Genotypic and phenotypic *M. tuberculosis* resistance: guiding clinicians to prescribe the correct regimens

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Introduction

Treating multidrug-resistant (MDR-) and extensively drug-resistant (XDR-) tuberculosis (TB) is a difficult task for any clinician: there are few therapeutic options, the treatment is very long (up to 2 years), complicated by frequent adverse events, and expensive [1, 2].-Recently, the debate in the scientific community has been focused on the role and contribution of the new (bedaquiline and delamanid) [3–7] and re-purposed anti-TB drugs (in particular, linezolid, clofazimine and carbapenems) [8–11]. Furthermore, the World Health Organization (WHO), in its 2016 revised MDR-TB guidelines, has recommended a 9–12 month "shorter regimen" for patients not previously exposed and with *Mycobacterium tuberculosis* (MTB) strains susceptible (or likely to be) to the drugs composing the regimen (with the exception of isoniazid) [12]. The regimen is composed of an initial 4–6-month phase of kanamycin, moxifloxacin, prothionamide, clofazimine, pyrazinamide, high dose isoniazid, and ethambutol followed by 5 months of moxifloxacin, clofazimine, pyrazinamide and ethambutol; it benefits from the use of isoniazid at high dose.

The WHO, in order to ensure that eligible patients have access to the shorter regimen recommends that (WHO-endorsed) rapid genetic tests are performed [12]. The clinician, ideally, has the results of the rapid tests in a few days and can, therefore, decide to initiate the shorter regimen, or, if the patient is not eligible, a longer regimen either standardised (according to each country's national recommendations) or individualised based on a number of drugs likely to be effective. After 4–8 weeks the drug susceptibility test (DST) for second-line drugs is made available and the clinician has the possibility to modify the regimen, and, if necessary, to shift from the shorter to the longer regimen.

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TABLE 1 Clinical interpretation of the rapid test for tuberculosis "GenoType MTBDR*sl* V2 (Hain Lifescience)"

Drug	Failing WT band	Developing mutation band	Mutation	Classification: final BCVs					Final interpretation for clinicians
				OFX-LFX	MFX	AM	CM	KM	
OFX	gyrA WT1	-	G88A	High	Indet				No OFX-LFX use, not informative for MFX
LFX		-	G88C	High	High[#]				No OFX-LFX use, not informative for MFX
MFX	gyrA WT2	gyrA MUT1	A90V	High	High				No OFX-LFX use, higher dosage of MFX?
		gyrA MUT2	S91P	High	High				No OFX-LFX use, higher dosage of MFX?
	gyrA WT3	gyrA MUT3A	D94A	High	High				No OFX-LFX use, higher dosage of MFX?
		gyrA MUT3B	D94N	High	High				No use of fluoroquinolones
		-	D94Y	High	High				No OFX-LFX use, higher dosage of MFX?
		gyrA MUT3C	D94G	High	High				No use of fluoroquinolones
		gyrA MUT3D	D94H	High	Indet				No OFX-LFX use, not informative for MFX
	gyrB WT	gyrB MUT1	N538D[¶]	Indet	Indet				Not informative
		gyrB MUT2	E540V[+]	Indet	Indet				Not informative
AM	rrs WT1	rrs MUT1	a1401g			High	High	High	No use of second-line injectables
CM		-	c1402t			Indet	High	High[#]	No CM use, not informative for AM- KM
KM	rrs WT2	rrs MUT2	g1484t			High	High	High[#]	No AM-CM use, not informative for KM
KM	eis WT1	-	g-37t					Minimal[#]	Not informative
	eis WT2	eis MUT1	c-14t					High	No use of kanamycin
		-	c-12t					Minimal[#]	Not informative
		-	g-10a					High	No use of kanamycin
	eis WT3	-	c-2a					Indet	Not informative

Modified from [13]. OFX: ofloxacin; LFX: levofloxacin; MFX: moxifloxacin, AM: amikacin; CM: capreomycin; KM: kanamycin; BCVs: best confidence values; Indet: indeterminate. [#]: the association of these mutation is based on nominal p-values only (putative, not corrected; refer to Miotto *et al.* [13]); [¶]: N499D; [+]: E501V.

