Title

Updating the approaches to define susceptibility and resistance to anti-tuberculosis agents: implications for diagnosis and treatment

Journal

European Respiratory Journal, 59(4)

ISSN

0903-1936

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Publication Date

2022-04-01

DOI

10.1183/13993003.00166-2022

Peer reviewed

EUROPEAN RESPIRATORY JOURNAL
EDITORIAL
ANTIMYCOBACTERIAL SUSCEPTIBILITY TESTING GROUP

Updating the approaches to define susceptibility and resistance to anti-tuberculosis agents: implications for diagnosis and treatment

Antimycobacterial Susceptibility Testing Group

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Shareable abstract (@ERSpublications)
Inappropriately high breakpoints have resulted in systematic false-susceptible AST results to anti-TB drugs. MIC, PK/PD and clinical outcome data should be combined when setting breakpoints to minimise the emergence and spread of antimicrobial resistance. https://bit.ly/3i43wb6

Cite this article as: Antimycobacterial Susceptibility Testing Group. Updating the approaches to define susceptibility and resistance to anti-tuberculosis agents: implications for diagnosis and treatment. *Eur Respir J* 2022; 59: 2200166 [DOI: 10.1183/13993003.00166-2022].

Received: 24 Jan 2022
Accepted: 5 March 2022

Approximately 85 000 deaths globally in 2019 were due to drug-resistant tuberculosis (TB), which corresponds to 7% of global deaths attributable to bacterial antimicrobial resistance [1]. Yet concerns have been mounting that drug-resistant TB was being underestimated because the approaches to define susceptibility and resistance to anti-TB agents had not kept up with those used for other major bacterial pathogens [2–9]. Here, we outline the recent, evidence-based initiatives spearheaded by the World Health Organization (WHO) and others to update breakpoints (traditionally referred to as critical concentrations (CCs)) that are used for phenotypic antimicrobial susceptibility testing (AST), also called drug susceptibility testing in the TB literature.

WHO commissioned five reports that considered studies in up to 16 languages from a wide diversity of global contributors to ensure that the compiled data were as comprehensive as possible. The first report consisted of a systematic review that covered publications relating to the CCs of the most important drugs for the treatment of multidrug-resistant (MDR) or rifampicin-resistant (RR) TB, including newly approved bedaquiline and delamanid [10]. The second report was an accompanying background document on the pharmacokinetics and pharmacodynamics (PK/PD) of those drugs, whereas the third presented the findings of a meta-analysis of clinical outcome data [11, 12]. The fourth was a systematic review of the CCs for the rifamycins and isoniazid [13]. Finally, WHO released its first official catalogue of resistance mutations to inform the interpretation of genotypic AST results [14, 15]. Together, these reports prompted WHO to make major changes to its recommendations for TB treatment (*e.g.* kanamycin is no longer recommended for the treatment of TB (figure 1b)) and AST, as discussed below [16].

For most antimicrobials with proven clinical efficacy at a specific dose, only the phenotypically wild-type (pWT) population of the bacterium in question is considered treatable (figure 1a) [17]. Thus, the main aim of the two systematic reviews commissioned by WHO was to evaluate the available minimum inhibitory concentration (MIC) data to assess whether existing CCs corresponded to epidemiological cut-off values (ECOFFs), which represent the upper end of the pWT MIC distribution (figure 1a) [10, 13]. This revealed limitations in both the quality and quantity of available MIC data in the TB field, in contrast to many other major bacterial pathogens [8, 9]. In fact, the data for most drug-medium combinations did not meet the criteria set out by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for setting ECOFFs [7, 9]. Faced with this situation and the critical, global need for AST guidance, WHO adopted a pragmatic approach and set 12 new CCs for second-line drugs based on systematic reviews of available existing data, while clearly highlighting that these decisions should be re-evaluated once additional data become available [7, 10]. Even using these less stringent criteria, three previously endorsed CCs had to be

