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In Vitro Susceptibility Testing of Tedizolid against Nontuberculous Mycobacteria

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ABSTRACT Tedizolid is a new oxazolidinone with improved *in vitro* and intracellular potency against *Mycobacterium tuberculosis*, including multidrug-resistant strains, and some species of nontuberculous mycobacteria (NTM) compared with that of linezolid. Using the current Clinical and Laboratory Standards Institute (CLSI)-recommended method of broth microdilution, susceptibility testing of 170 isolates of rapidly growing mycobacteria showed equivalent or lower (1- to 8-fold) MIC_{50} and/or MIC_{90} values for tedizolid compared with that for linezolid. The tedizolid MIC_{90} values for 81 isolates of *M. abscessus* subsp. *abscessus* and 12 isolates of *M. abscessus* subsp. *massiliense* were 8 μg/ml and 4 μg/ml, respectively, compared with linezolid MIC_{90} values of 32 μg/ml for both. The MIC_{90} values for 20 isolates of *M. fortuitum* were 2 μg/ml for tedizolid and 4 μg/ml for linezolid. Twenty-two isolates of *M. chelonae* had tedizolid and linezolid MIC_{90}s of 2 μg/ml and 16 μg/ml, respectively. One hundred forty-two slowly growing NTM, including 7/7 *M. marinum*, 7/7 *M. kansasii*, and 7/11 of other less commonly isolated species, had tedizolid MICs of ≤1 μg/ml and linezolid MICs of ≤4 μg/ml. One hundred isolates of *Mycobacterium avium* complex and eight *M. simiae* isolates had tedizolid MIC_{50}s of 8 μg/ml and linezolid MIC_{50}s 32 and 64 μg/ml, respectively. Nine *M. arupense* isolates had MIC_{50}s of 4 μg/ml and 16 μg/ml for tedizolid and linezolid, respectively. These findings demonstrate a greater *in vitro* potency of tedizolid than linezolid against NTM and suggest that an evaluation of tedizolid as a potential treatment agent for infections caused by selected NTM is warranted.

KEYWORDS oxazolidinones, susceptibility testing, tedizolid

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Nontuberculous mycobacteria (NTM) are responsible for a multiplicity of different types of infections, including respiratory, cutaneous, and systemic infections. Many species of NTM are multidrug resistant (1), emphasizing the urgent need for new antimicrobials with efficacies against these organisms.

Tedizolid phosphate is a novel oxazolidinone prodrug (TR-701) that is transformed in the serum into the active drug, tedizolid ([TZD] TR-700, formerly DA-7157) (2) with a broad range of activities against Gram-positive microorganisms, including mycobacteria. The mechanism of action of TZD is by the inhibition of protein synthesis. TZD binds to the 50S ribosome, apparently at a site near the 30S ribosome, which blocks the formation of the 70S initiation complex and, in turn, prevents protein synthesis (3). The supposition is that the major site of action of oxazolidinones is at the ribosomal peptidyltransferase center, and this unique mechanism of action eliminates the likelihood of cross-resistance with other antimicrobial classes (3).

Previous reports of *in vitro* and *in vivo* (intracellular) activities against *Mycobacterium tuberculosis*, including multidrug-resistant strains (4), and *Nocardia brasiliensis* have been published (5–7). A previously published study by Vera-Cabrera et al. showed *in vitro* activities of TZD against small numbers of several species of NTM (5). However, the

study did not include the differentiation of subspecies within the *M. abscessus* complex and did not include several more recently described species (5).

Previous investigators have also reported that TZD has enhanced *in vitro* activity against bacterial strains, including linezolid (LZD)-resistant strains of *Streptococcus pneumoniae* and methicillin-susceptible and -resistant coagulase-negative and -positive *Staphylococcus*, *Streptococcus pyogenes*, and *Streptococcus agalactiae* (2, 3, 8). With this superior activity in mind, we undertook a large study to evaluate the *in vitro* MICs of TZD compared with the MICs of LZD and other comparator antimicrobials against isolates of NTM.

(A portion of this study was presented at the first ASM Microbe meeting in Boston, MA, 16 to 20 June 2016 [9]).

