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Bedaquiline and linezolid MIC distributions and epidemiological cut-off values for *Mycobacterium tuberculosis* in the Latin American region

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Objectives: To describe the distributions of bedaquiline and linezolid MIC values for the *Mycobacterium tuberculosis* WT population and to define the corresponding epidemiological cut-offs (ECOFFs) in three Latin American countries.

Methods: MICs of bedaquiline and linezolid were determined by the resazurin microtitre assay (REMA). In phase 1, interlaboratory reproducibility was assessed using a panel of 10 fully susceptible *M. tuberculosis* strains. Phase 2 involved MIC determination for 248 clinical isolates from Argentina ($n = 58$), Brazil ($n = 100$) and Peru ($n = 90$) from patients who were treatment-naive for bedaquiline and linezolid. We then determined the ECOFFs for bedaquiline and linezolid by the eyeball method and the ECOFFinder statistical calculator.

Results: Phase 1: REMA MIC values in the three sites were either identical to each other or differed by one 2-fold dilution from the consensus value with the exception of a single value. Phase 2: the bedaquiline MIC range was 0.0039–0.25 mg/L for pan-susceptible and drug-resistant isolates combined. The linezolid MIC range was 0.062–0.5 mg/L for pan-susceptible isolates and 0.031–4 mg/L for drug-resistant isolates. ECOFFs were 0.125 mg/L for bedaquiline and 0.50 mg/L for linezolid.

Conclusions: REMA is reproducible and robust for the determination of bedaquiline and linezolid MIC distributions and ECOFF values when applied in laboratories of medium/low-resource countries. We suggest that WT MIC distributions for both drugs should be used as a monitoring tool to control the possible rapid emergence of resistance.

Introduction

The emergence of drug resistance in TB calls for the advent of improved treatment regimens and new drugs with different modes of action to avoid the generation of new drug resistance. Although the development of new TB drugs is overlong and expensive, a great achievement has been made in the global anti-TB drug pipeline.[1] Several FDA-approved candidates exhibit promising anti-TB activity *in vitro* as well as in clinical trials. Within this context, two anti-TB drugs have recently been introduced into the anti-TB drug armamentarium. Bedaquiline, formerly known as TMC207,[2] has now been evaluated in various clinical studies and approved by the US FDA and the EMA for treatment of MDR- and XDR-TB.[3–5] Linezolid, an oxazolidinone antibiotic used for the treatment of infections caused by Gram-positive bacteria,[6,7] has also demonstrated excellent activity against drug-resistant strains of *Mycobacterium tuberculosis* in both *in vitro* and animal studies. It has been introduced into the treatment of MDR-TB[8,9] and is currently being evaluated in Phase 3 clinical trials as a repurposed drug for the treatment of TB.[10]

Table 1. Bedaquiline and linezolid MIC values obtained for 10 fully susceptible *M. tuberculosis* clinical isolates and the H37Rv strain in reproducibility experiments performed in three Latin American laboratories

	Bedaquiline MIC (mg/L)				Linezolid MIC (mg/L)			
Isolate/strain	site 1	site 2	site 3	mode	site 1	site 2	site 3	mode
26054	0.031	0.062	0.062	0.062	0.500	0.250	0.250	0.250
26057	0.031	0.031	0.062	0.031	0.250	0.250	0.250	0.250
26065	0.031	0.062	0.062	0.062	0.250	0.250	0.250	0.250
26111	0.031	0.031	0.062	0.031	0.250	0.250	0.250	0.250
26133	0.031	0.031	0.062	0.031	0.250	0.250	0.250	0.250
26163	0.031	0.031	0.062	0.031	0.250	0.250	0.250	0.250
26167	0.031	0.031	0.062	0.031	0.250	0.250	0.250	0.250
26174	0.031	0.031	0.062	0.031	0.500	0.250	0.250	0.250
26181	0.015	0.015	0.062	0.015	0.250	0.125	0.250	0.250
26232	0.031	0.031	0.062	0.031	0.250	0.250	0.250	0.250
H37Rv	0.015	0.015	0.031	0.015	0.250	0.125	0.250	0.250

A sharp definition of susceptibility/resistance criteria for new and repurposed drugs is critical for the accurate and reproducible drug susceptibility testing (DST) of *M. tuberculosis*. Traditionally, the DST of *M. tuberculosis* was based on a critical concentration of the drug, defined as the lowest concentration inhibiting 95% of WT strains (i.e. those that have never been exposed to drugs), while not inhibiting strains considered as resistant (i.e. those obtained from patients not responding to treatment).[11] In many cases, critical concentrations were not determined based on evidence, but assumed by convention. The use of inadequate breakpoints can lead to low reproducibility of the results, which in turn can have undesired consequences for the treatment of patients.

