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

Antimycobacterial Susceptibility Testing Group

Collaborators are listed at the end of the article

Corresponding author: Claudio U. Köser (cuk21@cam.ac.uk)

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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

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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

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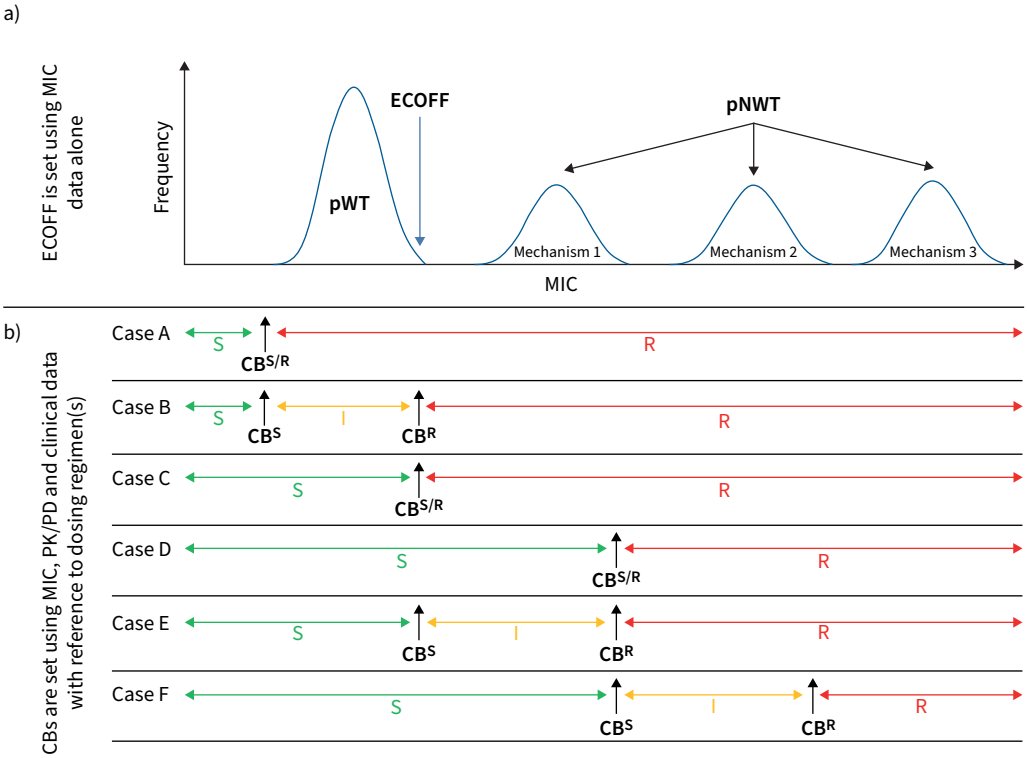

FIGURE 1 The European Committee on Antimicrobial Susceptibility Testing (EUCAST) approach for setting breakpoints compared with the World Health Organization (WHO). a) Four hypothetical minimum inhibitory concentration (MIC) distributions of an antibiotic for the same species. The distribution with the 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].

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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[f] **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**; N/A[f] **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

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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,

