC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
K48T	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
K48N	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
E107Q	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
TCC239TCCC	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
V9A	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
R140G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
CAA400CAAA	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
T142M	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
G132S	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
T114P	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
L159P	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
C72Y	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
S59P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
W119R	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
C14G	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
CG342C	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
V155G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
D12E	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
T142P	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H137P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
V139M	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
A171V	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
H71R	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
A102P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
D136E	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
T135N	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
E91!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
I133T	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
TC407TCC	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
G97R	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CGAGGA198C	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
K48E	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
L116V	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
L85P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
L116P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
T160P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
TGG422TGGG	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
G17V	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
E127!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
W68!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
G132C	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
A143G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
V131G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
Y34D	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
A134G	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
D8G	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
S164P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
H43P	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
Q10H	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
I5T	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GGCAATACCG402GG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
GC528GCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
ATAGTCCGGTGT192AT	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GGCCAGCGCGGCGCCACCGGTTACCGCCAGCG84GGCCAGCG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
CGACGG508C	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
G132V	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
AG56A	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
S164!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
GCC107GCCCC	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
Q10E	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
CGAGGAATAGTCCGGTGTGCCGGAGAAGTGGTCACCCGGGTCGATGTGGA198CGA	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
G105R	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H71P	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
C138G	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
S88!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
V7F	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
M1T	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
F58C	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
W119C	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
V163A	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
V155M	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H82D	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
V93G	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
D136Y	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
V130A	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
L19P	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
A46P	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
Y103C	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
G97C	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
C138!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
H57Q	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
CGG532CGGG	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
H71Q	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
G97A	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
S104G	*pncA*	1	1	1	0	891	1208	100%	100%	100%	100%	0.11%	100%	Inf	Uncert. Sig.		2) Assoc w RI
E37!	*pncA*	0	0	1	0	891	1208	100%	Undef	Undef	Undef	0.11%	100%	Undef	Uncert. Sig.		2) Assoc w RI
!187G	*pncA*	0	0	3	3	889	1205	50.0%	0%	0%	0%	0.34%	99.75%	Undef	Not Assoc w R		2) Assoc w RI
S67W	*pncA*	1	1	2	2	890	1206	50.0%	33.3%	0%	86.7%	0.22%	99.83%	Inf	Not Assoc w R		2) Assoc w RI
V157A	*pncA*	1	1	1	1	891	1207	50.0%	50.0%	0%	100%	0.11%	99.92%	Inf	Not Assoc w R		2) Assoc w RI
K96E	*pncA*	1	2	1	1	891	1207	50.0%	50.0%	0%	100%	0.11%	99.92%	1.3547	Not Assoc w R		2) Assoc w RI
G105D	*pncA*	1	1	1	1	891	1207	50.0%	50.0%	0%	100%	0.11%	99.92%	Inf	Not Assoc w R		2) Assoc w RI
T61P	*pncA*	1	2	1	1	891	1207	50.0%	50.0%	0%	100%	0.11%	99.92%	1.3547	Not Assoc w R		2) Assoc w RI
P62S	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
P69S	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
CGG76CGGG	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CGACGAGGAATAG201C	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
P54S	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
L156P	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
A102V	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
C14R	*pncA*	0	0	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
V9G	*pncA*	0	1	1	1	891	1207	50.0%	0%	0%	0%	0.11%	99.92%	0	Not Assoc w R		2) Assoc w RI
Y103H	*pncA*	1	2	1	2	891	1206	33.3%	33.3%	0%	86.7%	0.11%	99.83%	1.3535	Not Assoc w R		2) Assoc w RI
!187W	*pncA*	0	1	1	2	891	1206	33.3%	0%	0%	0%	0.11%	99.83%	0	Not Assoc w R		2) Assoc w RI
F58V	*pncA*	0	0	1	3	891	1205	25.0%	0%	0%	0%	0.11%	99.75%	Undef	Not Assoc w R		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
C138F	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
V155L	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
E181D	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
V169A	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
V183L	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
E174Q	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
C184G	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
S179C	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
A170P	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
H137R	*pncA*	0	2	0	2	892	1206	0%	0%	0%	0%	0%	99.83%	0	Not Assoc w R		2) Assoc w RI
F94V	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
Q141!	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
M1K	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
D63A	*pncA*	0	2	0	2	892	1206	0%	0%	0%	0%	0%	99.83%	0	Not Assoc w R		2) Assoc w RI
L27R	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
TGGTAGTCCG CCGCTTCGGC CAGGTAGTC12 5TGGTAGTC	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
CGGCCG496CG GCCGGGCCG	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
TCC239TC	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
AG172AGG	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
S66L	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
P62Q	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
TC167TCC	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
P69L	*pncA*	0	1	0	2	892	1206	0%	0%	0%	0%	0%	99.83%	0	Not Assoc w R		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CAATA400CAATAATA	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CG385CGG	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
TGG170TGGGG	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
D63G	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
CAA413CAAA	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
CTT288CT	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
V130M	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
T168I	*pncA*	0	2	0	2	892	1206	0%	0%	0%	0%	0%	99.83%	0	Not Assoc w R		2) Assoc w RI
AC307A	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
D63E	*pncA*	0	1	0	2	892	1206	0%	0%	0%	0%	0%	99.83%	0	Not Assoc w R		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
S59F	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
L116M	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
L116Q	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
G17A	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
Y41D	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
GCGAGCC55GC GAGCCCGAGCC	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
G24V	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
S65P	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
G55V	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
CTGGCG536C	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
CGGG86CGG	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GC526GCC	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
V21A	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
P77L	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
D49V	*pncA*	0	0	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	Undef	Not Assoc w R		2) Assoc w RI
S59Y	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
T153I	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI
A28T	*pncA*	0	1	0	1	892	1207	0%	0%	0%	0%	0%	99.92%	0	Not Assoc w R		2) Assoc w RI

Fluoroquinolones - Levofloxacin, Moxifloxacin and Ofloxacin

WGS-detected Group 1 mutations associated with resistance to levofloxacin, moxifloxacin and ofloxacin had a sensitivity for resistance detection of 93.6% (95% CI: 92.0– 95.0), 85.3% (95% CI: 83.0–87.4) and 96.4% (95% CI: 95.3–97.4), respectively. The expert rule for FLQ was applied following the WHO Mutation Catalogue approach. This expert rule requires any *gyr*A or *gyr*B mutation associated with LFX resistance to also be classified as resistant to MFX and OFX *(2, 3)*. For levofloxacin resistance determination, five mutations (D94G, D94N, A90V, D94Y and D94H) were classified as Group 1 and five mutations (S91P, D94A, G88C, N499D and D461N) were classified as Group 2 based on initial confidence grading. Of the remaining five mutations, two (A504V and G88A) were moved to Group 2 based on previous WHO guidance, two (E501D and D89N) were moved to Group 2 based on FLQ cross-resistance, and one (E501V) was moved to Group 2 based on WHO-endorsed gDST assay results. For moxifloxacin resistance, six mutations (D94G, D94N, D94Y, S91P, D94A and D94H) were classified into Group 1 and five mutations (E501D, A90V, G88C, N499D and D89N) were classified into Group 2 based on initial confidence grading. Of the remaining four mutations, three (E504V, D461N and G88A) were moved to Group 2 based on FLQ cross resistance and one (E501V) was moved to Group 2 based on WHO-endorsed gDST assay results. For ofloxacin, seven mutations (D94G, A90V, D94N, D94A, D94Y, S91P and D94H) were classified into Group 1 and six mutations (T500N, G88C, E501D, N499D, D461N and D89N) were classified into Group 2 based on initial confidence grading. Two FLQ Group 2 resistance-associated mutations (*gyr*B D461N in 6 resistant strains and *gyr*B A504V in 8 resistant strains) that were detected by WGS are not detected by the mWRDs used for diagnostic and drug susceptibility testing in India.

