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Commentary

What is the role of the EUCAST reference method for MIC testing of the *Mycobacterium tuberculosis* complex?

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Drug-resistant tuberculosis (TB) is estimated to account for approximately 30% of annual deaths due to antimicrobial resistance (AMR) [1]. This is mainly due to mortality of approximately 44% caused by multidrug-resistant (MDR) TB (defined by resistance to isoniazid and rifampicin) [2]. Therefore, accurately diagnosing AMR for TB is crucial not only to select the most effective regimen with the least side effects but also to minimize costs (e.g. the median cost to treat MDR-TB is \$6430 compared with \$973 for drug-susceptible TB) [2]. Owing to the slow growth rate of the *Mycobacterium tuberculosis* complex (MTBC), this is increasingly achieved using genotypic approaches [3]. However, phenotypic antimicrobial susceptibility testing (pAST) is still needed to correlate the presence of mutations and their phenotypic expression, especially for new anti-TB agents for which resistance mutations are unknown.

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Rationale for the EUCAST MIC reference method

Historically, several methods and culture media have been used for pAST of MTBC [3,4]. The corresponding breakpoints, which were traditionally referred to as critical concentrations (CC), were first set in the 1960s by the World Health Organization (WHO) and subsequently revised by the Clinical and Laboratory Standards Institute (CLSI) and WHO (Table 1) [5,6]. Although CC values were determined based on microbiological and clinical evidence, this was done only for certain drugs and media, which complicates the use of these values today [3]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) sets clinical breakpoints (CBs) by evaluating epidemiological cut-off values (ECOFFs), based on MIC distributions, pharmacokinetic/pharmacodynamic (PK/PD) and clinical outcome data together [7]. This approach has now been used for most antibacterial and antifungal agents, enabling high-quality pAST globally. The time has come to bring the same rigorous approach to the TB field.

In 2018, WHO redefined the CCs to correspond to ECOFFs and performed an extensive systematic literature review of MIC distributions for WHO-endorsed media (i.e. egg-based Löwenstein–Jensen (LJ), Middlebrook 7H10 or 7H11 synthetic solid media and BACTEC 960 MGIT liquid medium, see Table 1) [5]. This revealed that some CCs had been too high, resulting in the misclassification of some resistant strains as susceptible. In general, the quality and quantity of MIC data available for the majority of agents were insufficient to define ECOFFs according to the criteria adopted by EUCAST [7,8]. For example, MICs were often truncated because inappropriate concentration ranges were tested, which precluded a comprehensive assessment of the phenotypically wild-type MIC distributions [9,10]. In addition, systematic differences in the MIC distributions from different laboratories that supposedly used the same method became apparent, even for testing on 7H10 despite this method being standardized by CLSI [6]. This had gone largely unnoticed because of the lack of rigorously defined quality control (QC) ranges/targets [9].

The need for greater standardisation in the TB field was underscored when the new anti-TB agents -delamanid and bedaquiline- were submitted to the European Medical Agency (EMA) in 2012 and 2013, respectively. Since EMA routinely expects EUCAST to set CBs, EUCAST established a subcommittee for antimycobacterial susceptibility testing (AMST) in 2016 and its first task

was to develop a reference MIC method for MTBC. This was made possible by the strong involvement of the ESCMID study group of mycobacterial infections (ESGMYC). In 2019, AMST proposed a new protocol for broth microdilution (BMD) testing in Middlebrook 7H9 using a carefully standardised inoculum [11]. Following a public consultation, this protocol was validated in the four AMST laboratories. This new reference MIC method for MTBC was endorsed by EUCAST as the reference method to set QC ranges/targets, ECOFFs and CBs for the MTBC. As with other organisms, commercial methods, such as MGIT, could not be accepted as formal reference methods to avoid dependence on a single manufacturer that may go out of the business or decide to modify or discontinue a specific method. Instead, commercial methods have to be calibrated against the reference method. The choice of the reference method was based on a multicentre comparison of the intra- and interassay reproducibility of BMD with 7H10 as well as broader considerations, such as the labour requirements, costs, published MIC evidence and experiences with other bacteria (Table 2).

The reference method is described on the EUCAST website and is detailed in an accompanying article [11]. To ensure standardization, a QC target/range for H37Rv ATCC 27294 will be defined for each drug, as it is routinely done for other pathogens [9]. This will not only improve the reproducibility of MIC testing during routine clinical testing but also ensure comparability of MIC data from different clinical trials and scientific studies [9].

Role of other pAST methods

The EUCAST reference method is mostly suitable for specialized reference laboratories. Other non-commercial (e.g. LJ, 7H10 or 7H11) and commercial methods can be used instead for routine pAST or clinical trials as they may offer other distinct advantages (e.g. higher biosafety of MGIT, reduced labour requirements for Sensititre plates or lower cost of LJ, see Table 2) [5,10]. However, to interpret the results of surrogate methods using EUCAST CBs, these methods will need to be calibrated against the reference method. The calibration does not have to be carried out by each laboratory that would like to use a surrogate method. Rather, the calibration will be done once in a multicentre study according to the standard operating procedures provided by EUCAST. For other microbial pathogens, EUCAST considers a calibration successful if the surrogate method is equally reproducible (equal degree of random variation) and yields equivalent MICs (essential agreement; absence of systematic difference) compared with the reference method. For MTBC, however, EUCAST may accept methods that yield MICs that are systematically higher or lower, provided the difference is fully predictable, by introducing a conversion factor. This would allow for reference MICs to be predicted from the results of the surrogate method. This is needed for therapeutic drug monitoring using the PK/PD target that will be set based on the reference method.

