Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance

Second edition

World Health Organization

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Abbreviations and acronyms

Additional abbreviations and acronyms used are listed in Tables 4–5.

7H10	Middlebrook 7H10
7H11	Middlebrook 7H11
ALL data set	data set with all acceptable phenotypic drug-susceptibility testing results
AMK	amikacin
Assoc w R	associated with resistance
Assoc w R–interim	associated with resistance–interim
aR	algorithmically resistant
aS	algorithmically susceptible
aU	algorithmically uncertain
BDQ	bedaquiline
BMD	broth microdilution
bp	base pairs
CAP	capreomycin
CC	critical concentration
CFZ	clofazimine
CI	exact binomial confidence interval
DLM	delamanid
DST	drug-susceptibility testing
gDST	genotypic drug-susceptibility testing
pDST	phenotypic drug-susceptibility testing
EMB	ethambutol
ETO	ethionamide
FE-sig	significant with Fisher exact test
FQ	fluoroquinolone
HGVS	Human Genome Variation Society
indel	insertion/deletion
INH	isoniazid
KAN	kanamycin
lb	lower bound

LFX	levofloxacin
LJ	Löwenstein-Jensen
LoF	loss-of-function
LZD	linezolid
MGIT	BACTEC™ Mycobacterial Growth Indicator Tube™ 960
MIC	minimum inhibitory concentration
MODS	microscopic observation drug-susceptibility
MTBC	*Mycobacterium tuberculosis* complex
MFX	moxifloxacin
Not assoc w R	not associated with resistance
Not assoc w R–interim	not associated with resistance–interim
OFX	ofloxacin
OR	odds ratio
pDST	phenotypic drug susceptibility testing
PMD	pretomanid
PPV	positive predictive value
PTO	prothionamide
PZA	pyrazinamide
R	resistant or resistance
RIF	rifampicin
RPT	rifapentine
RRDR	rifampicin resistance-determining region
S	susceptible or susceptibility
SOLO	lone
STM	streptomycin
TB	tuberculosis
ub	upper bound
WGS	whole-genome sequencing
WHO data set	data set with WHO-endorsed phenotypic drug-susceptibility testing results

1 Introduction

A total of 1.6 million people died of tuberculosis (TB) in 2021, and approximately 10.6 million people developed active TB disease due to *Mycobacterium tuberculosis* complex (MTBC). Of the 10.6 million new cases, an estimated 450 000 had TB resistant to rifampicin (RIF), which requires rapid, accurate detection and characterization to initiate appropriate treatments *(1)*.

Detection of RIF resistance has improved significantly with the introduction of rapid molecular diagnostic tools that require less complex infrastructure and are simpler to perform than conventional phenotypic methods *(2)*. In 2012, globally, only 7% of individuals with bacteriologically confirmed TB were tested for RIF resistance *(3)*; by 2021, that proportion was 71% *(1)*. Over the same period, the number of individuals started on treatment for multi-drug- or RIF-resistant TB more than doubled, from 77 321 to 161 746, highlighting the central role of diagnostics in the TB response *(1,3)*. The molecular basis of RIF resistance in MTBC isolates is almost exclusively mutations in the RIF resistance-determining region (RRDR), an 81-base-pair fragment of the *rpoB* gene *(4)*. This knowledge and development of molecular tools that target RRDR have been critical to the delivery of new diagnostic solutions in the past decade *(5)*.

WHO recommends routine testing of all TB patients for resistance to RIF and isoniazid (INH), while resistance to the fluoroquinolones (FQs) is tested only when isolates are known to be RIF- or INH-resistant TB *(5)*. MTBC resistance mechanisms to INH and FQs are well understood, and molecular tools are commercially available for detecting mutations associated with phenotypic resistance to these drugs at a WHO-endorsed critical concentration (CC) *(6)*. After a long period of stagnation in innovation for TB treatment, the introduction of new drugs and repurposing of existing antimicrobial agents for the treatment of TB have significantly increased the potential for improved TB treatment. As resistance to new and repurposed drugs gradually increases in the community, however, concern has been raised about the lack of options for rapid detection of resistance to these new and repurposed drugs *(7–9)*. The recent WHO recommendation of several targeted next-generation sequencing assays for culture-free diagnosis of drug-resistant TB represents an important step towards improving the diagnostic landscape *(10)*. These assays provide sequences directly from sputum and reveal much more of the resistance-associated MTBC genome than traditional genotypic drug-susceptibility testing (gDST) assays *(2)*. They have been recommended for detecting resistance to up to 10 antibiotics simultaneously directly from clinical samples, but their sensitivity for predicting resistance to some drugs remains limited by incomplete understanding of the molecular basis of resistance *(10)*.

While allelic exchange experiments are the reference standard for demonstrating that a specific mutation is both necessary and sufficient to confer phenotypic resistance, these approaches are expensive, slow and technically demanding *(11)*. Therefore, association studies based on

whole-genome sequencing (WGS) and standardized phenotypic drug-susceptibility testing (pDST) data from large numbers of globally diverse MTBC isolates are indispensable for comprehensive investigations of the genetic basis of resistance, particularly in non-essential genes, where hundreds of loss-of-function (LoF) mutations can result in phenotypic resistance at a clinically relevant WHO CC *(12,13).*

A major obstacle to the development and diagnostic utility of sequence-based technologies and next-generation molecular diagnostics for gDST has been the lack of a standardized, comprehensive catalogue of mutations and their association with phenotypic drug resistance. In 2021, WHO published the first mutation catalogue, consisting of a high-quality, comprehensive list of confidence-graded MTBC genetic markers of phenotypic resistance *(14)*. The aim was to provide a resource that could be used to distinguish clinically relevant resistant variants (i.e. variants statistically associated with a resistant pDST result at a WHO CC) from those not associated with resistance and from those for which there are insufficient data to establish a meaningful association. While that catalogue helped to reduce continuing technical uncertainty about the number, identity and clinical interpretation of genomic resistance-determining regions for legacy drugs, data on graded mutations associated with new and repurposed drugs was very limited and so too was the representation of some geographical regions *(14)*.

The primary reason for revising the *Catalogue of mutations in* Mycobacterium tuberculosis *complex and their association with drug resistance*, published in 2021 (the first edition *(14)*) was to add sufficient new data on clinical MTBC isolates from global sources to the WHO database of genotypes and phenotypes to identify new genomic variants associated with phenotypic resistance to the new and repurposed TB therapeutics, while also improving detection of variants associated with resistance to all TB therapeutics and improving the geographical representation of data in the catalogue.

WHO held an expert consultation on 28 February, 1 March and 9 March 2023. All individuals who provided technical input were required to disclose any potential conflicts of interest, encompassing both financial and non-financial interests. Upon review no significant conflict of interests were identified. Participants' statements were summarized by the WHO at the start of the meeting and can be found in Annex 1.

For the analysis presented here, WGS and pDST data on the largest collection of multinational MTBC isolates to date (~52 000, up from ~38 000 analysed in 2021) were assembled to produce the *Catalogue of mutations in* Mycobacterium tuberculosis *complex and their association with drug resistance, second edition.* The second edition is intended to be a common, standardized reference for interpreting resistance to all first-line drugs (RIF, INH, ethambutol [EMB] and pyrazinamide [PZA]) and also to second-line drugs in group A (levofloxacin [LFX], moxifloxacin [MFX], bedaquiline [BDQ] and linezolid [LZD]), group B (clofazimine [CFZ]) and group C (delamanid [DLM], amikacin [AMK], streptomycin [STM], ethionamide [ETO] and prothionamide [PTO]). While kanamycin (KAN) and capreomycin (CAP) are no longer recommended for MDR-TB treatment by WHO, they are included in this analysis for historical context and because mutations associated with KAN resistance provide useful insights for interpreting some mutations that confer resistance to AMK *(15,16)*. Although WHO has not yet set a CC for pretomanid (PMD), this second edition provides some early guidance for interpretation of LoF mutations in the six genes required for activation

of this prodrug *(17,18)*. Guidance is also provided for rifapentine (RPT) *(4)*. This report on the second edition describes the revisions to the methods used to create the catalogue, the mutations identified and summaries of key findings for each drug. New areas for future research are also outlined. The report is intended to inform the development of new and improved molecular assays, based on sequencing or other methods, for comprehensive detection of resistance to TB drugs.

2 Overview of the second edition of the catalogue

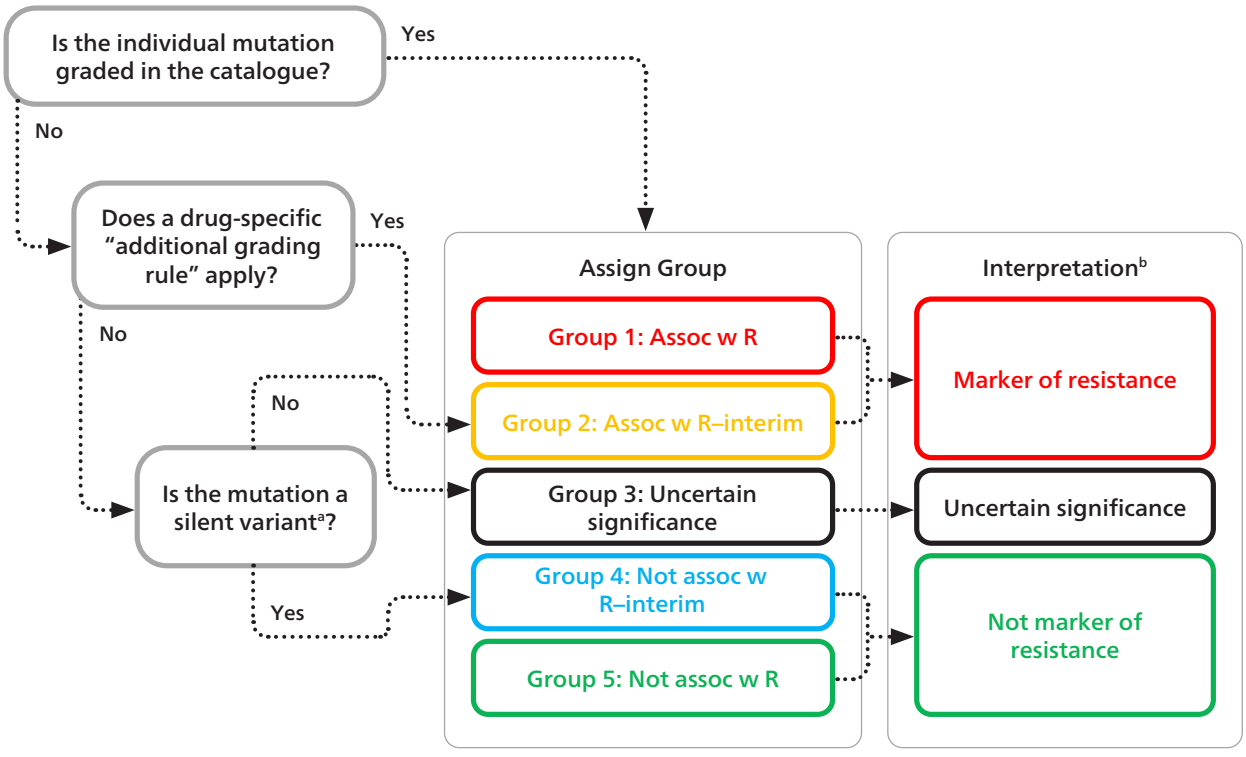
2.1 Understanding and using the catalogue

The second edition consists of three related elements:

- a list of graded variants (the catalogue master file and a file with the "genomic coordinates in VCF");
- evidence-based "additional grading rules", of which some are general and others drug-specific (Table 1); and
- evidence-based "other interpretation criteria" (Table 1).

These related elements can be used to interpret MTBC mutations detected with NGS or other gDST methods using the workflow demonstrated below (Fig. 1).

Fig. 1. Instructions for using the catalogue



[a] General "additional grading rule" (not shown in Table 1).

[b] "Other interpretation criteria", such as the level of resistance, must be considered for some Groups 1/2 variants (Table 1).

Table 1. Summary of "additional grading rules" and "other interpretation criteria" used in the second edition

Drug	Drug-specific additional grading rules[a]	Other interpretation criteria for Groups 1 and 2 mutations
RIF & RPT	Non-silent variants in RRDR of *rpoB*[b]	• Groups 1–5 classifications for RIF also apply to RPT.
INH	LoF in *katG*	• *katG* mutations assumed to confer high-level INH resistance. • *fabG1-inhA* mutations assumed to confer low-level INH resistance. • Multiple, genetically linked low-level resistance mutations have additive effects and should be considered to confer high-level INH resistance. • *fabG1-inhA* mutations confer cross-resistance to ETO and INH.
PZA	LoF in *pncA*	• If isolate identified as *M. canettii*, infer intrinsic PZA resistance.
LFX & MFX		• *gyrA* and *gyrB* mutations confer cross-resistance to LFX and MFX, but the level of LFX resistance is not stratified. • *gyrA* Gly88Cys, Asp94Asn, Asp94Gly, Asp94His and Asp94Tyr mutations assumed to confer high-level MFX resistance. • Remaining *gyrA* and *gyrB* mutations assumed to confer low-level MFX resistance. • Multiple, genetically linked low-level MFX resistance mutations have additive effects and should be considered to confer high-level MFX resistance.
BDQ & CFZ	LoF in *Rv0678* and *pepQ*	• *Rv0678* and *pepQ* mutations confer cross-resistance to BDQ and CFZ. • *Rv0678* mutations cannot confer resistance if genetically linked with LoF variants in *mmpL5* (epistasis).
DLM & PMD	LoF in *ddn*, *fbiA*, *fbiB*, *fbiC*, *fgd1*, and *Rv2983*	
AMK & KAN[c]		• *eis* promoter mutations cannot confer resistance if genetically linked with LoF variants in *eis* coding region (epistasis).
STM	LoF in *gid*	
ETO & PTO	LoF in *ethA*	• Groups 1–5 classifications for ETO also apply to PTO. • *fabG1-inhA* mutations confer cross-resistance to ETO and INH.
CAP[c]	LoF in *tlyA*	

For simplicity, the general "additional grading rule" whereby any novel silent variant is classified in Group 4 (Fig. 1) was not included in this table but applies to all genes for all drugs.

[a] When these drug-specific rules apply, the mutation in question is classified in Group 2 (Fig. 1).

[b] Changes at position 1346 in codon 449 of *rpoB* detected with Illumina sequencing, particularly if unfixed, may represent artefacts.

[c] No longer recommended for TB treatment.

Variants were graded if they occurred at an allele frequency of at least 75% in at least one isolate and if the pDST result was valid (see Section 5 for details). Specifically, these variants were stratified into one of five groups according to the amount and quality of evidence available to support the association statistically.

- Group 1: Associated with resistance (Assoc w R)
- Group 2: Associated with resistance–interim (Assoc w R–interim)
- Group 3: Uncertain significance
- Group 4: Not associated with resistance–interim (Not assoc w R–interim)
- Group 5: Not associated with resistance (Not assoc w R)

Groups 1 and 2 variants should be interpreted as markers of clinically relevant phenotypic resistance (i.e. mutations associated with phenotypic resistance at a WHO-endorsed CC), whereas Groups

4 and 5 variants are not markers of resistance (Fig. 1). The role of Group 3 mutations remains uncertain from the available evidence. Although the grading process for the second edition remained largely unchanged from that for the first edition, some new methods were introduced (Section 2.2), and considerably more isolates were analysed (Section 2.3). The performance of the resulting mutations listed as predictors of phenotypic resistance is summarized in Section 2.4.

Fig. 1 and Table 1 indicate how the three elements of the catalogue can be used to grade variants that were previously graded and those that were not. Some examples are given below to demonstrate the logic of the workflow:

- *katG* Ser315Thr and Ser315Ile mutations are graded as Group 1 and Group 2 mutations, respectively, and would, therefore, be interpreted as markers of INH resistance. In accordance with the "other interpretation criteria" (Table 1), they would be reported as conferring high-level INH resistance.
- The *katG* Gly33fs mutation is not listed in the catalogue but would be classified in Group 2 according to the *katG* LoF "additional grading rule" (Table 23) and considered to confer high-level INH resistance according to the "other interpretation criteria".
- The G>A mutation at position of 1350 of *rpoB*, resulting in a silent change at codon 450, is not included in the catalogue, but would be classified in Group 4 according to the general "additional grading rule" for RIF and RPT.
- The *ddn* Trp20* is in Group 2 for DLM and would also be reported as being in Group 2 for PMD by the LoF "additional grading rule" (Table 23).
- A sample with *gyrA* Ala90Val and Asp94Ala mutations would be reported as resistant to LFX. In contrast, the level of MFX resistance is stratified as low and high. Both variants are low-level resistance mutations. If they are not genetically linked (not phased according to the sequencing reads), or it is not clear whether they are genetically linked, they would be reported as "at least low-level resistant to MFX" (i.e. the sample is definitely low-level resistant, but high-level resistance due to another low-frequency mutation cannot be excluded, particularly at low sequencing coverage *(19)*). If the two mutations are genetically linked, their effects would be additive, and the sample would be reported as high-level resistant.

2.2 Main changes from the first edition of the catalogue

A detailed description of the methods used to create the second edition is provided in Section 5. The main improvements over the first edition are as follows.

- Data:
  - increased number and geographical diversity of MTBC isolates, including increased absolute number and prevalence of resistant isolates for most of the drugs considered (see Section 2.3); and
  - improved curation and prioritization of pDST results to increase the diversity of testing methods that could be included in analyses (see Sections 5.3 and 5.4).
- Bioinformatics pipeline:
  - improved pipeline for more appropriate handling of null calls and large deletions *(20)* (see Section 5.6); and

  - variants classified if they occurred at an allele frequency of ≥ 75% (previously ≥ 90%), with additional estimates of the impact of lowering the threshold of calling variants to ≥ 25% (see Table A.1 in the Annex).
- Association studies:
  - updated the list of target genes associated with resistance to all the drugs considered (see Table 21, p. 89) and revised the corresponding upstream regions (see Table 22 , p. 89);
  - revised the algorithm for Group 4/5 to reduce the risk of misclassifying mutations as not relevant for resistance and, consequently, of very major errors (Sections 5.7 and 5.8);
  - revised definition of LoF mutations (excluding inframe insertion/deletion [indel] mutations) to reduce the risk of overestimating resistance (Sections 3.4 and 5.6);
  - translated the Stata association algorithms into R in order to validate the association workflow code independently (quality control) and improve public access to the code (Section 5.2);
  - included new grading criteria: added new cross-resistance "additional grading rules" and added new evidence from laboratory mutation selection studies to improve genotype–phenotype associations (Sections 5.7 and 5.8). Notably, the catalogue now contains some mutations that confer resistance to BDQ and LZD that were identified only in laboratory-based selection experiments from at least two independent sites. These were clearly labelled as not occurring in our collection of clinical isolate data and were classified into Group 2 to reflect this; and
  - assessed the impact of epistasis for BDQ/CFZ and AMK/KAN (Sections 3.7 and 3.10).

A summary of the changes in the total number of variants by group and the justifications for those changes are provided in Fig. 2. A total of 226 mutations (green) were classified with higher certainty in the second edition; while group changes to 38 mutations (yellow) did not alter the final interpretation; and 22 mutations (red) were moved to Group 3 from other more certain groups. As relevant, these changes are discussed in more detail for each drug in Section 3.

Fig. 2. Changes (with explanations) in the classification of variants in the second edition as compared with the first edition

- Association now supported by WHO-endorsed assays
- Association now supported by ALL dataset only
- Association now supported by "relaxed" *pncA* thresholds
- Association from WHO dataset

- Association no longer supported by "relaxed" *pncA* thresholds
- Due to the switch from PPV|SOLO to PPV SOLO in neutral algorithm

Second edition \ First edition	Group 1: Assoc w R	Group 2: Assoc w R–interim	Group 3: Uncertain significance	Group 4: Not assoc w R–interim	Group 5: Not assoc w R	N/A
Group 1	167	53	18			8
Group 2	9	880	107			126
Group 3	4	7 (98)[a]	12581	3	8	21178
Group 4		(1)[a]	7	20	29	1 (12358)[b]
Group 5			36	5	136	182 (126)[b]

- Association no longer supported by data

- Algorithm pass 2 association no longer supported by data
- Inframe now excluded from LoF rule
- Association no longer supported by data
- Association no longer supported by "relaxed" *pncA* thresholds

- Now Group 4 by literature/ previous evidence

Yellow boxes denote number of variants downgraded within category (e.g. from Group 1 to Group 2); red boxes denote a downgrade across categories (e.g. from Group 1/2 to Group 3/4).

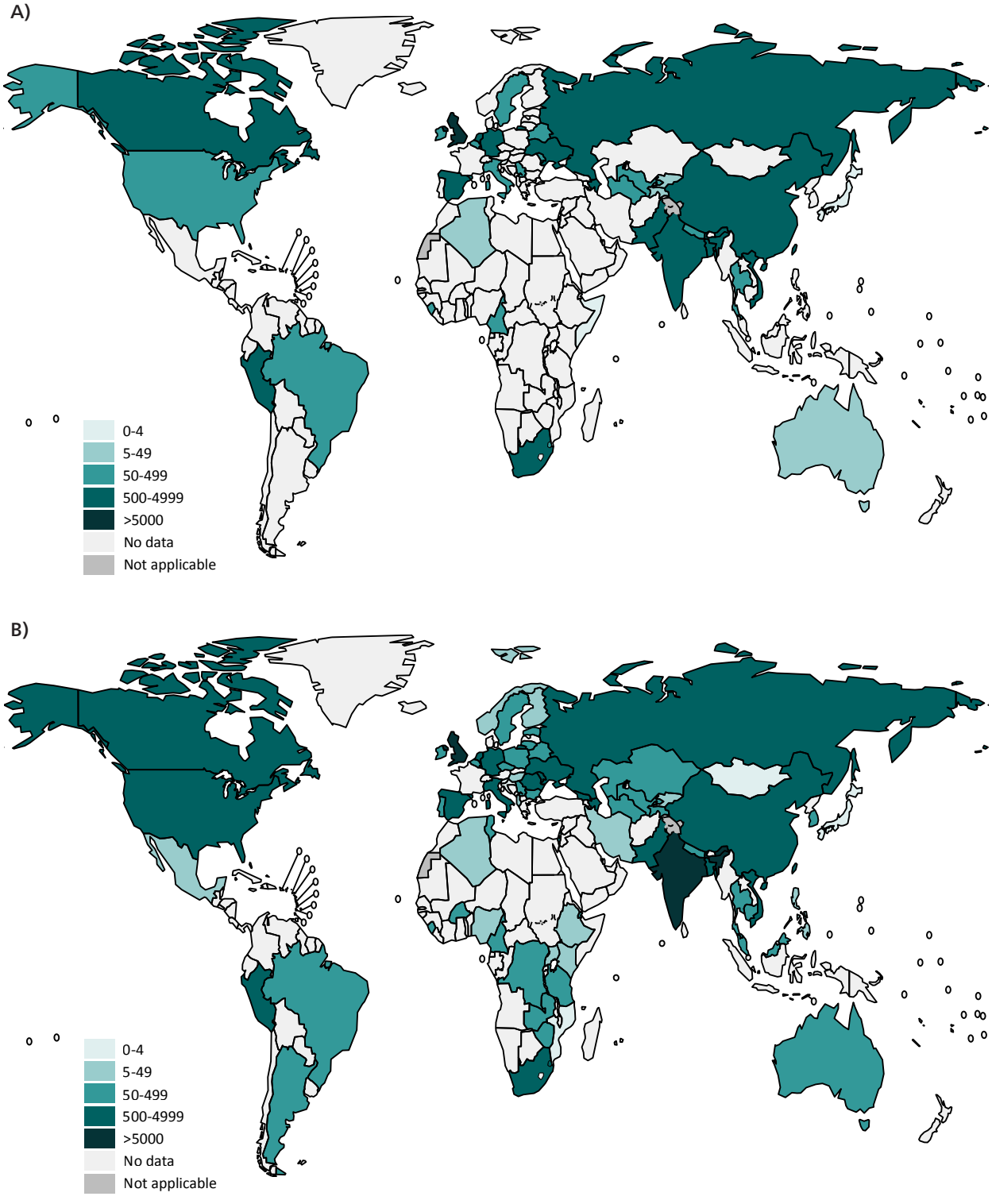
[a] Number of inframe mutations reported in brackets.

[b] Number of silent mutations reported in brackets.

2.3 Overview of clinical sample sources and types of pDST data

A total of 556 918 individual pDST results were collated for 64 622 MTBC isolates. Matching WGS data were available for 61 986 isolates. After prioritization and curation of the individual pDST data and quality control of processed sequencing data, data on 9 419 isolates were dropped from further consideration, leaving a total of 52 567 isolates to be used in the analyses for the second edition (up from ~38 000 for the first edition *(14)*). Sixty-seven countries contributed data for ≥ 5 MTBC isolates, 54 countries for ≥ 50 isolates, 21 for ≥ 500 isolates, and India and the United Kingdom contributed data for ≥ 5000 isolates (Fig. 3).

Fig. 3. Global origin and regional numbers of MTBC isolates included in the first edition (panel A) and the second edition (panel B)



The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

Approximately 52 000 isolates were considered for the second edition (Table 2). The number of isolates for which pDST results were available varied widely by drug, with as few as 11 803 isolates with pDST results for DLM in the WHO data set, which comprises pDST results obtained according to current or previous WHO-endorsed methods, to as many as 48 706 for INH in the ALL data set, which consists of the WHO data set plus other methods that are not WHO-endorsed but were deemed acceptable for inclusion (Sections 5.4 and 5.5). Of most significance to the analysis of genotypic–phenotypic associations for new and repurposed drugs, the second edition includes > 5800 new isolates with BDQ pDST and almost 7000 new isolates with LZD pDST in the ALL data set. There were substantial increases in the proportions of resistant isolates to every drug in the catalogue, except for ETO, for which more susceptible than resistant isolates with WHO phenotypes were included in the second edition, reducing the overall prevalence of resistance to ETO.

Table 2. Summary of pDST results included in the first and second edition, stratified by drug and data set

Drug	Dataset	First edition			Second edition			Variation
		Total	% R	(95% CI)	Total	% R	(95% CI)	Change % R
RIF	WHO	27063	24.9	(24.4–25.4)	35401	32.6	(32.1–33.1)	31
	ALL	34375	28.7	(28.2–29.2)	47730	35.3	(34.9–35.8)	23
INH	WHO	26727	31.6	(31.0–32.1)	34881	38.5	(38.0–39.0)	22
	ALL	34437	35.4	(34.9–35.9)	48706	43.0	(42.6–43.5)	21
EMB	WHO	23706	15.2	(14.8–15.7)	33240	19.8	(19.4–20.3)	30
	ALL	30708	16.0	(15.5–16.4)	45515	21.0	(20.6–21.3)	31
PZA	WHO	15903	14.6	(14.1–15.2)	19889	19.1	(18.6–19.7)	30
	ALL	15902	14.6	(14.1–15.2)	21319	20.8	(20.2–21.3)	42
LFX	WHO	10305	19.6	(18.8–20.4)	12441	22.0	(21.3–22.7)	12
	ALL	18277	17.0	(16.5–17.6)	27576	21.3	(20.8–21.8)	25
MFX	WHO	6904	15.8	(15.0–16.7)	8439	20.8	(19.9–21.7)	31
	ALL	13351	14.0	(13.4–14.6)	22783	17.7	(17.2–18.2)	26
BDQ	WHO	88	3.4	(0.7–9.6)	2165	41.7	(39.6–43.7)	1122
	ALL	8321	0.9	(0.7–1.1)	14135	7.3	(6.9–7.8)	736
LZD	WHO	1131	0.8	(0.4–1.5)	6825	2.0	(1.7–2.3)	152
	ALL	11018	1.1	(0.9–1.3)	18010	2.1	(1.9–2.3)	86
CFZ	WHO	3635	0.6	(0.4–0.9)	5027	4.3	(3.7–4.8)	576
	ALL	10179	1.2	(1.0–1.5)	14904	4.5	(4.2–4.9)	270
DLM	WHO	89	2.2	(0.3–7.9)	575	9.4	(7–11.8)	318
	ALL	7778	1.1	(0.8–1.3)	11803	2.1	(1.9–2.4)	103
AMK	WHO	8040	8.3	(7.7–8.9)	8958	12.5	(11.9–13.2)	52
	ALL	16978	7.6	(7.2–8.0)	24710	10.0	(9.7–10.4)	32
STM	WHO	9043	28.3	(27.4–29.3)	19747	39.3	(38.7–40.0)	39
	ALL	13984	33.1	(32.4–33.9)	26166	39.8	(39.2–40.4)	20
ETO	WHO	2184	40.5	(38.4–42.6)	5999	36.4	(35.2–37.6)	-10
	ALL	13918	21.3	(20.6–22.0)	20936	25.0	(24.4–25.6)	18
KAN[a]	WHO	7381	9.3	(8.7–10.0)	8014	20.1	(19.3–21.0)	116
	ALL	16154	9.2	(8.7–9.6)	24582	14.5	(14.1–15.0)	58
CAP[a]	WHO	9103	7.7	(7.2–8.3)	10025	13.1	(12.5–13.8)	70
	ALL	11526	8.4	(7.9–8.9)	17716	11.7	(11.2–12.1)	39

[a] No longer recommended for TB treatment.

2.4 Performance of mutations in the catalogue for predicting phenotypic resistance

One of the simplest ways to determine whether the mutation catalogue has captured most of the important genetic predictors of phenotypic resistance to a specific drug is to calculate the sensitivity, specificity and positive predictive value (PPV) of all of the Groups 1 and 2 mutations listed in the catalogue relative to the total number of phenotypically resistant isolates in the catalogue (Table 3). For the reasons given below, this is an oversimplification and the results should not be considered to represent the theoretical maximum sensitivity, specificity and PPV that could be achieved in clinical settings, where the prevalence of resistance depends on the location.

- Although we prioritized pDST results, the phenotypic reference standard was imperfect for some isolates. For example, some WHO-endorsed CCs had been set too high, and the CCs for interpreting broth microdilution (BMD) minimum inhibitory concentrations (MICs) have not been reviewed by WHO *(16,21–25)*. Moreover, use of data collected from many different laboratories is inherently more prone to error than use of data from smaller, well-controlled multi-centre studies specifically designed to assess the performance of gDST, and the specificity would probably be higher under the latter circumstances for at least some drugs.
- As we had no independent data set against which to validate results, we calculated the performance of the catalogue by making predictions for the same data set from which it was derived; consequently, the data may have been overfitted *(26,27)*. Independent validation against different data sets will still be necessary to better understand the performance of mutations that occur in both WHO and outside data sets; however, validating rare mutations will remain a challenge.
- Owing to transmission of a limited number of clones globally, particularly for drug-resistant TB, the prevalence of any specific mutation will impact sensitivity estimates. Clones with unclassified mutations that are globally rare but locally frequent can result in much lower sensitivity of the catalogue estimates in some settings. In contrast, in environments where transmission of clones with classified mutations is common, the sensitivity would be higher. Understanding local epidemiology is therefore important for using this catalogue and any WHO-endorsed gDST assays *(28–31)*. The pre-test probability of resistance to second-line drugs is one of the factors to be considered in this context.

For the reasons outlined above, we explored several alternative analytical scenarios with different assumptions to determine how sensitivity and specificity estimates are affected in our data set. Tables A.1–A.3 in the Annex show the results of those analyses. A more detailed discussion of the performance of the catalogue mutations for each drug is provided in Section 3.

Table 3 provides an overview of Groups 1–5 mutations in the second edition and their performance in predicting phenotypic resistance.

Table 3. Groups 1–5 mutations for the second edition and their performance for predicting phenotypic resistance

		Group 1: Assoc w R	Group 2: Assoc w R-interim	Group 3: Uncertain significance	Group 4: Not assoc w R-interim	Group 5: Not assoc w R
RIF	No. of variants identified	26	110	4484	2 (2568)	52 (32)
	Sens, spec, PPV (% [95% CI])	92.1 (91.7–92.5), 97.1 (96.9–97.3), 94.5 (94.2–94.9)	1.1 (1.0–1.3), 99.8 (99.8–99.9), 76.5 (70.8–81.6)			
	Combined performance	**93.3 (92.9–93.7), 96.9 (96.7–97.1), 94.2 (93.9–94.6)**				
INH	No. of variants identified	7	135	5404	11 (1671)	41 (16)
	Sens, spec, PPV (% [95% CI])	89.6 (89.2–90.0), 98.2 (98.1–98.4), 97.5 (97.2–97.7)	2.0 (1.8–2.2), 99.7 (99.6–99.7), 82.5 (78.9–85.7)			
	Combined performance	**91.6 (91.2–92.0), 97.9 (97.8–98.1), 97.1 (96.8–97.3)**				
EMB	No. of variants identified	13	0	4943	10 (2068)	50 (34)
	Sens, spec, PPV (% [95% CI])	81.1 (80.3–81.9), 91.6 (91.3–91.9), 71.9 (71.0–72.8)	0 (0–0), 100.0 (100.0–100.0), 0 (0–0)			
	Combined performance	**81.1 (80.3–81.9), 91.6 (91.3–91.9), 71.9 (71.0–72.8)**				
PZA	No. of variants identified	139	202	1465	20 (720)	17 (8)
	Sens, spec, PPV (% [95% CI])	63.5 (62.0–64.9), 98.6 (98.5–98.8), 92.4 (91.4–93.4)	14.6 (13.5–15.6), 99.2 (99.1–99.3), 82.9 (80.1–85.5)			
	Combined performance	**78.0 (76.8–79.2), 97.9 (97.6–98.1), 90.5 (89.5–91.4)**				
LFX	No. of variants identified	12	6	2016	2 (983)	19 (9)
	Sens, spec, PPV (% [95% CI])	83.6 (82.6–84.5), 97.3 (97.0–97.5), 89.2 (88.4–90.0)	1.2 (1.0–1.6), 99.6 (99.6–99.7), 48.3 (40.1–56.6)			
	Combined performance	**84.8 (83.9–85.7), 96.9 (96.7–97.1), 88.1 (87.3–89.0)**				
MFX	No. of variants identified	10	8	1775	2 (904)	15 (9)
	Sens, spec, PPV (% [95% CI])	84.8 (83.7–85.9), 94.0 (93.6–94.3), 75.2 (73.9–76.4)	0.9 (0.6–1.2), 99.5 (99.4–99.6), 29.5 (21.6–38.4)			
	Combined performance	**85.7 (84.6–86.8), 93.5 (93.2–93.9), 74.0 (72.7–75.2)**				
BDQ	No. of variants identified	5	81	947	5 (424)	1
	Sens, spec, PPV (% [95% CI])	26.3 (23.7–29.1), 99.4 (99.3–99.5), 78.2 (73.5–82.4)	23.0 (20.5–25.7), 99.3 (99.1–99.4), 72.0 (66.8–76.8)			
	Combined performance	**49.4 (46.3–52.5), 98.7 (98.5–98.9), 75.2 (71.8–78.4)**				
LZD	No. of variants identified	1	7	844	0 (71)	4 (1)
	Sens, spec, PPV (% [95% CI])	27.3 (22.8–32.1), 99.8 (99.8–99.9), 78.5 (70.4–85.2)	6.7 (4.4–9.7), 100.0 (99.9–100.0), 78.1 (60.0–90.7)			
	Combined performance	**34.0 (29.2–39.0), 99.8 (99.7–99.9), 78.4 (71.3–84.5)**				

		Group 1: Assoc w R	Group 2: Assoc w R-interim	Group 3: Uncertain significance	Group 4: Not assoc w R-interim	Group 5: Not assoc w R
CFZ	No. of variants identified	2	56	1256	0 (576)	11 (1)
	Sens, spec, PPV (% [95% CI])	4.3 (2.9–6.1), 99.8 (99.7–99.8), 46.0 (33.4–59.1)	12.7 (10.3–15.4), 98.9 (98.7–99.1), 36.0 (29.9–42.4)			
	Combined performance	**17.0 (14.2–20.0), 98.7 (98.5–98.9), 38.1 (32.6–43.8)**				
DLM	No. of variants identified	0	24	579	0 (334)	0
	Sens, spec, PPV (% [95% CI])	0 (0–1.5), 100.0 (100.0–100.0), 0 (0–0)	14.7 (10.6–19.7), 99.9 (99.8–99.9), 72.5 (58.3–84.1)			
	Combined performance	**14.7 (10.6–19.7), 99.9 (99.8–99.9), 72.5 (58.3–84.1)**				
AMK	No. of variants identified	2	2	1772	1 (343)	68 (2)
	Sens, spec, PPV (% [95% CI])	68.9 (67.0–70.7), 99.2 (99.0–99.3), 90.1 (88.7–91.4)	4.0 (3.2–4.8), 99.2 (99.0–99.3), 34.3 (28.8–40.1)			
	Combined performance	**72.8 (71.0–74.6), 98.3 (98.1–98.5), 82.8 (81.2–84.4)**				
STM	No. of variants identified	14	144	2342	1 (538)	15 (6)
	Sens, spec, PPV (% [95% CI])	72.1 (71.2–72.9), 97.6 (97.4–97.8), 95.2 (94.7–95.7)	7.6 (7.1–8.1), 96.5 (96.2–96.8), 58.8 (56.1–61.4)			
	Combined performance	**79.7 (78.9–80.5), 94.1 (93.7–94.4), 89.9 (89.3–90.5)**				
ETO	No. of variants identified	5	281	1944	0 (515)	2
	Sens, spec, PPV (% [95% CI])	45.8 (44.4–47.1), 94.0 (93.6–94.3), 71.7 (70.1–73.2)	29.1 (27.8–30.3), 91.9 (91.5–92.3), 54.6 (52.7–56.4)			
	Combined performance	**74.8 (73.6–76.0), 85.9 (85.3–86.4), 63.9 (62.7–65.1)**				
KAN[a]	No. of variants identified	6	2	1862	3 (353)	12 (2)
	Sens, spec, PPV (% [95% CI])	74.4 (73.0–75.9), 96.7 (96.5–97.0), 79.4 (78.0–80.8)	0.4 (0.3–0.7), 100.0 (99.9–100.0), 66.7 (44.7–84.4)			
	Combined performance	**74.9 (73.4–76.3), 96.7 (96.4–96.9), 79.3 (77.9–80.7)**				
CAP[a]	No. of variants identified	5	64	2273	1 (253)	52 (5)
	Sens, spec, PPV (% [95% CI])	61.2 (59.1–63.3), 98.0 (97.8–98.2), 80.4 (78.4–82.3)	4.9 (4.0–6.0), 99.8 (99.7–99.9), 76.1 (68.0–83.1)			
	Combined performance	**66.2 (64.1–68.2), 97.8 (97.6–98.1), 80.1 (78.1–81.9)**				

CI, exact binomial confidence interval; sens, sensitivity; spec, specificity.

