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MAJOR ARTICLE

Design of Multidrug-Resistant Tuberculosis Treatment Regimens Based on DNA Sequencing

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Background. Comprehensive and reliable drug susceptibility testing (DST) is urgently needed to provide adequate treatment regimens for patients with multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB). We determined whether next-generation sequencing (NGS) analysis of *Mycobacterium tuberculosis* complex isolates and genes implicated in drug resistance can guide the design of effective MDR/RR-TB treatment regimens.

Methods. NGS-based genomic DST predictions of *M. tuberculosis* complex isolates from MDR/RR-TB patients admitted to a TB reference center in Germany between 1 January 2015 and 30 April 2019 were compared with phenotypic DST results of mycobacteria growth indicator tubes (MGIT). Standardized treatment algorithms were applied to design individualized therapies based on either genomic or phenotypic DST results, and discrepancies were further evaluated by determination of minimal inhibitory drug concentrations (MICs) using Sensititre MYCOTBI and UKMYC microtiter plates.

Results. In 70 patients with MDR/RR-TB, agreement among 1048 pairwise comparisons of genomic and phenotypic DST was 86.3%; 76 (7.2%) results were discordant, and 68 (6.5%) could not be evaluated due to the presence of polymorphisms with yet unknown implications for drug resistance. Importantly, 549 of 561 (97.9%) predictions of drug susceptibility were phenotypically confirmed in MGIT, and 27 of 64 (42.2%) false-positive results were linked to previously described mutations mediating a low or moderate MIC increase. Virtually all drugs (99.0%) used in combination therapies that were inferred from genomic DST were confirmed to be susceptible by phenotypic DST.

Conclusions. NGS-based genomic DST can reliably guide the design of effective MDR/RR-TB treatment regimens.

Keywords. tuberculosis; DST; NGS; MDR-TB.

The emergence of antimicrobial resistance is challenging tuberculosis control in many parts of the world, including southern Africa, Asia, Eastern Europe, and South America. Globally, there were an estimated 484 000 (range, 417 000–556 000) incident cases of multidrug-resistant tuberculosis (MDR-TB; defined as resistance toward at least rifampicin and isoniazid) or rifampicin-resistant tuberculosis alone (RR-TB) in 2018 [1]. The number of MDR/RR-TB patients confirmed by laboratory diagnostics was only 38.6% of the total estimated burden of MDR/RR-TB in 2018, pointing to a substantial gap in case detection and diagnostic capacity to perform drug susceptibility testing (DST) [1].

Important advances in next-generation sequencing (NGS) technologies either by sequencing the entire genome of clinical *Mycobacterium tuberculosis* complex isolates (whole-genome sequencing) or by targeted sequencing of a set of resistance genes (amplicon sequencing) offer increasingly affordable high-resolution information on the genetic basis of antibiotic resistance [2]. Catalogues that provide standardized and comprehensive information for the interpretation of mutations as predictors of resistance to first- and second-line drugs have become available [3]. Thus, genomic DST (gDST) by NGS has the potential to overcome the diagnostic gap in MDR/RR-TB and to become a reliable alternative to phenotypic DST (pDST) [4–6].

An evaluation of more than 10 000 isolates of *M. tuberculosis* complex found genomic prediction of the susceptibility of *M. tuberculosis* complex to first-line drugs to be highly correlated with phenotypic susceptibility [6]. However, it remains unclear how accurately genomic analysis predicts phenotypic

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drug susceptibility to second-line, newer, or repurposed anti-tuberculosis (anti-TB) drugs used for the treatment of MDR/RR-TB.

To address this knowledge gap, we investigated the performance of gDST using a state-of-the-art mutation catalogue for *M. tuberculosis* complex with a cohort of well-characterized MDR/RR-TB patients at the Research Center Borstel hosting the National Reference Laboratory (NRL) of Mycobacteria in Germany. Our main aim in this study was to determine whether gDST alone can guide the design of an effective MDR/RR-TB treatment regimen, including only drugs with pDST-proven susceptibility.

METHODS

Study Population

Between 1 January 2015 and 30 April 2019, all tuberculosis patients at the Medical Clinic of the Research Center Borstel, Germany, receiving treatment against MDR/RR-TB were included in this study. The diagnoses were established using Xpert MTB/RIF (Cepheid, Sunnyvale, CA) or pDST in mycobacteria growth indicator tubes in the BACTEC MGIT 960 system (MGIT; Becton Dickinson, Sparks, MD).

Genomic Drug Susceptibility Testing

NGS served as the index test and was performed with Illumina Technology using Nextera XT library preparation kits (Illumina, San Diego, CA). Fastq files were submitted to the European nucleotide archive (accession number PRJEB38780) and analyzed with the MTBseq pipeline [7]. Genomic DST was performed blinded to the phenotypic susceptibility profile on the basis of a curated mutation catalogue used at the NRL for mycobacteria as of 10 December 2019 (Supplementary Table 1). Rationales for the interpretation and further investigations are described in the Supplementary Methods.

