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SUSCEPTIBILITY

In Vitro Susceptibility Testing of Omadacycline against Nontuberculous Mycobacteria

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ABSTRACT Infections caused by nontuberculous mycobacteria (NTM) are increasing globally. *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex are the most frequently encountered NTM, and oral treatment options are extremely limited for these pathogens, especially for the *M. abscessus* complex. In this study, the *in vitro* potency of omadacycline, a new tetracycline derivative, was tested against 111 isolates of NTM. MIC testing was performed as recommended by the Clinical and Laboratory Standards Institute against 70 isolates of rapidly growing mycobacteria (RGM), of which >90% were tetracycline resistant. These included *M. abscessus* subsp. *abscessus* (20 isolates), *M. abscessus* subsp. *massiliense* (3), *Mycobacterium chelonae* (15 isolates), *Mycobacterium immunogenum* (7 isolates), the *Mycobacterium fortuitum* group, including six doxycycline-resistant isolates (12 isolates), and the *Mycobacterium mucogenicum* group, including four doxycycline-resistant isolates (10 isolates). Forty-one isolates of slowly growing mycobacteria (SGM), including 16 isolates of MAC, were also tested. Omadacycline was active against all RGM species, with MIC_{50} ranges of 0.004 to 0.25 and 0.06 to 1 μg/ml for 80% and 100% inhibition, respectively. For *M. abscessus* subsp. *abscessus,* MIC_{50}s were 0.06 and 0.12 μg/ml with 80% and 100% inhibition, respectively. There was considerable trailing of the omadacycline endpoint with the RGM. MICs of tigecycline exhibited no trailing and were generally within 1 to 2 dilutions of the 100% inhibition omadacycline MICs. While there was no trailing observed in SGM, omadacycline MICs were higher (MIC range, 8 to >16 μg/ml; $n = 41$), as previously noted with tigecycline. This study supports further research of omadacycline, including clinical trials, for the treatment of RGM infections, especially *M. abscessus*.

KEYWORDS nontuberculous mycobacteria, omadacycline, susceptibility testing

Nontuberculous mycobacterial (NTM) infections are increasing in the United States and globally (1–4). The *Mycobacterium abscessus* complex and *Mycobacterium avium* complex (MAC) are the two most frequently encountered NTM complexes/species among clinical laboratories throughout the United States and are some of the most drug-resistant species (5–8).

Only a few antimicrobials, including macrolides (for isolates without a functional erythromycin resistance methylase [*erm*] gene), cefoxitin, imipenem, amikacin, and tigecycline, have been effective for treatment of *M. abscessus* complex infections (5). Previous studies have shown the glycylcycline tigecycline to be highly active against rapidly growing mycobacteria (RGM), including tetracycline-resistant isolates (9). Tigecycline has also been shown to be active clinically in an open-label multidrug study (10). However, gastrointestinal (GI) adverse events are common with tigecycline and its intravenous (i.v.)-only formulation often limits its dosage and frequency of usage. For *M. abscessus* complex, selection of antimicrobials is focused upon the presence or absence of a functional *erm* gene (5). Antimicrobial treatment regimens for *M. abscessus* routinely involve multiple injectable antimicrobials, including potentially

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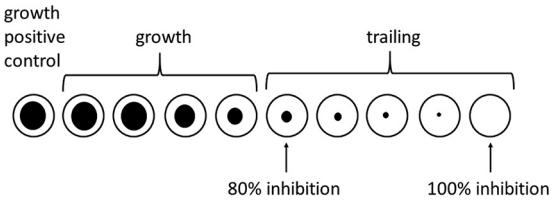

FIG 1 Graphic representation of trailing observed with omadacycline. The wells in which 80% inhibition and 100% inhibition were read are indicated with arrows.

toxic agents such as amikacin. Amikacin, imipenem, and cefoxitin are not available orally and may cause serious side effects (11). For other NTM, including MAC, treatment usually involves multiple antimicrobials, often also including i.v. agents, and can also be lengthy and difficult to withstand (12–14). Inhaled amikacin is approved (for MAC) for patients experiencing treatment failure (14).

