Epidemiological cutoffs for a 96-well broth microdilution plate for high-throughput research antibiotic susceptibility testing of *M. tuberculosis*: Supplemental Information

The CRyPTIC Consortium*

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Laboratory	Plate Design Location	Total	UKMYC5	UKMYC6
African Health Research Institute	South Africa	124	0	124
Brazil	Brazil	368	68	300
Centers for Disease Control and Prevention	United States	136	1	135
Centre for Tuberculosis, NICD	South Africa	1912	895	1017
Chinese Center for Disease Control and Prevention	China	2768	1409	1359
Hinduja Hospital and Foundation for Medical Research Mumbai	India	4992	2327	2665
Institute of Microbiology and Laboratory Medicine	Germany	1773	104	1669
Oxford University Clinical Research Unit	Vietnam	1509	679	830
Public Health Sweden	Sweden	457	100	357
San Raffaele Scientific Institute	Italy	2367	1134	1233
TORCH	South Africa	147	0	147
Taiwan Centers for Disease Control	Taiwan	171	171	0
Universidad Peruana Cayetano Heredia	Peru	3452	1077	2375
University of Capetown	South Africa	461	0	461
Total		20637	7965	12672

Table S1: Total number of samples collected by laboratory, split by microtitre plate design. This table can be reproduced online[?] .

Country	Samples
India	4992
Peru	3451
China	2767
South Africa	2638
Italy	1769
Vietnam	1509
Germany	1133
Sweden	457
Pakistan	408
Brazil	368
Nepal	302
Turkmenistan	256
Taiwan	171
Belarus	165
United States	136
Ukraine	30
Kyrgyzstan	28
Algeria	25
Tajikistan	19
Kazakhstan	4
Somalia	3
Unknown	1
South Georgia and the South Sandwich Islands	1
New Caledonia	1
Chile	1
Slovenia	1
Japan	1
Total	20,637

Table S2: Total number of samples grouped by country where collected[?]. Some laboratories only collected samples from within the country where they are based whilst others collected samples from several countries, hence this list is longer than Table S1.

Species	Number of isolates
M. tuberculosis	12348
M. bovis BCG	7
M. orygis	4
M. bovis bovis	2
M. bovis caprae	1

Table S3: All isolates contained a species belonging to the Mycobacterium complex[?], as determined by SNP-IT[?]. Since this was done by inspecting the genetics, this was only possible for isolates which had their whole genomes sequenced.

Lineage	Number of isolates	Proportion (%)
Lineage 1	692	5.6
Lineage 2	4358	35.3
Lineage 3	1065	8.6
Lineage 4	6227	50.4
Lineage 6	6	0.0

Table S4: The majority of *M. tuberculosis* isolates belonged to either lineage 2 or 4[?], as determined by SNP-IT[?].

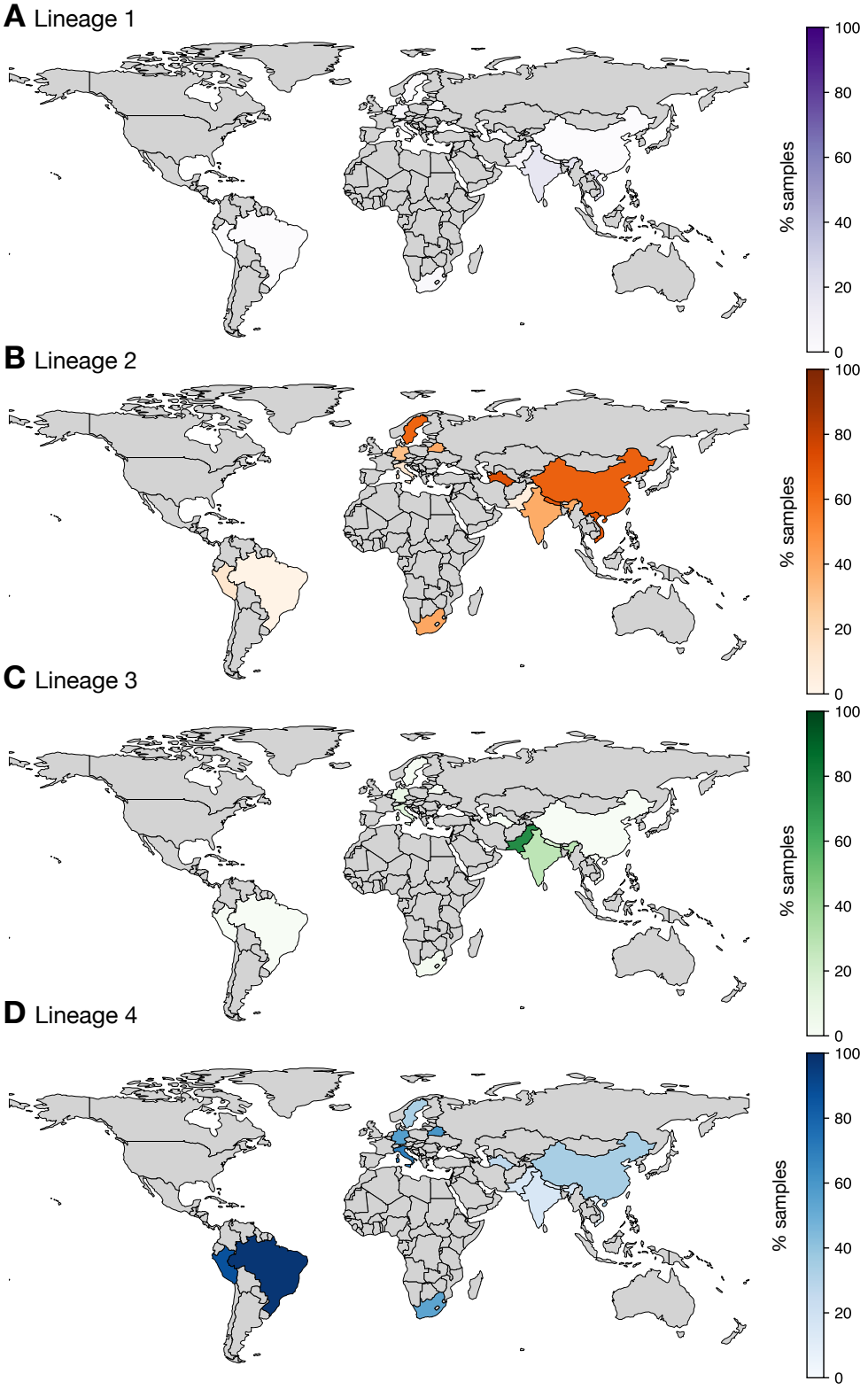

Figure S1: Choropleths showing the proportion of Lineages 1-4 in the isolates containing *M. tuberculosis* collected from each country[?]. Only countries where more than 100 samples were collected are shown and Lineage 6 is excluded due to the small number of samples.

Lineage Country	Lineage 1	Lineage 2	Lineage 3	Lineage 4	Total
Algeria	0	0	0	25	25
Belarus	0	40	0	62	102
Brazil	1	8	0	334	343
China	0	702	1	371	1074
Germany	25	218	60	400	703
India	261	581	425	239	1506
Italy	52	148	179	909	1288
Japan	0	0	0	1	1
Kyrgyzstan	0	25	0	3	28
Nepal	5	137	34	21	197
Pakistan	18	21	300	64	403
Peru	1	339	0	2303	2643
Slovenia	0	0	0	1	1
Somalia	0	1	0	1	2
South Africa	61	858	55	1171	2145
South Georgia and the South Sandwich Islands	0	0	0	1	1
Sweden	6	271	6	141	424
Tajikistan	0	15	0	4	19
Turkmenistan	0	85	1	32	118
Ukraine	0	21	0	9	30
Vietnam	262	888	4	134	1288
Total	692	4358	1065	6227	12342

Table S5: Distribution of *M. tuberculosis* lineages by country where collected[?] . For clarity only isolates with Lineages 1-4 are shown.

