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Clinical implications of molecular drug resistance testing for *Mycobacterium tuberculosis*: a 2023 TBnet/RESIST-TB consensus statement

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JD, CRHJr, and CLa conceptualised the study, acquired funding, and performed project administration. MJB, EC, DC, FC, VC, KD, AD, MRF, SG, MPG, MIG, LG, IK, CLi, AMM, FMa, MM, PM, BM-M, FMo, SN, NV, and AW reviewed the literature and created the consensus document. JD, BL, FvL, and CLa devised the methods. All authors contributed to writing of the original draft, and reviewing and editing, and JD, BM-M, and CLa were responsible for writing the final version.

For more on **TBnet** see http://www.tbnet.eu

For more on **RESIST-TB** see http://www.resisttb.org

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TBnet and RESIST-TB networks

Abstract

Drug-resistant tuberculosis is a substantial health-care concern worldwide. Despite culture-based methods being considered the gold standard for drug susceptibility testing, molecular methods provide rapid information about the *Mycobacterium tuberculosis* mutations associated with resistance to anti-tuberculosis drugs. This consensus document was developed on the basis of a comprehensive literature search, by the TBnet and RESIST-TB networks, about reporting standards for the clinical use of molecular drug susceptibility testing. Review and the search for evidence included hand-searching journals and searching electronic databases. The panel identified studies that linked mutations in genomic regions of *M tuberculosis* with treatment outcome data. Implementation of molecular testing for the prediction of drug resistance in *M tuberculosis* is key. Detection of mutations in clinical isolates has implications for the clinical management of patients with multidrug-resistant or rifampicin-resistant tuberculosis, especially in situations when phenotypic drug susceptibility testing is not available. A multidisciplinary

team including clinicians, microbiologists, and laboratory scientists reached a consensus on key questions relevant to molecular prediction of drug susceptibility or resistance to *M tuberculosis,* and their implications for clinical practice. This consensus document should help clinicians in the management of patients with tuberculosis, providing guidance for the design of treatment regimens and optimising outcomes.

Introduction

According to the latest estimates by WHO[1], tuberculosis affected 10·6 million people and caused 1·6 million deaths in 2021. That these deaths continue to occur despite the availability of curative antimicrobial regimens is a testimony to the difficulty of diagnosing disease and designing, administering, and monitoring tuberculosis treatment. The rise of tuberculosis resistance to isoniazid and rifampicin has seriously complicated treatment for the estimated 450 000 people each year who develop disease resistance to these first-line antimycobacterial agents.[1] Definitions of resistance, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis are shown in table 1.

Development of three essential capacities regarding tuberculosis treatment will be needed to bring the disease under control while minimising the emergence of resistance to currently available agents: optimising regimen composition, optimising regimen duration, and minimising toxicity from the components of the regimen. This consensus statement is directed at advancing the understanding of the first of these, namely optimising regimen composition.

Despite the attractiveness of a one-size-fits-all regimen strategy, having a single regimen that can be used for all patients with drug-resistant tuberculosis is difficult for several reasons. First, there will always be patients who cannot tolerate the usual agents and will need to have individualised regimens that address their intolerance. Second, development of resistance to the new agents, although hopefully minimised, can never be completely prevented; thus, some patients will require treatment with regimens that do not contain some of the more effective agents as the organisms causing their disease will be resistant to these agents. Third, in some patients, the use of concomitant medications, such as antiretroviral agents, will preclude the use of certain antimycobacterial compounds because of drug–drug interactions. Finally, compensatory mutations and epistasis render resistant clinical strains differentially susceptible to different second-line agents. Thus, prompt and accurate identification of the susceptibility pattern of a patient's *Mycobacterium tuberculosis* infecting strain is essential.

For many decades, *M tuberculosis* susceptibility testing has been done in specialised laboratories with culture-based methods. These methods are limited by the slow growth of the organisms, such that results are not available for many weeks after disease detection. This delay leads to uncertainty for clinicians during the first weeks of treatment, a crucial time period for getting the disease under control and preventing further tuberculosis transmission. Fortunately, some developments in rapid sequencing and molecular identification of critical resistance mutations have led to the ability to predict

drug susceptibility or resistance within hours to days after diagnosis with high diagnostic accuracy.[4,5]

We summarise progress made in developing tools to enable rapid identification of resistance to the agents used in tuberculosis treatment. As such, we update our consensus statement on this topic from 2016, with the substantial progress that has been made since publication.[6] Taken together, we believe that this updated consensus provides important and clinically useful guidance to aid the design of effective tuberculosis treatment regimens and to ensure optimal therapy outcomes.

Methods

This document has been produced by physicians, microbiologists, molecular biologists, and clinical epidemiologists of the TBnet (http://www.tbnet.eu) and RESIST-TB (http://www.resisttb.org) networks to reach a consensus in reporting standards in the clinical use of *M tuberculosis* molecular drug susceptibility testing (DST) results. Chapter leaders reviewed the available literature, and the targeted search for evidence included hand-searching journals and searching electronic databases including MEDLINE and PubMed. The search targeted articles published in English, before June 1, 2022, related to the evolution of drug resistance in clinical isolates, principles of phenotypic and genotypic drug resistance testing, and the impact of mutations detected by genotypic tests on phenotypic DST and clinical outcomes. In addition, relevant documents published by WHO were included. Consensus statements were developed in a stepwise approach, as previously described (panel 1).[6–10]

Evolution of drug resistance in clinical *M tuberculosis* isolates

M tuberculosis bacteria are continuing to develop resistance to both old and new drugs via spontaneous mutations of the bacterial chromosome.[11] Mutations include mostly single-nucleotide polymorphisms, but insertions and deletions can also occur. Horizontal gene transfer (eg, mediated by plasmids) has no role in the evolution of drug resistance in *M tuberculosis*.[12] The most common drug resistance mechanisms comprise the modification of the drug target itself, increased expression of the drug target, downregulation or abrogation of prodrug-activating enzymes, upregulation of drug efflux pumps, altered permeability, and phase variation.[12,13] The probability of acquiring drug resistance through spontaneous mutations and selection depends on the antibiotic (eg, the estimated frequency for rifampicin is 10^{-8}, and 10^{-6} for isoniazid).[14] Moreover, studies suggest that mutation rates can differ between strains of different *M tuberculosis* lineages, which influences the probability of acquiring drug-resistant mutations.[15–17]