New effective NTM antimicrobials are desperately needed, especially for patients who have pathogens that are or become macrolide or aminoglycoside resistant or for patients who become intolerant due to side effects of the antimicrobials. In previous studies, omadacycline showed pharmacokinetic advantages of higher and sustained concentrations in plasma, lung epithelial lining fluid, and alveolar cells of omadacycline compared to those of tigecycline (15). Moreover, the therapeutic potential of omadacycline is enhanced by its ability to be administered either i.v. or orally (16). In clinical trials, GI side effects are comparable to those in controls, providing a major advantage over tigecycline (15, 17, 18).

Omadacycline is the first agent from the novel class of aminomethylcyclines (17, 19). Its method of action is as a protein synthesis inhibitor which binds to the 30S ribosomal subunit in the mRNA translation complex and inhibits the binding of aminoacyl-tRNA to the mRNA-ribosome complex (17, 19). Omadacycline has shown potent activity against a wide variety of Gram-positive, Gram-negative, and atypical bacterial pathogens (20). Previous *in vitro* studies have shown that inhibition of protein synthesis occurs even in strains that express tetracycline efflux pumps and ribosomal protection mechanisms. Small studies previously showed activity against some species of RGM, including *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and *M. abscessus* complex (21, 22).

Thus, we undertook this study of the *in vitro* susceptibility to omadacycline of clinically significant species of NTM, including the most common drug-resistant groups, MAC and the *M. abscessus* complex.

RESULTS

Omadacycline MICs for RGM ranged from 0.03 to 1 μg/ml, while omadacycline MICs for the slowly growing mycobacteria (SGM) were 0.06 to >16 μg/ml, with MIC_{50}s of 8 to >16 μg/ml. Trailing was problematic with the majority of RGM isolates tested (Fig. 1 and 2). Therefore, omadacycline MICs were read at 80% (the point at which trailing began with little change in subsequent higher dilutions) and 100% inhibition with all of the RGM. For all RGM ($n = 70$), the range of omadacycline MIC_{50}s was 0.03 to 1 μg/ml at 100% inhibition; omadacycline MICs were one to three concentrations lower (MIC range = 0.004 to 0.5 μg/ml) when read at 80% inhibition.

The lowest omadacycline MICs (MIC_{50} = 0.12 at 100% and 0.015 μg/ml at 80% omadacycline) were seen with the three isolates of *M. abscessus* subsp. *massiliense*. Similar omadacycline MIC_{50} and MIC_{90}s (at 100% inhibition) were noted with *M. abscessus*

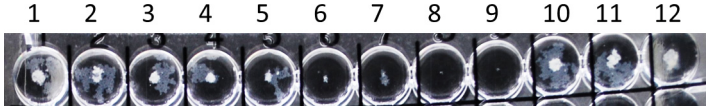

FIG 2 Demonstration of trailing endpoints with omadacycline. Wells 6 and 8 show 80% and 100% inhibition for omadacycline, respectively. Control growth is shown in well 12.

subsp. *abscessus* and the *M. fortuitum* group. Among the RGM, the highest omadacycline MIC_{90}s were seen with the isolates of *M. chelonae*, *Mycobacterium immunogenum*, and the *Mycobacterium mucogenicum* group (MIC_{90} = 0.5) (Table 1).

Among the 12 isolates of the *M. fortuitum* group, there were five doxycycline-resistant and seven doxycycline-susceptible isolates. The omadacycline MIC_{50}s and MIC_{90}s (0.12 and 0.25 μg/ml, respectively) were the same as for the total 12 isolates in the group (Table 1).

Likewise, four of the 10 isolates of the *M. mucogenicum* group were doxycycline resistant and six were doxycycline susceptible. The omadacycline MIC_{50}s and MIC_{90}s did not vary by more than one dilution between the two groups (Table 1). Importantly, this finding is much like those of earlier studies with tigecycline (9), which appears to have a similar or the same mechanism of action. Thus, this study shows that omadacycline was also effective *in vitro* against both doxycycline-resistant and -susceptible isolates of RGM.