Only majority variants (allele frequency ≥ 75%) are listed and used for calculations with the ALL data set. The number of silent mutations in each group is shown in parentheses.

[a] Drugs no longer recommended for TB treatment.

3 Results for individual drugs

3.1 Instructions for reading the mutation tables

As in the first edition, this section includes abridged mutation tables by drug. The searchable tables are available as supplementary material (WHO-UCN-TB-2023.5-eng.xlsx). The terms and abbreviations used in the mutation tables are listed in Tables 4–5. Below are the criteria that were used for grading mutations into different groups that relied on specific lone (SOLO) mutations associated with resistance (see Sections 5.7 and 5.8 for more details). The colour coding shown in parentheses was used in the tables to show whether the criteria for the initial confidence grading were met.

Group 1: Assoc w R

Mutations that met five criteria:

1. sum of resistant and susceptible isolates with the SOLO mutation (Present_SOLO_SR) ≥ 5 (red);
2. lower bound (lb) of 95% CI of PPV conditional on being SOLO (PPV|SOLO_lb) ≥ 25% (red);
3. odds ratio (OR) > 1, which always applies if criterion 4 is met (red);
4. OR SOLO > 1 (red); and
5. statistical significance of OR SOLO (OR SOLO_FE-sig) with Fisher exact false discovery rate-corrected (red).

Criteria 4 and 5 are merged in the "OR SOLO" column of the simplified tables in this report and are shown in red if both criteria were met.

Group 2: Assoc w R–interim

Mutations that met "relaxed" criteria for *pncA*:

1. resistant isolates with the SOLO mutation (Present_SOLO_R) ≥ 2 (yellow)
2. PPV ≥ 50% (yellow)

Group 3: Uncertain significance

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

Group 4: Not assoc with R– interim

Silent mutations that do not fulfil the requirements for other groups

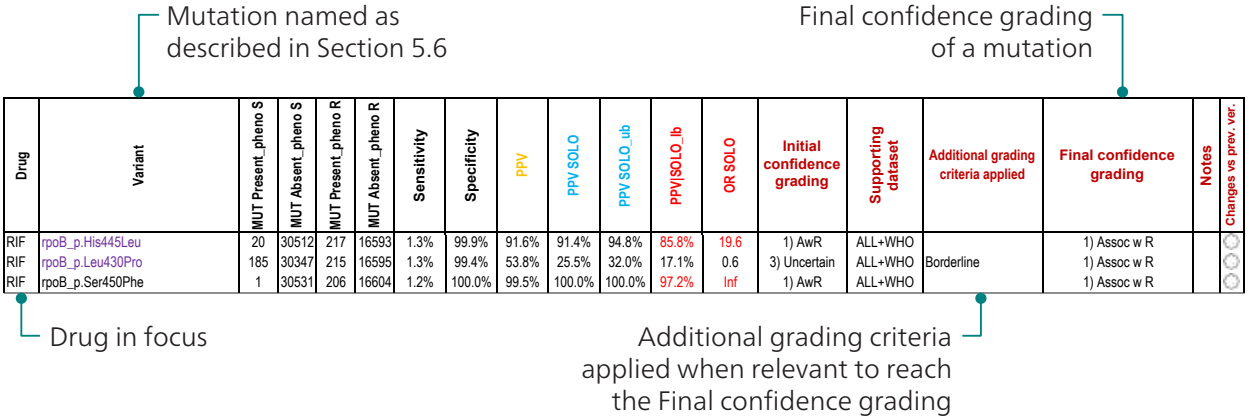
Mutations that meet "relaxed" criteria for *pncA*:

1. PPV SOLO < 40% (blue)
2. upper bound (ub) of 95% CI of PPV SOLO (PPV_SOLO_ub) < 75% (blue)

Group 5: Not assoc with R

Neutral mutations that were masked before use of the algorithm

Fig. 4. Example of an abridged variant classification table



Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	rpoB_p.His445Leu	20	30512	217	16593	1.3%	99.9%	91.6%	91.4%	94.8%	85.8%	19.6	1) AwR	ALL+WHO		1) Assoc w R		
RIF	rpoB_p.Leu430Pro	185	30347	215	16595	1.3%	99.4%	53.8%	25.5%	32.0%	17.1%	0.6	3) Uncertain	ALL+WHO	Borderline	1) Assoc w R		
RIF	rpoB_p.Ser450Phe	1	30531	206	16604	1.2%	100.0%	99.5%	100.0%	100.0%	97.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		

In the first example in Fig. 4, the drug considered is RIF. The variant is in the *rpoB* gene, the amino acid change is at codon 445 (MTBC codon numbering), and the change is from serine to leucine (this corresponds to codon 526 in the previous *Escherichia coli* nomenclature *(4,32)*). This variant was found in 20 phenotypically susceptible isolates and in 217 resistant isolates. The mutation was not found in 30 512 susceptible isolates or 16 593 resistant isolates.

The sensitivity, specificity and PPV represent the performance of this mutation in predicting a resistant phenotype in the data set. The next four columns indicate the statistical performance of this mutation when it occurs as a SOLO mutation in the genomic regions selected when assessing RIF resistance. The values given are the mid-point PPV and the corresponding lb and ub and the odds ratio for the SOLO mutation (OR SOLO).

The initial confidence grading for *rpoB* His445Leu was Group 1 because:

- present_SOLO_SR (see catalogue master file) was 210 and, consequently, ≥ 5;
- the PPV|SOLO_lb of 85.8% was ≥ 25%; and
- OR SOLO of 19.6 was > 1 and statistically significant.

As the initial confidence grading of the WHO and the ALL data sets was concordant for this mutation, the figures shown are for the ALL data set. Additional grading criteria were not applied to this mutation; therefore, the final confidence grading was unchanged. In contrast, the initial confidence grading for *rpoB* Leu430Pro was revised according to the additional grading rule

related to borderline RIF resistance mutations, which are shown in purple *(4, 33)*. More details can be found in the Section 5.

Table 4. Terms used in the mutation tables

Term used in the report	Description
ALL only	information only from the ALL data set
AwR	associated with resistance
AwRI	associated with resistance–interim
FQ X-R	fluoroquinolone cross-resistance
BDQ-CFZ X-R	bedaquiline-clofazimine cross-resistance
INH-ETO X-R	isoniazid-ethionamide cross-resistance
Inf	infinity
Lit.	information from the literature
NotAwR	not associated with resistance
NotAwRI	not associated with resistance–interim
Prev. WHO	previous WHO guidance
Uncert. Sig.	uncertain significance
WHO-end. gDST	WHO-endorsed genotypic drug susceptibility testing assay
Drug	name of drug
Interim on WHO	initial Group 2 classification by WHO was used
Pot. infl. PPV	potentially inflated positive predictive value
Selection	information from selection studies
Present_S	number of susceptible isolates with the mutation
Absent_S	number of susceptible isolates without the mutation
Present_R	number of resistant isolates with the mutation
Absent_R	number of resistant isolates without the mutation
Sensitivity	true positive rate of mutation
Specificity	true negative rate of mutation
PPV	positive predictive value of mutation
PPV|SOLO[a]	positive predictive value conditional on being SOLO
PPV SOLO[a]	positive predictive value of SOLO mutation
OR SOLO	odds ratio as SOLO mutation
Initial confidence grading	initial grouping of mutation
Supporting data set	data set(s) used to derive the initial confidence grading
Additional grading criteria	criteria used to change the initial confidence grading (e.g. previous WHO guidance or WHO-endorsed genotypic DST assays) to yield the final confidence grading
Final confidence grading	final grouping of mutation after relevant additional grading criteria were applied
Footnotes	additional details provided for specific variants
Changes vs ver.1	changes from first edition (Table 5)
Additional variables shown in the master file	**Description**
Tier	a-priori grouping of genomic regions; tiers 1 and 2
Algorithm pass	algorithm pass during which mutation was classified; 0, before algorithm (i.e. neutral mutation); 1, first pass; 2, second pass
Genome position	genomic position in H37Rv for indels, inter-genetic and ribosomal mutations
Present_SOLO_R	number of resistant isolates with the SOLO mutation
Present_SOLO_SR	total number of resistant and susceptible isolates with the SOLO mutation

Additional variables shown in the master file	Description
Sensitivity[a]	true positive rate of mutation
Specificity[a]	true negative rate of mutation
PPV[a]	positive predictive value of mutation
OR[a]	odds ratio of mutation
OR SOLO[a]	odds ratio of SOLO mutation
Sentivity SOLO[a]	true positive rate of SOLO mutation
Specificity SOLO[a]	true negative rate of SOLO mutation
OR SOLO_FE-sig	Fisher exact test for the false discovery rate-corrected *P* for the OR SOLO; TRUE = false discovery rate-corrected $P \leq 0.05$; FALSE = false discovery rate-corrected $P > 0.05$
Neutral masked	0 = not masked; 1 = masked
Previous WHO guidance	NGS Guide 2018, Level of resistance to INH or MFX, RIF CC guide 2021, Miotto et al. (PubMed 29284687) *(4, 6, 15, 19, 34)*
WHO-recommended genotypic DST assays	Abbott Real*Time* MTB RIF/INH *(35)*, BD MAX™ MDR-TB *(36)*, Cepheid Xpert® MTB/RIF *(37)*, Cepheid Xpert® MTB/RIF Ultra *(38)*, Cepheid Xpert® MTB/XDR *(39)*, Hain FluoroType® MTBDR VER 2.0 *(40)*, Hain GenoType MTBDR*plus* VER 2.0 *(41)*, Hain GenoType MTBDR*sl* VER 2.0 *(42)*, Molbio Truenat® MTB-RIF Dx *(43)*, Nipro Genoscholar™ NTM+MDRTB II *(44)*, Nipro Genoscholar™ PZA-TB II *(45)*, Roche Cobas® MTB-RIF/INH *(46)*.

[a] The lb and ub of the 95% CI for these figures are provided in additional columns.

Table 5. Symbols and abbreviations used in the mutation tables

Icon	Changes		
	Summary	from first edition	to second edition
	DOWN	Assoc w R	Uncertain significance
	DOWN	Assoc w R–interim	Uncertain significance
	DOWN	Not assoc w R	Uncertain significance
	DOWN	Not assoc w R–interim	Uncertain significance
	SWITCH	Assoc w R–interim	Not assoc w R–interim
	DOWN	Assoc w R	Assoc w R–interim
	DOWN	Not assoc w R	Not assoc w R–interim
	UP	Assoc w R–interim	Assoc w R
	UP	Not assoc w R–interim	Not assoc w R
	UP	Uncertain significance	Assoc w R
	UP	Uncertain significance	Assoc w R–interim
	UP	Uncertain significance	Not assoc w R
	UP	Uncertain significance	Not assoc w R–interim
	NEW	Not present	Not assoc w R
	NEW	Not present	Assoc w R
	NEW	Not present	Assoc w R–interim
	NEW	Not present	Not assoc w R–interim
	No change	Same classification	

The new symbols were added to the second edition to denote mutations that were new (NEW) or downgraded (DOWN), upgraded (UP), changed from being associated with resistance to not being associated (SWITCH) or remained the same in both catalogues. Because a new method of annotating indels was adopted for the second edition, comparison with the first edition was not possible for these variants.

3.2 Rifampicin and rifapentine

The *rpoB* Val170Phe and Ile491Phe remained the only mutations outside RRDR, spanning the *rpoB* codons 426–452 that are markers of RIF resistance (Table 6). Although some RRDR mutations (e.g. Ser450Tyr) were upgraded from Group 2 in the first edition to Group 1 in the second edition, this did not change final interpretation of the mutations, which means that the combined sensitivity of Groups 1 and 2 mutations in the two catalogues was identical at 93.3% (95% CI: 92.9–93.7) (Table A.1). The added sensitivity of the 110 Group 2 mutations that were almost exclusively classified according to the WHO-endorsed "additional grading rule" (any non-silent RRDR mutation was assumed to confer RIF resistance in the absence of evidence to the contrary (Table 1) *(4,33)*) increased the sensitivity by only 1.1% (95% CI: 1.0–1.3) from that of the 26 Group 1 mutations alone (Table 3). Lowering the variant frequency cut-off for calling Groups 1 and 2 mutations from 75% to 25% increased the combined sensitivity by 1.1% (Table A.1).

It should be noted that the WHO-endorsed GenoScreen Deeplex® Myc-TB assay based on Illumina sequencing does not call minority variants at a frequency below 10% at nucleotide 1346 of *rpoB* (genomic position 761152), because this position is considered to be "noisy" *(47)*. Moreover, an external quality assessment scheme for WGS questioned the validity of observed T to A changes at this position (*rpoB* Leu449Gln) *(48)*. Specifically, two laboratories reported this change in the "2018 FASTQ14" sequence file, and one of the two laboratories reported the same change in "2019 FASTQ14" (in all three cases, the allele frequency was reported to be < 10%) *(48)*.[1] Work is under way to investigate whether this represents a potential Illumina-specific artefact (e.g. linked to library preparation or sequencing) or whether alternative explanations, such as low-level contamination with DNA from other species, apply *(48)*. Any *rpoB* changes at this position identified by Illumina sequencing, particularly if unfixed, should be analysed carefully to avoid overcalling RIF resistance, as codon 449 falls within RRDR and is therefore subject to the associated "additional grading rule". It is not known whether this potential limitation extends to other sequencing technologies.

WHO has previously endorsed use of RIF as a surrogate for RPT, meaning that the Groups 1–5 classifications for RIF also apply to RPT *(4)*.

[1] Anthony R, personal communication, 2023.

Table 6. Abridged variant classification for RIF

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	rpoB_p.Ser450Leu	226	30643	10859	6002	64.4%	99.3%	98.0%	97.9%	98.2%	97.1%	234.4	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Asp435Val	17	30515	1154	15656	6.9%	99.9%	98.5%	98.8%	99.4%	97.5%	162.4	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.His445Asp	10	30522	608	16202	3.6%	100.0%	98.4%	98.4%	99.3%	96.8%	118.3	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.His445Tyr	11	30521	593	16217	3.5%	100.0%	98.2%	98.6%	99.4%	96.0%	128.2	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Asp435Tyr	93	30439	341	16469	2.0%	99.7%	78.6%	70.9%	76.5%	59.7%	4.5	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Leu452Pro	102	30430	281	16529	1.7%	99.7%	73.4%	64.9%	70.7%	56.1%	3.4	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Ser450Trp	5	30527	238	16572	1.4%	100.0%	97.9%	97.4%	99.1%	94.0%	68.9	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.His445Leu	20	30512	217	16593	1.3%	99.9%	91.6%	91.4%	94.8%	85.8%	19.6	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Leu430Pro	185	30347	215	16595	1.3%	99.4%	53.8%	25.5%	32.0%	17.1%	0.6	3) Uncertain	ALL+WHO	Borderline	1) Assoc w R		○
RIF	rpoB_p.Ser450Phe	1	30531	206	16604	1.2%	100.0%	99.5%	100.0%	100.0%	97.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.His445Arg	3	30529	136	16674	0.8%	100.0%	97.8%	98.1%	99.8%	92.1%	96.1	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.His445Asn	83	30449	126	16684	0.7%	99.7%	60.3%	33.7%	43.6%	21.6%	0.9	3) Uncertain	ALL+WHO	Borderline	1) Assoc w R		○
RIF	rpoB_p.Ile491Phe	88	30444	116	16694	0.7%	99.7%	56.9%	55.5%	63.0%	44.7%	2.3	1) AwR	ALL	Borderline	1) Assoc w R		○
RIF	rpoB_p.Val170Phe	2	30530	89	16721	0.5%	100.0%	97.8%	97.6%	99.9%	83.8%	73.0	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.His445Cys	8	30524	70	16740	0.4%	100.0%	89.7%	91.5%	96.8%	79.5%	19.8	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Gln432Pro	1	30531	69	16741	0.4%	100.0%	98.6%	98.1%	100.0%	89.7%	93.0	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Asp435Phe	4	30528	48	16762	0.3%	100.0%	92.3%	90.7%	97.4%	77.9%	17.8	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Gln432Lys	0	30532	46	16764	0.3%	100.0%	100.0%	100.0%	100.0%	83.9%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Ser441Leu	1	30531	38	16772	0.2%	100.0%	97.4%	94.4%	99.9%	72.7%	30.9	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Ser441Gln	2	30530	30	16780	0.2%	100.0%	93.8%	93.8%	99.2%	79.2%	27.3	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Gln432Leu	1	30531	28	16782	0.2%	100.0%	96.6%	94.1%	99.9%	71.3%	29.1	1) AwR	ALL+WHO		1) Assoc w R		○
RIF	rpoB_p.Phe433dup	0	30532	26	16784	0.2%	100.0%	100.0%	100.0%	100.0%	85.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
RIF	rpoB_p.His445Ser	7	30525	19	16791	0.1%	100.0%	73.1%	66.7%	86.7%	38.4%	3.6	3) Uncertain	ALL+WHO	Borderline	1) Assoc w R		○
RIF	rpoB_p.Thr444dup	0	30532	10	16800	0.1%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
RIF	rpoB_p.Gln432_Asp435delinsHis	0	30532	9	16801	0.1%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
RIF	rpoB_p.Ser450Tyr	0	30532	9	16801	0.1%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
RIF	rpoB_p.Asp435Gly	8	30524	148	16662	0.9%	100.0%	94.9%	50.0%	84.3%	9.9%	1.8	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.Met434Ile	2	30530	33	16777	0.2%	100.0%	94.3%	100.0%	100.0%	0.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.Leu430Arg	3	30529	32	16778	0.2%	100.0%	91.4%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.Asp435Glu	0	30532	21	16789	0.1%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.His445Gln	2	30530	21	16789	0.1%	100.0%	91.3%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.Asn437Asp	6	30526	15	16795	0.1%	100.0%	71.4%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.Asn438del	2	30530	13	16797	0.1%	100.0%	86.7%	83.3%	97.9%	51.6%	9.1	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○
RIF	rpoB_p.Gln429His	1	30531	13	16797	0.1%	100.0%	92.9%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	rpoB_p.Ser450Gln	0	30532	12	16798	0.1%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435Ala	6	30526	12	16798	0.1%	100.0%	66.7%	0.0%	70.8%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434Val	2	30530	12	16798	0.1%	100.0%	85.7%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.His445Gly	3	30529	11	16799	0.1%	100.0%	78.6%	70.0%	93.3%	34.8%	4.2	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Arg448Gln	0	30532	10	16800	0.1%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln429Leu	0	30532	10	16800	0.1%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu449Met	0	30532	10	16800	0.1%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431Gly	1	30531	10	16800	0.1%	100.0%	90.9%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.His445Pro	0	30532	9	16801	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ala451Val	6	30526	8	16802	0.0%	100.0%	57.1%	0.0%	70.8%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432Glu	0	30532	8	16802	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser450Met	1	30531	8	16802	0.0%	100.0%	88.9%	88.9%	99.7%	51.8%	14.5	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser428Arg	1	30531	7	16803	0.0%	100.0%	87.5%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asn437His	0	30532	6	16804	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435Asn	0	30532	6	16804	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431Arg	0	30532	6	16804	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Lys446Gln	1	30531	5	16805	0.0%	100.0%	83.3%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser428Thr	0	30532	5	16805	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser441Met	0	30532	5	16805	0.0%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser450Val	4	30528	5	16805	0.0%	100.0%	55.6%	0.0%	60.2%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427Ile	0	30532	5	16805	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Arg448Lys	0	30532	4	16806	0.0%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435His	0	30532	4	16806	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.His445Thr	0	30532	4	16806	0.0%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu430_Ser431insArg	1	30531	4	16806	0.0%	100.0%	80.0%	66.7%	99.2%	9.4%	3.6	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434_Asp435del	0	30532	4	16806	0.0%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434Arg	0	30532	4	16806	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser428Gly	1	30531	4	16806	0.0%	100.0%	80.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431_Gln432insArg	0	30532	4	16806	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431_Gln432insHis	0	30532	4	16806	0.0%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser441Ala	1	30531	4	16806	0.0%	100.0%	80.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427_Ser428del	1	30531	4	16806	0.0%	100.0%	80.0%	80.0%	99.5%	28.4%	7.3	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427Ala	1	30531	4	16806	0.0%	100.0%	80.0%	50.0%	98.7%	1.3%	1.8	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432His	0	30532	3	16807	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	rpoB_p.Gln436del	1	30531	3	16807	0.0%	100.0%	75.0%	100.0%	100.0%	19.4%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.His445Phe	0	30532	3	16807	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Phe433_Asp435del	0	30532	3	16807	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser428Ile	1	30531	3	16807	0.0%	100.0%	75.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431Asn	0	30532	3	16807	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser441Val	0	30532	3	16807	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser450Cys	5	30527	3	16807	0.0%	100.0%	37.5%	33.3%	77.7%	3.7%	0.9	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser450Gly	2	30530	3	16807	0.0%	100.0%	60.0%	50.0%	93.2%	6.8%	1.8	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427Ser	0	30532	3	16807	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr444Ile	0	30532	3	16807	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ala451Gly	0	30532	2	16808	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435_Gln436delinsGlu	1	30531	2	16808	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	3.6	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln429Pro	0	30532	2	16808	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432_Met434delinsLeu	0	30532	2	16808	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432del	0	30532	2	16808	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gly442Glu	0	30532	2	16808	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu430_Gln432del	0	30532	2	16808	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Lys446Arg	1	30531	2	16808	0.0%	100.0%	66.7%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Lys446Glu	1	30531	2	16808	0.0%	100.0%	66.7%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434Thr	0	30532	2	16808	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Phe433_Gln436del	1	30531	2	16808	0.0%	100.0%	66.7%	100.0%	100.0%	1.3%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431Thr	1	30531	2	16808	0.0%	100.0%	66.7%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Arg448Leu	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asn437_Asn438del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asn437Ile	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asn437Ser	3	30529	1	16809	0.0%	100.0%	25.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asn438His	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435_Gln436del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435del	1	30531	1	16809	0.0%	100.0%	50.0%	100.0%	100.0%	1.3%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asp435Leu	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432_Phe433del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432Asn	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln436_Asn437del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln436Arg	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	rpoB_p.Gln436Pro	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.His445_Lys446del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.His445_Lys446delinsGln	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu443_Lys446delinsProGln	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu443Phe	1	30531	1	16809	0.0%	100.0%	50.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu443Ser	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu443Trp	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu452Val	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Lys446Thr	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434_Asn437delinsIle	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434_Asp435insVal	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Phe425_Gly426del	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Pro439Ala	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Pro439Ser	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser431_Met434del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser441Trp	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser450_Leu452del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser450Ala	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427_Gln429del	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427_Gln429delinsLys	0	30532	1	16809	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427Pro	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr444Pro	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr444Ser	0	30532	1	16809	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Asn437Tyr	1	30531	0	16810	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln432_Met434del	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gln436Asn	1	30531	0	16810	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gly426_Thr427del	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Gly426Ser	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Leu452Met	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Met434Leu	1	30531	0	16810	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Pro439Leu	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Ser441Lys	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427Asn	2	30530	0	16810	0.0%	100.0%	0.0%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		
RIF	rpoB_p.Thr427Gly	1	30531	0	16810	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	RRDR	2) Assoc w R - Interim		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	rpoB_p.Val695Leu	71	30461	126	16684	0.7%	99.8%	64.0%	9.5%	18.5%	3.7%	0.2	3) Uncertain	ALL+WHO		3) Uncertain significance		
RIF	Rv2752c_p.Lys435Glu	33	23674	11	11491	0.1%	99.9%	25.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		
RIF	rpoB_p.Glu250Gly	49	23658	5	11497	0.0%	99.8%	9.3%	2.0%	10.9%	0.1%	0.0	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		
RIF	mtrB_p.Met517Leu	19355	4352	9872	1630	85.8%	18.4%	33.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv1129c_c.-28T>C	11233	12474	6324	5178	55.0%	52.6%	36.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	mtrB_p.Pro18Ser	4186	19521	4916	6586	42.7%	82.3%	54.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Glu1092Asp	1076	22631	1887	9615	16.4%	95.5%	63.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Gly594Glu	3984	19723	1064	10438	9.3%	83.2%	21.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_c.-61C>T	3347	20360	640	10862	5.6%	85.9%	16.1%	1.2%	1.7%	0.8%	0.0	5) NotAwR	WHO		5) Not assoc w R		
RIF	glpK_p.Val460Ala	3310	20397	404	11098	3.5%	86.0%	10.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	lpqB_p.Asp142Gly	3284	20423	403	11099	3.5%	86.1%	10.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	nusG_c.-138T>C	2918	20789	371	11131	3.2%	87.7%	11.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Ala172Val	2777	20930	366	11136	3.2%	88.3%	11.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	glpK_p.Val192fs	115	23592	173	11329	1.5%	99.5%	60.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Pro601Leu	1634	22073	172	11330	1.5%	93.1%	9.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Pro906Ala	249	23458	135	11367	1.2%	98.9%	35.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2477c_p.Gly41Glu	83	23624	127	11375	1.1%	99.6%	60.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2752c_p.Val300Ala	76	23631	101	11401	0.9%	99.7%	57.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2752c_p.Pro123Leu	206	23501	93	11409	0.8%	99.1%	31.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoA_p.Glu319Lys	412	23295	75	11427	0.7%	98.3%	15.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Ala621Thr	379	23328	66	11436	0.6%	98.4%	14.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv1129c_c.-48A>C	310	23397	64	11438	0.6%	98.7%	17.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv1129c_c.-49A>C	241	23466	44	11458	0.4%	99.0%	15.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_c.-261A>G	435	23272	44	11458	0.4%	98.2%	9.2%	1.3%	2.9%	0.4%	0.0	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2752c_p.Met31Ile	127	23580	37	11465	0.3%	99.5%	22.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2752c_p.Gly161Ser	100	23607	36	11466	0.3%	99.6%	26.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv1129c_c.-48A>G	266	23441	35	11467	0.3%	98.9%	11.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	mtrA_c.-162C>G	56	23651	28	11474	0.2%	99.8%	33.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv1129c_c.-9T>G	601	23106	28	11474	0.2%	97.5%	4.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoA_c.-68C>T	83	23624	27	11475	0.2%	99.6%	24.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	glpK_p.Glu209Lys	49	23658	24	11478	0.2%	99.8%	32.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	nusG_p.Pro34Leu	230	23477	24	11478	0.2%	99.0%	9.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Asp271Gly	226	23481	23	11479	0.2%	99.0%	9.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	nusG_p.Thr167Met	135	23572	17	11485	0.1%	99.4%	11.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
RIF	Rv1129c_c.-46C>G	49	23658	14	11488	0.1%	99.8%	22.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoA_c.-310T>A	71	23636	13	11489	0.1%	99.7%	15.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	glpK_p.Leu228Val	345	23362	11	11491	0.1%	98.5%	3.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoA_c.-124delC	141	23566	8	11494	0.1%	99.4%	5.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_p.Ile925Val	73	23634	8	11494	0.1%	99.7%	9.9%	1.4%	7.8%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2752c_p.Ala296Val	112	23595	6	11496	0.1%	99.5%	5.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	lpqB_p.Ser394Leu	206	23501	5	11497	0.0%	99.1%	2.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_p.Glu639Asp	55	23652	4	11498	0.0%	99.8%	6.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_p.Ser388Leu	56	23651	4	11498	0.0%	99.8%	6.7%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
RIF	glpK_p.Gly191dup	30	23677	2	11500	0.0%	99.9%	6.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_p.Lys944Glu	49	23658	2	11500	0.0%	99.8%	3.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	mtrB_p.Met260Val	107	23600	1	11501	0.0%	99.5%	0.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2477c_p.Thr372Lys	70	23637	1	11501	0.0%	99.7%	1.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Arg69Pro	72	23635	1	11501	0.0%	99.7%	1.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	glpK_p.Gly260Ala	42	23665	0	11502	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	nusG_p.Thr3Asn	46	23661	0	11502	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv1129c_c.-29A>G	65	23642	0	11502	0.0%	99.7%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_p.Asn381His	76	23631	0	11502	0.0%	99.7%	0.0%	0.0%	4.7%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoB_p.Asp853_Glu854dup	33	23674	0	11502	0.0%	99.9%	0.0%	0.0%	10.6%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
RIF	rpoC_p.Glu784Gln	58	23649	0	11502	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
RIF	Rv2752c_p.Ala273Val	108	23599	0	11502	0.0%	99.5%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		

Mutations shown in purple correspond to the seven WHO-endorsed borderline resistance mutations *(4,33)*. Silent mutations are not listed in the table but can be found in the catalogue master file.

3.3 Isoniazid

The combined sensitivity of Groups 1 and 2 resistance mutations for predicting phenotypic INH resistance was 91.6% (95% CI: 91.2–92.0), representing a gain of 0.6% over the first edition (Table A.1). Lowering the cut-off for calling Groups 1 and 2 mutations from 75% to 25% increased the combined sensitivity by only 0.5% (Table A.1). Two new Group 2 promoter mutations upstream of the *fabG1-inhA* operon (i.e. -17G>T and -8T>G, Table 7) were recognized by additional grading rules because they are detected with the WHO-endorsed FluoroType® MTBDR VER 2.0 assay *(40)* (see Section 5.8 for details of all additional grading rules applied). The remaining five mutations that confer resistance by over-expression of *inhA* (the shared target of INH and ETO) were -16A>G, -15C>T, -8T>A and -8T>C, which are upstream of *fabG1;* and the *fabG1* 609G>A Leu203Leu mutation that creates an alternative *inhA* promoter *(49,50)*. In light of this detailed mechanistic understanding, a new "additional grading rule" for cross-resistance was endorsed for the second edition (Table 7), whereby any *inhA* resistance mutation for INH was also recognized as a resistance mutation for ETO and vice versa (see Section 5.8) *(19)*. With this rule, *inhA* Ser94Ala, which was in Group 2 for ETO (Table 19), was also classified in Group 2 for INH. This is supported by transduction experiments for this mutation *(50)*.

Only six *katG* mutations (Met1?, Ser315Arg, Ser315Asn, Ser315Ile, Ser315Thr and Trp328Leu) were sufficiently frequent in this data set to be classified into Group 1 or 2 (Table 7). The WHO-endorsed "additional grading" rule was also used to classify 128 of the 135 Group 2 mutations, whereby any *katG* LoF mutation was assumed to result in an LoF phenotype and, consequently, INH resistance (Tables 1 and 23). In the second edition, inframe mutations were excluded from the additional grading rule because they are less likely to abolish *katG* function. All *katG* mutations were assumed to confer high-level INH resistance, whereas *inhA* mutations were considered to confer low-level resistance if they occurred in isolation. Genetically linked low-level *inhA* mutations (e.g. an upstream mutation with a coding mutation) have an additive effect, however, and confer high-level INH resistance *(4,19,51,52)*.

Potential role of rare variants

It is well recognized that various rare *katG* mutations do not meet the standard criteria used to classify resistance mutations in our analyses (Fig. 8) but which can be inferred by using mutations upstream of *ahpC* with the Xpert® MTB/XDR assay *(39,53)*. We explored their role in this data set by applying the "relaxed" criteria endorsed for *pncA* (Fig. 9) to *katG*. This increased the combined sensitivity of INH mutations by 1.2% while reducing the specificity and PPV by 0.2% (Table A.3). If it is assumed that any non-silent Group 3 coding mutation in *katG* (in RIF-resistant isolates) confers INH resistance, the sensitivity is increased by 1.7% at the cost of lowering the specificity by 0.4% and the PPV by 0.4% (Table A.3). These calculations indicate that only a small proportion of the lack of sensitivity in this data set was probably due to *katG* resistance mutations.

