International Journal of Infectious Diseases 80 (2019) S32–S35

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

‘Those who cannot remember the past are condemned to repeat it’: Drug-susceptibility testing for bedaquiline and delamanid

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ARTICLE INFO

Article history:
Received 9 January 2019
Received in revised form 18 February 2019
Accepted 20 February 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Tuberculosis
Drug resistance
Minimum inhibitory concentration
Breakpoint
Bedaquiline
Delamanid

ABSTRACT

Despite being fundamental to all treatment decisions, the breakpoints that define susceptibility and resistance to conventional anti-tuberculosis (TB) drugs were traditionally defined based on expert opinion as opposed to modern microbiological principles. As a result, the breakpoints for several key drugs (i.e. amikacin, levofloxacin, and moxifloxacin) were too high, resulting in the systematic misclassification of a proportion of resistant strains as susceptible. Moreover, a recent systematic review of clinical outcome data prompted the World Health Organization (WHO) to make significant changes to its treatment guidelines. For example, capreomycin and kanamycin are no longer recommended for TB treatment because their use correlates with worse clinical outcomes. This history notwithstanding, robust breakpoints still do not exist for bedaquiline and delamanid six years after their approval. This was compounded by the fact that access to both agents for drug-susceptibility testing had initially been restricted. It is incumbent upon the European Medicines Agency, the United States Food and Drug Administration, and WHO to ensure that drug developers generate the necessary data to set breakpoints as a prerequisite for the approval of new agents.

Drug-resistant tuberculosis (TB) is estimated to account for a quarter of annual deaths attributable to antimicrobial resistance (Review on Antimicrobial Resistance, 2016). Even if all patients diagnosed with rifampicin resistant or multidrug resistant (MDR) TB had received comprehensive drug-susceptibility testing (DST) and had had access to individualised treatment, a proportion would have received suboptimal regimens due to flawed DST results (WHO, 2018a). Furthermore, drugs with likely limited benefits (capreomycin and kanamycin) were widely used in accordance with World Health Organization (WHO) recommendations (WHO, 2018b).

The most fundamental reason for incorrect DST results is when the breakpoints that define phenotypic resistance are not sound, which are, in turn, used to define resistance on the genotypic level (Heyckendorf et al., 2018). In a landmark opinion piece from 2012, Ängeby et al. pointed out that, unlike most major bacterial pathogens, breakpoints to anti-TB drugs were traditionally set based on expert opinion rather than modern microbiological principles. In addition, neither the Clinical and Laboratory Standard Institute nor WHO had documented the evidence used to set or revise breakpoints comprehensively (Ängeby et al., 2012).

Yet, it was not until the publication of a systematic review of minimum inhibitory concentration (MIC) and sequencing data for second-line drugs commissioned by WHO that the extent of the problem became apparent (WHO, 2018c). Specifically, the breakpoints for several key anti-TB drugs (i.e. amikacin, levofloxacin, and moxifloxacin) were found to be too high, which meant that a proportion of resistant strains had been systematically misclassified as susceptible in routine laboratory practise (WHO, 2018c). In addition, WHO drug surveillance surveys underestimated the prevalence of resistance to these drugs, which could be partly compensated for by taking genotypic information into account (Zignol et al., 2016, 2018). In this context, it is important to appreciate that the majority of critical concentrations (CCs), which were redefined to correspond to epidemiological cut-offs (ECOFFs), would have had to be withdrawn if WHO had followed the guidelines by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) that apply to all other major bacterial pathogens (EUCAST, 2017). Instead, WHO rightly opted for a

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https://doi.org/10.1016/j.ijid.2019.02.027

pragmatic approach by proposing or maintaining CCs despite limited or poor-quality MIC data because otherwise no phenotypic DST would have been possible for several key drugs. Only where the evidence was very poor, were CCs withdrawn (e.g. for D-cycloserine on Löwenstein-Jensen medium (WHO, 2018c)). In effect, WHO made the best out of a limited evidence base that was the result of decades of limited investment and research in this area.

The most effective approach to avoid this issue in the future would be for strict regulators such as EUCAST, which sets clinical breakpoints (CBs) on behalf of the European Medicines Agency (EMA), to make the determination of the ECOFF and an accompanying quality control range/target requirements for the initial approval of an agent (Schön et al., 2019). In fact, EUCAST is currently taking steps towards this goal by selecting a non-commercial reference MIC method, for which it will set CBs and against which commercial or other non-commercial methods can be calibrated, as is the case for other bacteria (Schön, personal communication).

