Is the new WHO definition of extensively drug-resistant tuberculosis easy to apply in practice?

Received: 31 March 2021
Accepted: 22 April 2021

To the Editor:

The World Health Organization (WHO) recently endorsed a new definition of extensively drug-resistant tuberculosis (XDR-TB) and, for the first time, introduced the category of pre-XDR-TB [1]. Pre-XDR-TB is defined as multidrug resistance/rifampicin resistance (MDR/RR) in conjunction with resistance to any fluoroquinolone (levofloxacin or moxifloxacin), whereas the conditions for XDR-TB are now met by additional resistance to a group A drug (bedaquiline or linezolid) [1].

WHO also laid important scientific groundwork to support this transition (*e.g.* it revised the critical concentrations for phenotypic drug-susceptibility testing (pDST) of fluroquinolones and rifampicin) [2, 3]. We agree that the revisions to the definitions were needed but note that measuring resistance to these drugs comprehensively is not straightforward.

DST for fluroquinolones is an essential pre-condition for the initial selection of the most appropriate MDR-TB regimen, because of its predictive value for adverse outcomes and the high rates of fluroquinolone resistance (approximately 10–30%, depending on the setting) [4]. Therefore, fluoroquinolone resistance had already been a criterion for the old definition XDR-TB [1, 5]. Yet, despite the availability of pDST and rapid genotypic DST (gDST) solutions (figure 1), only 71% of the notified MDR/RR-TB cases globally were tested for fluoroquinolone resistance in 2019, with considerable variations among different regions [1, 6]. This is partly due to the fact that the only WHO-endorsed gDST assay (*i.e.* the GenoType MTBDR*sl* VER 2.0; Hain Lifescience) is relatively labour intensive and less reliable for direct testing of clinical samples [4]. The cartridge-based Xpert MTB/XDR (Cepheid), which is currently being evaluated by WHO, has the potential to narrow this diagnostic gap by enabling more decentralised testing [1]. Nevertheless, additional capacity for pDST and, potentially, targeted next-generation sequencing (tNGS) is needed given that a greater proportion of resistance to fluoroquinolones than rifampicin is due to low-frequency variants that are below the limit of detection of these assays [1, 6].

DST for bedaquiline and linezolid is more challenging for a number of related reasons. First, no rapid gDST assays exist for these agents as advocated by the End TB Strategy, which means that gDST is only possible with either tNGS or whole-genome sequencing, neither of which is currently available in the vast majority of countries with higher MDR-TB incidences (figure 1) [1, 6].

Second, even where gDST is routinely used, the interpretation of the results is hampered by the incomplete understanding of the genetic basis of resistance and/or the impact of mutations on the minimum inhibitory concentrations (MICs) [1, 6]. This is a particular challenge for bedaquiline, for which a large spectrum of resistance mutation is possible, whereas data from other bacteria indicate that the number of variants for linezolid is likely small [7].

Third, there is a lack of capacity for pDST [1]. A recent survey conducted by the European TB Reference Laboratory Network (ERLTB-net) and coordinated by the European Centre for Disease Prevention and

Shareable abstract (@ERSpublications)
The new definition of extensively drug resistant tuberculosis endorsed by WHO poses some challenges that must be addressed in a coordinated fashion by researchers, TB control stakeholders and assay developers https://bit.ly/3eAMU8B

Cite this article as: Alagna R, Cabibbe AM, Miotto P, *et al*. Is the new WHO definition of extensively drug-resistant tuberculosis easy to apply in practice? *Eur Respir J* 2021; 58: 2100959 [DOI: 10.1183/13993003.00959-2021].