Evidence on sensitivity and specificity of rapid drug susceptibility tests is available in the literature, but their interpretation in order to make clinical decisions is often difficult. Evidence on confidence to diagnose resistance based on both phenotypic and genotypic tests was scanty before the publication of the article by Miotto *et al.* [13] in this issue of the *European Respiratory Journal*. This study is pivotal in making the point on what is known and on directing future research. However, it is not yet of immediate application for clinicians managing MDR/XDR-TB cases.

We aim to provide simple criteria to help clinicians interpreting the available genetic tests before taking therapeutic decisions on their patients. They are based on the new mutation grading system presented by Miotto *et al.* [13] in this journal and on a review of the recent literature.

What is the information provided by WHO-recommended tests?

Xpert MTB/RIF and the next-generation assay Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) are fully automated nucleic acid amplification assays that detect MTB and mutations affecting the rifampicin resistance determining region (RRDR, codons 426–452) of the *rpoB* gene directly from clinical specimens. Real-time polymerase chain reaction (PCR) and melting temperature-based analysis are used by the two assays, respectively, to target the RRDR wild-type sequence (no mutant probes targeted).

Line probe assays (LPAs) are based on the PCR amplification of specific fragments of the MTB genome, followed by reverse hybridisation of the PCR products to oligonucleotide probes immobilised on nitrocellulose strips. Resistance is detected by lack of binding to wild type probes and also by binding to probes targeting specific mutations. Commercial LPAs include: GenoType MTBDR*plus* V2 (Hain Lifescience, Nehren, Germany) and Nipro NTM + MDRTB detection kit 2 (Nipro Corporation, Tokyo, Japan) for MTB rifampicin and isoniazid resistance determination (*rpoB* RRDR, *katG* region Ser315, *inhA* promoter); GenoType MTBDR*sl* V1 and V2 (Hain Lifescience) for identification of MTB mutations associated with fluoroquinolone (*gyrA* quinolone resistance determining region, QRDR, plus *gyrB* QRDR in version two) and second-line injectable drug (*rrs* region 1400, plus *eis* promoter in version two excluding the ethambutol resistance-conferring target *embB*, included in the previous version) resistance [14].

In 2016, the WHO endorsed the MTBDR*sl* V2, that includes the additional targets *eis* and *gyrB*, as initial test to detect resistance to fluoroquinolones and second-line injectables instead of phenotypic DST. This test's results would suffice to inform the critical decision to initiate (or not) the new shorter regimen *versus* the standard one [13]. The clinical interpretation of the test is summarised in table 1.