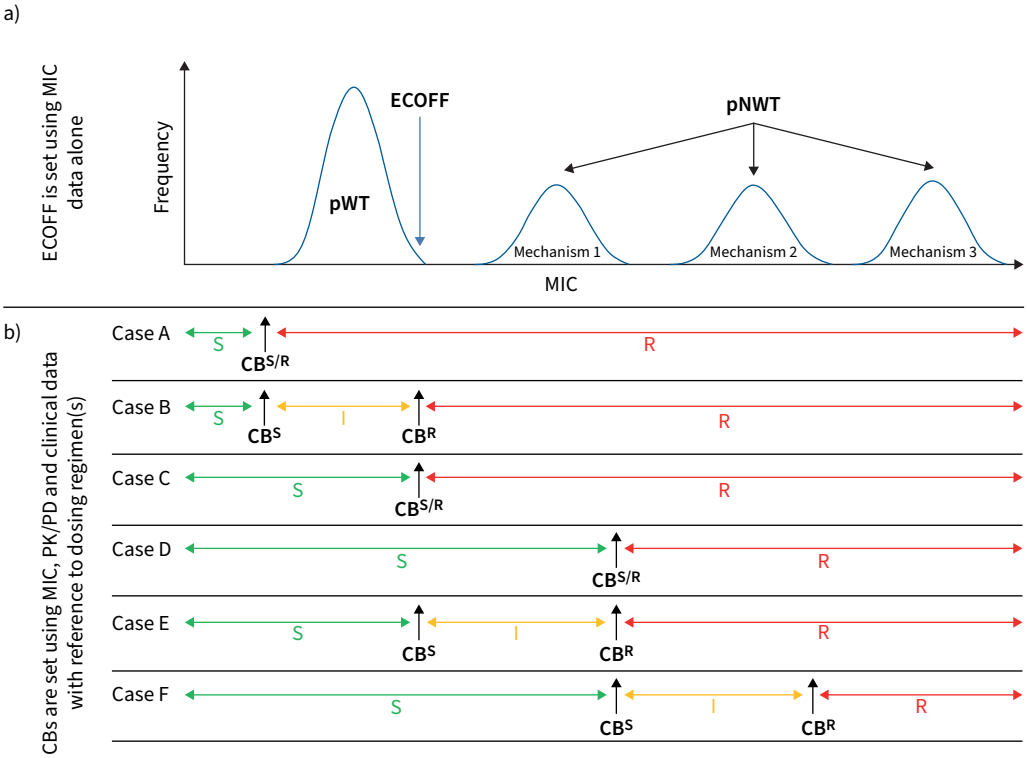

FIGURE 1 The European Committee on Antimicrobial Susceptibility Testing (EUCAST) approach for setting breakpoints compared with the World Health Organization (WHO). a) Four hypothetical minimum inhibitory concentration (MIC) distributions of an antibiotic for the same species. The distribution with the lowest MICs is typically the phenotypically wild-type (pWT) distribution, whereas the remaining three are phenotypically non-wild type (pNWT) with different underlying mechanisms. Notably, the upper end of the pWT distribution, which corresponds to the epidemiological cut-off value (ECOFF), does not automatically become a clinical breakpoint (CB), as shown in panel (b). Instead, pharmacokinetic/pharmacodynamic (PK/PD) and clinical data must be analysed to assess whether any of the represented populations are susceptible (S), susceptible at increased exposure (I), or resistant (R) [17, 24]. This may demonstrate that an agent offers no clinical benefits even for pWT strains at clinically attainable drug exposures, in which case the species in question would be deemed to be intrinsically resistant (case A). In 2018, WHO reached this conclusion for kanamycin and capreomycin after decades of clinical use globally, which prompted their withdrawal from clinical recommendations, although the underlying meta-analysis has attracted criticism [12, 16, 33, 34]. If a drug is clinically effective, one of five scenarios may apply. First, the pWT population may only be susceptible at increased exposure (case B). This uncommon approach is used to minimise the chance of clinicians prescribing the wrong regimen if a lower dose is commonly used for other pathogens. Second, the standard dosing regimen of the drug may be sufficient to treat only the pWT population (case C). This is the most common scenario when a drug is first approved and there is clinical outcome data to support its efficacy for the pWT population, whereas sufficient PK/PD and extensive clinical data in support of higher doses or treatment of pNWT isolates with resistance mechanisms are usually lacking. Gathering sufficient clinical outcome data for different pNWT populations is particularly challenging