Among the SGM tested ($n = 41$), omadacycline MICs for all species, including MAC, were generally similar, with MIC_{50}s and MIC_{90}s of >16 μg/ml except for five isolates of *Mycobacterium kansasii* which had an MIC_{50} of 8 μg/ml and one of the two isolates of *Mycobacterium marinum* (MIC = 4 μg/ml) (Table 2). Trailing was not a problem with the SGM. Therefore, all omadacycline MIC readings were at 100% inhibition.

All other MICs for the comparator antimicrobials were within expected ranges for the taxa of RGM and SGM tested (Tables 2 and 3). Tigecycline MICs were ≤ 1 μg/ml for all RGM isolates tested. Similar to a previous study (9), tigecycline MIC_{50}s ranged from 0.03 to 0.25 μg/ml, including in the doxycycline-resistant species.

Quality control was performed at each testing event. The CLSI acceptable range of MICs for *Staphylococcus aureus* ATCC 29213 was 0.12 to 1 μg/ml, and the MIC of omadacycline for *Escherichia coli* ATCC 25922 was 0.25 μg/ml. All 10 replicates of *S. aureus* ATCC 29213 and 15 isolates of *E. coli* ATCC 25922 had an omadacycline MIC within the CLSI acceptable ranges (23). All QC isolates tested with the comparator agents was within the CLSI acceptable range for *Mycobacterium peregrinum* ATCC 700686, *M. marinum* ATCC 927, and *S. aureus* ATCC 29213. Ten replicates of *M. peregrinum* ATCC 700686 had an MIC range for omadacycline of 0.06 to 0.12 μg/ml (mode = 0.12 μg/ml). Nine replicates of *M. abscessus* ATCC 19977^T were tested and had an omadacycline MIC range of 0.25 to 0.5 μg/ml (mode = 0.5 μg/ml). Eight replicates of *M. marinum* ATCC 927 had an omadacycline MIC range of 2 to 4 μg/ml. One isolate of *M. avium* ATCC 700898 had an omadacycline MIC of 8 μg/ml.

DISCUSSION

Previous small studies showed *in vitro* activity of omadacycline against RGM, including *M. chelonae*, the *M. fortuitum* group, and the difficult-to-treat isolates of the *M. abscessus* complex (21, 22, 24). To our knowledge, no *in vitro* MIC studies with omadacycline against other species of NTM have been published.

Previous MIC studies reported omadacycline MIC_{50}s of 1 and 2 μg/ml, respectively, for isolates of the *M. abscessus* complex (combining *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*) with species identification based upon *erm* gene sequence. In the study by Kaushik and colleagues, the MIC_{50} and MIC_{90}s for *M. abscessus* subsp. *abscessus* were one dilution higher than those for the combined grouping of *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* (Table 4) (21, 22). The *M. abscessus* complex MICs determined by Shoen and colleagues were read at 5 days after incubation at 37°C. As stated previously, the MICs in the present study, except for a single isolate of *M. chelonae*, were read at 3 days incubation at 30°C as per CLSI recommendations (25). It is known that the tetracyclines in general are unstable after extended incubation, and thus, these previously reported MICs may be higher than our MICs due to their longer incubation (11). Shoen et al. also tested 22 isolates of *M. chelonae* at 4 days after incubation at 32°C. These omadacycline MIC_{50} and MIC_{90}s were 0.125 and 0.25 μg/ml, respectively, and similar to our MICs. These MICs were

TABLE 1 MICs of omadacycline and comparator antimicrobials against 70 rapidly growing mycobacteria[a]

