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SUSCEPTIBILITY

Comparison of *In Vitro* Activity and MIC Distributions between the Novel Oxazolidinone Delpazolid and Linezolid against Multidrug-Resistant and Extensively Drug-Resistant *Mycobacterium tuberculosis* in China

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ABSTRACT Oxazolidinones are efficacious in treating mycobacterial infections, including tuberculosis (TB) caused by drug-resistant *Mycobacterium tuberculosis*. In this study, we compared the *in vitro* activities and MIC distributions of delpazolid, a novel oxazolidinone, and linezolid against multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) in China. Additionally, genetic mutations in 23S rRNA, *rplC*, and *rplD* genes were analyzed to reveal potential mechanisms underlying the observed oxazolidinone resistance. A total of 240 *M. tuberculosis* isolates were included in this study, including 120 MDR-TB isolates and 120 XDR-TB isolates. Overall, linezolid and delpazolid MIC_{90} values for *M. tuberculosis* isolates were 0.25 mg/liter and 0.5 mg/liter, respectively. Based on visual inspection, we tentatively set epidemiological cutoff (ECOFF) values for MIC determinations for linezolid and delpazolid at 1.0 mg/liter and 2.0 mg/liter, respectively. Although no significant difference in resistance rates was observed between linezolid and delpazolid among XDR-TB isolates ($P > 0.05$), statistical analysis revealed a significantly greater proportion of linezolid-resistant isolates than delpazolid-resistant isolates within the MDR-TB group ($P = 0.036$). Seven (53.85%) of 13 linezolid-resistant isolates were found to harbor mutations within the three target genes. Additionally, 1 isolate exhibited an amino acid substitution (Arg126His) within the protein encoded by *rplD* that contributed to high-level resistance to linezolid (MIC of >16 mg/liter), compared to a delpazolid MIC of 0.25. In conclusion, *in vitro* susceptibility testing revealed that delpazolid antibacterial activity was comparable to that of linezolid. A novel mutation within *rplD* that endowed *M. tuberculosis* with linezolid, but not delpazolid, resistance was identified.

KEYWORDS delpazolid, linezolid, multidrug-resistant tuberculosis, MIC, minimal inhibitory concentration

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex, is one of humankind's deadliest diseases (1). According to estimates by the World Health Organization, there were 10.4 million new incident cases and 1.67 million deaths due to TB in 2016 (1). Despite the decreases in incidence and mortality rates during the past decade (2), the emergence of drug-resistant TB, especially multidrug-resistant TB (MDR-TB) (defined as TB with *in vitro* resistance to rifampin and isoniazid) and extensively drug-resistant TB (XDR-TB) (defined as MDR-TB with *in vitro* resistance to any fluoro-

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quinolone and at least one of the second-line injectable drugs [kanamycin, amikacin, or capreomycin]), is creating major obstacles that will hinder disease control efforts worldwide (3). Due to emerging resistance to the two most potent first-line drugs, the treatment of MDR-TB requires more toxic, more costly, and less effective second-line treatment regimens, with poorer clinical outcomes than those achieved for drug-susceptible cases (3, 4). To make matters worse, additional resistance to fluoroquinolones and second-line injectable drugs leads to clinically almost incurable results for treatment of XDR-TB infections using current second-line regimens (4). Therefore, the epidemic of MDR- and XDR-TB highlights an urgent need for new antibiotics with improved safety, tolerability, and efficacy (5).

