Molecular tools for diagnosing drug resistance: Where have we got to?

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Funded by Wellcome

No disclosures

Xpert XDR – already outdated?

WHO recommended use of MTB/RIF Xpert in December 2010

If RIF-resistant, assume INH-resistant and therefore 'MDR'

If INH-resistant but not RIF-resistant, this will go undetected

Any mutation = Resistant

Table A2.4. Gene targets, codon regions and nucleotide sequences that determine presence of variants associated with drug resistance in the Xpert MTB/XDR test

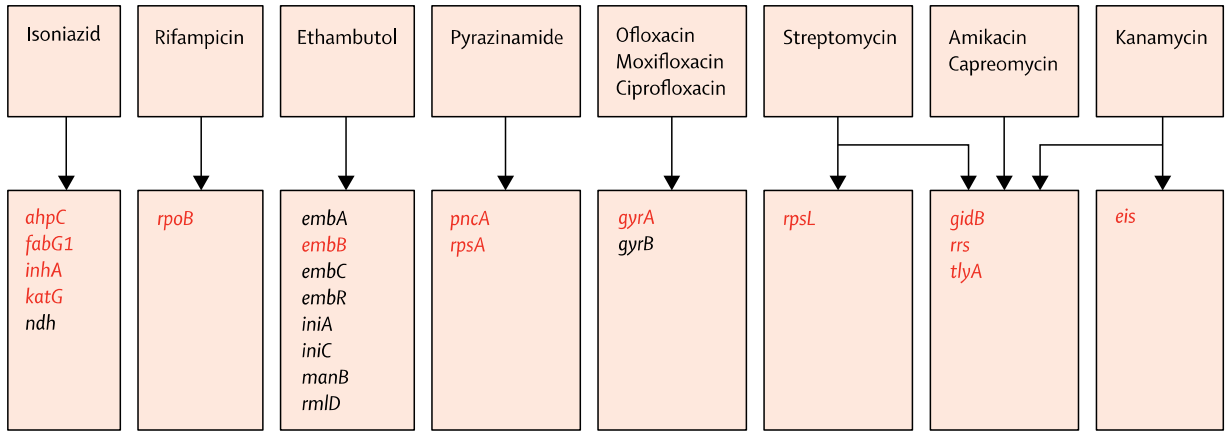
Drug	Gene target	Codon regions	Nucleotide
Isoniazid	*inh*A promoter	Not applicable	–1 to –32 intergenic region
	*kat*G	311–319	939–957
	fabG1	199–210	597–630
	oxyR-ahpC intergenic region	Not applicable	–5 to –50 intergenic region (or –47 to –92)[a]
Ethionamide	*inh*A promoter	Not applicable	–1 to –32 intergenic region
Fluoroquinolones	*gyrA*	87–95	261–285
	gyrB	531–544 (or 493–505)[a]	1596–1632
Amikacin, kanamycin, capreomycin	*rrs*	Not applicable	1396–1417
Amikacin, kanamycin	*eis* promoter	Not applicable	–6 to –42 intergenic region

World Health Organization

Sequencing based diagnostics

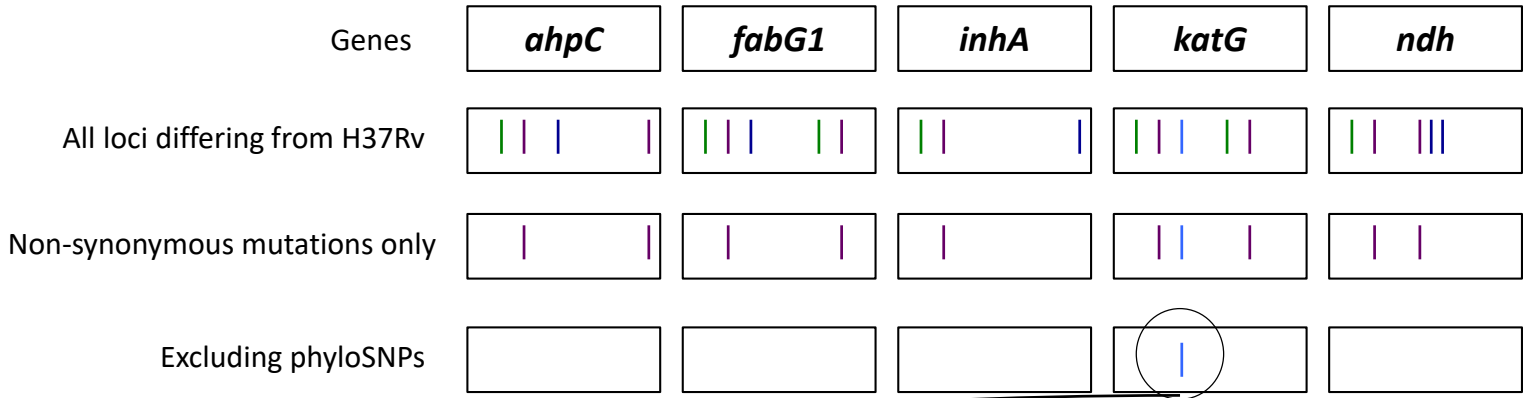
Platform/technology + knowledgebase/catalogue

Sequencing-based diagnostics - knowledgebase



Number of mutations remaining for each drug	Susceptible phenotype (%) (n=8978)	Resistant phenotype (%) (n=701)
0	7566 (84%)	33 (5%)
1	1111 (12%)	518 (74%)
2	211 (2%)	130 (19%)
3	61 (1%)	16 (2%)
4	21 (0%)	3 (0%)
5	6 (0%)	0 (0%)
6	1 (0%)	1 (0%)
7	0 (0%)	0 (0%)
8	1 (0%)	0 (0%)

Sample 1
Drug 1: Isoniazid - R



Conclusion: SNP | is classified as “resistance-determinant” for isoniazid in sample 1

