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Mbelele, Peter

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Whole genome sequencing-based drug resistance predictions of multidrug-resistant *Mycobacterium tuberculosis* isolates from Tanzania

Peter M. Mbelele[1,2*†], Christian Utpatel[3,4†], Elingarami Sauli[2], Emmanuel A. Mpolya[2], Beatrice K. Mutayoba[5,6], Ivan Barilar[3,4], Viola Dreyer[3,4], Matthias Merker[3,7], Margaretha L. Sariko[8], Buliga M. Swema[8], Blandina T. Mmbaga[8,9], Jean Gratz[10], Kennedy K. Addo[11], Michel Pletschette[6,12], Stefan Niemann[3,4], Eric R. Houpt[10], Stellah G. Mpagama[1,2,8,9] and Scott K. Heysell[10]

[1]*Kibong'oto Infectious Diseases Hospital (KIDH), Siha, Kilimanjaro, Tanzania;* [2]*Department of Global Health and Biomedical Sciences, School of Life Sciences and Bioengineering, Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania;* [3]*Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany;* [4]*German Center for Infection Research (DZIF) Tuberculosis Unit, Borstel, Germany;* [5]*Ministry of Health, National AIDS Control Program, Department of Preventive Services, Dodoma, Tanzania;* [6]*CIHLMU Center for International Health, University Hospital, LMU Munich, Germany;* [7]*Evolution of the Resistome, Research Center Borstel, Borstel, Germany;* [8]*Kilimanjaro Clinical Research Institute, Moshi, Tanzania;* [9]*Kilimanjaro Christian Medical University College, Moshi, Tanzania;* [10]*Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, USA;* [11]*Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana;* [12]*Division of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich (LMU), Munich, Germany*

*Corresponding author. E-mail: mbelelepeter@yahoo.com
†These authors made an equal contribution.

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Background: Rifampicin- or multidrug-resistant (RR/MDR) *Mycobacterium tuberculosis* complex (MTBC) strains account for considerable morbidity and mortality globally. WGS-based prediction of drug resistance may guide clinical decisions, especially for the design of RR/MDR-TB therapies.

Methods: We compared WGS-based drug resistance-predictive mutations for 42 MTBC isolates from MDR-TB patients in Tanzania with the MICs of 14 antibiotics measured in the Sensititre™ MycoTB assay. An isolate was phenotypically categorized as resistant if it had an MIC above the epidemiological-cut-off (ECOFF) value, or as susceptible if it had an MIC below or equal to the ECOFF.

Results: Overall, genotypically non-wild-type MTBC isolates with high-level resistance mutations (gNWT-R) correlated with isolates with MIC values above the ECOFF. For instance, the median MIC value (mg/L) for rifampicin-gNWT-R strains was >4.0 (IQR 4.0–4.0) compared with 0.5 (IQR 0.38–0.50) in genotypically wild-type (gWT-S, $P < 0.001$); isoniazid-gNWT-R >4.0 (IQR 2.0–4.0) compared with 0.25 (IQR 0.12–1.00) among gWT-S ($P = 0.001$); ethionamide-gNWT-R 15.0 (IQR 10.0–20.0) compared with 2.50 (IQR; 2.50–5.00) among gWT-S ($P < 0.001$). WGS correctly predicted resistance in 95% (36/38) and 100% (38/38) of the rifampicin-resistant isolates with ECOFFs >0.5 and >0.125 mg/L, respectively. No known resistance-conferring mutations were present in genes associated with resistance to fluoroquinolones, aminoglycosides, capreomycin, bedaquiline, delamanid, linezolid, clofazimine, cycloserine, or p-amino salicylic acid.

Conclusions: WGS-based drug resistance prediction worked well to rule-in phenotypic drug resistance and the absence of second-line drug resistance-mediating mutations has the potential to guide the design of RR/MDR-TB regimens in the future.

Introduction

Drug resistance in *Mycobacterium tuberculosis* complex (MTBC) isolates is fundamentally conferred through spontaneous point mutations in specific gene targets for an anti-tuberculosis drug.[1,2] A combination of these point mutations can result in multidrug-resistant tuberculosis (MDR-TB), defined as a TB caused by strains resistant to at least rifampicin and isoniazid,[3] and extensively drug resistant TB (XDR-TB), described as MDR-TB with additional resistance to at least one fluoroquinolone (levofloxacin, moxifloxacin, ofloxacin, gatifloxacin) and either bedaquiline or linezolid.[4] The global incidence of rifampicin-resistant or MDR-TB (RR/MDR-TB) has nearly doubled from 250 000 cases in 2010 to 463 000 cases in 2019. Despite Tanzania being a low-burden country, RR/MDR-TB notifications have risen from 34 in 2010 to 449 cases in 2019.[5] In patients with RR/MDR-TB the global treatment success rate is 57%, although success rates in Tanzania (83%) are relatively higher.[6] Mathematical models have shown that without optimal diagnostic and treatment solutions, RR/MDR-TB incidence will increase by 17% and mortality by 22% in 10 years, and it could become the dominant form of TB by 2050.[7,8] Relatively high rates of treatment success in Tanzania may in part be due to less-widespread genetic resistance in regionally circulating strains.

In order to improve RR/MDR-TB treatment outcomes, the WHO has transitioned from injectable to all-oral regimens composed of new drugs including bedaquiline and delamanid, as well as two repurposed drugs, clofazimine and linezolid.[3,9] Despite the high mycobactericidal activity of these regimens,[10] the MTBC genome remains vulnerable to development of new mutations that confer resistance to these drugs.[2] Phenotypic drug susceptibility testing (DST) by the proportion method on solid Löwenstein–Jensen (LJ) medium or the Mycobacterium Growth Indicator Tube (MGIT) liquid culture system are the gold standard.[11,12] With these methods, the MTBC isolate is deemed resistant based on growth in the presence of a single concentration of antibiotic, historically known as a critical concentration. This approach does not quantify the level of resistance, and therefore clinicians cannot adjust drug dosage when the patient is infected by a low-level resistant strain.[13] Alternatively, measuring the MIC, the lowest concentration of an anti-TB drug that inhibits 99% of the visible growth of MTBC isolates, may address these limitations.[14,15] The MIC is determined when MTBC strains are tested at multiple serial concentrations of anti-TB drugs that have been previously reported and employed clinically.[16,17] The MIC quantifies the level of resistance and may be able to resolve discordances between genotypic and phenotypic DST such as when a clinical breakpoint is greater than or equal to the tentative epidemiological cut-off value (ECOFF).[17,18] MIC testing can be done by commercially available microdilution platforms such as the Sensititre™ MycoTB assay (MycoTB; Trek Diagnostics, Cleveland, OH, USA).[14,19] The Sensititre™ MycoTB assay is customizable and can contain multiple different lyophilized anti-TB drugs at different concentrations, which, depending on the concentrations selected, can quantify elevated MICs that remain at or near the defined breakpoint.[14,20] Previous reports have demonstrated that Sensititre™ MycoTB assay results compare favourably to other culture-based DST methods.[14] Unfortunately, culture-based DST is prone to contamination and can take up to 8 weeks for definitive results, thereby limiting the scope for timely clinical action.[21,22]

Molecular methods such as the Xpert® MTB/RIF (Cepheid, USA) and line probe assays (LPA) (Hain Life-Science, Germany) are faster compared with culture-based DST, but they only target a small number of resistance-associated mutations in high fidelity regions for a few anti-TB drugs. For instance, while Xpert® MTB/RIF detects changes in target genes associated with rifampicin resistance, the LPA detects resistance to isoniazid, fluoroquinolones, aminoglycosides and capreomycin.[23,24] Importantly, Xpert® MTB/RIF and LPA do not interrogate mutations in genes previously linked with resistance to bedaquiline (*Rv0678*, *atpE*, and *pepQ*), delamanid (*ddn, fgd1, fbiA*, and *fbiC*), linezolid (*rrl* and *rplC*), clofazimine (*Rv0678, Rv1979c, pepQ*) or cycloserine (*alr, ddl, cyA*),[2,25,26] the key drugs recommended within the revised all-oral RR/MDR-TB treatment regimens.[9] Whole genome sequencing (WGS) can detect all putative resistance-associated mutations across the entire MTBC genome, overcoming these barriers.[18,27] However, much work remains in determining for certain drugs which putative resistance-associated mutations have an effect on the MIC and whether certain mutations confer lower or higher levels of phenotypic resistance. Therefore, this pilot study compared WGS resistance-associated mutations with the MICs of anti-TB drugs as measured by Sensititre™ MycoTB assay among MTBC isolates from patients treated for RR/MDR-TB in Tanzania.