The emergence of drug resistance to bedaquiline is already an ongoing threat.[12] The WHO Expert Group recommended that resistance to bedaquiline should be monitored through MIC assessment.[13] Resistance to linezolid in *M. tuberculosis* is still unusual, but can also occur. A study analysing 210 MDR strains found 1.9% of strains to be resistant to linezolid.[14] Thus, testing susceptibility to these drugs is required to support their clinical use. There are no reports on WT MIC distributions of bedaquiline and linezolid for *M. tuberculosis* in the Latin American region. The objective of this study was to describe the distributions of bedaquiline and linezolid MICs for the *M. tuberculosis* wild population and to define the corresponding epidemiological cut-offs (ECOFFs), i.e. the upper limits of these distributions, in three Latin American countries. This knowledge will contribute to the development of standard methods and protocols that will assist countries in establishing proper *in vitro* susceptibility testing for these drugs.

Materials and methods

Study sites

The study was coordinated by the Université catholique de Louvain, Laboratory of Medical Microbiology in Brussels, Belgium, and conducted in three participating sites: Instituto Nacional de Enfermedades Infecciosas Carlos G. Malbrán, Buenos Aires, Argentina; Núcleo de Tuberculose e Micobacterioses, Instituto Adolfo Lutz, São Paulo, Brazil; and National Reference Centre for Mycobacteria, Instituto Nacional de Salud, Lima, Peru.

Study design

The study was performed in two phases. Phase 1 was the validation of the resazurin microtitre assay (REMA) procedure to determine the range of MIC values of each drug using a panel of strains to assess the reproducibility of the results. The panel consisted of 10 fully susceptible, treatment-naive, *M. tuberculosis* strains with no known risk factor for drug resistance. The panel was sent to all participants, together with a detailed protocol, drugs and media to ensure the use of identical procedures and reagents in all settings. The *M. tuberculosis* laboratory strain H37Rv (ATCC 27294) was included as a control in the experiments. The reproducibility of REMA was assessed by cross-checking the results of the 10 strains blind-tested at each participating site. Phase 2 involved the testing of clinical isolates obtained at each site from patients who were treatment-naive for bedaquiline and linezolid according to laboratory records. The 248 isolates included in the study were recovered from patients originating from Argentina ($n = 58$), Brazil ($n = 100$) and Peru ($n = 90$). The isolates (one per patient) originated from diverse geographical locations in each participant country and had no record of epidemiological links to each other. The susceptibility pattern classified the isolates as 161 (64.9%) pan-susceptible, 29 (11.7%) MDR, 18 (7.3%) pre-XDR and 40 (16.1%) XDR. This work used collections of isolates whose numbers have been coded. Treatment history was obtained and handled blindly from the records of the participant laboratories without disclosing the patient's identity.

MIC determination by REMA

At each participating site, bedaquiline and linezolid MICs for *M. tuberculosis* were determined using the REMA, one of the methods previously endorsed and recommended by WHO as a non-commercial method for DST of *M. tuberculosis* and recommended by the US FDA in 2012 for bedaquiline testing.[3,15,16] Briefly, 2-fold serial dilutions were made in 7H9 medium supplemented with 0.1% casitone, 10% OADC and 0.5% glycerol in 96-well polystyrene plates. The concentration ranges were 1.0–0.0039 mg/L for bedaquiline and 4.0–0.015 mg/L for linezolid. A bacterial inoculum with a turbidity equivalent to that of a 1 McFarland standard diluted 1:20 was prepared for each strain. A positive control (bacteria without drug) and two negative controls (only medium and medium with drug) were included in each assay. To prevent evaporation during incubation, 200 μL of sterile distilled water was added to all perimeter wells. Plates were sealed and incubated at 37°C for 7 days before adding 30 μL of 0.01% resazurin to all wells and incubating for a further 24 h. The MIC value was determined as the lowest drug concentration that prevented growth and, therefore, a colour change from blue (oxidized state) to pink (reduced state). MIC values were scored for each isolate tested.