The oxazolidinones, a new class of synthetic antibiotics, exhibit good activity against Gram-positive pathogenic bacteria, including those resistant to other agents. Delpazolid (research code LCB01-0371), a novel oxazolidinone containing a cyclic amidrazone group, was evaluated for safety, tolerability, and pharmacokinetics in a recently completed phase I clinical trial (6). Preliminary studies have demonstrated that oxazolidinones inhibit the biosynthesis of bacterial proteins at an early stage of translation by binding to domain V of 23S rRNA (7). As a consequence, mutations in 23S rRNA and two ribosomal proteins, i.e., L3 (*rplC*) and L4 (*rplD*), are involved in the major mechanism employed by various pathogenic organisms conferring resistance to oxazolidinones (8, 9). Linezolid is efficacious in treating mycobacterial infections, including drug-resistant TB (10–13). A recent meta-analysis revealed that more than 90% of MDR-TB cases achieved culture conversion after treatment with individualized regimens containing linezolid, underscoring the excellent *in vivo* efficacy of the drug against MDR-TB (5). Recently, the novel oxazolidinone delpazolid was developed to produce improved antibacterial activity and safety (14, 15). *In vitro* studies and pharmacological evidence have indicated that this new agent is more active than linezolid against various Gram-positive bacteria (14); however, data regarding the *in vitro* activity of delpazolid against MDR- and XDR-TB are limited. In this study, we compared the *in vitro* activity and MIC distribution of the novel oxazolidinone delpazolid with those of linezolid against MDR- and XDR-TB in China. In addition, genetic mutations in 23S rRNA, *rplC*, and *rplD* were analyzed to explore potential mechanisms underlying *M. tuberculosis* oxazolidinone resistance.

RESULTS

Linezolid and delpazolid MICs for MDR- and XDR-TB isolates. The MICs of linezolid and delpazolid against *M. tuberculosis* isolates and the percentages of resistant strains are summarized in Table 1. Overall, the linezolid and delpazolid MIC_{90} values for *M. tuberculosis* isolates were 0.25 mg/liter and 0.5 mg/liter, respectively. Against MDR-TB, the MIC_{90} of delpazolid (MIC_{90}, 0.5 mg/liter) was lower than that of linezolid (MIC_{90}, 1.0 mg/liter). In contrast, the MIC_{90} of delpazolid (MIC_{90}, 1.0 mg/liter) against XDR-TB was 4-fold higher than the MIC_{90} of linezolid (MIC_{90}, 0.25 mg/liter).

We further analyzed the tentative epidemiological cutoff (ECOFF) values for linezolid and delpazolid. As shown in Fig. 1, the MIC distributions for linezolid and delpazolid were bimodal. Therefore, on the basis of visual inspection, we set tentative ECOFFs for MIC determinations at 1.0 mg/liter and 2.0 mg/liter for linezolid and delpazolid, respectively. Notably, the ECOFFs of linezolid were consistent with the breakpoints used for the determination of *in vitro* linezolid resistance in previous studies. When 1.0 mg/liter was used as the cutoff value, 8 (6.67% [8/120 isolates]) and 5 (4.17% [5/120 isolates]) MDR- and XDR-TB isolates, respectively, were resistant to linezolid. For delpazolid, resistance was noted for 1 (0.83% [1/120 isolates]) and 5 (4.2% [5/120 isolates]) MDR- and XDR-TB isolates, respectively. Although there was no significant difference in the resistance rates for linezolid and delpazolid among the XDR-TB isolates tested ($P > 0.05$), statistical analysis revealed that the proportion of linezolid-resistant isolates was significantly greater than the proportion of delpazolid-resistant isolates within the MDR group ($P = 0.036$). Of the 13 linezolid-resistant isolates, 6 (46.2%) were resistant to delpazolid, whereas the other 7 isolates were susceptible to delpazolid, including 1

TABLE 1 Distribution of *M. tuberculosis* isolates with different linezolid and delpazolid MIC values

