

1 **Title**

2 **Whole genome sequencing for drug resistance profile prediction in**
3 ***Mycobacterium tuberculosis***

4 **Authors**

5 Sebastian M. Gygli^{1,2,#}, Peter M. Keller^{3,#}, Marie Ballif⁵, Nicolas Blöchliger³, Rico Hömke^{3,4},
6 Miriam Reinhard^{1,2}, Chloé Loiseau^{1,2}, Claudia Ritter^{3,4}, Peter Sander^{3,4}, Sonia Borrell^{1,2},
7 Jimena Collantes Loo⁵, Anchalee Avihingsanon^{6,7}, Joachim Gnokoro⁸, Marcel Yotebieng⁹,
8 Matthias Egger^{5,10*}, Sébastien Gagneux^{1,2*§}, Erik C. Böttger^{3,4*§}

9 [#]equal contribution as first authors; ^{*}equal contribution as last authors; [§]corresponding
10 authors

11 **Affiliations**

12 ¹Swiss Tropical and Public Health Institute, Basel, Switzerland

13 ²University of Basel, Basel, Switzerland

14 ³Institute of Medical Microbiology, University of Zürich, Zürich, Switzerland

15 ⁴National Center for Mycobacteria, University of Zürich, Zürich, Switzerland

16 ⁵Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

17 ⁶HIV-NAT/Thai Red Cross AIDS Research Centre, Bangkok, Thailand

18 ⁷TB Research Unit, Department of Medicine, Faculty of Medicine, Chulalongkorn
19 University, Bangkok, Thailand

20 ⁸Centre de Prise en Charge de Recherche et de Formation, Yopougon, Abidjan, Côte d'Ivoire

21 ⁹The Ohio State University, College of Public Health, Columbus, Ohio, USA

22 ¹⁰Centre for Infectious Disease Epidemiology and Research, University of Cape Town, South
23 Africa

24 **Corresponding authors**

25 Prof. Erik C. Böttger, MD, University of Zurich, Institute of Medical Microbiology, National
26 Center for Mycobacteria, Gloriastrasse 28/30, 8006 Zurich, Switzerland; Phone: +41 44 634
27 2660; Fax: +41 44 634 49 06; email: boettger@imm.uzh.ch
28 Prof. Sébastien Gagneux, PhD, Swiss Tropical and Public Health Institute, Socinstrasse 57,
29 4002 Basel, Switzerland; Phone: +41 61 284 8369; Fax: +41 61 284 8101; email:
30 sebastien.gagneux@swisstph.ch

32 **One sentence summary**

33 Whole genome sequencing of clinical *M. tuberculosis* isolates accurately predicts drug
34 resistance profiles and may replace culture-based drug susceptibility testing in the future.

35 **Abstract**

36 Whole genome sequencing allows rapid detection of drug-resistant *M. tuberculosis* isolates.
37 However, high-quality data linking quantitative phenotypic drug susceptibility testing (DST)
38 and genomic data have thus far been lacking.

39 We determined drug resistance profiles of 176 genetically diverse clinical *M. tuberculosis*
40 isolates from Democratic Republic of the Congo, Ivory Coast, Peru, Thailand and
41 Switzerland by quantitative phenotypic DST for 11 antituberculous drugs using the BD
42 BACTEC MGIT 960 system and 7H10 agar dilution to generate a cross-validated phenotypic
43 DST readout. We compared phenotypic drug susceptibility results with predicted drug
44 resistance profiles inferred by whole genome sequencing.

45 Both phenotypic DST methods identically classified the strains into resistant/susceptible in
46 73-99% of the cases, depending on the drug. Changes in minimal inhibitory concentrations
47 were readily explained by mutations identified by whole genome sequencing. Using the
48 whole genome sequences we were able to predict quantitative drug resistance levels where
49 wild type and mutant MIC distributions did not overlap. The utility of genome sequences to
50 predict quantitative levels of drug resistance was partially limited due to incompletely
51 understood mechanisms influencing the expression of phenotypic drug resistance. The overall
52 sensitivity and specificity of whole genome-based DST were 86.8% and 94.5%, respectively.

53 Despite some limitations, whole genome sequencing has high predictive power to infer
54 resistance profiles without the need for time-consuming phenotypic methods.

55 **Introduction**

56 Timely and accurate drug susceptibility testing (DST) of *M. tuberculosis* isolates is vital to
57 prevent the transmission of multidrug-resistant strains (MDR – resistance to rifampicin and
58 isoniazid)[1]. However, the slow growth and stringent biosafety requirements of *M.*
59 *tuberculosis* make obtaining a full DST profile by culture-based techniques a matter of weeks
60 or months. In addition, culture-based DST is notoriously challenging for several drugs, e.g.
61 pyrazinamide and ethionamide due to poor drug solubility in commonly used culture media
62 [2].

63 Drug resistance in *M. tuberculosis* is mainly conferred by chromosomal mutations in a few
64 genes [3], making it possible to detect drug resistance by sequencing these genes or probing
65 them by molecular hybridisation [4]. Several commercial tests for the detection of resistance-
66 associated mutations are available, e.g. the GenoType MTBDRplus V2 (Hain Lifescience
67 GmbH, Nehren, DE) [5], the AID TB Resistance Line Probe Assay (AID GmbH, Strassberg,
68 DE) [6]. Line probe assays and the GeneXpert® system (Cepheid, Sunnyvale, CA, USA) are
69 endorsed by the World Health Organisation (WHO) the detection of rifampicin resistance as
70 surrogate marker for MDR [7]. These molecular tests demonstrate high sensitivities for drugs
71 with established target(s) of resistance and for which only a few mutations are responsible for
72 most resistance *in clinico* (e.g. rifampicin, isoniazid) [4]. However, molecular tests show low
73 sensitivity for heteroresistant strains (concomitant presence of wild type (wt) and resistant or
74 multiple different resistant variants in patient isolates), when frequencies of resistant variants
75 drop below 5-50 % [8, 9]. Furthermore, there are no commercially available tests for many
76 drugs currently/prospectively in use (e.g. bedaquiline, delamanid, linezolid, p-aminosalicylic
77 acid).

78 The past years have seen a wealth of genomic data on drug-resistant *M. tuberculosis* become
79 available [10, 11]. However, phenotypic DST data are lacking for most of the genetic data
80 sets. In addition, DST data are often limited as the strains were classified as susceptible or
81 resistant using only a single drug concentration. There is an urgent need to link genotypic and
82 phenotypic drug resistance readouts to obtain a better understanding of the mechanisms
83 influencing the evolution and spread of drug resistance in *M. tuberculosis*.

84 WGS of clinical isolates allows for accurate identification of established-resistance-
85 conferring chromosomal mutations [10, 12, 13] and may ensure adequate treatment in days

86 instead of months. We compared whole genome-based drug resistance profiles with two
87 culture-based quantitative DST methods for a total of 11 drugs, including all first-line drugs
88 (rifampicin, isoniazid, ethambutol, pyrazinamide, streptomycin) and an array of second-line
89 drugs (rifabutin, amikacin, kanamycin A, capreomycin, moxifloxacin, ethionamide).