	Main challenges	Assay	FQs		BDQ		LZD	
			Testing	Notes	Testing	Notes	Testing	Notes
Phenotypic DST	Implementation at community level; capital investment	**Liquid media**						
		BACTEC MGIT960 (BD)#	✓	RR-TB testing coverage <80%	✓	False S rate; Interim CC; limited capacity	✓	Limited capacity
		Sensititre MYCOTB AST Plate (Thermo Fisher)	✓	CCs proposed; limited capacity	x		x	
		Middlebrook 7H9 broth microdilution	✓	CCs proposed; limited capacity	✓	CC proposed; limited capacity	✓	CCs proposed; limited capacity
		Solid media						
		Löwenstein-Jensen proportion method#	✓	Interim CCs; RR-TB testing coverage <80%	x		x	
		Middlebrook 7H10 agar proportion method#	✓	RR-TB testing coverage <80%	x		✓	Limited capacity
		Middlebrook 7H11 agar proportion method#	✓	RR-TB testing coverage <80%	✓	False S rate; interim CC; limited capacity	✓	Limited capacity
Genotypic DST	Incomplete understanding of the molecular basis of resistance; capital investment	**Line probe assays**						
		AID TB FQ/EMB (Autoimmun Diagnostika)	✓		x		x	
		GenoType MTBDRs/ VER 2.0 (Hain Lifescience)#	✓		x		x	
		Genoscholar FQ+KM-TB II (Nipro Corporation)	✓		x		x	
		MolecuTech REBA MTB-XDR (YD Diagnostics)	✓		x		x	
		Real-time PCR						
		AccuPower XDR-TB (Bioneer)	✓		x		x	
		AllPlex MTB/MDR/XDRre Detection (Seegene)	✓		x		x	
		Amplitude-FQ-RV (Syntol)	✓		x		x	
		MeltPro FQ (Zeesan Biotech)	✓		x		x	
		Xpert MTB/XDR (Cepheid)¶	✓		x		x	
		Array						
		TB-test (BIOCHIP-IMB)	✓		x		x	
		Targeted next-generation sequencing						
		Deerplex Myc-TB (Genoscreen)	✓	Not widely available	✓	Not widely available	✓	Not widely available

FIGURE 1 Overview of options for genotypic and phenotypic drug-susceptibility testing (DST) of group A drugs for treating rifampicin-resistant tuberculosis (RR-TB). Commercial genotypic DST assays are only listed if they are approved for clinical use in at least one country (if a manufacturer has multiple assays on the market that are approved, only one is shown). Methods endorsed by the World Health Organization (WHO) are marked by # and the additional assay currently being reviewed by WHO is highlighted by ¶. BDQ: bedaquiline; CC: critical concentration; FQs: fluoroquinolones; S: susceptible; LZD: linezolid.

Control revealed that in 2019 only 61% and 32% of 28 participating TB laboratories tested for linezolid and bedaquiline, respectively (reassuringly, mostly reporting correct results) [8].

Fourth, pDST results for these drugs can also be difficult to interpret. On the one hand, the positive predictive value of pDST will be poor in settings where the true prevalence of resistance is low (*e.g.* in a setting with only susceptible isolates, approximately 1% of those isolates would be misclassified as resistant) [3]. On the other hand, it is becoming increasingly clear that pDST at the critical concentration does not detect mutations conferring only modest MIC increases reliably [9]. Whether such MIC increases are clinically relevant is not clear but if they are, MIC testing with a carefully validated and controlled method would be needed [3, 9, 10].

These challenges will adversely affect individual patient treatment. In addition, the rates of XDR-TB measured during surveillance studies will be strongly dependent on the method used, including the amount of retesting conducted (*i.e.* both over- and underreporting can be a problem for both gDST and pDST). This, in turn, will result in a worse understanding of countries' epidemiological profile of the most dangerous form of TB; lack of appropriate global prevention and control activities, such as equitable access to universal DST and anti-TB drugs regimens; lower capacity of healthcare providers and public health authorities in implementing appropriate national strategies; and limited efficiency in allocating health resources based on countries' shared experience [4, 5].

Given sufficient political will, the coronavirus disease 2019 pandemic has underlined that rapid technological advances are possible. There is a clear need for diagnostics in TB enabling universal DST access, and for rapid triage of people with XDR-TB. Ideally, a genome-based technology that is easy-to-use and rapid, and able to provide extensive coverage of genomic targets, is needed. WHO is due to publish updated target product profiles that reflect the revised needs for TB diagnostics. We call on assay developers, pharmaceutical companies and researchers, as well as funders and regulators, to renew their efforts to tackle the aforementioned questions in a coordinated fashion. We cannot afford to repeat the

mistakes of the past and risk the rapid emergence and spread of resistance to more group A drugs, thereby eroding the hard-won gains in the treatment of MDR-TB [1, 2].