Table 7. Abridged variant classification for INH

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
INH	katG_p.Ser315Thr	250	27505	16302	4649	77.8%	99.1%	98.5%	98.3%	98.5%	97.5%	336.7	1) AwR	ALL+WHO		1) Assoc w R	G	○
INH	inhA_c.-777C>T	161	27307	4449	16397	21.3%	99.4%	96.5%	87.8%	89.6%	83.7%	12.0	1) AwR	ALL+WHO		1) Assoc w R	F, L	○
INH	inhA_c.-154G>A	64	27404	745	20101	3.6%	99.8%	92.1%	70.1%	76.5%	61.1%	3.2	1) AwR	ALL+WHO		1) Assoc w R	F, H	○
INH	katG_LoF	11	27457	254	20592	1.2%	100.0%	95.8%	94.0%	98.0%	79.2%	21.1	1) AwR	ALL+WHO		1) Assoc w R	G	▲
INH	katG_p.Ser315Asn	5	27463	206	20640	1.0%	100.0%	97.6%	99.2%	100.0%	91.4%	170.3	1) AwR	ALL+WHO		1) Assoc w R	G	○
INH	katG_p.Met1?	0	27468	29	20817	0.1%	100.0%	100.0%	100.0%	100.0%	85.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R	G	▲
INH	katG_p.Ser315Arg	1	27467	14	20832	0.1%	100.0%	93.3%	100.0%	100.0%	64.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R	G	▲
INH	katG_p.Trp328Leu	0	27468	12	20834	0.1%	100.0%	100.0%	100.0%	100.0%	73.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R	G	○
INH	inhA_c.-770T>C	17	27451	388	20458	1.9%	99.9%	95.8%	41.7%	63.4%	19.4%	1.0	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	F, J	○
INH	inhA_c.-779G>T	26	27442	339	20507	1.6%	99.9%	92.9%	58.9%	71.9%	42.4%	1.9	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	F, N	▲
INH	inhA_p.Ser94Ala	27	27441	213	20633	1.0%	99.9%	88.8%	58.3%	72.4%	37.1%	1.9	3) Uncertain	ALL+WHO	INH-ETO X-R	2) Assoc w R - Interim	F	▲
INH	inhA_c.-770T>A	8	27460	166	20680	0.8%	100.0%	95.4%	70.6%	89.7%	36.1%	3.2	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	F, I	○
INH	katG_p.Ser315Ile	0	27468	20	20826	0.1%	100.0%	100.0%	100.0%	100.0%	71.5%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim	G	▲
INH	inhA_c.-770T>G	0	27468	19	20827	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	F, K	▲
INH	inhA_c.-778A>G	1	27467	0	20846	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	F, M	○
INH	katG_p.Ala480del	1	27467	5	20841	0.0%	100.0%	83.3%	50.0%	98.7%	1.3%	1.3	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Ala359_Gly362del	0	27468	3	20843	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Gly121del	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Gly124del	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Pro429del	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Thr380_Gly494delinsSer	0	27468	2	20844	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Ala480dup	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Asn133_Glu233delinsLys	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Gln50_Asn51insThr	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Glu195_Asn236del	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Glu233_Pro239del	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Glu67del	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Leu343_Ser346delinsArg	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Lys154dup	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Pro288_Glu289del	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
INH	katG_p.Pro29_Val30delinsLeu	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Thr380del	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Tyr353_Thr354insAsn	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Tyr390dup	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Val151_Tyr155delinsAsp	0	27468	1	20845	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Val23_Val30del	0	27468	1	20845	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Asp513_Leu514insHis	1	27467	0	20846	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	katG_p.Leu641_Gly644del	1	27467	0	20846	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
INH	Rv2752c_p.Val300Ala	68	21245	108	13266	0.8%	99.7%	61.4%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
INH	Rv2752c_p.Pro123Leu	175	21138	100	13274	0.7%	99.2%	36.4%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
INH	ndh_p.Arg268His	99	21214	84	13290	0.6%	99.5%	45.9%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		○
INH	Rv2752c_p.Met31Ile	140	21173	52	13322	0.4%	99.3%	27.1%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
INH	mshA_p.Asp218Ala	157	21156	27	13347	0.2%	99.3%	14.7%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		▬
INH	ndh_c.-70G>T	44	21269	27	13347	0.2%	99.8%	38.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		○
INH	Rv2752c_p.Ala296Val	101	21212	14	13360	0.1%	99.5%	12.2%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
INH	Rv2752c_p.Lys435Glu	32	21281	12	13362	0.1%	99.8%	27.3%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
INH	mshA_p.Arg443His	35	21278	8	13366	0.1%	99.8%	18.6%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
INH	katG_c.-354C>T	67	21246	3	13371	0.0%	99.7%	4.3%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		▬
INH	ndh_p.Gly313Arg	65	21248	2	13372	0.0%	99.7%	3.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		▬
INH	Rv1129c_c.-28T>C	9795	11518	7551	5823	56.5%	54.0%	43.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	katG_p.Arg463Leu	9704	11609	7507	5867	56.1%	54.5%	43.6%	4.2%	4.7%	1.9%	0.1	5) NotAwR	WHO		5) Not assoc w R		○
INH	Rv1258c_p.Glu194fs	3517	17796	5469	7905	40.9%	83.5%	60.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	mshA_p.Ala187Val	3361	17952	5236	8138	39.2%	84.2%	60.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
INH	ahpC_c.-88G>A	3214	18099	974	12400	7.3%	84.9%	23.3%	17.3%	30.3%	0.1%	0.3	5) NotAwR	WHO		5) Not assoc w R		○
INH	glpK_p.Val460Ala	2919	18394	753	12621	5.6%	86.3%	20.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv0010c_p.Ile87Met	2537	18776	703	12671	5.3%	88.1%	21.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	mshA_p.Asn111Ser	2163	19150	702	12672	5.2%	89.9%	24.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
INH	katG_c.-85C>T	494	20819	510	12864	3.8%	97.7%	50.8%	2.5%	4.3%	1.2%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
INH	Rv0010c_p.Ala26Val	844	20469	272	13102	2.0%	96.0%	24.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	ndh_p.Val18Ala	695	20618	235	13139	1.8%	96.7%	25.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV/SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
INH	Rv0010c_c.-141A>G	317	20996	185	13189	1.4%	98.5%	36.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	dnaA_p.Pro124Leu	474	20839	169	13205	1.3%	97.8%	26.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	inhA_c.-40C>T	604	20709	136	13238	1.0%	97.2%	18.4%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
INH	dnaA_c.-133G>T	572	20741	116	13258	0.9%	97.3%	16.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1129c_c.-9T>G	511	20802	109	13265	0.8%	97.6%	17.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv0010c_p.Tyr95Cys	494	20819	107	13267	0.8%	97.7%	17.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	dnaA_c.-32C>T	479	20834	100	13274	0.7%	97.8%	17.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1129c_c.-48A>C	280	21033	94	13280	0.7%	98.7%	25.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	dnaA_c.-58G>A	227	21086	66	13308	0.5%	98.9%	22.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	glpK_p.Leu228Val	291	21022	62	13312	0.5%	98.6%	17.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	katG_c.-278G>C	258	21055	58	13316	0.4%	98.8%	18.4%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		▲
INH	Rv1129c_c.-49A>C	229	21084	51	13323	0.4%	98.9%	18.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1129c_c.-48A>G	236	21077	43	13331	0.3%	98.9%	15.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv2752c_p.Gly161Ser	96	21217	41	13333	0.3%	99.5%	29.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲
INH	Rv0010c_p.Leu111fs	188	21125	32	13342	0.2%	99.1%	14.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1258c_p.Pro414Ser	117	21196	25	13349	0.2%	99.5%	17.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
INH	Rv0010c_p.Thr4Ile	56	21257	19	13355	0.1%	99.7%	25.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1258c_p.Gly88fs	49	21264	17	13357	0.1%	99.8%	25.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	dnaA_p.His156Arg	184	21129	10	13364	0.1%	99.1%	5.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv0010c_p.Thr121Ser	182	21131	10	13364	0.1%	99.1%	5.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	inhA_p.Val78Ala	55	21258	6	13368	0.0%	99.7%	9.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲
INH	glpK_p.Gly191dup	15	21298	3	13371	0.0%	99.9%	16.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	dnaA_c.-48G>A	53	21260	0	13374	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	inhA_c.-522C>G	45	21268	0	13374	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
INH	katG_p.Val469Leu	46	21267	0	13374	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
INH	Rv0010c_p.Ser82Pro	47	21266	0	13374	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv0010c_p.Thr40Ala	48	21265	0	13374	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1129c_c.-29A>G	65	21248	0	13374	0.0%	99.7%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
INH	Rv1258c_p.Glu243Ala	48	21265	0	13374	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
INH	Rv2752c_p.Ala273Val	108	21205	0	13374	0.0%	99.5%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
INH	katG_p.Ser315Gly	1	27467	29	20817	0.1%	100.0%	96.7%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gln127Pro	4	27464	23	20823	0.1%	100.0%	85.2%	50.0%	93.2%	4.3%	1.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Pro232Ser	1	27467	13	20833	0.1%	100.0%	92.9%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_c.-10A>C	1	27467	10	20836	0.0%	100.0%	90.9%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp142Gly	2	27466	10	20836	0.0%	100.0%	83.3%	87.5%	99.7%	40.0%	9.2	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly699Glu	1	27467	9	20837	0.0%	100.0%	90.0%	87.5%	99.7%	47.3%	9.2	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Tyr337Cys	1	27467	9	20837	0.0%	100.0%	90.0%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Ala109Val	0	27468	8	20838	0.0%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asn138Ser	0	27468	8	20838	0.0%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp189Gly	2	27466	8	20838	0.0%	100.0%	80.0%	66.7%	95.7%	22.3%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly279Asp	0	27468	8	20838	0.0%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Val423Ile	7	27461	8	20838	0.0%	100.0%	53.3%	22.2%	60.0%	2.8%	0.4	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asn138His	0	27468	7	20839	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asn138Asp	2	27466	6	20840	0.0%	100.0%	75.0%	66.7%	95.7%	22.3%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gln461Pro	3	27465	6	20840	0.0%	100.0%	66.7%	50.0%	93.2%	5.3%	1.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gln525Pro	1	27467	6	20840	0.0%	100.0%	85.7%	80.0%	99.5%	28.4%	5.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly297Val	1	27467	6	20840	0.0%	100.0%	85.7%	80.0%	99.5%	28.4%	5.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly299Ser	0	27468	6	20840	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Pro232Ala	0	27468	5	20841	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Thr344Pro	1	27467	5	20841	0.0%	100.0%	83.3%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Trp161Arg	2	27466	5	20841	0.0%	100.0%	71.4%	100.0%	100.0%	29.0%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_c.-185T>C	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Ala172Val	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp419His	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp94Ala	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp94Gly	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly118Ser	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Leu141Phe	1	27467	4	20842	0.0%	100.0%	80.0%	80.0%	99.5%	28.4%	5.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Thr271Ile	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Thr394Ala	1	27467	4	20842	0.0%	100.0%	80.0%	100.0%	100.0%	19.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
INH	katG_p.Trp149Arg	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Trp300Gly	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Tyr98Cys	0	27468	4	20842	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_c.-485_-484insTGTCT	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_c.-8G>A	1	27467	3	20843	0.0%	100.0%	75.0%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Arg104Gln	1	27467	3	20843	0.0%	100.0%	75.0%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Arg484His	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Arg571His	1	27467	3	20843	0.0%	100.0%	75.0%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp189Asn	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp311Gly	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp487Asn	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp735Ala	1	27467	3	20843	0.0%	100.0%	75.0%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gln439Arg	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly125Asp	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly285Val	2	27466	3	20843	0.0%	100.0%	60.0%	50.0%	93.2%	6.8%	1.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly491Ser	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Leu48Pro	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Thr271Pro	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Trp300Arg	0	27468	3	20843	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Ala476Val	2	27466	2	20844	0.0%	100.0%	50.0%	50.0%	93.2%	6.8%	1.3	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Arg128Trp	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Arg146Gly	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asn493Lys	1	27467	2	20844	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asn637Lys	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp189Ala	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Asp381Ala	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Glu588Gly	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly121Asp	2	27466	2	20844	0.0%	100.0%	50.0%	100.0%	100.0%	6.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly156Asp	1	27467	2	20844	0.0%	100.0%	66.7%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○
INH	katG_p.Gly234Arg	1	27467	2	20844	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	2.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
INH	katG_p.Gly699Arg	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Leu333Pro	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Leu427Pro	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Pro288His	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Ser160Pro	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Ser700Pro	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Thr322Ala	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
INH	katG_p.Tyr229Cys	0	27468	2	20844	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○

Individual LoF mutations in the coding regions of *katG* that are classified in Group 2 because of the associated additional grading rule (see Table 23 and Section 5.8) and silent mutations are not listed in this table but can be found in the catalogue master file.

[F] Low-level resistance (multiple, genetically linked low-level resistance mutations are additive and confer high-level resistance).

[G] High-level resistance.

[H] Alias fabG1_p.Leu203Leu.

[I] Alias fabG1_c.-8T>A.

[J] Alias fabG1_c.-8T>C.

[K] Alias fabG1_c.-8T>G.

[L] Alias fabG1_c.-15C>T.

[M] Alias fabG1_c.-16A>G.

[N] Alias fabG1_c.-17G>T.

[O] Group 2 by "relaxed" threshold (not endorsed).

3.4 Ethambutol

Thirteen non-synonymous mutations in *embB* and one intergenic mutation upstream of *embA* met the criteria for Group 1 mutations; no Group 2 mutations were identified (Table 8). The combined sensitivity of Group 1 mutations for predicting phenotypic EMB resistance was 81.1% (95% CI: 80.3–81.9), while the specificity was 91.6% (95% CI: 91.3–91.9) and the PPV 71.9% (95% CI: 71.0–72.8) (Table 3). The reduced specificity is probably due to the fact that many *embB* mutations confer MICs close to the CC, resulting in poor categorical agreement with pDST *(11,22,54–56)*. Indeed, the Clinical and Laboratory Standards Institute has an "inconclusive" category for EMB MICs equal to the CC on Sensititre MYCOTB plates *(21,57)*. Additionally, it is not clear whether the currently endorsed CCs are identical to the epidemiological cut-off values for EMB. Inappropriately high breakpoints may be responsible for high rates of misclassification of *embB* phenotypes and consequent discordance with gDST results, as was the case with borderline RIF resistance mutations *(21, 33)*. Notably, two markers of resistance in the first edition (*embA* -12C>T as Group 1 and *embB* Leu74Arg as Group 2) were downgraded to Group 3 in the second edition. As a result, EMB is one of only four drugs the sensitivity of which decreased slightly from that in the first edition (i.e. by 2.7%, as shown in Table A.1). Inclusion of mutations with a variant allele frequency below 75% increased the sensitivity by only 1% (Table A.1) suggesting a minimal role of heteroresistance.

Table 8. Abridged variant classification for EMB

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
EMB	embB_p.Met306Val	685	35020	3245	6236	34.2%	98.1%	82.6%	79.9%	81.5%	74.0%	22.4	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Met306Ile	1159	34546	1953	7528	20.6%	96.8%	62.8%	54.5%	56.7%	45.3%	5.5	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Gln497Arg	224	35481	999	8482	10.5%	99.4%	81.7%	73.0%	76.8%	58.8%	11.3	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Gly406Ala	199	35506	328	9153	3.5%	99.4%	62.2%	53.0%	59.0%	37.4%	4.4	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Asp354Ala	137	35568	301	9180	3.2%	99.6%	68.7%	64.2%	69.9%	49.9%	6.9	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Gly406Asp	212	35493	274	9207	2.9%	99.4%	56.4%	46.9%	53.2%	31.5%	3.4	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Tyr319Ser	47	35658	204	9277	2.2%	99.9%	81.3%	80.7%	85.7%	73.0%	16.1	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Gly406Ser	76	35629	187	9294	2.0%	99.8%	71.1%	61.1%	70.9%	34.8%	6.0	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Gln497Lys	51	35654	145	9336	1.5%	99.9%	74.0%	53.0%	65.4%	30.2%	4.3	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Met306Leu	50	35655	145	9336	1.5%	99.9%	74.4%	70.4%	79.2%	48.6%	9.1	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Asp328Tyr	5	35700	46	9435	0.5%	100.0%	90.2%	84.0%	95.5%	60.6%	19.9	1) AwR	ALL+WHO		1) Assoc w R		
EMB	embB_p.Tyr319Cys	12	26493	27	6533	0.4%	100.0%	69.2%	59.3%	77.6%	37.2%	5.9	1) AwR	WHO		1) Assoc w R		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
EMB	embB_p.Gly406Cys	28	35677	36	9445	0.4%	99.9%	56.3%	58.1%	73.0%	33.3%	5.2	1) AwR	ALL+WHO		1) Assoc w R		○
EMB	embA_c.-12C>T	173	35532	635	8846	6.7%	99.5%	78.6%	30.9%	41.1%	10.2%	1.8	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
EMB	embB_p.Leu74Arg	8	35697	21	9460	0.2%	100.0%	72.4%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
EMB	embC_c.-1188C>T	1498	25007	1403	5157	21.4%	94.3%	48.4%	5.4%	7.0%	2.7%	0.3	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		▲
EMB	embB_p.Gly156Cys	47	26458	45	6515	0.7%	99.8%	48.9%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		○
EMB	embA_p.Ala813Gly	47	26458	43	6517	0.7%	99.8%	47.8%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		○
EMB	embA_p.Pro639Ser	57	26448	16	6544	0.2%	99.8%	21.9%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		▬
EMB	embC_c.-20A>C	56	26449	16	6544	0.2%	99.8%	22.2%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		▬
EMB	embA_p.Val468Ala	57	26448	16	6544	0.2%	99.8%	21.9%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
EMB	embC_c.-1520C>A	71	26434	4	6556	0.1%	99.7%	5.3%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
EMB	ubiA_p.Val49Ile	72	26433	4	6556	0.1%	99.7%	5.3%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
EMB	embB_p.Ser1054Pro	31	26474	2	6558	0.0%	99.9%	6.1%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
EMB	embB_p.Val668Ile	23	26482	2	6558	0.0%	99.9%	8.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		○
EMB	aftB_p.Asp397Gly	5235	21270	3363	3197	51.3%	80.2%	39.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embC_p.Val981Leu	4076	22429	516	6044	7.9%	84.6%	11.2%	1.0%	1.7%	0.2%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_c.-565C>T	2287	24218	332	6228	5.1%	91.4%	12.7%	0.0%	70.8%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_p.Arg738Gln	3853	22652	327	6233	5.0%	85.5%	7.8%	1.1%	1.6%	0.5%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embB_p.Glu378Ala	3386	23119	251	6309	3.8%	87.2%	6.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	glpK_p.Val460Ala	3427	23078	248	6312	3.8%	87.1%	6.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	ubiA_p.Glu149Asp	3404	23101	247	6313	3.8%	87.2%	6.8%	0.0%	60.2%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_p.Thr270Ile	3246	23259	243	6317	3.7%	87.8%	7.0%	0.0%	84.2%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embA_p.Pro913Ser	3014	23491	226	6334	3.4%	88.6%	7.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_p.Asn394Asp	3000	23505	225	6335	3.4%	88.7%	7.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embR_p.Cys110Tyr	2931	23574	222	6338	3.4%	88.9%	7.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_c.-900C>T	945	25560	132	6428	2.0%	96.4%	12.3%	1.2%	2.4%	0.3%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embA_p.Val206Met	1689	24816	117	6443	1.8%	93.6%	6.5%	0.0%	84.2%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_c.-1743G>A	473	26032	99	6461	1.5%	98.2%	17.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embB_p.Thr1082Ala	108	26397	96	6464	1.5%	99.6%	47.1%	1.2%	6.7%	0.0%	0.1	5) NotAwR	WHO		5) Not assoc w R		▲
EMB	embC_c.-100C>T	255	26250	93	6467	1.4%	99.0%	26.7%	0.0%	2.1%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_c.-529T>C	107	26398	83	6477	1.3%	99.6%	43.7%	0.0%	4.6%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		▲
EMB	Rv2752c_p.Pro123Leu	210	26295	68	6492	1.0%	99.2%	24.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embC_p.Ala774Ser	141	26364	65	6495	1.0%	99.5%	31.6%	1.8%	6.3%	0.2%	0.1	5) NotAwR	WHO		5) Not assoc w R		○
EMB	Rv2752c_p.Val300Ala	114	26391	63	6497	1.0%	99.6%	35.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embC_c.-1193C>T	103	26402	36	6524	0.5%	99.6%	25.9%	1.2%	6.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
EMB	aftB_p.Lys522Arg	306	26199	29	6531	0.4%	98.8%	8.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	Rv2752c_p.Gly161Ser	113	26392	24	6536	0.4%	99.6%	17.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	Rv2752c_p.Met31Ile	147	26358	19	6541	0.3%	99.4%	11.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embA_p.Glu951Asp	125	26380	15	6545	0.2%	99.5%	10.7%	1.8%	6.3%	0.2%	0.1	5) NotAwR	WHO		5) Not assoc w R		▲
EMB	embC_c.-1419G>A	143	26362	15	6545	0.2%	99.5%	9.5%	1.7%	6.0%	0.2%	0.1	5) NotAwR	WHO		5) Not assoc w R		○
EMB	ubiA_p.Gly268Asp	143	26362	15	6545	0.2%	99.5%	9.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	Rv2477c_p.Val85Ile	84	26421	13	6547	0.2%	99.7%	13.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embC_c.-589C>G	88	26417	11	6549	0.2%	99.7%	11.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_p.Val104Met	299	26206	10	6550	0.2%	98.9%	3.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_p.Arg567His	375	26130	9	6551	0.1%	98.6%	2.3%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	ubiA_c.-32delG	713	25792	8	6552	0.1%	97.3%	1.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	glpK_p.Leu228Val	349	26156	7	6553	0.1%	98.7%	2.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	aftB_p.Ile327Val	194	26311	6	6554	0.1%	99.3%	3.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embB_p.Asn13Ser	192	26313	6	6554	0.1%	99.3%	3.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_c.-270G>A	64	26441	5	6555	0.1%	99.8%	7.2%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embR_p.Ala70Ser	120	26385	3	6557	0.0%	99.5%	2.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	Rv2752c_p.Ala296Val	113	26392	2	6558	0.0%	99.6%	1.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	Rv2477c_p.Arg86His	54	26451	1	6559	0.0%	99.8%	1.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embA_p.Pro383Ser	58	26447	1	6559	0.0%	99.8%	1.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embB_p.Arg213Gln	207	26298	1	6559	0.0%	99.2%	0.5%	0.0%	1.8%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embB_p.Gln139His	138	26367	1	6559	0.0%	99.5%	0.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embC_p.Leu661Ile	58	26447	1	6559	0.0%	99.8%	1.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embR_p.Cys372Gly	4	26501	0	6560	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embR_p.Phe376Leu	4	26501	0	6560	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	glpK_p.Gly260Ala	42	26463	0	6560	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	Rv2752c_p.Ala273Val	108	26397	0	6560	0.0%	99.6%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
EMB	embC_c.-1803G>C	58	26447	0	6560	0.0%	99.8%	0.0%	0.0%	7.0%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		▲
EMB	embA_p.Ala201Thr	136	26369	0	6560	0.0%	99.5%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
EMB	embA_p.Thr308Ala	46	26459	0	6560	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Silent mutations are not listed in this table but can be found in the catalogue master file.

3.5 Pyrazinamide

As in the first edition, only *pncA* mutations were classified as markers of PZA resistance in this analysis (Table 9). While only 139 *pncA* mutations were sufficiently frequent to meet the grading criteria for Group 1, resulting in a combined sensitivity of 63.5% (95% CI: 62.0–64.9) (Table 3), 49 of the 202 identified Group 2 mutations were classified according to the WHO-endorsed LoF additional grading rule (Tables 1 and 23), increasing the sensitivity to 78.0% (95% CI: 76.8–79.2). This represents a 4.3% increase in sensitivity over that in the first edition, with a negligible decrease in specificity and PPV (Table A.1). Notably, the inframe *pncA* Gly113_Leu116delinsVal indel, which was subject to the LoF grading rule in the first edition but was excluded when we changed the definition of LoF mutations (see Table 23, p. 91), was not associated with resistance on an interim basis (Group 4), confirming that inframe indels are less likely to be markers of resistance. Inclusion of mutations with variant allele frequencies below 75% increased the sensitivity by 2% (Table A.1), suggesting that mixed populations may play a role in observed PZA resistant phenotypes.

Most *M. bovis* isolates are intrinsically resistant to PZA because of the Group 1 *pncA* His57Asp mutation *(58)*. *M. canettii*, which is rarely found outside the Horn of Africa, is also intrinsically PZA-resistant *(59,60)*. The genetic basis of this phenotype is unclear, however, and most of the isolates described to date do not have a plausible *pncA* resistance mutation *(61,62)*. This intrinsic resistance is therefore best inferred by identification of *M. canettii* (Table 1). Ideally, identification should rely on multiple phylogenetically informative mutations in genes that are not under selection; in practice, however, the choice of markers depends on which parts of the MTBC genome are interrogated by an assay. The 138A>G synonymous mutation at codon 46 of *pncA*, which has been proposed as a marker for *M. canettii* in the literature *(62)*, was also observed in some lineage 4 MTBC isolates in this data set. As a result, it was not endorsed as a marker to infer intrinsic PZA resistance for *M. canettii* (i.e. it was classified as a Group 4 mutation instead). Nevertheless, a footnote was included for this synonymous mutation to indicate that *M. canettii* may be present, in order to minimize the possibility that intrinsic PZA resistance is missed by assays that analyse *pncA* but not other loci for differentiating MTBC (Table 9).

Potential role of rare variants

The sensitivity estimates for *pncA* mutations are based on "relaxed" grading criteria (Fig. 9) rather than the standard criteria used for all the other drugs (Fig. 8). In the first edition, an additional grading criterion was proposed, but not endorsed, whereby the sensitivity could be increased further by assuming that any coding *pncA* mutation, except silent and Group 4 or 5 mutations, in *pncA* was a valid marker of resistance if it occurred in genotypically RIF-resistant isolates. Inferring resistance is the principal approach used for the WHO-endorsed Genoscholar PZA-TB II assay, as it cannot differentiate most *pncA* mutations *(45,63,64)*. Use of this approach would increase the overall sensitivity by 2.6%, with an accompanying decrease in specificity of 0.7% and a decrease in the PPV of 2.4% (Table A.3). In settings where non-silent but still neutral *pncA* mutations are more frequent, the decrease in specificity and PPV might be considerably greater *(28)*. This assumption was therefore explored only for the analyses presented in Table A.3 and was not used to generate the master file results.

Table 9. Abridged variant classification for PZA

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_LoF	42	16780	573	3836	13.0%	99.8%	93.2%	93.6%	95.5%	89.8%	63.5	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.His57Asp	2	16820	166	4243	3.8%	100.0%	98.8%	98.8%	99.9%	95.6%	319.1	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_c.-11A>G	10	16812	141	4268	3.2%	99.9%	93.4%	93.3%	96.8%	88.1%	55.1	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His51Arg	5	16817	82	4327	1.9%	100.0%	94.3%	94.3%	98.1%	87.1%	63.7	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gln141Pro	5	16817	75	4334	1.7%	100.0%	93.8%	93.8%	97.9%	86.0%	58.2	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gln10Pro	5	16817	70	4339	1.6%	100.0%	93.3%	93.2%	97.8%	84.9%	53.5	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val139Ala	5	16817	59	4350	1.3%	100.0%	92.2%	91.9%	97.3%	82.2%	44.1	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gln10Arg	3	16819	57	4352	1.3%	100.0%	95.0%	94.6%	98.9%	85.1%	68.3	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gly132Ala	2	16820	57	4352	1.3%	100.0%	96.6%	96.6%	99.6%	88.1%	108.2	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His57Arg	5	16817	56	4353	1.3%	100.0%	91.8%	91.8%	97.3%	81.9%	43.3	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val131fs	0	16822	54	4355	1.2%	100.0%	100.0%	100.0%	100.0%	93.3%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Thr76Pro	3	16819	52	4357	1.2%	100.0%	94.5%	94.5%	98.9%	84.9%	66.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His51Asp	1	16821	48	4361	1.1%	100.0%	98.0%	98.0%	99.9%	89.1%	185.1	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gly97Asp	5	16817	46	4363	1.0%	100.0%	90.2%	95.7%	99.9%	61.9%	84.8	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu27Pro	0	16822	44	4365	1.0%	100.0%	100.0%	100.0%	100.0%	91.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp49Gly	2	16820	43	4366	1.0%	100.0%	95.6%	95.6%	99.5%	84.9%	82.8	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Thr135Pro	1	16821	43	4366	1.0%	100.0%	97.7%	97.7%	99.9%	88.0%	165.7	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Cys14Arg	1	16821	42	4367	1.0%	100.0%	97.7%	97.7%	99.9%	87.7%	161.8	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu4Ser	3	16819	42	4367	1.0%	100.0%	93.3%	93.0%	98.5%	80.9%	51.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His71Arg	2	16820	38	4371	0.9%	100.0%	95.0%	94.9%	99.4%	82.7%	71.2	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Glu173fs	0	16822	37	4372	0.8%	100.0%	100.0%	100.0%	100.0%	90.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Thr153fs	2	16820	35	4374	0.8%	100.0%	94.6%	94.6%	99.3%	81.8%	67.3	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.His71Tyr	2	16820	34	4375	0.8%	100.0%	94.4%	94.4%	99.3%	81.3%	65.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val7Gly	2	16820	34	4375	0.8%	100.0%	94.4%	94.1%	99.3%	80.3%	61.5	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Pro54Leu	2	16820	32	4377	0.7%	100.0%	94.1%	93.8%	99.2%	79.2%	57.6	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Trp68Gly	2	16820	32	4377	0.7%	100.0%	94.1%	94.1%	99.3%	80.3%	61.5	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu172Pro	0	16822	31	4378	0.7%	100.0%	100.0%	100.0%	100.0%	88.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ile133Thr	18	16804	29	4380	0.7%	99.9%	61.7%	61.7%	75.5%	46.4%	6.2	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu120Pro	0	16822	28	4381	0.6%	100.0%	100.0%	100.0%	100.0%	87.7%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ile5Ser	0	16822	27	4382	0.6%	100.0%	100.0%	100.0%	100.0%	87.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Asp63Ala	5	16817	26	4383	0.6%	100.0%	83.9%	83.3%	94.4%	65.3%	19.2	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Tyr103*	0	16822	26	4383	0.6%	100.0%	100.0%	100.0%	100.0%	86.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_c.-11A>C	0	16822	24	4385	0.5%	100.0%	100.0%	100.0%	100.0%	85.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ser59Pro	0	16822	24	4385	0.5%	100.0%	100.0%	100.0%	100.0%	85.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ile31Ser	4	16818	23	4386	0.5%	100.0%	85.2%	85.2%	95.8%	66.3%	22.0	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp8Gly	1	16821	22	4387	0.5%	100.0%	95.7%	95.7%	99.9%	78.1%	84.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ser67Pro	0	16822	22	4387	0.5%	100.0%	100.0%	100.0%	100.0%	83.9%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ala146Val	0	16822	21	4388	0.5%	100.0%	100.0%	100.0%	100.0%	83.9%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His71Pro	0	16822	20	4389	0.5%	100.0%	100.0%	100.0%	100.0%	83.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Trp68Cys	0	16822	20	4389	0.5%	100.0%	100.0%	100.0%	100.0%	83.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gly97Cys	1	16821	19	4390	0.4%	100.0%	95.0%	94.7%	99.9%	74.0%	69.0	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu151Ser	6	16816	19	4390	0.4%	100.0%	76.0%	75.0%	90.2%	53.3%	11.5	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Lys96Thr	1	16821	19	4390	0.4%	100.0%	95.0%	95.0%	99.9%	75.1%	72.8	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val125Gly	0	16822	19	4390	0.4%	100.0%	100.0%	100.0%	100.0%	82.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Asp12Ala	7	16815	18	4391	0.4%	100.0%	72.0%	72.0%	87.9%	50.6%	9.8	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp136Gly	6	16816	18	4391	0.4%	100.0%	75.0%	75.0%	90.2%	53.3%	11.5	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Trp68Arg	1	16821	18	4391	0.4%	100.0%	94.7%	94.7%	99.9%	74.0%	69.0	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Tyr34Asp	0	16822	18	4391	0.4%	100.0%	100.0%	100.0%	100.0%	81.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val180Phe	2	16820	18	4391	0.4%	100.0%	90.0%	90.0%	98.8%	68.3%	34.5	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Arg154Gly	7	16815	17	4392	0.4%	100.0%	70.8%	70.8%	87.4%	48.9%	9.3	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gly108Arg	1	16821	17	4392	0.4%	100.0%	94.4%	94.4%	99.9%	72.7%	65.1	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Thr47Ala	11	16811	17	4392	0.4%	99.9%	60.7%	60.7%	78.5%	40.6%	5.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ala146Thr	4	16818	15	4394	0.3%	100.0%	78.9%	78.9%	93.9%	54.4%	14.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu120Arg	0	16822	15	4394	0.3%	100.0%	100.0%	100.0%	100.0%	78.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Leu182Ser	4	16818	15	4394	0.3%	100.0%	78.9%	77.8%	93.6%	52.4%	13.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu4Trp	1	16821	15	4394	0.3%	100.0%	93.8%	93.8%	99.8%	69.8%	57.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Phe106fs	2	16820	15	4394	0.3%	100.0%	88.2%	88.2%	98.5%	63.6%	28.7	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val139Gly	0	16822	15	4394	0.3%	100.0%	100.0%	100.0%	100.0%	78.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp8Asn	0	16822	14	4395	0.3%	100.0%	100.0%	100.0%	100.0%	76.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Cys72Arg	0	16822	14	4395	0.3%	100.0%	100.0%	100.0%	100.0%	76.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Gln10His	0	16822	14	4395	0.3%	100.0%	100.0%	100.0%	100.0%	75.3%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Pro69Leu	5	16817	14	4395	0.3%	100.0%	73.7%	73.7%	90.9%	48.8%	10.7	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Thr142Ala	2	16820	14	4395	0.3%	100.0%	87.5%	86.7%	98.3%	59.5%	24.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val128Gly	0	16822	14	4395	0.3%	100.0%	100.0%	100.0%	100.0%	76.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ala134Val	1	16821	13	4396	0.3%	100.0%	92.9%	92.9%	99.8%	66.1%	49.7	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp12Gly	3	16819	13	4396	0.3%	100.0%	81.3%	81.3%	96.0%	54.4%	16.6	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gly97Ser	2	16820	13	4396	0.3%	100.0%	86.7%	86.7%	98.3%	59.5%	24.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Phe58Leu	4	16818	13	4396	0.3%	100.0%	76.5%	76.5%	93.2%	50.1%	12.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Thr142Met	1	16821	13	4396	0.3%	100.0%	92.9%	92.3%	99.8%	64.0%	45.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp129_Val131del	0	16822	12	4397	0.3%	100.0%	100.0%	100.0%	100.0%	73.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.His57Tyr	0	16822	12	4397	0.3%	100.0%	100.0%	100.0%	100.0%	73.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ile6Thr	0	16822	12	4397	0.3%	100.0%	100.0%	100.0%	100.0%	73.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Met175Val	3	16819	12	4397	0.3%	100.0%	80.0%	78.6%	95.3%	49.2%	14.0	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ser164Pro	1	16821	12	4397	0.3%	100.0%	92.3%	92.3%	99.8%	64.0%	45.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Thr160Pro	0	16822	12	4397	0.3%	100.0%	100.0%	100.0%	100.0%	71.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ala152fs	0	16822	11	4398	0.2%	100.0%	100.0%	100.0%	100.0%	71.5%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Gln122*	0	16822	11	4398	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Glu127_Asp129del	0	16822	11	4398	0.2%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_c.-5delG	3	16819	10	4399	0.2%	100.0%	76.9%	76.9%	95.0%	46.2%	12.7	1) AwR	ALL+WHO		1) Assoc w R		●
PZA	pncA_p.Asp49Ala	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Gly97Arg	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu19Pro	1	16821	10	4399	0.2%	100.0%	90.9%	90.9%	99.8%	58.7%	38.2	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Leu85Pro	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Lys96Arg	2	16820	10	4399	0.2%	100.0%	83.3%	83.3%	97.9%	51.6%	19.1	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Lys96Glu	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Met175Arg	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Phe94Leu	3	16819	10	4399	0.2%	100.0%	76.9%	75.0%	94.5%	42.8%	11.5	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Pro62Leu	1	16821	10	4399	0.2%	100.0%	90.9%	90.9%	99.8%	58.7%	38.2	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val180Ala	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val9Gly	0	16822	10	4399	0.2%	100.0%	100.0%	100.0%	100.0%	69.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Asp129fs	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Asp136fs	1	16821	9	4400	0.2%	100.0%	90.0%	90.0%	99.7%	55.5%	34.4	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Asp8Glu	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Cys138Arg	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Glu144fs	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Gly132Ser	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu85Arg	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Met1?	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Phe94Cys	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ser84fs	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Tyr103Cys	4	16818	9	4400	0.2%	100.0%	69.2%	69.2%	90.9%	38.6%	8.6	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val155Gly	1	16821	9	4400	0.2%	100.0%	90.0%	90.0%	99.7%	55.5%	34.4	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Val180Gly	0	16822	9	4400	0.2%	100.0%	100.0%	100.0%	100.0%	66.4%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ala102Pro	1	16821	8	4401	0.2%	100.0%	88.9%	87.5%	99.7%	47.3%	26.8	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His51Gln	1	16821	8	4401	0.2%	100.0%	88.9%	88.9%	99.7%	51.8%	30.6	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His51Pro	0	16822	8	4401	0.2%	100.0%	100.0%	100.0%	100.0%	63.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Leu159Arg	2	16820	8	4401	0.2%	100.0%	80.0%	80.0%	97.5%	44.4%	15.3	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Thr177Pro	1	16821	8	4401	0.2%	100.0%	88.9%	88.9%	99.7%	51.8%	30.6	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Thr76Ile	2	16820	8	4401	0.2%	100.0%	80.0%	87.5%	99.7%	40.0%	26.8	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val128fs	0	16822	8	4401	0.2%	100.0%	100.0%	100.0%	100.0%	63.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val139Met	0	16822	8	4401	0.2%	100.0%	100.0%	100.0%	100.0%	63.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val155Met	1	16821	8	4401	0.2%	100.0%	88.9%	88.9%	99.7%	51.8%	30.6	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val7Leu	0	16822	8	4401	0.2%	100.0%	100.0%	100.0%	100.0%	63.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_c.-12T>C	1	16821	7	4402	0.2%	100.0%	87.5%	87.5%	99.7%	47.3%	26.7	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Asp86fs	0	16822	7	4402	0.2%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Gln10*	0	16822	7	4402	0.2%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Gln141*	0	16822	7	4402	0.2%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ile90Ser	3	16819	7	4402	0.2%	100.0%	70.0%	70.0%	93.3%	34.8%	8.9	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Leu156fs	1	16821	7	4402	0.2%	100.0%	87.5%	87.5%	99.7%	47.3%	26.7	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Ser104Arg	0	16822	7	4402	0.2%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Tyr103His	0	16822	7	4402	0.2%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Tyr64*	1	16821	7	4402	0.2%	100.0%	87.5%	87.5%	99.7%	47.3%	26.7	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Val130fs	0	16822	7	4402	0.2%	100.0%	100.0%	100.0%	100.0%	59.0%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Asp12Glu	0	16822	6	4403	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.Asp63fs	1	16821	6	4403	0.1%	100.0%	85.7%	85.7%	99.6%	42.1%	22.9	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Gly105Asp	2	16820	6	4403	0.1%	100.0%	75.0%	75.0%	96.8%	34.9%	11.5	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Leu182Trp	0	16822	6	4403	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Phe13Ile	0	16822	6	4403	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Pro54Arg	0	16822	6	4403	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Ser66Pro	0	16822	6	4403	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Trp119*	0	16822	6	4403	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Arg154Met	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		●
PZA	pncA_p.Gly24Asp	1	16821	5	4404	0.1%	100.0%	83.3%	83.3%	99.6%	35.9%	19.1	1) AwR	ALL+WHO		1) Assoc w R		○
PZA	pncA_p.His51Tyr	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.His82Arg	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Lys96Gln	1	16821	5	4404	0.1%	100.0%	83.3%	83.3%	99.6%	35.9%	19.1	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Phe13Ser	1	16821	5	4404	0.1%	100.0%	83.3%	83.3%	99.6%	35.9%	19.1	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Thr47Pro	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Thr61fs	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Trp68*	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL+WHO		1) Assoc w R		▲
PZA	pncA_p.Lys48Thr	26	16796	20	4389	0.5%	99.8%	43.5%	43.5%	58.9%	28.9%	2.9	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
PZA	pncA_p.Asp126_Val130del	1	16821	12	4397	0.3%	100.0%	92.3%	66.7%	99.2%	9.4%	7.7	2) AwRI	ALL		2) Assoc w R - Interim		○
PZA	pncA_p.Ala146Glu	0	16822	8	4401	0.2%	100.0%	100.0%	100.0%	100.0%	63.1%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
PZA	pncA_p.Asp63Gly	5	16041	6	3780	0.2%	100.0%	54.5%	54.5%	83.3%	23.4%	5.1	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Leu116Pro	4	16818	6	4403	0.1%	100.0%	60.0%	42.9%	81.6%	9.9%	2.9	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Val7Ala	3	16819	6	4403	0.1%	100.0%	66.7%	62.5%	91.5%	24.5%	6.4	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▬
PZA	pncA_p.Asp12Asn	1	16045	5	3781	0.1%	100.0%	83.3%	80.0%	99.5%	28.4%	17.0	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Val125Phe	3	16043	5	3781	0.1%	100.0%	62.5%	62.5%	91.5%	24.5%	7.1	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		▬
PZA	pncA_p.Asp49Asn	1	16821	5	4404	0.1%	100.0%	83.3%	83.3%	99.6%	35.9%	19.1	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
PZA	pncA_p.His57Leu	1	16821	5	4404	0.1%	100.0%	83.3%	83.3%	99.6%	35.9%	19.1	1) AwR	ALL	ALL only	2) Assoc w R - Interim		●

