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Comparison of *In Vitro* Susceptibility of Delafloxacin with Ciprofloxacin, Moxifloxacin, and Other Comparator Antimicrobials against Isolates of Nontuberculous Mycobacteria

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ABSTRACT Nontuberculous mycobacterial (NTM) infections are increasing globally. *Mycobacterium avium* complex (MAC) and *M. abscessus* complex are the most commonly reported NTM. Oral treatment options are limited, especially for the *M. abscessus* complex. We tested delafloxacin, a new oral fluoroquinolone, against 131 isolates of NTM. Delafloxacin microdilution MICs were performed as recommended by the Clinical and Laboratory Standards Institute using cation adjusted Mueller-Hinton broth. The rapidly growing mycobacteria tested included *M. abscessus* subsp. *abscessus* ($n = 16$) and subsp. *massiliense* ($n = 5$), *M. chelonae* ($n = 11$), *M. immunogenum* ($n = 5$), *M. fortuitum* group ($n = 13$), *M. porcinum* ($n = 7$), *M. senegalense* ($n = 7$), *M. mucogenicum* group ($n = 5$), and *M. goodii* ($n = 1$). For the slowly growing NTM (SGM), *M. avium* ($n = 16$), *M. intracellulare* ($n = 13$), *M. chimaera* ($n = 9$), *M. arupense* ($n = 5$), *M. simiae* ($n = 5$), *M. lentiflavum* ($n = 4$), *M. kansasii* ($n = 6$), and *M. marinum* ($n = 3$) were tested. Delafloxacin was most active *in vitro* against the *M. fortuitum* and *M. mucogenicum* groups and *M. kansasii*, with MIC_{50} values of 0.12 to 0.5 μg/ml (MIC range, 0.001 to 4 μg/ml) compared to $\leq$0.06 to $>$4 μg/ml for ciprofloxacin and $\leq$0.06 to $>$8 μg/ml for moxifloxacin. For other SGM (including MAC), and the *M. abscessus*/*M. chelonae*, the delafloxacin MIC range was 8 to $>$16 μg/ml compared to ciprofloxacin and moxifloxacin of 0.5 to $>$4 μg/ml and $\leq$0.06 to 8 μg/ml, respectively. To our knowledge, this is the first MIC study with delafloxacin to use Clinical and Laboratory Standards Institute (CLSI) recommended methods. This study illustrates the potential utility of delafloxacin in treatment of infections due to some NTM.

KEYWORDS susceptibility, delafloxacin, quinolones,

The number of infections due to nontuberculous mycobacteria (NTM) is increasing in the United States and globally (1–4). Among the most commonly encountered rapidly growing mycobacterial (RGM) pathogens are *Mycobacterium abscessus*, most of the *M. fortuitum* group (including *M. fortuitum*, *M. senegalense*, and *M. porcinum*), the *M. mucogenicum* group (*M. mucogenicum*, *M. phocaicum*, and *M. aubagnense*), and the *M. chelonae*/*M. immunogenum* complex.

The *M. abscessus* bacteria are the most often encountered rapidly growing mycobacterial (RGM) species in pulmonary disease and are especially difficult to treat due to their resistance to most oral and intravenous (i.v.) antimicrobials (5, 6). This complex is also associated with skin and soft tissue infections (7).

The *M. fortuitum* group and *M. chelonae*/*M. immunogenum* complex are often associated with skin and soft tissue infections following traumatic injury, including postsurgery or postinjection (8, 9). These infections also present limited treatment options, especially among oral agents.

Among the slowly growing NTM (SGM), the *M. avium* complex (MAC) is most often encountered as a pulmonary pathogen. These species are also characterized by

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resistance to most of the first-line agents used for treatment of *M. tuberculosis*. Treatment requires the use of multiple drug regimens, often including an injectable or inhaled agent such as amikacin, especially for cavitary disease. *M. kansasii* is associated with pulmonary and occasionally skin and soft tissue infection and is the next most commonly seen SGM in the United States (10–12). Although *M. kansasii* is typically more susceptible to antimicrobials than MAC, additional oral treatment options are needed.

The fluoroquinolone antibiotics typically used to treat NTM infections include ciprofloxacin and moxifloxacin. However, most isolates of the *M. abscessus* and MAC groups are resistant to one or both of these fluoroquinolones. Fewer than 50% of isolates of the *M. chelonae/M. immunogenum* complex are susceptible to these agents, and resistance among other NTM species, including *M. marinum and M. kansasii*, appears to be increasing (13, 14). Mutational resistance to ciprofloxacin occurs readily, and because of this finding, fluoroquinolones should not be given as monotherapy (15).