Organism (*n*)	MIC type	MIC (μg/ml) of: OMC (100% inhibition)	OMC (80% inhibition)	DOX	MIN	TGC	AMK	FOX	SXT	LZD	CIP	MXF	IPM	CLR
M. abscessus subsp. *abscessus* (20)	Range	0.06–0.5	0.015–0.12	>8	4–>8	≤0.015–1	≤2–>64	32–64	>4/76	≤2–32	≥4	4–16	4–>32	≤2–>16
	50%	0.12	0.06	>8	>8	0.12	8	32	>4/76	16	≥4	8	8	≥16
	90%	0.25	0.12	>8	>8	0.25	16	32	>4/76	32	≥4	16	16	≥16
M. abscessus subsp. *massiliense* (3)	Range	0.06–0.25	0.015	>8	4–>8	0.06–0.25	8–32	32	>4/76	≤2–16	≥4	2–16	8–32	≤2
	50%	0.12	0.015	>8	>8	0.25	8	32	4/76	4	≥4	4	16	≤2
M. chelonae (15)	Range	0.03–0.5	0.004–0.25	>8	4–>8	0.015–0.5	≤2–32	>64	2/38–>4/76	≤2–16	0.25–>4	1–>16	8–>32	≤2
	50%	0.12	0.12	>8	>8	0.25	16	>64	≥4/76	8	2	2	≥32	≤2
	90%	0.5	0.25	>8	>8	0.5	32	>64	≥4/76	16	≥4	8	≥32	≤2
M. goodii (2)	Range	0.06	0.03	≤0.12	≤0.5	≤0.015–0.03	≤2	32–>64	≤1/19	≤2	≤0.12	≤0.06	≤2–4	16–32
M. wolinskyi (1)	Range	0.5	0.12	1	≤0.5	0.03	≤2	64	2/38	4	1	0.25	4	>32
M. immunogenum (7)	Range	0.03–0.5	0.008–0.5	1–>8	≤0.5–>8	0.03–0.5	4–16	>64	2/38–>4/76	4–32	0.5–>4	2–16	16–32	≤2
	50%	0.25	0.06	>8	>8	0.12	8	>64	≥4/76	8	2	8	16	≤2
	90%	0.5	0.5	>8	>8	0.5	16	>64	≥4/76	32	≥4	16	32	≤2
M. fortuitum group (12)	Range	0.06–0.25	0.015–0.25	≤0.12–>8	≤0.5–>8	≤0.015–0.12	≤2	≤16–>64	≤1/19–2/38	≤2–4	≤0.12–0.5	≤0.06–0.25	≤2–8	≤2–>8
	50%	0.12	0.06	1	≤0.5	0.03	≤2	32	≤1/19	≤2	0.25	0.12	≤2	≥8
	90%	0.25	0.12	≥8	≥8	0.12	≤2	>64	2/38	4	0.5	0.25	8	≥8
Doxycycline resistant[b] (5)	Range	0.06–0.25	0.03–0.25	4–>8	1–>8	≤0.015–0.03								
	50%	0.12	0.12	>8	4	0.03								
	90%	0.25	0.25	>8	>8	0.03								
Doxycycline susceptible (7)	Range	0.12–0.25	0.015–0.12	≤0.12–1	≤0.5	≤0.015–0.12								
	50%	0.12	0.06	≤0.12	≤0.5	0.03								
	90%	0.25	0.12	1	≤0.5	0.12								
M. mucogenicum group (10)	Range	0.12–1	0.03–0.5	≤0.12–>8	≤0.5–>8	≤0.015–0.25	≤2	≤16	≤1/19	≤2–4	≤0.12–2	0.25–1	≤2	≤2
	50%	0.25	0.12	0.25	≤0.5	0.06	≤2	≤16	≤1/19	≤2	0.5	0.25	≤2	≤2
	90%	0.5	0.25	≥8	≥8	0.25	≤2	≤16	≤1/19	≤2	2	0.5	≤2	≤2
Doxycycline resistant[b] (4)	Range	0.12–0.5	0.03–0.25	4–>8	4–>8	≤0.015–0.25								
	50%	0.12	0.03	>8	4	0.06								
Doxycycline susceptible (6)	Range	0.25–1	0.06–0.5	≤0.12–0.25	≤0.5	0.06–0.25								
	50%	0.25	0.12	≤0.12	≤0.5	0.06								
	90%	1	0.5	0.25	≤0.5	0.25								

[a]Because of the small number of isolates tested, *M. goodii* and *M. wolinskyi* MIC_{50} and MIC_{90} and *M. abscessus* subsp. *massiliense* MIC_{90} were not calculated. Tobramycin MICs are not reported for *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, *M. fortuitum* group, *M. mucogenicum* group, *M. goodii*, and *M. wolinskyi* per CLSI (36). The tobramycin MIC range, MIC_{50}, and MIC_{90} for *M. chelonae* were ≤2 μg/ml and for *M. immunogenum* were >8 μg/ml. *n*, number of isolates; OMC, omadacycline; DOX, doxycycline; MIN, minocycline; TGC, tigecycline; AMK, amikacin, FOX, cefoxitin; SXT, trimethoprim-sulfamethoxazole; LZD, linezolid; CIP, ciprofloxacin; MXF, moxifloxacin; IPM, imipenem; CLR, clarithromycin.