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Val131Gly	1	16821	5	4404	0.1%	100.0%	83.3%	83.3%	99.6%	35.9%	19.1	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
PZA	pncA_p.Val93Leu	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim		●
PZA	pncA_p.Leu159Pro	0	16822	5	4404	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Leu35Pro	3	16819	5	4404	0.1%	100.0%	62.5%	62.5%	91.5%	24.5%	6.4	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Phe13Leu	1	16821	5	4404	0.1%	100.0%	83.3%	75.0%	99.4%	19.4%	11.5	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Trp119Cys	1	16821	5	4404	0.1%	100.0%	83.3%	100.0%	100.0%	19.4%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▬
PZA	pncA_p.Leu116Arg	0	16046	4	3782	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Met175Thr	0	16046	4	3782	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Thr168Pro	0	16046	4	3782	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Trp119Arg	0	16046	4	3782	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Gly108Glu	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		▲
PZA	pncA_c.-11A>T	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Ala171Glu	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Ala46Glu	1	16821	4	4405	0.1%	100.0%	80.0%	80.0%	99.5%	28.4%	15.3	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Ala46Val	1	16821	4	4405	0.1%	100.0%	80.0%	80.0%	99.5%	28.4%	15.3	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Gly105Val	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▬
PZA	pncA_p.Gly162Asp	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Gly17Asp	1	16821	4	4405	0.1%	100.0%	80.0%	80.0%	99.5%	28.4%	15.3	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Gly78Asp	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Gly78Val	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.His137Pro	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Leu120Gln	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Lys96Asn	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Met175Ile	4	16818	4	4405	0.1%	100.0%	50.0%	50.0%	84.3%	15.7%	3.8	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Pro62Arg	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Ter187Trpext*?	1	16821	4	4405	0.1%	100.0%	80.0%	80.0%	99.5%	28.4%	15.3	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Thr61Pro	2	16820	4	4405	0.1%	100.0%	66.7%	60.0%	94.7%	14.7%	5.7	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Val131Phe	1	16821	4	4405	0.1%	100.0%	80.0%	80.0%	99.5%	28.4%	15.3	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Val44Gly	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Val7Phe	0	16822	4	4405	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Trp119Gly	0	16046	3	3783	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.Val21Gly	0	16046	3	3783	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		○
PZA	pncA_p.His43Pro	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		▲
PZA	pncA_p.Ile90Thr	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		○
PZA	pncA_c.-7T>C	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Ala143Gly	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▬
PZA	pncA_p.Ala3Glu	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Asp129Asn	1	16821	3	4406	0.1%	100.0%	75.0%	75.0%	99.4%	19.4%	11.5	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Asp8Ala	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Asp8Tyr	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Gly78Ser	2	16820	3	4406	0.1%	100.0%	60.0%	60.0%	94.7%	14.7%	5.7	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Gly97Val	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Leu159Val	2	16820	3	4406	0.1%	100.0%	60.0%	60.0%	94.7%	14.7%	5.7	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Phe106Ser	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Phe94Ser	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Pro54Gln	1	16821	3	4406	0.1%	100.0%	75.0%	75.0%	99.4%	19.4%	11.5	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Pro54Ser	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Pro62Ser	2	16820	3	4406	0.1%	100.0%	60.0%	75.0%	99.4%	14.7%	11.5	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Pro62Thr	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Thr100Pro	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Trp119Leu	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Tyr64Asp	2	16820	3	4406	0.1%	100.0%	60.0%	60.0%	94.7%	14.7%	5.7	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Val130Gly	0	16822	3	4406	0.1%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▬
PZA	pncA_p.Val163Ala	1	16821	3	4406	0.1%	100.0%	75.0%	75.0%	99.4%	19.4%	11.5	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Arg140Pro	0	16046	2	3784	0.1%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	WHO	Interim on WHO	2) Assoc w R - Interim		▲
PZA	pncA_p.Phe58Cys	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		▲
PZA	pncA_p.Thr142Pro	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		▲
PZA	pncA_p.Thr160Lys	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		●
PZA	pncA_p.Trp68Leu	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		▲
PZA	pncA_p.Val130Met	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL		2) Assoc w R - Interim		●

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Ala102Thr	2	16820	2	4407	0.0%	100.0%	50.0%	50.0%	93.2%	6.8%	3.8	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Ala171Pro	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Ala178_Ser179del	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Arg121Pro	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Asp136Val	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Asp49Glu	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Asp63_Ser67delinsGlu	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Asp8His	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Cys14Trp	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Cys72Tyr	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Gly132Asp	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Gly132Cys	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.His57Gln	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Ile90Asn	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Lys48Glu	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Phe106Leu	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Phe58Ser	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Pro54Thr	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Ser104_Gly108delinsArg	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Ser104Gly	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Ser104Ile	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Ter187Argext*?	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Thr114Pro	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Thr135Asn	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Thr142Lys	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Tyr103Asp	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Tyr34Ser	0	16822	2	4407	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	2) AwRI	ALL+WHO		2) Assoc w R - Interim		●
PZA	pncA_p.Tyr95Asp	2	16820	2	4407	0.0%	100.0%	50.0%	50.0%	93.2%	6.8%	3.8	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Val139Leu	1	16821	2	4407	0.0%	100.0%	66.7%	66.7%	99.2%	9.4%	7.6	2) AwRI	ALL+WHO		2) Assoc w R - Interim		○
PZA	pncA_p.Val155Ala	2	16820	2	4407	0.0%	100.0%	50.0%	50.0%	93.2%	6.8%	3.8	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Val93Ala	2	16820	2	4407	0.0%	100.0%	50.0%	50.0%	93.2%	6.8%	3.8	2) AwRI	ALL+WHO		2) Assoc w R - Interim		▲
PZA	pncA_p.Ile31Thr	2	16820	1	4408	0.0%	100.0%	33.3%	33.3%	90.6%	0.8%	1.9	3) Uncertain	ALL+WHO	Lit. (PMID 32571824)	2) Assoc w R - Interim		○
PZA	pncA_p.Ala171Val	6	16816	3	4406	0.1%	100.0%	33.3%	33.3%	70.1%	7.5%	1.9	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	Rv1258c_p.Gly88fs	37	16785	3	4406	0.1%	99.8%	7.5%	0.0%	10.3%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Phe81Val	5	16817	2	4407	0.0%	100.0%	28.6%	28.6%	71.0%	3.7%	1.5	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ala165del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ala28_Leu172del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ala3_Ile5del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Asp110_Asn112del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Asp129del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Asp136_Leu156del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Asp136Asn	3	16819	1	4408	0.0%	100.0%	25.0%	25.0%	80.6%	0.6%	1.3	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Glu107del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Gly132_Thr135del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Gly150_Leu151insAspAlaValArgAsnGly	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Gly17_Ala25del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Gly55del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Gly60_Thr61del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ile6_Val7del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Leu172Arg	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Leu19_Val21del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Lys48_Val155delinsMet	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Phe81_His82del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Pro54_Asp56del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ser18_Val44del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ser66del	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Tyr103Ser	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Val155dup	0	16822	1	4408	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ala79Val	2	16820	0	4409	0.0%	100.0%	0.0%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Ser74_Pro77del	1	16821	0	4409	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	pncA_p.Thr160_Ala171del	1	16821	0	4409	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Thr168_Val169del	1	16821	0	4409	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	pncA_p.Val7del	1	16821	0	4409	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
PZA	Rv3236c_p.Thr102Ala	2934	13112	1976	1810	52.2%	81.7%	40.2%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
PZA	clpC1_p.Val63Ala	1261	14785	241	3545	6.4%	92.1%	16.0%	11.1%	13.0%	8.4%	0.5	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
PZA	rpsA_p.Met432Thr	545	15501	158	3628	4.2%	96.6%	22.5%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 32143680)	4) Not assoc w R - Interim		●
PZA	Rv1258c_p.Pro414Ser	113	15933	14	3772	0.4%	99.3%	11.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
PZA	pncA_p.Ala102Val	11	16035	5	3781	0.1%	99.9%	31.3%	31.3%	58.7%	11.0%	1.9	4) NotAwRI	WHO		4) Not assoc w R - Interim		▲
PZA	pncA_p.Leu35Arg	30	16016	4	3782	0.1%	99.8%	11.8%	9.1%	24.3%	1.9%	0.4	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Phe58Val	12	16034	3	3783	0.1%	99.9%	20.0%	14.3%	42.8%	1.8%	0.7	4) NotAwRI	WHO		4) Not assoc w R - Interim		▲
PZA	pncA_p.Thr160Ala	3	16043	3	3783	0.1%	100.0%	50.0%	0.0%	70.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		▲
PZA	pncA_p.His137Arg	5	16041	2	3784	0.1%	100.0%	28.6%	28.6%	71.0%	3.7%	1.7	4) NotAwRI	WHO		4) Not assoc w R - Interim		▲
PZA	pncA_c.-3_-2insC	10	16036	1	3785	0.0%	99.9%	9.1%	0.0%	30.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Ile6Leu	18	16028	1	3785	0.0%	99.9%	5.3%	0.0%	18.5%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Val21Ala	6	16040	1	3785	0.0%	100.0%	14.3%	0.0%	52.2%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_c.-33G>A	5	16041	0	3786	0.0%	100.0%	0.0%	0.0%	52.2%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Ala79Thr	3	16043	0	3786	0.0%	100.0%	0.0%	0.0%	70.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Glu15Gly	3	16043	0	3786	0.0%	100.0%	0.0%	0.0%	70.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Gly113_Leu116delinsVal	3	16043	0	3786	0.0%	100.0%	0.0%	0.0%	70.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		◆
PZA	pncA_p.Ser66Leu	8	16038	0	3786	0.0%	100.0%	0.0%	0.0%	36.9%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Thr114Met	3	16043	0	3786	0.0%	100.0%	0.0%	0.0%	70.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Thr168Ile	3	16043	0	3786	0.0%	100.0%	0.0%	0.0%	70.8%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_p.Thr87Met	17	16029	0	3786	0.0%	99.9%	0.0%	0.0%	19.5%	0.0%	0.0	4) NotAwRI	WHO		4) Not assoc w R - Interim		○
PZA	pncA_c.138A>G	0	16822	3	4406	0.1%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		4) Not assoc w R - Interim	Q	○
PZA	PPE35_p.Leu896Ser	7025	9021	2563	1223	67.7%	56.2%	26.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
PZA	panD_c.-1937C>T	82	15964	164	3622	4.3%	99.5%	66.7%	0.0%	5.9%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
PZA	Rv3236c_p.Ala370Thr	516	15530	95	3691	2.5%	96.8%	15.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
PZA	PPE35_p.Pro822Ser	920	15126	92	3694	2.4%	94.3%	9.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
PZA	PPE35_p.Gly258Asp	470	15576	87	3699	2.3%	97.1%	15.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
PZA	Rv3236c_p.Val151Ala	425	15621	87	3699	2.3%	97.4%	17.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
PZA	PPE35_p.Thr712Pro	55	15991	37	3749	1.0%	99.7%	40.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
PZA	clpC1_p.Asp326Asn	428	15618	32	3754	0.8%	97.3%	7.0%	1.6%	3.3%	0.6%	0.1	5) NotAwR	WHO		5) Not assoc w R		●
PZA	Rv3236c_c.-520A>G	430	15616	32	3754	0.8%	97.3%	6.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
PZA	sigE_p.Arg8Trp	126	15920	23	3763	0.6%	99.2%	15.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
PZA	clpC1_p.Pro796Leu	178	15868	17	3769	0.4%	98.9%	8.7%	2.8%	6.5%	0.9%	0.1	5) NotAwR	WHO		5) Not assoc w R		○
PZA	PPE35_p.Ile15Met	171	15875	17	3769	0.4%	98.9%	9.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
PZA	PPE35_p.Gly51Glu	317	15729	15	3771	0.4%	98.0%	4.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
PZA	PPE35_p.Pro670Leu	286	15760	10	3776	0.3%	98.2%	3.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
PZA	PPE35_p.Ser948Ile	38	16008	0	3786	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲
PZA	rpsA_p.Ile70Leu	47	15999	0	3786	0.0%	99.7%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
PZA	Rv1258c_p.Glu243Ala	41	16005	0	3786	0.0%	99.7%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Individual LoF mutations in the coding regions of *pncA* that are classified in Group 2 because of the associated additional grading rule (see Table 23 and Section 5.8) and silent mutations, except for *pncA* 138A>G, are not listed in this table but can be found in the catalogue master file.

[Q] Consider that this variant might indicate that the isolate is *M. canettii*, which is intrinsically PZA-resistant.

3.6 Levofloxacin and moxifloxacin

The most significant change to fluoroquinolone (FQ) resistance mutations in the second edition is upgrading of *gyrA* Asp89Asn and three *gyrB* mutations (Ser447Phe, Asn499Thr and Asp461His; Tables 10 and 11). These mutations were Group 3 in the first edition but, with additional evidence, are now recognized as either Group 1 or 2 mutations for LFX and/or MFX, increasing the overall sensitivity of the combined Groups 1 and 2 mutations by 1.1% to 84.8% (95% CI: 83.9–85.7) for LFX and by 0.7% to 85.7% (95% CI: 84.6–86.8) for MFX when variants at allele frequencies ≥ 75% are called (Table A.1). Lowering the cut-off for calling variants to allele frequency of 25% increased the sensitivity of these mutations for predicting resistant phenotypes by approximately 4.5% for both drugs (Table A.1), which confirmed published evidence that heteroresistance plays an important role in FQ resistance *(65,66)*. All *gyrA* and *gyrB* mutations were classified as conferring low-level resistance to MFX, except for the high-level resistance *gyrA* mutations Gly88Cys, Asp94Asn, Asp94Gly, Asp94His and Asp94Tyr (Table 1). Genetically linked low-level resistance mutations probably have additive effects and should be considered high-level resistant (Table 1).

Table 10. Abridged variant classification for LFX

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
LFX	gyrA_p.Asp94Gly	131	21332	2128	3715	36.4%	99.4%	94.2%	94.2%	95.2%	92.5%	93.4	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrA_p.Ala90Val	157	21306	1311	4532	22.4%	99.3%	89.3%	88.7%	90.4%	85.1%	36.8	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrA_p.Asp94Ala	81	21382	441	5402	7.5%	99.6%	84.5%	81.1%	84.9%	75.1%	17.0	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrA_p.Asp94Asn	31	21432	375	5468	6.4%	99.9%	92.4%	92.0%	94.6%	88.2%	45.3	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrA_p.Ser91Pro	51	21412	274	5569	4.7%	99.8%	84.3%	83.8%	87.9%	77.0%	19.8	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrA_p.Asp94Tyr	19	21444	229	5614	3.9%	99.9%	92.3%	91.5%	94.8%	87.1%	41.2	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrA_p.Asp94His	7	21456	99	5744	1.7%	100.0%	93.4%	93.8%	97.7%	85.8%	56.7	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrB_p.Asp461Asn	18	21445	44	5799	0.8%	99.9%	71.0%	60.0%	74.3%	44.3%	5.5	1) AwR	ALL+WHO		1) Assoc w R		▲
LFX	gyrA_p.Gly88Cys	1	21462	43	5800	0.7%	100.0%	97.7%	97.4%	99.9%	86.5%	140.6	1) AwR	ALL+WHO		1) Assoc w R		○
LFX	gyrB_p.Asn499Thr	6	9633	12	2708	0.4%	99.9%	66.7%	62.5%	84.8%	35.4%	5.9	1) AwR	WHO		1) Assoc w R		▲
LFX	gyrA_p.Asp89Asn	9	21454	20	5823	0.3%	100.0%	69.0%	66.7%	83.5%	46.0%	7.4	1) AwR	ALL+WHO		1) Assoc w R		▲
LFX	gyrB_p.Asn499Asp	7	21456	19	5824	0.3%	100.0%	73.1%	77.8%	93.6%	43.0%	12.9	1) AwR	ALL+WHO		1) Assoc w R		▲
LFX	gyrB_p.Glu501Asp	33	21430	45	5798	0.8%	99.8%	57.7%	29.5%	45.2%	16.0%	1.5	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim		○
LFX	gyrB_p.Ala504Val	10	21453	32	5811	0.5%	100.0%	76.2%	37.5%	64.6%	15.2%	2.2	3) Uncertain	ALL+WHO	Prev. WHO	2) Assoc w R - Interim		○
LFX	gyrB_p.Ser447Phe	19	21444	21	5822	0.4%	99.9%	52.5%	50.0%	68.1%	28.8%	3.7	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
LFX	gyrB_p.Asp461His	4	21459	19	5824	0.3%	100.0%	82.6%	77.8%	97.2%	30.8%	12.9	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
LFX	gyrA_p.Gly88Ala	12	21451	16	5827	0.3%	99.9%	57.1%	45.0%	68.5%	21.8%	3.0	3) Uncertain	ALL+WHO	Prev. WHO	2) Assoc w R - Interim		○
LFX	gyrB_p.Glu501Val	1	21462	0	5843	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim		○
LFX	gyrA_p.Ala90Gly	23	9616	1	2719	0.0%	99.8%	4.2%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28137812)	4) Not assoc w R - Interim		○
LFX	gyrA_p.Arg252Leu	26	9613	0	2720	0.0%	99.7%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
LFX	gyrA_p.Glu21Gln	9541	98	2707	13	99.5%	1.0%	22.1%	3.1%	4.4%	0.2%	0.2	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrA_p.Gly668Asp	8388	1251	2489	231	91.5%	13.0%	22.9%	9.1%	41.3%	0.0%	0.5	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrA_p.Ser95Thr	8486	1153	2390	330	87.9%	12.0%	22.0%	0.0%	18.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
LFX	Rv1129c_c.-28T>C	4932	4707	1831	889	67.3%	48.8%	27.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
LFX	gyrA_p.Gly247Ser	680	8959	136	2584	5.0%	92.9%	16.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
LFX	glpK_p.Val460Ala	589	9050	45	2675	1.7%	93.9%	7.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
LFX	gyrA_p.Ala384Val	534	9105	43	2677	1.6%	94.5%	7.5%	50.0%	98.7%	0.0%	3.4	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrB_p.Met291Ile	519	9120	42	2678	1.5%	94.6%	7.5%	0.0%	84.2%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrA_c.-34C>T	118	9521	14	2706	0.5%	98.8%	10.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrA_p.Ala463Ser	58	9581	14	2706	0.5%	99.4%	19.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
LFX	Rv2752c_p.Pro123Leu	65	9574	13	2707	0.5%	99.3%	16.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
LFX	Rv1129c_c.-48A>C	126	9513	12	2708	0.4%	98.7%	8.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
LFX	gyrB_p.Val301Leu	90	9549	11	2709	0.4%	99.1%	10.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrA_p.Gln613Glu	92	9547	5	2715	0.2%	99.0%	5.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
LFX	gyrB_p.Pro94Leu	206	9433	5	2715	0.2%	97.9%	2.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
LFX	glpK_p.Cys29Tyr	72	9567	3	2717	0.1%	99.3%	4.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
LFX	gyrA_p.Thr80Ala	72	9567	3	2717	0.1%	99.3%	4.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲
LFX	gyrB_p.Gly520Ala	36	9603	1	2719	0.0%	99.6%	2.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲
LFX	gyrB_p.Ala403Ser	41	9598	1	2719	0.0%	99.6%	2.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Silent mutations are not listed in this table but can be found in the catalogue master file.

Table 11. Abridged variant classification for MFX

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
MFX	gyrA_p.Asp94Gly	260	18275	1692	2310	42.3%	98.6%	86.7%	86.4%	88.0%	83.8%	50.4	1) AwR	ALL+WHO		1) Assoc w R	G	○
MFX	gyrA_p.Ala90Val	452	18083	720	3282	18.0%	97.6%	61.4%	56.7%	59.8%	51.1%	7.2	1) AwR	ALL+WHO		1) Assoc w R	F	○
MFX	gyrA_p.Asp94Asn	53	18482	296	3706	7.4%	99.7%	84.8%	83.5%	87.5%	78.7%	25.3	1) AwR	ALL+WHO		1) Assoc w R	G	○
MFX	gyrA_p.Asp94Ala	172	18363	261	3741	6.5%	99.1%	60.3%	52.5%	58.1%	44.0%	5.4	1) AwR	ALL+WHO		1) Assoc w R	F	○
MFX	gyrA_p.Ser91Pro	68	18467	175	3827	4.4%	99.6%	72.0%	72.1%	78.1%	62.2%	12.5	1) AwR	ALL+WHO		1) Assoc w R	F	○
MFX	gyrA_p.Asp94Tyr	36	18499	171	3831	4.3%	99.8%	82.6%	81.9%	87.1%	74.7%	21.9	1) AwR	ALL+WHO		1) Assoc w R	G	○
MFX	gyrA_p.Asp94His	14	18521	70	3932	1.7%	99.9%	83.3%	85.9%	92.7%	72.7%	28.7	1) AwR	ALL+WHO		1) Assoc w R	G	○
MFX	gyrB_p.Glu501Asp	10	18525	61	3941	1.5%	99.9%	85.9%	79.1%	90.0%	62.2%	17.8	1) AwR	ALL+WHO		1) Assoc w R	F	○
MFX	gyrA_p.Gly88Cys	5	18530	33	3969	0.8%	100.0%	86.8%	85.7%	95.2%	69.7%	28.0	1) AwR	ALL+WHO		1) Assoc w R	G	○
MFX	gyrA_p.Asp89Asn	6	18529	20	3982	0.5%	100.0%	76.9%	76.9%	91.0%	56.4%	15.5	1) AwR	ALL+WHO		1) Assoc w R	F	▲
MFX	gyrB_p.Asn499Thr	15	18520	23	3979	0.6%	99.9%	60.5%	25.0%	52.4%	6.1%	1.6	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim	F	▲
MFX	gyrB_p.Ala504Val	10	18525	14	3988	0.3%	99.9%	58.3%	28.6%	71.0%	2.1%	1.9	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim	F	○
MFX	gyrB_p.Asn499Asp	5	18530	12	3990	0.3%	100.0%	70.6%	81.8%	97.7%	35.1%	20.9	1) AwR	ALL	ALL only	2) Assoc w R - Interim	F	○
MFX	gyrB_p.Asp461Asn	24	18511	12	3990	0.3%	99.9%	33.3%	12.0%	31.2%	2.4%	0.6	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim	F	○
MFX	gyrA_p.Gly88Ala	13	18522	11	3991	0.3%	99.9%	45.8%	41.2%	67.1%	15.4%	3.2	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim	F	○
MFX	gyrB_p.Ser447Phe	20	18515	7	3995	0.2%	99.9%	25.9%	13.6%	34.9%	2.8%	0.7	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim	F	▲
MFX	gyrB_p.Asp461His	4	18531	3	3999	0.1%	100.0%	42.9%	25.0%	80.6%	0.5%	1.5	3) Uncertain	ALL+WHO	FQ X-R	2) Assoc w R - Interim	F	▲
MFX	gyrB_p.Glu501Val	1	18534	0	4002	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	F	○
MFX	gyrA_p.Thr80Ala	17	6623	1	1741	0.1%	99.7%	5.6%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28137812)	4) Not assoc w R - Interim		○
MFX	gyrA_p.Ala90Gly	2	6638	0	1742	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28137812)	4) Not assoc w R - Interim		○
MFX	gyrA_p.Glu21Gln	6570	70	1737	5	99.7%	1.1%	20.9%	1.8%	3.4%	0.1%	0.3	5) NotAwR	WHO		5) Not assoc w R		○
MFX	gyrA_p.Gly668Asp	5921	719	1654	88	94.9%	10.8%	21.8%	0.0%	33.6%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
MFX	gyrA_p.Ser95Thr	5987	653	1598	144	91.7%	9.8%	21.1%	0.0%	26.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
MFX	Rv1129c_c.-28T>C	3897	2743	1399	343	80.3%	41.3%	26.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
MFX	gyrA_p.Gly247Ser	404	6236	59	1683	3.4%	93.9%	12.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
MFX	glpK_p.Val460Ala	762	5878	34	1708	2.0%	88.5%	4.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
MFX	gyrB_p.Met291Ile	720	5920	34	1708	2.0%	89.2%	4.5%	0.0%	84.2%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
MFX	gyrA_p.Ala384Val	734	5906	32	1710	1.8%	88.9%	4.2%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
MFX	gyrA_p.Ala463Ser	46	6594	25	1717	1.4%	99.3%	35.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
MFX	gyrA_c.-34C>T	171	6469	13	1729	0.7%	97.4%	7.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
MFX	Rv1129c_c.-48A>C	99	6541	8	1734	0.5%	98.5%	7.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
MFX	gyrB_p.Val301Leu	94	6546	8	1734	0.5%	98.6%	7.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
MFX	Rv2752c_p.Gly161Ser	49	6591	3	1739	0.2%	99.3%	5.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
MFX	gyrB_p.Pro94Leu	206	6434	3	1739	0.2%	96.9%	1.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
MFX	gyrA_p.Gln613Glu	87	6553	2	1740	0.1%	98.7%	2.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲

Silent mutations are not listed in this table but can be found in the catalogue master file.

[F] Low-level resistance (multiple, genetically linked low-level resistance mutations are additive and confer high-level resistance).

[G] High-level resistance.

3.7 Bedaquiline and clofazimine

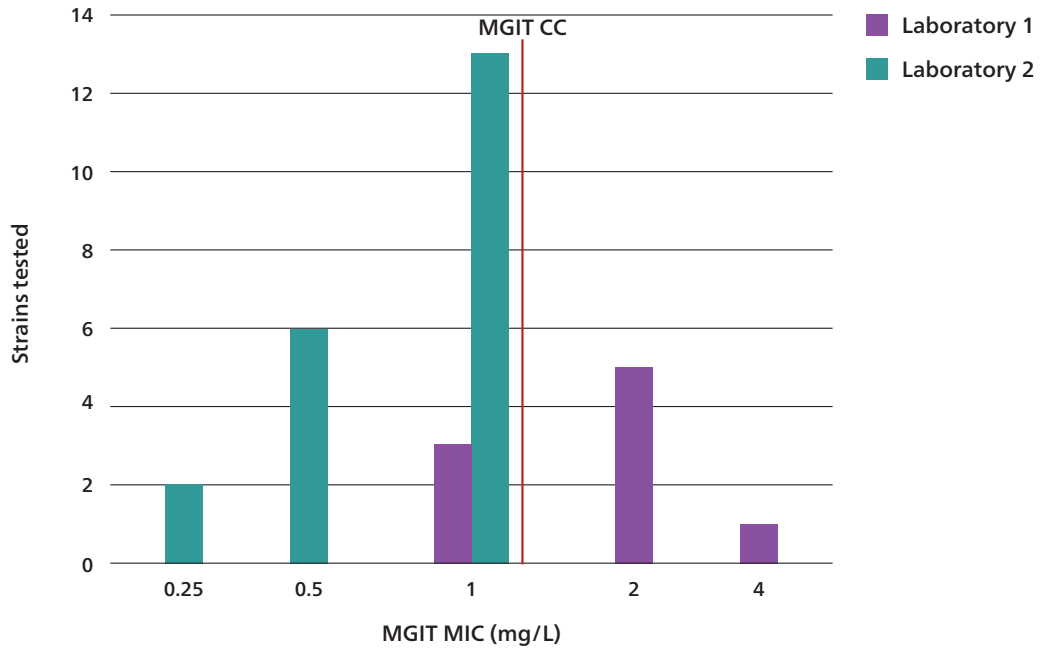
In the first edition, no mutations met Group 1 or 2 grading criteria for predicting BDQ or CFZ resistance. In the second edition, because of the addition of substantial new data, mutations in *Rv0678*, *atpE* and *pepQ* met criteria for Group 1 or 2 grading (Table 12), for a combined sensitivity of 49.4% (95% CI: 46.3–52.5), a specificity of 98.7% (95% CI: 98.5–98.9) and a PPV of 75.2% (95% CI: 71.8–78.4) (Table 3). Mutations with an allele frequency below 75% were found to play a major role in predicting BDQ phenotypes, as their inclusion increased the combined sensitivity of Groups 1 and 2 mutations by 10.2% to 59.6% (95% CI: 56.5–62.6) (Table A.1). Six *atpE* mutations (*atpE* Asp28Ala, Asp28Gly, Asp28Val, Glu61Asp, Ile66Met and Ala63Pro) were classified as Group 2 resistance mutations. Notably, two of these mutations (Ala63Pro and Ile66Met) occurred as SOLO mutations at sufficient frequency to meet thresholds for association with phenotypic resistance, indicating that *atpE* mutations can arise independently of *Rv0678* mutations. The remaining four *atpE* mutations met criteria based on in-vitro selection experiments, which also supported our additional grading rule that any LoF mutation in *Rv0678* and *pepQ* was assumed to confer BDQ resistance (Tables 1 and 23) *(67–74)*.

It should be noted that the contributing laboratory that supplied most of the BDQ pDST results for the WHO data set (referred to as laboratory 1 below) contributed only categorical results for isolates that were resistant on pDST at two independent laboratories (isolates that tested pDST resistant to BDQ in peripheral laboratories were referred to a reference laboratory for confirmatory testing at the WHO CC and were sequenced and contributed to the catalogue only if the initial R result was confirmed at the reference laboratory). Therefore, it is possible that the PPV|SOLO of the mutations in these isolates was inflated, given that no BDQ susceptible were submitted from this collection. For example, the grading of mutation *Rv0678* Met146Thr, which is frequent in Eswatini *(29)* but considered to be rare elsewhere, was potentially affected by this isolate collection. This mutation was assigned an initial confidence grading in Group 1 on the basis of a PPV|SOLO of 100% (95% CI: 72–100) in the WHO data set. This was calculated from 12 categorical pDST BACTEC™ Mycobacterial Growth Indicator Tube™ 960 (MGIT) results in the collection that included only resistant isolates. This laboratory also determined that the MGIT MIC range for 9 of the 12 *Rv0678* Met146Thr mutants as 1–4 mg/L (see result from laboratory 1 in Fig. 5), which, while still mainly resistant, represents a lower PPV|SOLO of 67% (95% CI: 30–93) when calculated by MIC result. Because our rules for addressing pDST data prioritized categorical pDST results over MIC data (see Sections 5.4 and 5.5), however, these MIC results were not considered in the WHO data set. Given these results, we also examined the BDQ MICs for 21 isolates with the same SOLO *Rv0678* Met146Thr mutation, but from a different laboratory. These isolates showed MGIT MICs of 0.25–1 mg/L in the susceptible range (see laboratory 2 in Fig. 5) but were not considered in the WHO data set, as MIC results were analysed only for the ALL data set.

Because of potentially inflated PPVs for these isolates, the working group decided to downgrade all Group 1 BDQ mutations that met the Group 1 criteria only on the basis of results from laboratory 1 to Group 2, and a footnote ([c]) was added to the BDQ mutation table (Table 12) to identify all 18 mutations with potentially elevated PPVs. It should be emphasized, however, that only three *Rv0678* mutations (i.e. Cys46Arg, Ile67Ser, Met146Thr) were ultimately downgraded from Group 1 to Group 2 to mitigate potential PPV inflation. The remaining 15 mutations would have had a

final confidence grading of Group 2 even without the data from laboratory 1 because they were covered by the *Rv0678* or *pepQ* LoF "additional grading rule" (10 mutations), their classification was based on the ALL data set only (three mutations) or was supported by in-vitro selection data (*atpE* Ala63Pro and Ile66Met).

Fig. 5. BDQ MICs for MTBC isolates with *Rv0678* Met146Thr



Published evidence suggests that *Rv0678* mutations confer resistance only if the corresponding efflux pump is functional. It appears that *mmpL5* LoF mutations, which may be frequent in some settings, such as Lima, Peru *(75)*, can confer a hyper-susceptible phenotype to BDQ and CFZ *(76)*. We evaluated this in our data set by determining that the BDQ PPV for combined Groups 1 and 2 mutations was significantly higher in isolates with silent or only Group 4 or 5 mutations in *mmpL5* (i.e. the corresponding protein was functional) as compared with isolates with LoF mutations in *mmpL5* (75% [95% CI: 71–79] vs. 0% [95% CI: 0–16]). Consequently, for assays that interrogate only *Rv0678*, inclusion of a disclaimer should be considered, acknowledging the possibility that epistasis affects the predictive power of *Rv0678* mutations (Table 1). Definitive identification of those isolates affected by epistasis is difficult, given the requirement to phase the *Rv0678* mutation results with *mmpL5* LoF mutations (see Section 3.10 for a discussion of this point for AMK). When epistasis could be identified, it was corrected for in our calculations of the predictive performance of *Rv0678* mutations in the second edition (Section 2.4 and in the Annex). Because this data set did not feature any *mmpS5* LoF mutants with BDQ pDST results, the potential role of those mutations in epistasis could not be investigated *(75,77,78)*.

Only pooled *Rv0678* LoF mutations and two individual *Rv0678* mutations (Glu49fs and Gly121Arg) could be classified as CZF resistance mutations in this data set (Table 13). As *Rv0678* and *pepQ* mutations are known to confer cross-resistance to BDQ and CFZ in selection experiments from independent laboratories, however, the working group decided that any Group 1 or 2 BDQ mutation in these two genes should also be classified as a Group 2 mutation for CFZ (Table 1).

In accordance with these findings for BDQ, epistasis caused by *mmpL5* LoF was considered also relevant for CFZ and was corrected (Section 2.4 and the Annex). The sensitivity and PPV for CFZ were significantly lower than those for BDQ (Table 3). The greater technical variability of pDST for CFZ probably explains these differences, but other explanations should be explored (e.g. whether some CCs for CFZ are too high).

Potential role of rare variants

The assumption that rare *Rv0678* promoter and coding mutations that meet the same "relaxed" grading criteria used for *pncA* (Fig. 9) confer resistance increased the sensitivity for BDQ by a further 9.2% while decreasing the specificity by only 0.1% (Table A.3). If it is assumed that all the coding mutations observed (except silent and Groups 4 and 5 mutations) confer BDQ resistance in RIF-resistant isolates, the combined sensitivity is increased by 15.5% to 75.1% and the specificity decreased by 0.7% (Table A.3). Taken together, these analyses suggest that detection of heteroresistance down to at least 25% is important for predicting BDQ resistance, that there is probably a wide variety of rare resistance-conferring mutations *(13)* in existing target genes and that revision of grading rules will be more informative than including new gene targets in the next version of the catalogue.