Drug	Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
LFX	D94G	*gyr*A	501	513	557	15	535	1006	97.4%	97.1%	95.6%	98.5%	51.01%	98.53%	78.506	Assoc w R		1) Assoc w R
LFX	D94N	*gyr*A	82	85	104	3	987	1018	97.2%	96.5%	92.6%	100%	9.53%	99.71%	28.192	Assoc w R		1) Assoc w R
LFX	A90V	*gyr*A	183	261	270	80	822	940	77.1%	69.6%	64.0%	75.1%	24.73%	92.16%	2.6829	Assoc w R		1) Assoc w R
LFX	D94Y	*gyr*A	59	61	70	4	1020	1018	94.6%	93.7%	87.6%	99.7%	6.42%	99.61%	29.442	Assoc w R		1) Assoc w R
LFX	D94H	*gyr*A	14	14	21	0	1070	1021	100%	100%	100%	100%	1.92%	100%	Inf	Assoc w R		1) Assoc w R
LFX	S91P	*gyr*A	28	41	45	14	1046	1007	76.3%	66.7%	52.4%	80.9%	4.12%	98.63%	2.0735	Assoc w RI		2) Assoc w RI
LFX	D94A	*gyr*A	44	80	78	37	1013	984	67.8%	54.3%	43.5%	65.2%	7.15%	96.38%	1.1872	Assoc w RI		2) Assoc w RI
LFX	G88C	*gyr*A	7	7	7	0	1084	1021	100%	100%	100%	100%	0.64%	100%	Inf	Assoc w RI		2) Assoc w RI
LFX	N499D	*gyr*B	3	3	4	0	1087	1021	100%	100%	100%	100%	0.37%	100%	Inf	Assoc w RI		2) Assoc w RI

Drug	Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
LFX	**D461N**	***gyr*B**	**2**	**3**	**6**	**1**	**1085**	**1020**	**85.7%**	**66.7%**	**13.3%**	**100%**	**0.55%**	**99.9%**	**1.8802**	**Assoc w RI**		**2) Assoc w RI**
LFX	**A504V**	***gyr*B**	**0**	**0**	**8**	**0**	**1083**	**1021**	**100%**	**Undef**	**Undef**	**Undef**	**0.73%**	**100%**	**Undef**	**Uncert. Sig.**	**Previous WHO guidance**	**2) Assoc w RI**
LFX	E501D	*gyr*B	1	7	14	6	1077	1015	70.0%	14.3%	0%	40.2%	1.28%	99.41%	0.1571	Not Assoc w RI	FQ cross-resistance	2) Assoc w RI
LFX	G88A	*gyr*A	0	1	4	1	1087	1020	80.0%	0%	0%	0%	0.37%	99.9%	0	Not Assoc w RI	Previous WHO guidance	2) Assoc w RI
LFX	E501V	*gyr*B	0	1	0	1	1091	1020	0%	0%	0%	0%	0%	99.9%	0	Not Assoc w R	WHO-endorsed gDST assay	2) Assoc w RI
LFX	D89N	*gyr*A	1	5	2	4	1089	1017	33.3%	20.0%	0%	55.1%	0.18%	99.61%	0.2335	Not Assoc w RI	FQ cross-resistance	2) Assoc w RI
MFX	D94G	*gyr*A	492	514	547	25	468	1073	95.6%	95.2%	93.3%	97.0%	53.89%	97.72%	51.274	Assoc w R		1) Assoc w R
MFX	D94N	*gyr*A	81	85	102	5	911	1094	95.3%	94.2%	89.2%	99.1%	10.07%	99.55%	24.318	Assoc w R		1) Assoc w R
MFX	D94Y	*gyr*A	54	61	65	9	947	1090	87.8%	85.7%	77.1%	94.4%	6.42%	99.18%	8.8792	Assoc w R		1) Assoc w R
MFX	S91P	*gyr*A	32	41	49	10	964	1089	83.1%	76.2%	63.3%	89.1%	4.84%	99.09%	4.0166	Assoc w R		1) Assoc w R
MFX	D94A	*gyr*A	46	80	81	34	933	1064	70.4%	57.5%	46.7%	68.3%	7.99%	96.9%	1.5429	Assoc w R		1) Assoc w R
MFX	D94H	*gyr*A	13	14	20	1	993	1098	95.2%	92.9%	79.4%	100%	1.97%	99.91%	14.375	Assoc w R		1) Assoc w R
MFX	**E501D**	***gyr*B**	**5**	**7**	**18**	**2**	**995**	**1097**	**90.0%**	**71.4%**	**38.0%**	**100%**	**1.78%**	**99.82%**	**2.7563**	**Assoc w RI**		**2) Assoc w RI**
MFX	A90V	*gyr*A	117	262	202	148	814	949	57.7%	44.2%	38.2%	50.1%	19.88%	86.51%	0.9407	Assoc w RI		2) Assoc w RI
MFX	**G88C**	***gyr*A**	**7**	**7**	**7**	**0**	**1006**	**1099**	**100%**	**100%**	**100%**	**100%**	**0.69%**	**100%**	**Inf**	**Assoc w RI**		**2) Assoc w RI**
MFX	N499D	*gyr*B	3	3	4	0	1009	1099	100%	100%	100%	100%	0.39%	100%	Inf	Assoc w RI		2) Assoc w RI
MFX	**D89N**	***gyr*A**	**3**	**5**	**4**	**2**	**1009**	**1097**	**66.7%**	**60.0%**	**17.1%**	**100%**	**0.39%**	**99.82%**	**1.6308**	**Assoc w RI**		**2) Assoc w RI**
MFX	A504V	*gyr*B	0	0	8	0	1005	1099	100%	Undef	Undef	Undef	0.79%	100%	Undef	Uncert. Sig.	FQ cross-resistance	2) Assoc w RI
MFX	D461N	*gyr*B	0	3	4	3	1009	1096	57.1%	0%	0%	0%	0.39%	99.73%	0	Not Assoc w RI	FQ cross-resistance	2) Assoc w RI