RESULTS

MICs for TZD were generally 1- to 4-fold less than the MICs for LZD. The rapidly growing mycobacteria (RGM) species and subspecies tested included *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *massiliense*, *M. fortuitum*, *M. porcinum*, *M. senegalense*, *M. chelonae*, *M. mucogenicum* group, *M. immunogenum*, *M. smegmatis*, and two other pigmented isolates identified as *M. obuense*. We also identified one small group of six "hybrid" *M. abscessus* isolates (which may represent a new subspecies within the *M. abscessus* complex) identified as *M. abscessus* subsp. *massiliense* (by the *erm* gene) and *M. abscessus* subsp. *abscessus* (by the *rpoβ* gene).

Eighty-one isolates of *M. abscessus* subsp. *abscessus* and 12 isolates of *M. abscessus* subsp. *massiliense* showed TZD MIC_{50}s of 4 μg/ml and 2 μg/ml, respectively, compared with MIC_{50}s of 16 μg/ml and 8 μg/ml, respectively, for LZD (these included isolates with mutational resistance to clarithromycin and amikacin). The TZD MIC_{90} for 81 isolates of *M. abscessus* subsp. *abscessus* was 8 μg/ml compared with 32 μg/ml for LZD (Table 1). Twelve isolates of *M. abscessus* subsp. *massiliense* showed an MIC_{50} of 2 μg/ml for TZD compared with 8 μg/ml for LZD, and a single isolate of *M. abscessus* subsp. *bolletii* exhibited a TZD MIC of 0.12 μg/ml and an LZD MIC of 0.5 μg/ml. The 6 hybrid isolates had MIC_{50}s of 0.5 μg/ml for TZD and 16 μg/ml for LZD.

One hundred forty-two isolates of slowly growing NTM were studied. Table 1 shows isolates, including 100 isolates of *Mycobacterium avium* complex (MAC), with TZD MIC_{90}s of >32 μg/ml compared with the LZD MIC_{90} of 64 μg/ml. The MIC ranges for the MAC isolates were 1 to >32 μg/ml and 2 to 128 μg/ml for TZD and LZD, respectively (these MAC isolates included isolates with known 23S rRNA gene clarithromycin mutational resistance).

Although the number of isolates tested for the other slowly growing NTM was smaller than the 100 isolates of MAC tested, most slowly growing species other than MAC in this study had TZD MICs of ≤8 μg/ml. Among the drug-resistant slowly growing NTM tested, both MAC and *M. simiae* had TZD MIC_{50}s equal to 8 μg/ml compared with LZD MIC_{50}s of 32 and 64 μg/ml, respectively (see Table 1). This study also identified two isolates of *M. terrae/algericum* complex with TZD MICs of 0.25 to 1 μg/ml compared with LZD MICs of 1 to 4 μg/ml.

Several other slowly growing NTM (SGM) species (not shown in Table 1 due to the low numbers tested) were included in this study. Two isolates of *M. lentiflavum* were identified with a TZD MIC range of 0.5 to 4 μg/ml compared with 8 to 32 μg/ml for LZD. There were also two *M. nebraskense* isolates with TZD MICs of 0.25 to 1 μg/ml and LZD MICs at 2 μg/ml. Two isolates of *M. paraffinicum* had a TZD range of 2 to 8 μg/ml compared with 16 to 32 μg/ml for LZD. Single isolates of *M. shimoidei and M. xenopi* each had TZD MICs of 0.25 μg/ml and 0.12 μg/ml, respectively, compared with LZD MICs of 2 μg/ml and 0.5 μg/ml, respectively. Additionally, one isolate of *M. interjectum* had a TZD MIC of 1 μg/ml in contrast to a LZD MIC of 16 μg/ml.

Table 1 also lists MICs of additional antimicrobials that were tested to confirm the susceptibility patterns of species and show a comparison of MICs to TZD, including intermediate breakpoints as currently recommended by the CLSI (10). As expected, the most active *in vitro* agents for the *M. abscessus* complex included amikacin, tigecycline,

TABLE 1 MIC values for tedizolid and comparative antimicrobials against isolates of nontuberculous mycobacteria