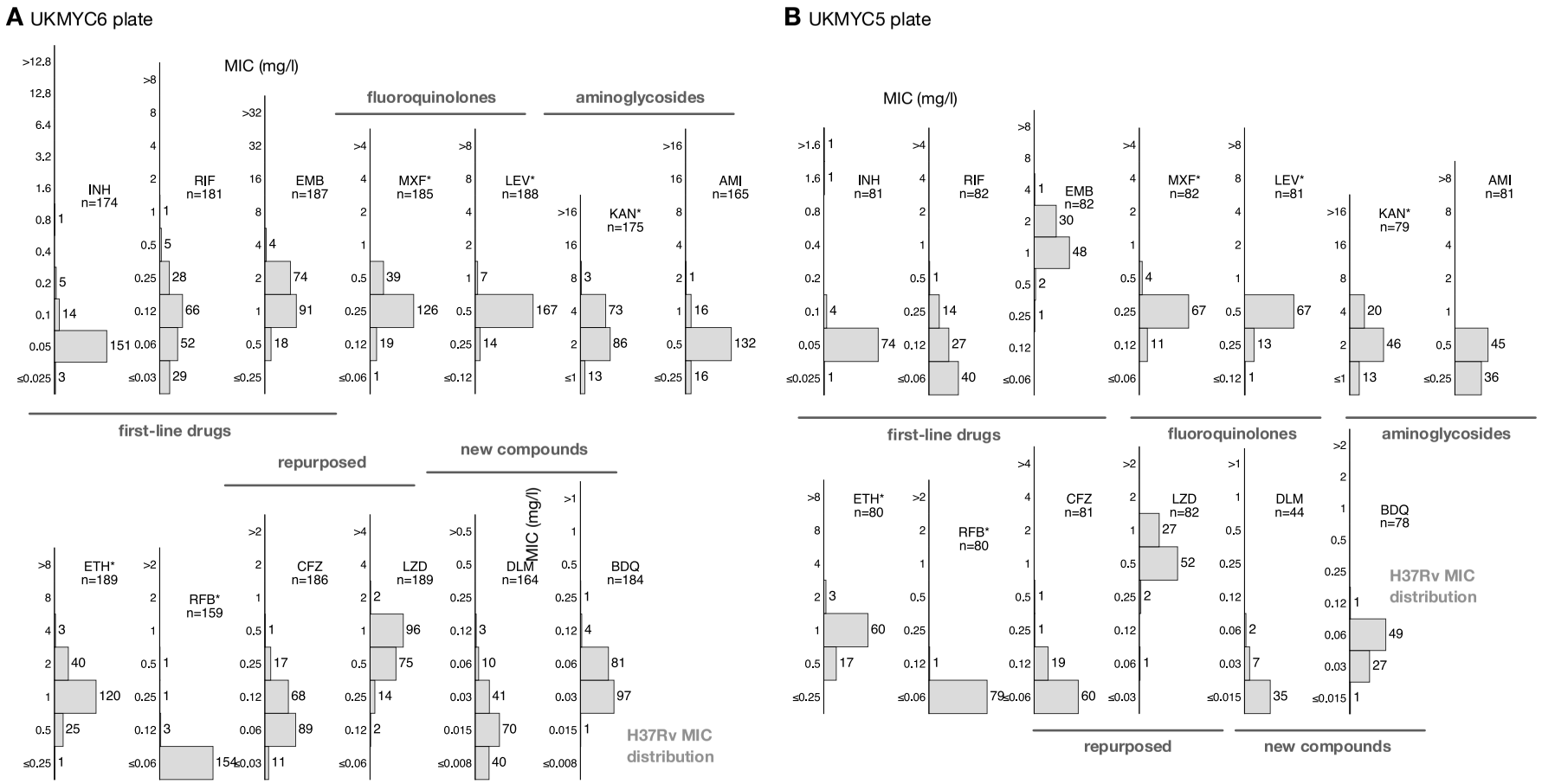

Figure S2: MIC distributions for the H37Rv reference strain on the (**A**) UKMYC6 and (**B**) UKMYC5 plates[?]. Data from eight and five laboratories, respectively, have been pooled. See Table S6 for the essential agreement by drug.

Drug	Plate design	H37Rv mode MIC	Essential agreement	Number of isolates
INH	UKMYC6	0.05 mg/L	94.9-96.6 %	(165-168)/174
RIF	UKMYC6	0.12 mg/L	80.7 %	146/181
EMB	UKMYC6	1 mg/L	97.9 %	183/187
MXF	UKMYC6	0.25 mg/L	99.5 %	184/185
LEV	UKMYC6	0.5 mg/L	100 %	188/188
KAN	UKMYC6	2 mg/L	90.9-98.3 %	(159-172/175
AMI	UKMYC6	0.5 mg/L	89.7-99.4 %	(148-164)/165
ETH	UKMYC6	1 mg/L	97.9 %	185/189
RFB	UKMYC6	$\leq$ 0.06 mg/L	–	–
CFZ	UKMYC6	0.06 mg/L	84.4-90.3 %	(157-168)/186
LZD	UKMYC6	1 mg/L	91.5 %	173/189
DLM	UKMYC6	0.015 mg/L	67.7-92.1%	(111-151)/164
BDQ	UKMYC6	0.03 mg/L	97.3 %	179/184
INH	UKMYC5	0.05 mg/L	96.3-97.5 %	(78-79)/81
RIF	UKMYC5	$\leq$ 0.06 mg/L	–	–
EMB	UKMYC5	1 mg/L	97.6 %	80/82
MXF	UKMYC5	0.25 mg/L	100 %	82/82
LEV	UKMYC5	0.5 mg/L	98.8 %	81/82
KAN	UKMYC5	2 mg/L	83.6-100 %	(66-79)/79
AMI	UKMYC5	0.5 mg/L	55.6-100 %	(45-81)/81
ETH	UKMYC5	1 mg/L	100%	80/80
RFB	UKMYC5	$\leq$ 0.06 mg/L	–	–
CFZ	UKMYC5	$\leq$ 0.06 mg/L	–	–
LZD	UKMYC5	0.5 mg/L	98.8 %	81/82
DLM	UKMYC5	$\leq$ 0.015 mg/L	–	–
BDQ	UKMYC5	0.06 mg/L	98.7 %	77/78

Table S6: The proportion of strains that lie within 1 dilution of the mode MIC of the standard H37Rv reference strain varies between drugs. Data for both plate designs are shown separately. For some of the drugs the mode is different on the two plates (LZD, BDQ) – the MIC distributions for these drugs are narrow with the majority of isolates contained within two doubling dilutions and hence it is difficult to define the mode. For other drugs the mode lies one well above the lowest dilution (INH, KAN, AMI, CFZ, DLM) on one or both of the plate designs, thereby introducing ambiguity into the essential agreement (EA). In these cases, the EA is calculated as a range that either includes or excludes the isolates in the bottom well. For yet other drugs, the mode lies in the lowest dilution (CFZ, RIF, RFB, DLM); the dilution range for CFZ, RIF and DLM was extended to lower values in the UKMYC6 plate design which partially resolved this issue. Five and eight laboratories contributed variable numbers of H37Rv readings to the UKMYC5 and UKMYC6 datasets, respectively. This study was not designed to determine the quality control ranges for H37Rv on the UKMYC series of plates, however this data could be used to guide future plate designs and experiments.