for TB given that multidrug regimens are always used, which may result in synergies or antagonism between one or more agents [27]. Nevertheless, the impact of individual mutations can be correlated with clinical outcomes, particularly for core drugs, provided that the studies are sufficiently powered [19, 25, 35, 36]. Third, the standard dosing regimen may also be sufficiently potent to treat strains with mechanism 1 but not strains with higher MICs because of mechanisms 2 and 3 (case D). Fourth, mechanism 1 may only be treatable at an increased exposure, as shown in case E. Finally, case F represents a hybrid between cases D and E. The current WHO definition of the critical concentration (CC) is effectively that of an ECOFF (*i.e.* it is set based on MIC data alone, taking genotypic information into consideration when relevant) even though the CC is actually used as a $CB^{S/R}$ (*i.e.* pWT strains are reported as susceptible and pNWT strains as resistant based on a limited review of clinical evidence and PK/PD data compared with other bacterial pathogens) [10, 12, 13, 16]. The only exception is moxifloxacin (table 1), for which the CC is used as a CB^{S} and the CB^{WHO}, as defined by WHO, is effectively a CB^{R} (case E), which may cause confusion with some clinicians who rarely treat TB. Moreover, this contradicts the assertion that an "intermediate" category, which is an alternative term to describe MIC increases that can be overcome by dose increases, does not exist for TB [10, 13, 24].

TABLE 1 Overview of changes to moxifloxacin breakpoints

Medium		Recommended moxifloxacin breakpoint (in mg·L^{-1}); daily dose (in mg)[¶]			
		CLSI	WHO		
		Since 2011 [37, 43, 44]	2008–2014 [45, 46]	2014–2018 [47]	Since 2018/19 [10, 48]
7H10	CC	0.5[+]; N/A[§]	–	**0.5**[f]; N/A **2; 400**[##]	**0.5**[¶¶]; **400 (standard dose)** in longer regimen or **400–800 (high dose)** in shorter regimen[++]
	CB^{WHO}	–	–	–	**2; 400–800 (high dose)** in longer regimen[++]
MGIT	CC	0.25[+]; N/A[§]	0.25; 400	**0.5**[f]; N/A **2; 400**[##]	**0.25**[¶¶]; **400 (standard dose)** in longer regimen or **400–800 (high dose)** in shorter regimen[++]
	CB^{WHO}	–	–	–	**1; 400–800 (high dose)** in longer regimen[++]