Phenotypic Drug Susceptibility Testing in Liquid Cultures

Phenotypic drug susceptibility testing in MGIT [8] was performed using World Health Organization (WHO)-recommended critical concentrations (CC). A critical proportion of 1% for all WHO first-line and group A-C second-line anti-TB drugs [9–11] except for pyrazinamide (10%) served as the reference standard. Recovery of isolates from sputum samples, species identification, and pDST were performed under routine laboratory conditions. More details on pDST are given in the Supplementary Methods.

The levels of resistance were determined by use of minimal inhibitory concentrations (MICs). MICs of the above-mentioned antimicrobials were determined using broth microdilution in TREK Sensititre MYCOTBI and UKMYC (Thermofisher, Waltham, MA) as described previously [12, 13]. MICs of pyrazinamide were determined using MGIT and MICs of meropenem were not determined. MIC values were categorized into susceptible (S) and resistant (R) per the Clinical and Laboratory Standards Institute (CLSI) document M24-A2 [14].

Treatment Algorithm

A standardized algorithm [15] (Supplementary Table 2) based on the latest WHO prioritization into group A, group B, and group C drugs [16] was applied using MGIT pDST results, MIC values, or gDST results as input to design personalized therapeutic regimens for each patient [17]. Genomic DST results of unknown impact on resistance were conservatively considered as being "resistant" and drugs were not considered for treatment regimens.

Statistics

Diagnostic accuracies with confidence intervals were calculated for MDR/RR-TB drugs with ≥5 of both phenotypic susceptible and resistant DST results. MIC distributions were normalized to the CLSI/WHO cutoff, $\log_2$-transformed, and rounded. Further statistical tests are described in the Supplementary Methods.

Ethics

The University of Lübeck Ethical Board approved the study protocol.

RESULTS

Study Population

Between 1 January 2015 and 30 April 2019, 70 patients with MDR/RR-TB had complete sets of NGS, MGIT, and MIC values for at least 1 *M. tuberculosis* isolate and were included in the final analysis (Figure 1). Most of the patients were male

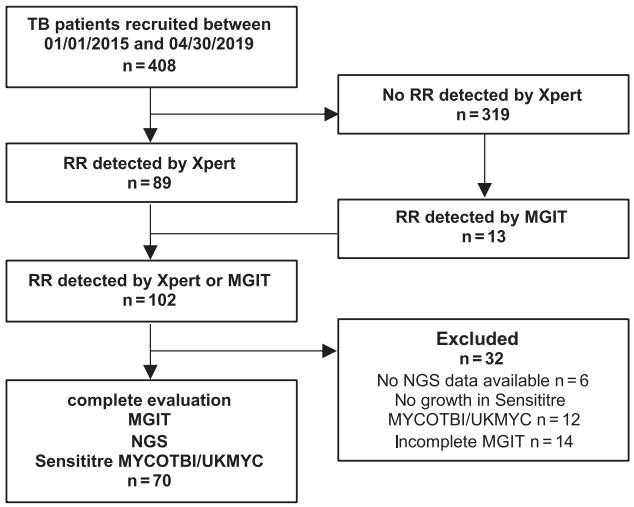

Figure 1. Patients flow chart. Flow chart showing the patients evaluated in this study. Abbreviations: NGS, next-generation sequencing; MDR-TB = multidrug-resistant tuberculosis; RIF = rifampicin; MIC = minimum inhibitory concentration; MGIT, mycobacteria growth indicator tube; RR, rifampicin resistance; TB, tuberculosis.

(68.6%), had a median age of 34.2 years (interquartile range [IQR], 27.1–43.7), and a median body mass index kg/m² of 20.2 (IQR, 18.0–23.2). Patients were born in Germany (n = 2), other European Union/European Economic Area countries (n = 8), other WHO European countries (n = 35), Africa (n = 15), and Asia (n = 10; Table 1).

For each patient, both genomic (gDST established by NGS = index test) and phenotypic (pDST established in MGIT = reference standard) susceptibility patterns were available for 15 antimicrobials (isoniazid, rifampicin, rifabutin, levofloxacin, moxifloxacin, bedaquiline, linezolid, clofazimine, cycloserine, ethambutol, delamanid, pyrazinamide, amikacin, prothionamide, and para-aminosalicylic acid [PAS]; Supplementary Table 3). In the complete dataset, only 2 MGIT results were missing, 1 for rifabutin and 1 for ethambutol.