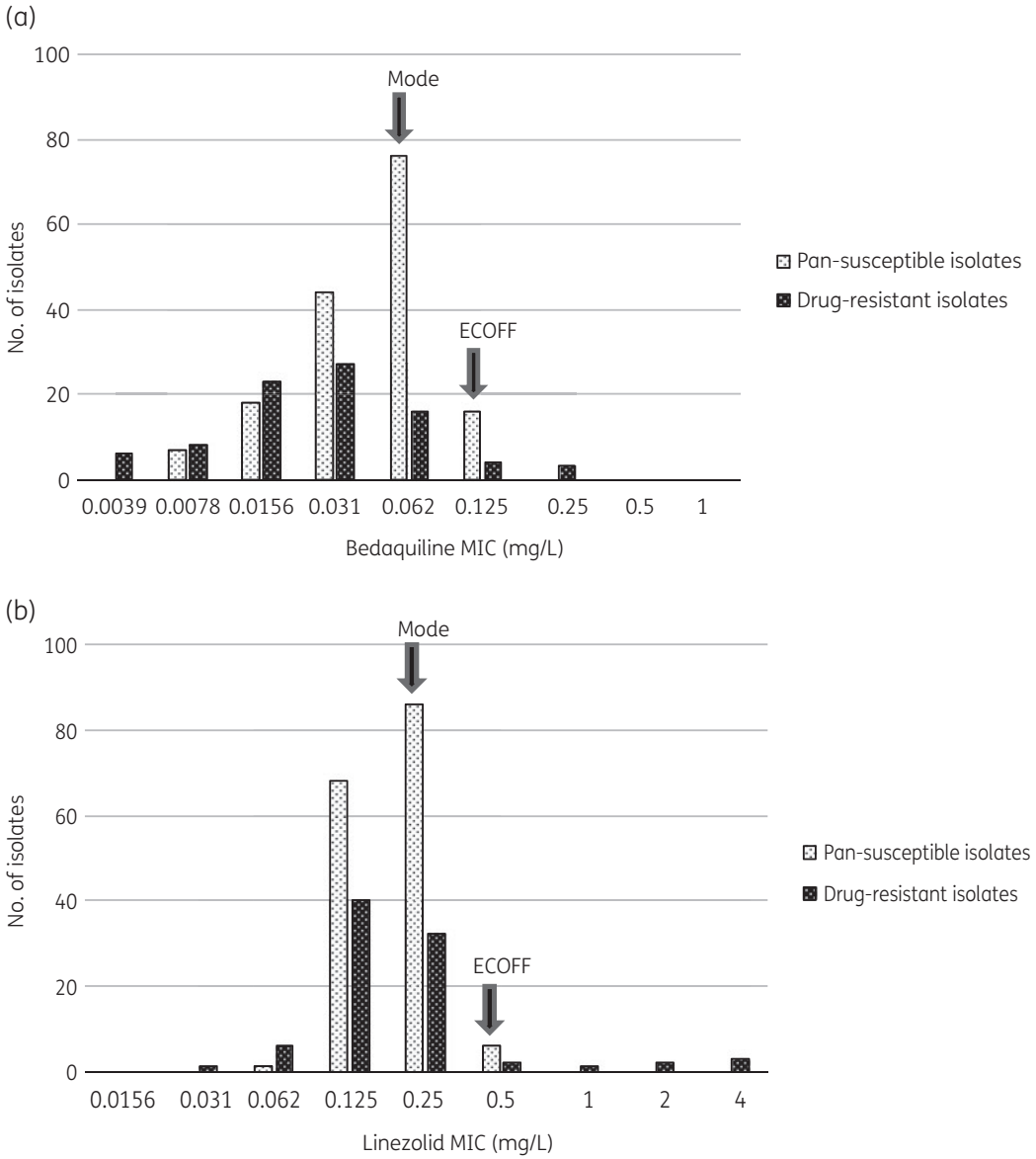

Figure 1. Distributions of MIC values of bedaquiline (a) and linezolid (b) for 248 clinical isolates from Argentina, Brazil and Peru. Strains were classified based on their susceptibility patterns. Values for pan-susceptible isolates (light bars) were used to determine ECOFF values by the eyeball method (marked with arrows). MIC values for drug-resistant isolates are represented by the dark bars.