Based on World Health Organization (WHO) surveillance data[#], approximately 90% of moxifloxacin-resistant isolates could have been misclassified as susceptible using the BACTEC Mycobacterial Growth Indicator Tube (MGIT) because the WHO critical concentration (CC) of 2 mg·L^{-1} was eight times higher than the epidemiological cut-off value (ECOFF) between 2014 and 2018. In practice, however, the rate of misclassification was far lower. First, many countries did not use moxifloxacin at all during this period and, consequently, did not use this CC. Second, even countries that prescribed moxifloxacin avoided or minimised the misclassifications because they completely or primarily relied on genotypic antimicrobial susceptibility testing, continued using the MGIT CC of 0.25 mg·L^{-1} in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, used a CC for another fluoroquinolone as surrogate for moxifloxacin resistance, or relied on 0.5 mg·L^{-1} as the breakpoint for the standard dose of moxifloxacin in combination with 2 mg·L^{-1} as the breakpoint for the high dose of moxifloxacin. The theoretical rate of false-susceptible results was lower using Middlebrook 7H10 medium because the WHO CC of 2 mg·L^{-1} was four rather than eight times higher than the ECOFF. The clinical breakpoints introduced by WHO (CB^{WHO}) are not recognised by CLSI. [#]: comparing MGIT results for 2 mg·L^{-1} moxifloxacin with 2 mg·L^{-1} ofloxacin in the WHO surveillance study, which is equivalent to testing the currently recognised levofloxacin CC of 1 mg·L^{-1} (*i.e.* 1.5 mg·L^{-1} tested in that study was also too high) [10, 20]. [¶]: changes to WHO breakpoint/dose combinations relative to the previous guidelines are highlighted in bold. Breakpoints that correspond to ECOFFs are underlined [10]. [+]: can be tested as surrogate for other fluoroquinolones [37, 43–45]. [§]: not applicable (N/A) as CLSI does not define doses for treatment. [f]: 0.5 mg·L^{-1} moxifloxacin in 7H10 and MGIT were recommended as surrogates for resistance to ofloxacin and levofloxacin. Because the ECOFF for moxifloxacin is 0.25 mg·L^{-1} in MGIT, this meant that some strains resistant to ofloxacin and levofloxacin were misclassified as susceptible [10]. In effect, the surrogate breakpoints at 0.5 mg·L^{-1} and moxifloxacin CCs at 2 mg·L^{-1} were set inconsistently for both media because the 7H10 data was extrapolated to MGIT, despite the systematic differences between both media [47]. [##]: the WHO-endorsed dosage for individualised multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) regimens was 400 mg [47]. However, operational research using a higher dosage of moxifloxacin (800 mg) in a standardised short-course MDR/RR-TB regimen was in progress, although not WHO-endorsed at the time [49]. [¶¶]: not recommended as surrogate for other fluoroquinolones [10]. [++]: levofloxacin is the preferred fluoroquinolone for the shorter all-oral bedaquiline-containing MDR/RR-TB regimen recommended by WHO in 2020, but high-dose moxifloxacin can be used instead. However, any moxifloxacin resistance, irrespective of the level, is an exclusion criterion for the shorter all-oral regimen (*i.e.* the CC is the relevant breakpoint) [48]. This exclusion criterion for moxifloxacin had also applied to the shorter amikacin-containing MDR/RR-TB regimen that was recommended by WHO between 2018 and 2020 [50]. High-dose moxifloxacin can only be used to treat low-level resistant strains as part of the longer MDR/RR-TB regimen, for which the CB^{WHO} is valid [48].

withdrawn owing to a clear lack of supporting evidence. This included the only CC for cycloserine available up to that point, which means that phenotypic AST is not possible and, consequently, patients with resistant strains are needlessly at risk of the sometimes severe side-effects of this drug [10, 16].

More importantly, two previous CCs for rifampicin, arguably amongst the most significant breakpoints in diagnostic microbiology, were found to be too high, leading to false-susceptible AST results for some isolates (*i.e.* very major diagnostic errors that increase the likelihood of treatment failure and selection of resistance to other drugs) [13, 18, 19]. In fact, the rifampicin CC for Middlebrook 7H10 medium was twice as high as the ECOFF for more than half a century [18]. The therapeutic impact of these diagnostic misclassifications was that some phenotypically non-wild type isolates were deemed treatable with the recommended 10 mg·kg^{-1} body weight per day dose despite a lack of PK/PD or clinical evidence (case D in figure 1b) [13, 18]. WHO, consequently, lowered the rifampicin CCs to the tentative ECOFFs based on data from the systematic review (case C in figure 1b) [13]. To minimise false-susceptible results, the WHO-endorsed CCs of three second-line drugs (amikacin, levofloxacin and moxifloxacin) were also lowered to their respective ECOFFs [10]. In the case of moxifloxacin, approximately 90% of resistant isolates could have been misclassified as susceptible using only the WHO CC of 2 mg·L^{-1} for BACTEC Mycobacterial Growth Indicator Tube system by Becton Dickinson that was valid between 2014 and 2018 [20]. Fortunately, a number of practical factors (table 1) meant that the clinical consequences of this incorrectly set CC were reduced considerably.