Materials and methods

Design, isolates and ethics

This cross-sectional study utilized 50 pre-treatment MTBC cultured isolates from adult patients aged ≥18 years, who were diagnosed with RR/MDR-TB in Tanzania. These 50 isolates were randomly selected from a list of 86 cultured isolates from patients who were enrolled to participate in a prospective cohort study in 2016 through 2018 (NCT03559582) at the Kibong'oto Infectious Diseases Hospital in Kilimanjaro region, Tanzania. At recruitment, RR/MDR-TB was confirmed using Xpert® MTB/RIF, and LPA including genotype MTBDR*plus* and MTBDR*sl*. Prior to study procedures, patients signed a witnessed written informed consent for a protocol approved by the National Institute for Medical Research in Tanzania, and the institutional review board of the University of Virginia in the USA (DMID #15-0100). After collecting the first sputum for culture in MGIT, all patients received RR/MDR-TB treatment in accordance with the Tanzania guideline for 2016 to 2018. The MIC testing and DNA extraction from pre-treatment MTBC cultured isolates were performed at the Kilimanjaro Clinical Research Institute in Tanzania. The isolates were stored at −80°C in trypticase soy broth supplemented with 10% glycerol until March 2019 when MTBC DNA was extracted and shipped to the Research Centre Borstel in Germany for WGS.

MIC testing by Sensititre™ MycoTB assay

The MIC of anti-TB drugs for cultured MTBC isolates was measured by the Sensititre™ MycoTB assay as described previously.[14,15,20] The Sensititre™ MycoTB assay was customized by Trek to be able to test 14 different first- and second-line anti-TB drugs per plate and sample: rifampicin, rifabutin, isoniazid, ethambutol, levofloxacin, moxifloxacin, kanamycin, amikacin, streptomycin, capreomycin, clofazimine, cycloserine, ethionamide, and p-amino salicylic acid. Individual drug concentrations tested per drug are shown in Table 1. In brief, suspensions of cultured isolates and the laboratory reference strain *M. tuberculosis* H37Rv (ATCC 27294) were

Table 1. Drug concentrations tested and epidemiological cut-off (ECOFF) values in Sensititre™ MycoTB assay

Anti-TB drugs	Tested concentrations (mg/L)	Tentative ECOFF (mg/L)
Isoniazid	0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0	0.25
Rifampicin	0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0	0.5 and 0.125
Rifabutin	0.125, 0.25, 0.5 and 1.0	0.125
Ethambutol	1.0, 2.0, 4.0, 8.0, 16.0 and 32.0	2.0 and 4.0
Streptomycin	0.25, 0.5, 1.0, 2.0, 4.0 and 8.0	2.0
Amikacin	0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0	2.0
Kanamycin	0.3, 0.6, 1.2, 2.5, 5.0, 10.0 and 20.0	2.5 and 5.0
Capreomycin	0.3, 0.6, 1.2, 2.5, 5.0, 10.0 and 20.0	2.5 and 5.0
Levofloxacin	0.125, 0.25, 1.0, 2.0, 4.0 and 8.0	1.0
Moxifloxacin	0.125, 0.25, 1.0, 2.0, 4.0 and 8.0	0.5
Clofazimine	0.06, 0.12, 0.25, 0.5, 1.0, 2.0 and 4.0	0.25 and 1.0
Ethionamide	0.6, 1.2, 2.5, 5.0, 10.0 and 20.0	5.0
Cycloserine[30]	4.0, 8.0, 16.0, 32.0, 64.0 and 128.0	64.0
p-Aminosalicylic acid	0.25, 0.5, 1.0, 2.0, 4.0 and 8.0	4.0

Each well of Sensititre® MycoTB assay was coated with a defined concentration per drug, at which an isolate was tested to determine the MIC. The ECOFF published by Ismail *et al.*[28] on Sensititre™ MycoTB assay categorized an isolate as resistant if had MIC above this value and as susceptible if had MIC at or below this ECOFF.

prepared and adjusted to 0.5 McFarland standard turbidity. A total of 100 μL of suspension was inoculated into each well of the Sensititre™ MycoTB assay, and was incubated aerobically at 37°C for up to 21 days. Unless it was contaminated in the first run, an isolate was tested only once. The MIC value was visually recorded by two independent readers at day 10 and at day 21 in case it was negative at day 10. A third opinion was sought if the MIC values reported by the two independent readers were different. The tentative ECOFF values published by Ismail *et al.*[28] on Sensititre™ MycoTB assay were used to categorize an isolate as susceptible to a drug, if its MIC value was at, or lower than the ECOFF, and resistant if it was above this ECOFF. For cycloserine, the published breakpoint derived from datasets, including a similar Tanzanian study population from the same study location, was used.[29,30]

WGS and bioinformatic analysis

DNA extraction

The MTBC isolates stored in trypticase soy broth supplemented with 10% glycerol were sub-cultured on glycerol containing LJ medium. The DNA was extracted from positive LJ slants using the cetyltrimethylammonium bromide protocol described previously.[31,32] Briefly, two loopfuls of bacteria cells were heat-killed and lysed using 50 μL of 10 mg/mL lysozyme and 75 μL of 10% SDS/proteinase K mixture (Promega Inc.). Then, 750 μL chloroform/isoamyl alcohol mix (24:1) was added to separate the aqueous DNA-containing layer. The genomic DNA (gDNA) was precipitated and washed using 5 M sodium chloride and 70% ethanol. The gDNA was dried, solubilized and protected from degradation by resuspending in 80 μL of 10× TE (100 mL Tris/HCl, pH 8.0 and 10 mL EDTA mixture) buffer and was frozen at −20°C before shipment to the Research Center Borstel in Germany for WGS and genomic analysis.

Whole genome sequencing

Libraries for next generation sequencing (NGS) were prepared from gDNA using a modified Nextera protocol.[33] Briefly, input DNA was fragmented by tagmentation and indexed adapters were added by reduced cycle amplification. DNA libraries were sequenced with 2 × 150 bp paired-end reads on an Illumina NextSeq 500 platform as instructed by the manufacturer (Illumina, San Diego, CA).

Bioinformatic analysis

FASTQ files (raw sequencing data) were analysed with MTBseq v1.0.3, a semi-automated bioinformatics pipeline for the analysis of MTBC isolates.[34] Briefly, reads were mapped to the *M. tuberculosis* H37Rv reference genome (GenBank ID: NC_000962.3), and alignments were refined with regard to base quality re-calibration and alignment corrections for possible PCR artefacts. WGS datasets with an average read coverage depth of ≤50-fold and coverage breadth of ≤95% as well as samples contaminated with other bacteria as detected by Kraken 2 were excluded.[35] Variants were called by changing the default variant detection parameters to read coverage of a minimum of two for each forward and reverse orientation, two reads of a phred score of at least 20 and 5% allele frequency. MTBseq was run with the default settings and with the additional parameter –low-freq_vars to allow the detection of low-frequency variants.

Phylogenetic classification of the strains was performed by MTBseq according to the presence of phylogenetically informative SNPs from the literature.[36–38]

Mutations such as short insertions/deletions (INDELS) and SNPs from a curated mutation catalogue employed at the Research Center Borstel (2020-05-10) were considered as resistance determinants.[39] Furthermore, unknown INDELS and stop codons in the following genes were also considered as resistance determinants: (a) *ethA* (ethionamide/prothionamide); (b) *pncA* (pyrazinamide); (c) *rpoB* (rifampicin, rifabutin); (d) Rv0678c (bedaquiline, clofazimine); (e) *ald* (cycloserine); (f) *katG* (isoniazid); (g) *gid* (streptomycin); and (h) *fbiC* and *ddn* (delamanid).[39] Nonsense mutations resulting in a STOP codon were indicated by an underscore '_' sign. Uncharacterized mutations were considered as unknown. The interpretation of SNPs and INDELS was performed without prior knowledge of the MIC results, and solely based on the mutation catalogue and global rule mentioned above.