Species (no. of isolates tested)	Intermediate breakpoint (μg/ml)	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
Rapidly growing species					
M. abscessus subsp. *abscessus* (81)	—[a]	Tedizolid	0.12–>32	4	8
	16	Linezolid	0.25–128	16	32
	32	Amikacin	2–>1024	16	32
	32–64	Cefoxitin	16–64	32	64
	2	Ciprofloxacin	0.5–>4	>4	>4
	4	Clarithromycin[b]	0.5–>16	>16	>16
	2–4	Doxycycline	8–>16	>16	>16
	8–16	Imipenem	4–>64	16	32
	2–4	Minocycline	2–>8	>8	>8
	2	Moxifloxacin	2–>8	8	>8
	—	Tigecycline[c]	0.03–0.5	0.25	0.5
	—	TMP-SMX[d]	≤0.25/4.75–8/152	4/76	8/152
M. abscessus subsp. *massiliense* (12)	—	Tedizolid	0.12–>32	2	4
	16	Linezolid	0.5–32	8	32
	32	Amikacin	4–>1024	16	64
	32–64	Cefoxitin	32–64	32	64
	2	Ciprofloxacin	1–>4	4	4
	4	Clarithromycin[b]	0.12–>128	0.5	2
	2–4	Doxycycline	0.25–>16	>16	>16
	8–16	Imipenem	8–32	8	16
	2–4	Minocycline	≤1–>8	>8	>8
	2	Moxifloxacin	2–>8	8	>8
	—	Tigecycline[c]	0.06–0.5	0.25	0.5
	—	TMP-SMX[d]	4/76–>8/152	4/76	>8/152
M. abscessus subsp. *massiliense*/*M. abscessus* subsp. *abscessus* hybrid[e] (6)	—	Tedizolid	0.25–>32	0.5	
	16	Linezolid	2–>128	16	
	32	Amikacin	16–64	16	
	32–64	Cefoxitin	16–64	32	
	2	Ciprofloxacin	4–>4	4	
	4	Clarithromycin[a]	0.12–16	1	
	2–4	Doxycycline	>16	>16	
	8–16	Imipenem	8–16	16	
	2–4	Minocycline	>8	>8	
	2	Moxifloxacin	4–>8	4	
	—	Tigecycline[c]	0.12–0.25	0.12	
	—	TMP-SMX[d]	2/38–8/152	4/76	
M. chelonae (22)	—	Tedizolid	0.25–4	1	2
	16	Linezolid	2–16	8	16
	32	Amikacin	8–32	16	32
	32–64	Cefoxitin	128–>128	>128	>128
	2	Ciprofloxacin	0.5–>4	4	>4
	4	Clarithromycin[a]	≤0.06–>128	1	2
	2–4	Doxycycline	2–>16	>16	>16
	8–16	Imipenem	8–64	16	32
	2–4	Minocycline	≤1–>8	>8	>8
	2	Moxifloxacin	1–>8	4	8
	—	Tigecycline[c]	0.06–0.5	0.25	0.5
	—	Tobramycin	≤1–2	2	2
	—	TMP-SMX[d]	1/19–>8/152	4/76	>8/152
M. mucogenicum group (9)	—	Tedizolid	0.06–4	1	
	16	Linezolid	0.5–8	1	
	32	Amikacin	≤0.5–4	1	
	32–64	Cefoxitin	4–16	16	
	2	Ciprofloxacin	0.25–>4	0.5	
	4	Clarithromycin[a]	0.25–2	1	
	2–4	Doxycycline	0.25–>16	16	
	8–16	Imipenem	≤2	≤2	
	2–4	Minocycline	1–>8	>8	

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TABLE 1 (Continued)