When CCs are too high, they may not only result in undertreatment of the patient based on phenotypic AST, but can also adversely affect the design and interpretation of genotypic AST methods that represent

the most viable option to scale up AST globally [18, 19, 21]. Between 2011 and 2014, for instance, the WHO-endorsed GenoType MTBDR*plus* VER 2.0 by Hain Lifescience was designed not to detect *rpoB* L452P because this mutation was not considered to be a rifampicin resistance mutation at that time [18, 19]. It took more than a decade for *eis* c-14t and *rrs* c1402t to be recognised as resistance mutations for amikacin [14, 15, 22]. Hence, the full potential of the GenoType MTBDR*sl* VER 2.0 was not exploited because these two mutations were only interpreted as markers for kanamycin and capreomycin resistance.

Another consequence of CCs that are too high is an unnecessarily high number of clinical isolates that are genotypically resistant (*i.e.* contain mutations associated with resistance) but test phenotypically susceptible. This has resulted in underestimates of the accuracy of genotypic methods when phenotypic AST has been used as a reference and, consequently, reduced the confidence in genotypic AST. This apparent discordance also obscured the fact that clinically relevant mutations for some drugs cannot be reliably confirmed by current phenotypic AST methods, even if the CC corresponds to the ECOFF,

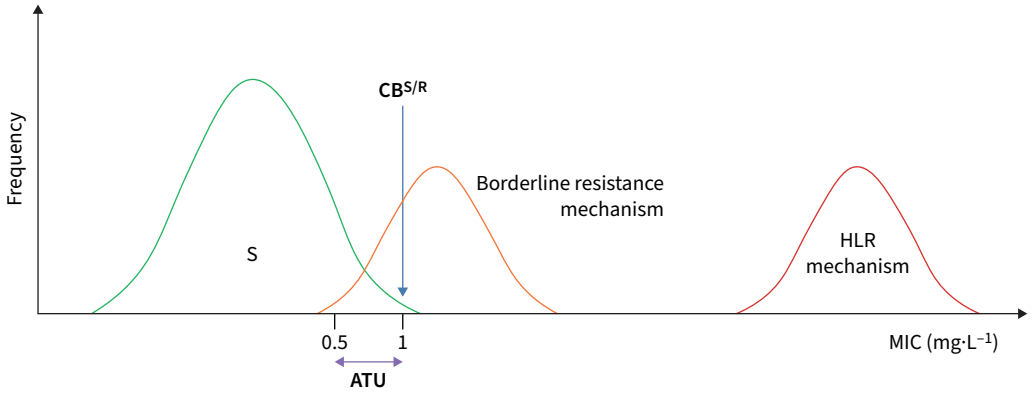

FIGURE 2 Strategies to minimise false-susceptible results by phenotypic antimicrobial susceptibility testing (AST) linked to borderline resistance mechanisms. Unlike the idealised scenario depicted in figure 1a, borderline resistance mechanisms exist with minimum inhibitory concentration (MIC) distributions that overlap with the susceptible distribution (*e.g.* the seven borderline rifampicin resistance mutations in *rpoB*) [10, 13, 37–39]. A clinical breakpoint (CB$^{S/R}$) that corresponds to the epidemiological cut-off value (ECOFF) (case C in figure 1b) intersects the MIC distributions of such mechanisms (at 1 mg·L^{-1} in the hypothetical example below). Even if such an isolate is tested multiple times in the same laboratory, it will variably test susceptible and resistant because of the inherent technical variability of phenotypic AST [18]. Four measures that are not mutually exclusive can be taken to decrease such false-susceptible results. First, the optimal solution would be to eliminate or at least minimise the degree of overlap between distributions by reducing the technical variability of MIC testing as much as possible, which was one of the reasons that prompted the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to develop its reference method and associated procedures to improve quality control [8, 9, 40, 41]. Second, EUCAST has introduced areas of technical uncertainty (ATUs) [24]. In this example, an MIC result of ⩽0.5 mg·L^{-1} would be reported as susceptible, whereas MICs of >1 mg·L^{-1} would be resistant. By contrast, an MIC result of 1 mg·L^{-1} would be "uncertain" as the isolate in question could not be unequivocally classified as either susceptible or resistant based on the single MIC result because of the overlapping MIC distributions (*i.e.* this applies to the borderline resistance mechanism but not high-level resistance (HLR) mechanism) [18]. Although the prevalence of borderline resistance in a particular setting can give an indication of which of these possibilities is more likely, other experimental results are needed to resolve this situation conclusively. For example, if the molecular basis of the borderline resistance mechanism is known and is detected, the isolate could be reported as resistant (*i.e.* a composite reference standard is used, as WHO recommends for rifampicin) [18, 19, 23]. In fact, the Clinical and Laboratory Standards Institute (CLSI) has set an "inconclusive" category for ethambutol for the Sensititre MYCOTB plate by Thermo Fisher Scientific, which appears to serve as an ATU to minimise false susceptibility due to *embB* mutations [37, 39]. Third, adopting interpretative reading, whereby the results of two antibiotics that share at least one resistance mechanism are analysed together, may be useful (*e.g.* if the MICs for bedaquiline and clofazimine are equal to or just above the CB$^{S/R}$, it is likely that the isolate in question has an *Rv0678* mutation) [38]. Finally, a surrogate agent could be tested that provides a better resolution between the relevant distribution (*e.g.* CLSI and EUCAST recommend pefloxacin as a surrogate for fluoroquinolone resistance in *Salmonella enterica*) [42].