Genomic Drug Susceptibility Patterns

We compared gDST predictions and pDST results for 1048 of 1050 (99.8%) datapoints (Figure 2). There was an overall concordance between gDST prediction as index test and pDST as reference standard in 904 of 1048 (86.3 %) and discordance in 76 of 1048 (7.2%) pairwise comparisons. In 68 of 1048 (6.5%) gDST analyses, we observed uncharacterized polymorphisms that were not considered to measure the accuracy of gDST (Figure 3A and B).

Overall, gDST correctly predicted phenotypic drug resistance in 89.5% for levofloxacin (95% confidence interval [CI]: 65.5–98.2), 88.2% for moxifloxacin (95% CI: 62.3–97.9), 93.8% for pyrazinamide (95% CI: 81.8–98.4), 100.0% for prothionamide (95% CI: 85.0–100.0), 87.5% for amikacin (95% CI: 46.7–99.3), 77.8% for PAS (95% CI: 40.2–96.1), and 100.0% for ethambutol (95% CI, 89.3–100.0) resistant cases (sensitivity; Table 2).

False gDST predictions of susceptibility, that is, false-negatives, occurred in 12 of 1048 (1.1%) pairwise comparisons (Figure 3A and B). Sequencing data were subsequently re-assessed without a variant frequency filter of 75% to increase the sensitivity and find every possible polymorphism. This revealed low-frequency polymorphisms in 5 (41.7%) of those 12 discrepant cases, that is, *ddn* S78P (delamanid), *ribD* -12 g/a (PAS), *pncA* M175V (pyrazinamide), and *gyrA* D94G (levofloxacin and moxifloxacin) at relative allele frequencies of 7%, 3%, 16%, and 3%, respectively. All of these polymorphisms were classified as being associated with resistance in the applied catalogue, except for *ddn* S78P. For the remaining isolates, no genomic resistance determinants could be identified in the resistance-associated regions investigated.

Phenotypic drug susceptibility was correctly predicted by gDST in 91.8% for levofloxacin (95% CI: 79.5–97.4), 88.2% for moxifloxacin (95% CI: 75.4–95.1), 95.2% for pyrazinamide (95% CI: 74.1–99.8), 80.8% for prothionamide (95% CI: 60.0–92.7), 95.2% for amikacin (95% CI: 85.6–98.7), 90.6% for PAS (95% CI: 78.6–96.5), and 30.4% for ethambutol (95% CI: 14.1–53.0) resistant cases (specificity; Table 2).

False gDST predictions of resistance were identified in 64 of 1048 (6.1%) pairwise comparisons (Figure 3A and B). These discordant results were largely (27 of 64, 45.3%) due to mutations known to mediate a low or moderate MIC increase, for instance, *embB* M306I (ethambutol, 5 cases); *gyrA* A90V, D94A (fluoroquinolones, 3 and 4 cases, respectively); or *rpoB* L430P, H445L, D435Y, L452P (rifampicin, rifabutin; 4, 5, 2, and 4 cases, respectively) [18]. Moreover, there were 4 canonical resistance-mediating mutations (2 *embB* M306V [ethambutol] and 2 *gyrA* D94N [fluoroquinolones]) in phenotypic susceptible isolates [18, 19].

The cumulative sensitivity and specificity of gDST to predict resistance for all 15 drugs were 96.7% (95% CI: 94.2–98.2) and 89.6% (95% CI: 86.8–91.8), respectively. The overall positive predictive value for predicting resistance in our study cohort was 84.7% (95% CI: 80.8–88.0), the negative predictive value was 97.9% (95% CI: 96.2–98.8). The evaluation of the diagnostic accuracy of Sensititre MYCOTB/UKMYC as potential index test using MGIT as the reference standard is presented in Supplementary Table 4.

The 68 uncharacterized polymorphisms were polymorphisms in any of the 92 interrogated resistance-implicating genes that were not contained in our knowledge database. For 60 of those (88.2%, or 60 of 1048 = 5.7% of total test results), pDST was susceptible. Thirty-two of those 60 (53.3%) polymorphisms were located in genes associated with resistance toward bedaquiline, clofazimine, cycloserine, or delamanid (Supplementary Table 5). In 8 of 68 (11.8%, or 8 of 1048 = 0.8% of total test results) uncharacterized polymorphisms, the respective *M. tuberculosis* complex isolate was phenotypically resistant to the corresponding drug; 1 of those 8 polymorphisms was located in the *ethA* gene of 3 prothionamide-resistant *M. tuberculosis* complex isolates (Figure 3A and B, Supplementary Table 5). None of the 68 isolates with uncharacterized polymorphisms had MIC values above the cutoffs of the respective drug [14] in Sensititre

Table 1. Baseline Demographics of Study Patients (n = 70)

Median age (IQR), years	34.2 (27.1–43.7)
Median body mass index (IQR) kg/m²	20.2 (18.0–23.2)
Male sex (%)	48 (68.6)
Rifampicin-resistant tuberculosis (%)	10 (1.4)
Multidrug-resistant tuberculosis (%) (no pre-XDR or XDR)	41 (60.0)
Pre-XDR (%)	21 (30.0)
XDR (%)	6 (8.6)
Region of origin	
Germany (%)	2 (2.9)
Other European Union/European Economic Area (%)	10 (14.3)
Other European World Health Organization region (%)	35 (50.1)
Africa (%)	15 (21.4)
Asia (%)	10 (14.3)

Baseline demographics of n=70 study patients. Abbreviations: IQR, interquartile range; XDR, extensively drug-resistant.