Walker *et al,* Lancet Inf Dis 2015

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

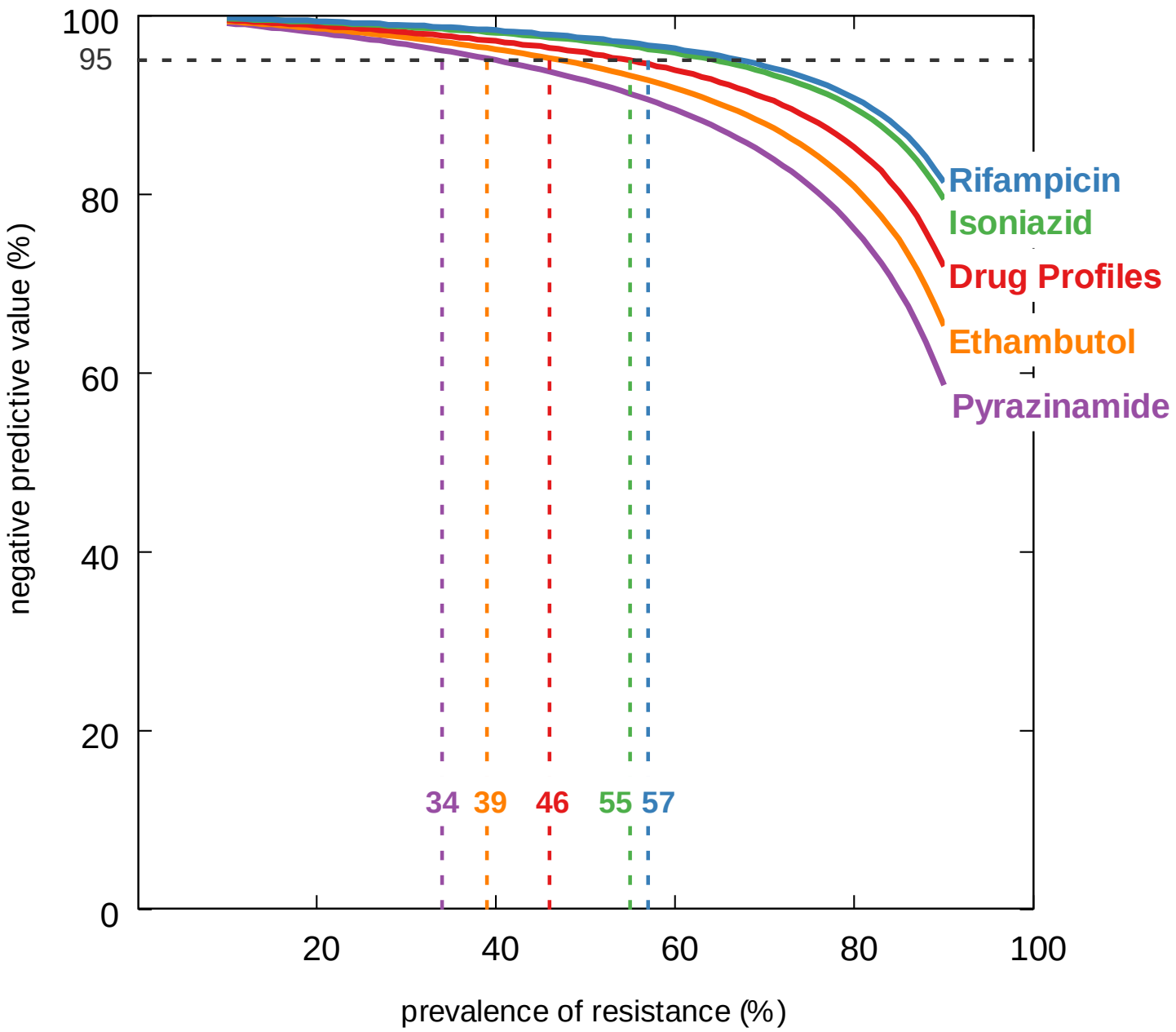
WGS

Table 2. Prediction of Phenotypes of Resistance or Susceptibility to Individual Drugs.*

Analysis and Drug	Resistant Phenotype					Susceptible Phenotype					Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Sensitivity, All†	Specificity, All†	NGP	RP
	R	S	U	F	Total	R	S	U	F	Total								
	number of isolates										*percent*							
WGS, all isolates																		
Isoniazid	3067	90	93	44	3294	65	6313	215	117	6710	97.1 (96.5–97.7)	99.0 (98.7–99.2)	97.9 (97.4–98.4)	98.6 (98.3–98.9)	93.1	94.1	4.7	32.9
Rifampin	2743	69	7	84	2903	85	6763	232	147	7227	97.5 (96.9–98.1)	98.8 (98.5–99.0)	97.0 (96.3–97.6)	99.0 (98.7–99.2)	94.5	93.6	4.6	28.7
Ethambutol	1410	81	94	55	1640	468	6835	781	70	8154	94.6 (93.3–95.7)	93.6 (93.0–94.1)	75.1 (73.0–77.0)	98.8 (98.5–99.1)	86.0	83.8	10.2	16.7
Pyrazinamide	863	82	117	77	1139	204	6146	197	108	6655	91.3 (89.3–93.0)	96.8 (96.3–97.2)	80.9 (78.4–83.2)	98.7 (98.4–99.0)	75.8	92.4	6.4	14.6

NEJM 2018

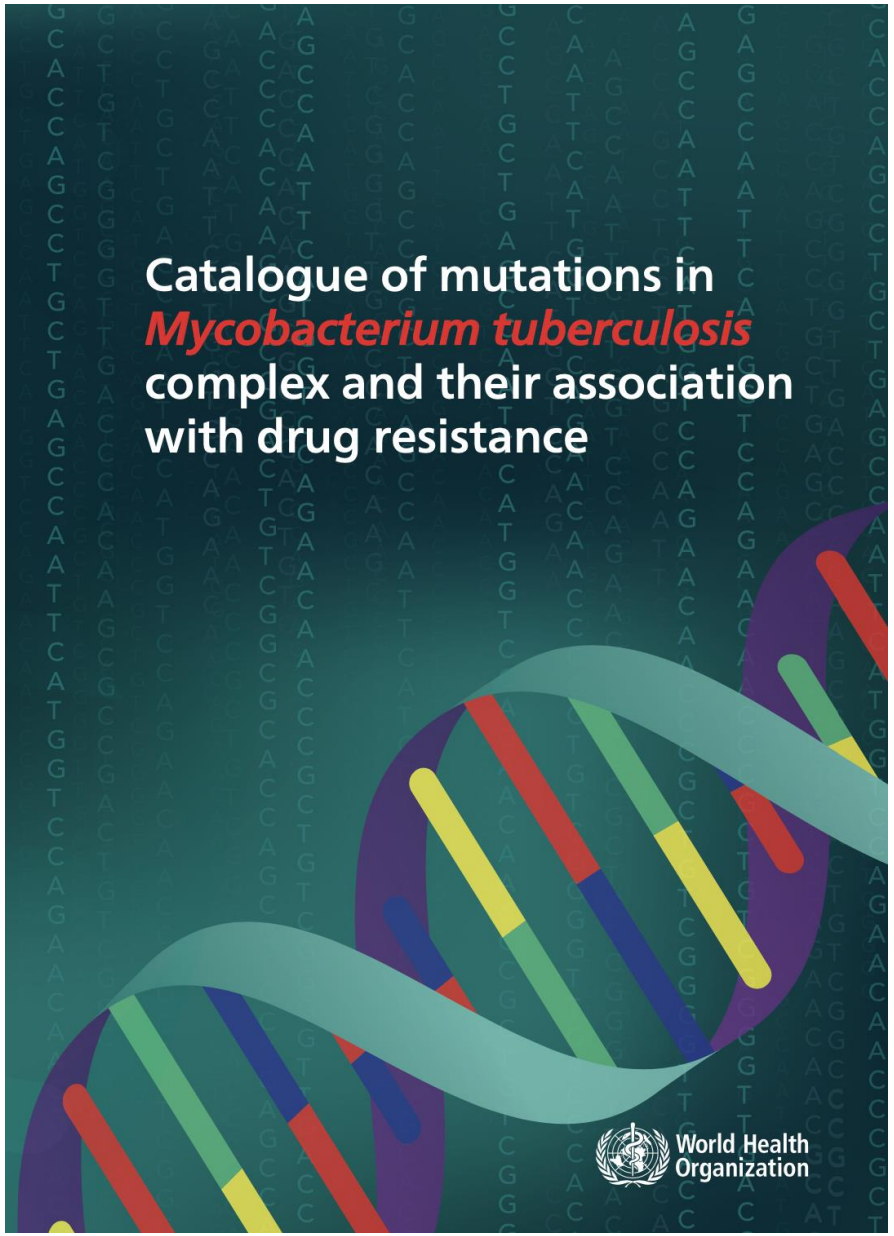
Ternary (R – S – U) vs. binary (R-S) predictions