Classification and drug[a]	No. (%) of strains with MIC of:														MIC_{50} (mg/liter)	MIC_{90} (mg/liter)
	<0.016 mg/liter	0.016 mg/liter	0.032 mg/liter	0.064 mg/liter	0.13 mg/liter	0.25 mg/liter	0.5 mg/liter	1 mg/liter	2 mg/liter	4 mg/liter	8 mg/liter	16 mg/liter	>16 mg/liter	Total		
MDR-TB																
Linezolid	4 (3.3)	6 (5.0)	24 (20.0)	49 (40.8)	11 (9.2)	9 (7.5)	6 (5.0)	3 (2.5)	7 (5.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	120	0.064	1
Delpazolid	2 (1.7)	3 (2.5)	3 (2.5)	6 (5.0)	13 (10.8)	32 (26.7)	53 (44.2)	6 (5.0)	1 (0.8)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	120	0.5	0.5
XDR-TB																
Linezolid	3 (2.5)	4 (3.3)	12 (10.0)	21 (17.5)	49 (40.8)	24 (20.0)	1 (0.8)	1 (0.8)	1 (0.8)	1 (0.8)	0 (0.0)	2 (1.7)	1 (0.8)	120	0.13	0.25
Delpazolid	1 (0.8)	2 (1.7)	2 (1.7)	3 (2.5)	5 (4.2)	35 (29.2)	57 (47.5)	9 (7.5)	1 (0.8)	2 (1.7)	1 (0.8)	0 (0.0)	2 (1.7)	120	0.5	1
Total																
Linezolid	7 (2.9)	10 (4.2)	36 (15.0)	70 (29.2)	60 (25.0)	33 (13.8)	7 (2.9)	4 (1.7)	8 (3.3)	1 (0.4)	0 (0.0)	2 (0.8)	2 (0.8)	240	0.064	0.25
Delpazolid	3 (1.3)	5 (2.1)	5 (2.1)	9 (3.8)	18 (7.5)	67 (27.9)	110 (45.8)	15 (6.3)	2 (0.8)	3 (1.3)	1 (0.4)	0 (0.0)	2 (0.8)	240	0.5	0.5

[a]XDR-TB, extensively drug-resistant tuberculosis; MDR-TB, multidrug-resistant tuberculosis.

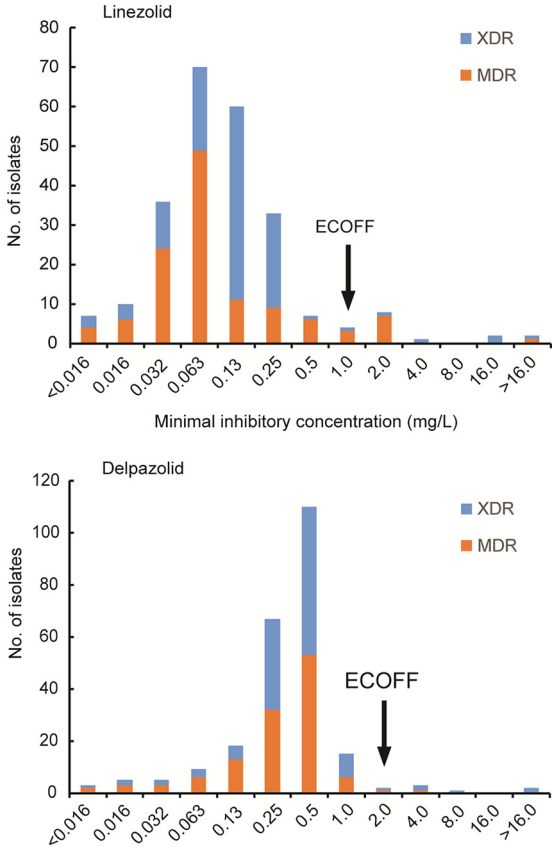

FIG 1 MIC distributions for MDR- and XDR-TB strains. The arrows indicate the proposed linezolid and delpazolid ECOFF values for *M. tuberculosis* isolates.

isolate with a MIC of 0.25 mg/liter, 3 with MICs of 0.5 mg/liter, 1 with a MIC of 1.0 mg/liter, and 2 with MICs of 2 mg/liter. In addition, 3 of 4 isolates with high-level resistance to linezolid (MICs of ≥8 mg/liter) belonged to the XDR-TB group, while 77.78% of isolates (7/9 isolates) with low-level resistance to linezolid belonged to the MDR-TB group.