This would ensure that MICs from the different phases of clinical trials are directly comparable, which is not always the case currently. Moreover, this would, by definition, avoid the problems that occurred when EUCAST first set CBs for bedaquiline and delamanid. Because EUCAST did not specify to which media its provisional bedaquiline CB applied, it effectively defined resistance inconsistently because the CB of 0.25 mg/L likely corresponds to the ECOFF on 7H11 but is two dilutions below the ECOFF in the Mycobacteria Growth Indicator Tube 960 (MGIT) system (EUCAST, 2015, 2018). As a result, *Mycobacterium tuberculosis* appeared intrinsically resistant on the latter but not the former medium (WHO, 2018c). This has recently been rectified by specifying that the CB applies to 7H10 and 7H11 only. Unfortunately, EUCAST has not yet clarified for which medium its delamanid CB is valid, despite clear evidence of systematic differences between media for delamanid (EUCAST, 2019; WHO, 2018c). In addition, EUCAST has not yet summarised the MIC, pharmacokinetic/pharmacodynamic (PK/PD), and clinical data underpinning these CBs as it does for other bacteria. By contrast, WHO stratified the MIC data for bedaquiline and delamanid by medium and, consequently, set appropriate interim CCs for 7H11 and MGIT, pending additional data from more laboratories to define robust ECOFFs according to EUCAST criteria (EUCAST, 2017; WHO, 2018c).

The difficulties with setting sound CBs are exacerbated by the fact that measuring clinical outcomes for TB is inherently challenging as TB is treated with multiple drugs over prolonged periods (Ängeby et al., 2012). Furthermore, the PK/PD drivers for efficacy of several drugs are poorly understood, which means that drug dosing may not be optimal (Gumbo et al., 2015). Nevertheless, these data are crucial for setting one or two clinical CBs per drug, as defined by EUCAST (Kahlmeter, 2015). Indeed, the ECOFF merely represents the upper end of the distribution of phenotypically wild type strains and is therefore the lowest possible CB, but not automatically the CB (i.e. it is possible to define ECOFFs even for compounds that have no clinical benefit). Two recent decisions illustrate the importance of evaluating clinical data. First, a recent meta-analysis commissioned by WHO showed that the use of capreomycin and kanamycin correlate with unfavourable treatment outcomes, which, assuming that biases were adequately controlled for, suggests that both drugs have no or only limited clinical benefits, although kanamycin likely plays a more important role in the shorter MDR-TB regimen (Collaborative Group for the Meta-Analysis of Individual Patient Data in MDR-TB treatment et al., 2018). Based on the data from that review, the updated WHO treatment guidelines no longer recommend capreomycin and kanamycin for treatment of MDR-TB (WHO, 2018b). Second, clinical evidence is even more important when defining CBs above the ECOFF. In 2014, WHO decided that even the standard dose of moxifloxacin (i.e. 400 mg daily) was sufficient to treat strains with MICs that were up to 8 times higher than what we now know corresponds to the ECOFF for this drug (i.e. 2 mg/L vs. 0.25 mg/L in MGIT). No evidence was provided for this decision (WHO, 2014). This change was reversed in 2018, when WHO extrapolated outcome data for low- and high-dose gatifloxacin to moxifloxacin to restrict the use of standard dose moxifloxacin to strains with MICs equal to or below the ECOFF (WHO, 2018c).

Unlike for antiviral drugs, the elucidation of resistance mechanisms for bacteria is not currently mandated as part of the approval process (Köser et al., 2015). However, thanks to the advances in next-generation sequencing it is now feasible to sequence the genomes of hundreds of mutants selected under different in vitro and in vivo conditions to elucidate most, if not all, resistance genes to a drug. For example, a recent study found that *cofC* (*Rv2983*) not only confers cross-resistance to nitroimidazoles but also renders mutants hypersusceptible to the decontamination agent malachite green (Rifat et al., 2018). Consequently, these mutants may evade detection on solid media that contain this agent. Had this been known earlier, regulators may have required for this to be formally labelled and may have recommended for media without this compound to be used to monitor patients treated with delamanid.

The resistance mechanisms to bedaquiline also have important practical implications. At the time of the approval of bedaquiline in 2012, only mutations in *atpE*, which encodes a subunit of the ATP synthase and represents the target of bedaquiline, were known. It took another two years for mutations in the transcriptional efflux pump repressor MmpR (Rv0678) to be shown to confer cross-resistance to bedaquiline and clofazimine and a further two years for *pepQ* to emerge as another mechanism for cross-resistance (Almeida et al., 2016; Köser et al., 2015). Again, EMA or the United States Food and Drug Administration (FDA) might have included information about this cross-resistance in the label of bedaquiline if these data had been available at the time of its approval (Köser et al., 2015). Moreover, the discovery of *mmpR* and *pepQ* were important since, unlike *aptE*, mutations in these genes confer only modest MIC increases, which means that their resulting MIC distributions are divided by the existing WHO CCs for bedaquiline (WHO, 2018c). Therefore, the reproducibility of phenotypic DST for these mechanisms is likely poor (i.e. there is a high likelihood that resistant strains are misclassified as susceptible because of the variation in testing alone) (WHO, 2018c). Unfortunately, interpreting the genotypic information for both of these non-essential resistance genes is also difficult, as the spectrum of resistance mutations is large (WHO, 2018c). This complicates individual patient treatment as well as efforts to study clinical outcome data for these mechanisms as part of the ongoing clinical trials.