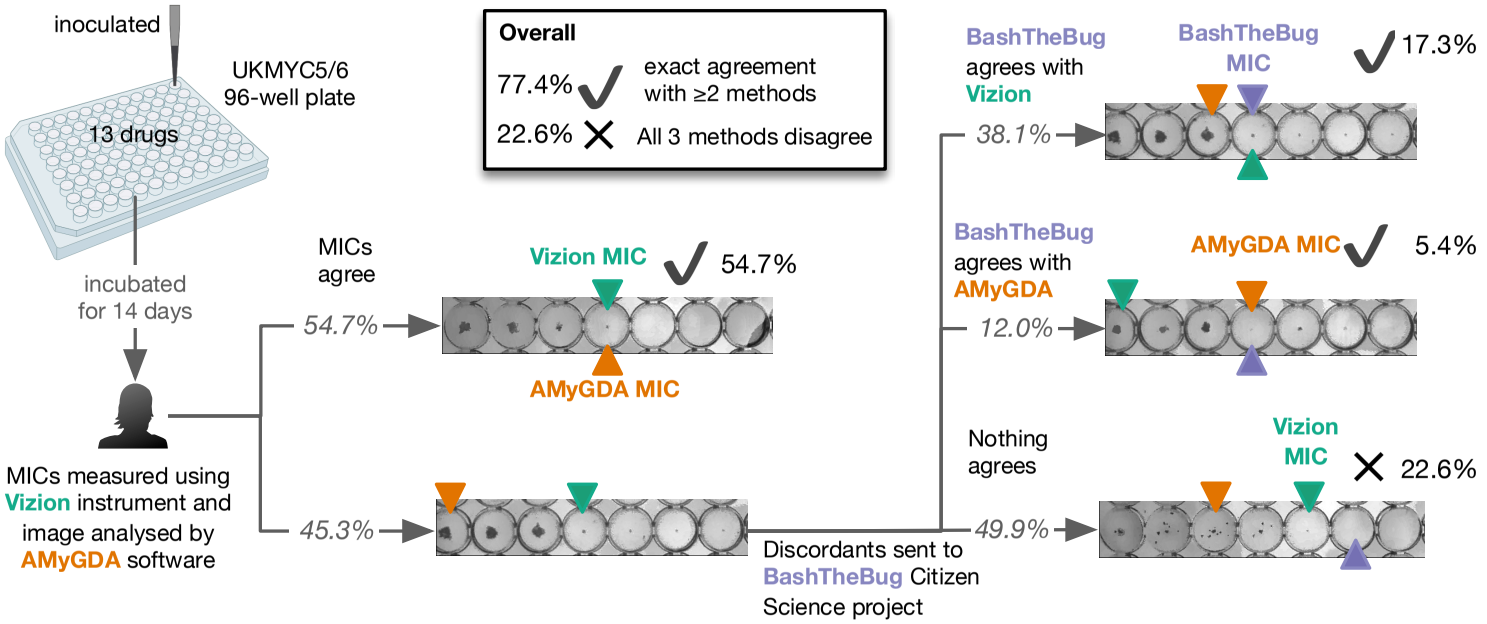

Figure S3: In our quality assurance workflow each minimum inhibitory concentration (MIC) was measured by two or three independent methods. (**A**) Following culture in a MGIT tube (Methods), a sample was inoculated onto a UKMYC plate. (**B**) After 14 days incubation, each plate was placed in a Thermo Fisher Sensititre Vizion Digital MIC Viewing System and the MICs were read by a trained laboratory scientist and stored in a central database[?] . A photograph of the plate was then taken using the Vizion instrument and the image uploaded to the same central database. (**C**) Each image was subsequently analysed centrally using the AMyGDA plate-reading software specifically developed for this purpose[?] . If the two MICs for a drug were identical (55% of all measurements), the process halts and that measurement is annotated as passing the control quality process. (**D**) If the MICs were different, an image of that drug doubling dilution series was uploaded to BashTheBug, a citizen science project. At least 11 different volunteers then measured the MIC and a consensus was taken. (**E**) Of the images sent to BashTheBug, the consensus MIC agreed with either the Vizion or AMyGDA reading in 39% and 12% of cases (Fig. S2). This resulted in a further 23% of the measurements passing the quality control process. (**F**) For the remaining 22% of measurements, all three MICs differed. These data were excluded from analyses.

Antibiotic	Passed QA (%)	Failed QA (%)
AMI	81.1	18.9
BDQ	78.6	21.4
CFZ	73.3	26.7
DLM	76.0	24.0
EMB	67.1	32.9
ETH	81.0	19.0
INH	88.7	11.3
KAN	84.4	15.6
LEV	71.5	28.5
LZD	68.3	31.7
MXF	63.5	36.5
RFB	91.7	8.3
RIF	80.6	19.4
Average	77.4	22.6

Table S7: The proportion of readings for each antibiotic where at least two methods agreed on the result and therefore have passed the quality control process. Cases where all three methods disagree fail the process. Only measurements where an image is available, a result was determined by the laboratory scientist and, if required, a consensus had been returned by the BashTheBug volunteers are included.

Figure S4: (Related to Figure S3) Randomly selected example images from different parts of the quality control workflow. (**A**) Six examples where the MIC measured by the laboratory scientist using the Vizion instrument and the AMyGDA software agree. (**B**) Six examples where the MIC measured by the laboratory scientist using the Vizion instrument and the consensus measured by the BashTheBug citizen scientists agree. In four cases AMyGDA assessed a lower MIC than the other methods as it incorrectly did not detect small growth in wells. In the remaining two cases, AMYGDA called a higher MIC since it incorrectly assessed growth due to artefacts. (**C**) Six examples where the MIC measured by AMyGDA and the BashTheBug consensus agree. The Vizion measurement appears to be either simply incorrect or the operator has assessed very small dots as growth, which is contrary to the CRyPTIC standard operating procedure. (**D**) Six examples where all three methods yield different MICs. These are a mixture of cases where e.g. all three methods call consecutive MICs and the tendency of the citizen scientists to be conservative in their assessment of growth can be seen.