Data management and statistical analysis

Demographic, and clinical data such as age, HIV status, prior history of exposure to anti-TB medications, weight (kg), height in metres, body mass index (kg/m^2), MIC values, and mutations were recorded in a clinical case report form. Data were entered in a Microsoft Excel 2018 Mac OS and cleaned before statistical analysis and visualization with R programming language (http://www.R-project.org). Patients whose isolate had poor quality of sequencing by WGS and those without MIC data were excluded from the final analysis. Resistance-associated variants were classified as previously defined by Heyckendorf *et al.*[18] For example, an isolate without mutations in resistance-associated genes or with only phylogenetic polymorphisms relative to the *M. tuberculosis* H37Rv reference sequence, with an MIC (mg/L) value at or below the ECOFF was defined as genotypically wild-type and phenotypically susceptible (gWT-S). Any isolate with a mutation known to result in an MIC (mg/L) above the highest breakpoint was considered as genotypically non-wild-type and phenotypically resistant (gNWT-R). Any isolate with a yet uncharacterized mutation about which

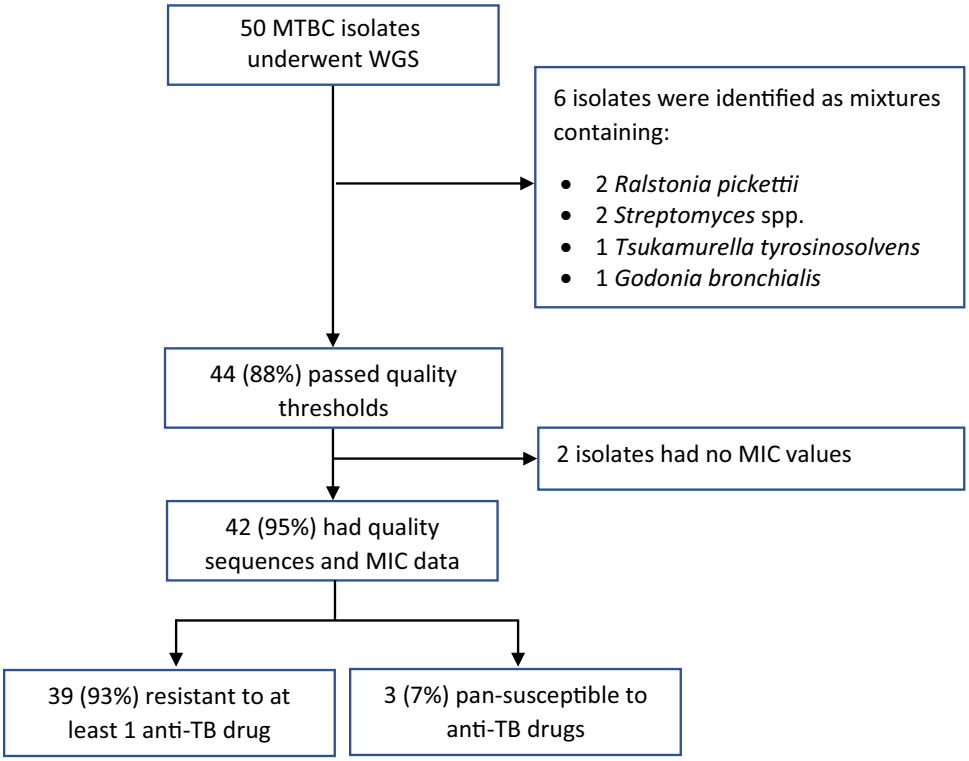

Figure 1. Sample selection based on WGS results and MIC data availability.

Table 2. Agreement of WGS drug resistance predictions of MTBC isolates at different epidemiological cut-off values (ECOFF) in Sensititre™ MycoTB assay

		Resistance prediction based on known mutations (gNWT-R) alone			Resistance prediction based on combined gNWT-R and unknown mutations (gNWT-U)		
Anti-TB drug	ECOFF (mg/L)	Agreement	Resistance predicted	Discordance	Agreement	Resistance predicted	Discordance
Rifampicin	0.5	95% (40/42)	95% (36/38)	5% (2/42)	95 (40/42)	95% (36/38)	5% (2/42)
	0.125	100% (42/42)	100% (38/38)	0% (0/42)	100% (42/42)	100% (38/38)	0% (0/42)
Rifabutin	0.125	100% (42/42)	100% (38/38)	0% (0/42)	100% (42/42)	100% (38/38)	0% (0/42)
Isoniazid	0.25	81% (34/42)	93% (27/29)	19% (8/42)	88% (37/42)	94% (30/32)	12% (5/42)
Ethambutol	2.0	57% (24/42)	88% (14/16)	42% (18/42)	74% (31/42)	73% (30/41)	26% (11/42)
	4.0	64% (27/42)	81% (13/16)	36% (15/42)	62% (26/42)	61% (25/41)	38% (16/42)
Streptomycin	2.0	81% (34/42)	67% (8/12)	19% (8/42)	71% (30/42)	50% (8/16)	29% (12/42)
Ethionamide	5.0	90% (38/42)	80% (4/5)	10% (4/42)	90% (38/42)	64% (7/11)	10% (4/42)
Levofloxacin	1.0	95 (40/42)	None	5% (2/42)	80% (34/42)	0% (0/8)	24% (10/42)
Moxifloxacin	0.5	90% (38/42)	None	10% (4/42)	80% (34/42)	13% (1/8)	24% (10/42)
Clofazimine	0.25	86% (36/42)	None	14% (6/42)	83% (35/42)	0% (0/1)	17% (7/42)
	1.0	97% (41/42)	None	2% (1/42)	98% (40/42)	0% (0/1)	5% (2/42)
Cycloserine	64.0	97% (41/42)	None	2% (1/42)	79% (33/42)	0% (0/8)	21% (9/42)
p-Amino salicylic acid	4.0	95% (40/42)	None	5% (2/42)	90% (38/42)	0% (0/2)	10% (4/42)
Kanamycin	2.5	81% (34/42)	None	19% (8/42)	83% (35/42)	60% (3/5)	17% (7/42)
	5.0	93% (39/42)	None	7% (3/42)	90% (38/42)	60% (3/5)	12% (5/42)
Capreomycin	2.5	90% (38/42)	None	10% (4/42)	79% (33/42)	0% (0/5)	21% (9/42)
	5.0	97% (41/42)	None	2% (1/42)	86% (36/42)	0% (0/5)	14% (6/42)
Amikacin	2.0	100% (42/42)	None	0% (0/42)	88% (37/42)	0% (0/5)	12% (5/42)

Table 3. Phenotypes of genotypically non-wild-type MTBC strains with unclear mutations (gNWT-U) in resistance-associated genes

Anti-TB drug	Genes	gNWT-U isolates	No. of R isolates	No. of S isolates	Mutation(s)
Levofloxacin ($n=8$)	*gyrA*	3	0	3	Q277R, A667D
	gyrB	2	1	1	G520A[c]
	eccB5	2	0	2	G267A, E257E
	eccC5	1	0	1	K835R
	gyrA	3	0	3	Q277R, A667D
Moxifloxacin ($n=8$)	*gyrA*	3	0	3	Q277R, A667D
	gyrB	2	1	1	G520A[c]
	eccB5	2	0	2	G267A, E257E
	eccC5	1	1	0	K835R
Cycloserine ($n=8$)	*PPE22*	5	0	5	V288G
	pykA[b]	3	0	3	R290R, P222L
Clofazimine ($n=1$)	*serB2*	1	0	1	V308V
p-Amino salicylic acid ($n=2$)	*thyA*	1	0	1	H51P
	folC	1	0	1	G226S
Ethionamide or prothionamide ($n=4$)	*ethA*	2	1	1	W391_, P284S1
	ethR	2	2	0	A168V
Streptomycin ($n=12$)	*rpsL*	10	7	3	K88M, L81L, G124S
	rrs	2	0	2	517c>t, 1472150a,
Isoniazid ($n=4$)	*katG*	4	4	0	G279D, G99E, N660D
Ethambutol ($n=25$)	*embR*	17	11	6	F376L, V289V
	embA[a]	1	1	0	G5D, V31I
	embB[a]	2	1	1	A19G
	ubiA[a]	1	1	0	M128L
	embC[a]	3	1	2	M257I, A322S

R, resistant isolate; S, susceptible isolate.
[a]Denotes combination with *embR* F376L.
[b]Denotes combination with *cycA* V110V ($n=1$).
[c]Denotes combination with *gyrA* F60Y ($n=1$) and *gyrA* I189M ($n=1$) mutations. Stop codons are indicated by an underlined space.

too little was known or no MIC values were available to make a judgement was considered to be genotypically non-wild-type and phenotypically unclear (gNWT-U).