Predicting entire profiles

NEJM 2018

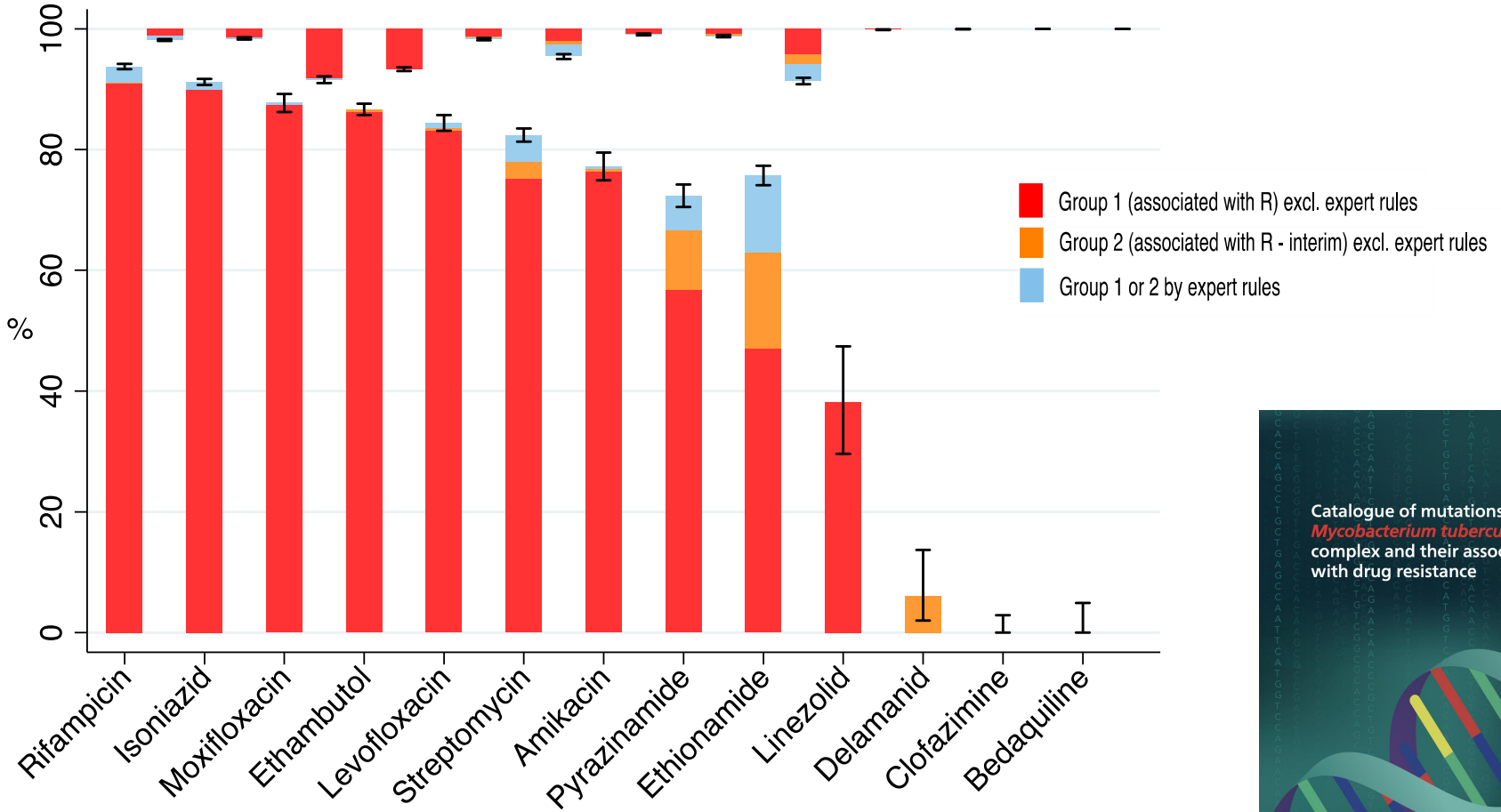
2021



~38,000 genomes from 40 countries

Different from other catalogues

- Expert rules
- Very conservative approach

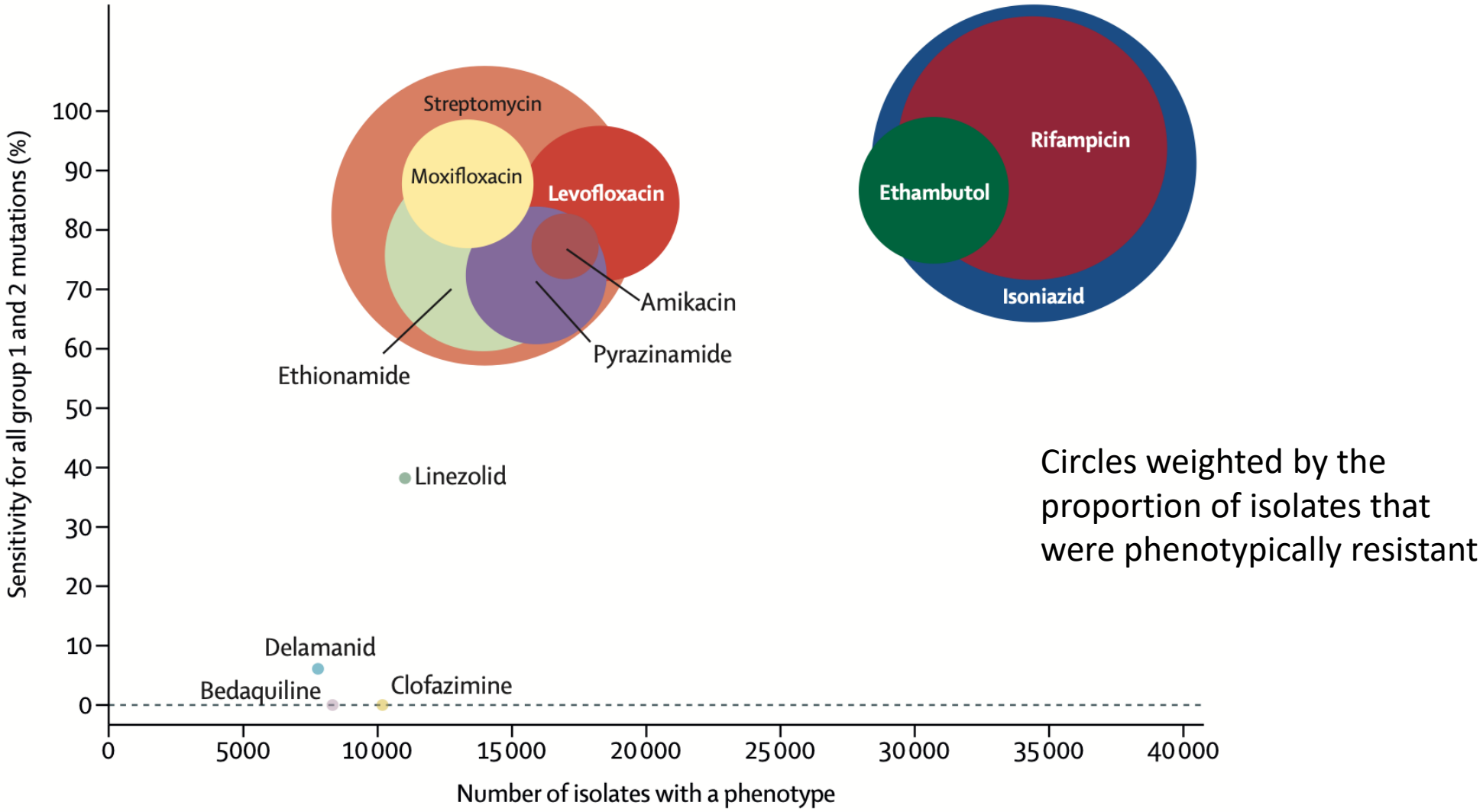


Walker *et al*, Lancet Microbe 2022

Performance of catalogue vs. evidence for individual mutations

drug	gene	mutation	characterisation	R	S	support
INH	katG	katG_S315T	R	2824	40	2182
INH	fabG	fabG_C-15T	R	587	47	187
INH	fabG	fabG_L203L	R	136	28	52
INH	katG	katG_V1A	R	4	0	3
INH	katG	katG_V423I	R	4	5	1
INH	inhA	inhA_C-100A	R	3	1	1
INH	inhA	inhA_T2A	R	3	8	1
INH	katG	katG_A109V	R	3	0	3
INH	katG	katG_E3G	R	3	0	1
INH	katG	katG_G491S	R	3	0	3
INH	katG	katG_L141F	R	3	1	1
INH	katG	katG_N493K	R	3	0	2
INH	katG	katG_P232S	R	3	0	1
INH	katG	katG_R484H	R	3	0	3
INH	katG	katG_W161R	R	3	0	3
INH	katG	katG_W191G	R	3	0	1