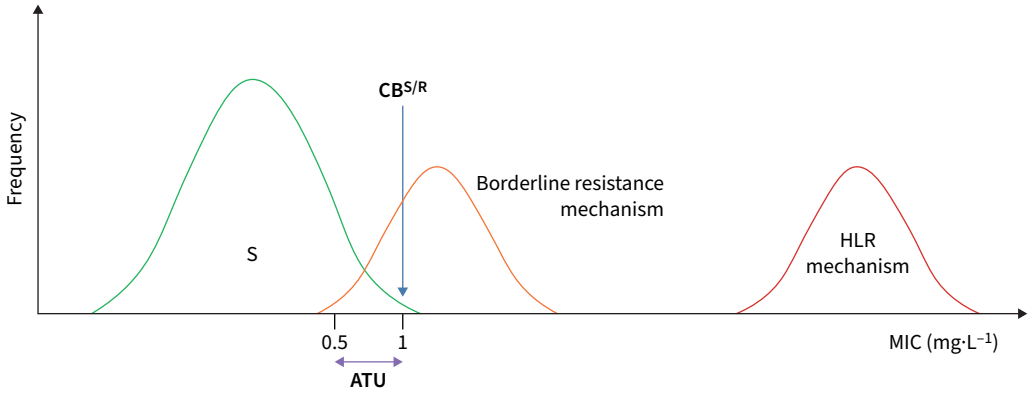

FIGURE 2 Strategies to minimise false-susceptible results by phenotypic antimicrobial susceptibility testing (AST) linked to borderline resistance mechanisms. Unlike the idealised scenario depicted in figure 1a, borderline resistance mechanisms exist with minimum inhibitory concentration (MIC) distributions 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].

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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].

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The Antimycobacterial Susceptibility Testing Group: Sophia B. Georghiou (FIND, Geneva, Switzerland), Timothy C. Rodwell (FIND, Geneva, Switzerland, and Dept of Medicine, University of California, San Diego, CA, USA), Alexei Korobitsyn (Global TB Programme, World Health Organization, Geneva, Switzerland), Said H. Abbadi (Dept of Microbiology, Faculty of Medicine, Suez University, Suez, Egypt), Kanchan Ajbani (Dept of Microbiology, P.D. Hinduja Hospital and Medical Research Centre, Mumbai, India), Jan-Willem Alffenaar (Sydney Institute for Infectious Diseases, University of Sydney, Sydney, Australia, Faculty of Medicine and Health, School of Pharmacy, The University of Sydney, Sydney, Australia, and Westmead Hospital, Sydney, Australia), David Alland (Dept of Medicine and the Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, NJ, USA), Nataly Alvarez (Unidad de Bacteriología y Micobacterias, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia), Sönke Andres (National and Supranational Reference Laboratory for Mycobacteria, Research Center Borstel, Borstel, Germany), Elisa Ardizzoni (Unit of Mycobacteriology, Dept of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium), Alexandra Aubry (Sorbonne Université, INSERM, Centre d'Immunologie et des Maladies Infectieuses, U1135, AP-HP, Hôpital Pitié-Salpêtrière, Centre National de Référence