90 **Material and methods**

91 ***M. tuberculosis* isolates**

92 The initial data-set consisted of 190 *M. tuberculosis* isolates. A subset of 61 strains was used
93 to establish the phenotypic DST methodology. These 61 strains were collected by the Swiss
94 National Center for Mycobacteria between 2004-2015, and represent a broad spectrum in
95 geographic origin and drug resistance profiles [14–16]. We then applied the quantitative
96 DST methodology to 125 clinical isolates from clinics participating in the International
97 Epidemiology Databases to Evaluate AIDS (IeDEA) [17] in Peru, Thailand, Ivory Coast and
98 the Democratic Republic of the Congo (supplementary [Table S3](#)). Thirteen strains had to be
99 excluded due to failed WGS (n = 4, failed library preparation due to low DNA quality),
100 irreproducible DST results (n = 1), no growth in the 7H10 agar dilution assay (n = 3),
101 duplication (n = 1), mixed cultures (n = 2, cross-contamination or patient infected with
102 multiple strains) or transmission clusters (n = 2). The final set consisted of 176 strains.

103 **Phenotypic DST**

104 MGIT 960- and 7H10 agar dilution-based phenotypic DST were performed as described
105 previously [14]. [Table 1](#) lists the epidemiological cut-offs (ECOFF) used [18], supplementary
106 [Table S2](#) the drug concentrations tested with the MGIT 960 and 7H10 agar-dilution assays
107 and [Table 2](#) the genes screened for mutations with WGS. Further details are available in the
108 supplementary materials.

109 **Data analysis**

110 The categorical agreement between the MIC determination by MGIT 960 and 7H10 agar
111 dilution was determined based on the ECOFFS ([Table 1](#)).

112 The numerical variation between the two methods was quantified as the geometric standard
113 deviation (SD, given with its standard error) of the ratio MIC MGIT 960/MIC agar dilution,
114 expressed as a number of 2-fold dilutions and denoted by σ . The geometric SD was computed

115 by fitting a log-normal distribution to the ratio MIC MGIT 960/MIC agar dilution as
116 implemented in the R package *fitdistrplus* (v.1.0-9) [19]. If the data was compatible with $\sigma =$
117 0, the geometric standard deviation could not be estimated and was defined as “not
118 applicable” (NA). The approach is a generalization of the Bland and Altman method [20],
119 taking censoring of the data into account. Strains for which the MGIT 960 MIC and 7H10
120 agar dilution MIC were both left-censored or both right-censored were excluded since no
121 information on the ratio could be derived.

122 Goodman and Kruskal’s gamma was used to quantify the rank correlation between the two
123 methods. No correlation could be calculated if the variance for either method was 0 (NA).

124 Distributions of wt and mutant MICs were analysed qualitatively based on the results of
125 7H10 agar dilution. We divided the dataset into two groups: drugs for which the MIC
126 distributions of wt and mutant strains did not overlap, and those for which MIC distributions
127 overlapped.

128 Sensitivities and specificities of WGS-based resistance profile inference were calculated
129 based on the 7H10 agar dilution results for all drugs, except pyrazinamide – for which the
130 MGIT 960 results were used, based on resistance/susceptibility at the WHO-defined critical
131 concentrations and the presence or absence of a putative resistance-associated mutation.

132 **Defining clinical breakpoints for high/low-level resistance**

133 The therapeutic window of a drug is defined as the maximal serum concentration which is
134 considered safe [21]. Mutations can increase the MIC beyond the therapeutic window and
135 render the drug clinically ineffective. Drugs may have large therapeutic windows beyond the
136 ECOFF. For these, MIC increases caused by mutations may still be within the therapeutic
137 window of a drug: these strains might still be treatable by increasing the drug dose. We
138 analysed the distribution of MICs of mutant strains, and assessed if cut-offs for low-level
139 (within the therapeutic window) and high-level (beyond the therapeutic window) resistance
140 were definable.

141 **WGS and single nucleotide polymorphism (SNP) calling**

142 WGS and data analysis was performed as previously described [22] and summarised in the
143 supplementary materials. The performance of WGS-based DST greatly depends on the

144 availability of robust markers of resistance. We therefore focussed on a set of high-
145 confidence resistance-associated genes [4, 12] ([Table 2](#)).

146 **Ethics**

147 Local institutional review board or ethics committee approval was obtained at all local study
148 sites. Informed consent was obtained where requested per local regulations. This project was
149 approved by the Swiss Ethics Committee on research involving humans (swissethics, Bern,
150 Switzerland).

151 **Results**

152 **Agreement between MGIT 960 and 7H10 agar dilution phenotypic DST**

153 [Table 3](#) and [Figure 2](#) summarize the agreement between the semi-quantitative/quantitative
154 MIC determination by MGIT 960 and 7H10 agar dilution in terms of classifying strains as
155 resistant or susceptible according to ECOFFs ([Table 1](#)). Agreement was high for all drugs,
156 except ethambutol (see below). For most drugs, the MGIT 960-based MICs were higher than
157 the 7H10 agar dilution-based MICs. MICs obtained using the two methods were within 1-2
158 two-fold dilution steps of each other. The classifications into resistant or susceptible
159 demonstrated high rank correlations ([Table 3](#) and [Figure 2](#)), except for capreomycin
160 (supplementary Figure S4) due to few resistant strains included in the study.

161 **WGS and *in silico* resistance profile prediction**

162 A total of 176 WGS with a median coverage of 67.6X (interquartile range (IQR) = 37.48)
163 were obtained. Median mapping percentage and percentage of genome covered were 98.7%
164 (IQR = 0.94) and 99.4% (IQR = 0.4), respectively. All major *M. tuberculosis* lineages, except
165 lineage 7, were represented in the study (L1 = 6, L2 = 36, L3 = 11, L4 = 123, L5 = 1, L6 = 1).
166 The strains showed a range of drug resistance profiles ([Figure 1](#)). Based on the set of
167 analysed genes ([Table 2](#)), 25 strains were predicted to be fully susceptible against all assayed
168 drugs, 59 strains were mono-/poly-resistant, 91 strains demonstrated MDR phenotypes and
169 two strains were extensively drug resistant (XDR: isoniazid, rifampicin, fluoroquinolone and
170 aminoglycoside resistant).

171 **Drug resistance profile prediction by WGS vs. phenotypic DST**

172 After exclusion of known phylogenetic markers not involved in resistance, WGS-based
173 prediction of drug resistance using a defined set of target genes (Table 2) was highly
174 congruent with the categorical classification based on the phenotypic DST for most drugs
175 (Table 3 and Figure 2). Based on the *in silico* resistance prediction, the MICs of mutant and
176 wt strains frequently followed a Gaussian distribution. However, the same resistance marker
177 may confer different MICs in different strains (supplementary Figures S1C, S2C, S3C,
178 S8C, S9C, S10C). In some cases, the increase in the MIC conferred by a certain resistance
179 mutation fell within the distribution of wt MIC (e.g. for *gidB*, *eis* promotor mutations,
180 supplementary Figures S3C, S6C).

181 **Distinct wt and mutant MIC distributions**

182 MIC distributions of wt and mutant strains were well separated for rifampicin, rifabutin,
183 isoniazid, kanamycin A, amikacin, capreomycin, streptomycin and pyrazinamide, indicating
184 that the resistance markers used had a high positive predictive power (88.9% overall positive
185 predictive power of resistance markers). For streptomycin, two strains harboured no
186 mutations in the target genes, yet demonstrated high-level phenotypic resistance
187 (supplementary Figure S3C).