because the MIC distributions of susceptible and resistant strains overlap based on current data (figure 2) [23]. This is the case for rifampicin and, therefore, WHO has adopted a composite reference standard to ensure that borderline *rpoB* resistance mutations are not missed (*i.e.* an isolate is now considered resistant to rifampicin if it tests resistant by phenotypic AST or harbours a recognised resistance mutation, provided that the pre-test probability is considered) [13, 18, 19, 23]. However, clear and user-friendly guidance on how to resolve discordances during routine clinical care is also needed for other drugs [23].

More fundamentally, MIC, PK/PD and clinical data should be fully integrated when setting breakpoints [11, 17]. In 2018, WHO endorsed a second breakpoint for moxifloxacin that is higher than the CC in support of high-dose moxifloxacin treatment as part of the longer individualised MDR/RR-TB regimen (table 1) [10]. A "susceptible at increased exposure" range thus was defined, though this specific terminology was not used in the report (case E in figure 1b) [24]. The primary justification for this decision relied on extrapolating clinical outcome data for high-dose gatifloxacin from a single study without data on drug exposure [10, 25]. It was not acknowledged that even high-dose gatifloxacin did not always overcome the low-level MIC increases conferred by *gyrA* A90V and similar mutations [10, 25]. Subsequent PK/PD modelling suggested that this second breakpoint might be clinically useful, but also reinforced the idea that low-level fluoroquinolone resistance is unlikely to be overcome by high-dose moxifloxacin in all patients because of patient-to-patient variability in the moxifloxacin exposure [26]. Nevertheless, given the potentially significant clinical value of using high-dose moxifloxacin when few other treatment options remain, this question should be prioritised for future review using additional data, including the recent studies using the hollow fibre infection model, to provide a more comprehensive and nuanced recommendation to clinicians [27–29]. Similarly, WHO has already announced that it would revisit the rifampicin breakpoint, should a higher dose of rifampicin be endorsed [13].