- Rare mutations – collectively enriched for resistance
- Presence of some mistakes can be accommodated
- Overall performance of a catalogue can be improved by their inclusion

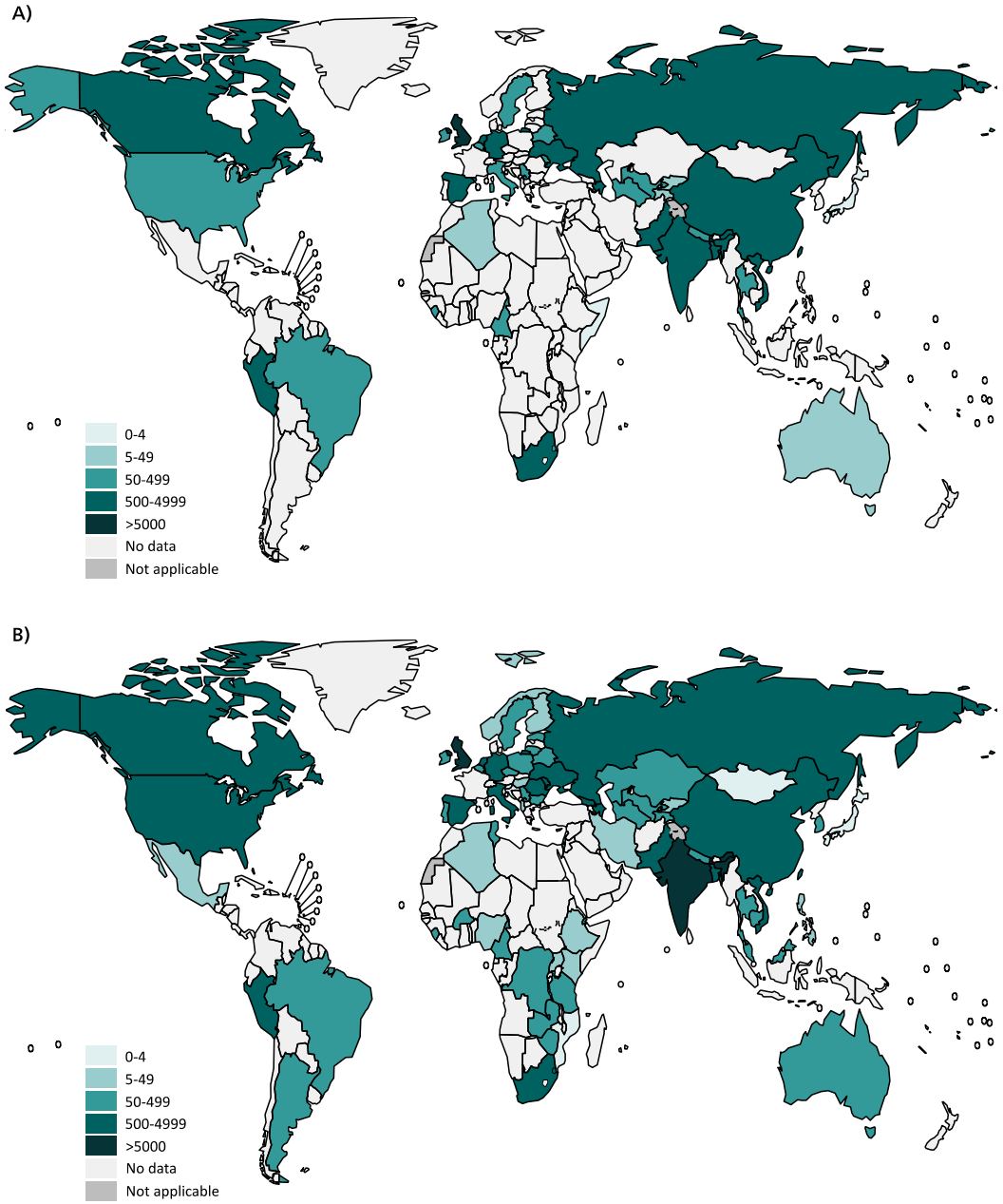


Circles weighted by the proportion of isolates that were phenotypically resistant