Drug	Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
MFX	**G88A**	***gyrA***	**0**	**1**	**3**	**2**	**1010**	**1097**	**60.0%**	**0%**	**0%**	**0%**	**0.3%**	**99.82%**	**0**	**Not Assoc w RI**	**FQ cross-resistance**	**2) Assoc w RI**
MFX	E501V	*gyrB*	0	1	0	1	1013	1098	0%	0%	0%	0%	0%	99.91%	0	Not Assoc w R	WHO-endorsed gDST assay	2) Assoc w RI
OFX	D94G	*gyrA*	509	513	565	6	662	879	99.0%	98.8%	97.9%	99.8%	46.05%	99.32%	168.96	Assoc w R		1) Assoc w R
OFX	A90V	*gyrA*	251	259	342	7	885	877	98.0%	97.3%	95.3%	99.3%	27.87%	99.21%	31.091	Assoc w R		1) Assoc w R
OFX	D94N	*gyrA*	84	85	106	1	1120	885	99.1%	98.8%	96.5%	100%	8.65%	99.89%	66.375	Assoc w R		1) Assoc w R
OFX	D94A	*gyrA*	72	80	107	8	1119	878	93.0%	90.0%	83.4%	96.6%	8.73%	99.1%	7.0617	Assoc w R		1) Assoc w R
OFX	D94Y	*gyrA*	61	61	72	2	1153	885	97.3%	96.8%	92.5%	100%	5.88%	99.77%	Inf	Assoc w R		1) Assoc w R
OFX	S91P	*gyrA*	39	41	57	2	1169	884	96.6%	95.1%	88.5%	100%	4.65%	99.77%	14.746	Assoc w R		1) Assoc w R
OFX	D94H	*gyrA*	14	14	21	0	1205	886	100%	100%	100%	100%	1.71%	100%	Inf	Assoc w R		1) Assoc w R
OFX	**T500N**	***gyrB***	**2**	**2**	**12**	**0**	**1213**	**887**	**100%**	**100%**	**100%**	**100%**	**0.98%**	**100%**	**Inf**	**Assoc w RI**		**2) Assoc w RI**
OFX	**G88C**	***gyrA***	**7**	**7**	**7**	**0**	**1219**	**886**	**100%**	**100%**	**100%**	**100%**	**0.57%**	**100%**	**Inf**	**Assoc w RI**		**2) Assoc w RI**
OFX	**E501D**	***gyrB***	**2**	**7**	**15**	**5**	**1211**	**881**	**75.0%**	**28.6%**	**0%**	**62.0%**	**1.22%**	**99.44%**	**0.291**	**Assoc w RI**		**2) Assoc w RI**
OFX	N499D	*gyrB*	3	3	4	0	1222	886	100%	100%	100%	100%	0.33%	100%	Inf	Assoc w RI		2) Assoc w RI
OFX	D461N	*gyrB*	2	3	6	1	1220	885	85.7%	66.7%	13.3%	100%	0.49%	99.89%	1.4508	Assoc w RI		2) Assoc w RI
OFX	**D89N**	***gyrA***	**2**	**5**	**3**	**3**	**1223**	**883**	**50.0%**	**40.0%**	**0%**	**82.9%**	**0.24%**	**99.66%**	**0.4813**	**Assoc w RI**		**2) Assoc w RI**

Linezolid

Only one mutation (C154R) in the linezolid resistance-associated gene, *rplC*, was classified as Group 1. The resulting sensitivity of 51.7% (95%CI: 38.2–65.1) for prediction of phenotypic resistance was low, and there were no false positive predictions. while the specificity of 100% (95%CI, 99.8% - 100.0%) and PPV of 100.0% (95%CI, 100.0% - 100.0%) were high because there were no False Positive.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
C154R	*rplC*	25	26	30	0	31	2050	100%	100%	100%	100%	49.18%	100%	Inf	Assoc w R		1) Assoc w R

Amikacin, Capreomycin and Kanamycin

Amikacin resistance-associated mutations were only identified and classified into Group 1, with a sensitivity of 93.3% (95%CI: 88.3–96.6) for prediction of phenotypic resistance. Group 1 mutations associated with capreomycin and kanamycin resistance had a sensitivity of 81.6% (95%CI: 74.8–87.2) and 82.5% (95%CI: 76.9–87.3), respectively. According to these data, AMK and KAN had similar sensitivities, while the sensitivity associated with prediction of CAP resistance was relatively low. For amikacin, only two resistance-associated mutations (A1401G and G1484T) in the *rrs* gene were classified as Group 1. For capreomycin, these same two *rrs* mutations (A1401G & G1484T) were classified as Group 1, while three additional *rrs* and *gid* mutations (C1402T, GT293G and E121Q) were classified as Group 2 based on initial confidence grading, including one deletion. The remaining two mutations (GCCCCC103GCCCC and GCCCCC352GCCCC), which were initially included in Group 5, moved to Group 2 as they were classified as Group 1 in initial confidence grading within the WHO Mutation Catalogue 2021 (Purple). For kanamycin, the same two resistance-associated mutations harboured in *rrs* for AMK and CAP (A1401G and G1484T), as well as three *eis* promoter mutations (G-10A, C-14T and G-37T), were classified into Group 1. An additional two mutations (*rrs* C1402T and *eis* promoter G-10C) were classified as Group 2 based on initial confidence grading. Lastly, one additional *eis* promoter mutation (CG-7C), initially classified as Group 5 was moved to Group 2 (Blue) based on previous WHO guidance *(2, 3).* Two SLID Group 2 resistance-associated mutations (*rrs* C1402T and *gid* E121Q) that were detected by WGS are not detected by the mWRDs used for diagnostic and drug susceptibility testing in India.

Drug	Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
AMK	A1401G	*rrs*	149	151	148	2	17	1945	98.7%	98.7%	96.9%	100%	89.7%	99.9%	8523.7	Assoc w R		1) Assoc w R
AMK	G1484T	*rrs*	5	5	5	0	161	1946	100%	100%	100%	100%	3.01%	100%	Inf	Assoc w R		1) Assoc w R
CAP	A1401G	*rrs*	128	151	128	22	38	1922	85.3%	85.3%	79.7%	91.0%	77.11%	98.87%	281.48	Assoc w R		1) Assoc w R
CAP	G1484T	*rrs*	5	5	5	0	161	1944	100%	100%	100%	100%	3.01%	100%	Inf	Assoc w R		1) Assoc w R