EUCAST strategy for setting CBs for MTBC

From now on, it will be the responsibility of pharmaceutical companies to present MIC data generated by the reference method, along with PK/PD and clinical outcome data to allow EUCAST to set ECOFFs and CBs when new drugs are to be approved by EMA. To define ECOFFs, it is important to note that all known MTBC lineages and major genotypes should be included to ensure that the resulting MIC distribution is as representative as possible of the global MTBC diversity. The first task for EUCAST will be to set definitive CBs for bedaquiline and delamanid based on reference method generated MIC values (i.e. the current CBs are provisional since the reference method and associated guidelines did not exist when these agents

Table 1

List of the critical concentrations (mg/L) recommended by WHO for phenotypic antimycobacterial susceptibility testing of isolates of *Mycobacterium tuberculosis* complex using the indirect proportion method

Antimicrobial agent	LJ	7H10	7H11	MGIT
Rifampicin	40	1	1	1
Isoniazid	0.2	0.2	0.2	0.1
Ethambutol	2	5	7.5	5
Pyrazinamide	—	—	—	100
Levofloxacin	2	1	—	1
Moxifloxacin	1	0.5	0.5	0.25
Bedaquiline	—	—	0.25	1
Linezolid	—	1	1	1
Clofazimine	—	—	—	1
Cycloserine	—	—	—	—
Delamanid	—	—	0.016	0.06
Imipenem–cilastatin	—	—	—	—
Meropenem	—	—	—	—
Amikacin	30	2	—	1
Streptomycin	4	2	2	1
Ethionamide	40	5	10	5
Prothionamide	40	—	—	2.5
P-aminosalicylic acid	—	—	—	—

Table 2
Criteria for the selection of a reference method for MIC testing on *Mycobacterium tuberculosis* complex

Criteria	7H9 BMD EUCAST	7H10	7H11	MGIT	LJ
Reproducibility	+++	+++	+++	+++	+
Media variability	—	—	—	—	+
Workload for preparation	+	++	++	+	++
Ease of use	++	+	+	+	+
Material cost per MIC concentration (Euro)	0.3–0.5	0.5–1	0.5–1	5–10	0.1–0.5
Similar reference method for other species?	Yes	No	No	No	No
Time to result (days)	7–14	21	21	7–14	28
Availability of different media distributors	Yes	Yes	Yes	No	Yes
Availability of a commercial kit	No	No	No	Yes	No
CLSI or WHO breakpoints available	No	Yes	Yes	Yes	Yes
Potential for future development	+++	++	++	+	+
Biosafety risk	++	++	++	+	+

BMD, broth microdilution; LJ, Löwenstein Jensen; MGIT, BACTEC 960 MGIT; —, low, +, acceptable, ++, high, +++, very high. CLSI, Clinical and Laboratory Standards Institute; WHO, World Health Organization.

were approved). The AMST network will facilitate the generation of necessary data to set CBs for traditional agents through transparent interactions with diagnostic companies and the pharmaceutical industry. For this extensive task, collaboration within the recently launched AMST general committee, to which representatives of all EU countries were invited, will be needed.

In summary, the overarching goal of AMST is to create a scientifically rigorous framework to set CBs for MTBC. To achieve this, global collaboration between varieties of stakeholders is necessary, ranging from microbiologists, molecular biologists, pharmacologists and clinicians to pharmaceutical and diagnostic companies and regulatory agencies. This is foremost in the interest of TB patients but also provides crucial information to those involved in the measurement of antimicrobial resistance and those struggling to develop new agents.

Transparency Declaration

C.U.K. is a consultant for the World Health Organization (WHO) Global TB Programme, the WHO Regional Office for Europe, The Global Alliance for TB Drug Development, Becton Dickinson, and the Foundation for Innovative New Diagnostics, which involved work for Cepheid, Hain Lifescience and WHO. C.U.K. is an unpaid advisor to GenoScreen. C.U.K. worked as a consultant for QuantuMDx. Hain Lifescience covered C.U.K.'s travel and accommodation to present at a meeting. Otsuka Novel Products GmbH has supplied C.U.K. with antibiotics for *in vitro* research. C.U.K. is collaborating with YD Diagnostics. The remaining authors declare that they have no conflicts of interest.

Author contributions

T.S. and C.U.K. designed the paper. J.W., M.V., S.G., G.K., C.G., F.M., G.L., J.T., K.v.I., M.J., D.G., D.M.C., M.S. and E.C. participated in the

discussion. T.S., C.U.K., S.G. and E.C. wrote a draft of the manuscript and all authors participated in the final version and revisions.

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