188 **Overlapping wt and mutant MIC distributions**

189 MIC distributions of wt and mutant strains overlapped for ethambutol, moxifloxacin and
190 ethionamide. For ethambutol and ethionamide, overlapping MIC distributions of wt and
191 mutant strains were associated with a large number of polymorphisms in resistance-
192 conferring genes (ethambutol resistance: 22 polymorphisms in *embB*, ethionamide resistance:
193 28 in *ethA*, 3 in *inhA*, 6 in *inhA* promoter). Solubility issues with ethionamide led to
194 quantitative differences in MGIT 960 vs. 7H10 agar dilution-based DST (Table 3, Figure 3).
195 The overlap in MIC distributions between wt and strains carrying an *embB* mutation was
196 reduced by adjusting the critical concentration for ethambutol resistance from 5 mg/L to 2.5
197 mg/L (MGIT 960). However, there was variability in the MICs for the same mutation (e.g.
198 MIC EmbB M306I/V in 7H10 agar dilution: 4-16 mg/L –supplementary Figure S2C).
199 Moxifloxacin resistance was rare (n = 9, MGIT 960, critical concentration 0.25 mg/L) and
200 MIC distributions of mutant strains partially overlapped with those of wt. Sensitivity of the
201 genome-based moxifloxacin resistance prediction was 80.0% (Table 4).

202 **Defining high-/low-level clinical breakpoint concentrations**

203 **Isoniazid**

204 Mutations in the promoter of *inhA* conferred low-level resistance <1 mg/L (7H10 agar
205 dilution), compared to strains harbouring mutations in *katG* or combinations of *inhA*
206 promoter and *katG* mutations which demonstrated MIC levels ranging from >1 mg/L to >32
207 mg/L in 7H10 agar dilution (supplementary [Figure S8C](#)). Defining clinical breakpoint
208 concentrations (CBC) for low- (≤ 1 mg/L for MGIT 960/7H10 agar dilution) and high-level
209 (>1 mg/L MGIT 960/7H10 agar dilution) isoniazid resistance is warranted.

210 **Rifampicin/Rifabutin**

211 Most mutations in *rpoB* increased the MIC for rifamycins beyond the therapeutic window
212 (peak serum concentration 10 mg/L [21, 23]). However, some rare *rpoB* mutations (e.g.
213 RpoB L452P, H445Y – supplementary [Figure 9C](#)) demonstrated MICs within the therapeutic
214 window. Defining low- and high-level CBC may thus be justified.

215 For rifampicin, CBC were $\leq 4/2$ mg/L for MGIT 960/7H10 agar dilution and $>4/2$ mg/L for
216 MGIT 960/7H10 agar dilution, respectively.

217 For rifabutin, our data suggests CBC for low- and high-level resistance of $\leq 0.4/0.25$ or 0.5
218 mg/L for MGIT 960/7H10 agar dilution and $>0.4/0.25$ or 0.5 mg/L for MGIT 960/7H10 agar
219 dilution, respectively.

220 Mutations in *rpoB* conferring resistance to rifampicin and rifabutin showed highly correlated
221 increases ([Figure 4](#)) of MICs beyond the therapeutic window for most *rpoB* mutations
222 ([Figure 3](#) and supplementary [Figure S9C & S10C](#)), indicating that both drugs are rendered
223 clinically ineffective and cannot substitute each other.

224 **Amikacin**

225 Few strains had mutations in the regions of *rrs* relevant for amikacin resistance or the *eis*
226 promoter (n=12). Mutations in *rrs* were associated with high-level (>128 mg/L in 7H10 agar
227 dilution) and mutations in the promoter region of *eis* with low-level level (2-4 mg/L in 7H10
228 agar dilution) amikacin resistance. Given the peak serum concentrations of amikacin (20-40
229 mg/L [21]), a CBC for low- (≤ 4 mg/L for MGIT 960/7H10 agar dilution) and high-level (4
230 mg/L for MGIT 960/7H10 agar dilution) amikacin resistance may be warranted.

231 **Streptomycin**

232 Certain mutations lead to MICs well beyond the peak serum concentrations [21] of
233 streptomycin (e.g. RpsL K43R, MIC 7H10 agar dilution >128 mg/L, supplementary Figure
234 S3C). On the other hand, *gidB* mutations increase the MIC only moderately (MIC 7H10 agar
235 dilution 1-4 mg/L, supplementary Figure 3C). Mutational combinations in *gidB*, *rrs*, *rpsL*
236 were common and produced a range of different MICs. However, there were mutations that
237 systematically lead to MICs beyond the therapeutic window, e.g. RpsL K43R. Defining low-
238 level (MGIT 960 \leq 4 mg/L, 7H10 agar dilution \leq 4-8 mg/L) and high-level CBC for
239 streptomycin resistance (MGIT 960 $>$ 4 mg/L, 7H10 agar dilution $>$ 4-8 mg/L) is warranted.

240 Discussion

241 We compared quantitative phenotypic DST with *in silico* or genomic resistance profile
242 prediction inferred from WGS using 176 clinical *M. tuberculosis* isolates.

243 The results of MGIT 960 and 7H10 agar dilution-based phenotypic DST methods were
244 highly correlated and suitable to separate susceptible from resistant variants. After exclusion
245 of known phylogenetic markers, genome-based resistance profile prediction displayed high
246 sensitivity and specificity for detecting resistance. Based on phenotypic DST results and
247 WGS, we were able to define CBC for high- and low-level resistance for isoniazid,
248 rifampicin, streptomycin and amikacin. Defining such breakpoints is important for preserving
249 efficacious drugs for treatment of resistant *M. tuberculosis* variants.

250 Our data suggest that the current WHO-defined critical concentration for phenotypic DST of
251 ethambutol by MGIT 960 (5 mg/L) is too high and may misclassify strains as susceptible
252 when compared to the 7H10 agar dilution-based classification. Given the low peak serum
253 concentrations for ethambutol, this may lead to mistreatment due to presumed ethambutol
254 susceptibility. After adjusting the critical concentration to 2.5 mg/L for MGIT 960, we
255 observed a strong improvement of the categorical agreement between MGIT 960- and 7H10
256 agar dilution-based classification.

257 The mutations identified by WGS had a high predictive power to classify strains as resistant.
258 However, the predictive power depends on a number of factors. For instance, the increase in
259 MIC conferred by an identical resistance mutation can vary greatly in different strains (e.g.
260 EmbB M306I/V, RpsL K88R). Such variation is clinically relevant if there is a significant
261 overlap between the MICs of mutant and wt strains, as was the case for ethionamide,
262 ethambutol and streptomycin (e.g. *gidB*) resistance mutations. Furthermore, it is difficult to

263 classify strains as resistant or susceptible if the MIC increase lies within the therapeutic
264 window of a drug. The overlap between MICs of mutant and wt strains is confounded by the
265 fact that we only screened for mutations in genes which had previously been associated with
266 drug resistance. We might thus have missed possible resistance-confering mutations in other
267 genes. Additionally, WGS will always produce distributions of coverages which in turn will
268 inevitably lead to certain regions in the genome suffering from low coverage, preventing the
269 detection of mutations. The inability to call mutations due to low coverage will therefore lead
270 to false negatives, reducing sensitivity. Furthermore, the strain genetic background [24], non-
271 mutational mechanisms (e.g. modulation of gene expression) [25], as well as drug efflux
272 mechanisms [26] may contribute to the variability in increase of the MIC conferred by
273 resistance mutations.