Drug	Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CAP	**C1402T**	***rrs***	**4**	**4**	**4**	**0**	**162**	**1944**	**100%**	**100%**	**100%**	**100%**	**2.41%**	**100%**	**Inf**	**Assoc w RI**		**2) Assoc w RI**
CAP	GT293G	*gid*	2	2	2	0	164	1944	100%	100%	100%	100%	1.2%	100%	Inf	Assoc w RI		2) Assoc w RI
CAP	**E121Q**	***gid***	**2**	**2**	**2**	**0**	**164**	**1944**	**100%**	**100%**	**100%**	**100%**	**1.2%**	**100%**	**Inf**	**Assoc w RI**		**2) Assoc w RI**
CAP	GCCCCC103 GCCCC	*gid*	0	6	7	69	159	1875	9.2%	0%	0%	0%	4.22%	96.45%	0	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
CAP	GCCCCC352 GCCCC	*gid*	0	1	1	44	165	1900	2.2%	0%	0%	0%	0.6%	97.74%	0	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
KAN	A1401G	*rrs*	146	148	148	2	76	1886	98.7%	98.7%	96.8%	100%	66.07%	99.89%	1811.6	Assoc w R		1) Assoc w R
KAN	G-10A	*eis*/ promoter	13	15	15	2	210	1885	88.2%	86.7%	69.5%	100%	6.67%	99.89%	58.345	Assoc w R		1) Assoc w R
KAN	C-14T	*eis*/ promoter	7	9	9	2	216	1885	81.8%	77.8%	50.6%	100%	4%	99.89%	30.544	Assoc w R		1) Assoc w R
KAN	G-37T	*eis*/ promoter	7	8	7	1	218	1886	87.5%	87.5%	64.6%	100%	3.11%	99.95%	60.56	Assoc w R		1) Assoc w R
KAN	G1484T	*rrs*	5	5	5	0	220	1887	100%	100%	100%	100%	2.22%	100%	Inf	Assoc w R		1) Assoc w R

Drug	Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
KAN	C1402T	*rrs*	3	3	4	0	221	1887	100%	100%	100%	100%	1.78%	100%	Inf	Assoc w RI		2) Assoc w RI
KAN	G-10C	*eis*/ promoter	6	11	6	5	219	1882	54.55%	54.55%	25.12%	83.97%	2.67%	99.74%	10.312	Assoc w RI		2) Assoc w RI
KAN	CG-7C	*eis*/ promoter	0	0	0	1	225	1886	0%	0%	0%	0%	0%	99.95%	Undef	Not Assoc w R		2) Assoc w RI

Streptomycin

The Group 1 mutations associated with streptomycin resistance had a sensitivity of 83.7% (95% CI: 81.4–85.9) for prediction of phenotypic resistance. In total, 15 resistance-associated mutations were harboured within three genes (*rps*L, *rrs* and *gid*). Four mutations (*rps*L K43R and K88R, *rrs* C517T and A514C) were classified into Group 1, while 11 mutations were classified in Group 2 based on initial confidence grading. Based on the expert rule followed in the WHO Mutation Catalogue that any nonsense or indel polymorphisms identified in *gid* should be considered STM-resistant, five mutations were moved from Group 3 or Group 5 categories to Group 2 (Blue). Of note, all Group 1 mutations had a high solo count within strains classified as phenotypically resistant with PPV | SOLO values of > 80% which supports a highly significant association with resistance *(2, 3)*.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
K43R	*rps*L	654	662	755	9	311	1037	98.8%	98.6%	97.7%	99.5%	70.83%	99.14%	272.59	Assoc w R		1) Assoc w R
K88R	*rps*L	69	69	82	0	985	1045	100%	100%	100%	100%	7.69%	100%	Inf	Assoc w R		1) Assoc w R
C517T	*rrs*	27	28	27	1	1040	1044	96.4%	96.4%	89.6%	100%	2.53%	99.9%	27.104	Assoc w R		1) Assoc w R
A514C	*rrs*	23	25	27	2	1040	1043	93.1%	92.0%	81.4%	100%	2.53%	99.81%	11.533	Assoc w R		1) Assoc w R
A1401G	*rrs*	21	48	121	29	946	1016	80.7%	42.0%	28.3%	55.7%	11.34%	97.22%	0.8353	Assoc w RI		2) Assoc w RI
A906G	*rrs*	16	19	19	3	1048	1042	86.4%	84.2%	67.8%	100%	1.78%	99.71%	5.3028	Assoc w RI		2) Assoc w RI
A908C	*rrs*	9	10	11	1	1056	1044	91.7%	90%	71.4%	100%	1.03%	99.9%	8.8977	Assoc w RI		2) Assoc w RI
K88M	*rps*L	8	8	8	0	1059	1045	100%	100%	100%	100%	0.75%	100%	Inf	Assoc w RI		2) Assoc w RI
C905G	*rrs*	5	5	6	0	1061	1045	100%	100%	100%	100%	0.56%	100%	Inf	Assoc w RI		2) Assoc w RI
P75R	*gid*	5	5	5	0	1062	1045	100%	100%	100%	100%	0.47%	100%	Inf	Assoc w RI		2) Assoc w RI
K88T	*rps*L	5	5	5	0	1062	1045	100%	100%	100%	100%	0.47%	100%	Inf	Assoc w RI		2) Assoc w RI
A908G	*rrs*	2	2	4	0	1063	1045	100%	100%	100%	100%	0.37%	100%	Inf	Assoc w RI		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
A514T	*rrs*	4	5	5	1	1062	1044	83.3%	80.0%	44.9%	100%	0.47%	99.9%	3.9322	Assoc w RI		2) Assoc w RI
A134E	*gid*	2	2	2	0	1065	1045	100%	100%	100%	100%	0.19%	100%	Inf	Assoc w RI		2) Assoc w RI
L79S	*gid*	2	5	3	3	1064	1042	50.0%	40.0%	0%	82.9%	0.28%	99.71%	0.6529	Assoc w RI		2) Assoc w RI
Q125!	*gid*	1	1	5	1	1062	1044	83.3%	50.0%	0%	100%	0.47%	99.9%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Q127!	*gid*	1	1	1	0	1066	1045	100%	100%	100%	100%	0.09%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
E103!	*gid*	1	2	1	1	1066	1044	50.0%	50.0%	0%	100%	0.09%	99.9%	0.9794	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
S136!	*gid*	0	3	0	3	1067	1042	0%	0%	0%	0%	0%	99.71%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
E40!	*gid*	0	1	0	1	1067	1044	0%	0%	0%	0%	0%	99.9%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Ethionamide