When suboptimal treatment regimens are used, drug-resistant *M tuberculosis* subpopulations can be selected in a patient.[18–21] The main reasons for ineffective therapy include improper intake of drug regimens, addition of single drugs to ineffective treatment regimens, inadequate drug supplies, quality of the anti-tuberculosis drugs, individual differences in pharmacokinetics and pharmacodynamics,[22] intrinsic strain differences, drug tolerance, and low-level resistance.[23,24] Some research highlights the heterogeneity of antibiotic concentrations within the tuberculous granuloma.[25] A lack of granuloma vascularisation and

diffusion barriers towards necrotic foci can lead to drastically altered pharmacodynamics and pharmacokinetic correlations of individual antibiotics as compared with blood plasma measurements.[25,26] The resulting heterogeneity of granuloma micro-environments in a single patient probably drives the microevolution of *M tuberculosis* subpopulations, which can harbour different drug resistance mutations and can coexist in separate lesions.[27–29] This intrapatient *M tuberculosis* diversity also influences the performance of drug resistance assays, and should therefore be considered in routine diagnostics and treatment.[29–31]

Diagnostic delays and misdiagnosis of patients with MDR tuberculosis can further amplify the evolution of resistance and virulence determinants in *M tuberculosis* strains.[32–34] Outbreaks of drug-resistant strains have been observed in different settings globally,[35–37] suggesting that these strains have few or no fitness costs, challenging the previous dogma of a transmission detriment of MDR *M tuberculosis* strains. However, no-cost or low-cost resistance mutations dearly exist, and secondary mutations can occur that compensate for the initial fitness defect of resistant mutants.[38–40] This molecular interaction is an example of epistasis, and might also influence antibiotic resistance and compensatory evolution in strains of different *M tuberculosis* lineages.[41,42] In multiple studies, compensatory mechanisms were suggested to be associated with an increased transmission risk, eventually leading to the expansion of MDR clones in different areas worldwide.[35–37,43]

Principles of phenotypic drug resistance testing

Mycobacterial phenotypic DST assesses the ability of the organism to grow in the presence of the antibiotic. The various phenotypic DST methods are detailed elsewhere.[44–46] The commonly used proportion method relies on the premise that if more than 1% of the organisms in a given population is resistant to the critical concentration of a drug, the population is defined as being resistant. These critical concentrations separate the susceptible populations from resistant ones, and are specific to the phenotypic DST method and media.

Mycobacterial phenotypic DST is technically difficult, and its interpretation is challenged by the molecular assays' detection of resistance mutations. Laboratory errors occur, and appropriate quality-management systems and proficiency testing are vital.[46–48] Phenotypic DST accuracy is also influenced by the prevalence of resistant strains and the level of this resistance. In settings where low-level resistant strains prevail, phenotypic DST can correlate less well with clinical outcomes, in contrast to settings where high-level resistance is common.[49] In isolates with low-level resistance, high doses of drugs such as rifampicin, isoniazid, and fluoroquinolones can be clinically effective.[50]

Two systematic reviews of the evidence supporting critical concentrations in phenotypic DST testing were done by WHO.[51,52] Although many of the current critical concentrations were reaffirmed, important changes were proposed, most notably that the critical concentration of rifampicin for testing on solid 7H10 medium and in Mycobacteria Growth Indicator Tube medium (Becton Dickinson, Franklin Lakes, NJ, USA) be lowered from 1 mg/L to 0·5 mg/L. WHO also introduced clinical breakpoints for moxifloxacin. Clinical breakpoints are the concentrations delineating strains that might still respond to therapy

with higher doses of the agent. The Clinical and Laboratory Standards Institute also defined clinical breakpoints for isoniazid.[45] Other important observations include few exploitable published data for critical concentrations of newer drugs such as bedaquiline;[53–55] and scarce data about reproducibility for these newer drugs. As more data become available, critical concentrations will need to be re-evaluated.

The reduced critical concentration of rifampicin could enable the detection of borderline mutations in the *rpoB* gene, which are considered to be of clinical relevance but only result in modest minimum inhibitory concentration increase. This reduced critical concentration of rifampicin would only reduce rather than eliminate the discordance between genotype and phenotype. Minimum inhibitory concentration distributions of bacterial populations with low-level resistance phenotypes might overlap with the minimum inhibitory concentration distributions of wild-type isolates, resulting in poor reproducibility of categorical phenotypic DST. This overlap could be also the case for other drugs like bedaquiline and levofloxadn (see section on molecular testing).

Although the use of critical concentrations to distinguish susceptible isolates from non-susceptible isolates is appealing as it is well standardised, more granular phenotypic DST data (such as minimum inhibitory concentrations with broth microdilution) could facilitate better understanding of the correlation between phenotypic DST, genotype, and treatment outcome—especially if associated with pharmacokinetic and pharmacodynamics data. Hence, quantitative drug susceptibility measures need to be implemented in diagnostic mycobacteriology.[56,57] The first objective of the newly formed European Committee on Antimicrobial Susceptibility Testing subcommittee on antimycobacterial susceptibility testing (EUCAST-AMST) was to set a reference method for minimum inhibitory concentration determination of *M tuberculosis.* This standardised method[58,59] will be used for both new and old anti-tuberculosis drugs to assess epidemiological cutoffs. This method will allow new compounds to be evaluated and compared, and will offer a basis for calibrating phenotypic DST methods used in the field, commertial or not, against a standard reference method.[60] In addition, the CRyPTIC Consortium[61] presented the epidemiological cutoffs for 13 anti-tuberculosis compounds, including bedaquiline and delamanid, from clinical isolates collected worldwide, for a more widespread adoption of the broth microdilution test. Finally, a 2022 WHO document provided a standardised methodology to facilitate the improvement of the broth microdilution plate method for clinical use, which represents a promising solution for comprehensive and quality-assured phenotypic DST.[62]

Principles of genotypic DST

The fundamental principle of genotypic DST is to predict phenotypic resistance on the basis of genotype (ie, the genetic variants detected). Drug resistance in *M tuberculosis* strains is due to variations of their genome (either single-nucleotide polymorphisms or insertions and deletions) mapping to spedfic genetic loci.[63] Silent mutations are usually not responsible for resistance, with few documented exceptions.[64] Importantly, not all mutations found at the genetic loci involved in drug resistance are responsible for a resistant phenotype, as phylogenetic markers and neutral polymorphisms can also be located in these regions.[65–69]

Whereas for several anti-tuberculosis drugs the association between sequence variations and phenotypic resistance development is well documented, our knowledge remains incomplete for others.[63] The frequency of resistance mutations observed in clinical *M tuberculosis* strains varies by resistance genes, local epidemiology, and *M tuberculosis* population structure in different geographical settings.[70,71] Generally, drug resistance in *M tuberculosis* is emerging at strain level, but there are a few exceptions where intrinsic resistance is found in strains of a specific phylogenetic clade.[65,72,73]