More effective antimicrobials (especially oral options) for treatment of NTM are desperately needed. Previous studies against of isolates, including Gram-positive, Gram-negative, anaerobic, and some atypical bacteria have shown good activity with delafloxacin (16–20). Moreover, delafloxacin has dual binding to DNA gyrase and topoisomerase IV and confers stability against target enzyme mutations often seen in Gram-positive bacteria, with delafloxacin, making it a better choice than other currently available fluoroquinolones (20, 21).

To our knowledge, only two studies of the activity of delafloxacin against NTM have been published. The first was a study of MAC isolates when the drug was known as WQ-3034 (22). However, this study used an agar dilution method in medium with an acidic pH (i.e., Middlebrook 7H11 medium), neither of which is recommended by the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing of NTM (13). The second study, by Gumbo and colleagues, used broth microdilution in Middlebrook medium to test multiple antibiotics, including delafloxacin, as potential agents for future use in the hollow-fiber model combination antibiotic studies against isolates of *M. abscessus* (23).

Our study aimed to study the activity of delafloxacin against commonly encountered clinically significant species of NTM using the CLSI-recommended broth microdilution method (13).

RESULTS

Recently published delafloxacin breakpoints for *Staphylococcus aureus* are $\leq$0.25 susceptible, 0.5 intermediate, and $\geq$1 resistant. No breakpoints are available yet for NTM (https://www.fda.gov/drugs/development-resources/delafloxacin-injection-and-oral-products). Delafloxacin MICs for the RGM ranged from 0.001 to >16 μg/ml, while the range for the SGM was 0.12 to >16 μg/ml. The lowest delafloxacin MIC_{50} values among the RGM were 0.12 to 2 μg/ml and were seen with isolates of the *M. fortuitum* group, including *M. fortuitum* (including four ciprofloxacin-resistant isolates with ciprofloxacin MICs of >4 μg/ml), *M. porcinum*, *M. senegalense*, and the *M. mucogenicum* group. Excluding the four ciprofloxacin-resistant isolates, the delafloxacin MIC range for *M. fortuitum* was 0.001 to 0.5 μg/ml, with 0.12 and 0.5 μg/ml for the MIC_{50} and MIC_{90}, respectively (see Table 1). Delafloxacin MICs for the four-ciprofloxacin resistant isolates of *M. fortuitum* were 2, 2, 4, and >16 μg/ml (see Table 1). The delafloxacin MIC for the single isolate of *M. goodii* was also low, at 0.06 μg/ml. For the 21 isolates of the *M. abscessus* complex (*M. abscessus* subsp. *abscessus, and M. abscessus* subsp. *massiliense*) and the 16 isolates of the *M. chelonae* complex (*M. chelonae* and *M. immunogenum*), delafloxacin MIC_{50} and MC_{90} values were >16 μg/ml (see Table 1).

Among the SGM, delafloxacin MIC_{50} values ranged from the lowest (MIC_{50}, 0.25 μg/ml) for six isolates of *M. kansasii* and MIC_{50} values of 1 μg/ml for 16 isolates of *M. avium*. Four isolates of *M. lentiflavum* had a delafloxacin MIC_{50} value of 2 μg/ml, and three isolates of *M. marinum* had delafloxacin MICs of 2 μg/ml. All 13 isolates of *M. intracellulare*

TABLE 1 MIC values (μg/ml) of delafloxacin and comparator antimicrobials against rapidly growing mycobacteria isolates[a]