Mutations conferring linezolid and delpazolid resistance. The entire 23S rRNA, *rplC*, and *rplD* genes were sequenced for all resistant isolates, to identify potential mutations associated with linezolid and delpazolid resistance. As shown in Table 2, the DNA sequence chromatograms demonstrated that 7 (53.85%) of 13 linezolid-resistant

TABLE 2 MICs and 23S rRNA, *rplC*, and *rplD* mutations for the 13 oxazolidinone-resistant clinical strains

	MIC (mg/liter)		Resistance genotype		
Strain	Linezolid	Delpazolid	23S rRNA[a]	*rplC*	*rplD*
XDR014	>16	>16	G2061T	WT	WT
MDR052	16	>16	G2061T	WT	WT
XDR042	>16	0.25	WT	WT	CGC377CAC (Arg126His)
XDR037	16	4	WT	TGC460CGC (Cys154Arg)	WT
XDR021	4	8	WT	TGC460CGC (Cys154Arg)	WT
MDR055	2	4	WT	TGC460CGC (Cys154Arg)	WT
MDR087	2	4	WT	CAC463GAC (His155Asp)	WT
XDR075	2	2	WT	WT	WT
MDR046	2	2	WT	WT	WT
MDR077	2	1	WT	WT	WT
MDR085	2	0.5	WT	WT	WT
MDR098	2	0.5	WT	WT	WT
MDR112	2	0.5	WT	WT	WT

[a]The nucleotide positions of the mutations are listed according to *Escherichia coli* numbering. WT, wild type.

isolates harbored mutations within the three target genes. The remaining 6 linezolid-resistant isolates (46.15%) exhibited wild-type sequences at all loci. The most frequently observed mutation (T460C; $n = 3$) was observed in the *rplC* gene and coded for a nonconservative amino acid substitution, Cys154Arg. In addition, 2 linezolid-resistant isolates exhibited a mutation at position 2061 of 23S rRNA, resulting in high-level resistance to both linezolid and delpazolid (MICs of ≥16 mg/liter). Interestingly, 1 isolate contained an amino acid substitution from Arg to His at codon 126 of the *rplD* gene, which contributed to high-level linezolid resistance (MIC, >16 mg/liter) but not delpazolid resistance (MIC, 0.25 mg/liter). Among the linezolid-susceptible isolates, we identified several synonymous single-nucleotide polymorphisms (SNPs) within *rplC*, including 1 isolate with Arg93Arg (AGG→AGA) and 1 isolate with Gly153Gly (GGA→GGG). In addition, two types of synonymous SNPs within the coding region of *rplD* were found among 4 isolates, including Gln89Gln (CAG→CAA; $n = 3$) and Gln47Gln (CAG→CAA; $n = 1$).

DISCUSSION

In this study, we first compared the *in vitro* activities of linezolid and delpazolid against MDR- and XDR-TB isolates. Our data demonstrated a delpazolid MIC_{90} of 0.5 mg/liter against severe forms of drug-resistant TB, similar to MIC_{90} values obtained for *Staphylococcus aureus* (MIC_{90}, 0.5 mg/liter) and *Streptococcus pneumoniae* (MIC_{90}, 1 mg/liter) (14). The most important finding of this study is that an ECOFF value of 2.0 mg/liter is suggested for delpazolid. On the basis of this ECOFF value, delpazolid showed antibacterial activity comparable to that of linezolid, while only 2.9% of drug-resistant TB strains exhibited resistance to this novel antimicrobial agent. Notably, the proportion of delpazolid-resistant isolates was significantly smaller than that of linezolid-resistant isolates within the MDR-TB group. Although linezolid has been reported to be one of the most potent antibiotics against infections caused by drug-resistant TB, the long-term use of linezolid produces high rates of adverse events, such as myelosuppression and peripheral neuropathy (5, 16). Previous experimental evidence showed that, compared with linezolid, delpazolid exhibited superior pharmacokinetic parameters and good safety profiles (14). In a recent clinical trial, Choi and colleagues demonstrated that LCB01-0371 was well tolerated in healthy male subjects after administration of multiple doses of up to 1,200 mg twice daily for 21 days (17). Therefore, the impressive *in vitro* effectiveness and favorable tolerability of delpazolid make it a promising candidate for use in combination treatment with other anti-TB drugs against MDR- and XDR-TB. In view of the prolonged administration needed for treatment of drug-resistant TB, additional trials are urgently needed to evaluate the efficacy and safety of delpazolid for the management of patients with drug-resistant TB.