274 The predictive power of mutations in target genes also depends on removing phylogenetic
275 markers not involved in resistance. Separating phylogenetic from resistance-associated
276 markers works well for essential (highly conserved) genes such as *rpoB*, *rpsL*, *rrs* but is
277 problematic in non-essential genes involved in the conversion of prodrugs into their active
278 forms like *pncA* (pyrazinamide), *ethA* (ethionamide) or in genes that generally exhibit higher
279 numbers of polymorphisms e.g. *embB*. Of note, the *embABC* operon is highly polymorphic,
280 harbouring more polymorphisms than expected by chance (mutations in *embABC* operon =
281 81, expected = 44.8, $p = 9.174\text{e-}07$, binomial test). Mutations conferring ethambutol resistance
282 [27] will therefore inevitably evolve in the presence of phylogenetic SNPs and may interact
283 epistatically to produce the variability in MICs we observed for wt strains and for the most
284 common ethambutol resistance markers *embB* M306I/V. The *embABC* operon is involved in
285 the biosynthesis of decaprenylphosphoryl- β -d-arabinose, which is an integral component of
286 the mycobacterial cell wall. The cell envelope interacts with the host immune system and the
287 high levels of diversity of these genes might be the product of diversifying selection due to
288 host immune pressure. The influence of polymorphisms in the *embABC* operon on MICs in
289 general is supported by the observation that sub-inhibitory concentrations of ethambutol
290 lower the MICs for isoniazid, rifampicin and streptomycin [28]. Even small changes in
291 activity of the decaprenylphosphoryl- β -d-arabinose biosynthetic and utilisation pathway
292 might thus alter cell wall permeability and influence MICs of several drugs.

293 Similarly, in the case of streptomycin resistance, the *RpsL* substitution K88R exhibited a
294 range in MICs from low to high-level resistance making it difficult to judge the susceptibility
295 of a strain harbouring this mutation based on the genotype. Streptomycin was the first

296 effective antituberculous drug discovered [29] and has been used for decades. The long-term
297 use has produced complex resistance profiles with multiple streptomycin resistance mutations
298 (e.g. in *gidB*, *rpsL*, *rrs*) occurring concomitantly, producing wide ranges of MICs.
299 Furthermore, many streptomycin resistant strains displayed MDR/XDR phenotypes.
300 Streptomycin resistance mutations are frequently found in backgrounds which have mutations
301 in genes affecting the information pathway (DNA -> RNA -> proteins) – e.g. *gyrA* (DNA
302 gyrase), *rpoB* (DNA-dependent RNA polymerase), *rrs* (ribosomal RNA). The simultaneous
303 presence of multiple resistance mutations may alter the adaptive landscape [30, 31]. In
304 addition, non-mutational processes (e.g. alteration of gene expression) may compensate for
305 fitness costs of drug resistance and at the same time alter the MIC for the drug [25]. This has
306 not been demonstrated for streptomycin resistance in *M. tuberculosis*, but it seems possible
307 that compensation of fitness costs in MDR phenotypes might alter the MIC for streptomycin
308 [30], considering that streptomycin is not part of the current standard treatment regimen and
309 selection for high-level streptomycin resistance is relaxed.

310 In conclusion, we demonstrate that MGIT 960 and 7H10 agar dilution-based phenotypic DST
311 provide highly congruent classifications of strains into resistant or susceptible. WGS has high
312 predictive power to infer resistance profiles without the need for time-consuming phenotypic
313 methods. Limitations due to overlapping distributions of wt and mutant MICs, varying MICs
314 for the same resistance mutations in different strains, presence of phylogenetic markers in
315 resistance-associated genes and rare resistance markers with low frequencies will likely be
316 resolved by on-going large-scale projects (e.g. ReSeqTB [32]) combining phenotypic DST
317 with WGS of thousands of *M. tuberculosis* isolates. Our findings, together with those of on-
318 going studies will pave the way for the replacement of phenotypic DST with drug resistance
319 profile prediction based on WGS in the coming years.

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334

335 **Conflict of interest**

336 Peter M. Keller reports travel grants by Copan Italia SpA outside of the submitted work. Erik
337 C. Böttger is a consultant for AID Diagnostics.

338

339 **Tables**

340

341

Antibiotic	ECOFF agar dilution (mg/L)	ECOFF MGIT 960 (mg/L)
Ethionamide	1	5
Ethambutol	2	5
Capreomycin	4	2.5
Streptomycin	0.5	1
Kanamycin A	2	2
Amikacin	1	1
Moxifloxacin	0.25	0.25
Isoniazid	0.125	0.1
Rifampicin	0.5	1
Rifabutin	0.0625	0.1
Pyrazinamide	NA	100

Table 1 Epidemiological cutoffs (ECOFF) used for
7H10 agar dilution and MGIT 960 phenotypic DST [14]

342	Drug	Target gene(s)
	Ethionamide	<i>ethA, inhA, inhA</i> promoter
343	Ethambutol	<i>embB</i>
	Capreomycin	<i>rrs, eis</i> promoter, <i>tlyA</i>
344	Streptomycin	<i>rrs, gidB, rpsL</i>
345	Kanamycin A	<i>rrs, eis</i> promoter
	Amikacin	<i>rrs, eis</i> promoter
346	Moxifloxacin	<i>gyrA</i>
347	Isoniazid	<i>katG, inhA</i> promoter
	Rifampicin/rifabutin	<i>rpoB</i>
348	Pyrazinamide	<i>pncA, pncA</i> promoter

349 Table 2 List of genes implicated in drug resistance in *M. tuberculosis* which were screened for
 350 polymorphisms by WGS. List adapted from [3, 21]

351	Antibiotic	n	Categorical agreement (%)	SD of $\log_2(\text{MIC MGIT 960}/\text{MIC}$ agar dilution)	γ
352	Ethionamide	56	95	1.9 ± 0.3	0.91
	Ethambutol	171	73	1.9 ± 0.5	0.94
353	Capreomycin	56	98	1.5 ± 0.5	0.65
	Streptomycin	56	93	1.5 ± 0.3	0.98
354	Kanamycin A	56	98	1.2 ± 0.2	0.8
	Amikacin	174	98	1.4 ± 0.6	1
355	Moxifloxacin	173	99	1 ± 0.2	1
	Isoniazid	173	96	1.2 ± 0.1	1
356	Rifampicin	174	99	NA	1
	Rifabutin	56	96	0.8 ± 0.1	0.98

357 Table 3 Summary statistics of the method agreement between 7H10 agar dilution- and MGIT 960-based
 phenotypic DST for all drugs assayed in this study

358	Drug	Sensitivity (%)	Specificity (%)
359	Ethionamide	75.0	92.9
	Ethambutol	89.6	94.2
360	Capreomycin	75.0	94
	Streptomycin	68.0	92.1
361	Kanamycin A	83.3	98.8
	Amikacin	63.6	96.9
	Moxifloxacin	80.0	90.2
	Isoniazid	93.6	96.8
	Rifampicin	100	94.0
	Rifabutin	98.9	94.0
	Pyrazinamide	80.8	88.9

362 Table 4 Sensitivity and specificity of the genome-based drug resistance profile prediction
 363 using the 7H10 agar dilution-based categorical classification as the gold standard for all
 364 drugs except pyrazinamide, for which the MGIT 960 categorical classification was used

362 **References**

363 1. World Health Organization. Treatment of tuberculosis: Guidelines [Internet]. 4th Editio. Treat. Tuberc.
364 Guidel. 2010. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK138741/#ch2.s3>.