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des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, Paris, France), Rossella Baldan (FIND, Geneva, Switzerland, and Institute for Infectious Diseases, University of Bern, Bern, Switzerland), Marie Ballif (Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland), Ivan Barilar (Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany, and German Center for Infection Research, Partner site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany), Erik C. Böttger (Institut für Medizinische Mikrobiologie, Nationales Zentrum für Mykobakterien, Universität Zürich, Zürich, Switzerland), Soumitesh Chakravorty (Cepheid, Sunnyvale, CA, USA, and New Jersey Medical School, Rutgers University, Newark, NJ, USA), Pauline M. Claxton (Scottish Mycobacteria Reference Laboratory, Directorate of Laboratory Medicine, Royal Infirmary of Edinburgh, Edinburgh, UK), Daniela M. Cirillo (Emerging Bacterial Pathogens Unit, IRCCS Ospedale San Raffaele, Milan, Italy), Iñaki Comas (Instituto de Biomedicina de Valencia IBV-CSIC, Spanish National Research Council, Valencia, Spain, and CIBER in Epidemiology and Public Health, Madrid, Spain), Chris Coulter (Queensland Mycobacterium Reference Laboratory, Pathology Queensland, Herston, Australia), Claudia M. Denkinger (FIND, Geneva, Switzerland, Division of Clinical Tropical Medicine, Centre of Infectious Diseases, Heidelberg University Hospital, Heidelberg, Germany, and German Centre for Infection Research (DZIF), partner site Heidelberg University Hospital, Heidelberg, Germany), Brigitta Derendinger (DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research/South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa), Edward P. Desmond (State Laboratories Division, Department of Health, Pearl City, HI, USA), Jurriaan E.M. de Steenwinkel (Dept of Medical Microbiology and Infectious Diseases, Erasmus University Medical Centre Rotterdam, Rotterdam, The Netherlands), Keertan Dheda (Centre for Lung Infection and Immunity, Division of Pulmonology, Dept of Medicine and UCT Lung Institute, and South African MRC/UCT Centre for the Study of Antimicrobial Resistance, University of Cape Town, Cape Town, South Africa, and Faculty of Infectious and Tropical Diseases, Dept of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, UK), Andreas H. Diacon (TASK, Cape Town, South Africa), David L. Dolinger (General Fluidics, Waltham, MA, USA), Kelly E. Dooley (Dept of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA), Matthias Egger (Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland, Centre for Infectious Disease Research and Epidemiology, University of Cape Town, Cape Town, South Africa, and Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK), Soudeh Ehsani (Joint Infectious Diseases Programme, Regional Office for Europe, World Health Organization, Copenhagen, Denmark), Maha R. Farhat (Dept of Biomedical Informatics, Harvard Medical School, Boston, MA, USA, and Pulmonary and Critical Care Medicine, Massachusetts General Hospital, Boston, MA, USA), Lanfranco Fattorini (Dept of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy), Iris Finci (Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany, and German Center for Infection Research, Partner site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany), Laure Fournier Le Ray (Sorbonne Université, INSERM, Centre d'Immunologie et des Maladies Infectieuses, U1135, Paris, France), Victoria Furió (Instituto de Biomedicina de Valencia IBV-CSIC, Spanish National Research Council, Valencia, Spain), Ramona Groenheit (Dept of Microbiology, Public Health Agency of Sweden, Solna, Sweden), Tawanda Gumbo (Praedicare Inc, Dallas, TX, USA), Scott K. Heysell (Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA, USA), Doris Hillemann (National and Supranational Reference Laboratory for Mycobacteria, Research Center Borstel, Borstel, Germany), Harald Hoffmann (Institute of Microbiology and Laboratory Medicine, Dept IML Red GmbH, WHO – Supranational Tuberculosis Reference Laboratory Munich-Gauting, Gauting, Germany, and SYNLAB Gauting, SYNLAB Human Genetics, Gauting, Germany), Po-Ren Hsueh (Depts of Laboratory Medicine and Internal Medicine, China Medical University Hospital, School of Medicine, China Medical University, Taichung, Taiwan, and Depts of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan), Yi Hu (School of Public Health and Key Laboratory of Public Health Safety, Fudan University, Shanghai, China), Hairong Huang (Beijing Chest Hospital, Capital Medical University, Beijing, China), Alamdar Hussain (National TB Reference Laboratory, National TB Control Program, Pakistan), Farzana Ismail (Centre for Tuberculosis, National and Supranational TB Reference Laboratory, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa, and Dept of Medical Microbiology, University of Pretoria, Pretoria, South Africa), Kiyohiko Izumi (Dept of Epidemiology and Clinical Research, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Kiyose, Japan), Tomasz Jagielski (Dept of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland), John L. Johnson (Case Western Reserve University and University Hospitals Cleveland Medical Center, Cleveland, OH, USA), Priti Kambli (Dept of Microbiology, P.D. Hinduja Hospital and Medical Research Centre, Mumbai, India), Koné Kaniga (Johnson & Johnson Global Public Health, Division of Janssen Pharmaceutica, Titusville, NJ, USA), G.H.R. Eranga Karunaratne (Dept of Microbiology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka, and Faculty of Science, Horizon Campus, Malabe, Sri Lanka), Meenu Kaushal Sharma (National Reference Centre for Mycobacteriology, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada), Peter M. Keller (Institute for Infectious Diseases, University of Bern, Bern, Switzerland), Ellis C. Kelly (Dept of Genetics, University of Cambridge, Cambridge, UK), Margarita Kholina (Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia), Mikashmi Kohli (FIND, Geneva, Switzerland), Katharina Kranzer (Dept of Clinical