In the past 15 years, rapid commercial genotypic DST assays have become available to detect drug resistance in clinical *M tuberculosis* strains by interrogating the presence of known resistance-conferring variants (table 2).[5,75–80] Performance of these genotypic DST tests, and implications for their use in different application scenarios are outlined in WHO guidelines.[81,82] The line probe assay GenoType MTBDR*sl* VER 2.0 (HAIN Lifescience/Bruker, Nehren, Germany) and the cartridge-based Xpert MTB/XDR (Cepheid, Sunnyvale, CA, USA) are the only assays currently endorsed for the detection of resistance to fluoroquinolones, thus allowing the identification of pre-extensively drug-resistant (pre-XDR) tuberculosis according to the updated definition.[3,82,83] Of note, the line probe assay Nipro Genoscholar PZA-TB II (Nipro Corporation, Osaka, Japan) is able to detect *pncA* mutations associated with pyrazinamide resistance.[82,84,85]

Knowledge about the genetic basis of resistance to new or repurposed drugs such as bedaquiline, linezolid, clofazimine, and delamanid remains poor, as is our capability for phenotypic DST.[86–88] No rapid molecular commercial assay exists for these drugs, and genotypic DST can be done by sequencing technologies only. In this context, the targeted sequencing assay Deeplex Myc-TB (Genoscreen, Lille, France) is able to provide genotypic DST for 13 drugs, including linezolid, bedaquiline, and clofazimine, by targeting the full sequences of 18 loci associated with drug resistance.[89,90] Preliminary studies show that, directly from clinical specimens, Deeplex Myc-TB can frequently be successful, reporting complete resistance predictions in sputum samples with acid-fast bacilli visible on microscopy.[89–91] Similar targeted next-generation sequencing approaches exist; however, they are not yet targeting new or repurposed drugs (Next Gen-RDST,[92] AmpliSeq TB[93]). Compared with whole-genome sequencing, targeted next-generation sequencing has the potential to decentralise sequencing-based genotypic DST for individual patient care, especially if coupled with versatile technological and bioinformatic platforms.[31,91,94–96] The different genotypic DST assays have varied turnaround times, ranging from 1 working day for Xpert and line probe assay under optimal conditions, to 7–10 working days for targeted next-generation sequencing under operational conditions, when applied to clinical specimens (table 3).

Notably, the technical success with a specimen depends on the number of bacilli, as a low inoculum increases the risk of technical failure. When genotypic DST assays are applied to a positive culture, the probability of obtaining a result increases, but so does the turnaround time.

Current genotypic DST assays cannot be used for treatment monitoring as they detect DNA from both live and dead bacteria, thus a positive result does not imply the viability of the

pathogen.[97] Similarly, the detection of heteroresistance in clinical specimens by the use of WHO-endorsed rapid molecular tests vary from below 10% to above 75%, depending on the assay and target considered.[83,98,99] By contrast, targeted next-generation sequencing assays allow for the detection of even minor subpopulations due to the high coverage they provide.[89,100,101] A cost evaluation of the workflow of whole-genome sequencing in eight laboratories in Europe and North America calculated the costs to be 7% cheaper than phenotypic DST.[102] In settings with a low tuberculosis burden, the routine use of Xpert shortened the time to tuberculosis diagnosis and treatment, whereas whole-genome sequencing shortened the time to DST and treatment modification when necessary, reducing treatment and hospitalisation costs. The combined use of these two methods was the most cost-effective option and allowed for faster appropriate treatment, and this subsequently reduces transmission, benefiting health, and reducing future treatment costs.[103] In India, molecular tests used in the diagnosis of MDR-tuberculosis, such as line probe assays and Xpert, were reported to have considerable advantages for the programmatic management of drug-resistant tuberculosis, including speed, standardisation of testing, potentially high throughput, and reduced laboratory biosafety requirements. They also appeared to be cost effective and helped in detecting missing cases.[104] Cost-effectiveness studies of sequencing tests in low-income settings with high tuberculosis burden are needed.

In 2022, a budget impact analysis was done to estimate the costs of introduction and routine use of next-generation sequencing in the National Tuberculosis Programme of Moldova, a country with a high burden of MDR tuberculosis.[105] According to the model, next-generation sequencing adoption would require expanded resources compared with conventional phenotypic DST. We are convinced that despite these initial investments, DST strategies using next-generation sequencing will also prove to be cost-effective. However, much needs to be done for the implementation of molecular DST, especially in countries of high tuberculosis burden. A recent study by the TBnet[106] documented substantial gaps in the availability of genotypic DST for anti-tuberculosis drugs in the European Region of WHO. These results are especially worrisome in view of the roll-out of the BPaLM regimen (a combination of bedaquiline, pretomanid, linezolid, and moxifloxacin), as many countries currently have no capacity for DST of key components of this regimen.

Relationship between results of genotypic and phenotypic drug resistance testing and clinical outcomes

There are different ways to study the clinical implications of drug resistance mutations. The simplest one is to show that in a case of treatment failure, there is associated drug resistance either phenotypically (ie, minimum inhibitory concentration increase) or genotypically (ie, appearance of a mutation) at the time of tuberculosis diagnosis. This technique was used in the 1960s to show that the phenotypic proportion method correctly identified strains associated with treatment failure.[107] It was also initially used to show that rifampicin resistance was associated with *rpoB* mutations.[108] For some genes (table 4), such a correlation has been shown.

However, for clinical use, the question is not only whether the mutation correctly identifies a strain with an elevated minimum inhibitory concentration, but also whether this mutation is associated with treatment failure, thus prompting the clinician to consider a change in the treatment regimen. Given that tuberculosis treatment relies on drug combinations, this second demonstration requires that the antibiotic showing a resistant phenotype associated with the mutation has an important impact on the outcome of the drug combination. This second demonstration is true for both drug-susceptible tuberculosis treatment regimens and MDR or rifampicin-resistant tuberculosis regimens, as shown in WHO's meta-analysis that indicated not all second-line drugs have the same impact on treatment outcome.[136] Hence, the drug resistance mutations that will have a strong effect on treatment outcome are those modulating the most effective antibiotics. Another factor to consider is the differential impact of mutations on the minimum inhibitory concentration as highlighted previously. Again, the probability of showing a differential impact on treatment outcome will depend on both the mutation-specific minimum inhibitory concentration effect and on the intrinsic activity of the drug. The more effective or mycobactericidal the drug, and the higher the impact of the mutation on the minimum inhibitory concentration, the more likely the effect on treatment outcome. We have reviewed studies linking the treatment outcome with the presence of a drug mutation (table 4). Of note, the lack of a clinical impact is by no means conclusive; it might only mean that although the mutation reduces the activity of the drug, other drugs in the regimen could facilitate clinical cure. The relationship between mutations and their clinical implications are discussed within the context of each drug type or class.