Antimicrobial (CLSI intermediate breakpoint)[b]	MIC of delafloxacin against (no. of isolates):										
	M. abscessus subsp. *abscessus* (16)	*M. abscessus* subsp. *massiliense* (5)	*M. chelonae* (11)	*M. immunogenum* (5)	*M. fortuitum*[f] (13)	*M. fortuitum* (CIP^S) (9)	*M. fortuitum* (CIP^R) (4)	*M. porcinum* (7)	*M. senegalense* (7)	*M. mucogenicum* group (5)	*M. goodii* (1)
DEL (NA)[d]											
MIC range	≥16	4 to >16	≥16	8 to >16	0.001 to >16	0.001–0.5	2 to >16	0.12–1	0.25–1	0.03–0.12	0.06
MIC_{50}	>16	>16	>16	>16	0.25	0.12	2	0.25	0.5	0.12	NA
MIC_{90}	>16	>16	>16	>16	2	0.5	NA	1	1	0.12	NA
CIP (2)											
MIC range	≥4	0.5 to >4	0.5 to >4	≤0.12–4	≤0.12 to >4	0.12–1	4 to >4	≤0.12–0.5	0.25–0.5	≤0.12–2	≤0.12
MIC_{50}	4	4	1	2	≤0.12	≤0.12	>4	≤0.12	0.5	0.25	NA
MIC_{90}	>4	>4	4	4	>4	1	NA	0.5	0.5	2	NA
MXF (2)											
MIC range	4 to >16	2 to >16	1 to >8	1 to >8	≤0.06–8	≤0.06–0.25	≤0.06–8	≤0.06–0.25	≤0.06–0.12	0.25–0.5	≤0.06
MIC_{50}	8	8	2	1	0.12	≤0.06	0.5	≤0.06	0.12	0.25	NA
MIC_{90}	16	>16	4	4	1	0.25	NA	0.25	0.12	0.5	NA
AMK (32)											
MIC range	4 to >64	8–16	≤2–64	4–16	≤2–4	≤2	≤2–4	≤2	≤2	≤2	≤2
MIC_{50}	8	8	8	8	≤2	≤2	≤2	≤2	≤2	≤2	NA
MIC_{90}	16	16	32	16	≤2	≤2	NA	≤2	≤2	≤2	NA
FOX (32–64)											
MIC range	32–64	16–32	>64	>64	≤16–64	≤16–64	32–64	≤16–32	≤16	≤16	32
MIC_{50}	32	32	>64	>64	32	32	64	≤16	≤16	≤16	NA
MIC_{90}	64	32	>64	>64	64	64	NA	32	≤16	≤16	NA
SXT (NA)[c]											
MIC range[e]	2/38 to >4/76	2/38–4/76	1/19–4/76	≤0.5/9.5 to >4/76	≤0.5/9.5–2/38	≤0.5/9.5–2/38	≤0.5/9.5–1/19	≤0.5/9.5–2/38	≤0.5/9.5–2/38	≤0.5/9.5	≤0.5/9.5
MIC_{50}	4/76	4/76	2/38	4/76	0.5–9.5	0.5–9.5	≤0.5/9.5	1/19	1/19	≤0.5/9.5	NA
MIC_{90}	>4/76	4/76	4/76	>4/76	2/38	2/38	NA	2/38	2/38	≤0.5/9.5	NA
LZD (16)											
MIC range	2–32	1–16	2–16	2–16	≤1-8	≤1–4	2-8	≤1–2	≤1–2	≤1	≤1
MIC_{50}	16	8	8	4	2	2	2	≤1	2	≤1	NA
MIC_{90}	32	16	8	16	4	4	NA	2	4	≤1	NA
IPM (8–16)											
MIC range	4 to >32	8 to >32	4–32	8–32	≤2–8	≤2–8	≤2–8	≤2 to >8	≤2–4	≤2–8	≤2
MIC_{50}	8	16	16	16	≤2	≤2	≤2	4	4	4	NA
MIC_{90}	32	>32	16	32	8	8	NA	8	4	8	NA
DOX (2–4)											
MIC range	>8	2 to >8	≤0.12 to >8	4 to >8	≤0.12 to >8	≤0.12 to >8	0.25 to >8	2–8	≤0.12	≤0.12 to >8	≤0.12
MIC_{50}	>8	>8	>8	>8	4	≤0.12	8	4	≤0.12	≤0.12	NA
MIC_{90}	>8	>8	>8	>8	>8	>8	NA	8	≤0.12	>8	NA
MIN (2–4)											
MIC range	>8	>8	≤1 to >8	>8	≤1 to >8	≤1 to >8	≤1 to >8	4 to >8	≤1	≤1 to >8	≤1
MIC_{50}	>8	>8	>8	>8	>8	≤1	≤1	4	≤1	≤1	NA
MIC_{90}	>8	>8	>8	>8	>8	>8	NA	>8	≤1	>8	NA
TGC (NA)[d]											
MIC range	0.06–0.5	0.06–0.25	≤0.03–0.12	≤0.03–0.12	≤0.03–0.06	≤0.03–0.06	≤0.03	≤0.03	≤0.03	≤0.03–0.25	≤0.03
MIC_{50}	0.12	0.12	0.06	0.12	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.06	NA
MIC_{90}	0.25	0.25	0.12	0.12	0.06	0.06	NA	≤0.03	≤0.03	0.25	NA
CLR (4)											
MIC range	≤2 to >16	≤2 to >16	≤2	≤2	≥16	≥16	≥16	≥16	1-≤2	≤2	8
MIC_{50}	≥16	≤2	≤2	≤2	≥16	>16	≥16	>16	≤2	≤2	NA
MIC_{90}	≥16	≥16[g]	≤2	≤2	≥16	>16	NA	>16	≤2	≤2	NA