Walker *et al*, Lancet Microbe 2022

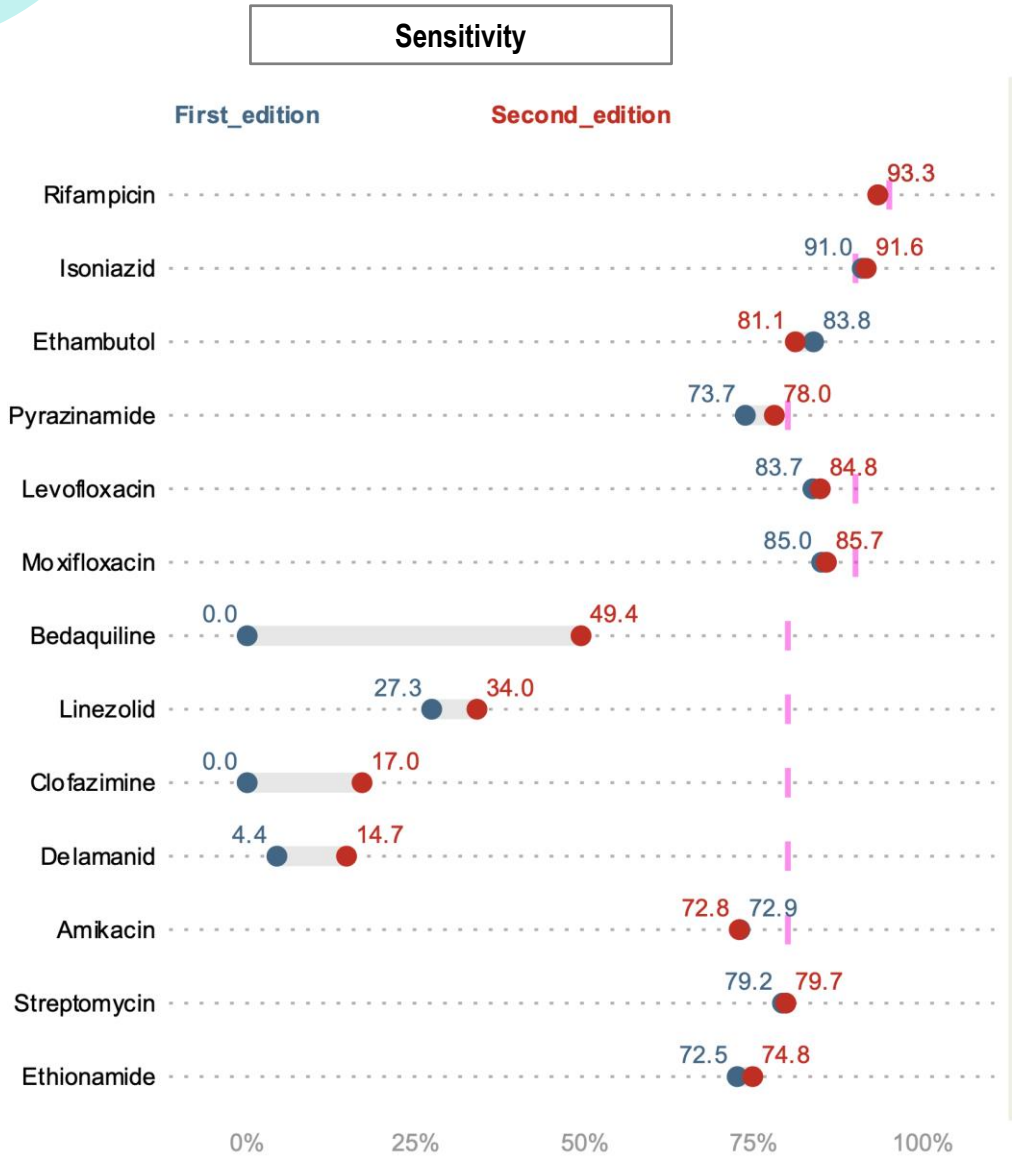
oucru

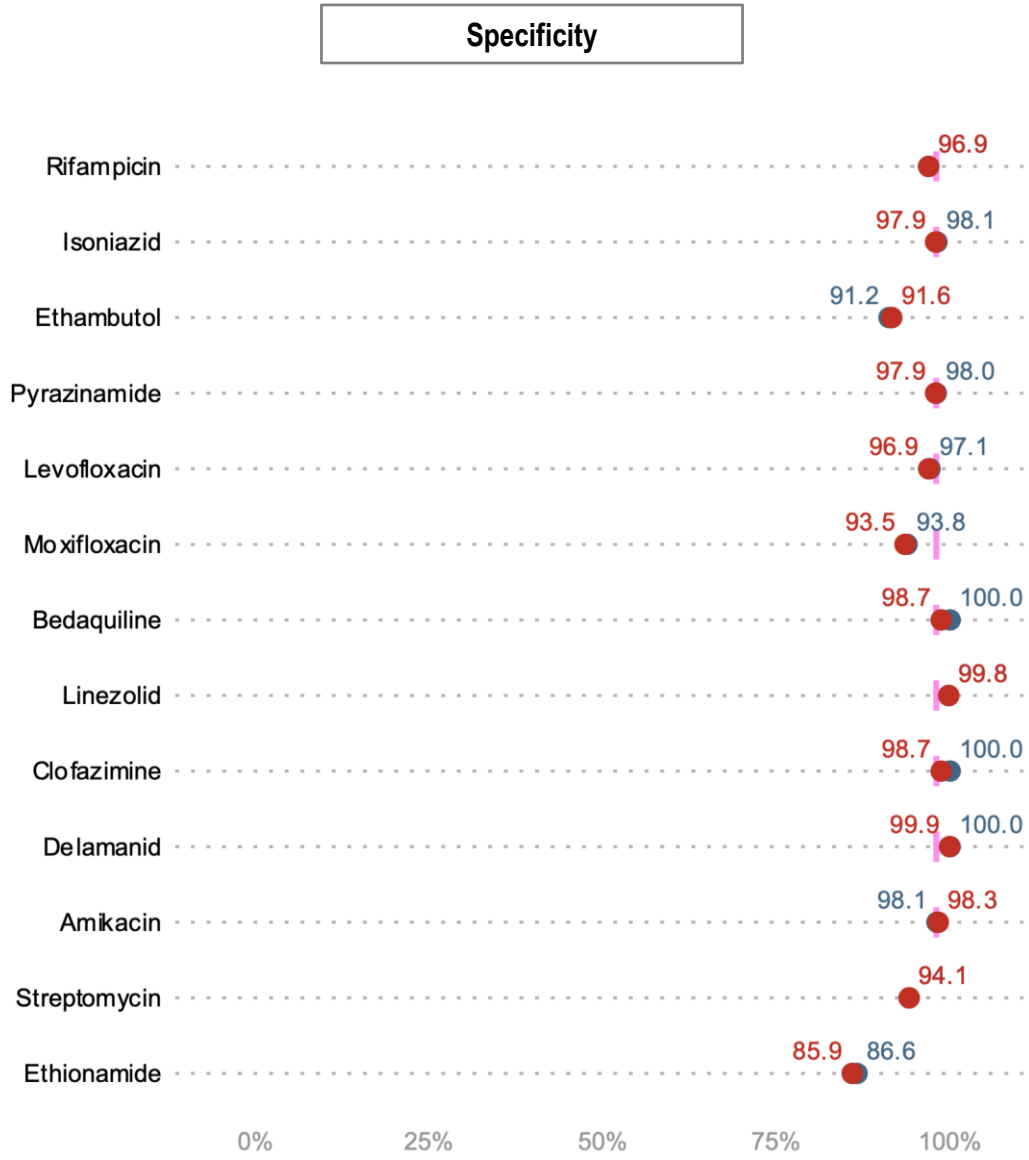
2023



~52,000 genomes from 67 countries

WHO catalogue V1 vs. V2 – yield from am extra 14,000 genomes





WHO catalogue V1 vs. V2 – yield from an extra 14,000 genomes

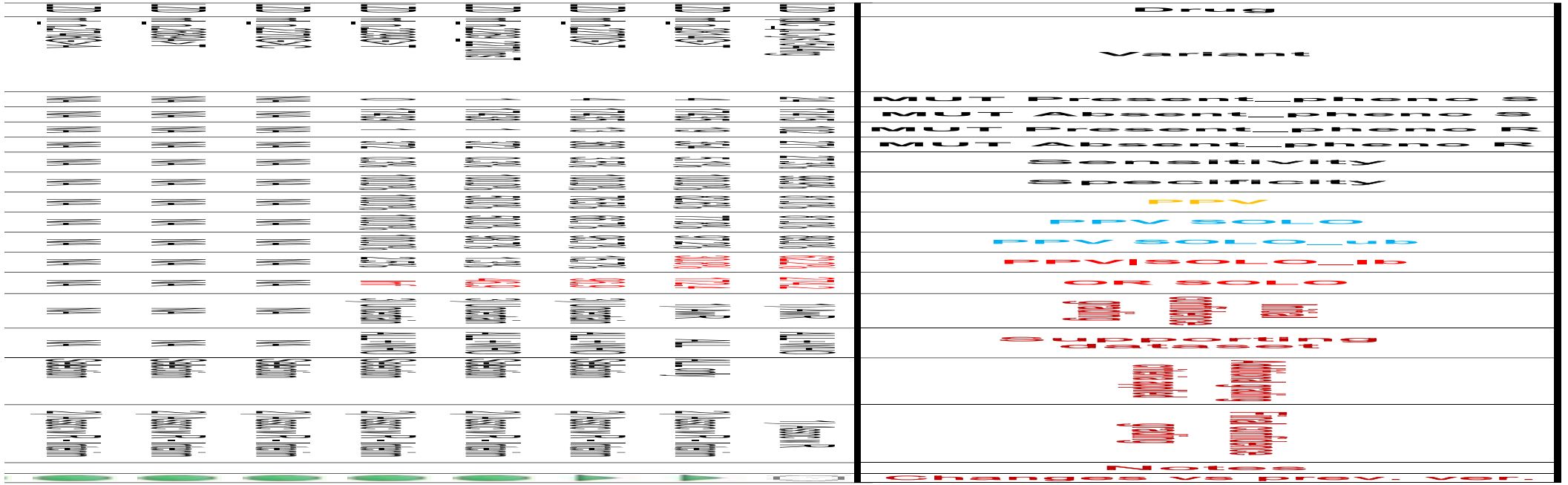
Bedaquiline

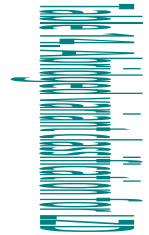
Table 12. Abridged variant classification for BDQ

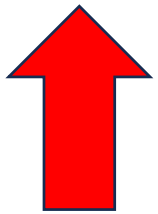
Drug	Variant	MUT Present_pheno S	MUT Absent_pheno S	MUT Present_pheno R	MUT Absent_pheno R	Sensitivity	Specificity	PPV	PPV SOLO	PPV SOLO_ub	PPV|SOLO_lb	OR SOLO	Initial confidence grading	Supporting dataset	Additional grading criteria applied	Final confidence grading	Notes	Changes vs prev. ver.
BDQ	Rv0678_LoF	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		●

WHO catalogue V1 vs. V2 – yield from an extra 14,000 genomes

Linezolid







WHO catalogue V1 vs. V2 – yield from an extra 14,000 genomes

Delamanid

Diarylquinolones (ATP synthetase inhibitors)