365 2. Domínguez J, Boettger EC, Cirillo D, Cobelens F, Eisenach KD, Gagneux S, Hillemann D, Horsburgh
366 R, Molina-Moya B, Niemann S, Tortoli E, Whitelaw A, Lange C, TBNET, RESIST-TB networks.
367 Clinical implications of molecular drug resistance testing for *Mycobacterium* tuberculosis: a
368 TBNET/RESIST-TB consensus statement. *Int. J. Tuberc. Lung Dis.* [Internet] 2016; 20: 24–42 Available
369 from: <http://www.ncbi.nlm.nih.gov/pubmed/26688526>.

370 3. Gygli SM, Borrell S, Trauner A, Gagneux S. Antimicrobial resistance in *Mycobacterium* tuberculosis:
371 mechanistic and evolutionary perspectives. *FEMS Microbiol. Rev.* [Internet] 2017; : 1–20 Available
372 from: <https://academic.oup.com/femsre/article-lookup/doi/10.1093/femsre/fux011>.

373 4. Deggim-Messmer V, Bloemberg G V., Ritter C, Voit A, Hönke R, Keller PM, Böttger EC. Diagnostic
374 Molecular Mycobacteriology in Regions With Low Tuberculosis Endemicity: Combining Real-time
375 PCR Assays for Detection of Multiple Mycobacterial Pathogens With Line Probe Assays for
376 Identification of Resistance Mutations. *EBioMedicine* [Internet] The Authors; 2016; 9: 228–
377 237 Available from: <http://dx.doi.org/10.1016/j.ebiom.2016.06.016>.

378 5. Nathavitharana RR, Hillemann D, Schumacher SG, Schlueter B, Ismail N, Omar SV, Sikhondze W,
379 Havumaki J, Valli E, Boehme C, Denkinger CM. Multicenter noninferiority evaluation of hain
380 GenoType MTBDRplus Version 2 and Nipro NTM+MDRTB line probe assays for detection of rifampin
381 and isoniazid resistance. *J. Clin. Microbiol.* 2016; 54: 1624–1630.

382 6. Ritter C, Lucke K, Sirgel FA, Warren RW, van Helden PD, Bottger EC, Bloemberg G V. Evaluation of
383 the AID TB Resistance Line Probe Assay for Rapid Detection of Genetic Alterations Associated with
384 Drug Resistance in *Mycobacterium* tuberculosis Strains. *J. Clin. Microbiol.* [Internet] 2014; 52: 940–
385 946 Available from: <http://jcm.asm.org/cgi/doi/10.1128/JCM.02597-13>.

386 7. WHO. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous
387 Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of
388 Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update [Internet]. Autom. Real-
389 Time Nucleic Acid Amplif. Technol. Rapid Simultaneous Detect. Tuberc. Rifampicin Resist. Xpert
390 MTB/RIF Assay Diagnosis Pulm. Extrapulm. TB Adults Child. Policy Updat. 2013. Available from:
391 <http://www.ncbi.nlm.nih.gov/pubmed/25473701>.

392 8. Engström A. Fighting an old disease with modern tools: characteristics and molecular detection methods
393 of drug-resistant *Mycobacterium* tuberculosis. *Infect. Dis. (London, England)* [Internet] 2015; 4235: 1–
394 17 Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26167849>.

395 9. Streicher EM, Bergval I, Dheda K, Böttger EC, Gey Van Pittius NC, Bosman M, Coetze G, Anthony
396 RM, Van Helden PD, Victor TC, Warren RM. *Mycobacterium* tuberculosis population structure
397 determines the outcome of genetics-based second-line drug resistance testing. *Antimicrob. Agents
398 Chemother.* 2012; 56: 2420–2427.

399 10. Coll F, McNerney R, Preston MD, Guerra-Assunção JA, Warry A, Hill-Cawthorne G, Mallard K, Nair
400 M, Miranda A, Alves A, Perdigão J, Viveiros M, Portugal I, Hasan Z, Hasan R, Glynn JR, Martin N,
401 Pain A, Clark TG. Rapid determination of anti-tuberculosis drug resistance from whole-genome
402 sequences. *Genome Med.* [Internet] 2015; 7: 51 Available from:
403 <http://genomemedicine.com/content/7/1/51>.

404 11. Walker TM, Kohl TA, Omar S V, Hedge J, Del C, Elias O, Bradley P, Iqbal Z, Feuerriegel S, Niehaus
405 KE, Wilson DJ, Clifton DA, Kapatai G, Ip CLC, Bowden R, Drobniowski FA, Allix-béguec C, Gaudin
406 C, Parkhill J, Diel R, Supply P, Crook DW, Smith EG, Walker AS, Ismail N, Niemann S. Whole-
407 genome sequencing for prediction of *Mycobacterium* tuberculosis drug susceptibility and resistance□: a
408 retrospective cohort study. 2015; 3099: 1–10.

409 12. Shea J, Halse TA, Lapierre P, Shudt M, Kohlerschmidt D, Van Roey P, Limberger R, Taylor J, Escuyer
410 V, Musser KA. Comprehensive Whole-Genome Sequencing and Reporting of Drug Resistance Profiles

411 on Clinical Cases of *Mycobacterium tuberculosis* in New York State. *J. Clin. Microbiol.* [Internet]
412 2017; 55: 1871–1882 Available from:
413 <http://jcm.asm.org/content/55/6/1871.full.pdf%0Ahttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=re>
414 ference&D=emex&NEWS=N&AN=616382615.

415 13. Colman RE, Anderson J, Lemmer D, Lehmkuhl E, Georghiou SB, Heaton H, Wiggins K, Gillece JD,
416 Schupp JM, Catanzaro DG, Crudu V, Cohen T, Rodwell TC, Engelthaler DM. Rapid Drug
417 Susceptibility Testing of Drug Resistant *Mycobacterium tuberculosis* Directly from Clinical Samples
418 using Amplicon Sequencing: A Proof of Concept Study. *J. Clin. Microbiol.* [Internet] 2016; 54:
419 JCM.00535-16 Available from: <http://jcm.asm.org/lookup/doi/10.1128/JCM.00535-16> <https://github.com/TGenNorth/SMOR>.

421 14. Springer B, Lucke K, Calligaris-Maibach R, Ritter C, Böttger EC. Quantitative drug susceptibility
422 testing of *Mycobacterium tuberculosis* by use of MGIT 960 and EpiCenter instrumentation. *J. Clin.*
423 *Microbiol.* 2009; 47: 1773–1780.

424 15. Stucki D, Ballif M, Egger M, Furrer H, Altpeter E, Battegay M, Droz S, Bruderer T, Coscolla M,
425 Borrell S, Zürcher K, Janssens J-P, Calmy A, Mazza Stalder J, Jaton K, Rieder HL, Pfyffer GE, Siegrist
426 HH, Hoffmann M, Fehr J, Dolina M, Frei R, Schrenzel J, Böttger EC, Gagneux S, Fenner L. Standard
427 Genotyping Overestimates Transmission of *Mycobacterium tuberculosis* among Immigrants in a Low-
428 Incidence Country. Carroll KC, editor. *J. Clin. Microbiol.* [Internet] 2016; 54: 1862–1870 Available
429 from: <http://jcm.asm.org/lookup/doi/10.1128/JCM.00126-16>.