Ethionamide resistance-associated mutations in Group 1 had a sensitivity, specificity and PPV of 47.4% (95%CI: 43.4–51.4), 97.7% (95%CI: 96.7–98.4), and 89.4% (95%CI: 85.7– 92.2) for prediction of phenotypic resistance, respectively. In total, 179 mutations were associated with ETO resistance. Out of these, only two promoter region mutations (*fab*G1 C-15T and *eth*A T-8C) were placed in Group 1, and 22 were placed in Group 2, based on initial confidence grading. The remaining number were moved to Group 2 based on additional grading criteria. Five of these mutations (M1T, G11V, G441GA and Q359! in *eth*A and GC753GCC in *eth*R) were moved based on WHO Mutation Catalogue classification (Purple), while the remaining 150 mutations were moved based on the expert rule that considers any premature stop codon and indel in *eth*A as associated with ETO resistance *(2, 3)*. Mutations bolded in the table below are those that fall outside of the inhA promoter and are therefore not detected by mWRDs targeting mutations associated with ETH resistance. Currently, as highlighted in sections above, these bolded mutations are therefore only detected by sequencing for surveillance of DR TB.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
C-15T	*fab*G1/ promoter	187	215	269	29	353	1461	90.3%	86.6%	82.0%	91.1%	43.25%	98.05%	27.641	Assoc w R		1) Assoc w R
T-8C	*eth*A	7	13	25	6	596	1485	80.7%	53.9%	26.8%	81.0%	4.03%	99.6%	2.9069	Assoc w R		1) Assoc w R
S266R	***inh*A**	**9**	**65**	**60**	**59**	**561**	**1432**	**50.4%**	**13.2%**	**5.2%**	**21.3%**	**9.66%**	**96.04%**	**0.4102**	**Assoc w RI**		**2) Assoc w RI**
L203L	***inh*A**	**10**	**19**	**18**	**9**	**603**	**1482**	**66.7%**	**52.6%**	**30.2%**	**75.1%**	**2.9%**	**99.4%**	**2.7308**	**Assoc w RI**		**2) Assoc w RI**
Y84D	*eth*A	9	10	9	1	612	1490	90.0%	90.0%	71.4%	100%	1.45%	99.93%	21.912	Assoc w RI		2) Assoc w RI
S94A	*eth*A	3	4	10	2	611	1489	83.3%	60.0%	17.1%	100%	1.61%	99.87%	7.311	Assoc w RI		2) Assoc w RI
G-17T	*eth*A	4	7	8	3	613	1488	72.7%	57.1%	20.5%	93.8%	1.29%	99.8%	3.2365	Assoc w RI		2) Assoc w RI
A341V	*eth*A	4	4	4	0	617	1491	100%	100%	100%	100%	0.64%	100%	Inf	Assoc w RI		2) Assoc w RI
CTTT365CTTTT	*eth*A	4	4	4	0	617	1491	100%	100%	100%	100%	0.64%	100%	Inf	Assoc w RI		2) Assoc w RI
R207C	*eth*A	3	4	5	1	616	1490	83.3%	75.0%	32.6%	100%	0.81%	99.93%	7.2565	Assoc w RI		2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CAAAA1243CAAA	***inh*A**	**7**	**14**	**10**	**7**	**611**	**1484**	**58.8%**	**50.0%**	**23.8%**	**76.2%**	**1.61%**	**99.53%**	**2.4288**	**Assoc w RI**		**2) Assoc w RI**
T342A	*eth*A	2	2	3	0	618	1491	100%	100%	100%	100%	0.48%	100%	Inf	Assoc w RI		2) Assoc w RI
W455!	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
Y276!	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
S390F	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
G43S	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
T88I	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
TGTAGGTGGG634TGTAGGTGGGGTAGGTGGG	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
TGCGC141TGCGCGC	*eth*A	2	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Assoc w RI		2) Assoc w RI
C137R	*eth*A	3	5	3	2	618	1489	60.0%	60.0%	17.1%	100%	0.48%	99.87%	3.6141	Assoc w RI		2) Assoc w RI
L134R	*eth*A	2	3	2	1	619	1490	66.7%	66.7%	13.3%	100%	0.32%	99.93%	4.8142	Assoc w RI		2) Assoc w RI
W21!	*eth*A	2	2	2	1	619	1490	66.7%	66.7%	13.3%	100%	0.32%	99.93%	Inf	Assoc w RI		2) Assoc w RI
G42D	*eth*A	2	3	2	1	619	1490	66.7%	66.7%	13.3%	100%	0.32%	99.93%	4.8142	Assoc w RI		2) Assoc w RI
G182D	*eth*A	2	2	2	2	619	1489	50.0%	50.0%	1%	99%	0.32%	99.87%	Inf	Assoc w RI		2) Assoc w RI
ATCGGCCCGACGAAATCCTCCGAGCCGGCGAATCTCGGC482ATCGGC	*eth*A	0	0	13	0	608	1491	100%	Undef	Undef	Undef	2.09%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TAGTTCTGATTCAG1382TAG	*eth*A	0	0	3	0	618	1491	100%	Undef	Undef	Undef	0.48%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
W69!	*ethA*	1	2	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACCCC826ACCC	*ethA*	1	2	2	1	619	1490	66.7%	50.0%	0%	100%	0.32%	99.93%	2.4071	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGC674AGCGC	*ethA*	1	2	2	1	619	1490	66.7%	50.0%	0%	100%	0.32%	99.93%	2.4071	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CAAAAA1048CAAAAAA	*ethA*	1	2	2	1	619	1490	66.7%	50.0%	0%	100%	0.32%	99.93%	2.4071	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GTTTT342GTTTTT	*ethA*	0	1	2	1	619	1490	66.7%	0%	0%	0%	0.32%	99.93%	0	Not Assoc w RI	Indel or premature stop codon (LoF)	2) Assoc w RI
CAAAAA1048CAAAA	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Y92!	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GTCTC695GTCTCTC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GC568GCC	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GTT861GT	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Q271!	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGGG1308TGGG	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GT1387G	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CA687CAA	*eth*A	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TG218TGG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
W391!	*eth*A	0	0	1	00	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACCCC826ACCCCC	*eth*A	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Q246!	*eth*A	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
M1T	*eth*A	1	1	3	5	618	1486	37.5%	16.7%	0%	46.5%	0.48%	99.66%	Inf	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
GTTTT342GTTT	*eth*A	1	3	2	2	619	1489	50.0%	33.3%	0%	86.7%	0.32%	99.87%	1.2027	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GC753GCC	*eth*R	0	1	2	3	619	1488	40.0%	0%	0%	0%	0.32%	99.8%	0	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
G11V	*eth*A	0	0	2	6	619	1485	25.0%	0%	0%	0%	0.32%	99.6%	Undef	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
C131!	*eth*A	1	2	1	1	620	1490	50.0%	50%	0%	100%	0.16%	99.93%	2.4032	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
W116!	*eth*R	1	1	1	1	620	1490	50.0%	50%	0%	100%	0.16%	99.93%	Inf	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AGGG386AGGGG	*eth*R	1	2	1	1	620	1490	50.0%	50%	0%	100%	0.16%	99.93%	2.4032	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
Y461!	*eth*A	0	0	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GC673GCC	*eth*A	0	1	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TC694TCC	*eth*R	0	0	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AGGGG865AGGG	*eth*A	0	0	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
W289!	*eth*A	0	0	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Y143!	*eth*A	1	2	1	2	620	1489	33.3%	33.3%	0%	86.7%	0.16%	99.87%	2.4016	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GGCGC756GGCGCGC	*eth*A	0	1	1	2	620	1489	33.3%	0%	0%	0%	0.16%	99.87%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GTT1035GT	*eth*A	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
W109!	*eth*A	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
C253!	*eth*A	0	0	0	5	621	1486	0%	0%	0%	0%	0%	99.66%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG88TG	*eth*A	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
ATT281AT	*eth*A	0	0	0	2	621	1489	0%	0%	0%	0%	0%	99.87%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CAGCCA687CA GCCAAGCCA	*ethA*	0	2	0	2	621	1489	0%	0%	0%	0%	0%	99.87%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TC1033T	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AGG631AG	*ethA*	0	2	0	2	621	1489	0%	0%	0%	0%	0%	99.87%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Y140!	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GT131GTT	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
G441GA	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
Q215!	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Q359!	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Mutation Catalogue 2021	2) Assoc w RI
GCC502GC	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Q347!	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGA316TGAGA	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Q269!	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GTC1392GTCTC	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CAAA141CAA	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CTTCTCG672C	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGGG1308TGGGGG	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CCA867C	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCC33GCC	*ethA*	0	1	0	2	621	1489	0%	0%	0%	0%	0%	99.87%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGCGC555TGCGCGC	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCC33GCCCC	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TCG167TCGCG	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GCC743GCCCC	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AAC1301AACAC	*ethA*	1	1	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TC313TCC	*ethA*	0	0	2	0	619	1491	100%	Undef	Undef	Undef	0.32%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
TG374TGG	*ethA*	0	0	2	0	619	1491	100%	Undef	Undef	Undef	0.32%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGGCCG61AGGCCGGCCG	*ethA*	0	0	2	0	619	1491	100%	Undef	Undef	Undef	0.32%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GTAGCCA1158G	*ethA*	0	0	2	0	619	1491	100%	Undef	Undef	Undef	0.32%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGGG1291AGG	*ethA*	1	1	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TC1154TCC	*ethA*	1	1	2	0	619	1491	100%	100%	100%	100%	0.32%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
S308!	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACC371ACCC	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCGCCG1063TCG	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG308TGGG	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGAG624TGAGGAG	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GACAGACAAAC1210G	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GAA300GAAA	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGCGC581AGCGCGC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
CGG172CG	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG697TGGG	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
K370!	*ethA*	0	1	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG710TGGG	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCGC676TCGCGC	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ATCGGCACTGATCACCTT324ATCGGCACTGATCACCTTCGGCACTGATCACCTT	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ATCTTC788ATC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Y235!	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TCGC1263TCGCGC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TC1187TCC	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GC81G	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
C27!	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CG215C	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GCC1128GCCCC	*eth*A	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CGGG84CGG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TC1391TCC	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
AGG902AGGG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGCG1038TGCGCG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TG1015TGG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TG778TGG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GAA906GAAA	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGGCG1433TG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
G103GC	*eth*R	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACGCG1356ACGCGCG	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CA801CAA	*eth*A	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCC1142GC	*eth*A	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
AG581AGG	*ethA*	0	0	1	0	620	1491	100%	Undef	Undef	Undef	0.16%	100%	Undef	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
G1152GC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CT1299CTT	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG872TG	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GA606G	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCC59GCCC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GA727G	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GTT1036GTTT	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
Q363!	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCCC345GCC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
ACAACGTCGAGGT23A	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG710TGGGG	*ethR*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
GCC1288GC	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
Y351!	*ethA*	1	1	1	0	620	1491	100%	100%	100%	100%	0.16%	100%	Inf	Uncert. Sig.	Indel or premature stop codon (LoF)	2) Assoc w RI
CTTTT111CTTTA	*ethA*	0	1	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AT779A	*ethA*	0	0	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TC1117TCC	*ethA*	0	1	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TCCC137TCCCC	*ethA*	0	1	1	1	620	1490	50.0%	0%	0%	0%	0.16%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGG241TGG	*ethA*	1	1	1	2	620	1489	33.3%	33.3%	0%	86.7%	0.16%	99.87%	Inf	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG761TG	*ethA*	0	2	0	2	621	1489	0%	0%	0%	0%	0%	99.87%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CGG977CG	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
ATGT1394ATGTGT	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GA286G	*ethR*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GT75GTT	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AGGATGGG245AGG	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GCC193GC	*mshA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CGG509CGGG	*mshA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Y155!	*mshA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG812TG	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TA816T	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
CA62C	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GAA906GA	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GT633GTT	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AG1043A	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AGG1037AGGG	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GGTGT948GGTGTGT	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GAA300GA	*ethA*	0	2	0	2	621	1489	0%	0%	0%	0%	0%	99.87%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
AGG1037AG	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Y286!	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
Y382!	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AGCCCCGCCACTGGACGCC1403AGCCCCGCCACTGGACGCCCCCGCCACTGGACGCC	*mshA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
S186!	*ethR*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGGG628TGGGG	*ethA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
AG94AGG	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GCGC879GCGCCGC	*mshA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TG878TGGCG	*mshA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TG878TGG	*mshA*	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGTCGATTCC286T	*ethA*	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
GGTG519GGTGTG	*msh*A	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TGG305TGGG	*eth*A	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
TAA1223TA	*eth*A	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
GT12G	*eth*A	0	0	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	Undef	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI
E274!	*eth*A	0	1	0	1	621	1490	0%	0%	0%	0%	0%	99.93%	0	Not Assoc w R	Indel or premature stop codon (LoF)	2) Assoc w RI