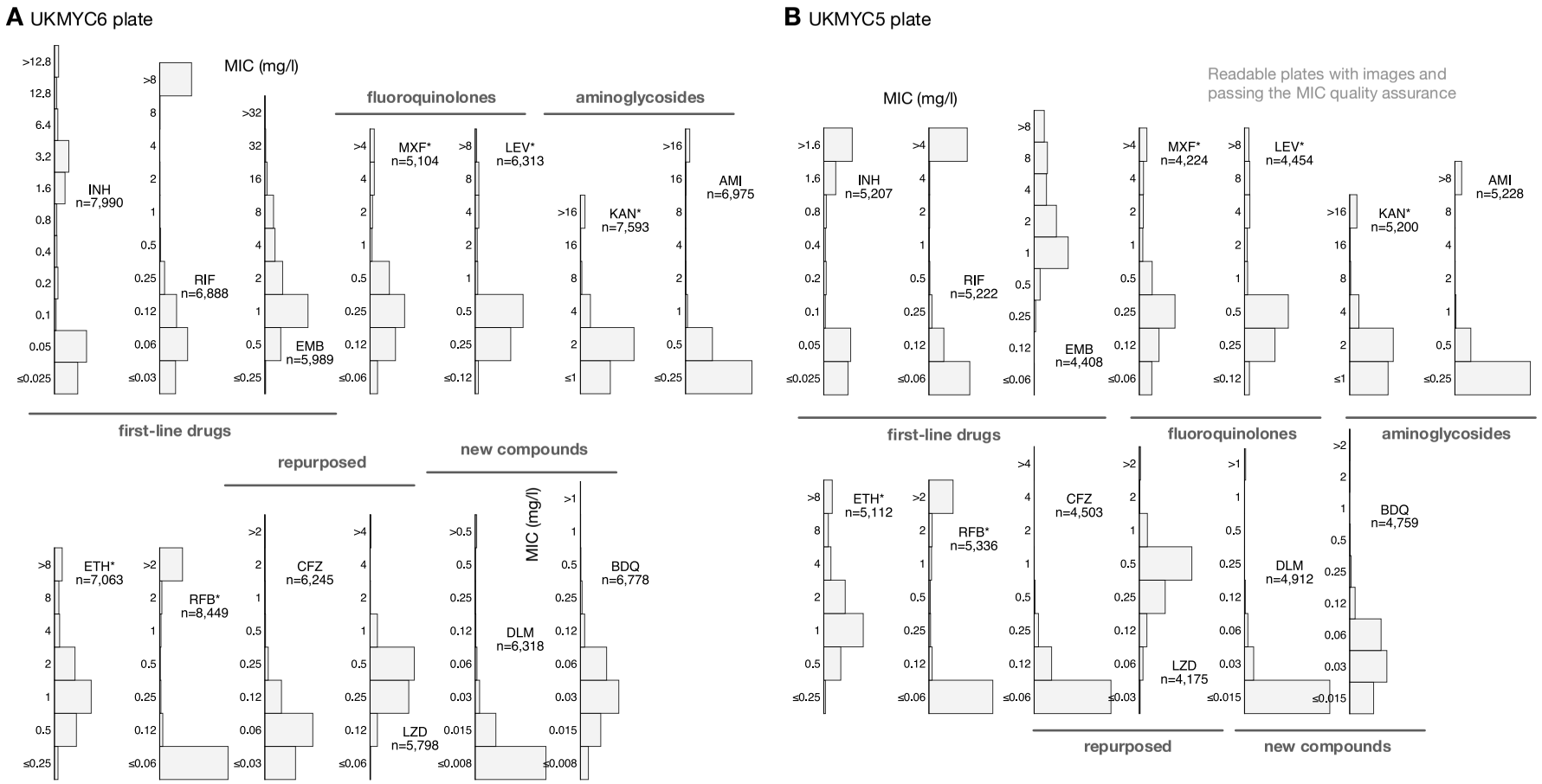

Figure S5: The histograms of the MICs, by drug, that passed the quality assurance process for the (**A**) UKMYC6 and (**B**) UKMYC5 plates[?]. Each MIC reading has therefore been confirmed by at least two independent methods. See the separate Supplemental TSV file for the numerical data.

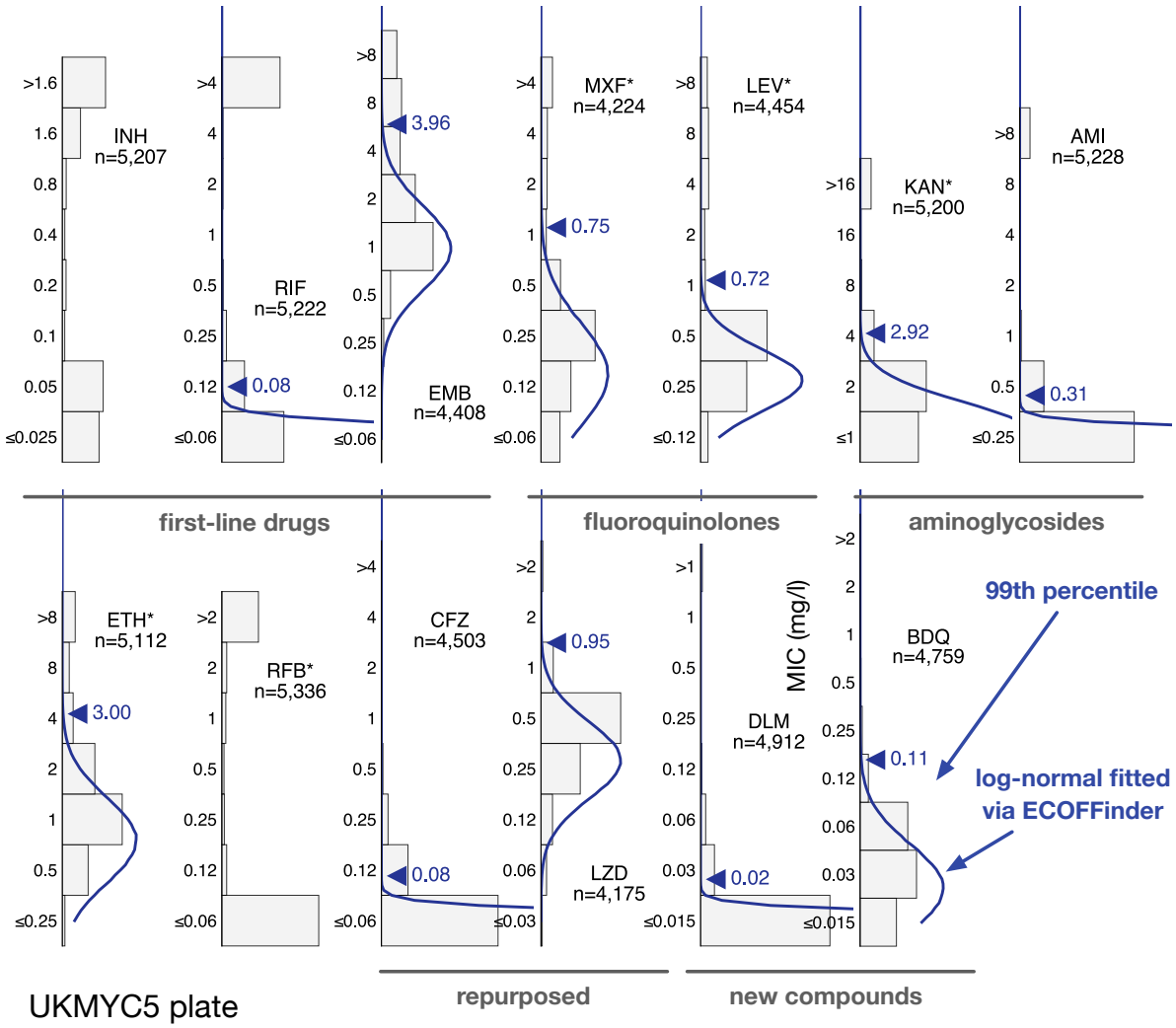

Figure S6: (Related to Figure 2) The MIC histograms for the 13 antibiotics on the UKMYC5 plate. Only MICs which have been confirmed by two independent measurement methods are shown. ECOFFinder was used to fit a log-normal distribution to each histogram; this is drawn in blue and the resulting 99th percentile is labelled. ECOFFinder was unable to fit a log-normal to isoniazid (INH) and rifabutin (RFB). See the separate Supplemental TSV file for the numerical data.