Another notable benefit of the use of molecular DST is proper initial distinguishing of patients with tuberculosis according to detected resistance patterns. This benefit is extremely important in countries with high MDR and XDR tuberculosis burden, with poor capacity for airborne infection isolation in single-occupant hospital rooms. Taking into consideration molecular DST results can facilitate an optimal distribution of isolation wards and prevent nosocomial transmission of drug-resistant *M tuberculosis* strains, even when it has low relevance in treatment decisions.

For isoniazid, *katG* 315 mutations have a higher negative impact on first-line treatment outcome than *inhA* mutations. Although not analysed in the meta-analysis of individual patient data due to low numbers in the dataset,[137] and not listed as a group C drug in recent WHO guidance[138], high-dose isoniazid is still used in children, in patients in whom an effective regimen cannot otherwise be constructed (due to lack of drug access, adverse events, resistance to other agents, etc), and in patients with low-level resistance predicted by *inhA* mutations and other mutations in *katG.*[118,139] Although high-dose isoniazid is still being used as part of an all-oral 9-month bedaquiline-based MDR or rifampicin-resistant tuberculosis regimen in countries like South Africa, and as part of the 9–11-month injectable-based regimen[140,141] previously endorsed by WHO in 2019 and 2020 guidance,[80,138] this regimen will soon be replaced by alternative 6-month pan-oral regimens like BPaLM.[142]

For rifampicin, more than 20 mutations in *rpoB* could have a negative effect on first-line treatment outcome, such as the Ile491Phe mutation found in Eswatini. As approximately 5% of mutations fall outside of the *rpoB* gene hotspot region, repeat genotypic or

phenotypic susceptibility testing should be considered in patients with genotypically rifampicin-susceptible tuberculosis who do not respond to rifampicin-based treatment at 2–3 months. It is also important to consider nonadherence and the possibility of heteroresistance, and to check the adequacy ofblood rifampicin concentrations.[143,144]

For fluoroquinolones, the *gyrA* mutations Asp94Asn, Asp94Gly, Ala90Val, and Asp94Ala have a negative effect on MDR or rifampicin-resistant tuberculosis treatment outcome, and that effect is closely linked to the minimum inhibitory concentration generated by the associated mutation.[119,126–130] Fluoroquinolone resistance implies pre-XDR tuberculosis. Results from the ZeNix trial[145] suggest that successful treatment of MDR or rifampicin-resistant tuberculosis can also be achieved in approximately 90% of patients treated for 6 months with bedaquiline (200 mg twice per day for 14 days, and 200 mg thrice per week thereafter), pretomanid (200 mg once per day), and linezolid (600 mg once per day), without fluoroquinolones. With a conventional treatment regimen that does not include all of the three BPaL drugs, fluoroquinolone resistance is associated with a poorer prognosis.[146] Treatment outcomes in fluoroquinolone-resistant MDR or rifampicin-resistant tuberculosis can possibly (but presently unclearly) be improved without these three medicines, if additional group C medicines are added to have at least five active drugs in the treatment regimen. For ethionamide, multiple *ethA* mutations throughout the gene have a negative effect on MDR tuberculosis treatment outcome.[119,126]

For bedaquiline, data are scarce about primary or acquired resistance in clinical cohorts and its effect on outcomes. In South Africa, the proportion of patients with a successful outcome was 72% (794/1103) among those with bedaquiline susceptibility compared with 57% (21/37) among those with bedaquiline resistance.[123] However, we know that addition of bedaquiline to a background regimen, or when used in combination with other drugs, is associated with a reduction in mortality and improved outcomes.[141,147] Thus, clinically significant resistance to bedaquiline would be expected to imply a worse prognosis. There are discrepant results regarding *Rv0678* mutations and treatment outcome, probably due to the low minimum inhibitory concentration increase. Unfortunately, phenotypic testing is required to ascertain the presence of bedaquiline resistance, as high-confidence mutations in the *atpE* and *Rv0678* genes are likely to predict less than 10% of phenotypically defined resistance.[148] After considering phenotypical cross-resistance with clofazimine[86,149] (which is quite frequent), a regimen containing four or five effective drugs would need to be constructed in patients with isolates resistant to bedaquiline.

For linezolid, *rrl* mutations 2814 g/t and 2270 g/t, and *rplC* mutation Cysl54Arg are associated with treatment failure. The high-confidence mutations currently predict approximately 75% of phenotypically defined resistance.[86] Thus, the addition of such mutations to standardised molecular testing platforms would be useful. When linezolid resistance occurs in combination with fluoroquinolone resistance, it defines XDR tuberculosis, and the design of an appropriate individualised regimen is required.

Although the phase 3 delamanid trial[150] did not show improved treatment outcomes, many authorities consider the drug useful for the treatment of patients with resistance beyond MDR or rifampicin-resistant tuberculosis. Mutations encoding drug resistance that predict

almost 90% of delamanid resistance involve six genes, and there is emerging evidence of acquired delamanid resistance.[151] Incorporation of genotypic testing into standardised molecular platforms would be useful. Pretomanid is now part of recommended 6-month pan-oral regimens, but there are no clear cutoffs, and critical concentration is not yet defined for performing phenotypic DST.[55] Nonetheless, a low proportion of phenotypically XDR tuberculosis isolates were found to be genotypically resistant to delamanid and pretomanid.[149]

For pyrazinamide, there are many mutations in *pncA* that can confer drug resistance. Mutations in *pncA* have been related to delayed sputum culture conversion; however, the effect on MDR or rifampicin-resistant tuberculosis treatment outcome has been variable.[119,129,135] For ethambutol, *embB* mutations do not appear to have an effect on treatment outcomes for MDR or rifampicin-resistant tuberculosis.[119]

For injectables such as amikacin, kanamycin, and capreomycin, the most commonly detected mutations are *rrs* 1401 a/g, 1402 c/t, 1484 a/t, and *eis* −14 c/t. No mutations have been shown to have an effect on treatment outcomes for MDR or rifampicin-resistant tuberculosis, which is consistent with the meta-analysis that showed these drugs have poor activity against MDR or rifampicin-resistant tuberculosis.[152] Nowadays, amikacin has little applicability but can still be widely used when access to bedaquiline and linezolid is restricted. It can also form part of the regimen in patients with XDR tuberculosis, or resistance beyond XDR tuberculosis when treatment options are scarce. Although an all-oral regimen is recommended by WHO,[142] many patients in tuberculosis-endemic countries do not yet have access to such regimens, making such recommendations unrealistic.[1,11]