P-aminosalicylic acid

The Group 1 mutations associated with PAS resistance had a sensitivity, specificity, and PPV of 43.1% (95%CI: 33.9–52.6), 99.5% (95%CI: 99.0–99.7), and 82.0% (95%CI: 70.9–89.5), respectively. In total, eight mutations were found in three PAS resistance-associated genes (*thy*A, *fol*C & *rib*D). Four mutations (*thy*A T22A, *fol*C I43T, *rib*D TCCCCC83TCCCC, and *fol*C R49W) were classified as Group 1, including one deletion. Four mutations (*thy*A R126G, R99!, and T22N, as well as *fol*C E40G) were classified as Group 2, including one nonsense mutation.

Mutations	Gene Name	Present SOLO_R	Present SOLO_SR	Present in R (TP)	Present in S (FP)	Absent in R (FN)	Absent in S (TN)	PPV	PPV | SOLO	PPV | SOLO_lb	PPV | SOLO_ub	Sensitivity	Specificity	OR | SOLO	INITIAL CONFIDENCE GRADING	Additional Grading Criteria	FINAL CONFIDENCE GRADING
T22A	*thy*A	30	37	30	7	87	1988	81.1%	81.1%	68.5%	93.7%	25.64%	99.65%	97.931	Assoc w R		1) Assoc w R
I43T	*fol*C	6	10	9	4	108	1991	69.2%	60.0%	29.6%	90.4%	7.69%	99.8%	27.653	Assoc w R		1) Assoc w R
TCCCCC83TCCCC	*rib*D	6	6	6	0	111	1995	100%	100%	100%	100%	5.13%	100%	Inf	Assoc w R		1) Assoc w R
R49W	*fol*C	5	5	5	0	112	1995	100%	100%	100%	100%	4.27%	100%	Inf	Assoc w R		1) Assoc w R
R126G	*thy*A	3	5	3	2	114	1993	60.0%	60.0%	17.1%	100%	2.56%	99.9%	26.224	Assoc w RI		2) Assoc w RI
E40G	*fol*C	2	2	2	0	115	1995	100%	100%	100%	100%	1.71%	100%	Inf	Assoc w RI		2) Assoc w RI
R99!	*thy*A	2	3	2	1	115	1994	66.7%	66.7%	13.3%	100%	1.71%	99.95%	34.678	Assoc w RI		2) Assoc w RI
T22N	*thy*A	2	3	2	1	115	1994	66.7%	66.7%	13.3%	100%	1.71%	99.95%	34.678	Assoc w RI		2) Assoc w RI