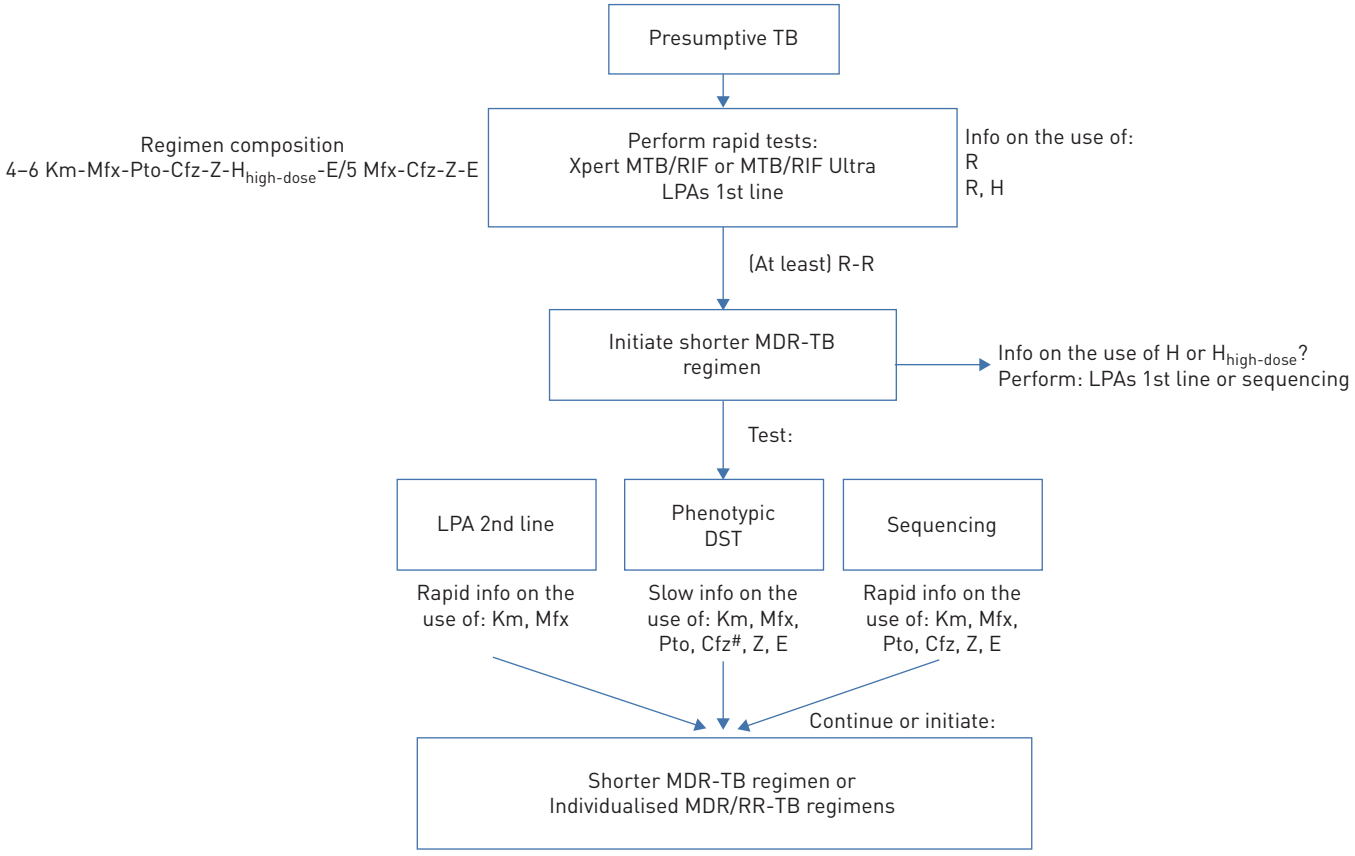

FIGURE 1 The multidrug-resistant (MDR) tuberculosis (TB) shorter regimen: schematic representation of a diagnostic algorithm. #: critical concentration not recommended. Km: kanamycin; Mfx: moxifloxacin; Pto: prothionamide; Cfz: clofazimine; Z: pyrazinamide; H$_{high-dose}$: high-dose isoniazid; E: ethambutol; R: rifampicin; LPA: line probe assay; R-R: rifampicin-resistant; DST: drug susceptibility testing.

Why is this study important?

Miotto *et al.* [13] developed an expert, consensus-driven, standardised approach for the interpretation of mutations in MTB associated with drug resistance, based on a systematic review of genotypic and phenotypic drug-resistance literature data and on a statistical procedure to grade mutations. Liquid- and solid-media conventional DST and their combination, and Wayne enzymatic assay for pyrazinamide, were considered the reference standards for the analysis [13].