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Research, London School of Hygiene and Tropical Medicine, London, UK, Dept of Infectious Diseases and Tropical Medicine, Ludwig Maximilian University of Munich, Munich, Germany, and Biomedical Research and Training Institute, Harare, Zimbabwe), Ian F. Laurenson (Scottish Mycobacteria Reference Laboratory, Directorate of Laboratory Medicine, Royal Infirmary of Edinburgh, Edinburgh, UK), Jason Limberis (Dept of Medicine, Division of Experimental Medicine, University of California San Francisco, San Francisco, CA, USA), S-Y. Grace Lin (Microbial Diseases Laboratory, California Department of Public Health, Richmond, CA, USA), Yongge Liu (Otsuka Pharmaceutical Development and Commercialization, Inc., Rockville, MD, USA), Alexandre López-Gavín (Hospital Clínic, Universitat de Barcelona, Barcelona, Spain), Anna Lyander (Clinical Genomics Stockholm, Science for Life Laboratory, Solna, Sweden, and School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Stockholm, Sweden), Diana Machado (Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, Lisbon, Portugal), Elena Martinez (Centre for Infectious Diseases and Microbiology – Public Health, Westmead Hospital and NSW Health Pathology, Sydney, Australia), Faisal Masood (National TB Reference Laboratory, National TB Control Program, Pakistan), Satoshi Mitarai (Dept of Mycobacterium Reference and Research, Research Institute of Tuberculosis, Japan Anti-tuberculosis Association, Kiyose, Japan), Nomonde R. Mvelase (Dept of Medical Microbiology, National Health Laboratory Service, Durban, South Africa, and School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa), Stefan Niemann (Molecular and Experimental Mycobacteriology, Research Center Borstel, Borstel, Germany, and German Center for Infection Research, Partner site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany), Vladyslav Nikolayevskyy (Dept of Infectious Diseases, Imperial College London, London, UK), Florian P. Maurer (National and Supranational Reference Laboratory for Mycobacteria, Research Center Borstel, Borstel, Germany, German Center for Infection Research, Partner site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany, and Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany), Matthias Merker (Evolution of the Resistome, Research Center Borstel, Borstel, Germany), Paolo Miotto (Emerging Bacterial Pathogens Unit, IRCCS Ospedale San Raffaele, Milan, Italy), Shaheed V. Omar (Centre for Tuberculosis, National and Supranational TB Reference Laboratory, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa, and Dept of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa), Ralf Otto-Knapp (German Central Committee against Tuberculosis, Berlin, Germany), Moisés Palaci (Núcleo de Doenças Infecciosas, Universidade Federal do Espírito Santo, Vitória, Brazil), Juan José Palacios Gutiérrez (Unidad de Referencia Regional de Micobacterias, Hospital Universitario Central de Asturias, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain), Sharon J. Peacock (Dept of Medicine, University of Cambridge, Cambridge, UK), Charles A. Peloquin (College of Pharmacy and Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA), Jennifer Perera (Dept of Microbiology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka), Catherine Pierre-Audigier (CMIP Institut Pasteur, Paris, France, and Laboratoire de Bactériologie, Hôpital Bichat-Claude Bernard, Paris France), Suporn Pholwat (Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA, USA), James E. Posey (Division of Tuberculosis Elimination, National Center for HIV, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA), Therdsak Prammananan (National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathum Thani, Thailand), Leen Rigouts (Unit of Mycobacteriology, Dept of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium), Jaime Robledo (Unidad de Bacteriología y Micobacterias, Corporación para Investigaciones Biológicas (CIB), Medellín, Colombia, and Escuela de Ciencias de la Salud, Universidad Pontificia Bolivariana (UPB), Medellín, Colombia), Neesha Rockwood (Dept of Microbiology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka, Dept of Infectious Diseases, Imperial College London, London, UK, and Wellcome Centre for Infectious Diseases Research in Africa, University of Cape Town, Cape Town, South Africa), Camilla Rodrigues (Dept of Microbiology, P.D. Hinduja Hospital and Medical Research Centre, Mumbai, India), Max Salfinger (University of South Florida College of Public Health and Morsani College of Medicine, Tampa, FL, USA), Marcos C. Schechter (Emory University School of Medicine, Dept of Medicine, Division of Infectious Diseases, Atlanta, GA, USA), Marva Seifert (Dept of Medicine, University of California, San Diego, CA, USA), Sarah Sengstake (Unit of Mycobacteriology, Dept of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium), Thomas Shinnick (Independent Consultant, Atlanta, GA, USA), Natalia Shubladze (National Reference Laboratory, National Center for Tuberculosis and Lung Diseases, Tbilisi, Georgia), Vitali Sintchenko (Sydney Institute for Infectious Diseases, University of Sydney, Sydney, Australia, and NSW Mycobacterium Reference Laboratory, Institute of Clinical Pathology and Medical Research, NSW Health Pathology, Sydney, Australia), Frederick Sirgel (DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research/South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa), Sulochana Somasundaram (National Institute for Research in Tuberculosis, Indian Council of Medical Research, Chennai, India), Timothy R. Sterling (Vanderbilt University Medical Center, Nashville, TN, USA), Andrea Spitaleri (Emerging Bacterial Pathogens Unit, IRCCS Ospedale San Raffaele, Milan, Italy, and Vita-Salute San Raffaele University, Milan, Italy), Elizabeth Streicher (DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research/South African