430 16. Bloomberg G V., Keller PM, Stucki D, Trauner A, Borrell S, Latshang T, Coscolla M, Rothe T, Hömke
431 R, Ritter C, Feldmann J, Schulthess B, Gagneux S, Böttger EC. Acquired Resistance to Bedaquiline and
432 Delamanid in Therapy for Tuberculosis. *N. Engl. J. Med.* [Internet] 2015; 373: 1986–1988 Available
433 from: <http://www.ncbi.nlm.nih.gov/pubmed/26559594>.

434 17. Egger M, Ekouevi DK, Williams C, Lyamuya RE, Mukumbi H, Braitstein P, Hartwell T, Gruber C, Chi
435 BH, Boulle A, Dabis F, Wools-Kaloustian K. Cohort Profile: The international epidemiological
436 databases to evaluate AIDS (IeDEA) in sub-Saharan Africa. *Int. J. Epidemiol.* [Internet] 2012; 41:
437 1256–1264 Available from: <https://academic.oup.com/ije/article-lookup/doi/10.1093/ije/dyr080>.

438 18. World Health Organization. Technical Report on critical concentrations for drug susceptibility testing of
439 medicines used in the treatment of drug-resistant tuberculosis [Internet]. 2018. Available from:
440 <http://www.who.int/iris/handle/10665/260470>.

441 19. Delignette-Muller ML, Dutang C. fitdistrplus: an R package for fitting distributions. *J. Stat. Softw.*
442 2015; 64: 1–34.

443 20. Martin Bland J, Altman D. STATISTICAL METHODS FOR ASSESSING AGREEMENT BETWEEN
444 TWO METHODS OF CLINICAL MEASUREMENT. *Lancet* [Internet] 1986; 327: 307–310 Available
445 from: <http://www.sciencedirect.com/science/article/pii/S0140673686908378>.

446 21. Böttger EC. The ins and outs of *Mycobacterium tuberculosis* drug susceptibility testing. *Clin. Microbiol.*
447 *Infect.* [Internet] 2011; 17: 1128–1134 Available from:
448 <http://linkinghub.elsevier.com/retrieve/pii/S1198743X14629396>.

449 22. Ghielmetti G, Coscolla M, Ruetten M, Friedel U, Loiseau C, Feldmann J, Steinmetz HW, Stucki D,
450 Gagneux S. Tuberculosis in Swiss captive Asian elephants: microevolution of *Mycobacterium*
451 tuberculosis characterized by multilocus variable-number tandem-repeat analysis and whole-genome
452 sequencing. *Sci. Rep.* [Internet] 2017; 7: 14647 Available from: <http://www.nature.com/articles/s41598-017-15278-9>.

454 23. Sekaggya-Wiltshire C, von Braun A, Lamorde M, Ledberger B, Buzibye A, Henning L, Musaazi J,
455 Gutteck U, Denti P, de Kock M, Jetter A, Byakika-Kibwika P, Eberhard N, Matovu J, Joloba M, Muller
456 D, Manabe YC, Kamya MR, Corti N, Kambugu A, Castelnovo B, Fehr JS. Delayed Sputum
457 Conversion in TB-HIV Co-Infected Patients with Low Isoniazid and Rifampicin Concentrations. *Clin.*
458 *Infect. Dis.* [Internet] 2018; Available from: <https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciy179/4919550>.

460 24. Fenner L, Egger M, Bodmer T, Altpeter E, Zwahlen M, Jaton K, Pfyffer GE, Borrell S, Dubuis O,

461 Bruderer T, Siegrist HH, Furrer H, Calmy A, Fehr J, Stalder JM, Ninet B, Bottger EC, Gagneux S.
 462 Effect of Mutation and Genetic Background on Drug Resistance in *Mycobacterium tuberculosis*.
 463 *Antimicrob. Agents Chemother.* [Internet] 2012 [cited 2013 Aug 9]; 56: 3047–3053 Available from:
 464 <http://www.ncbi.nlm.nih.gov/articlerender.fcgi?artid=3370767&tool=pmcentrez&rendertype=abstract>.
 465

466 25. Freihofer P, Akbergenov R, Teo Y, Juskeviciene R, Andersson DI, Böttger EC. Nonmutational
 467 compensation of the fitness cost of antibiotic resistance in mycobacteria by overexpression of *tlyA*
 468 rRNA methylase. *RNA* [Internet] 2016; 22: 1836–1843 Available from:
 469 <https://www.ncbi.nlm.nih.gov/pubmed/27698071>.

470 26. da Silva PEA, Machado D, Ramos D, Couto I, Von Groll A, Viveiros M. Efflux Pumps in
 471 Mycobacteria: Antimicrobial Resistance, Physiological Functions, and Role in Pathogenicity. In: Li X-
 472 Z, Elkins CA, Zgurskaya HI, editors. *Efflux-Mediated Antimicrob. Resist. Bact.* [Internet] Cham:
 473 Springer International Publishing; 2016. p. 527–559 Available from:
 474 <http://link.springer.com/10.1007/978-3-319-39658-3>.

475 27. Safi H, Lingaraju S, Amin A, Kim S, Jones M, Holmes M, McNeil M, Peterson SN, Chatterjee D,
 476 Fleischmann R, Alland D. Evolution of high-level ethambutol-resistant tuberculosis through interacting
 477 mutations in decaprenylphosphoryl-β-D-Arabinose biosynthetic and utilization pathway genes. *Nat.*
 478 *Genet.* [Internet] Nature Publishing Group; 2013; 45: 1190–1197 Available from:
 479 <http://dx.doi.org/10.1038/ng.2743>.