The authors identified 286 mutations classified as determining high, moderate, minimal, indeterminate, or "no-association" corrected confidence for predicting resistance to rifampicin (*rpoB*), isoniazid (*katG*, *inhA-mabA*, *furA*, *mshA*), ethionamide/prothionamide (*inhA-mabA*, *ethA*, *mshA*), ofloxacin/moxifloxacin/levofloxacin (*gyrA*, *gyrB*), pyrazinamide (*pncA*), streptomycin (*rpsL*, *rrs*, *gidB, tap, whiB7*), amikacin (*rrs*), capreomycin (*rrs*, *tlyA*) and kanamycin (*rrs*, *eis*, *whiB7*). The detection of these mutations can be used as rule-in criteria for predicting phenotypic drug resistance at current critical concentrations and, therefore, for guiding treatment decisions, developing molecular diagnostic DST assays, and interpreting the existing ones. This is the first study providing a standardised and statistical evidence-based approach to characterising drug resistance-associated MTB genetic mutations.

How to make clinical decisions based on the results of rapid tests and DST

Considering these graded mutations will enable the clinician to rule-in resistance with indisputable confidence (100% specificity), covering most of the resistant cases for at least the core drugs. Importantly, the use of this system will allow to overcome the well-known limitations of the conventional phenotypic testing for some drugs hampering an accurate and prompt choice of eligible regimens. TB laboratory staff and clinicians would simply investigate the presence of such mutations (by molecular approaches, *e.g.* LPAs) to include/exclude drugs for treatment (figure 1), considering the diagnostic performance reported in table 2.

TABLE 2 Diagnostic accuracy and sensitivity values for anti-tuberculosis drugs (graded mutations identified by Miotto *et al.* [13] were used within the new classification of the World Health Organization [12]).

Drug class	Drug (genomic region targeted)	Clinical interpretation of graded mutations as marker of resistance (high+moderate+minimal)	
		Diagnostic accuracy %	Sensitivity %
First-line	Rifampicin (*rpoB*)	94	90.5
	Isoniazid (*katG*, *inhA-mabA*, *furA*, *mshA*)	80.7	78.3
Second-line (group A)	Ofloxacin/levofloxacin (*gyrA*, *gyrB*)	90.9	81.7
	Moxifloxacin (*gyrA*, *gyrB*)	94	89.2
Second-line (group B)	Amikacin (*rrs*)	91.8	78.8
	Capreomycin (*rrs*, *tlyA*)	92	71.3
	Kanamycin (*rrs*, *eis*, *whiB7*)	87.7	68.3
	Streptomycin (*rpsL*, *rrs*, *gidB, tap, whiB7*)	76.4	61.3
Second-line (group C)	Ethionamide/prothionamide (*inhA-mabA*, *ethA*, *mshA*)	67.5	48.5
Second-line (group D)	Pyrazinamide (*pncA*)	76.8	53.1

Implications to use drugs at high dose in case of drug resistance

Isoniazid (INH)

If *inhA* mutations only are detected, even normal doses (*e.g.* 5 mg per day per kg body weight) of INH could be used; high doses (10 $mg \cdot kg^{-1}$ or more) are likely to be effective [15, 16]. If *katG* mutations only are detected, use of high doses is an option. Most *katG* mutations (other than 315 codon) confer moderate resistance (minimum inhibitory concentration (MIC) 1–5 $\mu g \cdot mL^{-1}$) that might be treated with higher doses of the drug; even the most common S315T variant leads to a variable range of resistance [17]. In the absence of additional mutations affecting the *inhA* gene (and *ethA* gene, so far uniquely detectable by sequencing approaches), ethionamide can be considered an option for the intensive phase of the shorter regimen. If *inhA* + *katG* mutations are concurrently detected, INH drug use should be avoided, since these patterns are linked to high resistance levels.

It would be crucial to determine whether the concurrent presence of *inhA* + *katG* mutations is associated with specific MTB genotypes as this might imply a limited use of shorter regimens in settings where these lineages are more represented.

Rifampicin–rifabutin (RMP-RFB)

If low level resistant *rpoB* mutations (MIC of 1–2 $\mu g \cdot mL^{-1}$) are identified, the clinician could consider either the use of high doses RMP (12–20 $mg \cdot kg^{-1}$) or a switch to RFB (for which susceptibility is common). Considering genotypes, D435Y, D435V, S441L, H445L plus other nucleotide variants, insertions and deletions affecting codons 435 and 445 are associated with low level RMP resistance and RFB susceptibility. Such mutations are included in the grading system developed by Miotto *et al.* [13] that, therefore, might be informative also for the use of RFB, although more data are needed. Nevertheless, critical concentrations used to determine RFB resistance are under evaluation, potentially leading to a redefinition of phenotypically resistant cases [18–21].