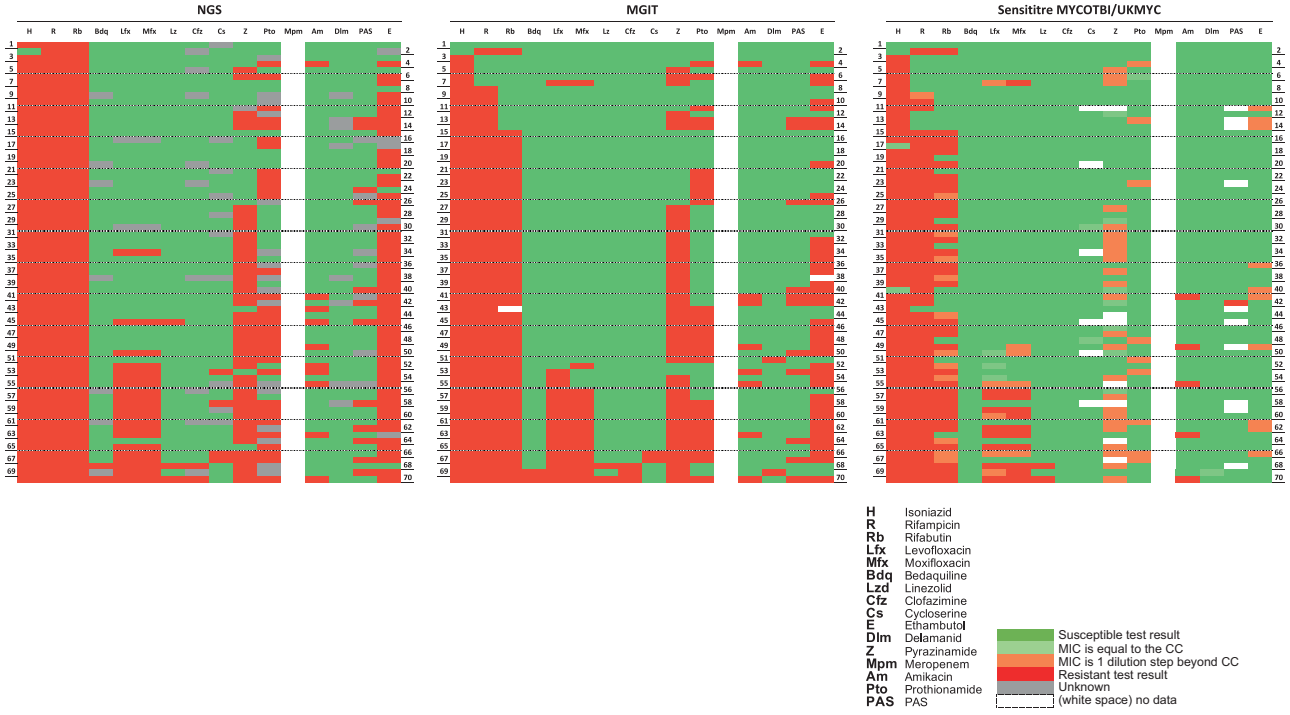

Figure 2. Phenotypic and genomic drug susceptibility testing (DST) data. Results of genomic prediction of DST by NGS, phenotypic DST in the MGIT 960 system, and phenotypic DST by broth microdilution assays (Sensititre MYCOTBI and/or UKMYC plates; MGIT for pyrazinamide) for 70 patients. Each row represents a patient, and each column represents a drug. Resistant test results are shown in red, and susceptible results are represented by green boxes. In the case of Sensititre MYCOTBI/UKMYC, MICs 1 level above the cutoff are displayed in light red, and MICs at the cutoff are displayed in light green. Polymorphisms without clear association to drug resistance are displayed in gray. In cases where no result was available, a white box was inserted. Abbreviations: CC, critical concentration; MGIT, mycobacteria growth indicator tube; MIC, minimal inhibitory concentration; NGS, next-generation sequencing; PAS, para-aminosalicylic acid.

MYCOTBI/UKMYC (Supplementary Table 3). However, to be on the side of caution, we did not include a drug in the algorithmic treatment regimen if an uncharacterized polymorphism was identified by gDST.