Drug resistance predicted by WGS, as well as concordance and discordance between WGS and Sensititre™ MycoTB assay were summarized as proportions. For non-parametric data such as MIC values as determined by Shapiro's test, median and the interquartile range (IQR) were calculated and data were compared using Mann–Whitney U (Wilcoxon rank sum) test. Relationships of resistance-associated variants and MIC for an individual drug were visualized using bar plots. The significance level was set at $\alpha=5\%$.

Data availability

The variants in resistance-associated genes and MIC values of the tested isolates are listed in Table S1 (available as Supplementary data at *JAC-AMR* Online). The raw sequence data (FASTQ files) were deposited in European Nucleotide Archive (ENA) at EMBL-EBI under the project accession number PRJE9680 (https://www.ebi.ac.uk/ena/browser/view/PRJEB9680). The distinct accession numbers of the analysed isolates are listed in Table S1.

Results

Patient characteristics

Of the 50 MTBC isolates, 29 (58%) were from male patients. The mean (SD) age was 43 (13) years and median body mass index was 17.4 (IQR 16.5–20.0) kg/m^2. Twenty-four patients (48%) had prior treatment for drug-susceptible TB, whereas 17 (34%) were living with HIV/AIDS and had a median absolute CD4+ count of 121 (IQR 76–236) cells/mm^3.

Drug resistance prediction in MTBC isolates

Among 50 MTBC isolates sequenced, 42 (84%) passed the sequencing quality thresholds and had complete MIC results, and hence were analysed. Of these 42 isolates, 36 (86%) were RR-TB whereas only 26 (60%) were MDR-TB detected by genotype MTBDR*plus*. All 42 isolates were identified as susceptible to fluoroquinolones and injectable aminoglycosides and cyclic peptide by the genotype MTBDR*sl*. From the eight excluded isolates, six were identified as mixtures of different bacteria with Kraken 2 and two had missing MIC data (Figure 1). WGS-derived genotypic drug-resistance prediction for MTBC isolates and the drug's ECOFF in Sensititre™ MycoTB assay are summarized in Table 2 and Table S1. The prediction of drug resistance was correctly made by WGS for streptomycin in 67% (8/12), for ethionamide in 80% (4/5), and for ethambutol in 81% (13/16) of isolates. For the drugs primarily used to treat RR/MDR-TB such as bedaquiline, fluoroquinolones and linezolid, no resistance-associated mutations were detected via WGS (Table 2). Nonetheless, Sensititre™ MycoTB

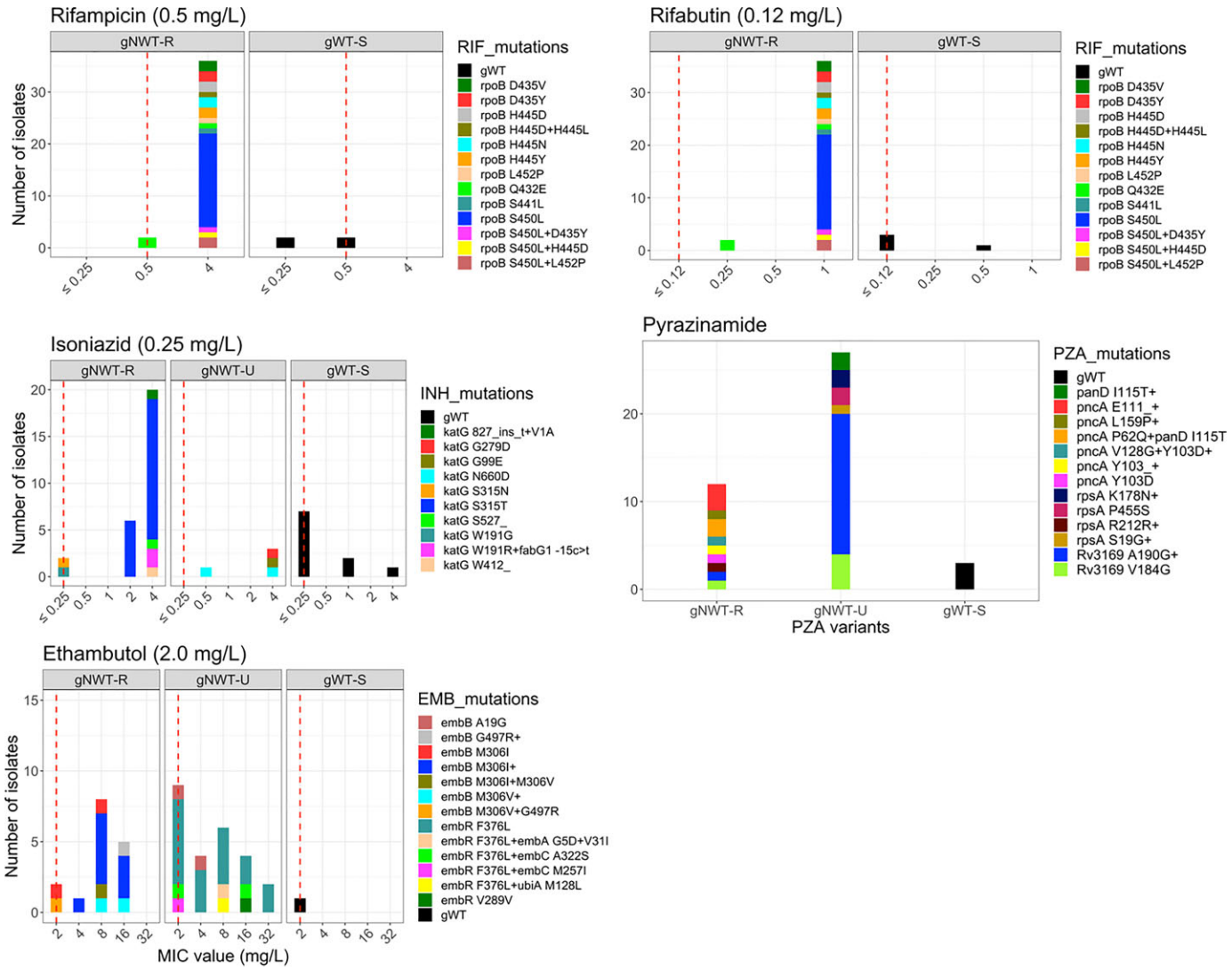

Figure 2. Distribution of MICs of first line anti-TB drugs in genotypically wild-type and non-wild-type isolates. The isolates with MIC (mg/L) values below the tentative epidemiological cut-off value (ECOFF) were defined as genotypically wild-type and phenotypically susceptible (gWT-S). Isolates with a mutation known to result in MIC (mg/L) increase above the highest breakpoint were considered as genotypically non-wild-type and phenotypically resistant (gNWT-R). Isolates with unclear mutations or a mutation for which too little was known or no MIC values were available to make a judgement were considered to be genotypically non-wild-type unclear (gNWT-U). MIC testing for pyrazinamide was not done. The underscore sign '_' in the *katG* gene indicates a stop codon. A plus (+) sign indicate presence of other gNWT-U gene-specific mutations shown in Table S1.

assay detected phenotypic resistance to levofloxacin ($n=2$), moxifloxacin ($n=4$), clofazimine ($n=1$), cycloserine ($n=1$), p-amino salicylic acid ($n=2$), kanamycin ($n=8$), capreomycin ($n=4$) and amikacin ($n=1$). Table 3 shows the number of genotypically non-wild-type MTBC isolates with unclear mutations (gNWT-U) in resistance-associated genes for drugs used to treat MDR-TB, such as fluoroquinolones, bedaquiline, delamanid and linezolid. Discordances between WGS and Sensititre™ MycoTB assay are shown in Table 2, using first gNWT-R, and were common when any mutation was considered as potentially predictive of phenotypic resistance (gNWT-R or gNWT-U). Ethambutol (both at ECOFF 2.0 and 4.0 mg/L) and streptomycin bore the highest discordance.