- **Bedaquiline**
- TBAJ-587 / TBAJ-876

Nitroimidazoles (DprE2 inhibitors, cell wall synthesis)

- Delamanid
- **Pretomanid**

Oxazolidinones (protein synthesis, acting on 50S ribosomal subunit)

- **Linezolid**
- Sutezolid
- Delpazolid

Benzothiazinones (DprE1 inhibitors, cell wall synthesis)

- BTZ-043
- OPC-167832 (Quabodepistat)
- PBTZ169
- TBA-7371

Oxaboroles (Leucyl t-RNA synthetase inhibitors)

- GSK3036656 (Ganfeborole)

Rv0678

see Almeida, AAC 2023

<u>Diarylquinolones (ATP synthetase inhibitors)</u>

- **Bedaquiline**
- TBAJ-587 / TBAJ-876

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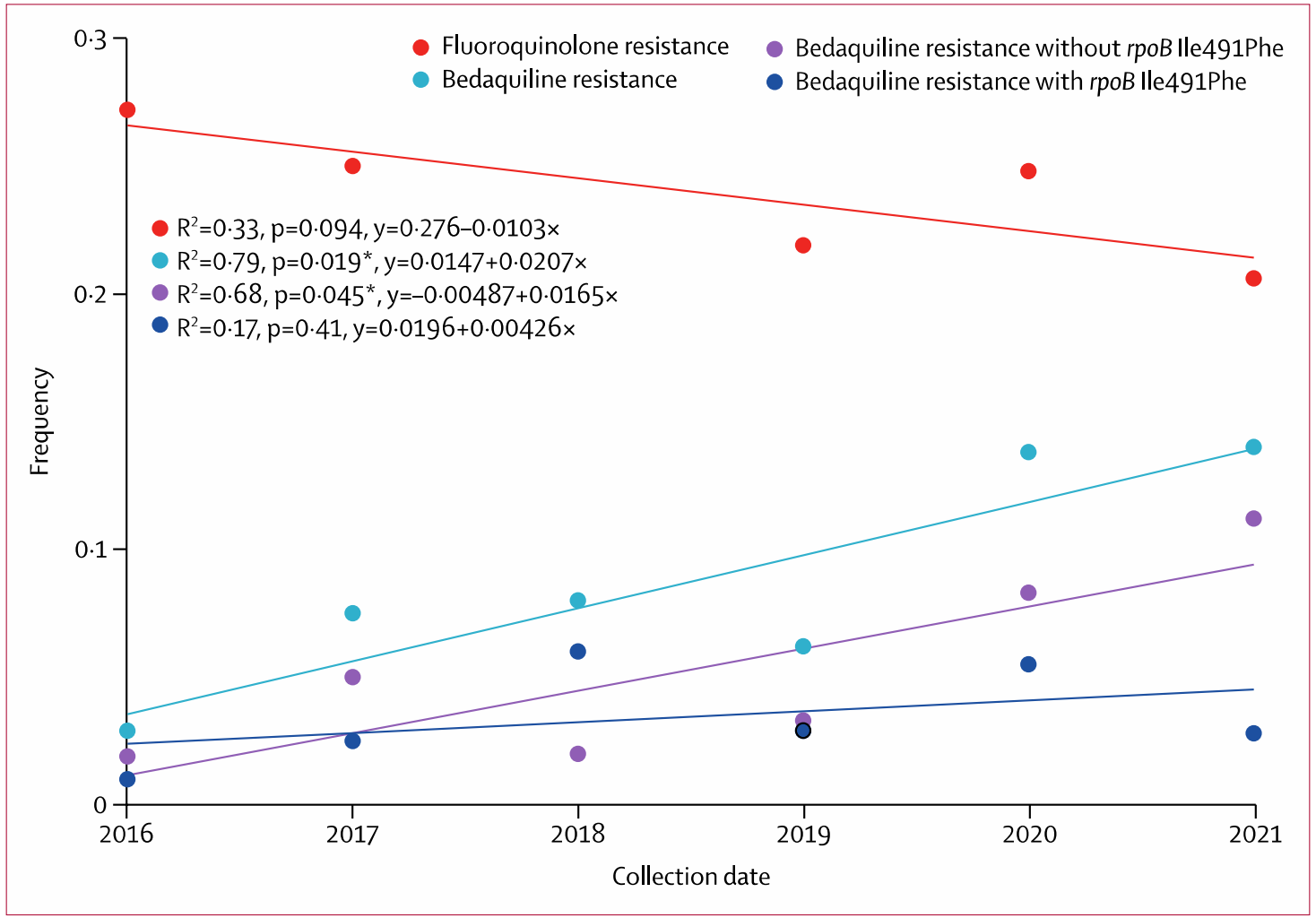
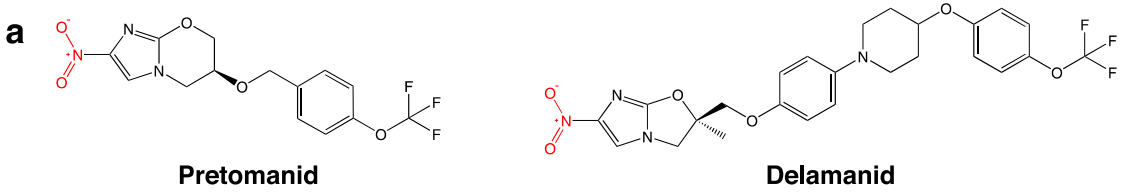
Rapidly emerging bedaquiline resistance - Mozambique

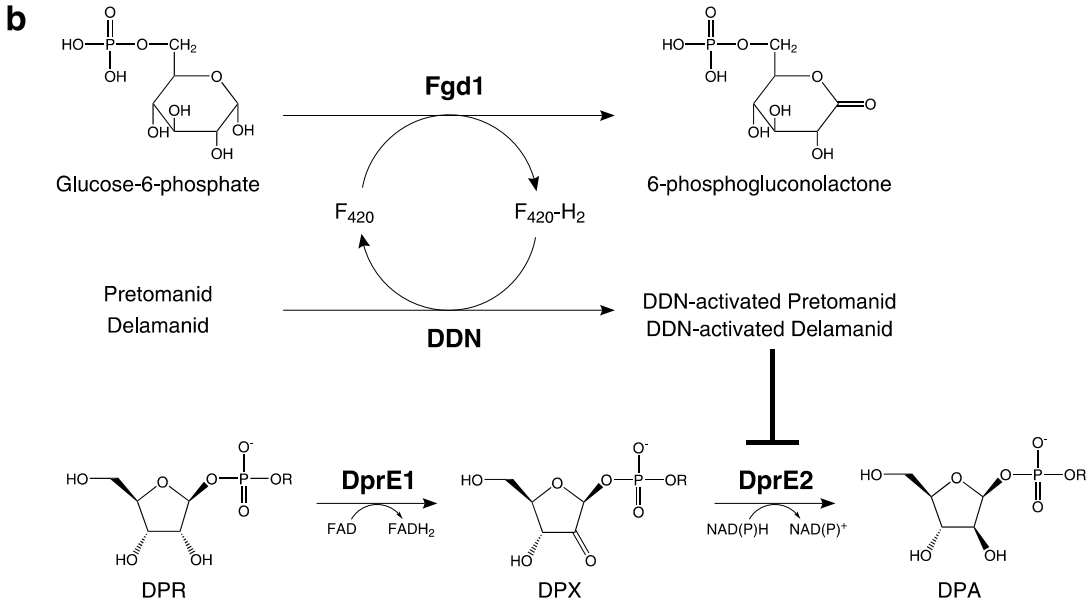


Non-essential genes and a long $t_{1/2}$, a perfect storm for *de novo* resistance?