Species (no. of isolates tested)	Intermediate breakpoint (μg/ml)	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
	2	Moxifloxacin	≤0.12–2	0.5	
	—	Tigecycline[c]	0.03–0.25	0.25	
	—	TMP-SMX[d]	≤0.25/4.75–0.5/9.5	≤0.25/4.75	
M. immunogenum (9)	—	Tedizolid	0.5–4	1	
	16	Linezolid	0.12–16	8	
	32	Amikacin	8–16	8	
	32–64	Cefoxitin	8–>128	>128	
	2	Ciprofloxacin	2–>4	4	
	4	Clarithromycin[a]	0.5–2	2	
	2–4	Doxycycline	>16	>16	
	8–16	Imipenem	16–64	16	
	2–4	Minocycline	>8	>8	
	2	Moxifloxacin	1–>8	8	
	—	Tigecycline[c]	0.06–0.5	0.25	
	—	Tobramycin	4–16	16	
	—	TMP-SMX[d]	4/76–>8/152	8/152	
M. fortuitum (20)	—	Tedizolid	0.25–2	1	2
	16	Linezolid	1–8	2	4
	32	Amikacin	≤1	≤1	≤1
	32–64	Cefoxitin	8–64	32	64
	2	Ciprofloxacin	≤0.12–0.25	≤0.12	≤0.12
	4	Clarithromycin[a]	≤0.06–128	32	64
	2–4	Doxycycline	≤0.12–>16	0.5	>16
	8–16	Imipenem	≤2–4	4	4
	2–4	Minocycline	≤1–>8	≤1	>8
	2	Moxifloxacin	≤0.25	≤0.25	≤0.25
	—	Tigecycline[c]	0.03–0.5	0.12	0.25
	—	TMP-SMX[d]	≤0.25/4.75–2/38	0.5/9.5	1/19
Slowly growing species					
M. avium complex (100)	—	Tedizolid	1–>32	8	>32
	16	Linezolid	2–128	32	64
	—	Amikacin[f]	2–>1024	32	128
	16	Clarithromycin	0.25–>128	2	8
	2	Moxifloxacin	0.25–>8	4	8
M. arupense (9)	—	Tedizolid	1–4	4	
	16	Linezolid	8–32	16	
	32	Amikacin	32–>1024	64	
	2	Ciprofloxacin	16–>16	>16	
	16	Clarithromycin	0.25–1	0.5	
	4	Doxycycline	4–>16	1	
	4	Ethambutol	≤0.5–8	>16	
	2	Moxifloxacin	>8	>8	
	2	Rifabutin	≤0.25–1	≤0.25	
	2	Rifampin	2–>8	8	
	—	TMP-SMX[d]	0.5/9/5–4/76	2/38	
M. kansasii (7)	—	Tedizolid	0.25–1	0.5	
	16	Linezolid	0.5–2	2	
	32	Amikacin	2–64	8	
	2	Ciprofloxacin	1–>16	2	
	16	Clarithromycin	≤0.06–1	0.25	
	4	Doxycycline	2–>16	16	
	4	Ethambutol	2–8	4	
	2	Moxifloxacin	≤0.12–0.5	0.25	
	2	Rifabutin	≤0.25–0.5	≤0.25	
	2	Rifampin	≤0.12–4	0.25	
	—	TMP-SMX[d]	≤0.12/2.38–0.5/9.5	≤0.12/2.38	

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TABLE 1 (Continued)

Species (no. of isolates tested)	Intermediate breakpoint (μg/ml)	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
M. simiae (8)	—	Tedizolid	1–>32	8	
	16	Linezolid	8–128	64	
	32	Amikacin	32–128	32	
	2	Ciprofloxacin	16–>16	16	
	16	Clarithromycin	8–>64	8	
	4	Doxycycline	>16	>16	
	4	Ethambutol	16–>16	>16	
	2	Moxifloxacin	4–>8	4	
	2	Rifabutin	8–>8	>8	
	2	Rifampin	>8	>8	
	—	TMP-SMX[d]	1/19–4/76	2/38	
M. marinum (7)	—	Tedizolid	0.25–1	1	
	16	Linezolid	1–4	1	
	32	Amikacin	≤1–4	≤1	
	2	Ciprofloxacin	4–16	8	
	16	Clarithromycin	0.25–1	0.5	
	4	Doxycycline	2–8	2	
	4	Ethambutol	≤0.5–4	4	
	2	Moxifloxacin	0.5–8	1	
	2	Rifabutin	≤0.25	≤0.25	
	2	Rifampin	≤0.12–1	0.5	
	—	TMP-SMX[d]	0.5/9.5–2/38	1/19	

[a]—, not determined.
[b]Clarithromycin MIC is the result of extended incubation (up to 14 days) to detect macrolide resistance induced by the *erm* gene.
[c]There is currently no CLSI-recommended breakpoint for tigecycline.
[d]TMP-SMX, trimethoprim sulfamethoxazole. There is no intermediate breakpoint for TMP-SMX; resistance is ≥4/76 μg/ml.
[e]There is currently no CLSI-recommended amikacin breakpoint for *M. avium* complex. A proposed resistance breakpoint associated with a 16S rRNA gene mutation (1408A→C) is >64 μg/ml (11).
[f]*M. abscessus* subsp. *massiliense* by *erm*/*M. abscessus* subsp. *abscessus* by *rpoB* gene (a "hybrid" subspecies or may represent a new species).

cefoxitin, and imipenem. Isolates of *M. abscessus* subsp. *massiliense* and a small group of *M. abscessus* subsp. *abscessus* with no functional *erm* gene were also susceptible to clarithromycin.