Overall, gNWT-R MTBC isolates correlated with MIC values above the ECOFF in Sensititre™ MycoTB assay (Table S1). For instance, the median MIC value (mg/L) was: for rifampicin-gNWT-R strains >4.0 (IQR 4.0–4.0) compared with 0.5 (IQR 0.38–0.50) in genotypically wild-type (gWT-S, $P<0.001$); for isoniazid-gNWT-R >4.0 (IQR 2.0–4.0) compared for 0.25 (IQR 0.12–1.00) among gWT-S ($P=0.001$); and for ethionamide-gNWT-R 15.0 (IQR 10.0–20.0) compared with 2.50 (IQR 2.50–5.00) among gWT-S ($P<0.001$). WGS correctly predicted resistance in 95% (36/38) and 100% (38/38) of the rifampicin-resistant isolates at an ECOFF value above 0.5 or 0.125 mg/L, respectively. The distribution of MIC values of anti-TB drugs for genotypically wild-type and non-wild-type isolates are shown in Figures 2–5.

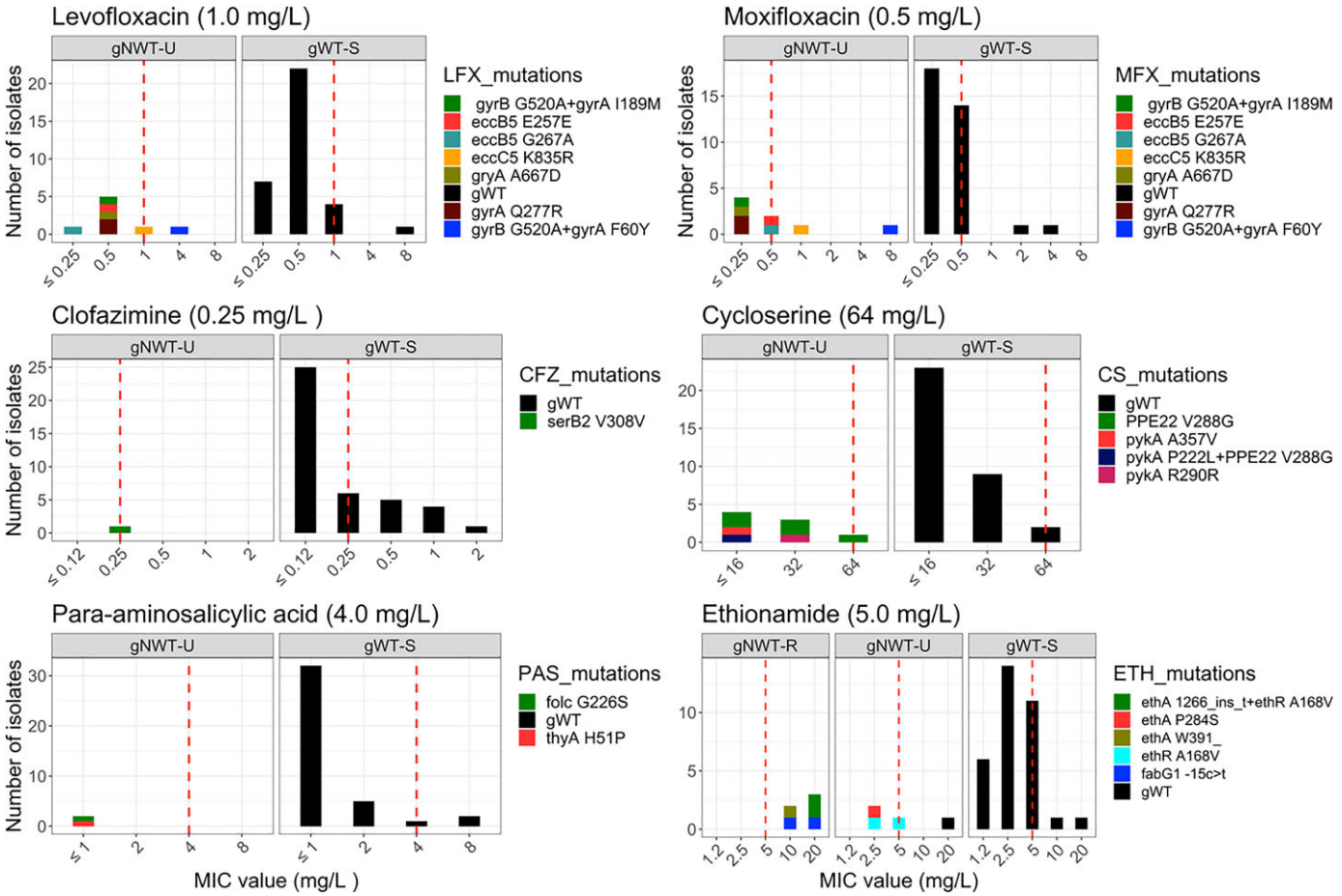

Figure 3. Distribution of MICs of second line anti-TB drugs (fluoroquinolones and add-on drugs) in genotypically wild-type and non-wild-type isolates. Tentative epidemiological cut-off (ECOFF) values are shown in brackets and indicated with the red dashed vertical lines. Isolates with MIC (mg/L) values below the tentative ECOFF were defined as genotypically wild-type and phenotypically susceptible (gWT-S). Isolates with a mutation known to result in an MIC (mg/L) increase above the highest breakpoint were considered as genotypically non-wild-type and phenotypically resistant (gNWT-R). Isolates with unclear mutations or a mutation for which too little was known or no MIC values were available to make a judgement were considered to be genotypically non-wild-type unclear (gNWT-U). The underscore sign '_' in the *ethA* gene indicates a stop codon.

Discussion

WGS compared favourably to phenotypic drug susceptibility testing using MIC values from the Sensititre™ MycoTB assay for predicting resistance in MTBC, with notable exceptions for some drugs. For drugs such as rifampicin, rifabutin, isoniazid and ethionamide, genotypically non-wild-type isolates had higher MIC values compared with wild-type isolates. Importantly, there were no mutations in genomic regions that confer resistance to bedaquiline, linezolid, fluoroquinolones, clofazimine and cycloserine, which comprised the bulk of the RR/MDR-TB treatment regimens.[25,26] This finding may further explain the comparatively higher rates of treatment success for MDR-TB in Tanzania.

Our findings also support the high predictive value of WGS to infer drug susceptible phenotypes measured by Sensititre™ MycoTB assay or MGIT that were reported from other studies in Romania[40] and Germany.[18] Moreover, previous reports from the same setting from the northeast[41,42] as well as from the northwest[43] of Tanzania showed that genotypic and phenotypic resistance for fluoroquinolones is less common than those reported in other MDR-TB endemic regions such as South Africa.[44] For example, since 2009, there has been only one patient report with extensively drug-resistant TB by phenotypic and genotypic information.[41] Additionally, and given the use of a customized plate with a lower range of MIC values, this study shows that even among phenotypically susceptible MTBC isolates, MICs were often well below the breakpoints for key drugs in the current empiric RR/MDR-TB treatment regimen.

The reasons for poor prediction of drug resistance in MTBC for certain drugs may be related to the phenotypic nature of isolates tested, laboratory methods used to predict resistance including consensus definitions for MIC breakpoints, and the approaches to bioinformatics analysis. For a few isolates with an elevated MIC of individual drugs such as levofloxacin, moxifloxacin, clofazimine and p-amino salicylic acid we did not identify a genotypic resistance determinant, either indicating a limitation of the mutation catalogue or a higher variability in the phenotypic assay.