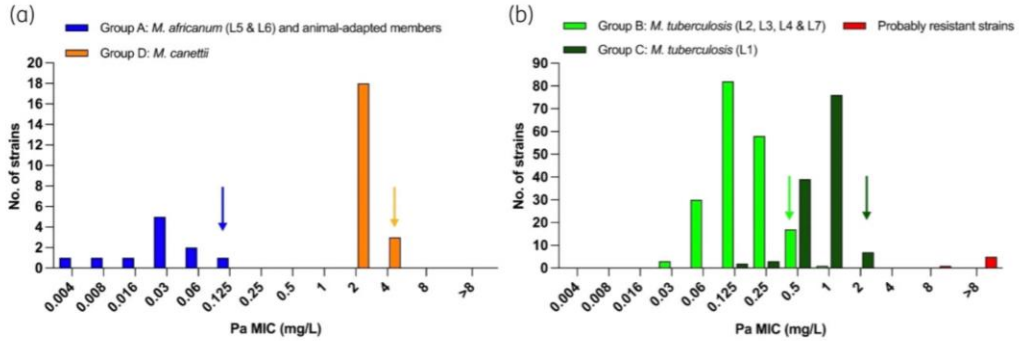
Barilar *et al,* Lancet Inf Dis 2024

Resistance to other ‘new’ drugs?





Abrahams *et al*
Nature Communications, June 2023



Bateson *et al*, *J Antimicrob Chemother* 2022

Delamanid:

*ddn W88** (premature stop codon)

sub-lineage 2 (TGG->TAG)

sub-lineage 4 (TGG->TGA); otherwise susceptible

Potential cross-resistance from LoF mutations in *ddn, fbiA, fbiB, fbiC, fgd1, Rv2983*

New regimens in trials

PAN-TB

Dlm Bdq OPC Szd

Pa Bdq OPC Szd

endTB

Bdq Lzd Mfx Pza

Bdq Lzd Cfz Pza Lfx

Bdq Dlm Lfx Lzd Pza

Dlm Cfz Lfx Lzd Pza

Dlm Cfz Mfx Pza

PARADIGM4TB

GSK656 Bdq Dlm Mxf

GSK656 Bdq Dlm Pza

GSK656 Bdq Dlm Lzd

GSK656 Bdq Pa Mxf

BTZ043 Bdq Dlm Mxf

BTZ043 Bdq Dlm Pza

BTZ043 Bdq Dlm Lzd

BTZ043 Bdq Pa Mxf

BTZ043 Bdq Mxf Pza

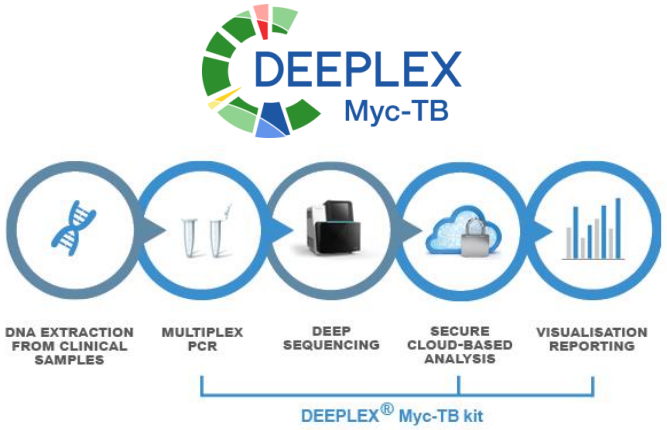
GSK656 BTZ043 Bdq Dlm

Bdq Dlm Mxf

OPC-167832 = Quabodepistat
GSK656 = Ganfeborole

WHO review of diagnostic accuracy of tNGS:

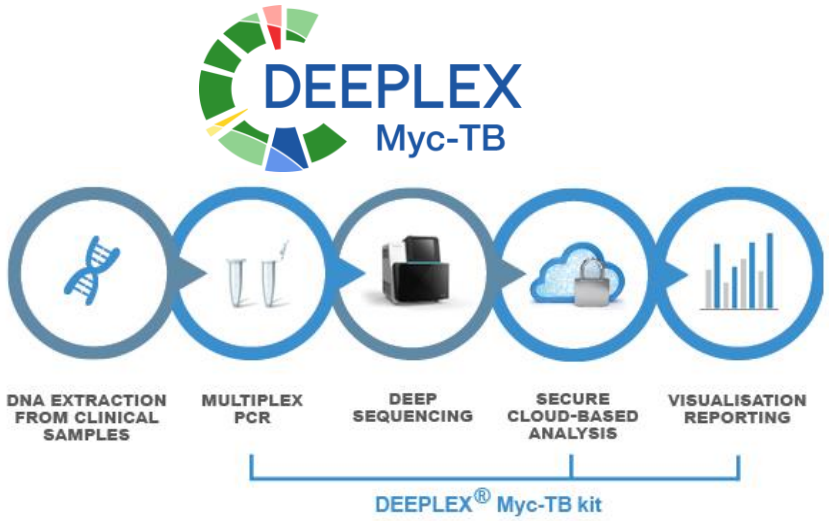
Market ready products with end-to-end workflows

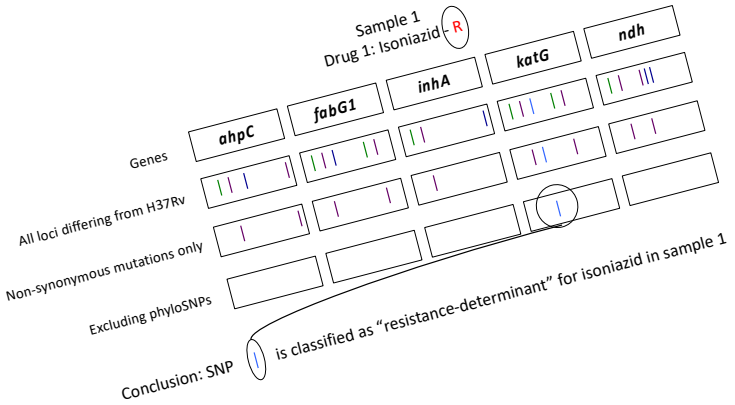


World Health Organization

WHO review of diagnostic accuracy of tNGS:

Market ready products with end-to-end workflows





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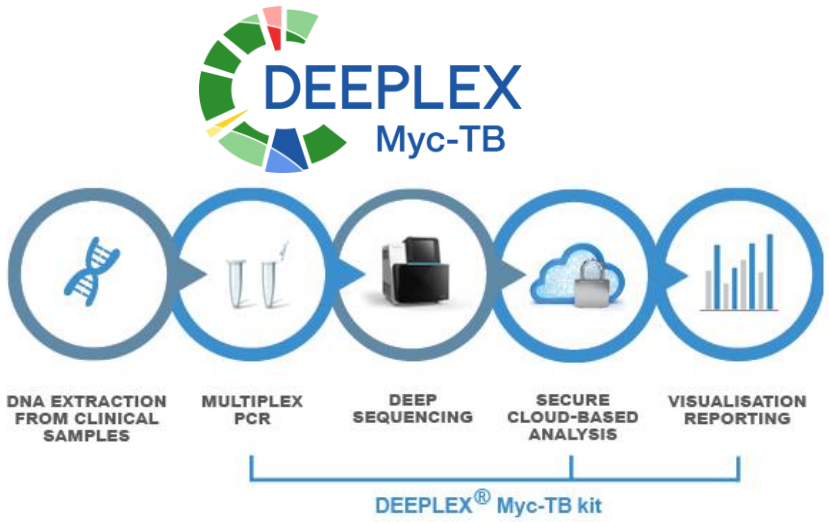


PICO 1

Should tNGS as the initial test be used to diagnose drug resistance in patients with bacteriologically confirmed pulmonary TB disease?