Antimicrobial agents

Pure bedaquiline powder was obtained through the NIH, USA, through the NIH AIDS Reagent Program (http://aidsreagent.org) and linezolid powder was obtained from Sigma–Aldrich (PZ0014-5MG). Linezolid and bedaquiline were both dissolved in DMSO. The stock solutions were made at a concentration of 1 mg/L and stored in small aliquots at −20°C. Frozen drug solutions were thawed once and discarded afterward and working solutions were not stored.

MIC distributions and ECOFF determination

We determined the mode and the ECOFFs for bedaquiline and linezolid by the conventional eyeball method, which consists of the visual inspection of histograms of WT MICs for single species. The mode was defined as the most frequent MIC value and the ECOFF as the upper MIC value for the WT distribution. Additionally, we applied a Microsoft Excel spreadsheet calculator (ECOFFinder program version XL 2000+, https://clsi.org/education/microbiology/ecoffinder/) for statistical bedaquiline and linezolid ECOFF determination using 97.5% endpoints for pan-susceptible isolates and the different groups of drug-resistant isolates.[17]

Results

Phase 1: validation of the REMA procedure

Table 1 shows the results for the 10 strains blind-tested in the participating laboratories. The mode of the MIC values obtained for each strain at the three sites was considered the consensus value for that strain. Linezolid MICs fell within comparable narrow ranges

Table 2. *M. tuberculosis* MIC parameters for bedaquiline and linezolid as determined by the ECOFFinder calculator (https://clsi.org/education/microbiology/ecoffinder/) in the whole study population and in subgroups of isolates classified according to drug susceptibility testing profiles

Parameter	All isolates ($n = 248$)	Pan-susceptible isolates ($n = 161$)	Drug-resistant isolates			
			all ($n = 87$)	MDR ($n = 29$)	pre-XDR ($n = 18$)	XDR ($n = 40$)
Bedaquiline						
MIC mode (mg/L)	0.062	0.062	0.031	0.016	0.016	0.031
$\log_2$ MIC mode	−4	−4	−5	−6	−6	−5
ECOFF 97.5% (mg/L)	0.250	0.125	0.125	0.125	0.125	0.125
observed >97.5% (%)	0	0	3.4	0	0	7.5
Linezolid						
MIC mode (mg/L)	0.250	0.250	0.125	0.250	0.125	0.125
$\log_2$ MIC mode	−2	−2	−3	−2	−3	−3
ECOFF 97.5% (mg/L)	0.500	0.500	0.500	0.500	0.250	0.250
observed >97.5% (%)	2.4	0	6.9	0	0	17.5

Table 3. Characteristics and results of various studies on bedaquiline and linezolid MIC distributions and ECOFF values for *M. tuberculosis*

Source	No. of isolates (a) or no. of observations (b)	MIC range (mg/L)	Microbiological method	ECOFF (c) or MIC_{90} (d) (mg/L)	Country/ region
Bedaquiline					
Torrea *et al.* (2015)[18]	47 (a)	0.03–1.0	MGIT960	1.0 (c)	miscellaneous
Keller *et al.* (2015)[19]	23 (a)	0.2–1.6	MGIT960	1.6 (d)	Europe
Kaniga *et al.* (2016)[20]	204/232 (b)[a]	0.015–0.12[a]	7H10/7H11 agar	–	–
Kaniga *et al.* (2016)[20]	207 (b)[a]	0.015–0.06[a]	7H9 broth	–	–
Jang *et al.* (2017)[21]	12 (a)	0.125–0.5	DAC	–	Korea
Ismail *et al.* (2018)[23]	378 (a)	0.125–0.25	7H9 broth	0.125 (c)	South Africa
Yang *et al.* (2018)[24]	420 (a)	≤0.03 to >4.0	7H9 broth	ND	Korea
this study	248 (a)	0.0039–0.25	REMA	0.125 (c)	Brazil, Peru, Argentina
Linezolid					
Alcalá *et al.* (2003)[9]	117 (a)	0.125–1.0	7H10 agar	1.0 (d)	Spain
Ermertcan *et al.* (2009)[25]	67 (a)	0.06–1.0	7H10 agar	0.5 (d)	Turkey
Schön *et al.* (2011)[26]	78 (a)	0.125–0.5	7H10 agar	0.5 (c)	Sweden
Yang *et al.* (2012)[27]	84 (a)	0.125–0.5	7H10 agar	0.25 (d)	China
Ahmed *et al.* (2013)[28]	102 (a)	0.25–2.0	7H10 agar	ND	Pakistan
Liu *et al.* (2015)[29]	61 (a)	0.125–1.0	7H10 agar	0.25 (d)	China
Patidar *et al.* (2015)[30]	90 (a)	0.125–1.0	REMA	ND	India
Yang *et al.* (2018)[24]	457 (a)	≤0.125–1.0	7H9 broth	ND	Korea
this study	248 (a)	0.031–4.0	REMA	0.5 (c)	Brazil, Peru, Argentina