Moxifloxacin (MFX)

If low level resistant *gyrA* mutations (MIC $\leqslant 1$ $\mu g \cdot mL^{-1}$) are identified, use of higher doses of MFX might be beneficial. Common *gyrA* mutations, such as A90 V, S91P, D94A and D94Y, are linked to low MIC levels and could enable the use of the drug at higher doses (800 $mg \cdot day^{-1}$) [22–25].

Implications for the use of the "shorter regimen"

The genetic tests can guide the clinician to identify the eligible patients for the shorter regimen or to decide for a traditional individualised regimen. This choice is made possible by our increasing knowledge of the genetic mechanisms that are the basis for MTB drug resistance and the study by Miotto *et al.* [13] represents a cornerstone.

High dosages of core anti-TB drugs, such as moxifloxacin, demonstrated their therapeutic efficiency [26]. On the other hand, the recent results from the STREAM stage 1 controlled trial has shown that adverse effects are possible; therefore, safety of higher doses needs to be assessed. [27]. While we promote the use of genetic tests to guide critical treatment decisions, we need to know more on the phenotypic meaning of

genetic mutations. MIC testing is required to link unequivocally these genetic mutations with phenotypic resistance levels examining different genetic backgrounds and large strain collections.

Given the wide information on drug resistance potentially provided by genetic investigation, any commercial molecular test based on sequencing of large genomic regions (or even better whole genome sequencing) would be decisive for guiding treatment of MDR- and XDR-TB cases.