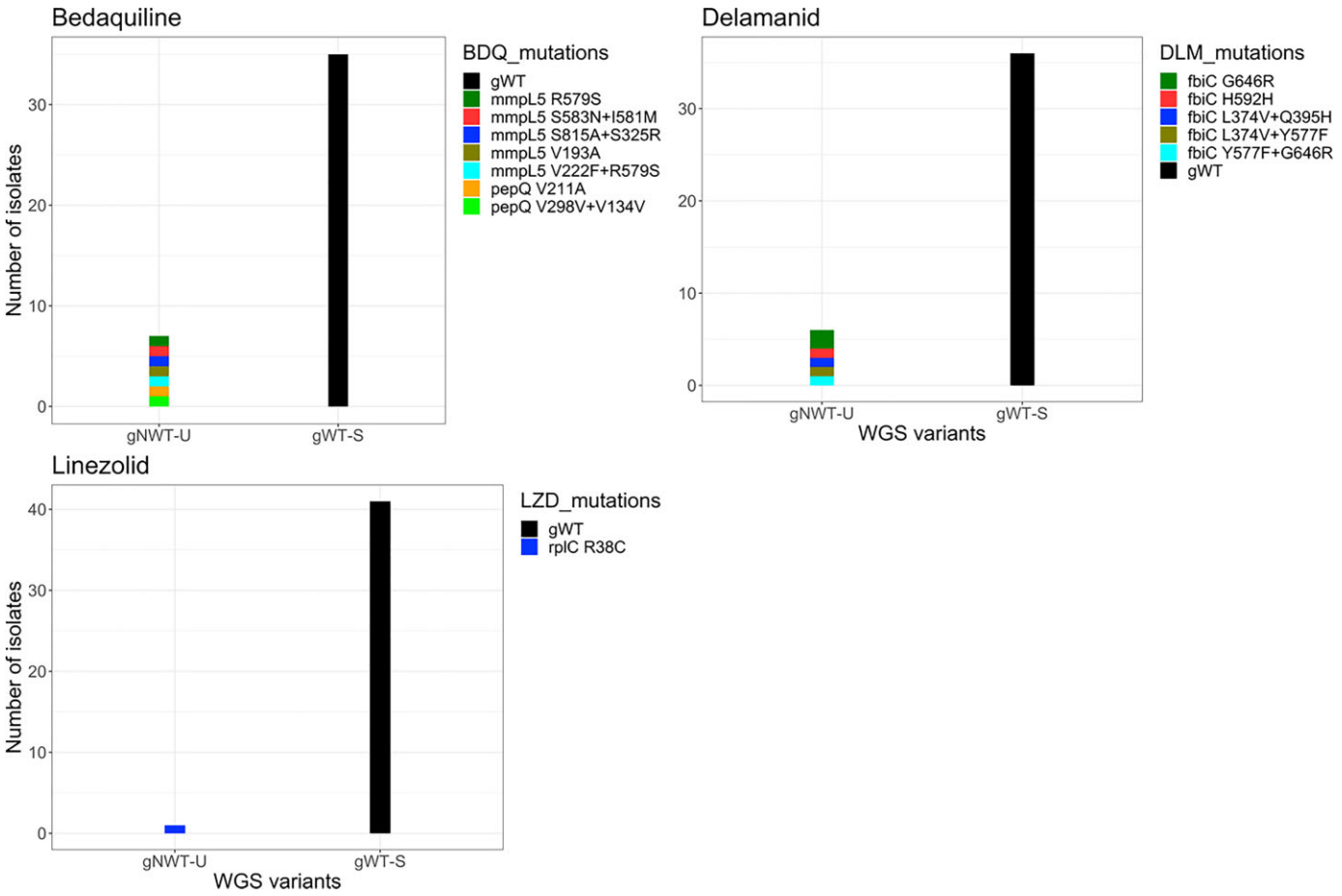

Figure 4. New and repurposed anti-TB drugs. Distribution of WGS resistance-associated variants in genotypically non-wild-type with unclear mutations (gNWT-U) and genotypically wildtype (gWT-S) isolates. There was no MIC testing for these drugs. Detailed lists of gNWT-U mutations are presented in Table S1.

As reported earlier, resistance-associated mutations predicted by WGS and the level of phenotypic resistance set by MIC values on Sensititre™ MycoTB assay in this study were often discordant for drugs such as moxifloxacin, ethionamide, aminoglycosides/cyclic peptides and ethambutol.[45,46] Certainly, these discrepancies could be due to limitations related to the Sensititre™ MycoTB assay,[17] given the absence of optimal and standardized criteria for interpreting and consensus definition for MIC breakpoints.[28,47] This assertion described by Schön *et al.*[17] argues that the Sensititre™ MycoTB assay contains MIC ranges that are unacceptable for these anti-TB drugs. The MIC ranges are either truncated at their lower-end relative to wild-type distributions or not defined for these drugs at all.[17] Because of this suboptimal definition, prior MIC breakpoints used by Ruesen *et al.*[40] in the Sensititre™ MycoTB assay were likely too high for moxifloxacin (1.0 mg/L) and amikacin (4.0 mg/L), compared with ECOFF values published by Ismail *et al.*,[28] which were used to interpret the MIC values in this study. Notably, higher breakpoints are likely to misinterpret drug-resistant MTBC strains as susceptible for drugs such as fluoroquinolones.[17,48] As a consequence, the WHO has recently lowered the critical concentrations for moxifloxacin and recently also for rifampicin in MGIT960 to 0.25 mg/L and 0.5 mg/L,[49] respectively. This also highlights the importance of defining a consensus for breakpoints in microdilution assays including Sensititre™ MycoTB assay.

Furthermore, even in a situation where a consensus definition for MIC breakpoints is determined, discordances may also be explained by an incomplete catalogue of drug resistance mutations currently used to predict resistance from the genotype, as has been shown before.[50,51] Moreover, large INDELS are not detectable by pipelines implemented for short-read (150–300 bp) sequencing experiments such as MTBseq, TB-Profiler, and PhyResSE, and further work could interrogate long-read sequencing with these isolates. Also, discrepancies can either represent a false-positive phenotypic result as the assay was only performed once or imprecise cutoffs to call phenotypic resistance. Discrepancies could also partially be attributed to the resistance-associated variant discovery cutoff for WGS, which was set at 5%. This threshold may lead to missing all potential mutations present at lower frequencies. Moreover, there were more discordances when MIC values were compared with a combination of known (gNWT-R) and unclear (gNWT-U) mutations. Collaborative databases such as ReSeqTB or CRyPTIC have been formed to overcome these limitations.[52,53]