MIC_{90}, lowest concentration of the drug at which 90% of the isolates are inhibited; ND, not determined; DAC, disc agarose channel system.
[a]Number of observations and MIC ranges of multiple determinations for strain H37Rv.

at the three sites, but bedaquiline MICs at site 3 were consistently higher than at the other two sites. In all, values for each individual strain obtained at the three sites were either identical to each other or differed in only one 2-fold dilution from the consensus value, except for a single bedaquiline MIC value (strain 26181, site 3), which exceeded by two 2-fold dilutions the mode for that strain. The bedaquiline and linezolid MIC values for *M. tuberculosis* H37Rv were 0.015 and 0.25 mg/L, respectively.

Phase 2: distributions of bedaquiline and linezolid MICs for clinical isolates

Considering all 248 clinical isolates, the overall MIC range of bedaquiline was 0.0039–0.25 mg/L, with similarly broad patterns of frequency for pan-susceptible and drug-resistant isolates. On the other hand, the MIC range of linezolid had an enclosed normal distribution between 0.062 and 0.5 mg/L for pan-susceptible isolates, and a particularly expansive pattern of frequency for the

distribution of drug-resistant isolates (0.031–4 mg/L). Based on the pan-susceptible group, the mode and the ECOFF were 0.062 and 0.125 mg/L for bedaquiline, and 0.25 and 0.50 mg/L for linezolid (Figure 1). The 97.5% statistical calculator yielded these same values for linezolid in the pan-susceptible group as well as in the whole study population. The mode for bedaquiline was also the same, while the ECOFF value of the whole population differed by only one 2-fold dilution from that of the pan-susceptible group (Table 2). Table 3 summarizes the overall values for bedaquiline and linezolid in our study compared with values from similar studies available in the literature.[9,18–29]

Discussion

The use of both solid media assays (egg-based Löwenstein–Jensen and agar-based 7H10 and 7H11) and commercial systems in liquid media (e.g. BACTEC MGIT) for determining MICs is time consuming. BACTEC MGIT also requires expensive specialized instrumentation and reagents. In 2002, we developed REMA, a simple, low-cost assay to rapidly determine the MICs of first- and second-line TB drugs for *M. tuberculosis*, which was shown to be highly sensitive and specific in a systematic review.[15] It is a robust microtitre-plate method that facilitates routine MIC determination in similar biosafety conditions as BACTEC MGIT. REMA also provides a flexible high-throughput platform for screening new and repurposed drugs at wide ranges of concentrations.[31,32]

Our group has previously found that bedaquiline MICs were reliably measured using REMA as shown in the study by Diacon *et al.*,[33] where 70% of the 43 baseline isolates had bedaquiline MICs $\leq$0.03 mg/L and 95% had bedaquiline MICs $\leq$0.12 mg/L (range 0.003–0.24 mg/L). Herein, we further show that REMA is reproducible for assessing bedaquiline and linezolid MIC distributions and ECOFF values when applied in laboratories of medium/low-resource countries. Bedaquiline and linezolid MIC values found in our study for *M. tuberculosis* H37Rv resulted close (one 2-fold dilution lower) to the published MIC values for this strain, further supporting the validity of the approach.[2] One participant site in our study tended to produce consistently higher bedaquiline MIC values than the other two sites. As reported by other authors,[18,19] these discrepancies could be ascribed to systematic differences in methodology.[22] In any case, the inter-laboratory differences observed in our study were modest as the mode varied $\leq$2-fold dilutions and the ECOFF varied by only one dilution among sites.