Thus, some mutations that compromise the activity of major drugs have an impact on treatment outcome. In this sense, a classification to stratify mutations in order of importance for regimen design and choice of drugs would be useful. For this, the CRyPTIC Consortium, ReSeqTB, contributors to WHO's surveillance programme, multinational tuberculosis researchers, and public health bodies provided whole-genome sequencing and associated anonymised metadata. Algorithms for identifying variants associated and not associated with resistant phenotypes were adapted from approaches developed by the multinational CRyPTIC Consortium, and the confidence-grading method developed the Seq&Treat project[153]. The consortium concluded that for levofloxacin, moxifloxacin, and ethambutol, the mutations' pooled sensitivity was more than 80%. Specificity was over 95% for all drugs except ethionamide (91·4%), moxifloxacin (91·6%), and ethambutol (93·3%). Only two resistance mutations were identified for bedaquiline, delamanid, clofazimine, and linezolid as prevalence of phenotypic resistance was low for these drugs. Finally, the WHO catalogue of mutations in *M tuberculosis* and their associations with drug resistance were published.[154] Based on the evidence presented in this review, we developed consensus recommendations that should help clinicans in the management of patients with TB, through the optimised use of molecular drug resistance testing for *M tuberculosis.* Consensus recommendations are shown in panel 2.

Conclusions

Molecular prediction of *M tuberculosis* drug susceptibility and resistance is currently revolutionising the management of patients with tuberculosis, especially in settings with a high burden of MDR or rifampicin-resistant tuberculosis. For the first time, in 2021, WHO issued a catalogue of mutations and their associations with antimicrobial resistance, to individualise and mirror the path taken almost two decades ago in the field of HIV infection. New technologies allow for targeted sequencing directly from sputum[90] or stool[155] specimens, providing the prospect of a much faster turnaround time for DST than for conventional bacteriological methods, but still allowing for the design of accurate MDR or rifampicin-resistant tuberculosis treatment regimens.[31] As new drugs are now being marketed, it is essential to ensure that *M tuberculosis* does not acquire resistance against these new compounds shortly after they become available. In tandem with the roll-out of reliable diagnostic technologies that meet WHO target product profiles[156] to comprehensively predict drug susceptibility and resistance, global availability of antimicrobial susceptibility testing[157] is equally important. Ideally, severe mutations in the *M tuberculosis* genome should already be known before marketing newer drugs, thus enabling the identification of emerging drug resistance early on. The tools are becoming available—now is the time to bring them to the places where they are needed most.

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Panel 1:

Stepwise consensus statements

- Step 1: preliminary proposals for key recommendations were drafted by the coordinating authors (JD, CL, CRHJr). All coauthors were asked to provide alternative statements
- Step 2: alternative statements were collected from coauthors
- Step 3: coauthors were asked to select one preferred statement among the alternative statements. The coauthors were masked to the vote
- Step 4: for each recommendation, the statement that received the most votes was selected for inclusion in the manuscript
- Step 5: all coauthors were asked to indicate their agreement, disagreement, or whether they preferred to abstain from a decision

Panel 2:

Consensus recommendations

1. Should rapid molecular testing for *Mycobacterium tuberculosis* drug resistance be the gold standard for detection of rifampicin resistance? When should it be done? Should it always be accompanied by phenotypic drug susceptibility testing (DST)?

Rapid molecular testing for the prediction of rifampicin resistance in *M tuberculosis* (by WHO-endorsed methods) should be done as part of the initial evaluations in the diagnosis of active tuberculosis. Of note, current routinely available rapid molecular testing methods for the prediction of rifampicin resistance in *M tuberculosis* are not covering all mutations in the *rpoB* gene that result in *M tuberculosis* drug resistance. Although not all detections of mutations in the *rpoB* gene have important implications for regions like Eswatini, these exceptions are of low clinical relevance in other geographical regions. Confirmatory culture-based DST for rifampicin should be done for genotypically susceptible isolates. Resistance inferred by the presence of unidentified mutations (eg, no wild type on rapid molecular testing) should be confirmed by culture-based DST. Identification of mutations recognised as markers of resistance (such as those listed in WHO's catalogue of mutations) should not be further confirmed by conventional culture-based DST.

19 (76%) agree, five (20%) disagree, and one (4%) abstain.

2. Should rapid molecular testing for *M tuberculosis* drug resistance be the gold standard for detection of isoniazid resistance? When should it be done? Should it always be accompanied by phenotypic DST?

Isoniazid resistance is the most common drug resistance of *M tuberculosis.* Rapid molecular testing for the prediction of isoniazid resistance in *M tuberculosis* (by WHO-endorsed methods) should be done as part of the initial evaluations in the diagnosis of active tuberculosis. Confirmatory culture-based DST for isoniazid should be done for genotypically susceptible isolates. Resistance inferred by the presence of unidentified mutations (eg, no wild type on rapid molecular testing) should be confirmed by culture-based DST. Identification of mutations recognised as markers of resistance (such as those listed in WHO's catalogue of mutations) should not be further confirmed by conventional culture-based DST.

19 (76%) agree, five (20%) disagree, and one (4%) abstain.

3. When is prediction of *M tuberculosis* drug resistance towards ethambutol and pyrazinamide recommended, and if so, by which methods?

Rapid molecular testing for the prediction of ethambutol and pyrazinamide resistance in *M tuberculosis* is not recommended as part of the initial evaluations in the diagnosis of active tuberculosis. However, due to the inherent limitations of culture-based DST for ethambutol and pyrazinamide,

the identification of mutations recognised as markers of resistance (such as those listed in WHO's catalogue of mutations) should rule out the need for further conventional culture-based DST.

20 (80%) agree, two (8%) disagree, and three (12%) abstain.

4. Which molecular methods are available for the prediction of second-line drug resistance to WHO group A, group B, and group C medicines?

The only molecular method available for prediction of resistance to all second-line drugs of WHO group A, group B, and group C is whole-genome sequencing. Amplicon sequencing (Deeplex [Genoscreen, Lille, France]) is available for prediction of resistance to fluoroquinolones, bedaquiline, linezolid, clofazimine, ethambutol, pyrazinamide, amikacin, streptomycin, and ethionamide. Line probe assays are available for prediction of resistance to fluoroquinolones, ethambutol, pyrazinamide, amikacin, and streptomycin. The Xpert MTB/XDR assay (Cepheid, Sunnyvale, CA, USA) can be used for prediction of resistance to isoniazid, fluoroquinolones, amikacin, and ethionamide.