Riccardo Alagna[1], Andrea Maurizio Cabibbe[1], Paolo Miotto[1], Francesca Saluzzo[1], Claudio Umberto Köser[2], Stefan Niemann[3,4], Sebastien Gagneux[5,6], Camilla Rodrigues[7], Paola Vittoria Maria Rancoita[8] and Daniela Maria Cirillo[1]

[1]IRCCS San Raffaele Scientific Institute, Milan, Italy. [2]Dept of Genetics, University of Cambridge, Cambridge, UK. [3]Molecular and Experimental Mycobacteriology, Priority Area Infections, Research Center Borstel, Borstel, Germany. [4]German Center for Infection Research (DZIF), Partner site Hamburg-Lübeck-Borstel-Riems, Germany. [5]Swiss Tropical and Public Health Institute, Basel, Switzerland. [6]University of Basel, Basel, Switzerland. [7]Dept of Microbiology, P. D. Hinduja Hospital and Medical Research Centre, Mumbai, India. [8]University Centre of Statistics in the Biomedical Sciences, Vita-Salute San Raffaele University, Milan, Italy.

Corresponding author: Daniela Maria Cirillo (cirillo.daniela@hsr.it)

P. Miotto, S. Niemann, S. Gagneux, C.U. Köser, C. Rodrigues and P.M.V. Rancoita are part of the New Diagnostic Working Group Task Force on NGS and DST of the STOP TB Partnership. D.M. Cirillo is co-chair of the of the New Diagnostic Working Group. The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official position of the STOP TB Partnership.

Conflict of interest: R. Alagna has nothing to disclose. A.M. Cabibbe has nothing to disclose. P. Miotto has nothing to disclose. F. Saluzzo has nothing to disclose. C.U. Köser is a consultant for Becton Dickinson, the Foundation for Innovative New Diagnostics, the Stop TB Partnership and the TB Alliance; has worked as a consultant for QuantuMDx, the World Health Organization (WHO) Global TB Programme, and WHO Regional Office for Europe; gave a paid educational talk for Oxford Immunotec; had travel and accommodation expensed covered by Hain Lifescience to present at a meeting; and is an unpaid advisor to BioVersys and GenoScreen. S. Niemann has nothing to disclose. S. Gagneux has nothing to disclose. C. Rodrigues has nothing to disclose. P.M.V. Rancoita has nothing to disclose. D.M. Cirillo has nothing to disclose.

References

1 Viney K, Nhat Linh N, Gegia M, *et al.* New definitions of pre-extensively and extensively drug resistant tuberculosis: update from the World Health Organisation. *Eur Respir J* 2021; 57: 2100361.
2 Köser CU, Maurer FP, Kranzer K. "Those who cannot remember the past are condemned to repeat it": drug-susceptibility testing for bedaquiline and delamanid. *Int J Infect Dis* 2019; 80: S32–S35.
3 Köser CU, Georghiou SB, Schön T, *et al.* On the consequences of poorly defined breakpoints for rifampin susceptibility testing of *Mycobacterium tuberculosis* complex. *J Clin Microbiol* 2021; 59: e02328-20.
4 Zignol M, Cabibbe AM, Dean AS, *et al.* Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study. *Lancet Infect Dis* 2018; 18: 675–683.
5 Veziris N, Bonnet I, Morel F, *et al.* Impact of the revised definition of extensively drug resistant tuberculosis. *Eur Respir J* 2021; in press [https://doi.org/10.1183/13993003.00641-2021].
6 Mohamed S, Köser CU, Salfinger M, *et al.* Targeted next-generation sequencing: a Swiss army knife for mycobacterial diagnostics? *Eur Respir J* 2021; 57: 2004077.
7 Kadura S, King N, Nakhoul M, *et al.* Systematic review of mutations associated with resistance to the new and repurposed *Mycobacterium tuberculosis* drugs bedaquiline, clofazimine, linezolid, delamanid and pretomanid. *J Antimicrob Chemother* 2020; 75: 2031–2043.
8 Farooq HZ, Cirillo D, Hillemann D, *et al.* Limited capability for testing *Mycobacterium tuberculosis* for susceptibility to new drugs. *Emerg Infect Dis* 2021; 27: 985–987.
9 Beckert P, Sanchez-Padilla E, Merker M, *et al.* MDR *M. tuberculosis* outbreak clone in Eswatini missed by Xpert has elevated bedaquiline resistance dated to the pre-treatment era. *Genome Med* 2020; 12: 104.
10 Nimmo C, Millard J, Brien K, *et al.* Bedaquiline resistance in drug-resistant tuberculosis HIV co-infected patients. *Eur Respir J* 2020; 55: 1902383.