Algorithm-based Design of an MDR/RR-TB Treatment Regimen

We applied a standardized MDR/RR-TB treatment algorithm (Supplementary Table 2) to the gDST predictions as well as to the pDST results. When comparing the gDST-based regimen to the pDST-based regimen, the overlap of drugs selected for the respective MDR/RR-TB treatment regimens was 84.9% (248 of 292 of gDST-based treatment decisions; Figure 4). Thirty-eight of 70 (54.3%) patients would have received exactly the same drug combination based on gDST and pDST results, respectively. There was no relevant difference in the overall distribution of WHO group A, B, and C drugs selected on the basis of gDST prediction and by phenotypic testing (Figure 4).

Concurrently, our approach of excluding drugs with an unclear gDST result due to mutations not classified in our database from treatment would have led to the administration of drugs with proven phenotypic resistance in only 3 of 292 cases (1.0% of drugs selected on the basis of NGS) or for 3 of 70 (4.3%) patients. One isolate was resistant toward PAS, which was reported as wild type by NGS. Two isolates had resistance to moxifloxacin/levofloxacin, of which 1 was reported as wild type and the other was only detected retrospectively with a 3% low-frequency mutation, that is, *gyrA* D94G.

DISCUSSION

In a cohort of 70 patients with MDR/RR-TB treated at a TB reference center in Germany, we found that NGS-based analysis of all genes known to be implicated in drug resistance could effectively guide the design of personalized MDR/RR-TB therapy regimens. This was achieved by the high concordance between gDST and pDST results and a conservative clinical treatment algorithm that included only anti-TB drugs that lacked any indication for genotypic resistance. Apparently discordant predictions of resistance by gDST were mainly based on mutations previously found to confer low-level resistance, for example, toward rifampicin or ethambutol [19, 20]. This corroborates the known limitations of pDST protocols to correctly detect low-level drug resistances by only testing at the currently endorsed critical concentrations [17, 21, 22]. When designing MDR/RR-TB treatment regimens based on gDST, only 1.0% of all selected drugs, including WHO group A and B medicines,