Table 12. Abridged variant classification for BDQ

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
BDQ	Rv0678_LoF	134	12817	424	611	41.0%	99.0%	76.0%	79.5%	82.9%	71.3%	81.1	1) AwR	ALL+WHO		1) Assoc w R	E	
BDQ	Rv0678_p.Glu49fs	26	12925	142	893	13.7%	99.8%	84.5%	85.7%	90.7%	77.6%	86.8	1) AwR	ALL+WHO		1) Assoc w R	E	
BDQ	Rv0678_p.Asp47fs	25	12926	61	974	5.9%	99.8%	70.9%	71.1%	80.5%	59.3%	32.6	1) AwR	ALL+WHO		1) Assoc w R	E	
BDQ	Rv0678_p.Ile67fs	35	12916	52	983	5.0%	99.7%	59.8%	76.1%	85.7%	48.2%	41.9	1) AwR	ALL+WHO		1) Assoc w R	E	
BDQ	Rv0678_p.Gly121Arg	1	12950	9	1026	0.9%	100.0%	90.0%	90.0%	99.7%	55.5%	113.6	1) AwR	ALL+WHO		1) Assoc w R	E	
BDQ	Rv0678_p.Leu117Arg	4	12947	8	1027	0.8%	100.0%	66.7%	66.7%	90.1%	34.9%	25.2	1) AwR	ALL+WHO		1) Assoc w R	E	
BDQ	Rv0678_p.Met146Thr	0	1249	11	890	1.2%	100.0%	100.0%	100.0%	100.0%	71.5%	Inf	1) AwR	WHO	Pot. infl. PPV	2) Assoc w R - Interim	C, E, O	
BDQ	Rv0678_p.Ile67Ser	0	12951	12	1023	1.2%	100.0%	100.0%	100.0%	100.0%	73.5%	Inf	1) AwR	ALL+WHO	Pot. infl. PPV	2) Assoc w R - Interim	C, E, O	
BDQ	Rv0678_p.Cys46Arg	1	12950	9	1026	0.9%	100.0%	90.0%	90.0%	99.7%	55.5%	113.6	1) AwR	ALL+WHO	Pot. infl. PPV	2) Assoc w R - Interim	C, E, O	
BDQ	pepQ_LoF	4	12947	8	1027	0.8%	100.0%	66.7%	63.6%	89.1%	30.8%	22.1	1) AwR	ALL	ALL only	2) Assoc w R - Interim	C	
BDQ	atpE_p.Ala63Pro	0	12951	7	1028	0.7%	100.0%	100.0%	100.0%	100.0%	47.8%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim	C	
BDQ	atpE_p.Ile66Met	0	12951	7	1028	0.7%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim	C	
BDQ	Rv0678_p.Ala36Val	0	12951	6	1029	0.6%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim	C, E, O	
BDQ	Rv0678_p.Asn70Asp	1	12950	6	1029	0.6%	100.0%	85.7%	85.7%	99.6%	42.1%	75.5	1) AwR	ALL	ALL only	2) Assoc w R - Interim	C, E, O	
BDQ	Rv0678_p.Leu32Ser	1	12950	5	1030	0.5%	100.0%	83.3%	83.3%	99.6%	35.9%	62.9	1) AwR	ALL	ALL only	2) Assoc w R - Interim	C, E, O	
BDQ	atpE_p.Glu61Asp	3	12948	2	1033	0.2%	100.0%	40.0%	50.0%	93.2%	5.3%	12.5	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim		
BDQ	atpE_p.Asp28Ala	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Selection	2) Assoc w R - Interim		
BDQ	atpE_p.Asp28Gly	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Selection	2) Assoc w R - Interim		
BDQ	atpE_p.Asp28Val	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Selection	2) Assoc w R - Interim		
BDQ	mmpL5_LoF	247	12704	0	1035	0.0%	98.1%	0.0%	0.0%	1.7%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	D	
BDQ	mmpL5_p.Ile948Val	1245	4	895	6	99.3%	0.3%	41.8%	7.7%	10.6%	2.0%	0.1	5) NotAwR	WHO	Lit. (PMID 28031270; 34503982)	4) Not assoc w R - Interim		
BDQ	Rv1979c_c.-129A>G	1238	11	893	8	99.1%	0.9%	41.9%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28031270; 34503982)	4) Not assoc w R - Interim		
BDQ	mmpL5_p.Thr794Ile	760	489	635	266	70.5%	39.2%	45.5%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28031270; 34503982)	4) Not assoc w R - Interim		
BDQ	Rv1979c_p.Asp286Gly	48	1201	71	830	7.9%	96.2%	59.7%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28031270; 34503982)	4) Not assoc w R - Interim		
BDQ	mmpS5_c.-74G>T	3	1246	36	865	4.0%	99.8%	92.3%	NA	NA	0.0%	NA	5) NotAwR	WHO	Lit. (PMID 28031270; 34503982)	4) Not assoc w R - Interim		
BDQ	mmpL5_p.Asp767Asn	509	740	146	755	16.2%	59.2%	22.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
BDQ	Rv0678_p.Leu142Arg	2	12949	7	1028	0.7%	100.0%	77.8%	50.0%	93.2%	6.8%	12.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Gly41Asp	0	12951	4	1031	0.4%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Ser53Pro	0	12951	4	1031	0.4%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Thr91Ile	0	12951	4	1031	0.4%	100.0%	100.0%	100.0%	100.0%	39.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Arg94Trp	1	12950	3	1032	0.3%	100.0%	75.0%	75.0%	99.4%	19.4%	37.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Asp47dup	0	12951	3	1032	0.3%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Gly65Glu	0	12951	3	1032	0.3%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Leu60Pro	0	12951	3	1032	0.3%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Pro48Leu	1	12950	3	1032	0.3%	100.0%	75.0%	75.0%	99.4%	19.4%	37.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Ser2Ile	1	12950	3	1032	0.3%	100.0%	75.0%	75.0%	99.4%	19.4%	37.6	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Ser63Gly	0	12951	3	1032	0.3%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Ala102Thr	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Ala99Pro	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Arg107Cys	1	12950	2	1033	0.2%	100.0%	66.7%	66.7%	99.2%	9.4%	25.1	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Arg109Pro	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Arg34Gln	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Arg34Trp	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Arg50Gln	1	12950	2	1033	0.2%	100.0%	66.7%	66.7%	99.2%	9.4%	25.1	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Asn70Ile	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Cys46Trp	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Gln115Pro	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Gly78Arg	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Ile67Leu	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Leu122Pro	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Leu35Trp	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Leu40Phe	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Leu60Gln	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Leu95Ser	1	12950	2	1033	0.2%	100.0%	66.7%	66.7%	99.2%	9.4%	25.1	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Met139Ile	1	12950	2	1033	0.2%	100.0%	66.7%	66.7%	99.2%	9.4%	25.1	3) Uncertain	ALL+WHO		3) Uncertain significance	0	
BDQ	Rv0678_p.Phe79Leu	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	0	

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
BDQ	Rv0678_p.Phe93Leu	1	12950	2	1033	0.2%	100.0%	66.7%	66.7%	99.2%	9.4%	25.1	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
BDQ	Rv0678_p.Ser151Pro	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
BDQ	Rv0678_p.Ser52Phe	0	12951	2	1033	0.2%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
BDQ	Rv0678_p.Ser63Arg	1	12950	2	1033	0.2%	100.0%	66.7%	66.7%	99.2%	9.4%	25.1	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
BDQ	Rv0678_p.Tyr157Ser	1	12950	2	1033	0.2%	100.0%	66.7%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○

Individual LoF mutations in the coding regions of *pepQ* and *Rv0678* that are classified in Group 2 because of the associated additional grading rule (see Table 23 and Section 5.8) and silent mutations are not listed in this table but can be found in the catalogue master file.

[C] Includes data from one site that only submitted resistant strains, which may have inflated the PPV.

[D] Abrogates effect of genetically linked Groups 1 and 2 *Rv0678* mutations.

[E] Can confer resistance only if genetically linked to a functional MmpL5.

[O] Group 2 by "relaxed" threshold (not endorsed).

Table 13. Abridged variant classification for CFZ

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
CFZ	Rv0678_LoF	68	4666	63	151	29.4%	98.6%	48.1%	46.6%	56.0%	35.3%	26.9	1) AwR	WHO		1) Assoc w R	E	●
CFZ	Rv0678_p.Glu49fs	30	14013	29	645	4.3%	99.8%	49.2%	50.0%	66.2%	26.4%	21.7	1) AwR	ALL+WHO		1) Assoc w R	E	▲
CFZ	Rv0678_p.Gly121Arg	1	14042	4	670	0.6%	100.0%	80.0%	80.0%	99.5%	28.4%	83.8	1) AwR	ALL	ALL only	2) Assoc w R - Interim	E	●
CFZ	Rv0678_p.Leu117Arg	9	14034	4	670	0.6%	99.9%	30.8%	30.8%	61.4%	9.1%	9.3	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	▲
CFZ	Rv0678_p.Cys46Arg	0	14043	3	671	0.4%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	●
CFZ	pepQ_LoF	3	14040	3	671	0.4%	100.0%	50.0%	33.3%	90.6%	0.6%	10.5	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim		●
CFZ	Rv0678_p.Ala36Val	1	14042	3	671	0.4%	100.0%	75.0%	100.0%	100.0%	9.4%	Inf	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	▲
CFZ	Rv0678_p.Asn70Asp	0	14043	2	672	0.3%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	●
CFZ	Rv0678_p.Met146Thr	36	14007	2	672	0.3%	99.7%	5.3%	5.4%	18.2%	0.6%	1.2	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	▲
CFZ	Rv0678_p.Leu32Ser	3	14040	1	673	0.1%	100.0%	25.0%	25.0%	80.6%	0.6%	7.0	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	▲
CFZ	Rv0678_p.Ile67Ser	1	14042	0	674	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	BDQ-CFZ X-R	2) Assoc w R - Interim	E	▲
CFZ	mmpL5_LoF	242	13801	7	667	1.0%	98.3%	2.8%	3.0%	6.4%	0.9%	0.6	3) Uncertain	ALL+WHO		3) Uncertain significance	D	○
CFZ	mmpL5_p.Ile948Val	4719	15	209	5	97.7%	0.3%	4.2%	0.0%	84.2%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	Rv1979c_c.-129A>G	4713	21	207	7	96.7%	0.4%	4.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	mmpL5_p.Thr794Ile	3782	952	179	35	83.6%	20.1%	4.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	mmpL5_p.Asp767Asn	1559	3175	49	165	22.9%	67.1%	3.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	fgd1_p.Val170Met	518	4216	10	204	4.7%	89.1%	1.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
CFZ	Rv1979c_p.Asp286Gly	665	4069	6	208	2.8%	86.0%	0.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	mmpL5_p.Phe696Leu	227	4507	4	210	1.9%	95.2%	1.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	fgd1_p.Lys270Met	108	4626	1	213	0.5%	97.7%	0.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
CFZ	Rv1979c_c.-389C>A	249	4485	1	213	0.5%	94.7%	0.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
CFZ	fbiA_p.Thr302Met	39	4695	0	214	0.0%	99.2%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
CFZ	Rv1979c_c.-327C>A	42	4692	0	214	0.0%	99.1%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		▲
CFZ	Rv0678_p.Ser53Pro	0	14043	4	670	0.6%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Ala102Thr	0	14043	2	672	0.3%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Arg50Gln	0	14043	2	672	0.3%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Leu142Arg	0	14043	2	672	0.3%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Leu35Trp	0	14043	2	672	0.3%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Ser151Pro	0	14043	2	672	0.3%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Ala99Pro	0	14043	1	673	0.1%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
CFZ	Rv0678_p.Asn70Ile	1	14042	1	673	0.1%	100.0%	50.0%	50.0%	98.7%	1.3%	20.9	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Leu40Phe	0	14043	1	673	0.1%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Pro48Leu	2	14041	1	673	0.1%	100.0%	33.3%	33.3%	90.6%	0.8%	10.4	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Ser52Phe	0	14043	1	673	0.1%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Tyr157Ser	0	14043	1	673	0.1%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Arg107Cys	1	14042	0	674	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Arg94Trp	1	14042	0	674	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Ile67Leu	1	14042	0	674	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Leu60Gln	1	14042	0	674	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Leu95Ser	2	14041	0	674	0.0%	100.0%	0.0%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Met139Ile	2	14041	0	674	0.0%	100.0%	0.0%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Phe93Leu	2	14041	0	674	0.0%	100.0%	0.0%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○
CFZ	Rv0678_p.Ser2Ile	1	14042	0	674	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance	P	○

Individual LoF mutations in the coding regions of *pepQ* and *Rv0678* that are classified in Group 2 because of the associated additional grading rule (see Table 23 and Section 5.8) and silent mutations are not listed in this table but can be found in the catalogue master file.

[D] Abrogates effect of genetically linked Groups 1 and 2 *Rv0678* mutations.

[E] Can only confer resistance if genetically linked to a functional MmpL5.

[P] Group 2 by "relaxed" threshold (not endorsed) and BDQ cross-resistance.

3.8 Linezolid

The *rplC* Cys154Arg mutation was the only marker of resistance in the first edition and remained the only Group 1 mutation with an associated sensitivity of 27.3% (95% CI: 22.8–32.1) (Table 3). The seven newly endorsed Group 2 mutations (Table 14) were in *rrl* and increased the combined sensitivity by 6.7% (95% CI: 4.4–9.7) (Table A.1). Six of the *rrl* mutations, three of which were not found in our data set (2270G>C, 2689A>T and 2746G>A), met the criteria as interim resistance markers according to the new in-vitro selection grading rule based on data from at least two independent laboratories *(79–84)*.[2] The combined sensitivity of all Groups 1 and 2 mutations was, however, still only 34.0% (95% CI: 29.2–39.0). Sensitivity increased by only 1.0% when variant allele frequency variants ≥ 25% were considered (Table A.1). The observed sensitivity is probably an underestimate, as the PPV of the pDST reference standard itself is likely to be low, because the estimated overall prevalence of LZD resistance is only 2.1% (95% CI: 1.9–2.3), rising to 3.0% (95% CI: 2.7–3.4) among genotypically RIF-resistant isolates (Table A.2) *(30)*. Alternatively, there may be unknown resistance mechanisms and novel gene targets for which we did not account in this analysis.

[2] Lee J, personal communication, 2023; Takaki A, Mitarai S, personal communication, 2023; Andres S, personal communication, 2023.

Table 14. Abridged variant classification for LZD

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
LZD	rplC_p.Cys154Arg	24	17415	102	271	27.3%	99.9%	81.0%	81.0%	89.0%	62.2%	274.2	1) AwR	ALL+WHO		1) Assoc w R		○
LZD	rrl_n.2814G>T	4	17435	19	354	5.1%	100.0%	82.6%	77.8%	97.2%	30.8%	172.4	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
LZD	rrl_n.2270G>T	4	17435	13	360	3.5%	100.0%	76.5%	66.7%	95.7%	15.7%	96.9	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim		▲
LZD	rrl_n.2269_2270insT	1	17438	1	372	0.3%	100.0%	50.0%	50.0%	98.7%	1.3%	46.9	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim		●
LZD	rrl_n.2299G>T	0	17439	1	372	0.3%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim		●
LZD	rrl_n.2270G>C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Selection	2) Assoc w R - Interim		●
LZD	rrl_n.2689A>T	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Selection	2) Assoc w R - Interim		●
LZD	rrl_n.2746G>A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Selection	2) Assoc w R - Interim		●
LZD	tsnR_p.Leu232Pro	6238	386	120	17	87.6%	5.8%	1.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
LZD	rplC_c.-452C>A	731	5893	3	134	2.2%	89.0%	0.4%	0.2%	1.1%	0.0%	0.1	5) NotAwR	WHO		5) Not assoc w R		▲
LZD	rrl_n.344C>T	225	6399	3	134	2.2%	96.6%	1.3%	0.9%	3.3%	0.1%	0.4	5) NotAwR	WHO		5) Not assoc w R		○
LZD	tsnR_p.Tyr147Cys	392	6232	2	135	1.5%	94.1%	0.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●

Silent mutations are not listed in this table but can be found in the catalogue master file.

3.9 Delamanid and pretomanid

The *ddn* Leu49Pro amino acid change was the only mutation that was sufficiently frequent in the first edition to be classified as a Group 2 mutation for DLM resistance, which remained the case for the second edition (Table 15). In contrast, pooled *ddn* LoF mutations (Table 23) met the criteria in the ALL data set for Group 2. Moreover, LoF mutations in the remaining five resistance genes considered to be associated with DLM resistance (*fbiA*, *fbiB*, *fbiC*, *fgd1* and *Rv2983*) were recognized as Group 2 mutations for DLM on the basis of laboratory selection, complementation and knockdown experiments conducted for DLM and/or PMD in at least two independent laboratories *(84–87)*. Despite addition of these new grading rules, the sensitivity of all Group 2 mutations for the second edition increased to only 14.7% (95% CI: 10.6–19.7) as compared with 4.4% (95% CI: 2.2–7.7) in the first edition (Table A.1). Heteroresistance did not appear to play a large role, as the sensitivity increased by only 0.4% when the cut-off for calling variants was reduced from an allele frequency of ≥ 75% to ≥ 25% (Table A.1).

WHO has not yet set a CC for PMD because of the different intrinsic susceptibility of different MTBC lineages; however, the issue should be settled in 2023 *(17,18)*. Standard analysis for PMD was therefore not possible for the second edition. Irrespective of whether lineage 1 isolates (which have intrinsically higher PMD MICs than lineages 2, 3, 4 and 7) should be considered susceptible or not *(17)*, there was sufficient evidence from several laboratories that LoF mutations in *ddn*, *fbiA*, *fbiB*, *fbiC*, *fgd1* and *Rv2983* confer cross-resistance to DLM and PMD and should, therefore, be interpreted as Group 2 interim resistance mutations for both DLM and PMD (Tables 1 and 23) *(84–87)*. LoF mutations have also been described in at least some of these genes in clinical isolates that have not been exposed to nitroimidazoles, indicating that intrinsic resistance to DLM and PMD is possible, although this appears to be rare globally *(17,88,89)*. As the relative increase in MIC for each drug differs for the six genes, and at least some mutations do not appear to affect the MIC for both agents, a general cross-resistance rule was not used for the second edition *(86,90)*. This question will be reviewed for the next version, once a full genotype/phenotype analysis is possible for PMD with a WHO-endorsed CC.

Potential role of rare variants

Application of "relaxed" thresholds to upstream and coding mutations in all tier-1 target genes for DLM increased the combined sensitivity for predicting DLM resistance by only 2.8%, without decreasing specificity, and increased PPV by 3.9% (Table A.3). The assumption that all Group 3 non-silent coding variants in the six tier-1 genes conferred resistance if they occurred in RIF-resistant isolates increased the combined sensitivity by 9.9% but reduced the specificity by 8.2% and the PPV by 56.2% (Table A.3). These results suggested the presence of some additional resistance mutations in tier 1 resistance genes but that they account for only a small portion of the reduced sensitivity and are less frequent than as-yet-unclassified neutral mutations in these genes. Most of the decrease in sensitivity as compared with pDST, which is similar among isolates that are susceptible rather than resistant to RIF, could be due either to unknown resistance genes or to random false-resistant pDST results, which may be

more frequent than the true prevalence of resistance for DLM. The latter possibility is plausible, as the approximate prevalence of resistance was only 2% by pDST, irrespective of whether the isolates were susceptible or resistant to RIF (Table A.2), probably resulting in an underestimate of the true sensitivity of gDST (as for LZD) *(30)*.

Table 15. Abridged variant classification for DLM

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
DLM	ddn_LoF	5	11389	19	230	7.6%	100.0%	79.2%	69.2%	90.9%	35.1%	111.4	1) AwR	ALL	ALL only	2) Assoc w R - Interim	R	●
DLM	ddn_p.Leu49Pro	2	11392	11	238	4.4%	100.0%	84.6%	100.0%	100.0%	54.6%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
DLM	fbiC_LoF	4	11390	3	246	1.2%	100.0%	42.9%	0.0%	60.2%	0.0%	0.0	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim	R	●
DLM	fbiA_LoF	1	11393	2	247	0.8%	100.0%	66.7%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim	R	●
DLM	fgd1_LoF	0	11394	2	247	0.8%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim	R	●
DLM	fbiB_LoF	1	11393	0	249	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim	R	●
DLM	Rv2983_LoF	1	11393	0	249	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO	Selection	2) Assoc w R - Interim	R	●
DLM	ddn_p.Tyr122_Met129del	0	11394	3	246	1.2%	100.0%	100.0%	100.0%	100.0%	29.2%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
DLM	ddn_p.Tyr29del	0	11394	2	247	0.8%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○
DLM	fbiA_p.Arg321Ser	0	11394	2	247	0.8%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance	O	○

Individual LoF mutations in the coding regions of *ddn*, *fbiA, fbiB, fbiC, fgd1* and *Rv2983*, which are classified in Group 2 because of the associated additional grading rule (see Table 23 and Section 5.8), and silent mutations are not listed in this table but can be found in the catalogue master file.

[R] Confer DLM-PMD cross-resistance.

[O] Group 2 by "relaxed" threshold (not endorsed).

3.10 Amikacin

In contrast to the first edition, *eis* -14C>T had a PPV|SOLO with a lower 95% CI bound of 20% in the ALL data set (see Table 17) and 19% in the WHO data set, which was below the 25% threshold required for Group 1 or 2 grading (Fig. 8). Previous allelic exchange experiments with this mutation, however, clearly showed a modest effect on AMK resistance *(15)*. To err on the side of caution and to remain consistent with other WHO-endorsed assays (Cepheid Xpert® MTB/XDR and Hain GenoType MTBDR*sl* VER 2.0 *(19, 39)*), it was decided to continue to classify *eis* -14C>T and *rrs* 1402C>T, which also did not meet the PPV|SOLO threshold, in Group 2 (see Table 17). Consequently, the same four mutations were considered resistance mutations in both catalogues.

The potential effect of epistasis was evaluated formally *(75)*. The AMK PPV for *eis* -14C>T was significantly higher when the *eis* coding region had only silent mutations rather than LoF mutations (Table 16). As expected, the difference in the equivalent KAN PPVs for *eis* -14C>T were more marked *(16)*. We found only a single isolate with one of the remaining four *eis* KAN resistance mutations and an *eis* LoF mutation, although there were 1 212 such *eis* mutants and only 293 *eis* -14C>T mutants (Table 16). It has been hypothesized that overrepresentation of LoF mutations that have evolved repeatedly after acquisition of *eis* -14C>T might be due to the higher fitness cost of this promoter mutation, which results in the greatest over-expression of *eis (75)*. Epistasis was, therefore, recognized for *eis* -14C>T in both AMK and KAN (Table 1). Assays to recognize this *eis* promoter mutation should include a disclaimer that this mutation could over-call resistance to AMK (KAN is no longer recommended for clinical use). Assays to recognize the *eis* promoter and coding region should, if possible, be refined to recognize samples that are potentially affected by epistasis (e.g. when all *eis* -14C>T reads are linked to an LoF mutation and no other AMK resistance mutation is present in *rrs*). This is difficult in practice (e.g. when sequencing reads are too short for phasing multiple *eis* mutations). In the absence of pDST results that prove definitively that an *eis* -14C>T mutant is susceptible to AMK because of epistasis, it is advisable to interpret *eis* -14C>T routinely as AMK resistant.

Correction for epistasis in this data set decreased the sensitivity by < 0.05% and increased the specificity by 0.2% and the PPV by 1.5% (Table A.1), illustrating the minor overall effect, as other mutations confer most of the AMK resistance. Thus, as the AMK resistance mutations did not differ between both catalogues, the slight difference in performance was due to epistasis. As expected, the corresponding effects of epistasis were more marked for KAN (Table A.1) *(16)*. Low-frequency resistance mutations increased the sensitivity to AMK by 2.6%, with negligible effects on the specificity and PPV (Table A.1).

Table 16. Impact of LoF mutations on PPV of *eis* mutations

Drug	*eis* mutation[a]	PPV (%, [95% CI]), n		
		With *eis* silent mutations only[b]	With *eis* LoF mutation[c]	Total *eis* mutants
AMK	-14C>T	34 (28–40), 241	2 (0–13), 41	282
KAN[d]	-37G>T	82 (76–87), 209	na, 0	209
	-14C>T	88 (83–92), 259	18 (7–35), 34	293
	-12C>T	44 (40–48), 664	na, 0	664
	-10G>A	69 (64–74), 331	0 (0–98), 1	332
	-8delC	86 (42–100), 7	na, 0	7

[a] Promoter mutation at a frequency of at least 90% with no other Group 1 or 2 mutation for the relevant agent at any frequency.

[b] No coding mutation at any frequency, except silent or Group 4 or 5 mutations.

[c] LoF mutation at a frequency of at least 90% (Table 23).

[d] Unlike for AMK (Table 17), an abridged variant table is not included in this report for KAN, as this drug is no longer recommended for TB treatment. The full list of KAN classifications can be found in the catalogue master file.

Table 17. Abridged variant classification for AMK

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
AMK	rrs_n.1401A>G	147	21831	1682	778	68.4%	99.3%	92.0%	92.6%	93.8%	89.8%	351.0	1) AwR	ALL+WHO		1) Assoc w R		○
AMK	rrs_n.1484G>T	5	21973	17	2443	0.7%	100.0%	77.3%	70.6%	89.7%	44.0%	21.6	1) AwR	ALL+WHO		1) Assoc w R		▲
AMK	eis_c.-14C>T	213	21765	90	2370	3.7%	99.0%	29.7%	36.0%	43.1%	20.3%	5.2	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	A	▬
AMK	rrs_n.1402C>T	14	21964	9	2451	0.4%	99.9%	39.1%	33.3%	59.0%	11.9%	4.5	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim		○
AMK	eis_LoF	240	21738	10	2450	0.4%	98.9%	4.0%	2.2%	5.4%	0.4%	0.2	3) Uncertain	ALL+WHO		3) Uncertain significance	B	○
AMK	whiB7_c.-178C>T	18	7755	0	1111	0.0%	99.8%	0.0%	0.0%	41.0%	0.0%	0.0	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		▬
AMK	whiB6_c.-75delG	7518	255	1092	19	98.3%	3.3%	12.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	ccsA_p.Ile245Met	3180	4593	617	494	55.5%	59.1%	16.2%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
AMK	whiB6_p.Thr51Pro	655	7118	216	895	19.4%	91.6%	24.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
AMK	rrs_n.514A>C	241	7532	99	1012	8.9%	96.9%	29.1%	0.6%	3.6%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		○
AMK	bacA_p.Ile273Thr	751	7022	68	1043	6.1%	90.3%	8.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	eis_c.-12C>T	620	7153	44	1067	4.0%	92.0%	6.6%	2.6%	4.4%	1.1%	0.2	5) NotAwR	WHO		5) Not assoc w R		○
AMK	rrs_n.517C>T	269	7504	43	1068	3.9%	96.5%	13.8%	2.9%	8.4%	0.2%	0.2	5) NotAwR	WHO		5) Not assoc w R		○
AMK	ccsA_p.Val27Ile	445	7328	39	1072	3.5%	94.3%	8.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
AMK	eis_c.-10G>A	278	7495	15	1096	1.4%	96.4%	5.1%	4.5%	7.9%	1.9%	0.3	5) NotAwR	WHO		5) Not assoc w R		▲
AMK	bacA_p.Ile603Val	219	7554	8	1103	0.7%	97.2%	3.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	whiB6_c.-82C>T	197	7576	8	1103	0.7%	97.5%	3.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
AMK	eis_p.Val163Ile	126	7647	7	1104	0.6%	98.4%	5.3%	0.9%	5.0%	0.0%	0.1	5) NotAwR	WHO		5) Not assoc w R		○
AMK	rrs_n.492C>T	229	7544	3	1108	0.3%	97.1%	1.3%	1.4%	4.0%	0.3%	0.1	5) NotAwR	WHO		5) Not assoc w R		○
AMK	whiB6_c.-75_-73delGCTinsCC	52	7721	2	1109	0.2%	99.3%	3.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	whiB6_c.-75_-73delGCTinsCG	48	7725	2	1109	0.2%	99.4%	4.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	whiB6_p.Arg107Cys	68	7705	2	1109	0.2%	99.1%	2.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		○
AMK	rrs_n.1050C>T	32	7741	1	1110	0.1%	99.6%	3.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.1208T>A	12	7761	1	1110	0.1%	99.8%	7.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.1217T>A	15	7758	1	1110	0.1%	99.8%	6.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.1223A>G	16	7757	1	1110	0.1%	99.8%	5.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.1507C>T	10	7763	1	1110	0.1%	99.9%	9.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.292G>A	11	7762	1	1110	0.1%	99.9%	8.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.533G>T	7	7766	1	1110	0.1%	99.9%	12.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●
AMK	rrs_n.534T>G	8	7765	1	1110	0.1%	99.9%	11.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		●

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
AMK	rrs_n.537G>A	8	7765	1	1110	0.1%	99.9%	11.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.555C>T	21	7752	1	1110	0.1%	99.7%	4.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.685G>A	20	7753	1	1110	0.1%	99.7%	4.8%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.726G>C	18	7755	1	1110	0.1%	99.8%	5.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.868T>C	16	7757	1	1110	0.1%	99.8%	5.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.871C>T	15	7758	1	1110	0.1%	99.8%	6.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.899A>G	22	7751	1	1110	0.1%	99.7%	4.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.936C>T	27	7746	1	1110	0.1%	99.7%	3.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.948A>T	26	7747	1	1110	0.1%	99.7%	3.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.958T>A	24	7749	1	1110	0.1%	99.7%	4.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1190G>A	33	7740	1	1110	0.1%	99.6%	2.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1211A>T	19	7754	1	1110	0.1%	99.8%	5.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.305T>A	21	7752	1	1110	0.1%	99.7%	4.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.306C>T	10	7763	1	1110	0.1%	99.9%	9.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.327T>C	33	7740	1	1110	0.1%	99.6%	2.9%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.358G>A	12	7761	1	1110	0.1%	99.8%	7.7%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.736A>T	17	7756	1	1110	0.1%	99.8%	5.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1277T>A	7	7766	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1302G>C	8	7765	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1319C>G	8	7765	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1327T>C	7	7766	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1332G>A	12	7761	0	1111	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1347A>G	12	7761	0	1111	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1407T>C	7	7766	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.261G>A	7	7766	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.499C>T	18	7755	0	1111	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.51T>C	8	7765	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.534T>C	4	7769	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.672T>A	8	7765	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.816A>G	5	7768	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
AMK	rrs_n.852T>C	7	7766	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.889C>T	11	7762	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.896G>A	11	7762	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.979T>A	3	7770	0	1111	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.983T>C	3	7770	0	1111	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	whiB7_p.Gly64fs	48	7725	0	1111	0.0%	99.4%	0.0%	0.0%	9.3%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1145A>G	7	7766	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1276T>C	12	7761	0	1111	0.0%	99.8%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1328C>T	9	7764	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.1414C>T	4	7769	0	1111	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.406G>A	16	7757	0	1111	0.0%	99.8%	0.0%	0.0%	97.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
AMK	rrs_n.906A>G	34	7739	0	1111	0.0%	99.6%	0.0%	0.0%	11.9%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
AMK	whiB6_p.Arg54Gln	51	7722	0	1111	0.0%	99.3%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
AMK	whiB7_c.-100T>C	86	7687	0	1111	0.0%	98.9%	0.0%	0.0%	4.6%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		

Silent mutations are not listed in this table but can be found in the catalogue master file.

[A] Can confer resistance only if genetically linked to a functional Eis.

[B] Abrogates effect of genetically linked Groups 1 and 2 *eis* mutations.

3.11 Streptomycin

The combined sensitivity of the mutations from the second edition for predicting STM resistance increased by only 0.5%, to 79.7% (95% CI: 78.9–80.5) over that in the first edition; a further 0.6% was gained by including variants with an allele frequency ≥ 25% (Table A.1). The latter marginal gain was expected, as the evolution of STM resistance often predates INH resistance, so that most STM resistance is transmitted *(91)*. The *rrs* G878A mutation, first implicated in STM resistance in the first edition, remained an interim resistance mutation (Table 18), consistent with recently published experimental data from MTBC *(92)*. Application of the WHO-endorsed LoF additional grading rule for *gid* resulted in classification of 137 of the 144 Group 2 mutations (Tables 1 and 23), which increased the sensitivity by 7.6% (95% CI: 7.1–8.1) as compared with Group 1 mutations alone (Table 3). The PPV of only 58.8% (95% CI: 56.1–61.4) was probably due to the small increases in MIC conferred by LoF *gid* mutations. The current CCs therefore divide the resulting MIC distributions at their lower end *(55,56,93–95)*. As for EMB, it is not clear how well the currently used CCs correspond to the epidemiological cut-off values, which may exacerbate the very major pDST error rate for this resistance mechanism *(30)*.

Table 18. Abridged variant classification for STM

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Final confidence grading	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
STM	rpsL_p.Lys43Arg	100	15508	4715	5653	45.5%	99.4%	97.9%	97.7%	98.2%	96.5%	116.9	1) AwR	ALL+WHO		1) Assoc w R		○
STM	rpsL_p.Lys88Arg	29	15579	1280	9088	12.3%	99.8%	97.8%	97.8%	98.6%	95.9%	77.2	1) AwR	ALL+WHO		1) Assoc w R		○
STM	gid_LoF	577	15031	938	9430	9.0%	96.3%	61.9%	60.5%	63.7%	45.0%	2.4	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	rrs_n.517C>T	54	15554	600	9768	5.8%	99.7%	91.7%	90.3%	92.8%	86.7%	14.9	1) AwR	ALL+WHO		1) Assoc w R		○
STM	rrs_n.514A>C	18	15590	550	9818	5.3%	99.9%	96.8%	96.5%	98.2%	91.3%	44.0	1) AwR	ALL+WHO		1) Assoc w R		○
STM	gid_p.Arg39fs	69	15539	130	10238	1.3%	99.6%	65.3%	60.2%	68.5%	45.3%	2.3	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	gid_p.Gln125*	20	15588	87	10281	0.8%	99.9%	81.3%	71.1%	83.6%	47.0%	3.7	1) AwR	ALL+WHO		1) Assoc w R		○
STM	rpsL_p.Lys88Met	0	15608	31	10337	0.3%	100.0%	100.0%	100.0%	100.0%	85.2%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
STM	gid_p.Glu99*	4	15604	23	10345	0.2%	100.0%	85.2%	88.0%	97.5%	65.1%	11.1	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	gid_p.Ala134Glu	0	15608	22	10346	0.2%	100.0%	100.0%	100.0%	100.0%	83.9%	Inf	1) AwR	ALL+WHO		1) Assoc w R		○
STM	gid_p.Val105Glu	2	15606	21	10347	0.2%	100.0%	91.3%	90.0%	98.8%	68.3%	13.6	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	gid_p.His48Gln	5	15603	20	10348	0.2%	100.0%	80.0%	81.8%	94.8%	56.3%	6.8	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	gid_p.Ser70Asn	3	15605	17	10351	0.2%	100.0%	85.0%	91.7%	99.8%	49.2%	16.6	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	gid_p.Gly73Glu	2	15606	14	10354	0.1%	100.0%	87.5%	91.7%	99.8%	54.6%	16.6	1) AwR	ALL+WHO		1) Assoc w R		●
STM	gid_p.Ala200Glu	2	15606	13	10355	0.1%	100.0%	86.7%	92.3%	99.8%	57.2%	18.1	1) AwR	ALL+WHO		1) Assoc w R		▲
STM	gid_p.Pro84Leu	36	15572	56	10312	0.5%	99.8%	60.9%	59.3%	70.1%	45.9%	2.2	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
STM	rrs_n.878G>A	5	15603	50	10318	0.5%	100.0%	90.9%	86.8%	95.6%	71.9%	10.0	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▬
STM	gid_p.Gly73Ala	14	15594	33	10335	0.3%	99.9%	70.2%	70.3%	84.1%	48.3%	3.6	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
STM	rpsL_p.Lys88Gln	4	15604	15	10353	0.1%	100.0%	78.9%	66.7%	92.5%	26.2%	3.0	3) Uncertain	ALL+WHO	Prev. WHO	2) Assoc w R - Interim		○
STM	gid_p.Asp67His	1	15607	9	10359	0.1%	100.0%	90.0%	100.0%	100.0%	51.8%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
STM	gid_p.Gly69Asp	4	15604	13	10355	0.1%	100.0%	76.5%	71.4%	91.6%	41.9%	3.8	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Pro75Arg	2	15606	11	10357	0.1%	100.0%	84.6%	77.8%	97.2%	40.0%	5.3	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Arg83dup	0	15608	2	10366	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Asp67_Gly71del	0	15608	2	10366	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Ala119_Glu120insAspGluIleValArgGlyArgAla	0	15608	1	10367	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Gly30_Pro38del	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Ile4_Pro38del	1	15607	1	10367	0.0%	100.0%	50.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Ile55dup	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Leu152_Arg154dup	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
STM	gid_p.Leu44_Asp46delinsHis	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Final confidence grading	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
STM	gid_p.Pro14_Gly42del	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
STM	gid_p.Pro84_Arg137del	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
STM	gid_p.Ser9_Ala25del	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
STM	gid_p.Val89_Arg102delinsGly	0	15608	1	10367	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
STM	gid_p.Arg118_Ser149del	3	15605	0	10368	0.0%	100.0%	0.0%	0.0%	70.8%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
STM	gid_p.Arg83_Glu92del	1	15607	0	10368	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
STM	rrs_n.492C>T	381	11493	43	7693	0.6%	96.8%	10.1%	NA	NA	0.0%	NA	5) NotAwR	WHO	Prev. WHO	4) Not assoc w R - Interim		
STM	rpsL_c.-165T>C	11673	201	7623	113	98.5%	1.7%	39.5%	3.9%	4.5%	1.3%	0.1	5) NotAwR	WHO		5) Not assoc w R		
STM	gid_p.Glu92Asp	2620	9254	4202	3534	54.3%	77.9%	61.6%	37.5%	75.5%	0.0%	1.6	5) NotAwR	WHO		5) Not assoc w R		
STM	Rv1258c_p.Glu194fs	2467	9407	4033	3703	52.1%	79.2%	62.0%	20.0%	71.6%	0.0%	0.6	5) NotAwR	WHO		5) Not assoc w R		
STM	gid_p.Leu16Arg	1630	10244	679	7057	8.8%	86.3%	29.4%	0.0%	28.5%	0.0%	0.0	5) NotAwR	WHO		5) Not assoc w R		
STM	glpK_p.Val460Ala	1732	10142	331	7405	4.3%	85.4%	16.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	bacA_p.Ile603Val	1673	10201	326	7410	4.2%	85.9%	16.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	gid_p.Tyr195His	152	11722	117	7619	1.5%	98.7%	43.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	whiB7_p.Gly64fs	421	11453	44	7692	0.6%	96.5%	9.5%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	whiB7_c.-100T>C	101	11773	36	7700	0.5%	99.1%	26.3%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	whiB7_c.-242G>C	76	11798	9	7727	0.1%	99.4%	10.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	rpsL_c.-125G>C	60	11814	7	7729	0.1%	99.5%	10.4%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	rrs_n.1328C>T	8	11866	1	7735	0.0%	99.9%	11.1%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	Rv1258c_p.Gly363Val	38	11836	1	7735	0.0%	99.7%	2.6%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	rrs_n.1327T>C	9	11865	0	7736	0.0%	99.9%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		
STM	Rv2477c_p.Thr372Lys	38	11836	0	7736	0.0%	99.7%	0.0%	NA	NA	0.0%	NA	5) NotAwR	WHO		5) Not assoc w R		

Individual LoF mutations in the coding regions of *gid*, which are classified in Group 2 because of the associated additional grading rule (see Table 23 and Section 5.8), and silent mutations are not listed in this table but can be found in the catalogue master file.