Resistance of clinical *M. tuberculosis* isolates to oxazolidinones has primarily been shown to be due to mutations in 23S rRNA and *rplC*, with G2061T and G2576T mutations in 23S rRNA having been shown to cause high-level resistance to linezolid (8). Consistent with previous reports (7), 2 strains with a G2061T mutation in 23S rRNA studied here demonstrated high-level resistance to both linezolid and delpazolid. In addition, we observed that mutations in *rplC* led to great diversity in linezolid susceptibility, such that the MIC values of strains ranged from 2 to 16 mg/liter. The *rplD* gene encodes ribosomal protein L4, the main portion of which is positioned close to the ribosomal peptidyl transferase center (PTC) (8). Many studies have associated *rplD* mutations with linezolid resistance in several bacterial species, such as *Staphylococcus epidermidis* and *Enterococcus faecium* (18, 19), while no study has reported the role of *rplD* in any *M. tuberculosis* linezolid resistance mechanism. In the present study, we first identified a novel mutation within the *rplD* gene that potentially confers linezolid resistance to *M. tuberculosis*. Compared with the greater frequency of the *rplC* mutation, the rarity of the *rplD* mutation in linezolid-resistant bacterial isolates is likely due to the fact that L3 residues are in close proximity to the PTC (8). Interestingly, the mutation in the L4 ribosomal protein causes decreased susceptibility only to linezolid and not to delpazolid, indicating that these two oxazolidinones may exhibit different binding sites

within the PTC. Further structural data on the PTC-oxazolidinone complex will extend our knowledge of the molecular mechanisms of oxazolidinone resistance.

Although multiple mutations conferring linezolid resistance have been identified, nearly one-half of linezolid-resistant strains in this study lacked target gene mutations. On one hand, in addition to the targets sequenced in our study, several other modifications of 23S rRNA may play important roles in the occurrence of linezolid resistance (20). In addition, 23S RNA mutations apparently confer high-level resistance, while efflux pumps and other mechanisms usually result in low-level resistance (7). Of note, all *M. tuberculosis* isolates in this study without detected genetic mutations exhibited low-level resistance (MICs of <4 mg/liter). Therefore, we hypothesize that the efflux-mediated mechanism may play an important role in these linezolid-resistant isolates. On the other hand, the poor correlation between genetic mutations and the linezolid resistance phenotype suggests that the current set of target genes is not suitable for prediction of linezolid resistance among MDR-TB isolates. Given the projected widespread future use of linezolid in the treatment of drug-resistant TB, there is an urgent need to broaden our knowledge of the mechanisms of linezolid resistance in *M. tuberculosis*, to provide a critical component for the development of favorable molecular diagnostic approaches.

We also acknowledge several obvious limitations of this study. First, the determination of critical concentrations for delpazolid should be based not only on the ECOFF value but also on pharmacokinetic/pharmacodynamic and clinical outcome data, as evaluated in prospective studies (21). Second, due to the small number of oxazolidinone-resistant *M. tuberculosis* isolates, the second MIC distribution peak was relatively unobvious, compared with the first peak, within the bimodal distribution of MIC values, which may undermine the reliability of tentative ECOFFs. Third, the novel *rplD* mutation that is potentially associated with resistance to linezolid but not delpazolid was not confirmed by further experimental evidence. Sequence analysis of *rplD* genes from a larger number of linezolid-resistant isolates should confirm our hypothesis. In addition, directed mutagenesis and heterologous expression studies will help us to conclusively link this mutation to linezolid resistance.

In conclusion, we first established an ECOFF value of 2.0 mg/liter for delpazolid. *In vitro* susceptibility tests revealed that delpazolid shows antibacterial activity comparable to that of linezolid, with only 2.9% of drug-resistant TB strains exhibiting resistance to this novel antimicrobial agent. In addition, nucleotide mutations of 23S rRNA yielded high-level resistance to both linezolid and delpazolid, while mutations of *rplC* led to great diversity in linezolid susceptibility. A novel mutation within *rplD* that conferred resistance to linezolid but not delpazolid was identified. Further studies are urgently needed to elucidate the role of *rplD* in the decreased susceptibility to linezolid observed for *M. tuberculosis* strains in this study.