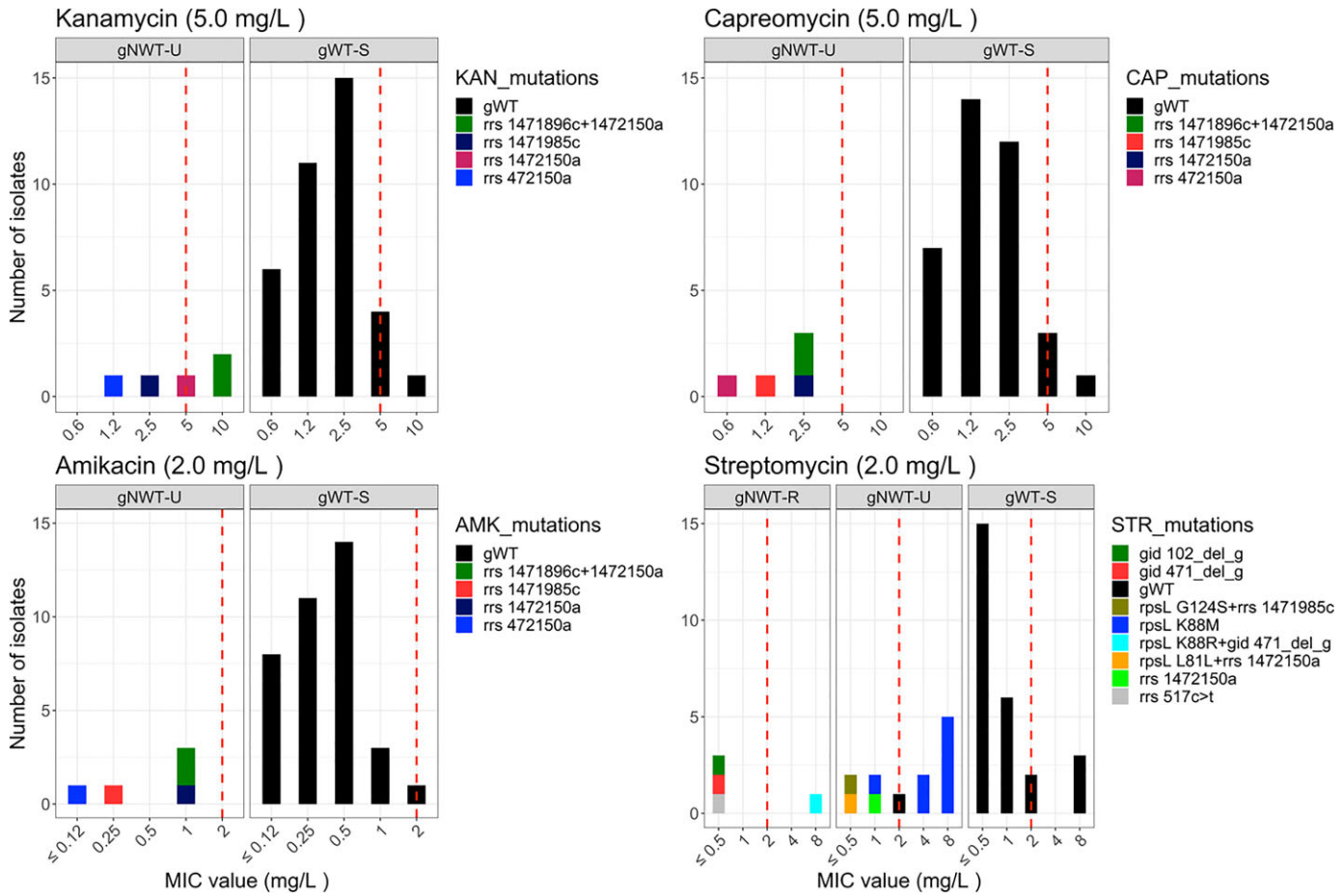

Figure 5. Injectables aminoglycosides and capreomycin. Distribution of MIC values for aminoglycosides and capreomycin in genotypically wild-type and non-wild-type isolates. Tentative epidemiological cut-off (ECOFF) values are listed in brackets and indicated by the red dashed vertical lines. Isolates with MIC (mg/L) values below the tentative ECOFF were defined as genotypically wild-type and phenotypically susceptible (gWT-S). Isolates with a mutation known to result in MIC (mg/L) increases above the highest breakpoint were considered as genotypically non-wild-type and phenotypically resistant (gNWT-R). Isolate with unclear mutations or a mutation for which too little was known or no MIC values were available to make a judgement were considered to be genotypically non-wild-type unclear (gNWT-U).

The study has other limitations. Firstly, the MIC values were derived from isolates of patients diagnosed for RR/MDR-TB, and including fully susceptible MTBC isolates would have aided in establishing local ECOFF values. Additionally, this would have helped determination of adequate MIC ranges of the custom Sensititre™ MycoTB assay. Secondly, the WGS and MIC testing were not done in real-time to guide clinical decision. As a result, some patients received RR/MDR-TB treatment based on Xpert® MTB/RIF results, but were fully susceptible by both WGS and Sensititre™ MycoTB assay. False-positive rifampicin resistance in MTBC isolates has been reported in sub-Saharan African countries including Rwanda,[54] and South Africa.[55] Lastly, there was no MIC testing for bedaquiline, delamanid and linezolid to compare WGS with the level of phenotypic resistance.

In conclusion, the WGS-based resistance prediction was in concordance with phenotypic resistance measured by the Sensititre™ MycoTB assay, except for poorly reproducible ethambutol and streptomycin phenotypes. In addition, the absence of second-line drug resistance-mediating mutations has the potential to guide the design of RR/MDR-TB regimens in the future. While mutations classified as gNWT-U were common, they were mostly found in isolates phenotypically susceptible to the drug of interest.

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Transparency declarations

None to declare.

Author contributions

P.M.M., C.U., E.A.M., E.S., S.G.M. and S.K.H. conceived the study, designed the work and interpreted the data. P.M.M., M.L.S., B.M.S. and C.U. acquired data. P.M.M., C.U., E.A.M., E.S. and S.G.M. analysed the data. P.M.M. and C.U. drafted the initial manuscript and P.M.M. responded to all co-authors' inputs. I.B., V.D., M.M., J.G., K.K.A., M.P., B.K.M., B.T.M., S.N., E.S., E.R.H., S.G.M. and S.K.H. reviewed the manuscript. All authors wrote, approved and agreed to be accountable for all scientific aspects in the final version of this manuscript.

Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online.

References

1 Hameed HMA, Islam MM, Chhotaray C *et al*. Molecular targets related drug resistance mechanisms in MDR-, XDR-, and TDR-Mycobacterium tuberculosis strains. *Front Cell Infect Microbiol* 2018; **8**: 1–21.

2 Dookie N, Rambaran S, Padayatchi N *et al*. Evolution of drug resistance in Mycobacterium tuberculosis: A review on the molecular determinants of resistance and implications for personalized care. *J Antimicrob Chemother* 2018; **73**: 1138–51.

3 World Health Organization. WHO Consolidated guidelines on drug-resistant tuberculosis treatment. *World Heal Organ* 2019: Licence: CC BY-NC-SA 3.0 IGO. https://apps.who.int/iris/handle/10665/311389.

4 World Health Organization. *Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27-29 October 2020*. 2021. https://apps.who.int/iris/handle/10665/338776.

5 National Tuberculosis and leprosy Programme. *The National Tuberculosis and leprosy Programme. Annual report of 2018*. 2018. papers2://publication/uuid/512EBCE8-D635-4348-A67D-22DD52988F4C.

6 World Health Organization. *Global tuberculosis report 2020*. 2020. https://www.who.int/publications/i/item/9789240013131.

7 Kendall EA, Azman AS, Cobelens FG *et al*. MDR-TB treatment as prevention: The projected population-level impact of expanded treatment for multidrug-resistant tuberculosis. *PLoS One* 2017; **12**: 1–16.

8 Mehra M, Cossrow N, Kambili C *et al*. Assessment of tuberculosis burden in China using a dynamic disease simulation model. *Int J Tuberc Lung Dis* 2013; **17**: 1186–94.

9 World Health Organization. *Rapid Communication: Key changes to treatment of multidrug- and rifampicin-resistant tuberculosis*. 2018. https://www.who.int/publications/i/item/WHO-CDS-TB-2018.18.

10 Mbelele PM, Mpolya EA, Sauli E *et al*. Mycobactericidal effect of different regimens measured by molecular bacterial load assay among people treated for multidrug-resistant tuberculosis in Tanzania. *J Clin Microbiol* 2021; **59**: e02927-20.

11 Lu P, Liu Q, Martinez L *et al*. Time to sputum culture conversion and treatment outcome of patients with multidrug-resistant tuberculosis: a prospective cohort study from urban China. *Eur Respir J* 2017; **49**: 1601558.

12 Chihota VN, Grant AD, Fielding K *et al*. Liquid vs. solid culture for tuberculosis: Performance and cost in a resource-constrained setting. *Int J Tuberc Lung Dis* 2010; **14**: 1024–31.

13 European Centre for Disease Prevention and Control. Handbook on tuberculosis laboratory diagnostic methods in the European Union – Updated 2018. *Stockholm* 2018: ECDC; 2018.

14 Heysell SK, Pholwat S, Mpagama SG *et al*. Sensititre MYCOTB MIC plate for testing mycobacterium tuberculosis susceptibility to first-And second-Line drugs. *Antimicrob Agents Chemother* 2015; **58**: 11–8.

15 Mpagama SG, Houpt ER, Stroup S *et al*. Application of quantitative second-line drug susceptibility testing at a multidrug-resistant tuberculosis hospital in Tanzania. *BMC Infect Dis* 2013; **13**: 432.

16 World Health Organization. *Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis*. 2018. https://www.who.int/publications/i/item/WHO-CDS-TB-2018.5.

17 Schön T, Matuschek E, Mohamed S *et al*. Standards for MIC testing that apply to the majority of bacterial pathogens should also be enforced for Mycobacterium tuberculosis complex. *Clin Microbiol Infect* 2019; **25**: 403–5.

18 Heyckendorf J, Andres S, Köser CU *et al*. What is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant tuberculosis. *Antimicrob Agents Chemother* 2018; **62**: e01550-17.