3.12 Ethionamide and prothionamide

ETO and PTO were considered to be equivalent for DST and are reported as ETO in this report. Thus, all Groups 1–5 classifications for ETO also apply to PTO (see Section 5.4). There are numerous mechanisms of resistance to ETO (Table 19), some of which include a wide diversity of potential resistance mutations distributed across gene targets, as they are non-essential genes *(96,97)*. Five Group 1 and 281 Group 2 mutations yielded a combined sensitivity of 74.8% (95% CI: 73.6–76.0), a gain of 2.3% over that in the first edition (Table A.1), but with an associated specificity of only 85.9% (95% CI: 85.3–86.4) and a PPV of 63.9% (95% CI: 62.7–65.1) (Table 3). Of those mutations, 258 were classified in Group 2 according to the WHO-endorsed additional grading rule that any LoF mutation in *ethA* (Table 1) should be assumed to confer ETO resistance. (This rule now excludes indels, in contrast to the first edition [Table 23]). In this analysis, an additional grading rule was applied to ensure that any *inhA* mutation that met the criteria for ETO resistance was also considered to confer resistance to INH and vice versa (see Table 1 and Section 3.3). Notably, the –154G>A mutation upstream of *inhA* (i.e. 609G>A in codon 203 of *fabG1*) was classified as a Group 2 mutation in the ALL data set, which is consistent with published allelic exchange data *(49)*. In contrast, this mutation is interpreted as a marker for INH resistance only in the Xpert® MTB/XDR assay, which should be updated *(39)*.

The low PPV (Table 3) observed is probably due mainly to the modest increases in MIC conferred by many ETO resistance mutations, resulting in a considerable overlap with the MIC distribution of susceptible isolates when these mutations occur alone *(55,56,98)*. Development of ETO resistance may be similar to that to EMB, evolving in a stepwise manner, as it is not uncommon for isolates to have many mechanisms with presumably additive effects *(54)*. For example, the most frequent Group 1 mutation, *inhA* -777C>T, commonly referred to as *fabG1* -15C>T, upstream of the *fabG1-inhA* operon, can co-occur with *ethA* mutations or the Group 2 *inhA* S94A, which mutation confers ETO and INH cross-resistance in transduction experiments *(50,52,55,99,100)*.

Table 19. Abridged variant classification for ETO

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
ETO	inhA_c.-777C>T	745	14742	2166	3046	41.6%	95.2%	74.4%	65.5%	67.9%	53.3%	9.2	1) AwR	ALL+WHO		1) Assoc w R	L	○
ETO	ethA_LoF	936	14551	1270	3942	24.4%	94.0%	57.6%	51.0%	53.7%	38.8%	3.8	1) AwR	ALL+WHO		1) Assoc w R		▲
ETO	ethA_p.Lys37fs	86	15401	109	5103	2.1%	99.4%	55.9%	49.4%	57.3%	40.7%	2.9	1) AwR	ALL+WHO		1) Assoc w R		▲
ETO	ethA_p.Met1?	49	15438	58	5154	1.1%	99.7%	54.2%	52.5%	62.7%	41.3%	3.3	1) AwR	ALL+WHO		1) Assoc w R		▲
ETO	ethA_p.Arg207Gly	24	15463	36	5176	0.7%	99.8%	60.0%	63.2%	75.6%	46.5%	5.1	1) AwR	ALL+WHO		1) Assoc w R		○
ETO	ethA_p.Tyr235fs	11	15476	26	5186	0.5%	99.9%	70.3%	68.6%	83.1%	50.7%	6.5	1) AwR	ALL+WHO		1) Assoc w R		▲
ETO	inhA_c.-154G>A	138	15349	309	4903	5.9%	99.1%	69.1%	46.2%	54.7%	26.3%	2.7	1) AwR	ALL	ALL only	2) Assoc w R - Interim	H	○
ETO	inhA_c.-770T>C	164	15323	186	5026	3.6%	98.9%	53.1%	34.6%	55.7%	2.4%	1.6	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	J	○
ETO	inhA_c.-779G>T	139	15348	122	5090	2.3%	99.1%	46.7%	11.5%	22.2%	1.9%	0.4	3) Uncertain	ALL+WHO	INH-ETO X-R	2) Assoc w R - Interim	N	▲
ETO	inhA_p.Ser94Ala	24	15463	118	5094	2.3%	99.8%	83.1%	60.7%	78.5%	26.3%	4.7	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
ETO	inhA_c.-770T>A	20	15467	64	5148	1.2%	99.9%	76.2%	33.3%	70.1%	2.8%	1.5	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	I	○
ETO	ethA_p.Asn379Asp	84	15403	61	5151	1.2%	99.5%	42.1%	40.9%	49.8%	30.9%	2.1	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
ETO	ethA_c.-7T>C	67	15420	53	5159	1.0%	99.6%	44.2%	43.5%	53.4%	32.1%	2.3	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
ETO	ethA_p.Ser390Phe	5	15482	19	5193	0.4%	100.0%	79.2%	79.2%	92.9%	57.8%	11.3	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
ETO	ethA_p.Ala341Val	7	15480	17	5195	0.3%	100.0%	70.8%	71.4%	88.7%	45.1%	7.4	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
ETO	ethA_p.Leu35Arg	2	15485	12	5200	0.2%	100.0%	85.7%	81.8%	97.7%	48.2%	13.4	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
ETO	ethA_p.Ser57Tyr	1	15486	11	5201	0.2%	100.0%	91.7%	90.9%	99.8%	58.7%	29.8	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
ETO	inhA_c.-770T>G	1	15486	11	5201	0.2%	100.0%	91.7%	66.7%	99.2%	9.4%	6.0	3) Uncertain	ALL+WHO	INH-ETO X-R	2) Assoc w R - Interim	K	▲
ETO	ethA_p.Thr88Ile	1	15486	9	5203	0.2%	100.0%	90.0%	88.9%	99.7%	51.8%	23.8	1) AwR	ALL	ALL only	2) Assoc w R - Interim		○
ETO	ethA_p.Val202Gly	1	15486	7	5205	0.1%	100.0%	87.5%	87.5%	99.7%	47.3%	20.8	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
ETO	ethA_p.Cys403Tyr	0	15487	6	5206	0.1%	100.0%	100.0%	100.0%	100.0%	54.1%	Inf	1) AwR	ALL	ALL only	2) Assoc w R - Interim		▲
ETO	inhA_c.-778A>G	1	15486	0	5212	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO	WHO-end. gDST	2) Assoc w R - Interim	M	○
ETO	ethA_p.Val398dup	3	15484	36	5176	0.7%	100.0%	92.3%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Pro378Leu	60	15427	34	5178	0.7%	99.6%	36.2%	39.0%	50.8%	23.7%	1.9	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Arg150_Pro160del	3	15484	33	5179	0.6%	100.0%	91.7%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Tyr32Asp	14	15473	15	5197	0.3%	99.9%	51.7%	48.1%	68.1%	28.7%	2.8	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Gly11Val	1	15486	14	5198	0.3%	100.0%	93.3%	85.7%	99.6%	42.1%	17.9	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Ala222_Ile338del	0	15487	7	5205	0.1%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Asn287_Leu333del	8	15479	4	5208	0.1%	99.9%	33.3%	11.1%	48.2%	0.3%	0.4	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Gly184del	0	15487	3	5209	0.1%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		▼

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
ETO	ethA_p.Thr2_Met41del	0	15487	3	5209	0.1%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Thr323dup	5	15482	3	5209	0.1%	100.0%	37.5%	37.5%	75.5%	8.5%	1.8	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ala248dup	0	15487	2	5210	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ala252_Asp300del	0	15487	2	5210	0.0%	100.0%	100.0%	100.0%	100.0%	15.8%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ala20dup	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ala237dup	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Asp357del	0	15487	1	5211	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Glu318_Ile339del	1	15486	1	5211	0.0%	100.0%	50.0%	50.0%	98.7%	1.3%	3.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Gly299_Val310del	0	15487	1	5211	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ile339del	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ile339dup	0	15487	1	5211	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Leu129_Val312del	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Leu194_Ala195delinsPro	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Lys30_Ser31insArg	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Lys370_Tyr382del	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Met233_Thr236delinsIle	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Pro160del	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ser251_Ala252insGly	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Trp116_Cys137del	0	15487	1	5211	0.0%	100.0%	100.0%	NA	NA	NA	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Val85del	0	15487	1	5211	0.0%	100.0%	100.0%	100.0%	100.0%	2.5%	Inf	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Glu223_Lys224del	2	15485	0	5212	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.His201_Lys370del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Ile81_His102del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Leu344del	1	15486	0	5212	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Lys224dup	1	15486	0	5212	0.0%	100.0%	0.0%	NA	NA	0.0%	NA	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Met263_Phe320delinsIle	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Phe431_Thr435del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Pro284_Leu344del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Pro297_Asn388del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		
ETO	ethA_p.Thr366_Tyr369del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		

Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
ETO	ethA_p.Tyr32dup	2	15485	0	5212	0.0%	100.0%	0.0%	0.0%	84.2%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	ethA_p.Val188_Ser251del	1	15486	0	5212	0.0%	100.0%	0.0%	0.0%	97.5%	0.0%	0.0	3) Uncertain	ALL+WHO		3) Uncertain significance		▼
ETO	mshA_p.Ala187Val	1563	2227	987	1183	45.5%	58.8%	38.7%	6.7%	8.6%	2.5%	0.1	5) NotAwR	WHO		5) Not assoc w R		▲
ETO	mshA_p.Asn111Ser	173	3617	62	2108	2.9%	95.4%	26.4%	3.7%	8.4%	0.9%	0.1	5) NotAwR	WHO		5) Not assoc w R		▲

Individual LoF mutations in the coding regions of *ethA*, which are classified in Group 2 by the associated additional grading rule (see Table 23 and Section 5.8), and silent mutations are not listed in this table but can be found in the catalogue master file.

[H] Alias fabG1_p.Leu203Leu.

[I] Alias fabG1_c.-8T>A.

[J] Alias fabG1_c.-8T>C.

[K] Alias fabG1_c.-8T>G.

[L] Alias fabG1_c.-15C>T.

[M] Alias fabG1_c.-16A>G.

[N] Alias fabG1_c.-17G>T.

4 Future research priorities

The mutation catalogue will be updated and revised regularly according to need and emerging evidence. The following research areas have been identified as priorities for future catalogues.

Types of data to be analysed

Allelic exchange, enzymatic, lineage and MIC data:

- Current WHO treatment guidelines indicate that the levels of resistance to INH and MFX have notable implications for treatment. For example, high-dose INH may be useful for low-resistance isolates, and isolates with high-level resistance mutations to MFX cannot be treated with high-dose MFX, even as part of a longer, individualized regimen *(5)*. The assumption that all *katG* mutations confer high-level INH resistance should be investigated. Moreover, more MICs are required on the rarer FQ resistance mutations. For example, the limited MIC data for *gyrA* Gly88Cys suggest that this mutation causes high-level resistance, which is not in agreement with enzymatic measurements *(15,101)*.
- Some mutations result in only modest increases in MIC that are difficult to classify from categorical pDST data (e.g. *rrs* 1402C>T and some *inhA* promoter mutations already included in WHO-endorsed gDST assays). Ideally, the shape and particularly the mode of the MIC distribution of individual mutations should be reviewed to identify potential borderline resistance mechanisms *(30,33)*. Moreover, areas of technical uncertainty, as defined by the European Committee on Antimicrobial Susceptibility Testing, may be necessary to minimize very major pDST errors *(4,21, 29,102)*. Inclusion of additional allelic exchange or selection data may be relevant in this context.
- Lineage effects (e.g. lineage 3 for FQs and lineage 1 for PMD and PZA *(16,17,103)*) and homoplasy, which may be a signal for selection, should be explored systematically *(64,104)*.

A more strategic approach to collecting data, commissioning additional testing and interpreting the findings to maximize the usefulness of gDST:

- To support this approach, a global TB sequencing knowledgebase has been established at WHO, in collaboration with FIND, as a repository of associated phenotypic–genotypic data sets, with user-friendly dashboards and features for browsing and querying. The portal will support gathering of data contributions by global partners for future updates of the catalogue. It is expected to be ready for use by the end of 2023 and available at TB sequencing knowledgebase. Potential data contributors who seek more information are invited to contact the WHO Global TB Programme (tbsequencing@who.int) .

- Susceptible and resistant pDST must be collected to avoid inflated PPVs and potential false associations with resistance. Given the frequency of *Rv0678* Met146Thr in Eswatini, additional unbiased results for this mutation are particularly important *(29)*.
- Group 3 mutations that may result in the largest potential gain in sensitivity should be a priority, particularly if they are homoplasic.
- Additional isolates are required from countries and MTBC lineages that are currently underrepresented.
- Exceptions to the "additional grading rules" (Table 1) that may result in significant harm in some settings should be studied. For example, *rpoB* Thr427Ala may not confer RIF resistance despite being in the RRDR *(33,105)*.

Grading criteria

- A comprehensive analysis of PMD is warranted as soon as a WHO-endorsed CC becomes available *(17)*.
- The validity of the breakpoints used should be assessed, particularly if dosing of antibiotics changes (e.g. if a higher dose of RIF is endorsed *(4)*).
- Prioritization of categorical pDST results over MIC results obtained with the same method and exclusion of discordant pDST results may have to be reconsidered, as became apparent in the analysis of *Rv0678* Met146Thr.
- The calculations in Table A.3 show that rare resistance mutations in non-essential genes probably play an important role for some key drugs (e.g. in *Rv0678* for BDQ). Less stringent grading criteria, akin to the "relaxed" thresholds already endorsed for *pncA* (Fig. 9), might have to be endorsed.
- Alternative approaches, such as regression analyses, will be required to classify markers of resistance that, by definition, have a low PPV|SOLO (e.g. compensatory mechanisms, such as in *ahpC*, or resistance mutations that usually occur in combination).
- Selection of genes and corresponding regulatory regions should be revised in the light of the latest scientific evidence. For example, the role of *Rv1979c* in BDQ and CFZ resistance has been questioned, whereas *Rv1453* may be involved in CFZ resistance *(13,106,107)*. Moreover, *dprE2* (*Rv3791*) was recently found to be the shared target of DLM and PMD, *fusA1* (*Rv0684*) may have to be considered for KAN/AMK, and *mshC* (*Rv2130c*) may be relevant for ETO/INH~~, and~~ *(84,108)*.

Bioinformatics pipeline

- Large insertions, such as an IS*6110* insertion that can cause resistance to several drugs, including BDQ, cannot be detected reliably with the current pipeline *(109,110)*. Moreover, some small inframe changes are sometimes reported as two frameshifts, resulting in inappropriate application of the LoF additional grading rule, e.g. *katG* changes in samples "2020 DNA6" and "2020 DNA10" from an external quality assessment scheme in 2020 *(48)*.
- So far, only *aftA*, *fabG1* and *furA* have been analysed at nucleotide instead of amino acid level, because mutations in these genes could affect the expression of downstream resistance genes

(49,54,111). Such analysis might have to be extended to other translated genes if there is evidence of selection of synonymous mutations (e.g. if they are homoplasic) *(112–114)*.

- The assumption that different nucleotide changes that result in the same amino acid substitution have the same effect may have to be reconsidered *(64,115)*.
- The pipeline should be adapted for analysis of next-generation sequencing data from genomic technologies other than Illumina, such as those from Oxford Nanopore Technologies.

Guidance should be developed on what, if any, confirmatory testing should be conducted if a marker for resistance is found and how discordant results should be resolved if an isolate is found to be susceptible by pDST (i.e. the extent to which a composite reference standard should be endorsed for individual patient treatment *(30,33)*). The relative contributions of the following factors should be considered for each mechanism and/or mutation.

- It is well understood that traditional gDST assays can yield false-resistant or false-susceptible results because of inherent limitations in the underlying technology (e.g. some mutations are missed, even though they are targeted, and low bacillary loads may affect the accuracy *(30,116–118)*). Limitations specific to certain technologies for next-generation sequencing are also possible (Section 3.2). Manufacturers of sequencing technology, assay developers and users should notify WHO and other relevant parties of suspected problems (e.g. positions that are more prone to sequencing errors). The outcomes of the investigation of such issues should be shared with users in a timely manner *(116,119)*.
- The reproducibility of pDST, the accuracy of the breakpoint used and the prevalence of resistance should be evaluated.
- Studies should be conducted on whether a mutation results in MICs close to the breakpoint.
- The classification of some mutations according to additional grading rules or to previous WHO decisions may be incorrect. For example, a nonsense mutation one codon before the actual stop codon of *katG* is unlikely to confer INH resistance. Such exceptions could be excluded from the additional grading rules by adding them to Group 4 or 5, depending on the quality of the evidence.
- Investigation should be made of whether epistasis can confound the interpretation of a mutation in genes other than *Rv0678* and the *eis* promoter region. For example, if *mymA* (*Rv3083*) is naturally overexpressed in some isolates, it could counteract an LoF mutation in *ethA*, although this has not been described to date *(97)*.

5 Methods

5.1 Overview

Four primary components were essential for developing the second edition.

1. **High-quality pDST**

   DST data were curated manually to ensure that the best available phenotypic data were used as a reference standard for genotypic/phenotypic associations. As different DST methods were used during the period over which the data were collected and as WHO-endorsed CCs changed with time, the phenotypic methods were rank-ordered from WHO-endorsed methods (highest ranking) to non-endorsed methods (lowest); among WHO-endorsed methods, they were ranked from most recent (highest) to oldest (lowest). For data on isolates with multiple phenotypes, a hierarchy was used, in which the most recent WHO-endorsed DST methods were ranked highest, and older or non-WHO methods were ranked lower. A large data set on BMD DST was also included, which contributed substantial data on new and repurposed drugs. As the method and criteria for its interpretation have not been reviewed or endorsed by WHO, findings based on these data alone were classified as "interim" associations in the catalogue.

2. **High-quality, standardized WGS for generating unbiased sequence data**

   Only WGS data that were generated with Illumina instruments were included in the analysis. These next-generation sequencing platforms are currently the most widely used globally. This ensures that the raw sequencing output data are standardized to the greatest extent possible among the various Illumina next-generation sequencing instruments. The output file of raw sequencing reads was used as the starting-point for the bioinformatics analyses.

3. **A comprehensive bioinformatics pipeline for variant detection and annotation**

   To ensure that mutations were identified uniformly in all the raw sequencing data, a single bioinformatic pipeline based on the "clockwork" architecture (https://github.com/iqbal-lab-org/clockwork) *(120)* was used to process the data through quality checks, align the reads to the *M. tuberculosis* H37Rv reference genome and identify variants. The pipeline was designed to maximize variant detection of both single nucleotide polymorphisms and small insertions and deletions up to 15 bp in length. The pipeline also generated sequencing depth and coverage values for each sample, so that quality control filters could be applied further downstream. For the second edition, we also identified large-scale deletions of up to 100 kbp with an additional open access tool *(121)*.

4. **A validated algorithmic approach for statistically associating variants with phenotypes**

   The matched and curated phenotype and genotype data were then processed in a multistage algorithm that was first used to identify neutral mutations (not associated with resistance). Masking the neutral mutations from further analysis, it then identified MTBC isolates with SOLO mutations, defined as the single remaining mutation among candidate genes (selected by the working group as having the highest pre-test probability of being associated with drug resistance to a specific drug, Table 21). All mutations and corresponding phenotypes were then analysed statistically for confidence grading, including determining odds ratios (ORs) and PPVs for association of the variant with phenotypic resistance. As the data set was large and heterogeneous, strict criteria were applied, and, when applicable, the bounds of the 95% CI were considered with the relevant statistics.

The bioinformatics pipeline for identifying variants and the algorithms for identifying variants "associated with" and "not associated with" resistant phenotypes was adapted from approaches developed by the CRyPTIC Consortium *(22)*, and the confidence grading method was developed for the Seq & Treat project *(6,122)*. All these elements were described in detail in the first edition *(14)*.

Methods were refined and optimized for the second edition during a series of meetings of an international panel of expert advisors in sequencing, bioinformatics, biostatistics and mycobacteriology. Methods were proposed, adapted and finalized in webinars and e-mail communication due to travel restrictions during the coronavirus disease 2019 pandemic. Details of the methods and changes are described below.

5.2 Revised computational architecture

One of the secondary but still critical goals of the second edition of the mutation catalogue was to ensure a more centralized, efficient, sustainable workflow for future revisions of the catalogue, with minimal hands-on work. For the first edition *(14)*, all the phenotypic and genotypic data and all the computational processes, including bioinformatics processing of the sequencing data and statistical grading of the variants, were based on a restricted-access, academic high-performance computing cluster. For the second edition, all the data and computational processes were transferred to a WHO-supervised cloud service and adapted to run on a public cloud service provider. A new relational database schema was developed to house the data in the WHO cloud. The schema was designed to include information ranging from samples and associated sequencing data, to genotype calls identified after bioinformatics processing. A complete relational model for the required variables for downstream bioinformatics and statistical analyses helped to form a reliable, accessible, transparent and reproducible service. PostgreSQL was chosen as the open-source relational database management system. We incorporated tables from the open-source BioSQL database format (https://github.com/biosql/biosql) and used the associated suite of tools to load the reference genome and annotation in the associated table. Then, we included additional tables for the specific study of genetic variants and their association with phenotypes. The full database table definition (which we called GenPhenSQL) is available for review and dissemination at https://github.com/GTB-tbsequencing/mutation-catalogue-2023/tree/main/Final%20Result%20Files. For the second edition, the GenPhenSQL database was used to provide

all inputs for the downstream computational analyses, starting with bioinformatics processing of the sequencing data.

5.3 Data sources

As for the first edition, data were aggregated from various sources: legacy data sets from publications and consortium initiatives and direct submissions in response to public calls for contributions by the WHO Global Tuberculosis Programme. The minimum acceptable phenotypic data included the pDST method and a categorical result (resistant, susceptible or intermediate), but MIC ranges were also collected in tabular or simple text formats when available. pDST and MIC data were collected in tabular format and were inserted into the GenPhenSQL database as specific extract-transform-load scripts built with Python/Pandas. We synchronized the GenPhenSQL daily with the International Nucleotide Sequence Database Collaboration databases to connect pDST and MIC submissions linked to publicly available sequencing data. We used the public National Center for Biotechnology Information ENTREZ application programming interface for BioSample and Sequence Read Archive databases for that purpose. Consequently, our extract–transform–load script could accept pDST or MIC submissions with sample identification labels from the National Center for Biotechnology Information or the European Nucleotide Archive directly. We also accepted direct private sequencing data submission. We recorded the origin of the sequencing data (i.e. private or publicly available) in GenPhenSQL so that the analytical workflow could correctly retrieve the raw data at runtime. Whenever possible, submissions linked directly to publicly available sequencing data were preferred. When contributors preferred private, direct submission of sequencing data, we accepted only raw FASTQ files, so that all samples could be processed with the same bioinformatics pipeline. In a few cases in which only BAM files were available, we converted the BAM back to FASTQ format with SAMtools.

5.4 Curation of pDST data

All pDST results associated with MTBC isolates for which WGS data were also available were collated and analysed. Categorical (resistant, susceptible or intermediate) and/or MIC pDST data were considered. Intermediate categorical pDST results were converted to binary results (R or S) or excluded according to additional grading rules approved by the working group. MIC data were converted into categorical binary results (resistant or susceptible) according to CCs appropriate to the pDST method used, as described below. We then stratified the pDST data into eight categories according to the level of WHO endorsement of the method, as described in detail below and summarized in Fig. 6.

Category 1. pDST methods currently endorsed by WHO (WHO CURRENT)

Categorical pDST results for Löwenstein-Jensen (LJ), Middlebrook 7H10 (7H10), Middlebrook 7H11 (7H11) and MGIT were regarded as "current" if the CCs in the latest published WHO DST manual *(123)* were used, with the following exceptions. We used the updated RIF CCs from 2021 *(4)*. Results for ofloxacin (OFX) and KAN were regarded as "current" if they were based on the

CCs from the 2018 WHO technical report *(15)*. KAN was still included, even though it is no longer recommended for TB treatment, as the data provide useful insights into the effect of *eis* promoter mutations and *rrs* 1402C>T on AMK *(15,16)*. Similarly, although WHO no longer recommends testing for OFX to ensure that it is not used clinically, pDST for OFX resistance at concentration *x* is equivalent to testing LFX at *x*/2, as OFX consists of equal amounts of the active L-isomer of OFX (i.e. LFX) and the largely inactive D-isomer, as reflected in the CCs for the two drugs *(15)*. For this reason and because OFX was still widely tested in WHO-endorsed media, pDST results for OFX and LFX were pooled and reported as LFX results in the mutation tables in this report. Similarly, pDST results for ETO and PTO were pooled and reported as ETO. For MFX, we also considered non-WHO CCs that were lower than previous WHO CCs but above the current WHO CC, as these non_WHO_CC_SR results were considered more accurate than results with previous WHO CCs *(15)*. In some cases, we had to assume that pDST was conducted by the proportion method with the correct critical proportion, as this information was not collected systematically from all contributors. In practice, this assumption was probably correct for the majority of results (i.e. only a minority of testing on LJ may have been done with the resistance ratio or absolute concentration method). Microscopic observation drug-susceptibility (MODS) results with a CC of 1 mg/L RIF or 0.4 mg/L INH were also considered to be "current" *(124,125)*.

Category 2. pDST methods previously endorsed by WHO (WHO PAST)

This category included pDST results for LJ, 7H10, 7H11, MGIT or BACTEC™ 460 obtained either with outdated WHO CCs or simply reported to have been based on WHO CCs without providing the drug concentration tested, in which case it was not clear which WHO CC was followed *(126–128)*. It was assumed that the proportion method with the correct critical proportion was used.

Category 3. Other pDST methods

This category consisted primarily of a very large genotypic and pDST data set from the CRyPTIC Consortium, which used novel BMD plates manufactured by Thermo Fisher for pDST. Although Clinical and Laboratory Standards Institute CCs exist for RIF, INH and EMB for the MYCOTB(I) BMD plate, which is also manufactured by Thermo Fisher, BMD plates and the corresponding CCs are not endorsed by WHO. Two different CRyPTIC plates were used to produce raw MIC data, namely the UKMYC5 and UKMYC6 plates. MIC data were translated into binary resistant or susceptible results with the CCs listed in Table 20, and the pDST results were given the code MYCOTB_MIC in the data set. The same CCs were also used to translate MICs from the MYCOTB(I) plates into categorical pDST *(16)*. The rationale and derivation of these CCs can be found elsewhere *(22)*. Category 3 also included pDST results derived with methods for which it was unclear whether they met either current or previous WHO guidelines, either because no information on the CC was provided, or, a CC was available, but it was not clear whether 7H10 or 7H11 had been used. In addition, resistance results from LJ, 7H10, 7H11, MGIT or BACTEC™ 460 from categorical or MIC data with a breakpoint above the latest WHO CC were considered and given the Non_WHO_CC_R code. Susceptible results from LJ, 7H10, 7H11, MGIT or BACTEC™ 460 from categorical or MIC data with a breakpoint below or equal to the latest WHO CC were included with the Non_WHO_CC_S code.

Table 20. Drugs tested with two CRyPTIC BMD plate designs and corresponding CC used to translate MIC data into binary resistant or susceptible categories

Group	Drug	Concentration range (mg/L)		CC used for interpretation as R or S (mg/L)[a]
		UKMYC5	UKMYC6	
First-line	RIF	0.06–4	0.03–8	0.5
	INH	0.025–1.6	0.025–12.8	0.1[b]
	EMB	0.125–8	0.25–32	4[c]
Group A	LFX	0.125–8	0.12–8	1
	MFX	0.6–4	0.06–4	1
	BDQ	0.016–2	0.008–1	0.25
	LZD	0.03–2	0.06–4	1
Group B	CFZ	0.06–4	0.03–2	0.25
Group C	DLM	0.016–1	0.008–0.5	0.125
	AMK	0.25–8	0.25–16	1
	ETO	0.25–8	0.25–8	4
Other[d]	KAN	1–16	1–16	4

[a] Neither method nor CC is endorsed by WHO, and some of the CCs have been questioned *(16,22–25)*.

[b] Equivalent to the Clinical and Laboratory Standards Institute CC of 0.12 mg/L for the MYCOTB plate *(57)*.

[c] Identical to the Clinical and Laboratory Standards Institute CC of 4 mg/L for the MYCOTB plate on the assumption that the "inconclusive" concentration of 4 mg/L corresponds to an area of technical uncertainty, as defined by the European Committee on Antimicrobial Susceptibility Testing *(21,57)*.

[d] Drug no longer endorsed for TB treatment.

Category 4. Excluded pDST results

This category included all pDST results that did not fit into categories 1–3 and were excluded from the analysis.

5.5 Prioritization of pDST results

As the reference pDST methods and the CCs used to evaluate genotype/phenotype associations could significantly affect the outcomes of our statistical analyses, we completed two separate association analyses, first using only data with pDST results in categories 1 and 2 (WHO CURRENT and PAST) – which we considered to be more conservative but also less inclusive – and then using pDST data from categories 1, 2 and 3 (ALL) – which we considered less conservative and more inclusive. After completing independent association calculations, we compared and contrasted the resulting associations stratified by pDST category and reported both in the ALL and WHO (WHO CURRENT and PAST) columns of the master table of mutation associations.

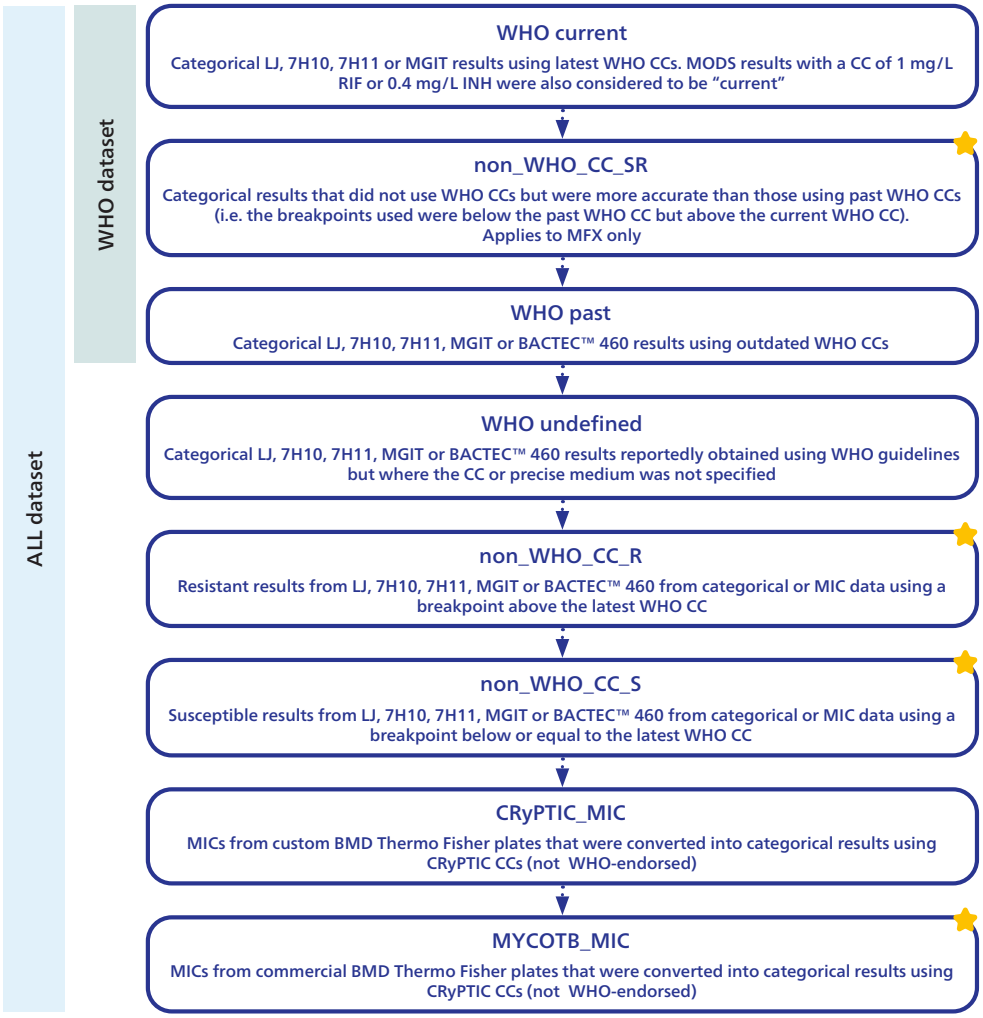
For many MTBC isolates, pDST results were obtained with more than one method. In these cases, we ordered them by priority to include only the pDST data most recently endorsed by WHO according to the following hierarchy:

- category 1 > category 2 > category 3

- Within the same category, solid media pDST results were given higher priority than those in liquid media, because the latter are more likely to miss key, clinically relevant *rpoB* mutations *(129)*.
- Liquid media methods within the same category were prioritized as follows: MGIT > MODS > BACTEC™ 460 > CRyPTIC, as BACTEC™ 460 are no longer used, and MGIT has undergone more validation than MODS.
- Solid media methods in the same category were prioritized as follows: 7H10 > LJ > 7H11, because there is generally more supporting evidence for 7H10 CCs.

This hierarchical organization enabled creation of standardized data sets that included only isolates that had been tested with the same methods, e.g. only currently WHO-endorsed methods (WHO) or any acceptable DST method (ALL) (Fig. 6). While there was probably more pDST variation and phenotype noise in the ALL data set, the number of isolates in that data set was significantly larger, which was at times critical for detecting a statistically significant relation between genotype and observed phenotype. If an isolate was tested several times with the same method (same media and same CC) but with discrepant results, all the discordant results were excluded, and concordant results were considered. The same rule was applied to binarized MIC data.

Fig. 6. Summary of pDST stratification and hierarchy



Stars denote changes from the approach used for the first edition.

5.6 Variant analysis

Bioinformatics pipeline

Although the bioinformatics pipeline for variant detection was adapted to run on a public cloud provider for the second edition, most of the steps in the analysis remained the same for the two catalogues. Paired reads were mapped with Burrows-Wheeler Aligner to the same reference (GenBank NC_000962.3), and PCR duplicates were removed with SAMtools. When more than one sequencing run was available for a single isolate, alignments for each pair were merged before genotyping. For genotyping, a standard caller (*Freebayes*) was used to detect single nucleotide polymorphisms and small indels, and another caller (*delly*), capable of detecting large deletions, was also used. For quality control, the taxonomy of aligned reads was assigned with *kraken*; global sequencing statistics metrics were collected with SAMtools, and gene-specific sequencing statistics were calculated with *mosdepth*. At the end of bioinformatics processing, four types of information were inserted into the GenPhenSQL database: all genotype calls, the global sequencing metrics, the per-gene sequencing metrics, and the taxonomy assignments of aligned reads. A scheme of the pipeline is provided in Annex Fig. A.1.

Variant callers

Genotype calls were generated with two different callers, which handle small-scale events separately: *Freebayes* for genotypes typically shorter than 15 bp and *delly* for large-scale deletions. All variants found in more than 20% of the reads (fraction of supporting reads) were considered and inserted into the GenPhenSQL. *Freebayes* is a haplotype-based Bayesian genotype caller and can therefore handle many consecutive nucleotide changes, ensuring that point mutations that affect the same codon are appropriately accounted for at the annotation step. Before insertion into GenPhenSQL, all variant coordinates were normalized with *bcftools norm*; variants that spanned several nucleotides were not decomposed into single variants in order to preserve the correctness of the annotation. Large-scale deletions were inserted next to the other genotype calls according to the coordinates of the deletion, as determined by *delly*. For overall quality control of per-sample sequencing, we recorded the median sequencing depth for the full genome as well as the percentage of the genome covered at 15×, 20×, 25× and 30×. We included samples in the analysis if their median depth was greater than 15× and at least 95% of the reference genome had been sequenced at least 20 times. We collected the same metrics for each gene and considered that a gene was missing from a sample if at least one of its nucleotides had been sequenced fewer than 10 times.