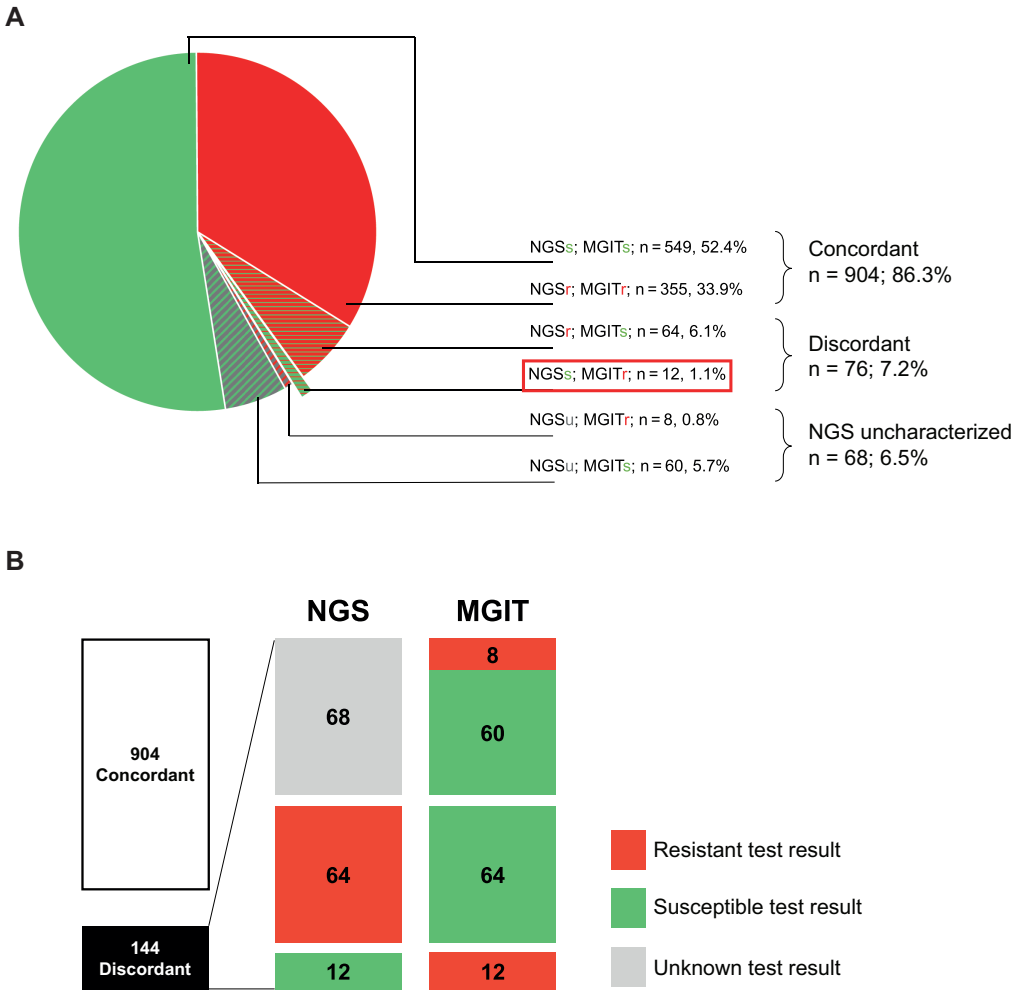

Figure 3. Distribution of drug susceptibility test (DST) results by genomic NGS and phenotypic drug susceptibility testing (MGIT). *A,* Concordant and discordant test results and results of phenotypic testing with unclassified NGS results. Red box: false gDST predictions of susceptibility *B,* Overview of discrepant test results between DST prediction by NGS and phenotypic DST by MGIT. Susceptible test results are shown in green, and resistant test results are shown in red. Mutations of unknown relevance are displayed in gray. Abbreviations: MGIT, mycobacteria growth indicator tube; NGS, next-generation sequencing.

would have been administered even though pDST results documented drug resistance. Thus, our approach supplies clinicians with a high degree of certainty when designing MDR-TB treatment regimens on the basis of gDST results as long as they are administering drugs for which no genetic resistance marker has been identified.

Recently, gDST has been shown to predict phenotypic susceptibility to first-line anti-TB agents with an accuracy of more than 98% [6]. However, the diagnostic accuracy of gDST for predicting pDST results for second-line antibiotics has not yet reached the WHO target product profile requirements of 95% specificity and 90% sensitivity [23, 24]. The main reasons for the reduced accuracy of gDST for MDR-TB drugs are knowledge gaps in the mutation catalogues [20, 24], clinical breakpoint artifacts in routine clinical diagnostics [17, 25], and poor reproducibility of individual pDSTs [11, 26]. To overcome these limitations, we developed a unique approach that conservatively applies only antibiotics in the absence of any potential genomic resistance determinant. This also allowed full coverage of group A and group B anti-TB medicines in the WHO priority list, which featured moxifloxacin, levofloxacin, bedaquiline, linezolid, clofazimine and cycloserine. Consequently, our study translates NGS-based resistance gene profiling into clinically meaningful designs of treatment regimens based on standardized algorithms following WHO drug prioritizations.