MATERIALS AND METHODS

Ethics statement. The protocols applied in this study were approved by the Ethics Committee of Beijing Chest Hospital, Capital Medical University. All of the patients provided signed informed consent forms prior to their enrollment in this study.

Bacterial strains. A total of 120 MDR-TB strains and 120 XDR-TB strains were randomly selected from the Tuberculosis BioBank maintained at the National Clinical Laboratory on Tuberculosis. These strains were obtained from consecutive patients who sought health care in Beijing Chest Hospital between January 2017 and October 2017. Each *M. tuberculosis* strain was isolated from a unique patient. The drug susceptibility profiles were retrospectively reviewed using *in vitro* drug susceptibility testing (DST) results determined in the National Clinical Laboratory on Tuberculosis. Tests for first- and second-line antituberculosis drug susceptibilities were performed using the absolute concentration method with Löwenstein-Jensen (L-J) medium containing the corresponding anti-TB drugs, as reported previously (22).

MIC determinations. The microplate alamarBlue assay (MABA), which employs alamarBlue reagent for the determination of growth, was performed to determine the MICs of MDR- and XDR-TB against linezolid and delpazolid (7). Prior to *in vitro* susceptibility testing, the strains were recovered on L-J medium after incubation for 4 weeks at 37°C. Briefly, fresh bacterial clones were harvested from the surface of L-J slants. After vigorous mixing for 1 min on a vortex mixer, a suspension of each *M. tuberculosis* strain was prepared in sterile saline solution and adjusted to a density of 1.0 McFarland

standard. The inoculum was further diluted 1:20 with Middlebrook 7H9 broth containing 10% Middlebrook oleic-albumin-dextrose-catalase (OADC) enrichment supplement (containing oleic acid and bovine serum albumin along with sodium chloride, dextrose, and catalase). Next, 100 μl of this inoculum was added to wells of 96-well plates containing 100 μl of antimicrobial serial dilutions in broth per well, to yield a highest final concentration of 16 mg/liter. After 7 days of incubation at 37°C, 70 μl of alamarBlue solution was added to each well, plates were further incubated for 24 h at 37°C, and then color changes were read by visual inspection. The results were interpreted by two independent individuals and inconsistent MIC values were read again by a third individual, to avoid potential bias. The MIC was defined as the lowest concentration of antimicrobial agent that prevented a color change from blue to pink. The standard strain H37Rv served as a control in the MABA assay. The final concentrations of linezolid and delpazolid in the test panel ranged from 0.016 mg/liter to 16 mg/liter. The MIC breakpoint for linezolid was defined as 1.0 mg/liter on the basis of a previous report (7). For delpazolid, we set tentative ECOFFs for MIC determination using the MABA method (23).

DNA sequencing. Extraction of genomic DNA from *M. tuberculosis* strains was performed with freshly cultured bacteria, as reported previously (7). Crude DNA served as the template for PCR amplification to generate gene fragments from isolates exhibiting oxazolidinone resistance. Sequencing of PCR products was performed using the Sanger method, with primers designed to be specific for 23S rRNA, *rplC*, or *rplD*. The primers used in this study are listed in Table S1 in the supplemental material and were synthesized by Tsingke Biotech Co. (Beijing, China). The 50-μl reaction mixtures were prepared as follows: 25 μl of 2× PCR mixture (Genstar, Beijing, China), 0.2 μM each primer, and 4 μl of template DNA. PCR cycling consisted of 94°C for 5 min and then 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were submitted to Tsingke Biotech Co. for DNA sequencing. The sequencing results were analyzed by alignment against corresponding sequences of the reference *M. tuberculosis* strain H37Rv (ATCC 27294).