[b]Doxycycline-resistant isolates include isolates with intermediate and resistant MICs (≥2 μg/ml).

TABLE 2 MICs of omadacycline and comparator antimicrobials against 25 slowly growing mycobacteria other than *Mycobacterium avium* complex[a]

Mycobacterium species (*n*)[b]	MIC type	MIC (μg/ml) of:										
		OMC	DOX	MIN	AMK	SXT	LZD	CIP	MXF	CLR	RFB	RIF
M. arupense (6)	Range	1–>16	0.5–16	0.5–8	≤2–64	≤0.5/9.5–4/76	≤1–32	>4	8–>16	≤0.06–4	≤0.25–0.5	0.5–>2
	50%	>16	8	8	16	2/38	16	>4	>16	1	≤0.25	2
	90%	>16	16	8	64	4/76	32	>4	>16	4	0.5	2
M. simiae (7)	Range	>16	≥16	≥8	8–64	2/38–4/76	16–>32	2–>4	2–>8	2–4	>2	>2
	50%	>16	>16	≥8	16	4/76	≥32	>4	4	4	>2	>2
	90%	>16	>16	≥8	64	4/76	≥32	>4	>8	4	>2	>2
M. kansasii (5)	Range	4–>16	0.12–16	≤0.25–>8	≤2–8	≤0.5/9.5–2/38	≤1–32	≤0.12–4	≤0.06–2	0.12–1	≤0.25	≤0.25–>2
	50%	8	4	2	8	≤0.5/9.5	2	1	0.12	0.25	≤0.25	2
	90%	16	16	>8	8	2/38	32	4	2	1	≤0.25	>2
M. marinum (2)	Range	4–16	1–2	0.5–1	≤2	≤0.5/9.5	≤1–2	4	0.5	0.25–0.5	≤0.25	1
M. lentiflavum (3)	Range	4–>16	4–16	4–16	4	≤0.5/9.5–1/19	8–16	0.5–2	0.5–1	0.12–0.5	≤0.25	>2
	50%	>16	8	8	4	1/19	8	2	1	0.25	>2	≤0.25
M. paraffinicum (2)	Range	8–>16	4–8	4	≤2–4	1/19	16–32	0.25–>4	0.25–1	0.25–1	≤0.25	2

[a]OMC, omadacycline; DOX, doxycycline; MIN, minocycline; AMK, amikacin; SXT, trimethoprim sulfamethoxazole; LZD, linezolid; CIP, ciprofloxacin; MXF, moxifloxacin; CLR, clarithromycin; RFB, rifabutin; RIF, rifampin.
[b]The MIC_{50} and/or MIC_{90} was not calculated for species with <5 isolates tested.

similar to those for our isolates of the *M. fortuitum* group (MIC_{50} and MIC_{90}s of 0.125 and 0.5 μg/ml compared to 0.12 and 0.25 μg/ml, respectively) which they also tested. However, MICs for these isolates were read after only 48 h incubation in their study (21).

A separate report of testing the type strain of *M. abscessus* subsp. *abscessus* (CIP 104536, ATCC 19977) in the Netherlands using CLSI guidelines showed omadacycline and tigecycline MICs of 4 μg/ml when the strain was tested in duplicate at 35°C in cation-adjusted Mueller-Hinton broth (24). These MICs are in contrast to an MIC of 1 μg/ml for both agents reported by Kaushik and colleagues and our finding of omadacycline MICs of 0.25 to 0.5 μg/ml with this strain. The tigecycline MIC range for ATCC 19977^T in our laboratory was 0.12 to 0.5 μg/ml.