(Continued on next page)

TABLE 1 (Continued)

Antimicrobial (CLSI intermediate breakpoint)[b]	MIC of delafloxacin against (no. of isolates):										
	M. abscessus subsp. *abscessus* (16)	*M. abscessus* subsp. *massiliense* (5)	*M. chelonae* (11)	*M. immunogenum* (5)	*M. fortuitum*[f] (13)	*M. fortuitum* (CIP^S) (9)	*M. fortuitum* (CIP^R) (4)	*M. porcinum* (7)	*M. senegalense* (7)	*M. mucogenicum* group (5)	*M. goodii* (1)
TOB (4)											
MIC range	NA	NA	≤2	4 to >8	NA	NA	NA	NA	NA	NA	NA
MIC_{50}	NA	NA	≤2	>8	NA	NA	NA	NA	NA	NA	NA
MIC_{90}	NA	NA	≤2	>8	NA	NA	NA	NA	NA	NA	NA

[a]NA = not applicable.

[b]DEL, delafloxacin; AMK, amikacin; FOX, cefoxitin; SXT, trimethoprim sulfamethoxazole; LZD, linezolid; CIP, ciprofloxacin; IPM, imipenem; MXF, moxifloxacin; DOX, doxycycline; MIN, minocycline; TGC, tigecycline; CLR, clarithromycin; TOB, tobramycin.

[c]There are no intermediate breakpoints for SXT; the resistance breakpoint is >64 μg/ml.

[d]The CLSI has not addressed breakpoints for tigecycline (TGC) or delafloxacin (DEL).

[e]MIC_{90} values were not calculated for groups with <5 isolates.

[f]Four isolates were known to be quinolone resistant.

[g]Isolate was mutationally macrolide resistant.

and all nine isolates of *M. chimaera* had MIC_{50} values of $\geq$8 μg/ml, in contrast to those isolates of *M. avium*. Additionally, five isolates each of *M. arupense and M. simiae* had delafloxacin MC_{50} values of $\geq$16 μg/ml (see Table 2).

The MICs for comparator antimicrobials were within the expected ranges (see Tables 1 and 2) as defined by the CLSI (24, 25).

Quality control (QC) was performed at each testing event. The CLSI acceptable range of MICs for *S. aureus* ATCC 29213 was 0.001 to 0.008 μg/ml and for *Escherichia coli* ATCC 25922 was 0.008 to 0.03 μg/ml (24, 25). All 10 replicates of *S. aureus* ATCC 29213 and eight replicates of *E. coli* were within the acceptable ranges. All QC isolates tested with the comparator agents were within the CLSI acceptable ranges for *M. peregrinum* ATCC 700686 (for RGM), *M. marinum* ATCC 927 (for SGM), and *S. aureus* ATCC 29213. Seven replicates of *M. peregrinum* ATCC 700686 had a delafloxacin MIC range of 0.12 to 0.25 μg/ml. Six replicates of *M. marinum* had a delafloxacin MIC range of 4 to 16 μg/ml, and all nine replicates of *M. abscessus* ATCC 19977T had delafloxacin MICs of $>$16 μg/ml.

DISCUSSION

In this study, we compared the *in vitro* activity (MICs) of a new fluoroquinolone, delafloxacin, with those of ciprofloxacin and moxifloxacin and a battery of the typically tested comparator antimicrobials for each organism type.