For *M. chelonae* and *M. immunogenum*, the most active *in vitro* agents included LZD, clarithromycin, and tigecycline, with only *M. chelonae* isolates susceptible to tobramycin. Among the *M. fortuitum* group, the most active *in vitro* agents included LZD, imipenem, moxifloxacin, ciprofloxacin, tigecycline, amikacin, and trimethoprim sulfamethoxazole (TMP-SMX) (see Table 1).

Among the most frequently seen slowly growing species other than MAC (*M. simiae*, *M. arupense*, *M. kansasii*, and *M. marinum*), low MICs for clarithromycin and rifabutin were observed for most isolates except for *M. simiae*, which is uniformly resistant to rifabutin.

The only antimicrobials recommended for reporting by the CLSI against isolates of MAC include clarithromycin, amikacin, LZD, and moxifloxacin. For MAC, most isolates in this cohort showed susceptibility to clarithromycin. Because several known MAC isolates were included with high MICs (>64 μg/ml) and a 16S rRNA gene mutation, the amikacin MICs were higher than generally seen (11). At this time, the CLSI has not addressed an amikacin MIC breakpoint for MAC. However, an amikacin resistance breakpoint of >64 μg/ml corresponding to isolates with a mutation in the 16S rRNA gene has been proposed to the CLSI (11).

Quality control. The manufacturer's acceptable range of MICs for *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 was 0.25 to 1 μg/ml. All 34 isolates of *S. aureus* ATCC 29213 and 10 isolates of *E. faecalis* had TZD MICs within the acceptable range (see Table 2).

DISCUSSION

Linezolid has been an important addition to the armamentarium of antimicrobials used in the treatment of NTM (1, 4, 12–14). The introduction of TZD provides another

TABLE 2 MICs and MIC ranges of reference strains tested against tedizolid

Organism	Acceptable MIC range (μg/ml)	No. of values at an MIC (μg/ml) of:		
		0.25	0.5	1
Staphylococcus aureus ATCC 29213	0.25–1	8	25	1
Enterococcus faecalis ATCC 29212	0.25–1	0	10	0
Mycobacterium avium ATCC 700898	NA[a]	2	3	5
Mycobacterium smegmatis ATCC 19420	NA	4	0	0
Mycobacterium peregrinum ATCC 700686	NA	2	23	8
Mycobacterium marinum ATCC 927	NA	0	0	4

[a]NA, not available.

potential antimicrobial with an efficacy against these organisms, and early MIC studies performed with bacterial species show that TZD has a 4- to 16-fold greater potency than LZD against some bacteria, including LZD-resistant organisms (2, 15, 16). The higher MICs of TZD compared with those of LZD among many of the NTM emphasizes the need for careful species identification prior to the selection of treatment options. A 2006 *in vitro* study by Vera-Cabrera et al. included 57 isolates of the *M. fortuitum* group (including the 3rd biovariant group, *M. peregrinum/senegalense* group, and *M. fortuitum*) with a TZD MIC range of ≤0.25 to 64 μg/ml (MIC_{90}, 4 μg/ml) and an LZD MIC range of 0.5 to >64 μg/ml (MIC_{90}, 16 μg/ml) (5). Our study included 26 isolates of the *M. fortuitum* group with similar MIC results to those of the Vera-Cabrera et al. study, although the numbers for members of the former third biovariant group of *M. fortuitum* (*M. porcinum*, *M. senegalense*, *M. houstonense*, and *M. septicum*) were small. The previous 2006 study tested only 14 isolates of *M. abscessus* with an MIC_{50} and an MIC_{90} of 4 μg/ml for TZD compared with an MIC_{50} and an MIC_{90} of 64 μg/ml for LZD, and isolates were not differentiated into subspecies (current subspecies designations were unknown at the time) as they were in this study of 81 isolates of *M. abscessus* subsp. *abscessus* (MIC_{50}, 4 μg/ml and MIC_{90}, 8 μg/ml for TZD; MIC_{50}, 16 μg/ml and MIC_{90}, 32 μg/ml for LZD). Also included in this study were 12 isolates of *M. abscessus* subsp. *massiliense* (not described in the 2006 study), TZD (MIC_{50}, 2 μg/ml and LZD MIC_{50}, 8 μg/ml), and one isolate of *M. abscessus* subsp. *bolletii* (MIC of 0.12 μg/ml for TZD compared with 0.5 μg/ml for LZD). The 2006 study also reported 17 isolates of *M. chelonae* complex, but again the species were not differentiated (i.e., *M. chelonae* and *M. immunogenum*) as in this study (5).