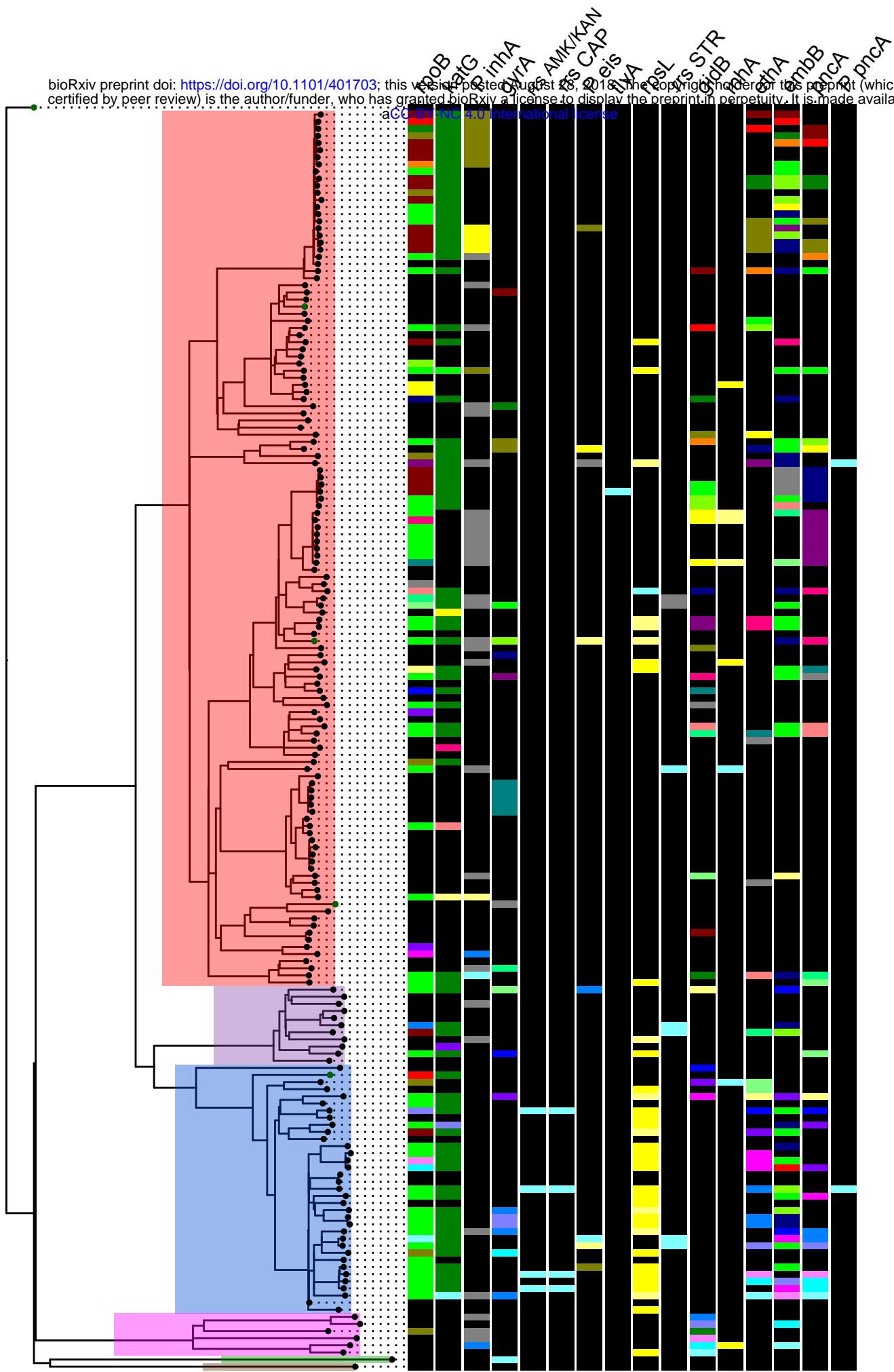
480 28. Jagannath C, Reddy VM, Gangadharam PRJ. Enhancement of drug susceptibility of multi-drug resistant
 481 strains of *Mycobacterium tuberculosis* by ethambutol and dimethyl sulphoxide. *J. Antimicrob.*
 482 *Chemother.* [Internet] 1995; 35: 381–390 Available from: <https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/35.3.381>.

484 29. Schatz A, Bugle E, Waksman SA. Streptomycin, a Substance Exhibiting Antibiotic Activity Against
 485 Gram-Positive and Gram-Negative Bacteria. *Exp. Biol. Med.* 1944; 55: 66–69.

486 30. Moura de Sousa J, Balbontín R, Durão P, Gordo I. Multidrug-resistant bacteria compensate for the
 487 epistasis between resistances. de Visser A, editor. *PLOS Biol.* [Internet] 2017; 15: e2001741 Available
 488 from: <http://dx.plos.org/10.1371/journal.pbio.2001741>.

489 31. Borrell S, Teo Y, Giardina F, Streicher EM, Klopper M, Feldmann J, Muller B, Victor TC, Gagneux S.
 490 Epistasis between antibiotic resistance mutations drives the evolution of extensively drug-resistant
 491 tuberculosis. *Evol. Med. Public Heal.* [Internet] 2013 [cited 2013 May 30]; 2013: 65–74 Available from:
 492 <http://emph.oxfordjournals.org/cgi/doi/10.1093/emph/eot003>.

493 32. Starks AM, Avilés E, Cirillo DM, Denkinger CM, Dolinger DL, Emerson C, Gallarda J, Hanna D, Kim
 494 PS, Liwski R, Miotto P, Schito M, Zignol M. Collaborative Effort for a Centralized Worldwide
 495 Tuberculosis Relational Sequencing Data Platform: Figure 1. *Clin. Infect. Dis.* [Internet] 2015; 61:
 496 S141–S146 Available from: <https://academic.oup.com/cid/article-lookup/doi/10.1093/cid/civ610>.



0.005 Figure 1 Maximum likelihood phylogeny of 176 *M. tuberculosis* strains based on 20510 variable positions. Reference strains labeled with green tip labels. Main lineages are highlighted as follows: red L4, purple L3, blue L2, pink L1, green L6, brown L5. Scale bar indicates number of substitutions per site. Phylogeny rooted on *M. canettii*. Colored bars indicate resistance mutations per gene and within a distinct column (gene); each colored bar represents a distinct mutation. Black bars indicate no mutation, i.e. wt.