The shortcomings in the regulatory process were compounded by the fact that, until recently, it had been challenging to obtain pure bedaquiline and delamanid. For bedaquiline, applications have to be made to the National Institute of Health in the United States. Until 2018 the substance was released for research use only and is still restricted to 20 mg and 40 mg per year for national and WHO supra-national reference laboratories, respectively. Commercial entities and regional laboratories did not have access to the pure compound. This meant that only a small proportion of the 24,659 patients who had been treated with bedaquiline under programmatic conditions in 40 high TB and MDR-TB burden countries received DST results (DR-TB Scale-Up Treatment Action Team). Pure delamanid was initially only available for research use and upon signing a study specific memorandum of understanding. Because of this, the Swiss national reference laboratory had to resort to using crushed bedaquiline and delamanid for DST when investigating acquired bedaquiline and delamanid resistance in a

patient with extensively drug resistant (XDR) TB (Bloemberg et al., 2015; Keller et al., 2015). This lack of quality-controlled routine DST capacity is concerning as there is some pre-existing resistance to both bedaquiline and delamanid (i.e. natural resistance that is not due to selection by either drug or clofazimine (WHO, 2018c)). Moreover, there is increasing evidence for the rapid selection of *mmpR* mutations, particularly in the context of weak background regimens (Bloemberg et al., 2015; Zimenkov et al., 2017).

The recent decision by WHO to reclassify bedaquiline as a group A drug means that bedaquiline should be used for all MDR-TB patients who are treated with the longer MDR-TB regimens (WHO, 2018b). In 2017, 139,114 patients were started on MDR-TB treatment (out of an estimated 558,000 new cases) (WHO, 2018a). If all patients currently diagnosed with MDR-TB were initiated on bedaquiline containing regimens, the number of patients receiving bedaquiline would quintuple. Although delamanid roll-out has lagged behind, several cohorts of XDR-TB patients have received the drug. Consequently, global DST capacity needs to increase rapidly to provide universal DST for all patients receiving the new drugs (Cox et al., 2018). Specifically, baseline and follow-up isolates need to undergo DST. This will reduce the likelihood of transmitted resistance and will minimise acquired resistance to other group A drugs used in a regimen, such as linezolid.

In summary, despite the consensus that antibiotic resistance represents a key driver for poor treatment outcomes, the standards to define resistance that apply to all other major bacterial pathogens have not been followed for traditional anti-TB drugs (Ängeby et al., 2012; Kahlmeter, 2015). By commissioning two landmark systematic reviews, WHO has taken important steps to rectify this issue, but a more concerted effort is needed by the wider TB field and funders. Six years after the approval of bedaquiline and delamanid, data to define robust CBs based on EUCAST criteria are still lacking. We call on the manufacturers of both drugs to support activities to rectify this shortcoming. Moreover, it is incumbent upon EMA and FDA to ensure that history does not repeat itself when they approve novel anti-TB agents. In addition, because WHO effectively controls the market size for novel drugs, it should adopt a more muscular approach to ensure that pure, quality-controlled compounds are available immediately to all laboratories wishing to implement phenotypic DST. Furthermore, standard operating procedures for DST, a standardised validation protocol, and a set of resistant reference strains need to be made available at the time of approval of an agent, as opposed to with long delays (WHO, 2018d). Finally, external quality assurance schemes are urgently needed to ensure high-quality DST results.

Acknowledgement

The quote in the title is from George Santayana's *The Life of Reason: Reason in Common Sense* (Scribner's, 1905: 284).

Conflicts of interest

C.U.K. is a consultant for the World Health Organization (WHO) Regional Office for Europe, QuantuMDx Group Ltd., and the Foundation for Innovative New Diagnostics, which involves work for the Cepheid Inc., Hain Lifescience and WHO. C.U.K. is an advisor to GenoScreen. The European Society of Mycobacteriology awarded C.U.K. the Gertrud Meissner Award, which is sponsored by Hain Lifescience. The Bill & Melinda Gates Foundation, Janssen Pharmaceutica, and PerkinElmer covered C.U.K.'s travel and accommodation to present at meetings. The Global Alliance for TB Drug Development Inc. and Otsuka Novel Products GmbH have supplied C.U.K. with antibiotics for *in vitro* research. C.U.K. is collaborating with YD Diagnostics. F.P.M. is a consultant for the WHO Regional Office for Europe. The German Society for Hygiene and Microbiology awarded F.P.M. the BD Diagnostics Award, which is sponsored by BD. The Bill & Melinda Gates Foundation and Janssen Pharmaceutica covered K.K.'s travel and accommodation to present at meetings.

Funding sources

This study received no external funding.

Ethical approval

Ethical approval was not required for this study.

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