The two previous studies showed tigecycline and omadacycline MIC_{50} and MIC_{90}s to be 3 to 4 dilutions higher than those in the present study. The differences between this study and the study by Shoen et al. may be explained, as noted above, by the longer incubation and differences in incubation temperatures in their study (21). However, the differences between the study by Kaushik et al. and this study cannot be attributed to differences in incubation parameters, since their incubation times and temperatures were the same as ours. Instead, possible reasons for differences could be related to the study population or geographical origin of the isolates (22). Furthermore, there was no mention of trailing endpoints in either study, and these higher MICs could have been related to that problem, as we noted with the omadacycline MICs in the present study.

The current CLSI document (25) states that mycobacterial colonies should be transferred to tubes of sterile water containing glass beads. The suspension should be vor-

TABLE 3 MICs of omadacycline and comparator antimicrobials against 16 isolates of *Mycobacterium avium* complex[a]

MIC type	MIC (μg/ml) of:				
	OMC	AMK	LZD	MXF	CLR
Range	0.06–>16	4–>64	2–>32	0.12–4	≤0.06–4
50%	>16	8	16	1	1
90%	>16	32	>32	4	2

[a]OMC, omadacycline; AMK, amikacin; LZD, linezolid; MXF, moxifloxacin; CLR, clarithromycin.

TABLE 4 Comparison of three studies of rapidly growing mycobacteria against omadacycline and tigecycline

Study	No. of isolates tested	Incubation temp/time	Species or complex	MIC (μg/ml) of:			
				Omadacycline		Tigecycline	
				50%	90%	50%	90%
Shoen et al. (21)	24	37°C/5 days	*M. abscessus* subsp. *abscessus*	1	2	1	2
	22	32°C/4 days	*M. chelonae*	0.125	0.25	0.06	0.25
	20	37°C/2 days	*M. fortuitum* group	0.125	0.5	0.25	0.5
Kaushik et al. (22)	16	30°C/3 days	*M. abscessus* subsp. *abscessus*	2	4	1	2
	12	30°C/3 days	*M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *massiliense-bolletii*	1	2	1	2
This study	20	30°C/3 days	*M. abscessus* subsp. *abscessus*	0.12 (0.06)	0.25 (0.12)	0.12	0.25
	3	30°C/3 days	*M. abscessus* subsp. *massiliense*	0.12 (0.015)		0.25	
	15	30°C/3 days	*M. chelonae*	0.12 (0.12)	0.5 (0.25)	0.25	0.5
	12	30°C/3 days	*M. fortuitum* group	0.12 (0.06)	0.25 (0.12)	0.03	0.12

texed and large clumps allowed to settle with the supernatant used for the inoculum to avoid clumping of the organisms in broth. Clumping may also cause trailing, leading to higher MIC reads (Fig. 1 and 2).

It should also be noted that our research reference laboratory at the University of Texas Health Science Center at Tyler (UTHSCT) has more than 40 years of experience in antimicrobial susceptibility testing of NTM, with more than 20 years of experience in the susceptibility testing of tigecycline. Moreover, our lower tigecycline MICs have been corroborated by other investigators, including a 2008 study in Spain (26). During this time, we have rarely seen isolates of RGM with tigecycline MICs of $>1\ \mu$g/ml with our clinical isolates. Our MICs in this study are similar to those previously published by our laboratory and in the Spanish study (9, 26).

Isolates with trailing endpoints present a problem in reading/interpretation of MICs. Since the CLSI has not yet addressed MICs of omadacycline, we recommend that extreme care be taken in reading the MICs, disregarding obvious trailing, which is usually seen as a "ghost-like" or faint button exhibiting much less growth than the positive control. Just as is recommended for difficult-to-read MIC wells, including MICs for trimethoprim-sulfamethoxazole, which are also read at 80% inhibition, we also recommend adjusting the MIC panel on the mirrored light box with overhead illumination so that the wells in which the trailing occurs are in the most brightly lit area.