Bedaquiline MICs, critical concentrations and ECOFFs vary amply with the different methodologies. Using MGIT for evaluation, Torrea *et al.*[18] and Keller *et al.*[19] reported ECOFFs $\geq$1.0 mg/L, matching the interim critical concentration recommended by WHO for MGIT (1.0 mg/L).[34] For 7H11 agar WHO adopted a much lower interim critical concentration (0.25 mg/L), identical to that set by the EUCAST for agar-based media.[35] The higher concentrations needed for the MGIT assay have been ascribed to bedaquiline interaction with either labware plastics or Tween 80 concentrations in broth.[22] The MICs observed for the clinical isolates analysed in our study cover a rather narrow range with an ECOFF set as low as 0.125 mg/L, identical to that reported by Ismail *et al.*[23] for 7H9 broth microdilution. Our results may reflect the fact that most people living in the participating Latin American countries have hardly yet been exposed to this novel drug. Other authors[21,24] reported either narrower or much broader ranges (Table 3).

The linezolid MIC distribution for the *M. tuberculosis* clinical isolates in our study (0.031–4 mg/L) was within the range of values observed in other reports (Table 3). As expected, the MIC range was clearly broader than that of bedaquiline, probably because linezolid has been longer in use and, in most countries including those participating in our study, is approved for treating common infections. Even though our laboratory records showed no evidence of any of the isolates in the study being previously exposed to linezolid, it has been used for the compassionate treatment of MDR- and XDR-TB in Brazil and Argentina for several years.[36–38] Thus, it is not surprising that the highest MIC values (1–4 mg/L) obtained in our study corresponded to XDR isolates. We cannot rule out the possibility that those patients had been infected with MDR or XDR *M. tuberculosis* bacilli that were already resistant to linezolid or even had been treated with this drug for non-mycobacterial diseases. Further studies are necessary to investigate those isolates with MIC values above the ECOFF for the presence of mutation in *rplC* (encoding the ribosomal protein L3) or *rrl* (the 23S rRNA gene).[39,40]

Our work has two flaws. First, it was performed in only three countries and values are not wholly representative of the Latin American region. To our knowledge, however, this is the first study on bedaquiline and linezolid ECOFFs performed with *M. tuberculosis* strains that are circulating in Latin America. It provides a reliable picture of MIC distributions and ECOFF values in three countries in the region, further endorsing the utility of REMA for bedaquiline and linezolid MIC determination. Two of the participating countries contribute significantly to the TB pandemic. Brazil is in the top 30 list of high TB burden countries (TB prevalence 42/100 000) and Peru is in the top 30 MDR-TB burden countries.[41] Second, we did not perform isolate genotyping and thus were unable to verify either strain diversity or dependence of the MIC distributions on isolate lineage. Although we cannot overcome this limitation, clustering among isolates is unlikely because, to our knowledge, no epidemiological link existed among them.

It should be noted that our study fulfils the following requirements set by EUCAST (http://www.eucast.org/mycobacteria/): (i) MICs determined with methods calibrated to an internationally agreed standard method for broth microdilution; (ii) large numbers of MICs ($n \geq 100$); (iii) MICs from different places ($\geq$3); and (iv) MICs performed by many investigators ($\geq$3).[24]

This study was endorsed by the Pan American Health Organization, which is assisting the national TB reference laboratories in the Latin American region to improve data quality and ensure that appropriate standardization and methodology are in place for *M. tuberculosis* laboratory techniques. Results of this work will contribute to the drafting of protocols and the implementation of appropriate DST methods for the screening of anti-TB drugs in the Latin-American region and will encourage other countries in the region and the rest of the world to perform equivalent studies.

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Transparency declarations

None to declare.

References

1 Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens. *Nat Rev Drug Discov* 2013; **12**: 388–404.

2 Andries K, Verhasselt P, Guillemont J *et al*. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science* 2005; **307**: 223–7.

3 *Prescribing Information for Sirturo (Bedaquiline) Tablets*. Titusville, NJ, USA: Janssen Therapeutics, 2012. http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/204384s000lbl.pdf.