Delafloxacin is the first anionic fluoroquinolone approved by the Food and Drug Administration (FDA) for the treatment of acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia (CABP) (17, 26–28). However, to our knowledge, until now, no studies have been undertaken in the United States to determine the *in vitro* activity of multiple species of NTM to delafloxacin using the current CLSI recommendations (13). As previously mentioned, a single study in Japan by Tomioka and colleagues (22) when the antimicrobial was known as WQ-3034, was performed comparing it to other quinolones, including ciprofloxacin (but not moxifloxacin) on 20 isolates each of *M. avium* and *M. intracellulare*. Using the agar dilution method with Middlebrook 7H11 (as previously mentioned, an antimicrobial susceptibility method and medium not recommended by the CLSI), the ciprofloxacin and WG-3034 MIC_{50} values were both 3.13 μg/ml with *M. avium*. The *M. avium* MIC_{90} values were 25 μg/ml for WQ-3034 compared to 12.5 μg/ml for ciprofloxacin. In comparison, the MIC_{50} values for both WQ-3034 and ciprofloxacin were 25 μg/ml, and the MIC_{90} values were 25 and 50 μg/ml, respectively, with *M. intracellulare*. The broth microdilution method (not used with the MAC isolates), using 7HSF (7H11 medium without malachite green) showed delafloxacin MICs of 1 μg/ml, compared to 0.25 μg/ml for levofloxacin, against 45 isolates of *M. tuberculosis* (22).

A more recent study by Gumbo and colleagues (23) tested multiple antibiotics including delafloxacin in Middlebrook (acidic medium) against 19 clinical isolates of *M. abscessus* and the *M. abscessus* reference type strain. Delafloxacin MICs were all $>$128 μg/ml, similar to our findings in alkaline medium (CAMHB).

Our study MICs were determined using the CLSI-recommended broth microdilution method with CAMHB (13). Because CAMHB has a pH of 7.2 to 7.4, our delafloxacin MICs were higher than might be expected in acidic broth (e.g., Middlebrook 7H9 broth) (13). However, as stated previously, this did not prove to be true in a study of the *in vitro* activity of delafloxacin in acidic medium against *M. abscessus* (23) and with *M. kansasii* (S. Srivastava, unpublished data).

Despite the pH of our test media, several species/groups of RGM, including the *M. fortuitum* and *M. mucogenicum* groups, demonstrated low MICs. Among the SGM, some isolates of the MAC, *M. kansasii*, *M. marinum*, *M. lentiflavum*, and *M. arupense* demonstrated low MICs.

Delafloxacin exhibits antimicrobial activity by inhibiting the two major enzymes for replication, DNA gyrase and topoisomerase IV (16). Delafloxacin has a greater affinity for DNA gyrase in comparison with other fluoroquinolones, and it is this finding that

TABLE 2 MIC values (μg/ml) of delafloxacin and comparator antimicrobials against slowly growing nontuberculous mycobacteria isolates[a]

Antimicrobial (CLSI intermediate breakpoint)[b,c]	*Mycobacterium avium* complex (includes all 3 species) (38)	*M. intracellu-lare* (13)	*M. avium* (16)	*M. chimaera* (9)	*M. arupense* (5)	*M. simiae* (5)	*M. lentiflavum* (4)	*M. kansasii* (6)	*M. marinum* (3)
DEL (NA)									
MIC range	0.12 to >16	8 to >16	0.12 to >16	16 to >16	2–16	≥16	0.5–16	0.25–4	2
MIC_{50}	16	>16	1	>16	8	>16	2	0.25	2
MIC_{90}	>16	>16	>16		16	>16		4	
CIP (2)									
MIC range	0.5 to >4	4 to >4	0.5 to >4	>4	>4	4 to >4	0.5–4	0.5 to >4	2–4
MIC_{50}	>4	>4	4	>4	>4	4	2	1	4
MIC_{90}	>4	>4	>4	>4	>4	>4		>4	
MXF (2)									
MIC range	≤0.06–8	1–8	≤0.06–4	2–4	8 to >16	4–8	0.5–2	≤0.06–1	0.5–1
MIC_{50}	2	4	0.5	2	>16	4	1	≤0.06	1
MIC_{90}	4	4	4	4	>16	8		1	
AMK (32)									
MIC range	≤2 to >64	4–32	≤2 to >64	4–16	≤2 to >64	4–8	4–8	≤2–8	≤2
MIC_{50}	8	8	8	8	>64	8	4	≤2	≤2
MIC_{90}	32	32	32	16	>64	8		8	
CLR (16)									
MIC range	≤0.06 to >32	1–4	≤0.06 to >32	1–4	0.12–2	2–4	0.12–1	≤0.06–1	0.5–1
MIC_{50}	2	2	1	2	0.5	4	0.25	0.12	0.5
MIC_{90}	4	4	4	4	2	4		1	
SXT (NA)									
MIC range	NA	NA	NA	NA	≤0.05/9.5–4/76	1/19–4/76	1/19–2/38	≤0.5/9.5–2/38	≤0.5/9.5–1/19
MIC_{50}	NA	NA	NA	NA	1/19	2/38	1/19	≤0.5/9.5	≤0.5/9.5
MIC_{90}	NA	NA	NA	NA	4/76	4/76		2/38	
LZD (16)									
MIC range	≤1–32				≤1–16	8–32	8−16	≤1–16	≤1–4
MIC_{50}	16				≤1	16	8	≤1	≤1
MIC_{90}	32				16	32		16	
DOX (2–4)									
MIC range	NA				2–8	>16	4 to >16	1–16	1–4
MIC_{50}	NA				8	>16	8	1	1
MIC_{90}	NA				8	>16		16	
RFB (NA)									
MIC range	NA				≤0.25	≥2	≤0.25–0.5	≤0.25	≤0.25
MIC_{50}	NA				≤0.25	>2	≤0.25	≤0.25	≤0.25
MIC_{90}	NA				≤0.25	>2		≤0.25	
RMP									
MIC range	NA				0.5 to >2	>2	>2	≤0.25 to >2	≤0.25–0.5
MIC_{50}	NA				>2	>2	>2	≤0.25	≤0.25
MIC_{90}	NA				>2	>2		>2	