Considering this complex history, regulators and developers of diagnostics and drugs should fully embrace modern microbiological principles to define breakpoints and associated dosing regimens (figure 1) [17, 30, 31]. To this end, the two systematic reviews provide unprecedented detail about the underlying reasons and scientific evidence for all new recommendations by WHO, to facilitate external scrutiny and to encourage more research where the available evidence was limited [10, 13]. Where possible, these efforts should be coordinated between major regulators to minimise the burden to developers of drugs and AST devices (*e.g.* by recognising a single reference method against which all commercial AST methods are validated) [9]. It would also be beneficial if common AST terminology were adopted to avoid confusion. For instance, the meaning of "clinical breakpoint" differs between regulators (figure 1) and adopting the "area of technical uncertainty" for TB needs further consideration (figure 2) [10, 13]. Regulators should also review if the use of surrogate drugs can minimise false-susceptible results to provide clarity for assay developers about which agents to invest in (*e.g.* whether levofloxacin should be tested as the representative fluoroquinolone and whether kanamycin should be used as a surrogate for amikacin resistance (figure 2)) [22]. Although there has been a great deal of progress in the past 5 years, proactive action by the entire TB community is required to develop an updated AST framework given that the In Vitro Diagnostic Medical Device Regulation will come into effect in the European Union in May 2022. Because previous diagnostic approvals will not be automatically recognised, industry will have to invest to keep its AST devices on the market. We have an obligation to those infected and affected by TB to learn from past experiences and to make the most of this unique window of opportunity [7, 18, 32].

Acknowledgements: We thank Andrew Vernon and others who are acknowledged in the respective WHO reports covered in this viewpoint for sharing relevant data.

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Conflict of interest: D. Alland receives research support and royalty payments from Cepheid, a diagnostic company that makes tests for tuberculosis. A. Aubry and N. Veziris work in a laboratory that received a grant from Janssen outside the scope of this work. R. Baldan, I. Comas, C.M. Denkinger, D.L. Dolinger, S.B. Georghiou, C.U. Köser and T.C. Rodwell are or were consultants or employees of FIND, the global alliance for diagnostics, a not-for-profit foundation that supports the evaluation of publicly prioritised tuberculosis assays and the implementation of WHO-approved (guidance and prequalification) assays using donor grants. FIND has product evaluation agreements with several private sector companies that design diagnostics for tuberculosis and other diseases. These agreements strictly define FIND's independence and neutrality with regard to these private sector companies. D.L. Dolinger works for General Fluidics and provides consulting services for Médecins Sans Frontières, Molbio Diagnostics and Partners in Health. D.L. Dolinger worked for QuantuMDx and PhAST. T. Gumbo is founder and CEO of Praedicare Inc, a pre-clinical contract research organisation and is a founder of Praedicare Africa Pvt Ltd, a clinical contract research organisation. K. Kaniga is a full-time employee of Johnson & Johnson Global Public Health. C.U. Köser is a consultant for the TB Alliance. C.U. Köser's consulting work for Becton Dickinson involves a collaboration with Janssen and Thermo Fisher Scientific. C.U. Köser is collaborating with PZA Innovation. C.U. Köser worked as a consultant for QuantuMDx, the Stop TB Partnership, the WHO Global TB Programme, and the WHO Regional Office for Europe. C.U. Köser gave a paid educational talk for Oxford

Immunotec. Hain Lifescience covered C.U. Köser's travel and accommodation to present at a meeting. C.U. Köser is an unpaid advisor to BioVersys and GenoScreen. Y. Liu is an employee of Otsuka Pharmaceutical Development & Commercialization Inc, USA. V. Nikolayevskyy is employed by QIAGEN Manchester Ltd. S.V. Omar has received funding to prepare and provide training for Janssen Pharmaceutica activities. T.C. Rodwell is a cofounder, board member, and shareholder of Verus Diagnostics, a company that was founded with the intent of developing diagnostic assays. Verus Diagnostics was not involved in any way with data collection, analysis or publication of the results, and T.C. Rodwell has not received any financial support from Verus Diagnostics. University of California, San Diego (UCSD) Conflict of Interest office has reviewed and approved T.C. Rodwell's role in Verus Diagnostics. T.C. Rodwell is a coinventor of a provisional patent for a TB diagnostic assay (provisional patent 63/048.989). T.C. Rodwell is also a coinventor on a patent associated with the processing of TB sequencing data (European patent application number 14840432.0 and USSN 14/912,918), and has agreed to "donate all present and future interest in and rights to royalties from this patent" to UCSD to ensure that he does not receive any financial benefits from this patent. P. Supply is a consultant for Genoscreen. G. Theron's research group has received funding and/or in-kind donations in the last 5 years *via* his employer from Bruker Hain Lifesciences, Cepheid, LumiraDx, FIND, Biopromic, Newmark Diagnostics, Hemocue, Boditech and Copan. N. Veziris received travel support from Becton Dickinson for attending the Union Conference in 2018 outside the scope of this work. All other authors have nothing to disclose.