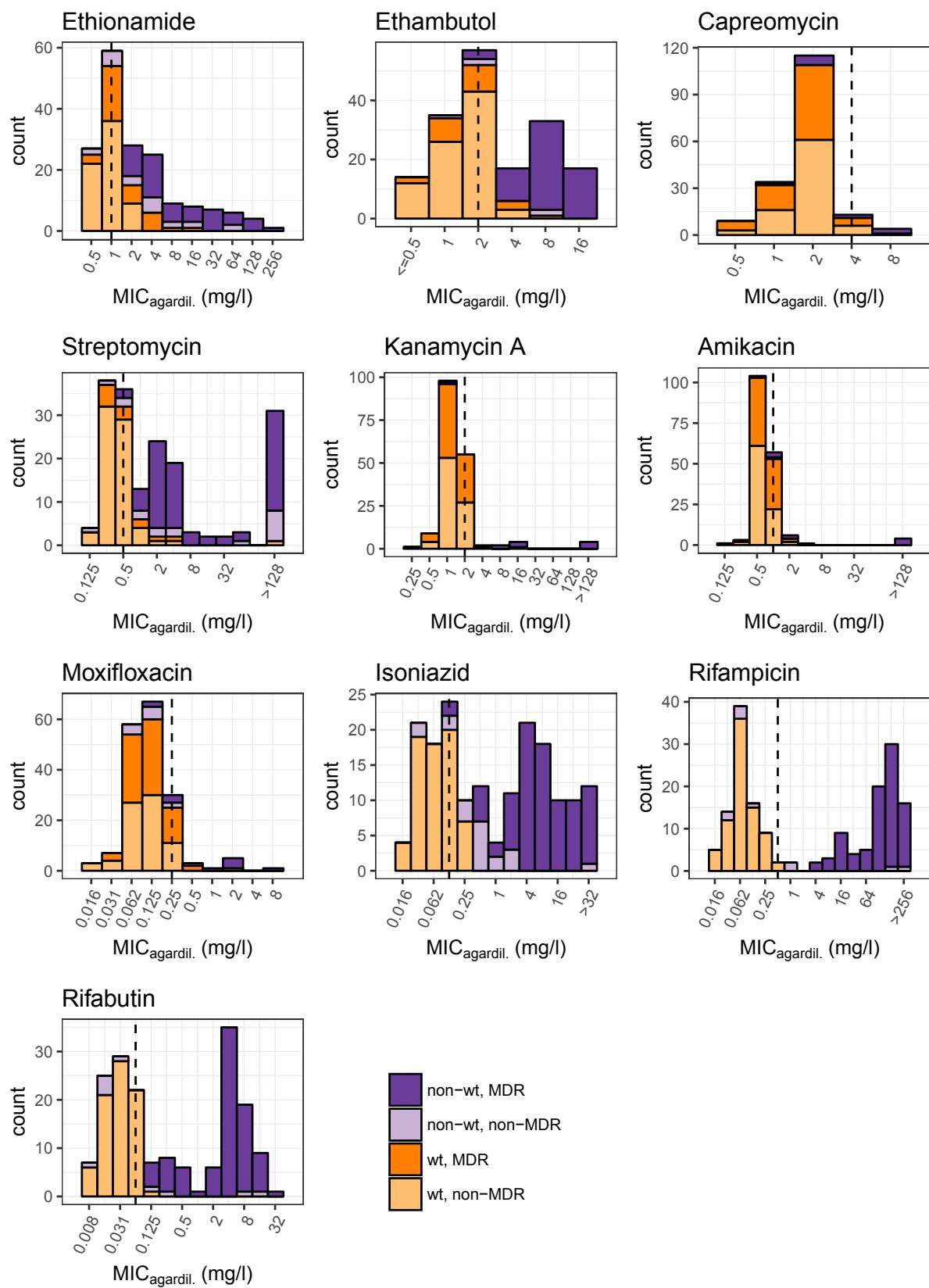


Figure 2 Histograms of MICs (7H10 agar dilution) for all drugs assayed in this study

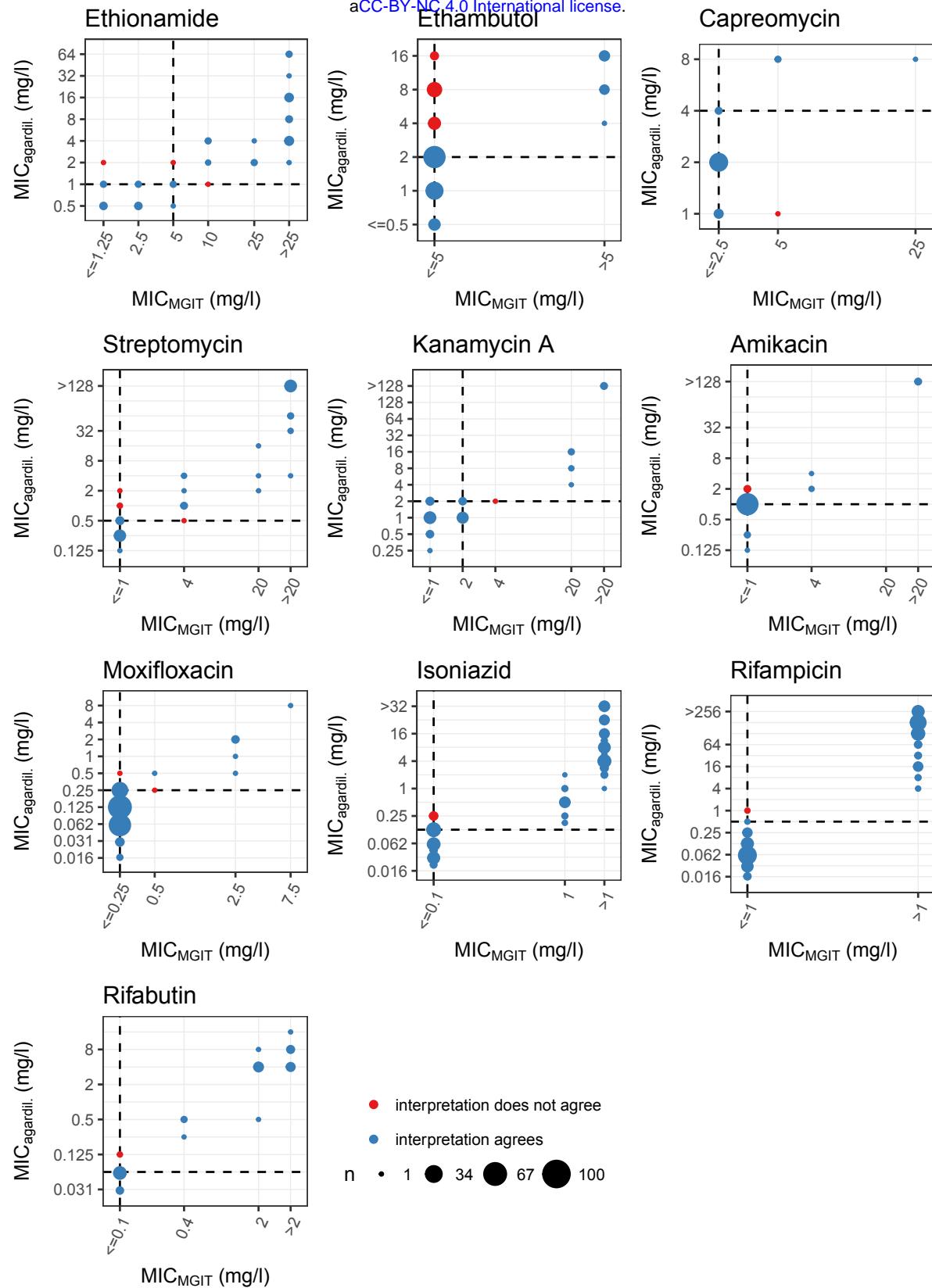


Figure 3 Method agreement between phenotypic DST performed with MGIT 960 and 7H10 agar dilution represented as Bland-Altman plots for all drugs tested in this study.

n ● 4 ● 8 ● 12 ● 16

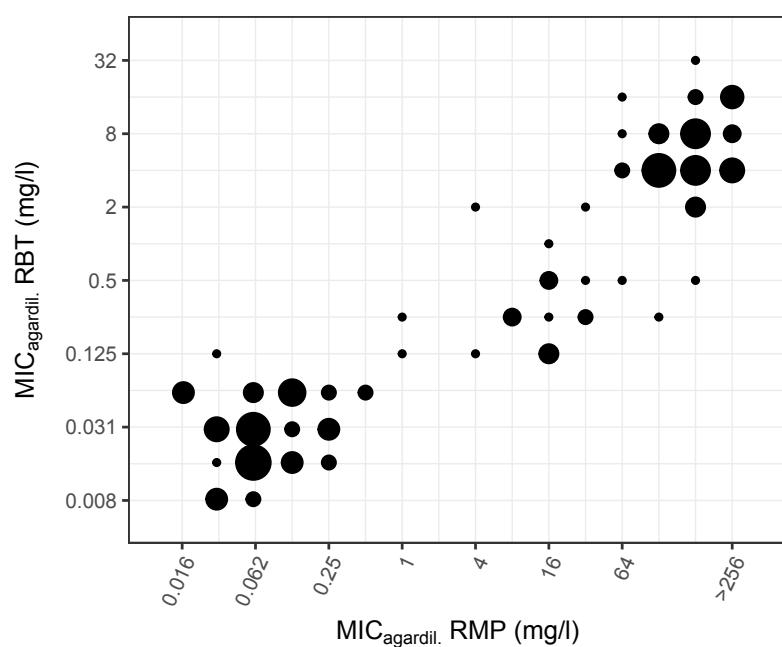


Figure 4 Correlation between 7H10 agar dilution MICs for rifampicin and rifabutin