23 (92%) agree, one (4%) disagree, and one (4%) abstain.

5. In which patients, on which specimens, and at which time after diagnosis of tuberculosis should molecular testing for the prediction of resistance to second-line anti-tuberculosis medicines be done?

Additional molecular testing for the prediction of drug resistance to second-line anti-tuberculosis medicines in *M tuberculosis* should be done as part of the evaluations in the diagnosis of active tuberculosis, as soon as resistance to rifampicin has been suggested by molecular methods, or when rifampicin resistance has been identified by phenotypic DST, on the earliest available specimen. This timing is especially important in patients from countries with a high burden of multidrug-resistant or rifampicin-resistant tuberculosis. Since phenotypic DST for several key second-line anti-tuberculosis medicines is not universally available, implementation of molecular testing for the prediction of drug resistance to second-line anti-tuberculosis medicines in *M tuberculosis* has high priority for the control of drug-resistant tuberculosis.

25 (100%) agree.

6. How fast can results of molecular drug susceptibility prediction be provided?

Under ideal circumstances, results that predict susceptibility or resistance of *M tuberculosis* towards rifampicin, isoniazid, fluoroquinolones, amikacin, and ethambutol can be obtained within 1 working day. Results of amplicon sequencing and whole-genome sequencing to predict resistance against second-line anti-tuberculosis medicines can be obtained within 2–3 working days. Under operational conditions, once a sample has arrived at the laboratory, rapid molecular testing results can be obtained within 1–2 working days, line probe assay results within 2–4 working days, and amplicon

sequencing (Deeplex) and whole-genome sequencing results within 7–10 working days.

23 (92%) agree, one (4%) disagree, and one (4%) abstain.

7. How should the results of molecular DST be reported by the laboratory to the clinicians?

Results of molecular DST should be reported on standardised forms by the laboratory to the clinicians, including interpretation on the confidence of drug resistance prediction and on the level of drug resistance, when agreed-upon definitions for high-level or low-level resistance are available.

25 (100%) agree.

8. After starting a patient on treatment for tuberculosis based on molecular results, should the treatment regimen be changed if phenotypic DST provides a discrepant result?

In case of discrepant results between the genotypic prediction and phenotypic testing of *M tuberculosis* drug resistance, results should be discussed between the clinician and microbiologist to identify the cause of the discrepancy and the relevance for clinical decision making. Low-level drug resistance that is not identified by routine phenotypic DST might be identified by genotypic testing. At the same time, genotypic analysis might identify mutations that confer drug resistance, which leads to a low level of phenotypic drug resistance that could potentially be overcome by high dosages of medicines in clinical practice. By contrast, phenotypic DST might identify drug resistance caused by other, yet unknown, mechanisms not detected by genotypic analysis.

25 (100%) agree.

Table 1:

WHO definitions of drug resistance in tuberculosis

	2006 WHO definitions[2]	**2021 WHO definitions[3]**
RR tuberculosis	Rifampicin	Rifampicin
MDR tuberculosis	Rifampicin; isoniazid	Rifampicin; isoniazid
Pre-extensively drug-resistant tuberculosis	Not defined	MDR or RR tuberculosis; fluoroquinolone *
Extensively drug-resistant tuberculosis	MDR or RR tuberculosis; a fluoroquinolone *; second-line injectable drugs †	MDR or RR tuberculosis; a fluoroquinolone *; and bedaquiline or linezolid, or both

RR=rifampicin-resistant. MDR=multidrug-resistant.

* Fluoroquinolone (ie, levofloxacin or moxifloxacin).

† Second-line injectable drugs (ie, amikacin, capreomycin, and kanamycin).

Table 2:

Summary of assays currently available for genotypic DST in tuberculosis, and their genetic targets

	Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA)	**Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA)**	**Truenat MTB-Rif Dx (MolBio Diagnostics, Goa, India)**	**GenoType MTBDR*plus* VER 2.0 (HAIN Lifescience/ Bruker, Nehren, Germany)**	**Nipro NTM+MDRTB detection kit 2 (Nipro Corporation, Osaka, Japan)**	**GenoType MTBDRs*l* VER 2.0 (HAIN Lifescience/ Bruker, Nehren, Germany)**	**Xpert MTB/XDR (Cepheid, Sunnyvale, CA, USA)**	**Nipro Genoscholar PZA-TB II (Nipro Corporation, Osaka, Japan)**	**Deeplex Myc-TB[*] (Genoscreen, Lille, France)**	**Next Gen-RDST[*] (Illumina, San Diego, CA, USA)**	**AmpliSeq TB[*]**
Technology	Cartridge-based real-time PCR and molecular beacons	Cartridge-based real-time PCR and sloppy molecular beacons	Chip-based real-time PCR	Line probe assay	Line probe assay	Line probe assay	Cartridge-based real-time PCR and sloppy molecular beacons	Line probe assay	tNGS	tNGS	tNGS
Genotypic DST approach	Negative hybridisation of wild-type probes	Mutation-specific melting temperature shifts analysis	Melting temperature shifts analysis	Negative hybridisation of wild-type probes or positive hybridisation of specific mutation probes	Negative hybridisation of wild-type probes or positive hybridisation of specific mutation probes	Negative hybridisation of wild-type probes or positive hybridisation of specific mutation probes	Mutation-specific melting temperature shifts analysis	Negative hybridisation of wild-type probes	Specific identification of mutations by sequencing	Single molecule-overlapping reads	Specific identification of mutations by sequencing
First-line											
Rifampicin (RR)	*rpoB* RRDR	*rpoB* RRDR	*rpoB* RRDR	*rpoB* RRDR	*rpoB* RRDR	..	..	..	*rpoB* fragment (incl. cod. 170,491, and RRDR)	*rpoB* RRDR	*rpoB* full gene
Isoniazid (MDR)	..	..	..	*katG* codon 315; *inhA* promoter region	*katG* codon 315 region; *inhA* promoter region	..	*katG* codon 315 region; *inhA* promoter region; *ahpC* promoter region; *fabG1* codon 203 region	..	*katG* fragment (including codon 315); *ahpC* fragment (including promoter region); *inhA* fragment (including promoter region, and codon 94); *fabG1* fragment (including promoter region)	*katG* fragment (including codon 315); *inhA* promoter region	*katG* full gene; *inhA* full gene
Group A											

	Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA)	**Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA)**	**Truenat MTB-Rif Dx (MolBio Diagnostics, Goa, India)**	**GenoType MTBDR*plus* VER 2.0 (HAIN Lifescience/ Bruker, Nehren, Germany)**	**Nipro NTM+MDRTB detection kit 2 (Nipro Corporation, Osaka, Japan)**	**GenoType MTBDRs*l* VER 2.0 (HAIN Lifescience/ Bruker, Nehren, Germany)**	**Xpert MTB/XDR (Cepheid, Sunnyvale, CA, USA)**	**Nipro Genoscholar PZA-TB II (Nipro Corporation, Osaka, Japan)**	**Deeplex Myc-TB* (Genoscreen, Lille, France)**	**Next Gen-RDST* (Illumina, San Diego, CA, USA)**	**AmpliSeq TB***
Levofloxacin and moxifloxacin (pre-XDR)	..	..	..	..	..	*gyrA* QRDR; *gyrB* QRDR	*gyrA* QRDR; *gyrB* QRDR	..	*gyrA* QRDR; *gyrB* QRDR	*gyrA* QRDR	*gyrA* full gene
Bedaquiline (XDR)	..	..	..	..	..	..	..	..	*Rv0678* full gene	..	..
Linezolid (XDR)	..	..	..	..	..	..	..	..	*rplC* fragment; *rrl* fragment	..	..
Group B											
Clofazimine	..	..	..	..	..	..	..	..	*Rv0678* full gene	..	..
Cycloserine	..	..	..	..	..	..	..	..	..	..	..
Terizidone	..	..	..	..	..	..	..	..	..	..	..
Group C											
Ethambutol	..	..	..	..	..	..	..	..	*embB* fragment (including codon 306)	..	*embB* full gene
Delamanid	..	..	..	..	..	..	..	..	..	..	..
Pretoman id	..	..	..	..	..	..	..	..	..	..	..
Pyrazinamide	..	..	..	..	..	..	..	*pncA* full gene	*pncA* full gene	..	*pncA* full gene
Imipenem-cilastatin	..	..	..	..	..	..	..	..	..	..	..
Meropenem	..	..	..	..	..	..	..	..	..	..	..
Amikacin	..	..	..	..	..	*rrs* position 1401, 1402, and 1484	*rrs* 1400 region	..	*rrs* full gene	*rrs* 1400 and 1484 regions	..
Streptomycin	..	..	..	..	..	..	..	..	*rpsL* fuIl gene; *gidB* full gene	..	*rpsL* full gene
Ethionamide and prothionamide	..	..	..	*inhA* promoter region	..	..	*inhA* promoter region	..	*inhA* fragment (including promoter region, and	..	..

	Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA)	Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA)	Truenat MTB-Rif Dx (MolBio Diagnostics, Goa, India)	GenoType MTBDR*plus* VER 2.0 (HAIN Lifescience/ Bruker, Nehren, Germany)	Nipro NTM+MDRTB detection kit 2 (Nipro Corporation, Osaka, Japan)	GenoType MTBDR*sl* VER 2.0 (HAIN Lifescience/ Bruker, Nehren, Germany)	Xpert MTB/XDR (Cepheid, Sunnyvale, CA, USA)	Nipro Genoscholar PZA-TB II (Nipro Corporation, Osaka, Japan)	Deeplex Myc-TB* (Genoscreen, Lille, France)	Next Gen-RDST* (Illumina, San Diego, CA, USA)	AmpliSeq TB*
									codon 94); *fabG1* fragment (including promoter region); *ethA* full gene		
P-aminosalicylic acid	..	..	..	..	..	..	..	..	..	..	..
No longer recommended											
Kanamycin	..	..	..	..	..	*rrs* position 1401, 1402, and 1484; *eis* promoter region	*rrs* 1400 region; *eis* promoter region	..	*rrs* full gene; *eis* fragment (including promoter region)	*rrs* 1400 and 1484 regions; *eis* promoter region	*eis* full gene
Capreomycin	..	..	..	..	..	*rrs* position 1401, 1402, and 1484	*rrs* 1400 region	..	*rrs* full gene; *tlyA* full gene	*rrs* 1400 and 1484 regions	..

The table focuses on WHO-endorsed methods, and main tNGS assays currently available. Drugs are grouped according to WHO.[74] MDR, pre-XDR, and XDR tuberculosis are defined according to WHO.[3] Target regions are indicative; for detailed positions covered please refer to the specific manufacturers' instruction manuals. tNGS=targeted next-generation sequencing. DST=drug susceptibility testing. RR=rifampicin resistance. MDR=multidrug-resistant, pre-XDR=pre-extensively drug-resistant. XDR=extensively drug-resistant. RRDR=rifampicin resistance-determining region. QRDR=quinolone resistance-determining region.

*None of the targeted next-generation sequencing approaches are currently endorsed by a WHO recommendation.

Table 3:

Time-to-result of different genotypic tests under optimal and operational conditions

	Under optimal conditions	Under operational conditions*
Xpert (Cepheid, Sunnyvale, CA, USA)	1 working day	1–2 working days
Line probe assay	1 working day	2–4 working days
Amplicon sequencing (Deeplex, [Genoscreen, Lille, France])	2–3 working days	7–10 working days
Whole-genome sequencing	2–3 working days	7–10 working days

* Once sample has arrived at the laboratory.

Table 4:

Genomic mutations of Mycobacterium tuberculosis associated with phenotypic drug resistance and their effect on treatment outcome67,109,110