This *in vitro* study showed activity of omadacycline against RGM, including isolates of *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, *M. chelonae*, *M. immunogenum*, the *M. fortuitum* group, the *M. mucogenicum* group, *Mycobacterium goodii*, and *Mycobacterium wolinskyi*. Trailing was common among most RGM isolates tested, and thus, we read those MICs at 80% inhibition (ignoring faint buttons of growth, much as trimethoprim-sulfamethoxazole MICs are read), and 100% inhibition. The 80% inhibitory MICs are given in Table 1. Upon careful analysis, we noted that these 80% reads usually varied by $\leq$2 concentrations. Moreover, the omadacycline MIC_{50}s and MIC_{90}s with both 80% and 100% inhibition were $\leq 0.5\ \mu$g/ml against all of the RGM tested except for six isolates of the *M. mucogenicum* group, with an MIC_{90} of 1 μg/ml at 100% inhibition and 0.5 μg/ml at 80% inhibition.

MICs for omadacycline were generally comparable to the tigecycline MICs for most RGM isolates (Table 1). However, unlike with omadacycline, no trailing was obvious with tigecycline. Tigecycline MIC_{50}s were within one dilution of the omadacycline MIC_{50} (100% inhibition) except for the MIC_{50}s of the *M. fortuitum* group and the *M. mucogenicum* group, which were two dilutions less, with tigecycline MIC_{50}s of 0.03 μg/ml and 0.06 μg/ml, respectively, compared to omadacycline MIC_{50}s of 0.12 μg/ml and 0.25 μg/ml, respectively (Table 1). Furthermore, as shown in Table 1, and similar to our earlier findings in the *in vitro* study of tigecycline, the omadacycline MIC_{50}s and MIC_{90}s

were the same or within one dilution for isolates of both doxycycline-resistant and doxycycline-susceptible isolates of the *M. fortuitum* group and the *M. mucogenicum* group, respectively.

Although there was no trailing of MICs with the omadacycline with the SGM, omadacycline MICs were similar to our previous tigecycline MIC against the SGM (9). Omadacycline MIC_{50}s and MIC_{90}s were higher than those of the RGM, and MICs were not within the CLSI-recommended MIC range for doxycycline-susceptible isolates (9, 25). The MAC isolates in this study had omadacycline MIC_{50}s of $>16\,\mu$g/ml (Table 3), and the other SGM exhibited omadacycline MIC_{50}s of $\geq 8\,\mu$g/ml, although fewer than 10 isolates of each species other than MAC were tested (Table 2). Additional experiments to elucidate reasons for the higher MICs with omadacycline with the SGM were beyond the scope of this study.

Previous structural studies of omadacycline demonstrated that the aminomethyl substituent modification at the C-9 position on the tetracycline molecule allows omadacycline to overcome the usual tetracycline resistance mechanisms, including ribosomal protection proteins and efflux mechanisms that characterize the older tetracyclines (16, 17, 19, 27). Tigecycline, a glycylcycline tetracycline, is also known to circumvent these common mechanisms (20). However, since tigecycline is available only for parenteral use and its use is limited by serious adverse events, including nausea, vomiting, and increased mortality related to dosage (10, 20, 28), omadacycline provides an attractive oral option, although it can also be administered intravenously (29). Moreover, the most frequently observed GI adverse events, including nausea and vomiting, were only 14.9 and 8.3%, respectively, in a recent safety study of omadacycline with both oral and intravenous dosing (30).

In a 2017 comparative study of intravenous omadacycline (100 mg every 12 h for two doses followed by 100 mg every 24 h for six doses) and 100 mg tigecycline given i.v. followed by 50 mg every 12 h for six doses in healthy nonsmoking male and female adult subjects, the incidence of nausea with omadacycline was 2.4%, compared to 47.6% in subjects treated with tigecycline. There were no incidences of vomiting with omadacycline, compared to 14.3% in subjects to whom tigecycline was administered. Also, 9.5% of the subjects given tigecycline discontinued treatment because of nausea, compared to 0% of subjects given omadacycline (15).