Data analysis. Comparisons of the rates of resistance of *M. tuberculosis* isolates to linezolid and delpazolid were performed using Pearson's chi-square test, with P values of <0.05 being considered significant. All statistical analyses were carried out using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00165-18.

SUPPLEMENTAL FILE 1, XLSX file, 0.1 MB.

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REFERENCES

1. World Health Organization. 2017. Global tuberculosis report 2017. WHO/HTM/TB/2017.23. World Health Organization, Geneva, Switzerland. http://apps.who.int/iris/bitstream/handle/10665/259366/9789241565516-eng.pdf;jsessionid=2CDE1255E5813851E3CBC49470A41260?sequence=1.
2. Gunther G. 2014. Multidrug-resistant and extensively drug-resistant tuberculosis: a review of current concepts and future challenges. Clin Med (Lond) 14:279–285. https://doi.org/10.7861/clinmedicine.14-3-279.
3. Falzon D, Mirzayev F, Wares F, Baena IG, Zignol M, Linh N, Weyer K, Jaramillo E, Floyd K, Raviglione M. 2015. Multidrug-resistant tuberculosis around the world: what progress has been made? Eur Respir J 45:150–160. https://doi.org/10.1183/09031936.00101814.
4. Caminero JA, Sotgiu G, Zumla A, Migliori GB. 2010. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infect Dis 10:621–629. https://doi.org/10.1016/S1473-3099(10)70139-0.
5. Sotgiu G, Centis R, D'Ambrosio L, Alffenaar JW, Anger HA, Caminero JA, Castiglia P, De Lorenzo S, Ferrara G, Koh WJ, Schecter GF, Shim TS, Singla R, Skrahina A, Spanevello A, Udwadia ZF, Villar M, Zampogna E, Zellweger JP, Zumla A, Migliori GB. 2012. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. Eur Respir J 40:1430–1442. https://doi.org/10.1183/09031936.00022912.
6. Jeong JW, Jung SJ, Lee HH, Kim YZ, Park TK, Cho YL, Chae SE, Baek SY, Woo SH, Lee HS, Kwak JH. 2010. In vitro and in vivo activities of LCB01-0371, a new oxazolidinone. Antimicrob Agents Chemother 12:5359–5362. https://doi.org/10.1128/AAC.00723-10.
7. Zhang Z, Pang Y, Wang Y, Liu C, Zhao Y. 2014. Beijing genotype of *Mycobacterium tuberculosis* is significantly associated with linezolid resistance in multidrug-resistant and extensively drug-resistant tuberculosis in China. Int J Antimicrob Agents 43:231–235. https://doi.org/10.1016/j.ijantimicag.2013.12.007.
8. Long KS, Vester B. 2012. Resistance to linezolid caused by modifications at its binding site on the ribosome. Antimicrob Agents Chemother 56:603–612. https://doi.org/10.1128/AAC.05702-11.
9. Beckert P, Hillemann D, Kohl TA, Kalinowski J, Richter E, Niemann S, Feuerriegel S. 2012. *rplC* T460C identified as a dominant mutation in linezolid-resistant *Mycobacterium tuberculosis* strains. Antimicrob Agents Chemother 56:2743–2745. https://doi.org/10.1128/AAC.06227-11.
10. Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, Via LE, Goldfeder LC, Kang E, Jin B, Park H, Kwak H, Kim H, Jeon HS, Jeong I, Joh JS, Chen RY, Olivier KN, Shaw PA, Follmann D, Song SD, Lee JK, Lee D, Kim CT, Dartois V, Park SK, Cho SN, Barry CE, III. 2012. Linezolid for treatment of chronic extensively drug-resistant tuberculosis. N Engl J Med 367:1508–1518. https://doi.org/10.1056/NEJMoa1201964.
11. Murray S, Mendel C, Spigelman M. 2016. TB Alliance regimen development for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 20:38–41. https://doi.org/10.5588/ijtld.16.0069.
12. Conradie F, Diacon AH, Everitt D, Mendel C, Niekerk C, Howell P, Comins K, Spigelman M. 2017. The NIX-TB trial of pretomanid, bedaquiline and linezolid to treat XDR-TB. Abstr Conf Retroviruses Opportunistic Infect, Seattle, Washington, 13 to 16 February 2017.
13. Cox H, Ford N. 2012. Linezolid for the treatment of complicated drug-resistant tuberculosis: a systematic review and meta-analysis. Int J Tuberc Lung Dis 16:447–454. https://doi.org/10.5588/ijtld.11.0451.