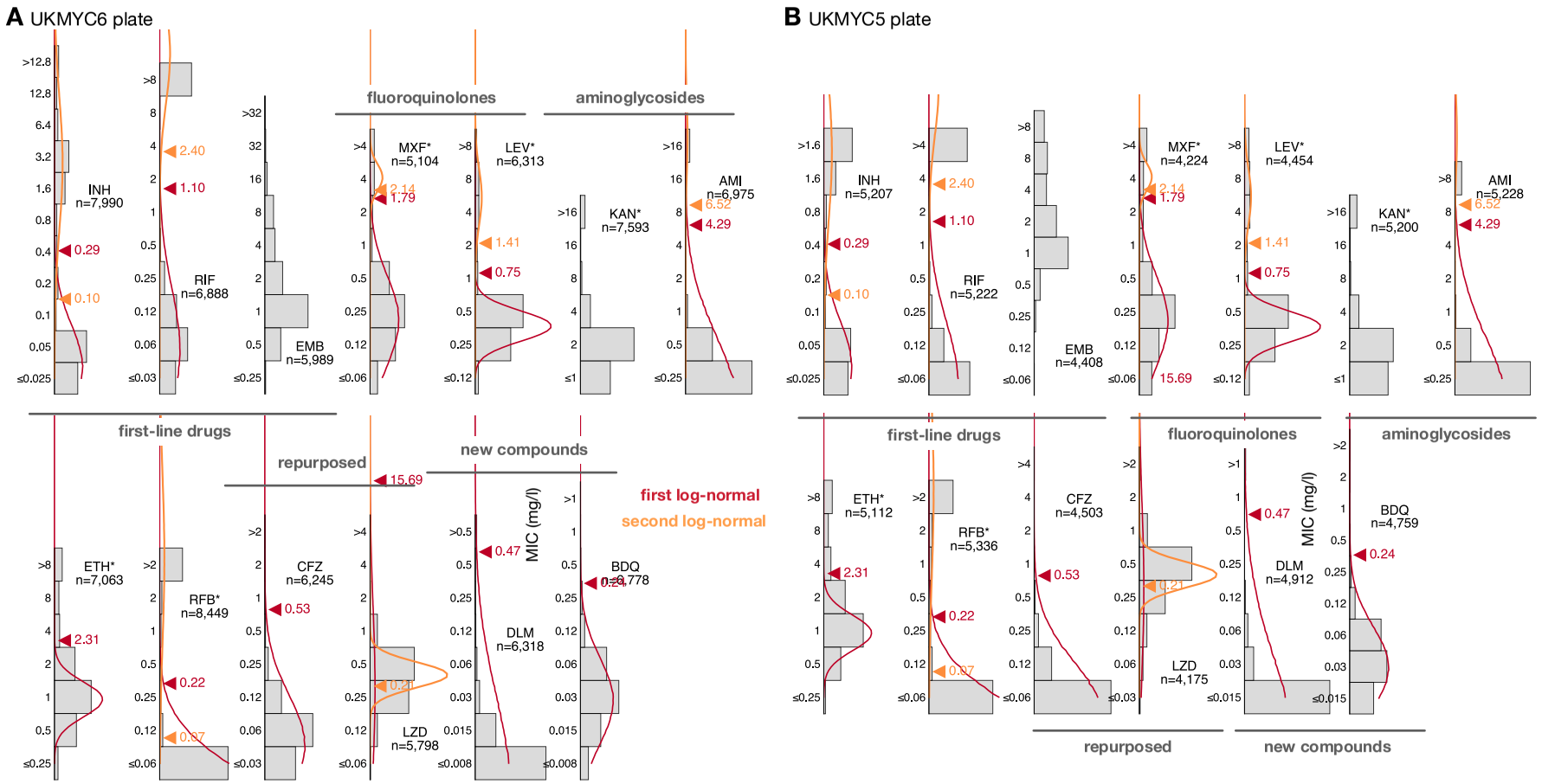

Figure S7: Two log-normal distributions were simultaneously fitted to the MIC histograms from each plate design for each drug using interval regression. The log-normal distribution with the smaller mean is coloured red and the other is coloured orange. The method fails to converge for kanamycin (KAN) and the variance of the second log-normal distribution is occasionally much larger than the range of the data (DLM, ETH)

Laboratory	Plate Design Location	Total	UKMYC5	UKMYC6
Brazil	Brazil	126	48	78
Centre for Tuberculosis, NICD	South Africa	592	333	259
Chinese Center for Disease Control and Prevention	China	463	463	0
Hinduja Hospital and Foundation for Medical Research Mumbai	India	365	365	0
Institute of Microbiology and Laboratory Medicine	Germany	439	8	431
Oxford University Clinical Research Unit	Vietnam	526	370	156
San Raffaele Scientific Institute	Italy	411	109	302
Universidad Peruana Cayetano Heredia	Peru	1039	354	685
University of Capetown	South Africa	342	0	342
Total		4303	2050	2253

Table S8: Total number of genetically wild-type (gWT) isolates collected by laboratory, split by microtitre plate design.

PLATEDESIGN DRUG	UKMYC5	UKMYC6	Total
AMI	1840	1667	3507
BDQ	1666	1631	3297
CFZ	1586	1414	3000
DLM	1687	1361	3048
EMB	1505	1354	2859
ETH	1832	1680	3512
INH	1813	1890	3703
KAN	1834	1790	3624
LEV	1587	1447	3034
LZD	1483	1297	2780
MXF	1477	1117	2594
RFB	1959	2119	4078
RIF	1810	1568	3378

Table S9: Number of genetically wild-type MICs by drug that have passed the quality assurance process. Note that only definite numerical MICs have been included.