4 EMA. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002614/WC500163209.pdf.

5 ClinicalTrials.gov. *Identifier NCT02454205. An Open-Label RCT to Evaluate a New Treatment Regimen for Patients with Multi-Drug Resistant Tuberculosis (NEXT)*. 2015. https://clinicaltrials.gov/ct2/show/study/NCT02454205.

6 Till M, Wixson RL, Pertel PE. Linezolid treatment for osteomyelitis due to vancomycin-resistant *Enterococcus faecium*. *Clin Infect Dis* 2002; **34**: 1412–4.

7 Yanagihara K, Kaneko Y, Sawai T *et al*. Efficacy of linezolid against methicillin-resistant or vancomycin-insensitive *Staphylococcus aureus* in a model of hematogenous pulmonary infection. *Antimicrob Agents Chemother* 2002; **46**: 3288–91.

8 Fortún J, Martín-Dávila P, Navas E *et al*. Linezolid for the treatment of multidrug-resistant tuberculosis. *J Antimicrob Chemother* 2005; **56**: 180–5.

9 Alcalá L, Ruiz-Serrano MJ, Pérez-Fernández-Turégano C *et al*. In vitro activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* that are susceptible or resistant to first-line antituberculosis drugs. *Antimicrob Agents Chemother* 2003; **47**: 416–7.

10 ClinicalTrials.gov. *Identifier NCT02333799. A Phase 3 Study Assessing the Safety and Efficacy of Bedaquiline Plus PA-824 Plus Linezolid in Subjects With Drug Resistant Pulmonary Tuberculosis*. https://clinicaltrials.gov/ct2/show/NCT02333799. 2015.

11 WHO. *Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*. Geneva, Switzerland: WHO, 2008. http://www.who.int/tb/challenges/mdr/programmatic_guidelines_for_mdrtb/en/.

12 Somoskovi A, Bruderer V, Hömke R *et al*. A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment. *Eur Respir J* 2015; **45**: 554–7.

13 WHO. *The Use of Bedaquiline in the Treatment of Multidrug-Resistant Tuberculosis: Interim Policy Guidance*. Geneva, Switzerland: WHO, 2013. http://apps.who.int/iris/bitstream/10665/75146/1/9789241548441_eng.pdf.

14 Richter E, Rüsch-Gerdes S, Hillemann D. First linezolid-resistant clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2007; **51**: 1534–6.

15 Martin A, Portaels F, Palomino JC. Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. *J Antimicrob Chemother* 2007; **59**: 175–83.

16 WHO. *Noncommercial Culture and Drug-Susceptibility Testing Methods for Screening Patients at Risk for Multidrug-Resistant Tuberculosis: Policy Statement*. WHO/HTM/TB/2011.9. Geneva, Switzerland: WHO, 2011.

17 Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect* 2006; **12**: 418–25.

18 Torrea G, Coeck N, Desmaretz C *et al*. Bedaquiline susceptibility testing of *Mycobacterium tuberculosis* in an automated liquid culture system. *J Antimicrob Chemother* 2015; **70**: 2300–5.

19 Keller PM, Hömke R, Ritter C *et al*. Determination of MIC distribution and epidemiological cutoff values for bedaquiline and delamanid in *Mycobacterium tuberculosis* using the MGIT 960 system equipped with TB eXiST. *Antimicrob Agents Chemother* 2015; **59**: 4352–5.

20 Kaniga K, Cirillo DM, Hoffner S *et al*. A multilaboratory, multicountry study to determine bedaquiline MIC quality control ranges for phenotypic drug susceptibility testing. *J Clin Microbiol* 2016; **54**: 2956–62.

21 Jang JC, Jung YG, Choi J *et al*. Bedaquiline susceptibility test for totally drug-resistant *Mycobacterium tuberculosis*. *J Microbiol* 2017; **55**: 483–7.

22 Lounis N, Vranckx L, Gevers T *et al*. In vitro culture conditions affecting minimal inhibitory concentration of bedaquiline against *M. tuberculosis*. *Med Mal Infect* 2016; **46**: 220–5.

23 Ismail NA, Omar SV, Joseph L *et al*. Defining bedaquiline susceptibility, resistance, cross-resistance and associated genetic determinants: a retrospective cohort study. *EBioMedicine* 2018; **28**: 136–42.