Support statement: As current or former employees or consultants for FIND, the work of R.B. Baldan, I. Comas, C.M. Denkinger, D.L. Dolinger, S.B. Georghiou, C.U. Köser and T.C. Rodwell on the systematic reviews, including this viewpoint, was supported by Unitaid (grant 2019-32-FIND MDR), BMGF (grant OPP1105925), the German Federal Ministry of Education and Research through KfW, the Dutch Ministry of Foreign Affairs, the Australian Department of Foreign Affairs and Trade, and UK aid from the British people. N. Alvarez and J. Robledo are funded by MinCiencias, Colombia (number 221389666216 CT-783-2018). A. Aubry and N. Veziris work at the Centre National de Reference des Mycobactéries, which receives an annual grant from Santé Publique France and have received research grants from Janssen for studies on bedaquiline. P. Claxton and I.F. Laurenson are funded through National Services Scotland. I. Comas was supported by PID2019-104477RB-I00 from the Spanish Science Ministry and by ERC (CoG 101001038). M. Egger is supported by the Swiss National Science Foundation (grant number 320030_153442 and 189498) and the US National Institutes of Health, National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Cancer Institute, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart, Lung, and Blood Institute, the National Institute on Alcohol Abuse and Alcoholism, the National Institute of Diabetes and Digestive and Kidney Diseases, the Fogarty International Center, and the National Library of Medicine: Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919. M.R. Farhat is supported by NIH NIAID R01AI155765. S.K. Heysell was funded by NIH NIAID grants R01 AI137080 and U01 AI150508. T. Jagielski was supported by a DAINA grant (number 2017/27/L/NZ6/03279) from the National Science Centre, Poland. J.L. Johnson was supported by contracts NO1-AI95383 and NO1-AI-70022 of the US National Institutes of Health. P.M. Keller was supported by Innosuisse 36198.1 IP-LS. C.U. Köser is a research associate at Wolfson College and visiting scientist at the Department of Genetics, University of Cambridge. The Federal Government of Germany supported C.U. Köser as part of his work for the European Laboratory Initiative, WHO Regional Office for Europe. C.U. Köser was further supported by the Royal Society of Tropical Medicine and Hygiene and the National Institute for Health Research Cambridge Biomedical Research Centre and received an observership by the European Society of Clinical Microbiology and Infectious Diseases to the EUCAST Development Laboratory for Bacteria (Växjö, Sweden), hosted by Gunnar Kahlmeter and Erika Matuschek. D. Machado and M. Viveiros are funded in part by Fundação para a Ciência e a Tecnologia, Portugal (PTDC/BIA-MIC/30692/2017, UID/Multi/04413/2020 and DL57/CEECIND/0256/2017). S. Niemann is supported by the German Center for Infection Research, Excellenz Cluster Precision Medicine in Chronic Inflammation EXC 2167, Leibniz Science Campus Evolutionary Medicine of the LUNG (EvoLUNG). S.V. Omar has received funding to prepare and provide training for Janssen Pharmaceutica activities. L. Rigouts is supported by the Belgian Directorate General for Development. T.C. Rodwell was additionally funded in part by FIND and NIH NIAD, grants: P30 AI036214 and R21 AI135756. T. Schön is funded by the Swedish Heart and Lung Foundation and the Swedish Research Council. T.R. Sterling has received funding from the US National Institutes of Health and the Centers for Disease Control and Prevention. G. Theron and R. Warren are supported by baseline funding from the South African Medical Research Council. R.J. Wilkinson receives funding from the Wellcome Trust (203135) and from the Francis Crick Institute, which is supported by Cancer Research UK (FC0010218), UKRI (FC0010218) and the Wellcome Trust (FC0010218). The views expressed here are those of the authors and do not necessarily correspond to those of their respective employers.

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