Bedaquiline

Bedaquiline resistance association analysis was carried out by conducting phenotypic and genotypic DST (see Methodology below for detailed methods), and then comparing WGS-detected mutations against those published in peer-review literature as associated with bedaquiline resistance. By this method, 12 isolates were classified as resistant to the drug based on detection of 12 distinct mutations; one in each isolate. Eleven of the 12 mutations were located in the Rv0678 gene, while one was located in the *atp*E gene. The 12 isolates were not associated with a specific cluster of BDQ resistance, as evidenced by the strainwise distinct mutation profiles and widespread geographic distribution across eight states and one Union Territory (Table 4). Only four of the 12 isolates determined by the literature method to be genotypically BDQ-resistant were resistant according to pDST, while the remaining eight were phenotypically susceptible. The pDST-resistant strains harbored mutations in the Rv0678 (R50W, T33A, D15G, S53P) and *atp*E genes (I66M). Results are presented in Table 4 below without performance or statistical data due to the low overall sample size that did not support such analyses.

Table 4. Phenotypic and genotypic drug susceptibility profiles of Indian Mutation Catalogue strains harboring mutations published as associated with bedaquiline resistance

S. No	pDST	gDST (WGS) Mutations	Gene/ RvID
1	Susceptible	D88G	Rv0678
2	Susceptible	V1A	Rv0678
3	Susceptible	S53L	Rv0678
4	Susceptible	A59V	Rv0678
5	Susceptible	G11G	Rv0678
6	Resistant	R50W	Rv0678
7	Resistant	T33A	Rv0678
8	Resistant	I66M	*atp*E/ Rv1305
9	Susceptible	D15G	Rv0678
10	Susceptible	S52F	Rv0678
11	Resistant	S53P	Rv0678
12	Susceptible	I108T	Rv0678

Methodology

Outline

The survey underlying this catalogue involved phenotype-genotype correlation for MTB isolates and processed sputum specimens collected from across India and was multi-layered. In brief, it included:

a) Generation of high-quality, raw, whole genome sequencing reads from an Illumina Miseq instrument,
b) Use of a novel, validated bioinformatics pipeline for variant sequencing data interrogation, annotation, resistance determination, and lineage prediction *(20),*
c) Completion of phenotypic culture and DST by WHO-endorsed methods (LJ solid culture and BACTEC MGIT 960 liquid culture),
d) Repetition of sequencing and phenotypic DST for strains with initial discordance between gDST and pDST testing for any anti-TB drug, and
e) Resistance classification of variants into "Associated with" and "Not associated with" resistance categories, with confidence grading followed per adapted procedures outlined above in the Initial Confidence Grading and Additional Confidence Grading sections, and by the WHO Mutation Catalogue.

Sample Collection

From 2018 to 2021, a national survey of MTB samples collected from persons with presumed rifampicin-, multidrug- and/ or isoniazid-resistant tuberculosis (and from a small proportion of those with drug-sensitive tuberculosis) from 28 States and 4 Union Territories of India was conducted by ICMR-National Institute for Research in Tuberculosis. A quota sampling method was used for collection of samples, with statewise distribution based on 2017 DR-TB burden estimates. Coordinating sites for sample collection are listed below (Table 4). Sampling was not statistically representative of the geographic areas and is therefore not generalizable to the subnational or national drug-resistant TB epidemics.

Table 4. Coordinating Sites for Sample Collection

S. No	States / UTs	Laboratories
1	Andaman & Nicobar	STDC & IRL Chennai
2	Andhra Pradesh	STDC & IRL Visakhapatnam, DFIT Nellore
3	Arunachal Pradesh	STDC Naharlagun, Through IRL Guwahati
4	Assam	STDC & IRL Guwahati
5	Bihar	STDC & IRL Patna, Bhagalpur and Dharbhanga
6	Chandigarh	STDC & IRL Chandigarh
7	Chhattisgarh	STDC & IRL Raipur
8	Delhi	NRL NITRD, NDTB Centre, STDC & IRL AIIMS
9	Goa	JJ Hospital Mumbai
10	Gujarat	STDC & IRL Ahmedabad, Jamnagar
11	Haryana	STDC & IRL Karnal
12	Himachal Pradesh	STDC & IRL Dharampur
13	Jammu	STDC & IRL Srinagar
14	Jharkhand	STDC & IRL ITKI
15	Karnataka	NRL NTI, STDC & IRL Bengaluru, KIMS Hubli
16	Kashmir	STDC & IRL Srinagar
17	Kerala	STDC & IRL Thiruvananthapuram
18	Madhya Pradesh	NRL BMHRC Bhopal, STDC & IRL Indore
19	Maharashtra	STDC & IRL Nagpur, STDC & IRL Pune, JJ Hospital Mumbai, GTB Sewri, Aurangabad
20	Manipur	IRL Guwahati
21	Meghalaya	IRL Guwahati
22	Mizoram	IRL Guwahati
23	Nagaland	IRL Guwahati
24	Odisha	NRL RMRC Bhubaneswar, STDC & IRL Cuttack
25	Puducherry	STDC & IRL Puducherry
26	Punjab	STDC & IRL Patiala
27	Rajasthan	STDC & IRL Ajmer, SMS Jaipur
28	Sikkim	NRL NITRD Delhi
29	Tamil Nadu	NRL NIRT Chennai, STDC & IRL Chennai, STDC & IRL Madurai, STDC & IRL Puducherry
30	Telangana	STDC & IRL Hyderabad
31	Tripura	IRL Guwahati
32	Uttar Pradesh	NRL NJILOMD (Jalma) Agra, STDC & IRL Agra, STDC IRL Lucknow, BHU Varanasi
33	Uttarakhand	STDC & IRL Dehradun
34	West Bengal	STDC & IRL Kolkata, NBMC

Abbreviations: STDC (State TB Training and Demonstration Centre), IRL (Intermediate TB Reference Laboratory), NRL (National TB Reference Laboratory). All other abbreviations are proper names and refer to the institutes and organizations listed.

Phenotypic Drug Susceptibility Testing

Phenotypic DST was performed using Mycobacterial Growth Indicator Tubes (MGIT) in BACTEC^{TM} MGIT^{TM} 960 system (BD, Franklin Lakes, NJ, USA) using the 2018 WHO-recommended critical concentrations (Rifampicin (1.0µg/ml), Isoniazid (0.1µg/ml), Ethambutol (5.0µg/ml), Pyrazinamide (100µg/ml), Levofloxacin (1.5µg/ml), Moxifloxacin (0.5µg/ml), Ofloxacin (2.0µg/ml), Linezolid (1.5µg/ml), Amikacin (1.0µg/ml), Capreomycin (2.5µg/ml), Kanamycin (2.5µg/ml), Streptomycin (1.0µg/ml), Ethionamide (5.0µg/ml), and P-aminosalicylic acid (4.0µg/ml) *(29)*. Critical concentrations from 2018 were used as phenotypic testing began before release of the updated '2021 WHO Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)'. Future MGIT-based DST will use the updated critical concentrations to maximize detection of phenoptyic susceptibility and resistance. For circumstances in which genotypic and phenotypic results were discordant, pDST was repeated.