Variant annotation

One benefit of using a relational model for computation was that variant annotation (which relies on a given set of coordinates for a reference genome) could be performed independently from identification of genotypes in samples. In practice, this meant that only iterative extraction of new entries from the "variant" table of GenPhenSQL was necessary to perform the annotation. Annotation was performed with *SnpEff,* and the same reference genome and was configured with

the GFF annotation file for the entry GCF_000195955.2_ASM19595v2 and the bacterial genetic code (translation table 12 in GenBank). To correctly annotate *dnaA* (*Rv0001*), the first gene on the linearized sequence, and its promoter variants at the end of the linear sequence, we created a modified sequence whereby nucleotides located at the end of a reference were collated in front. Only variants on the *dnaA* promoter were annotated with that modified reference. From the raw output of the annotation returned by *SnpEff*, a custom Python script curated some of issues that have been identified, such as incorrect Human Genome Variation Society (HGVS) nomenclature, in order to improve compatibility with bioinformatic interpretation tools over that in the first edition of the catalogue *(130)*, and normalized the information for insertion into GenPhenSQL. The relation modelled between variants and annotation was "many-to-many", as one variant could have had different annotation values with respect to different genes, and an annotation could be associated with more than one variant because of redundancy of the genetic code, such as when different nucleotide changes lead to the same missense variant. After normalization and insertion into the database, the annotation table included the predicted effect of the detected variant (e.g. missense or synonymous) on the gene or protein considered, as well as the tentative HGVS nomenclature annotation as provided by *SnpEff* for that effect. With respect to the annotation of deletion variants detected by *delly*, one annotation entry was inserted for every gene that fell entirely within the range of the deletion. The "predicted_effect" value was set as "feature_ablation".

Data extraction

The rules for setting the hierarchy and prioritization for categorizing pDST and MIC data were based on the large-scale data analysis, open-source engine, *Apache Spark* and its Python implementation (*PySpark*). Quality control per sample and per gene was also assured with *PySpark*, as was transformation of the raw information on variant annotation inserted into the GenPhenSQL.

The aim of variant curation was to link genotypes identified in samples unambiguously to predicted changes in the tiered genes included in the resistance association list (Table 21). For each resistance gene, relevant promoter and/or upstream regions were defined according to the primary transcriptional start site (Table 22). Two pieces of information for each variant were determined from the inserted *SnpEff* output for statistical grading: the predicted effect and the variant category. Depending on the context, the final unit of study for the grading algorithm could be a protein encoded by the gene (for missense, frameshift or in-frame insertion or deletion included in a coding sequence) or relative to the gene or mRNA itself (for non-coding genes, synonymous or intergenic variants). All possible values and examples are listed in Table 23.

Table 21. Candidate resistance genes

Drug	Tier 1	Tier 2	References
INH	*fabG1* (*Rv1483*), *inhA* (*Rv1484*), *katG* (*Rv1908c*), *furA* (*Rv1909c*), *ahpC* (*Rv2428*)	*dnaA* (*Rv0001*), *Rv0010c*, *mshA* (*Rv0486*), *hadA* (*Rv0635*), *Rv1129c*, *Rv1258c*, *ndh* (*Rv1854c*), *Rv2752c*, *glpK* (*Rv3696c*)	*49,111,131–141*
RIF	*rpoB* (*Rv0667*)	*nusG* (*Rv0639*), *rpoC* (*Rv0668*), *Rv1129c*, *Rv2477c*, *Rv2752c*, *lpqB* (*Rv3244c*), *mtrB* (*Rv3245c*), *mtrA* (*Rv3246c*), *rpoA* (*Rv3457c*), *glpK*	*6,76,135,136,139–142*
EMB	*aftA* (*Rv3792*), *embC* (*Rv3793*), *embA* (*Rv3794*), *embB* (*Rv3795*), *ubiA* (*Rv3806c*)	*embR* (*Rv1267c*), *Rv2477c*, *Rv2752c*, *glpK*, *aftB*	*54,76,135,13–141,143*
PZA	*pncA* (*Rv2043c*), *clpC1* (*Rv3596c*), *panD* (*Rv3601c*)	*Rv1258c*, *rpsA* (*Rv1630*), *sigE* (*Rv1221*), *PPE35* (*Rv1918c*), *Rv3236c*	*6,137,139,144–146*
FQ	*gyrB* (*Rv0005*), *gyrA* (*Rv0006*)	*Rv1129c*, *Rv2477c*, *Rv2752c*, *glpK*	*6,76,136,140,141*
BDQ	*mmpL5* (*Rv0676c*), *mmpS5* (*Rv0677c*), *Rv0678*, *atpE* (*Rv1305*), *pepQ* (*Rv2535c*)	*Rv1979c*, *lpqB*, *mtrB*, *mtrA*	*13,76*
LZD	*rplC* (*Rv0701*), *rrl* (*MTB000020*)	*tsnR* (*Rv1644*)	*13,76*
CFZ	*mmpL5*, *mmpS5*, *Rv0678*, *Rv1979c*, *pepQ*	*fgd1* (*Rv0407*), *fbiC* (*Rv1173*), *Rv2983*, *fbiA* (*Rv3261*), *fbiB* (*Rv3262*)	*13,84,147*
DLM	*fgd1*, *ddn* (*Rv3547*), *fbiC*, *Rv2983*, *fbiA*, *fbiB*	*ndh*	*86,148*
AMK	*rrs* (*MTB000019*), *eis* (*Rv2416c*), *whiB7* (*Rv3197A*)	*ccsA* (*Rv0529*), *bacA* (*Rv1819c*), *Rv2477c*, *whiB6* (*Rv3862c*)	*15,76,139,149*
STM	*rpsL* (*Rv0682*), *Rv1258c*, *rrs*, *whiB7*, *gid* (*Rv3919c*)	*bacA*, *Rv2477c*, *glpK*	*6,76,137,149,150*
ETO	*mshA*, *fabG1*, *inhA*, *ethA* (*Rv3854c*)	*Rv0565c*, *ndh*, *Rv3083*, *ethR* (*Rv3855*)	*49,97,133,134,138,139,151, 152*
KAN	*rrs*, *eis*, *whiB7*	*ccsA*, *bacA*, *Rv2477c*, *whiB6*	*6,76,139,149*
CAP	*rrs*, *tlyA* (*Rv1694*)	*ccsA*, *rrl*, *bacA*, *Rv2680*, *Rv2681*, *whiB6*	*6,76,135,139,153*

Table 22. Upstream/promoter regions of candidate resistance genes

Gene	Upstream/ promoter regions	Primary transcriptional start site	Reference
aftB	1–129	4268914	*154*
ahpC	1–93	2726151	*131*
atpE	1–51	1461045	*154*
bacA	1–81	2064758	*155*
ccsA	1–191	619751	*155*
clpC1	1–106	4040759	*155*
ddn	1–51	3986844	*155*
dnaA	1–314	4411270	*155*
eis	1–84	*2715365*	*156*
embA	1–86	4243233	*155*
embC	1–1982[a]		
embR	1–103	1417399	*155*
ethA	1–51	4327473	*155*
ethR	1–26	4327505	*154*
fbiA	1–138	3640456	*155*
fbiC	1–127	1302855	*155*

Gene	Upstream/ promoter regions	Primary transcriptional start site	Reference
fgd1	1–51	490783	*155*
gid	1–79	4408230	*157*
glpK	1–52	4139756	*155*
gyrA	1–35		
gyrB	1–108	5183	*155*
hadA	1–51	731930	*155*
inhA	1–813[b]	1673440	*155*
katG	1–532[c]	2156592	*158*
mmpS5	1–85[d]	778965	*155*
mshA	1–669	574730	*155*
mtrA	1–376	3627674	*155*
mtrB	1–50	3626821	*155*
ndh	1–96	2103087	*155*
nusG	1–201	734104	*155*
panD	1–51 & 1838–1949	4046179	*155*
pepQ	1–51	2860418	*155*
pncA	1–51	2289241	*155*
PPE35	1–122	2170683	*155*
rplC	1–51 & 323–503	800357	*154*
rpoA	1–536	3878992	*154*
rpoB	1–263	759595	*155*
rpoC	1–45		
rpsA	1–100	1833493	*155*
rpsL	1–234	781377	*155*
rrl	1–51		
rrs	1–151	1471746	[3]
Rv0010c	1–156		
Rv0565c	1–78	657497	*155*
Rv1129c	1–51	1254510	*154*
Rv1258c	1–58	1407347	*155*
Rv1979c	1–470	2223583	*154*
Rv2477c	1–88	2784079	*155*
Rv2680	1–153	2996003	*155*
Rv2681	1–2		
Rv2752c	1–51 & 934–984	3067124	*155*
Rv2983	1–51	3339118	*155*
Rv3083	1–51	3448504	*155*
Rv3236c	1–51 & 488–538	3613603	*155*
sigE	1–51	1364413	*155*
tlyA	1–51 & 185–236	1917755	*155*
tsnR	1–51		
ubiA	1–51		
whiB6	1–126	4338596[e]	
whiB7	1–404	3569032	*159*

[3] Cortes T, personal communication, 2023.

[a] Includes *aftA*, which was analysed at the nucleotide level.

[b] Includes *fabG1*, which was analysed at the nucleotide level.

[c] Includes *furA*, which was analysed at the nucleotide level.

[d] Includes the upstream region for *Rv0678* and its transcriptional start site at 778990 *(155)*.

[e] Transcriptional start site unknown; 4338596 was used instead, as a G insertion at this position is known to affect *whiB6* expression *(160)*.

Table 23. Predicted effects and variant categories used in the SOLO association algorithm

Predicted effect	Variant category format	Example of variant category values
feature_ablation[a,b]	Constant value	deletion
frameshift[a]	Proteic HGVS	p.Arg158fs
inframe_deletion	Proteic HGVS	p.Ala136_Ala139del p.Lys766_Asp770delinsAsn
inframe_insertion	Proteic HGVS	p.Cys46_Asp47insGly
initiator_codon_variant[c]	Nucleotidic HGVS	c.3G>A
missense_variant	Proteic HGVS	p.Ala277Thr
non_coding_transcript_exon_variant	Nucleotidic HGVS	n.1016G>T
start_lost[a]	Proteic HGVS	p.Met1?
stop_gained[a]	Proteic HGVS	p.Trp36*
stop_lost	Proteic HGVS	p.Ter215Argext*?
stop_retained_variant[c]	Nucleotidic HGVS	c.1044G>A
synonymous_variant[c]	Nucleotidic HGVS	c.1212G>A
upstream_gene_variant	Nucleotidic HGVS	c.-134G>T c.-30_-29insGCG

[a] Assumed to be an LoF mutation. Unlike in the first edition, inframe indels were excluded from this category because they are less likely to cause a LoF phenotype.

[b] Assigned when an entire gene was deleted.

[c] Considered to be a silent variant.

The *PySpark* extract-transform-load script for generating the input for the SOLO and grading algorithm was developed to abide by the following rules:

- Mutations in promoter and upstream regions were analysed because of their potential to alter the expression of downstream resistance genes. Therefore, all mutations in these regions were interpreted with the *nucleotidic HGVS* system, even if they overlapped with upstream protein-coding genes that were not considered to be resistance genes.
- Different genetic changes in resistance genes (Table 21) that had the same protein-altering effect were grouped and interpreted with the standard protein-based naming system, *proteic HGVS*. Three resistance genes (*aftA*, *fabG1* and *furA*) were analysed with the *nucleotidic HGVS* system, because variants in these regions can potentially affect the expression of downstream resistance genes (i.e. they were considered part of the upstream/promoter regions of *embC*, *inhA* and *katG* respectively, Table 22) *(49,54,111)*.
- Genetic changes that cause a shift in the reading frame at the same position in the genetic code were also grouped and interpreted with the *proteic HGVS* system.
- Synonymous changes, which do not alter the protein, were treated individually and interpreted with a nucleotide-based naming system (*nucleotidic HGVS*).

- The predicted effects "synonymous_variant", "initiator_codon_variant" (mutations that change a start codon to another start codon in bacteria) and "stop_retained_variant" were all treated as silent in the SOLO and grading algorithms (Table 23).
- Consecutive point mutations that affect several nucleotides were analysed together only if they occurred in the same codon and resulted in a protein-altering effect. Point mutations on different codons were always treated individually within their respective codon. For instance, one rare, consecutive point change in *rpoB* (variant occurring at genomic position 761138 that leads to replacement of the nucleotide sequence CCACA by GTCCC) was interpreted as three different units: one synonymous change in codon 444, one missense variant in codon 445 (p.His445Ser) and one missense variant on codon 446 (p.Lys446Gln).
- Consecutive nucleotide changes in non-coding sequences were evaluated separately.
- Leading amino acids encoded by non-canonical start codons in bacteria were corrected to methionine. Although these non-canonical start codons encode amino acids other than methionine everywhere else in the gene sequence, translation is always initiated with a special tRNA bound to formylmethionine *(161–163)*.

The transformation scripts for these methods are available at https://github.com/GTB-tbsequencing/mutation-catalogue-2023.

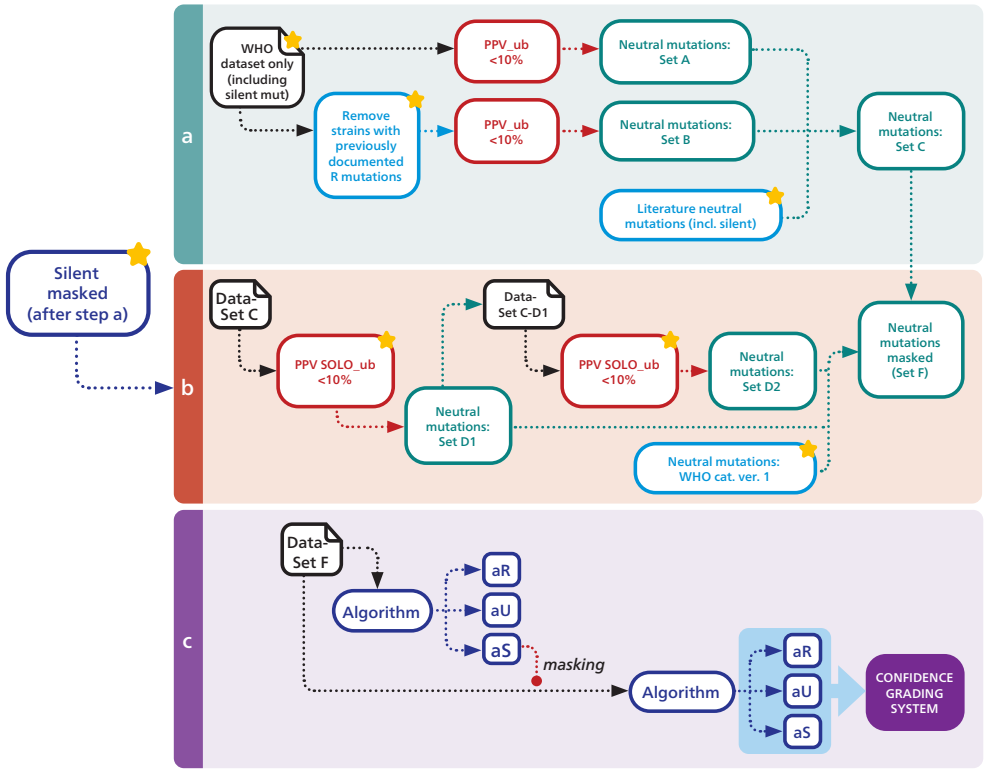
5.7 Association studies

SOLO – an algorithmic method for identifying SOLO mutations

The second edition of individual mutations associated independently with resistance was produced with a method that was applied to MTBC in 2015 *(12)* and adapted in 2021 to produce the first mutation catalogue *(14)*. The approach resembles the "definite defectives" algorithm used in group testing, pioneered in 1943 *(164,165)*. For MTBC genomic data, it is used to characterize the effects of specific mutations in various genes and promoters that are considered highly likely to be linked to expression of phenotypic resistance. Deviations from the published method *(12)* are described below.

The algorithm, presented in Fig. 7, is used to characterize variants as resistance-determining (algorithmically resistant or "aR") or as consistent with susceptibility (algorithmically susceptible or "aS"). Silent mutations were assumed to be neutral ("aS") and were masked before step b. Any non-silent variant found to be the only variant in the relevant resistance regions (i.e. a "SOLO" variant) in a phenotypically resistant isolate was classified as "aR". Any non-silent variant that was found, either overall or as a SOLO variant, only in phenotypically susceptible isolates was classified as "aS". Any non-silent mutation that could not be characterized as either "aR" or "aS", such as a mutation that never appeared as a SOLO mutation and was never seen in either phenotypically susceptible or phenotypically resistant isolates, was classified as algorithmically uncertain ("aU"). The resulting SOLO mutations were further classified with the grading algorithm (Section 5.8).

Fig. 7. Steps in the SOLO algorithm for determining neutral mutations (a and b) and for quantifying associations between genotype and phenotype



Stars denote changes from the approach used in the first edition.

Before running the algorithm, the raw data were prepared for analysis.

1. Selection of candidate genes and promoters

The success of the algorithmic approach requires exploration of relatively short genetic sequences. In the method published in 2015, the range was 1–8 genes and up to an arbitrarily defined 100 bp upstream, which was curtailed if it ran into an adjacent coding sequence *(12)*. The target genes (Table 21) and upstream and promoter regions were further refined from the first edition (Table 22). To minimize the effect of excessive genomic variation on the algorithm, we retained the division of candidate genomic regions into two tiers from the first edition, although the classification of some genes was adjusted (e.g. *ubiA* was upgraded to tier 1 from tier 2). Tier 1 comprised gene sequences and associated promoters that were considered most likely to contain resistance mutations. Tier 2 included the remaining candidate genes and associated promoters, considered to have a lower, but still reasonable pre-test probability of containing resistance mutations.

2. Sequencing defects and fraction of supporting reads

When sequencing defects that could not be explained by deletion calls generated by *delly* or *Freebayes* were detected, the information was inserted into the grading algorithm, as were all variants identified in at least 25% of reads for a particular sample. This ensured that running the algorithm with ≥ 75% as the cut-off for calling mutations did not result in false SOLO mutations.

For example, a *gyrA* mutation with a variant frequency of 80% could not be a SOLO if it co-occurred with another *gyrA* mutation with a frequency of 30% in the same sample.

3. Quality control

Sample mislabelling is relatively common and can lead to spurious results in algorithm-based analyses. One way of mitigating such error is to exclude all isolates with a previously well-established resistance mutation but a susceptible phenotype in the data set, i.e. isolates in which a susceptible phenotype is not credible. With this logic, MTBC isolates that had a *katG* Ser315Thr mutation for INH resistance or a *rpoB* Ser450Leu mutation for RIF resistance but were recorded as having "susceptible" phenotypes for the corresponding drug in the data were excluded from further consideration, on the assumption that these mutations were best explained by sample mislabelling. A separate analysis was performed only for these two mutations, which included all the isolates, phenotypically susceptible and resistant alike, to ensure accurate associations for *katG* Ser315Thr and *rpoB* Ser450Leu.

4. Initial identification of neutral mutations

As the aim of the algorithmic approach is to identify specific SOLO mutations associated with resistance, a preliminary step should be to identify as many "neutral" (i.e. not associated with resistance) mutations as possible and to mask them to downstream analyses. Isolates for which there were category 1 and 2 pDST results (forming the WHO data set) were analysed separately in four ways to identify neutral mutations. In each case, the quality control steps (see above) were implemented, and uncertain base calls were masked. Any mutation with a lower bound of 95% CI of PPV or PPV_SOLO < 10% was considered neutral. To ensure a conservative approach, phenotypes unique to category 3 (i.e. not WHO-endorsed) were not used to identify neutral mutations.

Neutral mutations were identified in a stepwise fashion, described below and shown in Fig. 7a and 7b.

(i) $\mathrm{PPV} = \frac{\mathrm{Present\_R}}{\mathrm{Present\_R} + \mathrm{Present\_S}}$ was computed for each mutation.

(ii) The PPV was computed for each mutation after removal of isolates that contained one of a few previously documented resistance mutations, defined as follows:

   a. any mutation, excluding indels, classified as Group 1/2 in the first edition;
   b. all non-synonymous mutations and indels in RRDR (codons 426–452 *rpoB*-RIF);
   c. any LoF mutation in non-essential tier 1 genes (Table 21) in which at least some LoF mutations are known to confer resistance; and
   d. any mutation, except silent ones, in *Rv0678* (for BDQ and CFZ).

(iii) A list of mutations identified specifically as neutral from evidence in the literature for RIF, INH, EMB and PZA *(107)*, and for BDQ *(166, 167)* was appended to the results of (i) and (ii), and neutral mutations were masked from all downstream analyses, with silent mutations not classified as neutral in (i) or (ii).

(iv) $\mathrm{PPV\_SOLO} = \frac{\mathrm{Present\_SOLO\_R}}{\mathrm{Present\_SOLO\_R + Present\_SOLO\_S}}$ was computed for each mutation that remained as the only (SOLO) mutation in a set of candidate genes after masking the neutral mutations identified in steps (i), (ii) and (iii).

(v) PPV_SOLO was computed for each mutation that remained as the only SOLO mutation in a set of candidate genes after masking of the neutral mutations identified in steps (i)–(iv).

A list of mutations identified specifically as neutral in the first edition was then appended to the results, and neutral mutations were masked from all downstream analyses.

Running the algorithm to identify mutations associated with resistance

The algorithm was run separately for each category of pDST data (WHO and ALL). In each instance, the quality control steps outlined above were first applied, then the neutral mutations identified in Fig. 7a and 7b were masked, with silent mutations, before the final algorithm was run in two iterations (Fig. 7c).

Prioritizing gene targets

As the algorithm approach used was hierarchical, tier-1 target gene sequences (with a higher probability of association with phenotypic resistance) were investigated first. For a resistant phenotype linked to an "aR" or "aU" mutation, no additional sequence was analysed. Tier-2 gene sequences were investigated for the remaining data. Two passes of the algorithm were performed for tier-1 sequences (Fig. 7c), whereby mutations characterized as "aS" in the first pass were masked in the second pass in order to characterize further the now solitary mutations as "aS" or "aR". Tier-2 sequences were assessed only after the second pass of tier-1 sequences. Only one pass of the algorithm was performed for tier-2 sequences because of the lower probability of finding resistance mutations in those targets.

Validation and optimization of the SOLO algorithm

It is our long-term goal to produce iterative improvements to the WHO mutation catalogue as sufficient new data are accumulated, or at least annually. For this work to be sustainable, we must ensure that the methods used to create the catalogue are accurate and as simple, efficient and cost-effective as possible. The genotypic and phenotypic associations included in the first edition were produced with a SOLO algorithm that was programmed in Stata. For the second edition, we sought to reduce the dependence of future work on the catalogue on proprietary or licensed software (e.g. Stata) and therefore asked an independent expert to review the entire Stata code set and to recreate it in R. This provided independent coding of the SOLO algorithm to validate the revised Stata code outputs, all of which were cross-checked to ensure that both codes provided the same intermediate and final statistical outputs. In the final Stata and R versions of the SOLO algorithm, all outputs were effectively identical. Future catalogues will probably be run on the open-source R version of the SOLO algorithm. For the second edition, both the R and the Stata version are available online (https://github.com/GTB-tbsequencing/mutation-catalogue-2023).

Strengths and limitations of the algorithmic approach

The strength of the SOLO algorithmic approach is that it is known to perform well for building catalogues *(12)*. Its theoretical basis in group testing provides additional confidence *(164)*. By focusing on a set of candidate sequences with a high probability of being associated with phenotypic resistance, the chances of correctly identifying SOLO "aR" mutations are enhanced, although the risk of not addressing additional relevant sequences remains. In the original method, all phylogenetically deep-rooted mutations were masked; in both catalogues, however, they were masked only if there was sufficient evidence of their neutrality. This analysis may therefore be more vulnerable to confounding due to MTBC population structure but may be less prone to misclassification that leads to arbitrary masking of the wrong mutations.

While it was assumed in the first edition that mutation calls with a fraction of supporting reads below a threshold (formerly 0.9, now 0.75) were wild-type, and thus effectively removed from the analysis, in the second edition we kept all such mutations for analysis. By propagating the uncertainty associated with their presence, we ensured that other mutations in the same isolate and tier were not spuriously classified as SOLO mutations. This helped to prevent misclassification of mutations as SOLO in the presence of potential other mutations, albeit with less strong support.

Statistical support for resistance mutations

The numbers of resistant and susceptible isolates with and without a mutation were collated into a 2×2 contingency table in order to assess genotypic/phenotypic associations, from which ORs were computed in a Fisher exact test, with corresponding *P* values according to the hypergeometric distribution and CIs computed with a normal approximation. To control for multiple testing, a Benjamini-Hochberg correction was used, with a false discovery rate of 5%. The set of hypotheses was assumed to contain all mutations except those determined to be neutral in the first edition, those determined to be neutral in the literature *(107,166,167)*, and silent ones. This correction was applied because only variants that fit into one of the above categories could be classified as not associated with resistance before the data were seen, while the status of the remaining ones (including those that were not finally classified with the algorithm) was determined only after the analysis. The same statistical procedure was applied to the ORs for SOLO mutations (OR SOLO), whereby only isolates with a SOLO mutation were counted instead of all isolates with a mutation and compared with the corresponding numbers of isolates without the mutation:

$$\text{OR SOLO} = \frac{\text{Present\_SOLO\_R}}{\text{Absent\_R}} \Big/ \frac{\text{Present\_SOLO\_S}}{\text{Absent\_S}}$$

PPV|SOLO was calculated for all mutations as the number of times the mutation was observed as a SOLO with a resistant phenotype, divided by the sum of that number and the number of times the mutation was observed with a susceptible phenotype. PPV_SOLO was calculated for all mutations as the number of times the mutation was observed as a SOLO with a resistant phenotype divided by the sum of that number and the number of times the mutation was observed as a SOLO with a susceptible phenotype:

$$\mathrm{PPV|SOLO} = \frac{\mathrm{Present\_SOLO\_R}}{\mathrm{Present\_SOLO\_R} + \mathrm{Present\_S}}$$

$$\mathrm{PPV\ SOLO} = \frac{\mathrm{Present\_SOLO\_R}}{\mathrm{Present\_SOLO\_R} + \mathrm{Present\_SOLO\_S}}$$

95% CIs were obtained with the Clopper-Pearson method. These statistical metrics were then used to stratify and prioritize mutations in the confidence grading algorithm described below.

Because of the quality control steps taken before these analyses, mutations at *katG* S315T and at *rpoB* S450L would necessarily have appeared to be perfectly associated with resistance to INH and RIF, respectively. To generate real-world data for these mutations, a separate analysis was performed in which mutations to INH and RIF were reanalysed without removing probably mislabelled isolates. Only the results for *katG* Ser315Thr and *rpoB* Ser450Leu were retained from that analysis and substituted for the results for those mutations in the main analysis.

5.8 Confidence grading

Once variants that were and were not associated with resistant phenotypes were identified with the SOLO algorithm and relevant association statistics were generated, as described in "Association studies" above, a set of consensus statistical thresholds and additional grading rules for confidence grading and ranking the observed MTBC mutations were applied to stratify the associations into five Groups according to the strength of the evidence for a genotype–phenotype association and the prioritization (according to level of support) of the phenotypic method used (see "Prioritization of pDST results" above):

Group 1: Assoc with R

Group 2: Assoc with R–interim

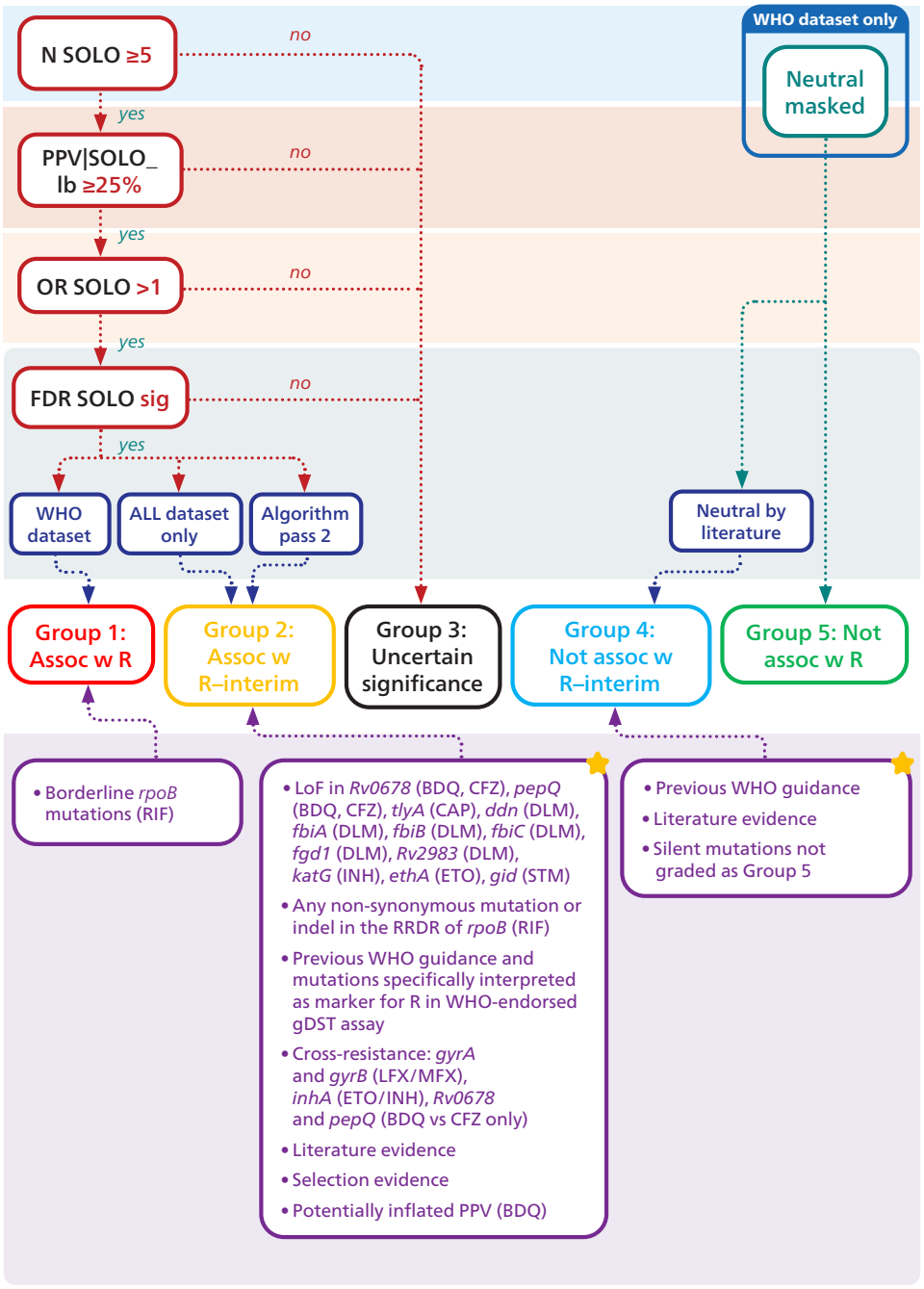
Group 3: Uncertain significance

Group 4: Not assoc with R–interim

Group 5: Not assoc with R

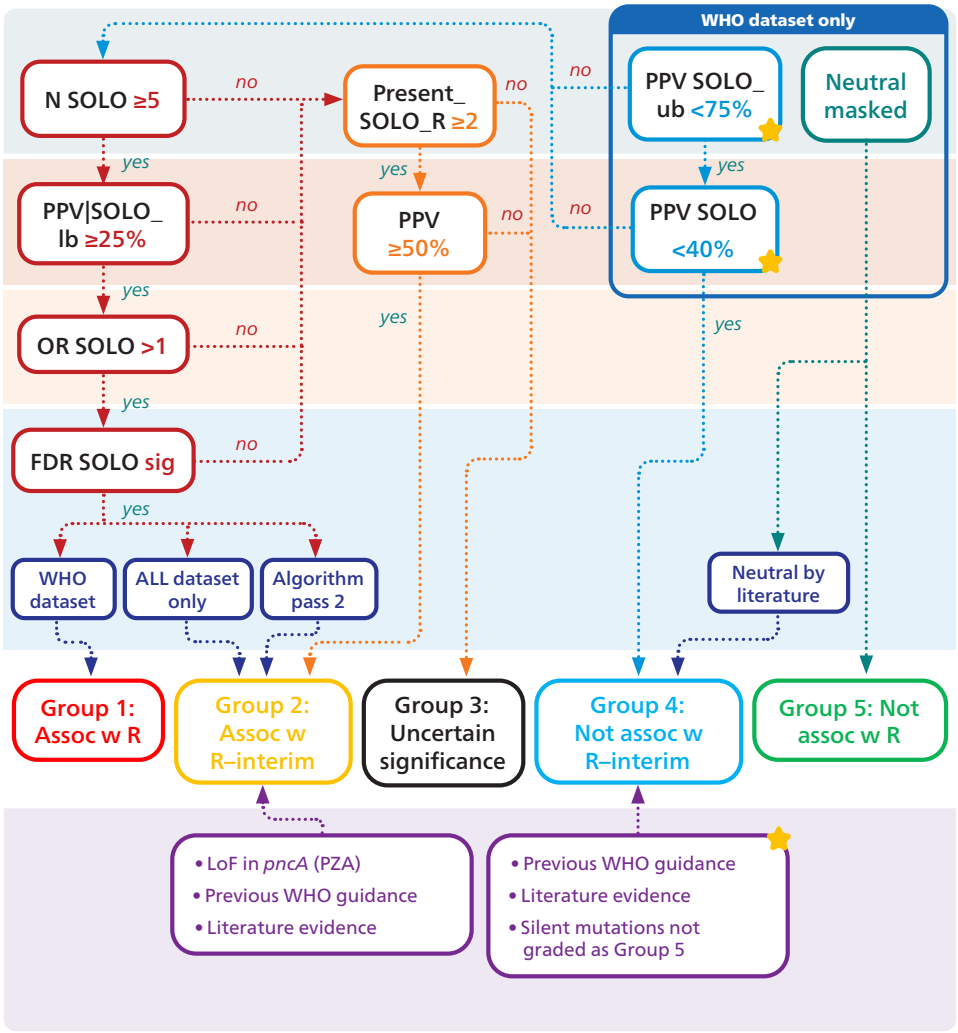
Grading criteria (see specific criteria below) were applied equally to all mutations for all drugs, as shown in Fig. 8. As individual mutations associated with PZA resistance are both found less frequently and distributed more broadly among genes such as *pncA* than other resistance mutations (such as those in *rpoB*), they required special consideration. Therefore, we applied "relaxed" grading criteria with less stringent thresholds to identify additional, infrequent mutations associated and not associated with resistance to PZA in the *pncA* gene only (Fig. 9).

Fig. 8. Standard criteria for grading variants



Stars denote changes from the approach used for the first edition.

Fig. 9. "Relaxed" criteria for grading PZA variants



Stars denote changes from the approach used for the first edition.

General principles of the grading approach and presentation of mutation tables

- While most mutations were identified from the data set that we analysed, some that were assigned to Group 1, 2 or 4 were identified from the literature and from published WHO documents, according to additional grading rules. Any mutations in the tables from these sources are clearly marked as such. All mutations not flagged as such were processed in the algorithm and then classified according to the grading criteria detailed below. The additional grading rules were based on the available evidence and will be revised as new evidence emerges.
- The grading criteria used to stratify mutations into Group 2 or 4 were generally more permissive than those for Group 1 or 5 or identified solely with pDST methods that have not been endorsed by WHO.
- Group 3 mutations could not be classified from the available data. They comprise all mutations that do not fall into Group 1, 2, 4 or 5.

- While the MICs of the mutations analysed in this data set were not evaluated to determine whether the observed mutations were associated with high or low MICs to specific drugs, WHO previously endorsed specific mutations associated with "high" or "low" MICs to INH and MFX *(4,15,19)*. These mutations are therefore flagged as "high" or "low" in the tables in the relevant report (see Table 1).

Criteria for initial confidence grading

Group 1: Assoc w R

Mutations that met five criteria:

1. number of resistant and susceptible isolates with the SOLO mutation (Present_SOLO_SR) ≥ 5
2. lower bound of 95% CI of PPV conditional on being SOLO (PPV|SOLO_lb) ≥ 25%
3. OR > 1 (always applies if criterion 4 is met)
4. OR SOLO mutation > 1
5. statistical significance of OR as SOLO mutation (OR SOLO_FE-sig) with Fisher exact test for false discovery rate, false discovery rate-corrected $P \leq 0.05$

Group 2: Assoc w R–interim

Mutations that met "relaxed" criteria for *pncA*:

1. resistant isolates with the SOLO mutation (Present_SOLO_R) ≥ 2
2. PPV ≥ 50%

Group 3: Uncertain significance

All mutations that did not meet the criteria for inclusion in Group 1, 2, 4 or 5.