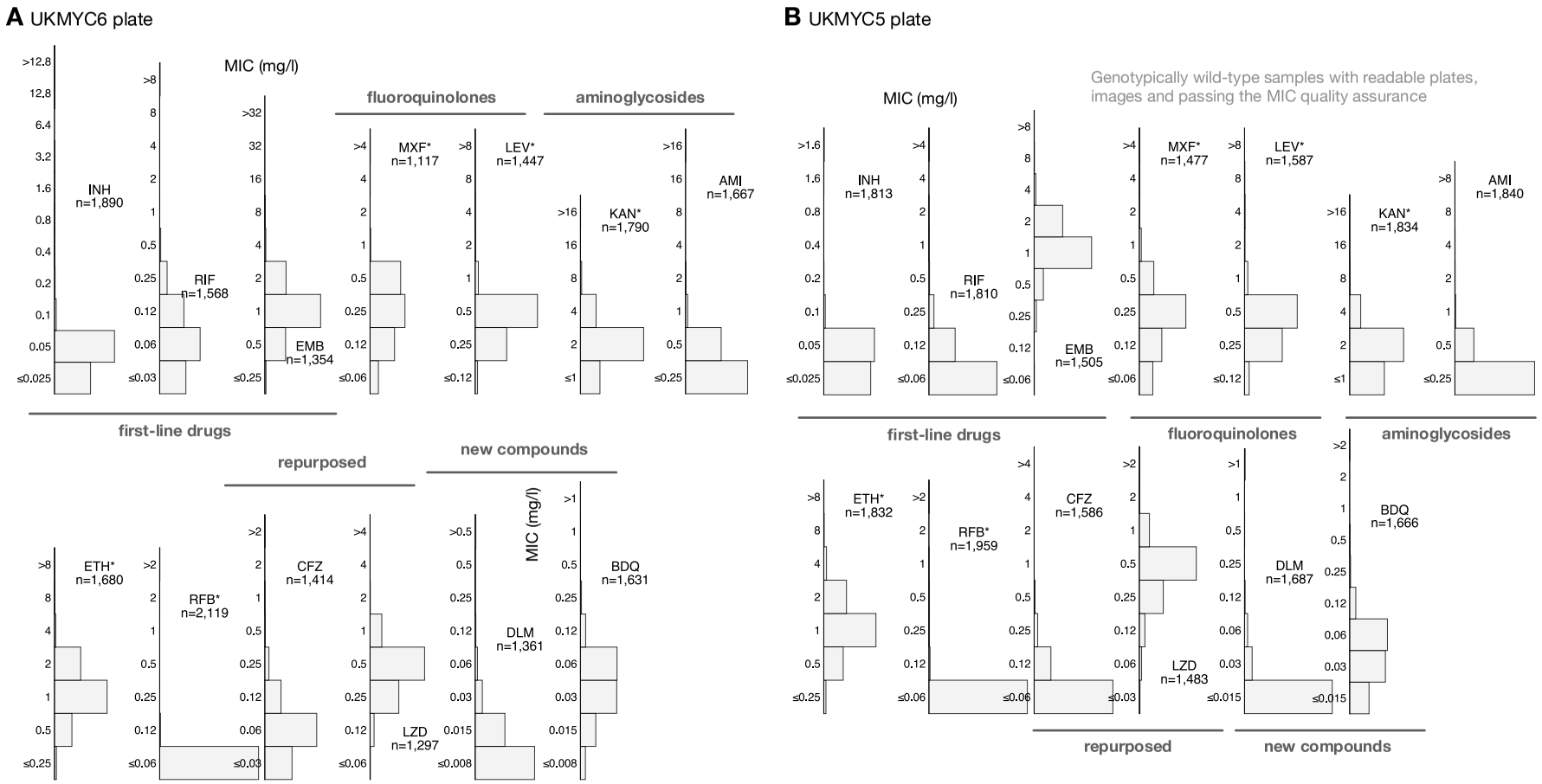

Figure S8: The histograms of all MICs that passed the quality assurance process and are *genotypically wild-type* for the (**A**) UKMYC6 and (**B**) UKMYC5 plates. Each MIC reading has therefore been confirmed by at least two independent methods.

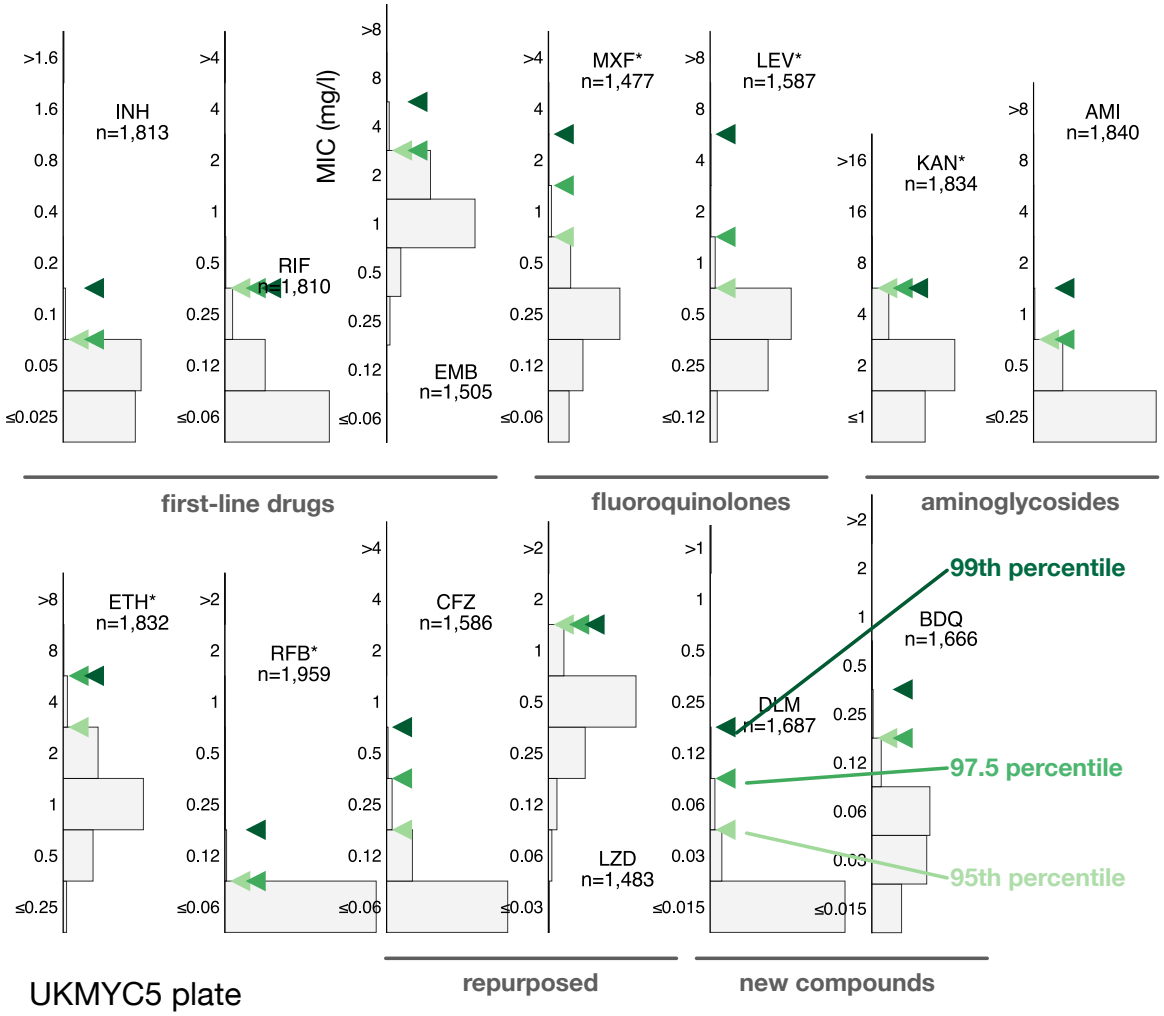

Figure S9: (Related to Figure 3) Direct measurement of ECOFF/ECVs from the gWT population on the UKMYC5 plate. To illustrate the sensitivity to the precise percentile used in the definition, the 95th, 97.5th and 99th percentiles are all labelled.