An important aspect of NGS-based DST is the unbiased detection of mutations that mediate only low or moderate MIC increase and that often test phenotypically susceptible [22]. This is of particular relevance for mutations that confer elevated MICs below current CCs, which may still be associated

Table 2. Diagnostic Accuracy of Genotypic Drug Susceptibility Testing by Next-Generation Sequencing Compared With Liquid Culture (mycobacteria growth indicator tube - MGIT) as the Reference Standard

Genotype (Next-Generation Sequencing)	Resistant Phenotype (MGIT)				Susceptible Phenotype (MGIT)				Phenotype Not Applicable				Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive Predictive Value, % (95% CI)	Negative Predictive Value, % (95% CI)
	R	U	S	Total	R	U	S	Total	R	U	S	Total				
Rifampicin	64	0	0	64	6	0	0	6	0	0	0	0	[a]	[a]	[a]	[a]
Rifabutin	56	0	0	56	13	0	0	13	1	0	0	1	100.0 (92.0–100.0)	0.0 (0.028.3)	81.2 (69.6–89.2)	n/a
Isoniazid	68	0	0	68	1	0	1	2	0	0	0	0	[a]	[a]	[a]	[a]
Bedaquiline	1	1	0	2	1	6	61	68	0	0	0	0	[b]	[b]	[b]	[b]
Levofloxacin	17	0	2	19	4	2	45	51	0	0	0	0	89.5 (65.5–98.2)	91.8 (79.5–97.4)	81 (57.4–93.7)	95.7 (84.3–99.3)
Moxifloxacin	15	0	2	17	6	2	45	53	0	0	0	0	88.2 (62.3–97.9)	88.2 (75.4–95.1)	71.4 (47.7–87.8)	95.7 (84.3–99.3)
Linezolid	2	0	0	2	1	0	67	68	0	0	0	0	[b]	[b]	[b]	[b]
Clofazimine	2	1	0	3	0	8	59	67	0	0	0	0	[b]	[b]	[b]	[b]
Cycloserine[c]	2	0	0	2	2	10	56	68	0	0	0	0	[b]	[b]	[b]	[b]
Pyrazinamide	45	0	3	48	1	1	20	22	0	0	0	0	93.8 (81.8–98.4)	95.2 (74.1–99.8)	97.8 (87.0–99.9)	87.0 (65.3–96.6)
Prothionamide[c]	28	3	0	31	5	13	21	39	0	0	0	0	100.0 (85.0–100.0)	80.8 (60.0–92.7)	84.8 (67.3–94.3)	100.0 (80.8–100.0)
Amikacin	7	0	1	8	3	0	59	62	0	0	0	0	87.5 (46.7–99.3)	95.2 (85.6–98.7)	70.0 (35.4–91.9)	98.3 (89.9–99.9)
Delamanid	0	0	2	2	0	8	60	68	0	0	0	0	[b]	[b]	[b]	[b]
Para-aminosalicylic acid [c]	7	2	2	11	5	6	48	59	0	0	0	0	77.8 (40.2–96.1)	90.6 (78.6–96.5)	58.3 (28.6–83.5)	96.0 (85.1–99.3)
Ethambutol[c]	41	1	0	42	16	4	7	27	1	0	0	1	100.0 (89.3–100.0)	30.4 (14.1–53.0)	71.9 (58.3–82.6)	100.0 (56.1–100.0)
Overall	355	8	12	375	64	60	549	673	2	0	0	2	96.7 (94.2–98.2)	89.6 (86.8–91.8)	84.7 (80.8–88.0)	97.9 (96.2–98.8)