Group 4: Not assoc with R–interim

Silent mutations not classified as neutral in steps a and b of the neutral algorithm (Fig. 7a and 7b).

Mutations that met "relaxed" criteria for *pncA*:

1. PPV SOLO < 40%
2. upper bound of 95% CI of PPV SOLO < 75%

To ensure a conservative approach, phenotypes unique to category 3 (i.e. not WHO-endorsed) were not used to identify Group 4 or 5 mutations.

Group 5: Not assoc with R

Neutral mutations that were masked before use of the algorithm (see "Initial identification of neutral mutations").

Additional grading rules applied for final confidence grading

When the initial confidence grades of mutations selected according to statistical thresholds conflicted with strong evidence before the analysis, additional grading rules and established precedent were applied. As far as possible, the grading was based on the available data, and such changes were kept to a minimum. When additional criteria were used to override the initial confidence grading, the mutation in question was annotated with the specific criterion applied in the mutation tables. The basis for these changes and the abbreviations for each criterion are described below (the rules relevant for classifying mutations that are not graded in this catalogue are also included in Table 1).

Group 1 (Assoc w R):

1. Four "borderline" *rpoB* resistance mutations (Leu430Pro, His445Asn, His445Ser and Ile491Phe) were assigned a final grading of Group 1 because previous WHO guidance explicitly states that these mutations are valid markers of resistance, do not require confirmation by pDST and that their detection overrules a susceptible pDST result *(4)*. Without this reclassification, their final confidence grading would have been Group 3, except for Ile491Phe, which would have been Group 2.

Group 2 (Assoc w R–interim):

1. All only: tier-1 mutations that met the criteria for Group 1 during initial confidence grading only because the ALL data set contained methods that were not endorsed by WHO.
2. Pass 2: tier-1 mutations that met the criteria for Group 1 only during initial confidence grading in pass 2 of the algorithmic method (i.e. after masking mutations classified as neutral during pass 1).
3. Tier 2: probably-2 mutations that met the criteria for Group 1 during the initial confidence grading (i.e. based on algorithmic decisions about tier 1-mutations). This did not apply to any of the mutations in this analysis.
4. WHO precedent ("prev. WHO"): Two LFX mutations (*gyrA* Gly88Ala and Ala504Val) and one STM mutation (*rpsL* Lys88Gln) were recognized as Group 2 in accordance with WHO precedents *(6,168)*.
5. WHO-endorsed genotypic DST assays ("WHO-end. gDST"): any mutations specifically interpreted as markers of resistance in one or more of the WHO-endorsed assays listed in Table 4.
6. RRDR additional grading rule ("RRDR"): Any non-synonymous mutation or indel in the RRDR of *rpoB (4)*.
7. FQ cross-resistance additional grading rule ("FQ X-R"): Any *gyrA* or *gyrB* mutation that was ultimately graded into Group 1 or 2 for LFX but initially graded into Group 3 for MFX was upgraded to Group 2 for MFX and vice versa.

8. INH-ETO cross-resistance additional grading rule ("INH-ETO X-R"): Any *inhA* or *fabG1* mutation that was ultimately graded into Group 1 or 2 for INH but initially graded into Group 3 for ETO was upgraded to Group 2 for ETO and vice versa *(19,49,50)*.
9. BDQ-CFZ cross-resistance additional grading rule ("BDQ-CFZ X-R"): Any *Rv0678* or *pepQ* mutation that was ultimately graded into Group 1 or 2 for BDQ but initially graded into Group 3 for CFZ was upgraded to Group 2 for CFZ *(70,73,74)*.
10. Selection experiment additional grading rule ("Selection"): specific mutations in *atpE (67–69,71,72)* or *rrl (79–84)* found to confer resistance during in-vitro or animal selection in MTBC in at least two laboratories.[4] This two-laboratory threshold was also met for pooled LoF mutations in *ddn*, *fbiA*, *fbiB*, *fbiC*, *fgd1* and *Rv2983* for DLM and/or PMD *(84–87)*.
11. LoF additional grading rule ("LoF"): Any premature stop codon (i.e. nonsense mutation), gene deletion (feature_ablation), frameshift or start-loss mutation in the coding regions of *ethA* (ETO), *gid* (STM), *katG* (INH), *pncA* (PZA), *Rv0678* (BDQ, CFZ), *pepQ* (BDQ, CFZ), *ddn* (DLM), *fbiA* (DLM), *fbiB* (DLM), *fbiC* (DLM), *fgd1* (DLM), *Rv2983* (DLM) and *tlyA* (CAP) was considered a Group 2 mutation for that drug. The mechanisms by which LoF mutations in these genes confer resistance is well understood, and, except for *Rv0678*, no epistatic interaction is known that could render an isolate with a LoF mutation in one of these genes susceptible *(64,93,94,97,145,169–171)*.
12. Literature evidence additional grading rule ("Lit."): This rule was used to support classification of the *pncA* Ile31Thr mutation into drug susceptibility 2 for PZA *(64)*.
13. Potentially inflated PPV (Pot. infl. PPV): This rule was used to downgrade all Group 1 BDQ SOLO mutations from laboratory 1 into Group 2.
14. Interim on WHO: If the initial confidence grading was Group 2 for the WHO data set but Group 1 for the ALL data set, the Group 2 classification was used.

Group 4 (Not assoc w R-interim):

1. Silent mutations not classified as neutral in steps a and b of the neutral algorithm.
2. Previous WHO guidance: mutations previously documented as "not associated with R" *(6, 34)* that did not meet the criteria for Group 1, 2 or 5.
3. Literature evidence additional grading rule ("Lit."): mutations previously documented as "Not associated with R" that are frequent in some settings were placed in Group 4:
    a. Mutations that could not be classified as neutral in the WHO data set but that were classified as neutral in the literature for RIF, INH, EMB and PZA *(107)*, and for BDQ *(166,167)* according to pDST data and were consequently masked before use of the algorithmic method; and
    b. *gyrA* Thr80Ala and Ala90Gly, because these are frequent in the Uganda genotype *(6,28)*.

[4] Lee J, personal communication, 2023; Takaki A, Mitarai S, personal communication, 2023; Andres S, personal communication, 2023.

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Annex 1. Further information

The performance of the first edition was compared with that of the second edition for majority variants (allele frequency ≥ 75%) in the ALL data set. The latter figures correspond to the "combined performance" numbers in Table 3 of the main text. The role of minority variants (allele frequency ≥ 25%) was calculated for the second edition only.

Table A1.1. Comparison of the performance of the first and second edition and impact of minority variants

Drug	Catalogue, threshold for variants (%)	Sensitivity, specificity, PPV (% [95% CI])	Changes in sensitivity, specificity, PPV (%)
RIF	First, 75	93.3 (92.9–93.7), 96.9 (96.7–97.1), 94.2 (93.9–94.6)	
	Second, 75	93.3 (92.9–93.7), 96.9 (96.7–97.1), 94.2 (93.9–94.6)	0, 0, 0
	Second, 25	94.4 (94.0–94.7), 96.8 (96.6–97.0), 94.2 (93.8–94.5)	1.1, -0.1, 0
INH	First, 75	91.0 (90.6–91.4), 98.1 (97.9–98.2), 97.3 (97.1–97.5)	
	Second, 75	91.6 (91.2–92.0), 97.9 (97.8–98.1), 97.1 (96.8–97.3)	0.6, -0.2, -0.2
	Second, 25	92.1 (91.7–92.4), 97.9 (97.7–98.0), 97.0 (96.8–97.2)	0.5, 0.0, -0.1
EMB	First, 75	83.8 (83.1–84.6), 91.2 (90.9–91.5), 71.6 (70.7–72.4)	
	Second, 75	81.1 (80.3–81.9), 91.6 (91.3–91.9), 71.9 (71.0–72.8)	-2.7, 0.4, 0.3
	Second, 25	82.1 (81.3–82.9), 91.4 (91.1–91.7), 71.7 (70.9–72.6)	1, -0.2, -0.2
PZA	First, 75	73.7 (72.4–75.0), 98.0 (97.8–98.2), 90.8 (89.8–91.7)	
	Second, 75	78.0 (76.8–79.2), 97.9 (97.6–98.1), 90.5 (89.5–91.4)	4.3, -0.1, -0.3
	Second, 25	80.0 (78.8–81.2), 97.7 (97.5–98.0), 90.3 (89.4–91.2)	2, -0.2, -0.2
LFX	First, 75	83.7 (82.7–84.6), 97.1 (96.9–97.3), 88.7 (87.8–89.5)	
	Second, 75	84.8 (83.9–85.7), 96.9 (96.7–97.1), 88.1 (87.3–89.0)	1.1, -0.2, -0.6
	Second, 25	89.2 (88.4–90.0), 96.7 (96.4–96.9), 87.9 (87.0–88.7)	4.4, -0.2, -0.2
MFX	First, 75	85.0 (83.8–86.1), 93.8 (93.4–94.1), 74.5 (73.2–75.8)	
	Second, 75	85.7 (84.6–86.8), 93.5 (93.2–93.9), 74.0 (72.7–75.2)	0.7, -0.3, -0.5
	Second, 25	90.3 (89.4–91.2), 93.2 (92.8–93.5), 74.0 (72.7–75.2)	4.6, -0.3, 0
BDQ	First, 75	0 (0–0.4), 100.0 (100.0–100.0), 0 (0–0)	
	Second, 75	49.4 (46.3–52.5), 98.7 (98.5–98.9), 75.2 (71.8–78.4)	49.4, -1.3, 75.2
	Second, 25	59.6 (56.5–62.6), 98.4 (98.2–98.6), 75.0 (71.9–77.9)	10.2, -0.3, -0.2
LZD	First, 75	27.3 (22.8–32.1), 99.8 (99.8–99.9), 78.5 (70.4–85.2)	
	Second, 75	34.0 (29.2–39.0), 99.8 (99.7–99.9), 78.4 (71.3–84.5)	6.7, 0, -0.1
	Second, 25	35.0 (30.2–40.1), 99.8 (99.7–99.8), 75.7 (68.6–81.9)	1.0, 0, -2.7
CFZ	First, 75	0 (0–0.5), 100.0 (100.0–100.0), 0 (0–0)	
	Second, 75	17.0 (14.2–20.0), 98.7 (98.5–98.9), 38.1 (32.6–43.8)	17.0, -1.3, 38.1
	Second, 25	21.3 (18.2–24.5), 98.4 (98.2–98.6), 38.9 (33.9–44.1)	4.3, -0.3, 0.8

Drug	Catalogue, threshold for variants (%)	Sensitivity, specificity, PPV (% [95% CI])	Changes in sensitivity, specificity, PPV (%)
DLM	First, 75	4.4 (2.2–7.7), 100.0 (99.9–100.0), 84.6 (54.6–98.1)	
	Second, 75	14.7 (10.6–19.7), 99.9 (99.8–99.9), 72.5 (58.3–84.1)	10.3, -0.1, -12.1
	Second, 25	15.1 (10.9–20.1), 99.8 (99.7–99.9), 62.3 (49.0–74.4)	0.4, -0.1, -10.2
AMK	First, 75	72.9 (71.1–74.6), 98.1 (97.9–98.3), 81.3 (79.6–82.9)	
	Second, 75	72.8 (71.0–74.6), 98.3 (98.1–98.5), 82.8 (81.2–84.4)	-0.1, 0.2, 1.5
	Second, 25	75.4 (73.7–77.1), 98.2 (98.0–98.4), 82.2 (80.6–83.8)	2.6, -0.1, -0.6
STM	First, 75	79.2 (78.4–80.0), 94.1 (93.7–94.5), 89.9 (89.2–90.5)	
	Second, 75	79.7 (78.9–80.5), 94.1 (93.7–94.4), 89.9 (89.3–90.5)	0.5, 0, 0
	Second, 25	80.3 (79.5–81.1), 94.0 (93.6–94.4), 89.8 (89.2–90.4)	0.6, -0.1, -0.1
ETO	First, 75	72.5 (71.3–73.7), 86.6 (86.1–87.2), 64.4 (63.2–65.7)	
	Second, 75	74.8 (73.6–76.0), 85.9 (85.3–86.4), 63.9 (62.7–65.1)	2.3, -0.7, -0.5
	Second, 25	76.1 (74.9–77.2), 85.6 (85.0–86.1), 63.8 (62.6–65.0)	1.3, -0.3, -0.1
KAN[a]	First, 75	75.1 (73.6–76.5), 96.5 (96.3–96.8), 78.7 (77.2–80.0)	
	Second, 75	74.9 (73.4–76.3), 96.7 (96.4–96.9), 79.3 (77.9–80.7)	-0.2, 0.2, 0.6
	Second, 25	77.2 (75.8–78.5), 96.6 (96.3–96.8), 79.2 (77.8–80.5)	2.3, -0.1, -0.1
CAP[a]	First, 75	66.4 (64.3–68.4), 97.8 (97.6–98.1), 80.1 (78.2–82.0)	
	Second, 75	66.2 (64.1–68.2), 97.8 (97.6–98.1), 80.1 (78.1–81.9)	-0.2, 0, 0
	Second, 25	69.3 (67.3–71.3), 97.6 (97.3–97.8), 79.2 (77.3–81.1)	3.1, -0.2, -0.9

[a] Drugs no longer recommended for TB treatment.

The performance of the second edition for minority variants in the ALL data set (these figures are also included in Table A.1) was stratified by the genotypic RIF result (pDST could not be used as it was not available for all isolates). Results for RIF are not shown, as stratifying them by genotypic RIF resistance would not have yielded meaningful results.

Table A1.2. Impact of rifampicin resistance on the performance of the second edition

Drug	Genotypic RIF results	% R (95% CI)	Sensitivity, specificity, PPV (% [95% CI])	Changes in sensitivity, specificity, PPV (%)
INH	R+S	43.0 (42.6–43.5)	92.1 (91.7–92.4), 97.9 (97.7–98.0), 97.0 (96.8–97.2)	
	R	93.5 (93.2–93.9)	96.7 (96.4–97.0), 73.1 (70.5–75.7), 98.1 (97.9–98.3)	4.6, -24.8, 1.1
	S	14.2 (13.8–14.6)	74.8 (73.5–76.0), 98.9 (98.8–99.0), 92.0 (91.0–92.9)	-17.3, 1.0, -5
EMB	R+S	21.0 (20.6–21.3)	82.1 (81.3–82.9), 91.4 (91.1–91.7), 71.7 (70.9–72.6)	
	R	56.8 (56.0–57.6)	86.0 (85.2–86.7), 56.9 (55.7–58.1), 72.4 (71.6–73.3)	3.9, -34.5, 0.7
	S	2.3 (2.1–2.5)	32.1 (28.6–35.8), 99.4 (99.3–99.4), 54.1 (49.1–59.0)	-50, 8.0, -17.6
PZA	R+S	20.8 (20.2–21.3)	80.0 (78.8–81.2), 97.7 (97.5–98.0), 90.3 (89.4–91.2)	
	R	58.4 (57.2–59.6)	88.7 (87.6–89.7), 86.3 (85.0–87.6), 90.1 (89.1–91.0)	8.7, -11.4, -0.2
	S	4.9 (4.5–5.2)	36.0 (32.5–39.6), 99.9 (99.8–99.9), 93.2 (89.6–95.9)	-44, 2.2, 2.9
LFX	R+S	21.3 (20.8–21.8)	89.2 (88.4–90.0), 96.7 (96.4–96.9), 87.9 (87.0–88.7)	
	R	36.0 (35.3–36.8)	92.6 (91.8–93.2), 93.0 (92.4–93.5), 88.1 (87.2–88.9)	3.4, -3.7, 0.2
	S	3.6 (3.3–4.0)	49.6 (44.9–54.3), 99.6 (99.5–99.7), 83.0 (78.0–87.3)	-39.6, 2.9, -4.9
MFX	R+S	17.7 (17.2–18.2)	90.3 (89.4–91.2), 93.2 (92.8–93.5), 74.0 (72.7–75.2)	
	R	35.1 (34.2–36.0)	93.3 (92.5–94.1), 82.6 (81.7–83.5), 74.4 (73.1–75.6)	3, -10.6, 0.4
	S	2.6 (2.3–2.9)	54.9 (49.2–60.5), 99.3 (99.1–99.4), 66.3 (60.2–72.0)	-35.4, 6.1, -7.7
BDQ	R+S	7.3 (6.9–7.8)	59.6 (56.5–62.6), 98.4 (98.2–98.6), 75.0 (71.9–77.9)	
	R	14.2 (13.3–15.0)	61.8 (58.7–64.9), 96.6 (96.1–97.1), 75.0 (71.8–78.0)	2.2, -1.8, 0
	S	1.0 (0.8–1.3)	31.6 (21.4–43.3), 99.9 (99.8–100.0), 75.0 (56.6–88.5)	-28, 1.5, 0.1
LZD	R+S	2.1 (1.9–2.3)	35.0 (30.2–40.1), 99.8 (99.7–99.8), 75.7 (68.6–81.9)	
	R	3.0 (2.7–3.4)	44.9 (39.0–50.9), 99.6 (99.4–99.7), 75.7 (68.6–82.0)	9.9, -0.2, 0
	S	1.0 (0.8–1.3)	3.4 (0.7–9.5), 100.0 (99.9–100.0), 75.0 (19.4–99.4)	-31.6, 0.2, -0.7
CFZ	R+S	4.5 (4.2–4.9)	21.3 (18.2–24.5), 98.4 (98.2–98.6), 38.9 (33.9–44.1)	
	R	6.3 (5.7–6.9)	31.5 (27.2–36.2), 96.8 (96.3–97.2), 39.6 (34.4–45.0)	10.2, -1.6, 0.7
	S	3.1 (2.7–3.5)	3.6 (1.7–6.8), 99.7 (99.6–99.8), 31.0 (15.3–50.8)	-17.7, 1.3, -7.9
DLM	R+S	2.1 (1.9–2.4)	15.1 (10.9–20.1), 99.8 (99.7–99.9), 62.3 (49.0–74.4)	
	R	2.4 (2.0–2.8)	16.0 (9.9–23.8), 99.8 (99.6–99.9), 63.3 (43.9–80.1)	0.9, 0, 1
	S	2.0 (1.7–2.3)	14.3 (8.8–21.4), 99.8 (99.7–99.9), 61.3 (42.2–78.2)	-0.8, 0, -1
AMK	R+S	10.0 (9.7–10.4)	75.4 (73.7–77.1), 98.2 (98.0–98.4), 82.2 (80.6–83.8)	
	R	17.0 (16.4–17.7)	80.2 (78.5–81.8), 96.5 (96.1–96.8), 82.4 (80.7–83.9)	4.8, -1.7, 0.2
	S	1.7 (1.5–2.0)	19.8 (14.5–26.1), 99.9 (99.8–99.9), 76.5 (62.5–87.2)	-55.6, 1.7, -5.7
STM	R+S	39.8 (39.2–40.4)	80.3 (79.5–81.1), 94.0 (93.6–94.4), 89.8 (89.2–90.4)	
	R	77.7 (76.8–78.5)	85.0 (84.2–85.8), 76.5 (74.7–78.2), 92.6 (92.0–93.2)	4.7, -17.5, 2.8
	S	14.9 (14.4–15.5)	64.3 (62.4–66.3), 97.0 (96.7–97.3), 79.0 (77.1–80.8)	-16, 3, -10.8
ETO	R+S	25.0 (24.4–25.6)	76.1 (74.9–77.2), 85.6 (85.0–86.1), 63.8 (62.6–65.0)	
	R	38.6 (37.7–39.5)	77.9 (76.6–79.1), 72.5 (71.5–73.5), 64.1 (62.8–65.3)	1.8, -13.1, 0.3
	S	7.4 (6.8–7.9)	63.8 (60.0–67.4), 96.8 (96.4–97.2), 61.6 (57.9–65.2)	-12.3, 11.2, -2.2
KAN[a]	R+S	14.5 (14.1–15.0)	77.2 (75.8–78.5), 96.6 (96.3–96.8), 79.2 (77.8–80.5)	
	R	25.1 (24.4–25.9)	82.3 (81.0–83.6), 92.8 (92.3–93.3), 79.3 (77.9–80.7)	5.1, -3.8, 0.1
	S	2.5 (2.2–2.8)	17.6 (13.4–22.5), 99.8 (99.7–99.9), 71.4 (59.4–81.6)	-59.6, 3.2, -7.8
CAP[a]	R+S	11.7 (11.2–12.1)	69.3 (67.3–71.3), 97.6 (97.3–97.8), 79.2 (77.3–81.1)	
	R	16.6 (15.9–17.2)	72.8 (70.7–74.8), 96.3 (95.9–96.7), 79.7 (77.7–81.5)	3.5, -1.3, 0.5
	S	2.4 (2.0–2.8)	23.6 (16.9–31.4), 99.7 (99.5–99.8), 64.2 (49.8–76.9)	-45.7, 2.1, -15

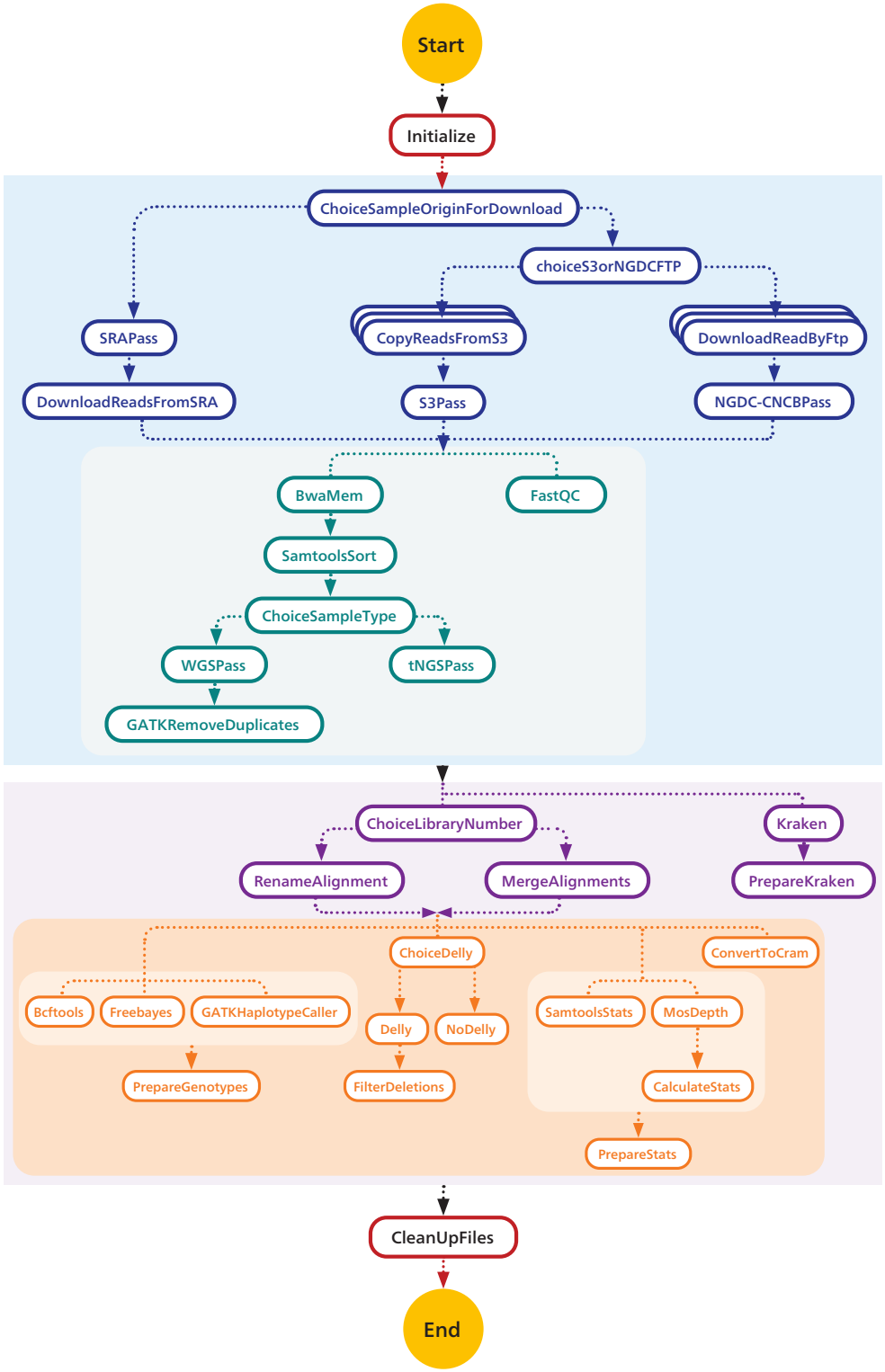
[a] Drugs no longer recommended for TB treatment.

We explored the extent to which rare Group 3 mutations in the following nonessential genes that did not meet the standard grading thresholds (Fig. 8) might account for the "missing" sensitivity as compared with pDST: INH (*katG*), PZA (*pncA*), BDQ and CFZ (*Rv0678*), and DLM (*ddn*, *fbiA*, *fbiB*, *fbiC*, *fgd1*, and *Rv2983*). We used two approaches. First, we extended the "relaxed" grading criteria that were WHO-endorsed in the first edition to classify rare *pncA* mutations into Group 2 (Fig. 9) to the remaining genes of interest (coding and upstream regions). The mutations that met these "relaxed" criteria were highlighted in footnotes in the catalogue master file. The resulting sensitivities, specificities and PPVs are shown in the "1+2+3 relaxed" entries in the table below. As the "relaxed" criteria were already approved for *pncA*, there is no "1+2+3 relaxed" entry for PZA (i.e. the mutations in question were already in Group 2 and therefore included under "Groups 1+2"). Secondly, we assumed that any coding mutation (upstream regions excluded) in the above genes, except silent and Groups 4/5 mutations, might also be valid markers of resistance in isolates that are genotypically resistant to RIF. The resulting figures are shown as "1+2+3 coding" entries. All calculations were conducted with a 25% allele frequency as the threshold for calling mutations in the ALL data set. **These results are only for information available at this time**, and the same limitations for e.g. sampling and overfitting discussed in Section 2.4 apply to these calculations. A more detailed discussion of these findings is provided in the relevant sections for drugs in Section 3.

Table A1.3. Potential role of rare Group 3 mutations for predicting resistance to INH, PZA, BDQ and DLM

Drug	Genotypic RIF results	% R (95% CI)	Mutation Groups	Sensitivity, specificity, PPV (% [95% CI])	Changes in sensitivity, specificity, PPV relative to Group 1+2 (%)
INH	R+S	43.0 (42.6–43.5)	1+2	92.1 (91.7–92.4), 97.9 (97.7–98.0), 97.0 (96.8–97.2)	
			1+2+3 relaxed	93.3 (93.0–93.6), 97.7 (97.5–97.9), 96.8 (96.6–97.1)	1.2, -0.2, -0.2
			1+2+3 coding	93.8 (93.4–94.1), 97.5 (97.3–97.7), 96.6 (96.4–96.9)	1.7, -0.4, -0.4
	R	93.5 (93.2–93.9)	1+2	96.7 (96.4–97.0), 73.1 (70.5–75.7), 98.1 (97.9–98.3)	
			1+2+3 relaxed	97.6 (97.4–97.9), 71.8 (69.1–74.4), 98.0 (97.8–98.3)	0.9, -1.3, -0.1
			1+2+3 coding	98.8 (98.7–99.0), 65.3 (62.4–68.0), 97.6 (97.4–97.9)	2.1, -7.8, -0.5
	S	14.2 (13.8–14.6)	1+2	74.8 (73.5–76.0), 98.9 (98.8–99.0), 92.0 (91.0–92.9)	
			1+2+3 relaxed	77.0 (75.7–78.2), 98.8 (98.7–98.9), 91.5 (90.5–92.4)	2.2, -0.1, -0.5
PZA	R+S	20.8 (20.2–21.3)	1+2	80.0 (78.8–81.2), 97.7 (97.5–98.0), 90.3 (89.4–91.2)	
			1+2+3 coding	82.6 (81.5–83.7), 97.0 (96.7–97.3), 87.9 (86.8–88.9)	2.6, -0.7, -2.4
	R	58.4 (57.2–59.6)	1+2	88.7 (87.6–89.7), 86.3 (85.0–87.6), 90.1 (89.1–91.0)	
			1+2+3 coding	91.8 (90.8–92.6), 81.6 (80.1–83.0), 87.5 (86.4–88.5)	3.1, -4.7, -2.6
	S	4.9 (4.5–5.2)	1+2	36.0 (32.5–39.6), 99.9 (99.8–99.9), 93.2 (89.6–95.9)	
BDQ	R+S	7.3 (6.9–7.8)	1+2	59.6 (56.5–62.6), 98.4 (98.2–98.6), 75.0 (71.9–77.9)	
			1+2+3 relaxed	68.8 (65.8–71.6), 98.3 (98.1–98.5), 76.6 (73.7–79.3)	9.2, -0.1, 1.6
			1+2+3 coding	75.1 (72.4–77.7), 97.7 (97.4–97.9), 71.9 (69.1–74.5)	15.5, -0.7, -3.1
	R	14.2 (13.3–15.0)	1+2	61.8 (58.7–64.9), 96.6 (96.1–97.1), 75.0 (71.8–78.0)	
			1+2+3 relaxed	71.0 (68.0–73.8), 96.4 (95.9–96.9), 76.6 (73.7–79.4)	9.2, -0.2, 1.6
			1+2+3 coding	78.6 (75.8–81.1), 94.9 (94.3–95.4), 71.8 (68.9–74.5)	16.8, -1.7, -3.2
	S	1.0 (0.8–1.3)	1+2	31.6 (21.4–43.3), 99.9 (99.8–100.0), 75.0 (56.6–88.5)	
			1+2+3 relaxed	40.8 (29.6–52.7), 99.9 (99.7–99.9), 75.6 (59.7–87.6)	9.2, 0, 0.6
CFZ	R+S	4.5 (4.2–4.9)	1+2	21.3 (18.2–24.5), 98.4 (98.2–98.6), 38.9 (33.9–44.1)	
			1+2+3 relaxed	24.2 (21.0–27.6), 98.3 (98.1–98.5), 40.6 (35.8–45.6)	2.9, -0.1, 1.7
			1+2+3 coding	29.4 (26.0–33.0), 97.7 (97.4–97.9), 37.6 (33.5–41.9)	8.1, -0.7, -1.3
	R	6.3 (5.7–6.9)	1+2	31.5 (27.2–36.2), 96.8 (96.3–97.2), 39.6 (34.4–45.0)	
			1+2+3 relaxed	35.5 (31.0–40.3), 96.6 (96.2–97.1), 41.4 (36.3–46.6)	4, -0.2, 1.8
			1+2+3 coding	44.4 (39.6, 49.2), 95.2 (94.6, 95.7), 38.0 (33.7, 42.4)	12.9, -1.6, -1.6
	S	3.1 (2.7–3.5)	1+2	3.6 (1.7–6.8), 99.7 (99.6–99.8), 31.0 (15.3–50.8)	
			1+2+3 relaxed	4.8 (2.5–8.3), 99.7 (99.5–99.8), 32.4 (18.0–49.8)	1.2, 0, 1.4
DLM	R+S	2.1 (1.9–2.4)	1+2	15.1 (10.9–20.1), 99.8 (99.7–99.9), 62.3 (49.0–74.4)	
			1+2+3 relaxed	17.9 (13.3–23.2), 99.8 (99.7–99.9), 66.2 (53.7–77.2)	2.8, 0, 3.9
			1+2+3 coding	25.0 (19.8–30.8), 91.6 (91.0–92.1), 6.1 (4.7–7.7)	9.9, -8.2, -56.2
	R	2.4 (2.0–2.8)	1+2	16.0 (9.9–23.8), 99.8 (99.6–99.9), 63.3 (43.9–80.1)	
			1+2+3 relaxed	17.6 (11.3–25.7), 99.8 (99.6–99.9), 65.6 (46.8–81.4)	1.6, 0, 2.3
			1+2+3 coding	37.0 (28.3–46.3), 80.4 (79.3–81.5), 4.4 (3.2–5.8)	21, -19.4, -58.9
	S	2.0 (1.7–2.3)	1+2	14.3 (8.8–21.4), 99.8 (99.7–99.9), 61.3 (42.2–78.2)	
			1+2+3 relaxed	18.0 (11.9–25.6), 99.8 (99.7–99.9), 66.7 (49.0–81.4)	3.7, 0, 5.4

Fig. A1.1. Scheme of the bioinformatics pipeline for detecting variants in the second edition



Annex 2. Conflict of interest assessment

WHO held an expert consultation on 28 February, 1 March and 9 March 2023. All individuals who provided technical input were required to disclose any potential conflicts of interest, encompassing both financial and non-financial interests.

The candidates' DOI forms and information retrieved from the internet, were examined by WHO staff members Nazir Ismail and Carl-Michael Nathanson to assess whether there were, or might be, actual or perceived conflicts of interest and, if so, whether a management plan was required. This evaluation process, and resultant management plans, were based on the Guidelines for declaration of interests (WHO experts) and the WHO handbook for guideline development (2nd edition).

Both financial and non-financial interests were considered. A "significant" conflict of interest would include:

- "intellectual bias", where an individual may have repeatedly and publicly taken a position on an issue under review, which may affect the individual's objectivity and independence in the global policy development process;
- involvement in research or publication of materials related to issues under review; and
- a financial interest above US$ 5000.

Developers of any assay are never involved in the process of policy development – such involvement is automatically considered a conflict of interest.

Upon review no significant conflict of interest were identified. Participants' statements were summarized by the WHO at the start of the meeting.

The review findings are summarized in Table A2.1.

Table A2.1. Declarations of interests

Participant	Interest declared	Conclusion
Heidi Albert	FIND has several clinical research projects to evaluate multiple new diagnostic tests against published Target Product Profiles that have been defined through consensus processes.	Conflict of interest not significant
Uladzimir Antoneka	None declared	No conflict of interest
Arnold Bainomugisa	None declared	No conflict of interest
Leonid Chindelevitch	None declared	No conflict of interest
Daniela Cirillo	None declared	No conflict of interest
Francesc Coll	Consulting and employment, Next Gen Diagnostics in the past	Conflict of interest not significant
Rebecca Colman	None declared	No conflict of interest
Iñaki Comas	None declared	No conflict of interest
Sarah Cook-Scalise	None declared	No conflict of interest
Chris Coulter	None declared	No conflict of interest
James Dawson	None declared	No conflict of interest
Maha Farhat	None declared	No conflict of interest
Philip Fowler	Consulting - Scientific and Technical consultant for GPAS Ltd	Conflict of interest not significant
Sophia Georghiou	None declared	No conflict of interest
Patricia Hall-Eidson	None declared	No conflict of interest
Zahra Hasan	None declared	No conflict of interest
Harald Hoffmann	None declared	No conflict of interest
Zamin Iqbal	Grant to support development and pilot of a cloud-based global TB genomic surveillance tool, and testing in Buenos Aires.	Conflict of interest not significant
George Kasule	None declared	No conflict of interest
Claudio Köser	Consulting for BD and TB Alliance, remuneration not declared, advisor for Cepheid in 2022 pro bono, collaboration with Janssen, PZA Innocation and Thermo Fisher with no remuneration	Conflict of interest not significant
Sanjana Kulkarni	Not declared, observer	N/A
Sacha Laurent	None declared	No conflict of interest
Marguerite Massinga Loembé	Grant (acting as project scientific lead for ASLM who is one of the grant recipient) from EDCTP Grant (acting as project scientific lead for ASLM who is the grant recipient) from FIND/BMGF	Conflict of interest not significant
Heather McLaughlin	Not declared, observer	N/A
Alberto Mendoza	None declared	No conflict of interest
Matthias Merker	None declared	No conflict of interest
Paolo Miotto	None declared	No conflict of interest
Satoshi Mitarai	Research grant from Roche diagnostics KK, ceased in 2021	Conflict of interest not significant
Stefan Niemann	Consultation for Illumina, 3700 Euro reimbursement, ended 2022	Conflict of interest not significant
Jamie Posey	None declared	No conflict of interest
Leen Rigouts	None declared	No conflict of interest
Camilla Rodrigues	Research grant from FIND	Conflict of interest not significant

Participant	Interest declared	Conclusion
Timothy Rodwell	Co-inventor on a patent involving the processing of sequencing data for the purposes of detecting drug resistant TB mutations. All rights to future exploitation of and potential income from the patent have been transferred to UCSD	Conflict of interest not significant
Anita Suresh	None declared	No conflict of interest
Swapna Uplekar	None declared	No conflict of interest
Shaheed Vally Omar	Reserarch grant from Janssen Pharma to assess prevalence of BDQ resistance using WGS	Conflict on interest not significant
Wayne van Gemert	None declared	No conflict of interest
Timothy Walker	None declared	No conflict of interest
Zhao Yanlin	None declared	No conflict of interest
Danila Zimenkov	None declared	No conflict of interest

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