[a]NA, not applicable.

[b]DEL, delafloxacin; CIP, ciprofloxacin; MXF, moxifloxacin; AMK, amikacin; CLR, clarithromycin; SXT, trimethoprim sulfamethoxazole, LZD, linezolid; DOX, doxycycline; RFB, rifabutin; RMP, rifampin.

[c]There are no intermediate breakpoints for SXT, RFB, and RMP. The resistance breakpoints are >64 μg/ml, >4 μg/ml, and ≥2 μg/ml, respectively. The CLSI has not addressed breakpoints for DEL. There is no intermediate breakpoint for inhaled AMK; resistance is ≥128 μg/ml. MIC_{90} values were not calculated for groups with <5 isolates.

along with its specific structural characteristics, including shape, size, and weak polarity, and the large hetero-aromatic ring are thought to increase potency to the agent even with resistance to other fluoroquinolones (18).

Since prior studies with delafloxacin with other organisms have demonstrated MICs consistently 3- to 5-fold lower than comparator fluoroquinolones (ciprofloxacin, moxifloxacin), our hypothesis was that this could be true with NTM (18). However, in the current study, the delafloxacin MICs for the RGM were generally 3 to 4 times higher than those of the comparator fluoroquinolones, moxifloxacin and ciprofloxacin. For the SGM, comparisons to other fluoroquinolones were more difficult since most isolates were also resistant (i.e., greater than the highest concentration tested), but most MICs were at least equivalent Table 2). Previous studies have shown ciprofloxacin MICs to be comparable to those obtained in this study (29).

The absence of a base group at C7 that gives delafloxacin an anionic character at neutral pH is unique in comparison to the other fluoroquinolones. This structural change allows delafloxacin to enter bacterial cells more readily and increases its activity in the acidic environment of the infection site with corresponding decreases in MICs, especially among Gram-positive bacteria. It is this characteristic that initially evoked consideration of the CLSI-currently recommended susceptibility testing medium/method used for NTM (18). Since the CLSI recommends testing of antimicrobials in cation-adjusted Mueller-Hinton broth, at pH 7.2 to 7.4, this study was performed under those conditions (13). Modifications of methods and medium selection would require extensive work in multicenter studies and are beyond the scope of this study. However, as previously stated, Gumbo and colleagues recently showed that delafloxacin MICs for *M. abscessus* were not lower in acidic media than in alkaline media, suggesting that repeat testing in acidic media would be unnecessary (23).

Because ciprofloxacin has been shown to have a high mutational frequency among some of the RGM, it would be important to demonstrate improved activity with another quinolone such as delafloxacin that might offer an option for treatment regimens (15). We performed delafloxacin MICs on four isolates of *M. fortuitum* which were known to have a gyrase mutation due to previous quinolone therapy (unpublished data from our laboratory). The MICs are shown in Table 1. The delafloxacin MIC_{50} value for the ciprofloxacin-resistant isolates was 2 μg/ml, compared to 0.12 μg/ml for the nine ciprofloxacin-susceptible isolates. Further investigation of these results was beyond the scope of this study.