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Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa), Philip Supply (Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 – UMR 9017 – CIIL – Center for Infection and Immunity of Lille, F-59000 Lille, France), Erik Svensson (International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark), Elisa Tagliani (Emerging Bacterial Pathogens Unit, IRCCS Ospedale San Raffaele, Milan, Italy), Sabira Tahseen (National TB Reference Laboratory, National TB Control Program, Pakistan), Akiko Takaki (Dept of Mycobacterium Reference and Research, Research Institute of Tuberculosis, Japan Anti-tuberculosis Association, Kiyose, Japan), Grant Theron (DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research/South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa), Gabriela Torrea (Unit of Mycobacteriology, Dept of Biomedical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium), Armand Van Deun (Independent Consultant, Leuven, Belgium), Jakko van Ingen (Radboudumc Center for Infectious Diseases, Dept of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands), Annelies Van Rie (Tuberculosis Omics Research Consortium, Family Medicine and Population health, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium), Dick van Soolingen (Tuberculosis Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands), Roger Vargas Jr (Dept of Biomedical Informatics, Harvard Medical School, Boston, MA, USA, and Center for Computational Biomedicine, Harvard Medical School, Boston, MA, USA), Amour Venter (TASK, Cape Town, South Africa), Nicolas Veziris (Sorbonne Université, Centre d'Immunologie et des Maladies Infectieuses (Cimi-Paris), UMR 1135, Département de Bactériologie, Hôpital Saint-Antoine, Centre National de Référence des Mycobactéries, APHP, Sorbonne Université, Paris, France), Cristina Villellas (Janssen Research and Development, Beerse, Belgium), Miguel Viveiros (Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, Lisbon, Portugal), Robin Warren (DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research/South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa), Shu'an Wen (Beijing Chest Hospital, Capital Medical University, Beijing, China), Jim Werngren (Dept of Microbiology, Public Health Agency of Sweden, Solna, Sweden), Robert J. Wilkinson (Dept of Infectious Diseases, Imperial College London, London, UK, Wellcome Centre for Infectious Diseases Research in Africa, University of Cape Town, Cape Town, South Africa, and The Francis Crick Institute, London, UK), Caie Yang (Dept of Clinical Laboratory, The Eighth Medical Center of People's Liberation Army General Hospital, Beijing, China), F. Ferda Yılmaz (Ege University, Faculty of Pharmacy, Dept of Pharmaceutical Microbiology, Bornova, İzmir, Turkey), Tingting Zhang (Beijing Chest Hospital, Capital Medical University, Beijing, China), Danila Zimenkov (Center for Precision Genome Editing and Genetic Technologies for Biomedicine, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia), Nazir Ismail (Global TB Programme, World Health Organization, Geneva, Switzerland), Thomas Schön (Dept of Infectious Diseases, Kalmar County Hospital, Linköping University, Kalmar, Sweden, Unit of Infection and Inflammation, Dept of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden, and Dept of Infectious Diseases, Linköping University Hospital, Linköping, Sweden) and Claudio U. Köser (Dept of Genetics, University of Cambridge, Cambridge, UK). Thomas Schön and Claudio U. Köser contributed equally.

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