14. Jeong JW, Jung SJ, Lee HH, Kim YZ, Park TK, Cho YL, Chae SE, Baek SY, Woo SH, Lee HS, Kwak JH. 2010. *In vitro* and in vivo activities of LCB01-0371, a new oxazolidinone. Antimicrob Agents Chemother 54:5359–5362. https://doi.org/10.1128/AAC.00723-10.
15. Kim TS, Choe JH, Kim YJ, Yang CS, Kwon HJ, Jeong J, Kim G, Park DE, Jo EK, Cho YL, Jang J. 2017. Activity of LCB01-0371, a novel oxazolidinone, against *Mycobacterium abscessus*. Antimicrob Agents Chemother 61:e02752-16. https://doi.org/10.1128/AAC.02752-16.
16. Bolhuis MS, Tiberi S, Sotgiu G, De Lorenzo S, Kosterink JG, van der Werf TS, Migliori GB, Alffenaar JW. 2015. Linezolid tolerability in multidrug-resistant tuberculosis: a retrospective study. Eur Respir J 46:1205–1207. https://doi.org/10.1183/13993003.00606-2015.
17. Choi Y, Lee SW, Kim A, Jang K, Nam H, Cho YL, Yu KS, Jang IJ, Chung JY. 2018. Safety, tolerability and pharmacokinetics of 21 day multiple oral administration of a new oxazolidinone antibiotic, LCB01-0371, in healthy male subjects. J Antimicrob Chemother 73:183–190. https://doi.org/10.1093/jac/dkx367.
18. Wong A, Reddy SP, Smyth DS, Aguero-Rosenfeld ME, Sakoulas G, Robinson DA. 2010. Polyphyletic emergence of linezolid-resistant staphylococci in the United States. Antimicrob Agents Chemother 54:742–748. https://doi.org/10.1128/AAC.00621-09.
19. Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN. 2010. Assessment of linezolid resistance mechanisms among *Staphylococcus epidermidis* causing bacteraemia in Rome, Italy. J Antimicrob Chemother 65:2329–2335. https://doi.org/10.1093/jac/dkq331.
20. Mendes RE, Deshpande LM, Jones RN. 2014. Linezolid update: stable *in vitro* activity following more than a decade of clinical use and summary of associated resistance mechanisms. Drug Resist Updat 17:1–12. https://doi.org/10.1016/j.drup.2014.04.002.
21. Yu X, Wang G, Chen S, Wei G, Shang Y, Dong L, Schon T, Moradigaravand D, Parkhill J, Peacock SJ, Koser CU, Huang H. 2016. Wild-type and non-wild-type *Mycobacterium tuberculosis* MIC distributions for the novel fluoroquinolone antofloxacin compared with those for ofloxacin, levofloxacin, and moxifloxacin. Antimicrob Agents Chemother 60:5232–5237. https://doi.org/10.1128/AAC.00393-16.
22. Pang Y, Zong Z, Huo F, Jing W, Ma Y, Dong L, Li Y, Zhao L, Fu Y, Huang H. 2017. *In vitro* drug susceptibility of bedaquiline, delamanid, linezolid, clofazimine, moxifloxacin, and gatifloxacin against extensively drug-resistant tuberculosis in Beijing, China. Antimicrob Agents Chemother 61:e00900-17. https://doi.org/10.1128/AAC.00900-17.
23. Pang Y, Zheng H, Tan Y, Song Y, Zhao Y. 2017. *In vitro* activity of bedaquiline against nontuberculous mycobacteria in China. Antimicrob Agents Chemother 61:e02627-16. https://doi.org/10.1128/AAC.02627-16.