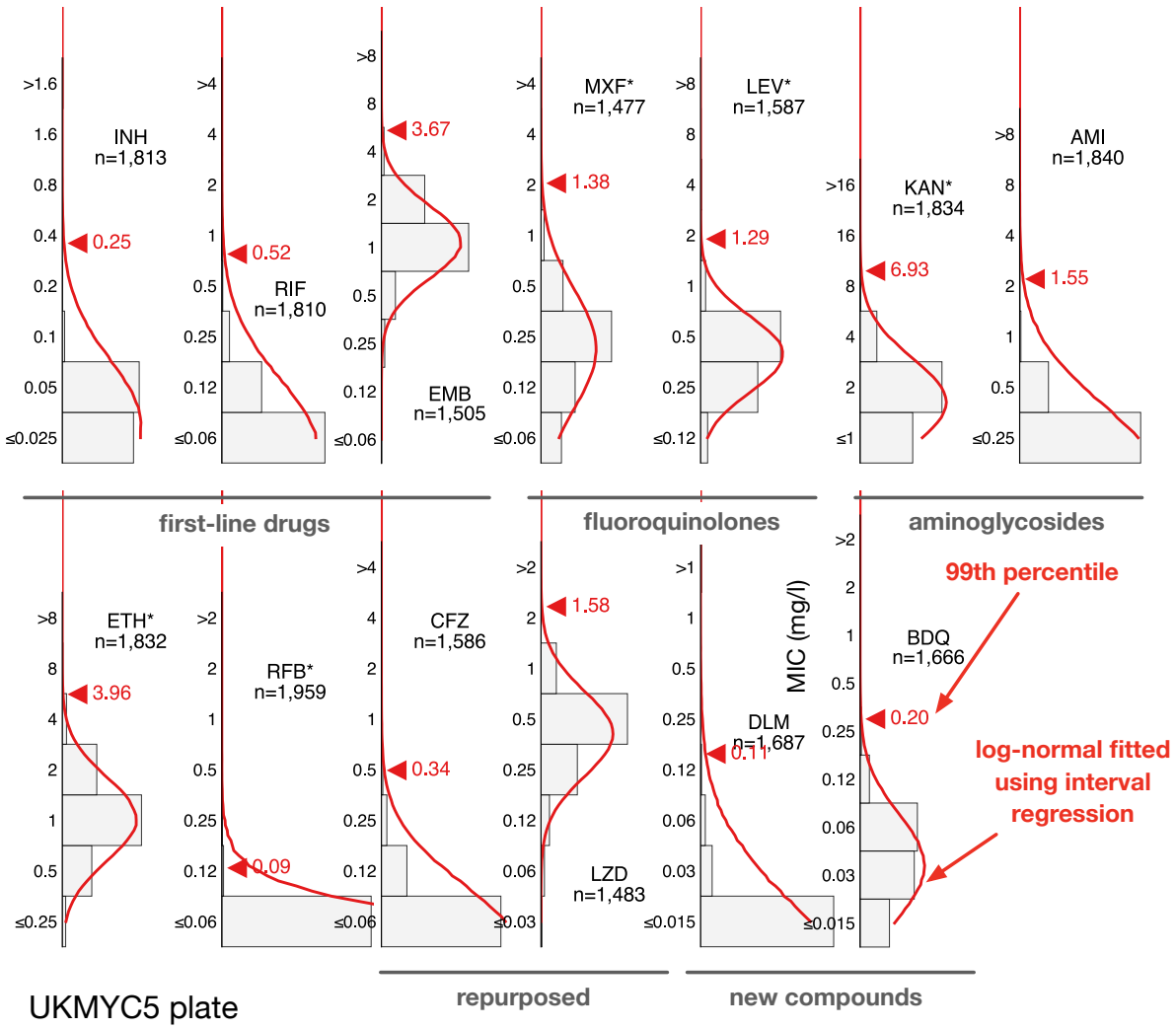

Figure S10: (Related to Figure 4) Interval regression is able to fit a log-normal distribution to the MIC histograms of the genetically wild-type isolates for the 13 drugs on the UKMYC5 plate. Data from both plate designs were considered simultaneously, hence the resulting distributions are those the algorithm considers to best describe both the UKMYC6 (Fig. 4) and UKMYC5 data sets.

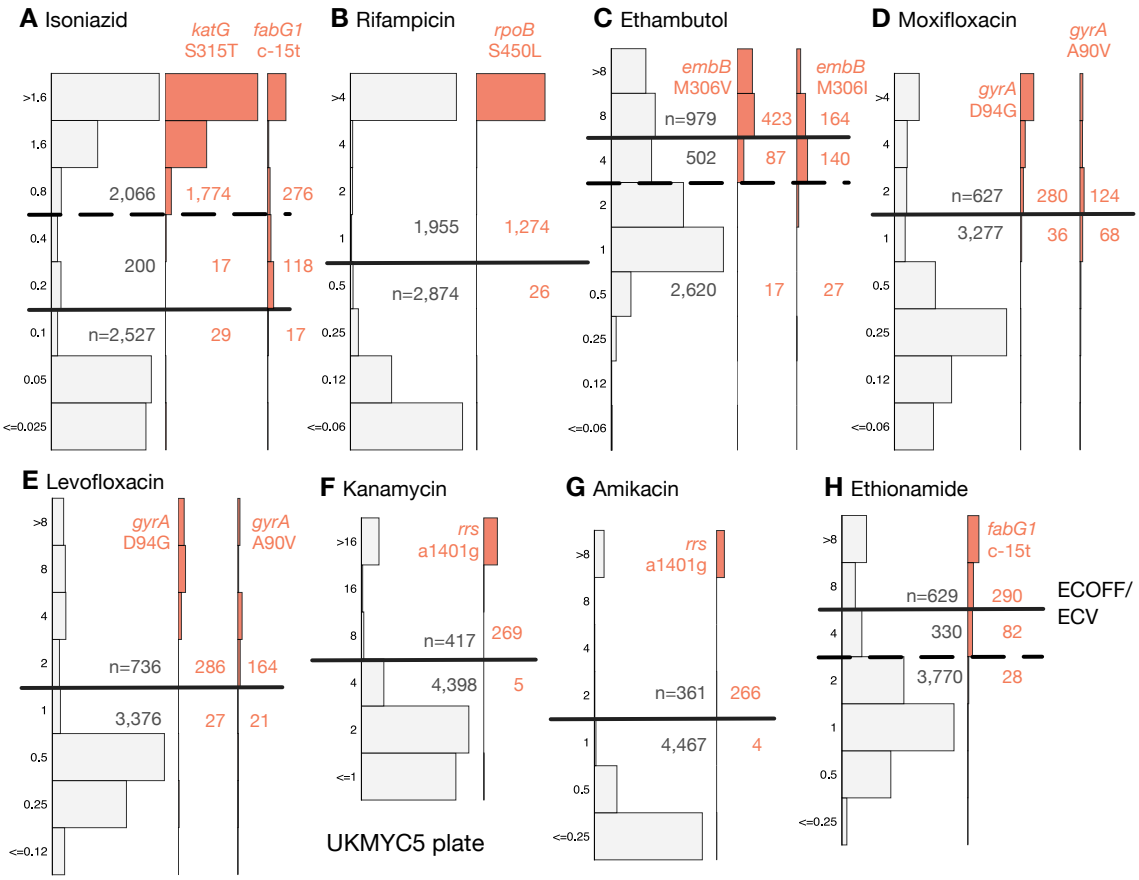

Figure S11: (Related to Figure 6) The MICs of isolates containing genetic variants known to confer resistance to different drugs tend to lie above the proposed ECOFF/ECV on the UKMYC6 plate. The number of isolates lying above and below the ECOFF/ECV is annotated. The dashed line indicates the margin of a proposed borderline category for isoniazid, ethambutol and ethionamide. The same analysis repeated on the UKMYC5 dataset is Figure 6 in the main body of the manuscript.