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References

1 Migliori GB, Sotgiu G, Gandhi NR, *et al.* Drug resistance beyond extensively drug-resistant tuberculosis: individual patient data meta-analysis. *Eur Respir J* 2013; 42: 169–179.
2 Diel R, Vandeputte J, de Vries G, *et al.* Costs of tuberculosis disease in the European Union: a systematic analysis and cost calculation. *Eur Respir J* 2014; 43: 554–565.
3 Sotgiu G, Pontali E, Centis R, *et al.* Delamanid (OPC-67683) for treatment of multi-drug-resistant tuberculosis. *Expert Rev Anti Infect Ther* 2015; 13: 305–315.
4 Pontali E, Sotgiu G, D'Ambrosio L, *et al.* Bedaquiline and MDR-TB: a systematic and critical analysis of the evidence. *Eur Respir J* 2016; 47: 394–402.
5 Pontali E, D'Ambrosio L, Centis R, *et al.* Multidrug-resistance tuberculosis and beyond: an updated analysis of the current evidence on bedaquiline. *Eur Respir J* 2017; 49: 1700146.
6 Borisov SE, Dheda K, Enwerem M. Effectiveness and safety of bedaquiline-containing regimens in the treatment of multidrug and extensively drug-resistant tuberculosis: a multicentre study. *Eur Respir J* 2017; 49: 1700387.
7 Pontali E, Sotgiu G, Tiberi S, *et al.* Cardiac safety of bedaquiline: a systematic and critical analysis of the evidence. *Eur Respir J* 2017; 50: 1701462.
8 Sotgiu G, Pontali E, Migliori GB. Linezolid to treat MDR-/XDR-tuberculosis: available evidence and future scenarios. *Eur Respir J* 2015; 45: 25–29.
9 Dalcolmo M, Gayoso R, Sotgiu G, *et al.* Effectiveness and safety of clofazimine within a standard multidrug-resistant tuberculosis regimen in Brazil: first nation-wide report on over 2,500 cases. *Eur Respir J* 2017; 49: 1602445.
10 Tiberi S, Payen MC, Sotgiu G, *et al.* Effectiveness and safety of meropenem/clavulanate-containing regimens in the treatment of multidrug and extensively drug-resistant tuberculosis. *Eur Respir J* 2016; 47: 1235–1243.
11 Tiberi S, Sotgiu G, D'Ambrosio L, *et al.* Comparison of effectiveness and safety of imipenem/clavulanate- versus meropenem/clavulanate-containing regimens in the treatment of MDR- and XDR-TB. *Eur Respir J* 2016; 47: 1758–1766.
12 Falzon D, Schunemann HJ, Harausz E, *et al.* World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update. *Eur Respir J* 2017; 49: 1602308.
13 Miotto P, Tessema B, Tagliani E, *et al.* A standardised method for interpreting the association between mutations and phenotypic drug-resistance in *Mycobacterium tuberculosis*. *Eur Respir J* 2017; 50: 1701354.
14 Gilpin C, Korobitsyn A, Weyer K. Current tools available for the diagnosis of drug-resistant tuberculosis. *Ther Adv Infectious Dis* 2016; 3: 145–151.
15 Rieder HL, Van Deun A. Rationale for high-dose isoniazid in the treatment of multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis* 2017; 21: 123–124.
16 Jacobson K R, Theron D, Victor T C, *et al.* Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape Province, South Africa. *Clin Infect Dis* 2011; 53: 369–372.
17 Cambau E, Viveiros M, Machado D, *et al.* Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. *J Antimicrob Chemother* 2015; 70: 686–696.
18 van Ingen J, Aarnoutse R, de Vries G, *et al.* Low-level rifampicin-resistant *Mycobacterium tuberculosis* strains raise a new therapeutic challenge. *Int J Tuberc Lung Dis* 2011; 15: 990–992.
19 ElMaraachli W, Slater M, Berrada ZL, *et al.* Predicting differential rifamycin resistance in clinical *Mycobacterium tuberculosis* isolates by specific rpoB mutations. *Int J Tuberc Lung Dis* 2015; 19: 1222–1226.
20 Jamieson FB, Guthrie JL, Neemuchwala A, *et al.* Profiling of rpoB mutations and MICs for rifampin and rifabutin in *Mycobacterium tuberculosis*. *J Clin Microbiol* 2014; 52: 2157–2162.
21 Schön T, Juréen P, Chryssanthou E, *et al.* Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact? *J Antimicrob Chemother* 2013; 68: 2074–2077.
22 Kambli P, Ajbani K, Sadani M, *et al.* Correlating minimum inhibitory concentrations of ofloxacin and moxifloxacin with gyrA mutations using the genotype MTBDRsl assay. *Tuberculosis (Edinb)* 2015; 95: 137–141.
23 Willby M, Sikes RD, Malik S, *et al.* Correlation between GyrA substitutions and ofloxacin, levofloxacin, and moxifloxacin cross resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2015; 59: 5427–5434.
24 Chien J-Y, Chien S-T, Chiu W-Y, *et al.* Moxifloxacin improves treatment outcomes in patients with ofloxacin-resistant multidrug resistant tuberculosis. *Antimicrob Agents Chemother* 2016; 60: 4708–4716.
25 Chien J-Y, Chiu W-Y, Chien S-T, *et al.* Mutations in gyrA and gyrB among fluoroquinolone- and multidrug-resistant *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother* 2016; 60: 2090–2096.
26 Xu Y, Wu J, Liao S, *et al.* Treating tuberculosis with high doses of anti-TB drugs: mechanisms and outcomes. *Ann Clin Microbiol Antimicrob* 2017; 16: 67.
27 The 48th Union World Conference on Lung Health, Guadalajara, Mexico. STREAM clinical trial results provide vital insight into nine-month treatment regimen for multidrug-resistant tuberculosis. http://guadalajara.worldlunghealth.org/media/conference-news/updates/stream-clinical-trial-results-provide-vital-insight-into-nine-month-treatment-regimen-for-multidrug-resistant-tuberculosis Date last accessed: November 5, 2017.