	Gene	High-confidence mutations associated with drug resistance	Sensitivity of the high-confidence mutations as predictors of phenotypic drug susceptibility[111]	Drug mutations for which a dose increase might be considered to overcome resistance at standard dosing*	Effect on treatment outcome
First-line drug					
Rifampicin	*rpoB*	Most commonly Ser450Leu but >20 other mutations described	93·8%	Asp435Tyr, His445Leu†, Leu452Pro, Leu430Pro, His445Asn, Ile491Phe	*rpoB* mutations associated with unfavourable first-line regimen treatment outcome; no difference between disputed and undisputed mutations;[49,112,114] *rpoB* mutations have no effect on MDR treatment outcome[115]
Isoniazid	*inhA-mabA*	−15 c/t+Ilel94Thr, −15 c/t+Ser94Ala	91·2%‡	−15 c/t	*inhA* mutations increase relapse after first-line treatment and reduce early bactericidal activity of isoniazid 5 mg/kg; *inhA* −15 c/t mutation has more of an effect than other mutations in *inhA*[116,118]; *inhA* mutations have no effect on MDR treatment outcome (short or long regimen)[115,119,120]
Isoniazid	*katG*	Ser315Ile, Ser315Asn, Ser315Thr	91·2%‡	..	*katG* 315 mutations are associated with unfavourable first-line regimen treatment outcome (treatment failure or death) and relapse, have more of an effect than *inhA* mutations,[116,115] and have no effect on MDR treatment outcome (short or long regimen)[115,120]
Group A drug					
Bedaquiline	*Rv0678*	Gln22Leu,Thr33Ala, Ser63Arg, Ile67PheSer, Arg72Trp, Arg135Gly, Leu136Pro	0%§	l85ins_Gln†	Some studies show a negative effect of initial bedaquiline resistance in relation to *Rv0678* mutations on treatment outcome whereas others do not; the emergence of *Rv0678* mutations during treatment is associated with worse treatment outcome[121–123]
Bedaquiline	*atpE*	Asp28Gly, Asp28Val, Ala63Pro	0%§	..	Emergence of *atpE* mutations during treatment is not always associated with worse treatment outcome[124,125]
Bedaquiline	*pepQ*	Insufficient data	0%§	..	Unknown
Levofloxacin and moxifloxacin	*gyrA*	Gly88Cys, Asp94Gly, Asp94His, Asp94Asn, Asp94Tyr	~85%	Asp89Asn, Ala90Val, Ser91Pro, Asp94Ala	*gyrA* 94 mutations delay sputum conversion of MDR tuberculosis regimen; *gyrA* mutations are selected in case of ofloxacin treatment failure and can predict MDR tuberculosis treatment outcome as efficiently as phenotypic drug susceptibility testing; for *gyrA* mutations associated with unfavourable MDR tuberculosis treatment outcome, the higher the associated fluoroquinolone minimum inhibitory concentration, the higher the negative effect on treatment outcome (failure or relapse Asp94Asn>Asp94Gly>Ala90Val>Asp94Ala)[116,126–130]
Levofloxacin and moxifloxacin	*gyrB*	..	..	Asp461His†, Asp461Asn†, Asp499Asp†, Ala504Val†	The clinical effect of *gyrB* mutations on MDR tuberculosis treatment outcome has not been shown but has been shown in a murine model[119,131]
Linezolid	*rplC*	Cys154Arg	38·2%¶	..	*rplC* mutations are selected in case of linezolid regimen treatment failure[132,133]
Linezolid	*rrl*	2299 g/t, 2814 g/t	38·2%¶	..	*rrl* mutations are selected in case of linezolid regimen treatment failure[132,133]

	Gene	High-confidence mutations associated with drug resistance	Sensitivity of the high-confidence mutations as predictors of phenotypic drug susceptibility[111]	Drug mutations for which a dose increase might be considered to overcome resistance at standard dosing*	Effect on treatment outcome
Group B drug					
Clofazimine	*Rv0678*	Gln22Leu,Thr33Ala, Ser63Arg, Ile67PheSer, Arg72Ter, Gly25Asp, Leu44Pro, Arg135Gly, Leu136Pro, Ser68Arg, >30 mutations described	0%	..	Unknown
Clofazimine	*pepQ*	Insufficient data	..	..	Unknown
Cycloserine	*alr*	−8 c/t, Met319Thr, Tyr364Asp, Tyr364Cys, Arg373Leu,Arg373Gly	..	..	Unknown
Amikacin	*rrs*	1401 a/g, 1484 a/t	77·3%‖	1402 c/t	*rrs* mutations are selected in case of kanamycin or capreomycin treatment failure, and predict 4-month sputum culture conversion as efficiently as phenotypic drug susceptibility testing; *rrs* mutations are not associated with MDR tuberculosis treatment outcome[119,126,129,134]
Amikacin	*eis*	..	77·3%‖	−14 c/t	*eis* mutations are not associated with MDR tuberculosis treatment outcome[119,129]
Group C drug					
Streptomycin	*rpsL*	Lys43Arg, Lys43Thr, Lys88Gln, Lys88Arg	82·4%**	..	*rpsL* mutations predict 4-month sputum culture conversion as efficiently as phenotypic drug susceptibility testing[134]
Streptomycin	*rrs*	514 a/c, 514 a/t, 462 c/t, 513 c/t, 517 c/t	82·4%**	..	Unknown
Delamanid	*fbiA*	Asp49Tyr, Lys250Stop	6·1%††	..	Unknown
Delamanid	*ddn*	Trp88Stop	6·1%††	..	Unknown
Ethambutol	*embB*	Me306lle, Met306Val, Asp354Ala, Gly406Asp, Gly406Cys, Gly406Ser, Gln497Arg	86·7%††	..	*embB* mutations have not been associated with M DR tuberculosis treatment outcome in one study[119]
Ethambutol	*embC-embA*	−8 c/t, −12 c/t, −16 c/t (often in linkage with *embB* mutations)	86·7%††	..	Unknown
Ethionamide and prothionamide	*inhA*	−15 c/t, Ser94Ala	75·7%§§	..	*inhA* mutations have no impact on MDR tuberculosis treatment outcome (short or long regimen)[115,119,120]
Ethionamide and prothionamide	*ethA*	Pooled frameshifts and premature stop codons	75·7%§§	..	*ethA* mutations are selected in case of ethionamide treatment failure, and are associated with unfavourable MDR tuberculosis treatment outcome[119,126]
Imipenem and meropenem	Unknown	Insufficient data	..	..	Unknown

	Gene	High-confidence mutations associated with drug resistance	Sensitivity of the high-confidence mutations as predictors of phenotypic drug susceptibility[111]	Drug mutations for which a dose increase might be considered to overcome resistance at standard dosing*	Effect on treatment outcome
P-aminosali cylic acid	*folC*	Glu153Ala, Glu153Gly, Ser150Gly, Phe152Ser, Ile43Thr, Ile43Ala, Glu40Gly	..	..	Unknown
P-aminosali cylic acid	*ribD*	−12 g/a	..	..	Unknown
P-aminosali cylic acid	*thyA*	..	..	..	*thyA* mutations are selected in case of p-aminosalicylic acid treatment failure[126]
Pyrazinamide	*pncA*	>300 mutations described	72·3%	Val180Ile†,Ala170Val†, Asp110GIy†, Ser65Ala†, Glu37Val†	*pncA* mutations are associated with delayed sputum culture conversion of MDR tuberculosis treatment; the negative impact of *pncA* mutations on MDR tuberculosis treatment outcome has been shown in one study but not in two others[119,129,135]

MDR=multidrug resistant.

*Agent should not count among active drugs.

†Additional data needed.

‡When *inhA-mabA* and *katG* are studied.

§When *Rv0678, atpE*, and *pepQ* are studied.

¶When *rplC* and *rrl* are studied.

//When *rrs* and *eis* are studied.

**When *rpsL* and *rrs* are studied.

††When *fbiA* and *ddn* are studied.

‡‡When *embB* and *embC-embA* are studied.

§§When *inhA* and *ethA* are studied.

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