	Sensitivity	Specificity	Negative predictive value	Positive predictive value	Indeterminate rate
Rifampicin_composite	93.1 (87.0 - 99.2)	96.2 (88.6 - 100)	84.8 (78.4 - 91.1)	99.0 (96.5 - 100)	12.0 (10.5-13.6)
Isoniazid	95.8 (92.8 - 98.7)	97.0 (95.1 - 98.9)	90.9 (87.7 - 94.0)	98.9 (98.2 - 99.6)	14.6 (13.0 - 16.2)
Levofloxacin	94.2 (88.4 - 99.9)	96.2 (93.4 - 98.9)	96.8 (95.7 - 97.9)	93.2 (88.5 - 97.9)	9.2 (7.8 - 10.7)
Moxifloxacin	95.6 (92.4 - 98.7)	96.3 (93.2 - 99.5)	97.4 (96.2 - 98.7)	94.4 (90.6 - 98.3)	9.3 (7.9 - 10.9)
Pyrazinamide_composite	88.4 (85.2 - 91.7)	98.5 (97.1 - 100)	87.7 (80.6 - 94.9)	98.7 (97.5 - 99.9)	17.6 (14.6 - 20.8)
Ethambutol_composite	95.8 (94.0 - 97.6)	99.3 (98.2 - 100)	93.4 (89.3 - 97.4)	99.5 (98.8 - 100)	16.3 (13.6 - 19.2)

PICO 2

Should tNGS as the initial test be used to diagnose drug resistance in patients with bacteriologically confirmed rifampicin-resistant pulmonary TB disease?

	Sensitivity	Specificity	Negative predictive value	Positive predictive value	Indeterminate rate
Isoniazid	96.5 (93.8 - 99.2)	95.8 (91.8 - 99.8)	75.7 (67.1 - 84.4)	99.6 (99.1 - 100)	14.6 (13.0 - 16.2)
Levofloxacin	95.8 (90.4 - 100)	96.0 (93.1 - 98.9)	97.5 (96.2 - 98.8)	94.2 (89.6 - 98.9)	9.2 (7.8 - 10.7)
Moxifloxacin	96.5 (93.6 - 99.5)	95.2 (91 - 99.4)	97.4 (95.6 - 99.2)	94.7 (90.9 - 98.6)	9.3 (7.9 - 10.9)
Pyrazinamide_composite	90.0 (86.8 - 93.2)	98.6 (96.8 - 100)	84.6 (75.2 - 94.0)	99.3 (98.4 - 100)	17.6 (14.6 - 20.8)
Bedaquiline	67.9 (42.6 - 93.2)	97.0 (94.3 - 99.7)	99.4 (98.6 - 100)	62.2 (46.5 - 77.8)	16.7 (13.7 - 20.1)
Linezolid	68.9 (38.7 - 99.1)	99.8 (99.6 - 100)	99.8 (99.4 - 100)	93.0 (84.0 - 100)	15.1 (13.1 - 17.3)
Clofazimine	70.4 (34.6 - 100)	96.3 (93.2 - 99.3)	99.2 (98.1 - 100)	44.2 (12.4 - 75.9)	11.6 (9.5 - 14.0)
Amikacin	87.4 (74.5 - 100)	99.0 (98.4 - 99.6)	98.0 (96.0 - 100)	82.0 (57.0 - 100)	17.8 (15.6 - 20.2)
Ethambutol_composite	96.7 (95.0 - 98.4)	98.4 (96.1 - 100)	88.8 (81.2 - 96.3)	100 (99.0 - 100)	20.6 (17.3 - 24.2)
Streptomycin	98.1 (96.1 - 100)	75.0 (59.5 - 90.5)	90.8 (82.0 - 99.7)	94.8 (92.8 - 96.8)	18.8 (16.1 - 21.8)

World Health Organization

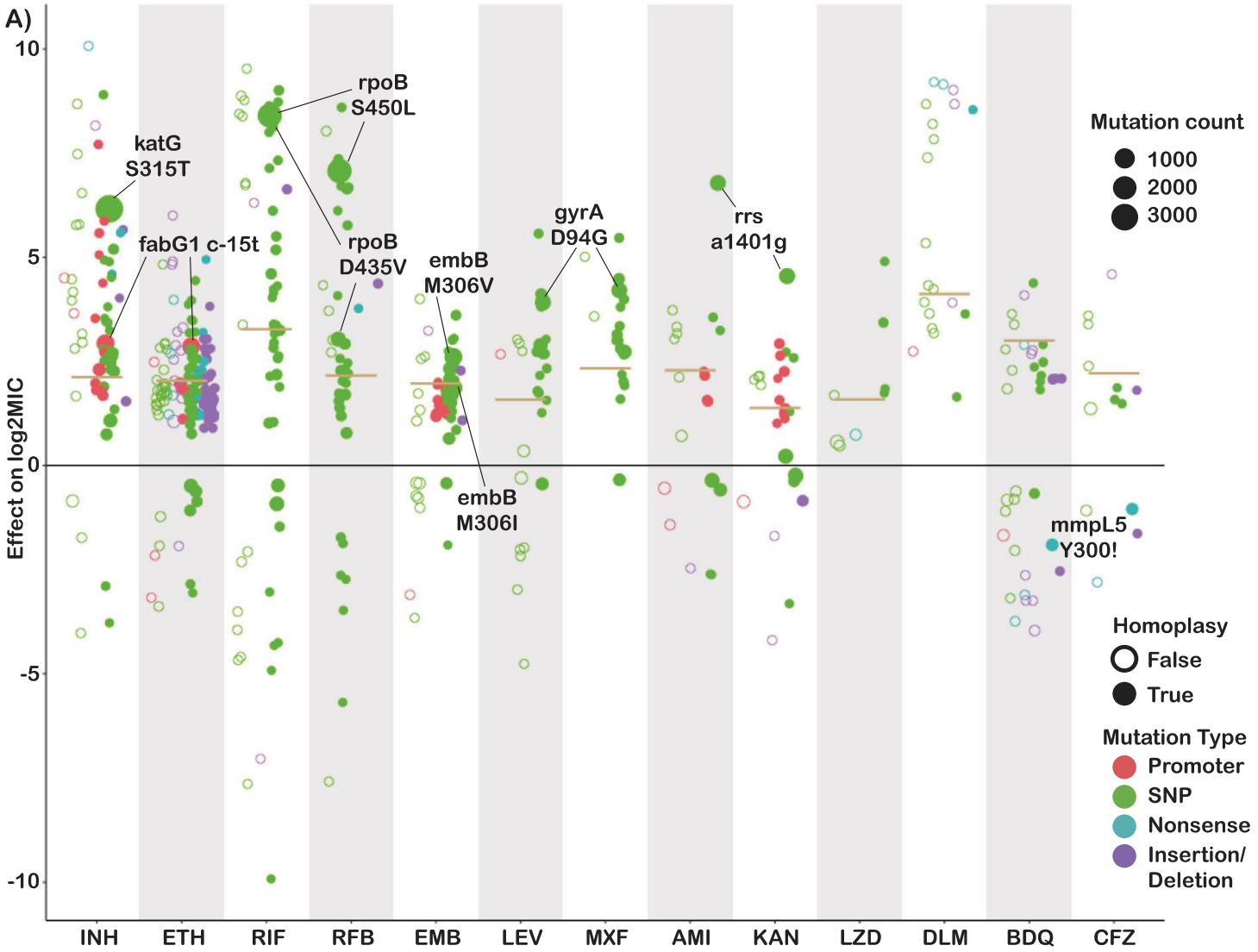
Use of targeted next-generation sequencing to detect drug-resistant tuberculosis

Rapid communication, July 2023

"In people with bacteriologically confirmed pulmonary TB disease, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol rather than culture-based phenotypic drug susceptibility testing.
(Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide],low [rifampicin, fluoroquinolones and ethambutol])"

"In people with bacteriologically confirmed rifampicin-resistant pulmonary TB disease, targeted NGS technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin rather than culture-based phenotypic drug susceptibility testing.
(Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate[ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin], very low [amikacin])"

The future?



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