Delafloxacin provides a safe oral or intravenous therapeutic option for treatment of some species of NTM. The bioavailability of oral dosing is 58.8%. Although no delafloxacin clinical trials with patients with NTM have been reported, the most common adverse events in treatment of bacterial infections have been nausea, vomiting, or diarrhea. In a bacterial (not NTM) clinical trial of acute bacterial skin and skin structure infections (duration of treatment, 5 to 14 days) comparing delafloxacin to tigecycline, the patients given tigecycline had more gastrointestinal events (mostly nausea) (16). Moreover, delafloxacin has demonstrated no clinically significant increase in QTc in comparison to moxifloxacin, and phototoxicity does not appear to be a concern compared to other fluoroquinolones (16).

MATERIALS AND METHODS

Isolates. A total of 131 isolates of NTM submitted to the Mycobacteria/Nocardia Research Laboratory at the University of Texas Health Science Center at Tyler, Texas, from 2018 to 2019 were tested against delafloxacin and other comparative antimicrobials (see Table 1); 106 (81%) of the isolates were of respiratory origin, while the remaining 25 isolates (19%) were from skin and soft tissue, blood, body fluids, etc.5

The RGM included 16 *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, 11 *M. chelonae*, 5 *M. immunogenum*, 27 *M. fortuitum* group (13 *M. fortuitum* and 7 each *M. porcinum* and *M. senegalense*), and 5 *M. mucogenicum* group (*M. mucogenicum and M. phocaicum*). The SGM species included 5 *M. simiae*, 3 *M. marinum*, 6 *M. kansasii*, 4 *M. lentiflavum*, 5 *M. arupense*, 16 *M. avium*, 13 *M. intracellulare*, and 9 *M. chimaera*).

Identification. All isolates of NTM were identified by gene sequencing as indicated for each species/group. For the RGM, sequencing of region 5 of the *rpo*β gene and the *erm*(41) gene (for the *M.*

abscessus) was performed using previously recommended criteria for identification, including the CLSI recommendations (30, 31). The SGM species were identified using partial 16S rRNA gene sequencing along with the CLSI interpretive criteria (30).

Antimicrobial susceptibility testing. Isolates were tested by broth microdilution in cation-adjusted Mueller-Hinton broth (CAMHB), pH 7.3, using doubling dilutions of antimicrobials as recommended by the CLSI. Repeat testing of isolates was performed if the delafloxacin or comparator MICs were discrepant with other isolate MICs within that taxa. However, testing in duplicate or triplicate was not performed with all isolates. Delafloxacin concentrations were 0.005 to 16 μg/ml for RGM and SGM in frozen panels manufactured by Thermo Fisher (Westlake, OH). MICs for the RGM were read after incubation at 30°C for 3 to 5 days until sufficient growth was evident in the growth control well. The slowly growing NTM were read after incubation at 35°C for 7 to 14 days when sufficient growth was evident in the growth control wells.

Comparator antimicrobials included amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, minocycline, moxifloxacin, tigecycline, trimethoprim-sulfamethoxazole (TMP-SMX), and tobramycin (for *M. chelonae* and *M. immunogenum*) for the RGM. For the SGM (except MAC), the antimicrobials included amikacin, ciprofloxacin, clarithromycin, doxycycline, linezolid, moxifloxacin, rifabutin, rifampin, and TMP-SMX (see Table 1). Comparator antimicrobials for MAC included amikacin, clarithromycin, linezolid, and moxifloxacin as recommended by the Clinical and Laboratory Standards Institute (CLSI). The CLSI-recommended breakpoints are listed in Table 2. All comparator antimicrobial MICs were read on panels manufactured by Thermo Fisher.

Quality control. Quality control of susceptibility testing was performed each week of testing using the CLSI-recommended strains *M. peregrinum* ATCC 700686 (RGM) and *M. marinum* ATCC 927 (SGM) for the comparator antimicrobials and *Staphylococcus aureus* ATCC 29213 as recommended by the CLSI for delafloxacin (13, 24, 25). Additional quality control for delafloxacin was performed using *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 and the above mycobacterial reference strains.

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