Detected and/or predicted resistance is considered a positive test result, and susceptibility is considered a negative test result. Uncharacterized mutations were not considered in the performance calculation.

Abbreviations: CI, confidence interval; MGIT, mycobacteria growth indicator tube; R, resistant, S, susceptible, U, unknown.

[a]Performance not calculated as drug resistance was an inclusion criterion.

[b]Performance not calculated due to low prevalence of resistance.

[c]With reservation as no reliable critical concentrations/breakpoints exist for cycloserine, para-aminosalicylic acid, ethambutol, and protionamide, drug susceptibility testing in mycobacteria growth indicator tube is not recommended.

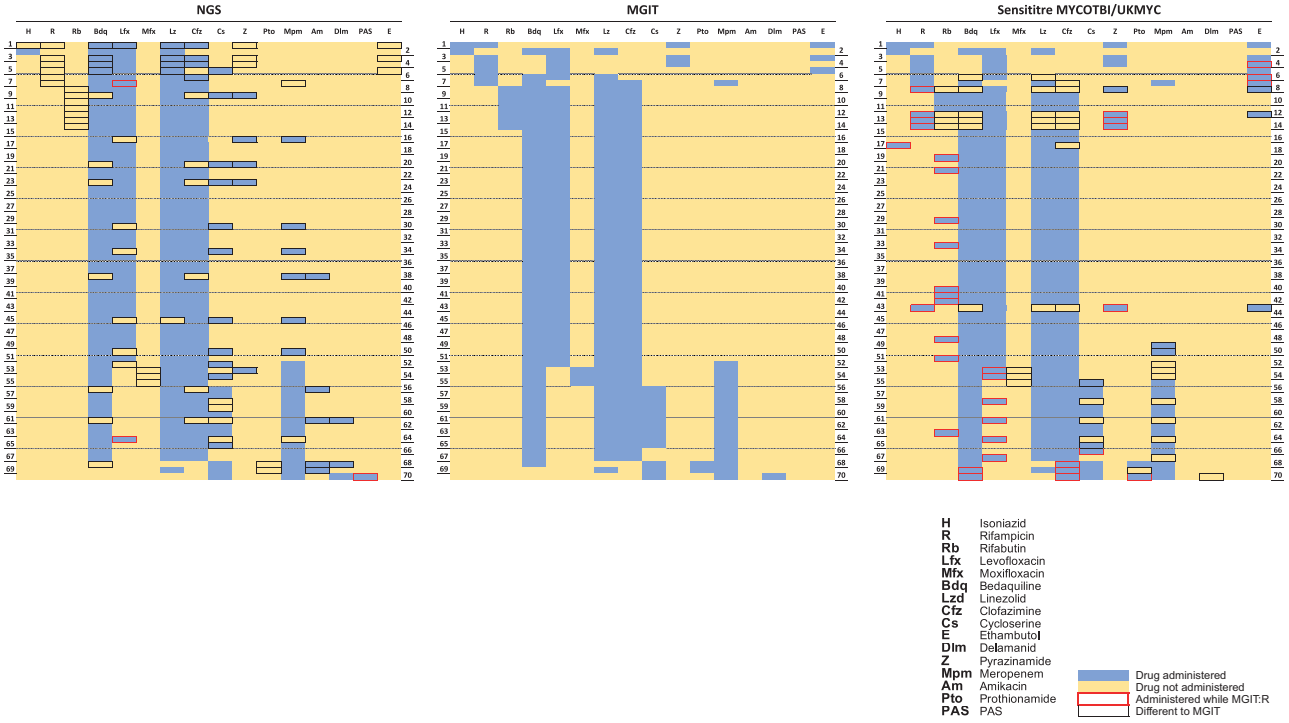

Figure 4. Algorithm-derived treatment regimens based on different methods of drug susceptibility testing. Regimens were based on respective results of NGS, MGIT, and minimal inhibitory concentration (by Sensititre MYCOTBI and/or UKMYC; MGIT for pyrazinamide). Differences in the resulting therapy regimes compared with MGIT are highlighted by black frames. Red frames indicate treatment with a drug that tested resistant in MGIT. Columns indicate data for 16 drugs for each patient Meropenem was selected as per treatment algorith, irrespective of the unavailability of DST. Abbreviations: MGIT, mycobacteria growth indicator tube; NGS, next-generation sequencing; para-aminosalicylic acid.

with worse clinical outcome [27–29]. Moreover, our MIC data corroborate previous findings in other bacterial pathogens, indicating that the chance for misclassification based on pDST (suggesting susceptibility) is higher in genetic backgrounds that confer resistance levels close to the CC or that overlap with the MIC distribution of wild-type strains [30]. In these cases, gDST data are crucial and pDST perhaps even detrimental. A more gradual characterization of antimicrobial susceptibility also opens the way to adjust drug doses rather than excluding drugs entirely from therapeutic regimens [17, 31, 32]. For only a few mutations, the clinical implications of low-level resistance on the treatment outcome have been studied convincingly. However, recent reports of emerging resistance to bedaquiline, clofazimine, and delamanid suggest that low-level drug resistance is becoming an increasingly relevant challenge regarding both DST and clinical interpretation [33–36].

In our data, mutations that cause only moderate MIC increases below or close to the CC were the main drivers for discrepancies between gDST and pDST, putting into question the suitability of pDST with current CCs as a reference "gold" standard for some drugs. In contrast, genetically undetermined drug resistances point to knowledge gaps in the NGS interpretation pipeline and support the conclusion that mutation catalogues are not yet complete, requiring regular reassessments and timely updates.

This study has several limitations. We used binary phenotypic susceptibility data based on current and, in the case of cycloserine, PAS, and prothionamide, historic CCs as the reference method. We tried to accommodate for the binary pDST results by using MIC data. This was well suited to indicate shifts in the absolute levels of drug resistance. Results from this study highlight the dilemma to find a gold standard for the definition of "drug resistance."

All patients were from a single center in Germany. Although the patient population was international (>90% of patients were foreign-born), it is unclear whether the results can be applied to other regions of the world where the population of *M. tuberculosis* complex strains causing MDR/RR-TB could differ. Subsequent prospective studies that implement a gDST-based therapy design are needed to confirm our results. Furthermore, the clinical relevance of uncharacterized mutations with an as yet unknown effect on drug resistance, needs to be further assessed in larger cohorts. Here, the vast majority of isolates with uncharacterized mutations would have been judged as phenotypically susceptible and did not show significant MIC increases. This means that high priority-drugs that were initially excluded with our conservative treatment

design can likely be included upon confirmation of drug susceptibility by pDST.

Also, standardized drug susceptibility testing to meropenem was not available due to the intrinsic instability of the drug in aqueous solution. After the study was completed, pretomanid, a new medicine for the treatment of extensively drug-resistant tuberculosis, was licensed. Pretomanid was not evaluated in this study. Our NGS pipeline and chosen thresholds for variant frequency and coverage to identify mutations in the *M. tuberculosis* genome have been shown to be highly reproducible in comparison to other available pipelines [37].

Despite these limitations, the data presented are highly conclusive and strongly support the suitability of gDST for the design of MDR/RR-TB drug regimens. Our conservative prediction of drug susceptibility by NGS is highly encouraging as NGS can potentially provide a comprehensive antibiotic resistance report more quickly than conventional culture-based methods [38, 39]. Of note, in some cases, gDST by NGS could successfully be applied directly to *M. tuberculosis* complex DNA extracted and enriched from sputum specimens, providing drug resistance reports within a few days [39–41]. Given the lack of diagnostic infrastructure for comprehensive, timely, and reliable second-line anti-TB pDST testing in many high-burden countries [42], these results strongly support the implementation of gDST in lower- and middle-income countries.

CONCLUSIONS

Genomic DST based on NGS showed high concordance to pDST results and effectively guided the design of antimicrobial combination therapies for MDR/RR-TB patients. Virtually all drugs (99.0%) used in combination therapies that were inferred from gDST were confirmed to be susceptible by pDST. Rapid genome sequencing linked with the developed mutation catalogue provides a powerful tool for timely initiation of rational, individualized MDR/RR-TB treatment regimens.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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