19 Rancoita PMV, Cugnata F, Gibertoni Cruz AL *et al*. Validating a 14-drug microtiter plate containing bedaquiline and delamanid for large-scale research susceptibility testing of mycobacterium tuberculosis. *Antimicrob Agents Chemother* 2018; **62**: 1–15.

20 Lee J, Armstrong DT, Ssengooba W *et al*. Sensititre MYCOTB MIC plate for testing mycobacterium tuberculosis susceptibility to first-And second-Line drugs. *Antimicrob Agents Chemother* 2014; **58**: 11–8.

21 van Zyl-Smit RN, Binder A, Meldau R *et al*. Comparison of quantitative techniques including Xpert MTB/RIF to evaluate mycobacterial burden. *PLoS One* 2011; **6**: e28815.

22 World Health Organization. *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis*. https://apps.who.int/iris/handle/10665/275469.

23 Mbelele PM, Aboud S, Mpagama SG *et al*. Improved performance of Xpert MTB/RIF assay on sputum sediment samples obtained from presumptive pulmonary tuberculosis cases at Kibong'oto infectious diseases hospital in Tanzania. *BMC Infect Dis* 2017; **17**: 1–7.

24 Mbelele PM, Mohamed SY, Sauli E *et al*. Meta-narrative review of molecular methods for diagnosis and monitoring of multidrug-resistant tuberculosis treatment in adults. *Int J Mycobacteriology* 2018; **7**: 299–309. doi: 10.4103/ijmy.ijmy_135_18.

25 Ramirez LMN, Vargas KQ, Diaz G. Whole genome sequencing for the analysis of drug resistant strains of mycobacterium tuberculosis: A systematic review for bedaquiline and delamanid. *Antibiotics* 2020; **9**: 1–14.

26 Coll F, Phelan J, Hill-Cawthorne GA *et al*. Genome-wide analysis of multi- and extensively drug-resistant Mycobacterium tuberculosis. *Nat Genet* 2018; **50**: 307–16.

27 Katale BZ, Mbelele PM, Lema NA *et al*. Whole genome sequencing of Mycobacterium tuberculosis isolates and clinical outcomes of patients treated for multidrug-resistant tuberculosis in Tanzania. *BMC Genomics* 2020; **21**: 1–15.

28 Ismail NA, Ismail F, Joseph L *et al*. Epidemiological cut-offs for Sensititre susceptibility testing of Mycobacterium tuberculosis: interpretive criteria cross validated with whole genome sequencing. *Sci Rep* 2020; **10**: 1–7.

29 Yu X, Zeng X, Shi W *et al*. Validation of cycloserine efficacy in treatment of multidrug-resistant and extensively drug-resistant tuberculosis in Beijing, China. *Antimicrob Agents Chemother* 2018; **62**: e01824-17.

30 Deshpande D, Alffenaar JWC, Köser CU *et al*. D-Cycloserine Pharmacokinetics/Pharmacodynamics, Susceptibility, and Dosing Implications in Multidrug-resistant Tuberculosis: A Faustian Deal. *Clin Infect Dis* 2018; **67**: S308–16.

31 Somerville W, Thibert L, Schwartzman K *et al*. Extraction of Mycobacterium tuberculosis DNA: a Question of Containment. *J Clin Microbiol* 2005; **43**: 2996–7.

32 Van Soolingen D, Hermans PWM, De Haas PEW *et al*. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *J Clin Microbiol* 1991; **29**: 2578–86.

33 Baym M, Kryazhimskiy S, Lieberman TD *et al*. Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One* 2015; **10**: e0128036.

34 Kohl TA, Utpatel C, Schleusener V *et al*. MTBseq: A comprehensive pipeline for whole genome sequence analysis of Mycobacterium tuberculosis complex isolates. *PeerJ* 2018; **2018**: 1–13.

35 Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol* 2019; **20**: 1–13.

36 Coll F, McNerney R, Guerra-Assunção JA *et al*. A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. *Nat Commun* 2014; **5**: 4–8.

37 Homolka S, Projahn M, Feuerriegel S *et al*. High resolution discrimination of clinical mycobacterium tuberculosis complex strains based on single nucleotide polymorphisms. *PLoS One* 2012; **7**: e39855.

38 Merker M, Blin C, Mona S *et al*. Evolutionary history and global spread of the Mycobacterium tuberculosis Beijing lineage. *Nat Genet* 2015; **47**: 242–9.

39 Grobbel H-P, Merker M, Köhler N *et al*. Design of multidrug-resistant tuberculosis treatment regimens based on DNA sequencing. *Clin Infect Dis* 2021; **73**: 1194–202.

40 Ruesen C, Riza AL, Florescu A *et al*. Linking minimum inhibitory concentrations to whole genome sequence-predicted drug resistance in Mycobacterium tuberculosis strains from Romania. *Sci Rep* 2018; **8**: 1–8.

41 Lyakurwa D, Lyimo J, Mleoh L *et al*. Successful treatment of XDR-TB patient in Tanzania: report of the first XDR-TB patient. *Trop Doct* 2019; **49**: 224–6.

42 Mpagama SG, Heysell SK, Ndusilo ND *et al*. Diagnosis and Interim Treatment Outcomes from the First Cohort of Multidrug-Resistant Tuberculosis Patients in Tanzania. *PLoS One* 2013; **8**: e62034.

43 Kidenya BR, Mshana SE, Fitzgerald DW *et al*. Genotypic drug resistance using whole-genome sequencing of Mycobacterium tuberculosis clinical isolates from North-western Tanzania. *Tuberculosis* 2018; **109**: 97–101.

44 Dheda K, Limberis JD, Pietersen E *et al*. Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *Lancet Respir Med* 2017; **5**: 269–81.

45 Foongladda S, Banu S, Pholwat S *et al*. Comparison of TaqMan W Array Card and MYCOTB TM with conventional phenotypic susceptibility testing in MDR-TB. *Int J Tuberc Lung Dis* 2016; **20**: 1105–12.

46 Chen X, He G, Wang S *et al*. Evaluation of Whole-Genome Sequence Method to Diagnose Resistance of 13 Anti-tuberculosis Drugs and Characterize Resistance Genes in Clinical Multi-Drug Resistance Mycobacterium tuberculosis Isolates From China. *Front Microbiol* 2019; **10**: 1–10.

47 Faksri K, Kaewprasert O, Ong RTH *et al*. Comparisons of whole-genome sequencing and phenotypic drug susceptibility testing for Mycobacterium tuberculosis causing MDR-TB and XDR-TB in Thailand. *Int J Antimicrob Agents* 2019; **54**: 109–16.

48 Ängeby K, Juréen P, Kahlmeter G *et al*. Challenging a dogma: antimicrobial susceptibility testing breakpoints for Mycobacterium tuberculosis. *Bull World Health Organ* 2012; **90**: 693–8.

49 World Health Organization. *Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)*. 2021. https://www.who.int/publications/i/item/technical-report-on-critical-concentrations-for-drugsusceptibility-testing-of-isoniazid-and-therifamycins-(rifampicin-rifabutin-and-rifapentine).

50 Ngo TM, Teo YY. Genomic prediction of tuberculosis drug-resistance: Benchmarking existing databases and prediction algorithms. *BMC Bioinformatics* 2019; **20**: 1–9.

51 van Beek J, Haanperä M, Smit PW *et al*. Evaluation of whole genome sequencing and software tools for drug susceptibility testing of Mycobacterium tuberculosis. *Clin Microbiol Infect* 2019; **25**: 82–6.

52 Starks AM, Aviles E, Cirillo DM *et al*. Collaborative Effort for a Centralized Worldwide Tuberculosis Relational Sequencing Data Platform. *Clin Infect Dis* 2015; **61**: S141–6.

53 The CRyPTIC Consortium and the 100 000 Genomes Project abstract. Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing. *N Engl J Med* 2018; **379**: 1403–15.

54 Ngabonziza JCS, Decroo T, Migambi P *et al*. Prevalence and drivers of false-positive rifampicin-resistant Xpert MTB/RIF results: a prospective observational study in Rwanda. *Lancet Microbe* 2020; **1**: e74–83.

55 Theron G, Venter R, Smith L *et al*. False-positive xpert MTB/RIF results in retested patients with previous tuberculosis: Frequency, profile, and prospective clinical outcomes. *J Clin Microbiol* 2018; **56**: e01696-17.