DNA Isolation

Genomic DNA was extracted from LJ-amplified isolates of MTB using the CTAB method and purified using the Genomic DNA Clean and Concentrator kit (ZYMO Research, Irvine, CA, USA). DNA quality and quantity were measured using a NanoDrop instrument (Thermo Fisher Scientific, Waltham, MA, USA) and the Qubit dsDNA Assay (Invitrogen, Waltham, MA, USA).

Whole Genome Sequencing

DNA libraries were prepared using NexteraXT DNA Library Preparation and Index kits (Illumina, San Diego, CA, USA). Average library sizes measured ~850 bp on the Bioanalyzer 2100 System (Agilent Technologies, Santa Clara, CA, USA), normalized in equimolar concentrations and loaded for WGS using the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA). The 2 × 251 cycles of paired-end read sequencing were performed on a Miseq sequencer (Illumina, San Diego, CA).

Quality Control

All NIRT testers were trained and deemed competent using standardized and/ or retrospectively collected and appropriately stored isolates before testing survey samples. All procedures were validated for accuracy, precision, and intertester agreement against established

acceptability criteria. Once sequencing data were available for survey samples, coverage depth and breadth were calculated after alignment to the H37Rv reference genome. Kraken analysis provided the top species for each isolate along with relatedness percentages for any other species *(21)*. After applying filter quality in variant calling as described in Variant Calling and Resistance Prediction below, the following criteria was used to remove low quality isolates from downstream analysis:

- If breadth coverage was < 85%, sequences were removed from further analysis as these isolates were considered to be contaminated.
- If isolates showed contamination with genomes from other species during the Kraken analysis, they were also removed from further downstream analytics to ensure MTB-specific genomes were analyzed for variant calling and resistance determination.

Variant Calling and Resistance Prediction

The genomes that passed quality control were analysed using CamNIRTResPred, an in-house, validated, genomic analytics pipeline *(20)*. In total, 2112 whole genome sequences from tested isolates passed quality control with reads of at least 60bp and a minimum base quality of 20. These sequences were filtered using Trimmomatic v0.36 (LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:60) *(22)*. A reference index for the H37Rv reference genome (NC_000916.3) using bwa v0.7.12 and the samtools faidx option were used to prepare the fasta reference index *(23, 24)*. After quality control, readers were mapped to the H37Rv reference genome (NC_000916.3) using bwa v0.7.12 mem with default parameters. Sorting, duplicate removal, and indel mapping correction was done using picard v2.2.4 and GATK v3.5 *(25, 26)*. Variants were identified using Samtools v1.3.1 and bcf tools v1.3.1 with parameters (samtools mpileup -d 8000 -t DP -B -u -g -m 4 and bcftools call -m -v -o) *(27)*.

To filter quality variants the following metrics were applied: base quality >50, mapping quality >30, read depth >5 and at least one read mapping in either direction. Variants supported by >75% of the mapped reads were classified as homozygous sites and those with <75% mapped reads were classified as heterozygous sites. The lineages of the isolates were then predicted using RD-analyzer *(28)*. Finally, the filtered variants were annotated and compared against a database of mutations containing published resistance-associated mutations for first- and second-line antituberculosis drugs to predict association with resistance.

Statistical Analysis

Based on genotype and phenotype comparison (Table 5), the number of drug-resistant and drug-susceptible samples with and without mutations were merged into 2x2 contingency tables. Sensitivity, Specificity, PPVs and ORs were calculated with corresponding CIs and Fisher's exact test, with *p* values according to the hypergeometric distribution. To control multiple testing, a Benjamini-Hochberg correction procedure *(30)* was used with a False Discovery Rate (FDR) of 5%. For SOLO mutation counts, only isolates with a single (SOLO) mutation were counted, instead of all isolates with a given mutation, and compared with the corresponding numbers of isolates without the mutation. The same statistical procedure was applied to the PPVs and ORs for SOLO mutations (PPV SOLO and OR SOLO), respectively, per the WHO Mutations Catalogue and shown below.

- PPV SOLO $= \dfrac{\text{Present SOLO\_R}}{\text{Present SOLO\_R + Present in S}}$

- OR SOLO $= \dfrac{\text{Present SOLO\_R}}{\text{Absent in R}} \Big/ \dfrac{\text{Present SOLO\_S}}{\text{Absent in S}}$

Table 5. Phenotype - Genotype Comparison Conditions

Diagnostic List	Phenotype Results	Genotype Results
True Positive (TP)	R	R
False Positive (FP)	S	R
False Negative (FN)	R	S
True Negative (TN)	S	S

All analyses were done in R v.4.1.0, MedCalc, v.19.2.6. The graphical representation was done in Tableau Desktop v.2022.2. *(31–33).*

Executive Summary

In conclusion, this catalogue documents MTB-specific mutations graded into five categories of TB drug-resistance association, based on comparison of genotypic and phenotypic data for a large collection of strains collected across India. Using the quota sampling method, a total of 3167 MTBC isolates were collected across 25 States and 4 Union Territories, of which 2112 isolates were selected for downstream analysis based on availability of quality genotypic (whole genome sequencing) and phenotypic (TB culture) drug susceptibility testing data. The downstream analysis identified and querried 8825 mutations based on the 2021 WHO Mutations Catalogue approach (*2*), which included mutation grading into 'Groups' based on their degree of evidence-based association with TB drug resistance. In the initial grading, 45 mutations were classified into Group 1 (associated with resistance) and 118 mutations were classified into Group 2 (associated with interim resistance). After applying the expert rules outlined in the WHO Mutations Catalogue, an additional 520 mutations were placed within Group 2, yielding a total of 683 mutations associated with resistance (Group 1 and Group 2 combined) to at least one antituberculosis drug.

As the number of strains characterized and added to the Indian Mutation Catalogue database increases, mutations currently classified within Groups 3, 4 and 5 may move to Groups 1 or 2. This additional data will be of particular value, as in some cases, the number of included strains in this 2022 version was too low to apply performance and statistical analyses or to achieve the established Group classification threshold. Regardless, this document serves as the first standardized resource for interpretation of mutations associated with drug-resistant TB in India to guide surveillance and disease control efforts nationally.

Data Availability Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: [to be added post-clearance upon achievement of Indian national approvals].

Ethics Statement

This project was reviewed in accordance with CDC human research protection procedures and was determined to be research (Human Subject Research Tracking No. 2017-461), but CDC investigators did not interact with human subjects or have access to identifiable data or

specimens for research purposes. The project was also reviewed and approved by the Ethical Committee of ICMR National Institute for Research in Tuberculosis, Chennai, Indian Council of Medical Research (Institutional Ethics Committee No. 2015019), and the Revised National Tuberculosis Control Programme, Indian Ministry of Health and Family Welfare. Informed consent was waived because activities involved only routine samples collected for the National TB Elimination Program (NTEP).

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC.

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