For the slowly growing species in this study, only *M. marinum* (7 isolates), *M. kansasii* (7 isolates), *M. nebraskense* (2 isolates), *M. algericum/terrae* group (2 isolates), and one each of *M. shimoidei*, and *M. xenopi*, and *M. interjectum* had MICs of ≤1 μg/ml to TZD (see Table 1 for isolates with MICs of ≥ 5 μg/ml). Vera-Cabrera et al. also reported an MIC range of ≤0.25 to 0.5 μg/ml among 8 isolates of *M. kansasii* (5), similar to the MIC range of 0.25 to 1 μg/ml reported here. Additionally, the 2006 study showed a single isolate of *M. terrae* complex (not identified to the species level) with a TZD MIC of 1 μg/ml compared with 16 μg/ml with LZD (5). Six isolates of *M. simiae* in the 2006 study exhibited a TZD MIC range of 1 to 8 μg/ml and an LZD MIC range of 8 to 32 μg/ml (MIC_{50}s were not given [5]) compared with the TZD MIC range of 1 to >32 μg/ml (MIC_{50}, 8 μg/ml) and an LZD MIC range of 8 to 128 μg/ml (MIC_{50}, 64) of eight isolates in this study.

Vera-Cabrera and colleagues reported only 13 isolates of MAC with a TZD MIC_{90} of 8 μg/ml (5) compared with an MIC_{90} of 64 μg/ml in this study of 100 isolates of MAC. The MIC range of TZD was 1 to 8 μg/ml in the 2006 study (5) compared with the MIC range of 1 to >32 μg/ml reported here.

TZD has a high oral bioavailability and a longer half-life (11.0 h versus 5.0 h for LZD), thus allowing the clinician to easily modify the route from intravenous to oral and to use once-daily dosing, encouraging more patient compliance and outpatient usage (17, 18). Moreover, although long-term usage has not been assessed, TZD appears to be better tolerated than LZD, especially in regard to hematological adverse events,

including thrombocytopenia (3, 15). No apparent dose-related toxicity has been observed with short-term (≤7 days) administration of TZD so far (16).

TZD has been shown to be more active than LZD when evaluating the ability to decrease CFU of bacterial species, including *Staphylococcus aureus*, *Listeria monocytogenes*, and *Legionella pneumophila*, in cultured macrophages or human umbilical vein endothelial cells (8, 19). Although the intracellular activity of TZD has not yet been studied in NTM, Vera-Cabrera et al. showed that TZD was more active at inhibiting the intracellular growth of *Nocardia* than LZD (6). Additionally, the intracellular concentration of TZD is at least 10- to 15-fold higher than the extracellular concentration in contrast to the intracellular concentration of LZD, which is equivalent to the extracellular concentration (8, 15, 19). Previous studies also showed excellent penetration of TZD through the epithelial lining into the fluid of the lungs, suggesting that TZD may be useful in the setting of pneumonia (20). Other studies have shown a superior distribution of TZD in the interstitial fluid of adipose and muscle tissues, making TZD a potential therapeutic option for skin and soft tissue infections (15).

Previous studies in healthy adults have shown that TZD half-life values are approximately 2-fold higher than those of LZD, and TZD is rapidly absorbed with nearly complete oral bioavailability with 200-mg doses of tedizolid phosphate (16). Studies also suggest that the 200-mg dose of tedizolid phosphate (150 mg TZD equivalent) has favorable pharmacokinetic, safety, and efficacy profiles and thus was selected for therapeutic dosing (3, 16, 17, 20).