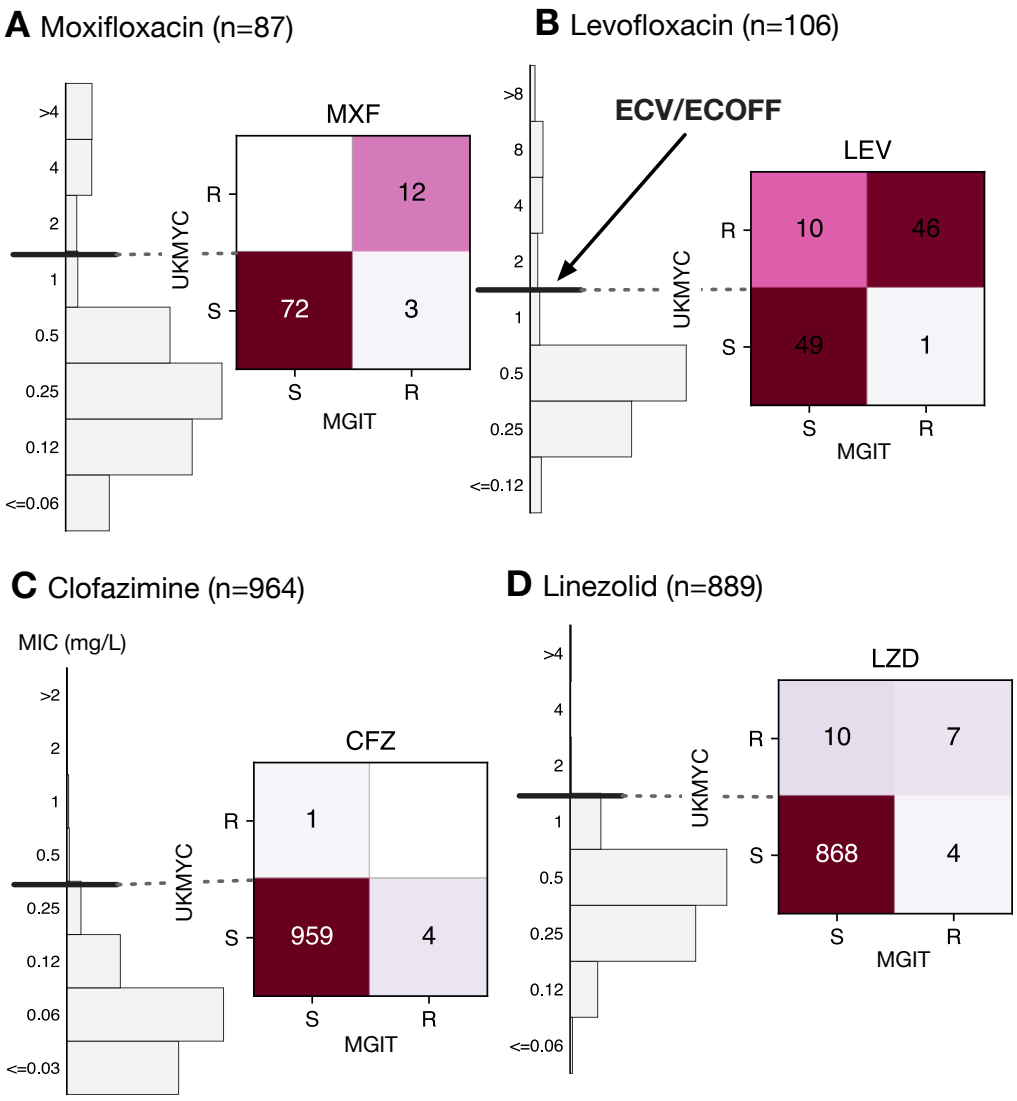

Figure S12: (Related to Figure 7) There is reasonable agreement between the phenotypes measured by the UKMYC plates (assuming the ECOFF/ECVs) and the MGIT960. There are comparatively few isolates for the fluoroquinolones and very few resistant isolates for clofazimine and linezolid.

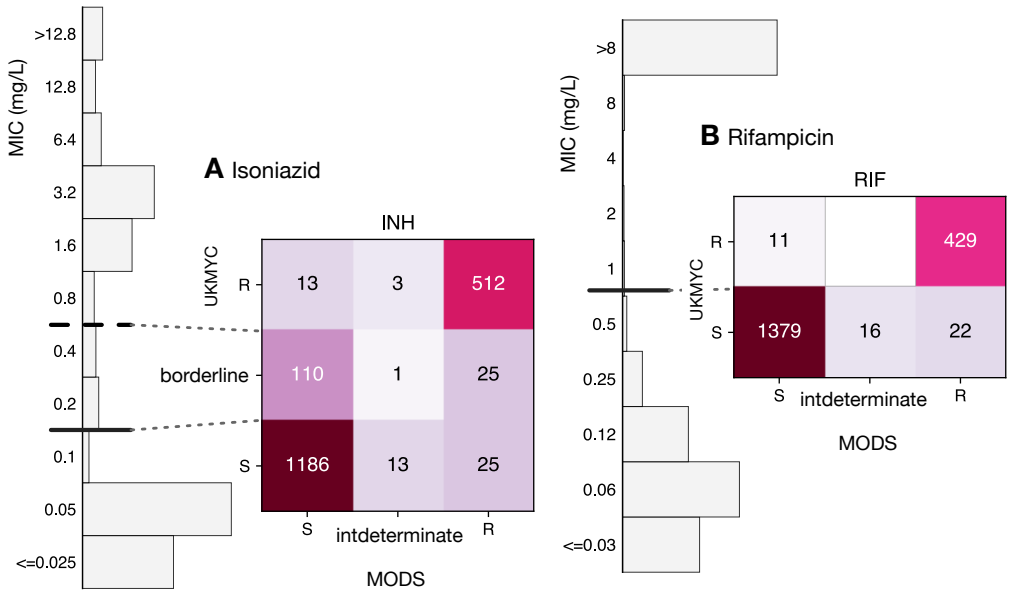

Figure S13: There is reasonable agreement between the phenotypes measured by the UKMYC plates (assuming the ECOFF/ECVs) and the MODS assay[?]. Only isoniazid and rifampicin were tested.

Drug	Number of isolates	Sensitivity (%)	Specificity (%)	Categorical agreement (%)	MD (%)	VMD (%)
INH	1516	93.4	97.0	95.0	6.6	3.0
RIF	1456	96.5	96.6	96.6	3.5	3.4
EMB	961	91.4	91.9	91.6	8.6	8.1
MXF	87	80.0	NaN	NaN	20.0	NaN
LEV	106	97.9	83.1	89.6	2.1	16.9
KAN	1262	76.2	99.1	96.4	23.8	0.9
AMI	1175	84.3	99.3	98.0	15.7	0.7
ETH	954	63.0	97.0	86.9	37.0	3.0
LZD	889	63.6	98.9	98.4	36.4	1.1
CFZ	964	NaN	99.9	NaN	NaN	0.1

Table S10: Comparing the binary phenotypes derived from a UKMYC plate results to MGIT960. The sensitivity and specificity were calculated ignoring any borderline categorisation.

Drug	Number of isolates	Sensitivity (%)	Specificity (%)	Categorical agreement (%)	MD (%)	VMD (%)
INH	1888	95.3	98.9	97.8	4.7	1.1
RIF	1857	95.1	99.2	98.2	4.9	0.8

Table S11: Comparing the binary phenotypes derived from a UKMYC plate results to MODS$^{?}$. The sensitivity and specificity were calculated ignoring any borderline categorisation.

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