In the same healthy-subject study, the pattern and time course of tigecycline concentration in plasma, epithelial lining fluid, and alveolar cells were similar to those seen in subjects given omadacycline. However, the tigecycline concentrations were lower than those of omadacycline, suggesting pharmacokinetic advantages of higher and sustained concentrations of omadacycline compared to tigecycline (15). Plasma levels of omadacycline have previously shown a maximum concentration in serum (C_{max}) of $2.12 \pm 0.68\,\mu$g/ml compared to a tigecycline C_{max} of $0.98 \pm 0.21\,\mu$g/ml following a 100-mg dose of omadacycline and a 50-mg dose of tigecycline (15).

Omadacycline was approved by the Food and Drug Administration (FDA) in 2018 for use in acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia (27, 31). Although no indication for mycobacterial infections has been FDA approved, its use against NTM infections is promising. *In vitro* MIC studies against NTM and the safety and efficacy profile of omadacycline suggest a potential efficacious addition to the RGM treatment armamentarium.

Previous genomic analysis of the *M. abscessus* complex has revealed the presence of many potential antimicrobial resistance determinants, including multiple tetracycline efflux genes (32). Additional studies with different species of NTM are needed to assess the activity of omadacycline against isolates that express the antibiotic resistance mechanisms that have been previously reported to be overcome by omadacycline, such as resistance genes for ribosomal protection, including *tet*M, *tet*O, and *tet*S, and tetracycline efflux genes, including *tet*K and *tet*L (17, 29). Further research with omadacycline focusing on tetracycline resistance mechanisms and more dynamic models, such as animal models and/or the hollow fiber model, may be warranted.

MATERIALS AND METHODS

MIC testing was performed in cation-adjusted Mueller-Hinton broth (CAMHB) as described and recommended by the Clinical and Laboratory Standards Institute (CLSI) (25). Comparator antimicrobials included amikacin (AMK), cefoxitin (FOX), ciprofloxacin (CIP), clarithromycin (CLR), doxycycline (DOX), imipenem (IPM), linezolid (LZD), minocycline (MIN), moxifloxacin (MXF), tigecycline (TGC), and trimethoprim sulfamethoxazole (TMP-SMX) for the RGM ($n = 70$). Comparator antimicrobials included amikacin, clarithromycin, linezolid, and moxifloxacin for the MAC ($n = 16$) and amikacin, ciprofloxacin, clarithromycin, doxycycline, linezolid, moxifloxacin, rifabutin, rifampin, and TMP-SMX for the other SGM ($n = 25$) (Tables 1 to 3) (25).

Species identification. Isolates of NTM were identified by gene sequencing as indicated for each species/group as previously described. For the RGM, sequencing of region 5 of the *rpoβ* gene and the *erm*(41) gene (for the *M. abscessus* complex) was performed using previously recommended criteria for identification, including CLSI recommendations (33, 34). The SGM species were identified using partial 16S rRNA gene sequencing along with the CLSI interpretive criteria (33, 35).

Antimicrobial susceptibility testing. Seventy isolates of RGM were tested in CAMHB with incubation at 30°C for 3 days (one isolate of *M. chelonae* required 6 days for adequate growth in broth). These included six each of doxycycline-susceptible and doxycycline-resistant isolates of the *M. fortuitum* group and four of 10 doxycycline-resistant isolates of the *M. mucogenicum* group. Additionally, 41 SGM MICs were determined in CAMHB containing oleic acid-albumin-dextrose-catalase (OADC) and incubated at 35°C for 7 to 8 days. All MIC testing was performed in accordance with the current CLSI guidelines (25, 36).

Quality control. Quality control (QC) was performed with each susceptibility testing event. QC for RGM and SGM comparator antimicrobials was performed using CLSI guidelines for interpretive criteria for acceptable ranges with *M. peregrinum* ATCC 700686, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 (25, 36).

Omadacycline QC was performed using the same three QC isolates as for comparator agents, including *S. aureus* ATCC 29213 10 times, and *E. coli* ATCC 25922 15 times. The omadacycline QC with *M. peregrinum* ATCC 700686 was performed with 10 replicates. In addition, QC was performed with *M. marinum* ATCC 927 eight times, *M. avium* ATCC 700898 one time, and *M. abscessus* ATCC 19977^T nine times.

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