The *in vitro* MICs of TZD obtained in this and previous studies, along with the once-daily lower dosage for TZD and the potential for fewer and less serious adverse events associated with TZD compared with LZD, emphasize the potential for TZD in the treatment of infections caused by some species of NTM (3, 8, 17, 19, 20). Considering the data from this study and depending upon the determination of susceptibility breakpoints for TZD compared with those currently accepted for LZD against NTM, this new oxazolidinone may provide an effective therapeutic agent for the treatment of infections caused by NTM.

MATERIALS AND METHODS

Isolates. Three-hundred twelve isolates of nontuberculous mycobacteria (NTM) submitted to the Mycobacteria/Nocardia research laboratory at the University of Texas Health Science Center at Tyler (UTHSCT) from 2014 to 2015 were tested against TZD, LZD, and other comparative antimicrobials (see Table 1). These isolates included 100 isolates of MAC, 42 isolates of other slowly growing NTM (7 *M. kansasii*, 8 *M. simiae*, 9 *M. arupense*, 7 *M. marinum*, 2 each of *M. lentiflavum*, *M. nebraskense*, *M. algericum/terrae* group, and *M. paraffinicum*, and 1 each of *M. xenopi*, *M. interjectum*, *and M. shimoidei*), 100 *M. abscessus* complex (81 isolates of *M. abscessus* subsp. *abscessus*, 12 isolates of *M. abscessus* subsp. *massiliense*, six isolates [sometimes considered a "hybrid" subspecies] identified as *M. abscessus* subsp. *massiliense* [by the *erm* gene] and *M. abscessus* subsp. *abscessus* [by *rpoβ* gene], and one isolate of *M. abscessus* subsp. *bolletii*), and 70 isolates of other RGM (26 *M. fortuitum* group composed of 20 *M. fortuitum*, two each *M. porcinum* and *M. houstonense*, and one each of *M. septicum*, *M. senegalense*, and *M. goodii*, 22 *M. chelonae*, nine *M. mucogenicum* group [including *M. mucogenicum* and *M. phocaicum*], nine *M. immunogenum*, one *M. smegmatis*, and two other pigmented RGM identified as *M. obuense*).

Identification. All isolates of NTM were identified by gene sequencing as indicated for each species/group. For the RGM, sequencing of the *rpoβ* gene and the *erm* gene (for the *M. abscessus* complex) was performed using previously recommended criteria for identification, including the Clinical and Laboratory Standards Institute (CLSI) recommendations (21, 22). The slowly growing NTM species were identified using partial 16S rRNA gene sequencing. Again, the CLSI interpretive criteria were used (22).

Antimicrobial susceptibility testing. Isolates were tested by broth microdilution in cation-adjusted Mueller-Hinton broth using doubling dilutions of antimicrobials (TZD concentrations were 0.008 to 32 μg/ml) according to the CLSI-recommended procedure (10). Antimicrobial concentrations for some antimicrobials varied due to the use of multiple lot numbers of panels. MICs for the RGM were read after incubating at 30°C for 3 to 5 days until sufficient growth was evident in the control well. Clarithromycin was read initially and again after an extended incubation up to 14 days to determine inducible resistance (10). The slowly growing NTM were read after incubating 35°C for 7 to 14 days when sufficient growth was evident in the control well. For TZD and LZD, pinpoint growth in the well was not considered growth according to the manufacturer's instructions (personal communication, Merck).

RGM antimicrobials that were compared with TZD included LZD, amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, minocycline, moxifloxacin, tigecycline, trimethoprim sulfamethoxazole, and tobramycin (for *M. chelonae* only). For the slowly growing NTM except MAC (for which only the CLSI-recommended agents, LZD, clarithromycin, amikacin, and moxifloxacin were tested),

comparative antimicrobials included LZD, amikacin, clarithromycin, doxycycline, ethambutol, rifabutin, rifampin, and TMP-SMX (Table 1). The CLSI-recommended breakpoints are listed in Table 1 (10).

Quality control. Quality control of susceptibility testing was performed weekly using the CLSI-recommended strain of *Mycobacterium peregrinum* ATCC 700686 for the comparative antimicrobials and *Staphylococcus aureus* ATCC 29213 for TZD (10). In a search for an alternate quality control strain, additional quality control for TZD was performed using *Enterococcus faecalis* ATCC 2912, *Mycobacterium smegmatis* ATCC 19420, *M. marinum* ATCC 927, and *M. avium* ATCC 700898 (see Table 2).

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