24 Yang JS, Kim KJ, Choi H *et al*. Delamanid, bedaquiline, and linezolid minimum inhibitory concentration distributions and resistance-related gene mutations in multidrug-resistant and extensively drug-resistant tuberculosis in Korea. *Ann Lab Med* 2018; **38**: 563–8.

25 Ermertcan S, Hosgor-Limoncu M, Erac B *et al*. In vitro activity of linezolid against *Mycobacterium tuberculosis* strains isolate from Western Turkey. *Jpn J Infect Dis* 2009; **62**: 384e5.

26 Schön T, Juréen P, Chryssanthou E *et al*. Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2011; **15**: 502–9.

27 Yang C, Lei H, Wang D *et al*. In vitro activity of linezolid against clinical isolates of *Mycobacterium tuberculosis*, including multidrug-resistant and extensively drug-resistant strains from Beijing, China. *Jpn J Infect Dis* 2012; **65**: 240–2.

28 Ahmed I, Jabeen K, Inayat R *et al*. Susceptibility testing of extensively drug-resistant and pre-extensively drug-resistant *Mycobacterium tuberculosis* against levofloxacin, linezolid, and amoxicillin-clavulanate. *Antimicrob Agents Chemother* 2013; **57**: 2522–5.

29 Liu H, Wang Y, Liu N *et al*. *In vitro* activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* from Shenyang, north of China. *Indian J Med Microbiol* 2015; **33** Suppl: 164–5.

30 Patidar V, Hemvani N, Chitnis DS. Evaluation of drug susceptibility to rifampicin, INH and linezolid using resazurine microtitre assay. *Int J Curr Microbiol App Sci* 2015; **4**: 404–12.

31 Aguilar-Ayala DA, Cnockaert M, André E *et al*. In vitro activity of bedaquiline against rapidly growing nontuberculous mycobacteria. *J Med Microbiol* 2017; **66**: 1140–3.

32 Maltempe FG, Caleffi-Ferracioli KR, do Amaral RCR *et al*. Activity of rifampicin and linezolid combination in *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* 2017; **104**: 24–9.

33 Diacon AH, Donald PR, Pym A *et al*. Randomized pilot trial of eight weeks of bedaquiline (TMC207) treatment for multidrug-resistant tuberculosis: long-term outcome, tolerability, and effect on emergence of drug resistance. *Antimicrob Agents Chemother* 2012; **56**: 3271–6.

34 WHO. *Technical Report on Critical Concentrations for Drug Susceptibility Testing of Medicines Used in the Treatment of Drug-Resistant Tuberculosis*. WHO/CDS/TB/2018.5. Geneva, Switzerland: WHO, 2018. http://www.who.int/iris/handle/10665/260470.

35 EUCAST. *Definitions of Clinical Breakpoints and Epidemiological Cut-Off Values. Clinical Breakpoints Table V.5.0*. http://www.eucast.org/clinical_breakpoints/.

36 Arbex MA, Bonini EH, Kawakame Pirolla G *et al*. Effectiveness and safety of imipenem/clavulanate and linezolid to treat multidrug and extensively drug-resistant tuberculosis at a referral hospital in Brazil. *Rev Port Pneumol* 2016; **22**: 337–41.

37 Abbate E, Vescovo M, Natiello M *et al*. Successful alternative treatment of extensively drug-resistant tuberculosis in Argentina with a combination of linezolid, moxifloxacin and thioridazine. *J Antimicrob Chemother* 2012; **67**: 473–7.

38 Palmero D, González Montaner P *et al*. First series of patients with XDR and pre-XDR TB treated with regimens that included meropenem-clavulanate in Argentina. *Arch Bronconeumol* 2015; **51**: e49–52.

39 Beckert P, Hillemann D, Kohl TA *et al*. *rplC* T460C identified as a dominant mutation in linezolid-resistant *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* 2012; **56**: 2743–5.

40 Zimenkov DV, Nosova EY, Kulagina EV *et al*. Examination of bedaquiline- and linezolid-resistant *Mycobacterium tuberculosis* isolates from the Moscow region. *J Antimicrob Chemother* 2017; **72**: 1901–6.

41 WHO. *Global Tuberculosis Report 2017*. ISBN 978-92-4-156551-6. Geneva, Switzerland: WHO.