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Activities of Dual Combinations of Antibiotics Against Multidrug-Resistant Nontuberculous Mycobacteria Recovered from Patients with Cystic Fibrosis

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Patients with cystic fibrosis (CF) are at risk for recurrent pulmonary infections due to increased viscosity of airway secretions, leading to persistent colonization with pathogenic bacteria, including nontuberculous mycobacteria (NTM). Extensive antibiotic use for treatment of infections has led to increasing antimicrobial resistance, which is a significant barrier to the treatment of NTMs. We examined the *in vitro* activity of several antibiotics against a selection of the most drug-resistant clinical isolates of *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium avium* complex recovered from CF patients at our institution, as well as paired combinations of antibiotics against a subset of *M. abscessus* strains, to determine whether they exhibit synergy in inhibiting bacterial growth. Most isolates displayed resistance to at least six of the nine antibiotics tested for which phenotypic interpretation is available, and elevated minimum inhibitory concentrations (MICs) were observed for many of the other drugs. The major exception was clofazimine, which had relatively low MICs for most isolates across all species. When synergy testing was performed by using paired combinations of drugs, clofazamine and clarithromycin exhibited 100% synergy for all combinations tested, as did amikacin, with the exception of one isolate. These results suggest that synergistic antibiotic combinations are capable of overcoming drug resistance *in vitro*, and laboratories might consider implementation of synergy testing in multidrug-resistant (MDR)-NTM organisms to guide treatment decisions in the setting of extensive antimicrobial resistance.

Keywords: nontuberculous mycobacteria, antibiotic synergy, clofazimine

Introduction

Cystic fibrosis (CF) was once considered a disease with an extremely poor prognosis, from which most patients died in early childhood. However, improved treatment options have led to a steady increase in life expectancy, with the median survival now at 39 years.[1] Improved survival has resulted in additional challenges, as patients are at high risk for development of recurrent bacterial infections due to abnormal airway secretions that are difficult to clear, leading to chronic inflammation and progressive pulmonary colonization with pathogenic bacteria. Nontuberculous mycobacteria (NTM), some of which can cause clinically significant infections, are also commonly detected in the sputum of these patients.[2,3] Differentiating between colonization and infection with these organisms is one of the challenges that physicians face in treating this patient population.[4] At our institution, a tertiary medical center, the most frequently isolated NTM species from CF patients are *Mycobacterium abscessus*, *Mycobacterium chelonae*, and the *Mycobacterium avium complex* (MAC). This reflects similar findings from other studies, in which *M. abscessus* and MAC represented up to 56% and 72% of NTMs recovered from CF patients, respectively.[2,5]

In addition, copious use of antibiotics for treatment of common bacterial pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* collaterally exposes colonizing NTM organisms to various antibiotics, thus promoting selection of drug resistant strains. Treatment of NTM infections in CF patients is a growing challenge, as strains that are resistant to multiple antibiotics are increasingly common.[6] The treatment regimens for resistant NTM pulmonary infections generally involve several months of therapy with multiple antibiotics, many of which are poorly tolerated and associated with significant cytotoxic effects.[7]

Susceptibility testing of NTMs to antibiotics based on determination of minimum inhibitory concentration (MIC) is often performed only at reference laboratories, where

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individual antibiotics are tested by using either a commercially available microbroth dilution assay or a laboratory-developed test. In most instances, an MIC is determined for each drug tested and an interpretation of susceptible, intermediate, or resistant is assigned. For those antibiotics for which phenotypic interpretations are not available, only an MIC is provided. Few laboratories perform *in vitro* testing to determine activity of combinations of currently used antibiotics. For patients with multidrug-resistant (MDR) NTM infections, few treatment options remain when resistance is identified to nearly all antibiotics for which interpretations exist, and the MICs for the remaining drugs are at or above the test range. In this setting, the development of a synergistic combination regimen may have significant impact on providing effective treatment. Recent studies have identified *in vitro* synergistic activity from dual antibiotic combinations against NTMs[8–10] and proposed usage of combination therapy for more effective treatment of NTM infections.

In this study, we examined the *in vitro* activity of several antibiotics against a selection of the most drug-resistant clinical isolates of *M. abscessus*, *M. chelonae*, and MAC recovered from CF patients at our institution, as well as paired combinations of antibiotics against a subset of *M. abscessus* strains, to determine whether dual antibiotic combinations exhibit synergy in inhibiting their growth. In addition to the antibiotics commonly used for treatment of NTMs such as macrolides, aminoglycosides, and fluoroquinolones, we have also included nonstandard antibiotics such as clofazamine, dapsone, and rifampin, as they were previously shown to have potent *in vitro* activity against various nontuberculous species.[6] Although some synergy testing with these drugs had been done by prior investigators, the number of antibiotic combinations tested were limited and they have not been extensively studied with MDR-NTM strains such as those utilized in this study,[6] thus providing an opportunity to investigate an expanded number of *in vitro* combinations against drug-resistant strains.

Materials and Methods

Strains and antibacterial activity

A total of 41 MDR NTM isolates were used in this study, including rapidly growing (*M. abscessus*, $n=19$; *M. chelonae*, $n=5$) and slow-growing (MAC, $n=17$) species. Mycobacterial speciation for MAC was performed by using DNA probes according to the manufacturer's instructions (Accuprobe; Hologic, Inc., San Diego, CA). Identification of *M. abscessus* and *M. chelonae* was done by using either 16S rDNA sequencing in tandem with biochemical confirmation of species by sodium citrate biochemical testing for isolates recovered before 2015 or MALDI Tof MS for more recent isolates using the Bruker Microflex LT (MicroFlex LT; Bruker, Bremen, Germany) and Bruker Biotyper software (Version 2.0). Subspeciation within the complexes for *M. abscessus* and *M. chelonae* was not performed, as this is not routinely done as part of the standard of care in the clinical mycobacteriology lab (Johns Hopkins, Baltimore, MD). All isolates were part of an archived collection recovered from the sputum of CF patients for whom previous antimicrobial susceptibility was incomplete or had not been obtained. All species and strains were initially grown on Lowenstein Jensen agar slants and Middlebrook 7H11 agar plates (Hardy Diagnostics, Santa Maria, CA). For all assays, incubation times and temperatures were adjusted by species (3 to 5 days at 30°C for *M. abscessus* and *M. chelonae*; 14 days at 35°C for MAC). All *M. abscessus* isolates were incubated for 14 days total to detect the presence of inducible macrolide resistance. Antimicrobial susceptibility testing was performed for all isolates by using a commercially available microbroth dilution assay according to the manufacturer's instructions (Trek Sensititre®; Thermo Scientific, Oakwood Village, OH). The target inoculum for all susceptibility tests was $\sim 10^5$ CFU/ml, which was verified by determination of viable counts. All assays were performed in duplicate. Additional or nonstandard antibiotics, including clofazimine and dapsone (Sigma-Aldrich, St. Louis, MO), were tested separately with all NTM species and strains by using a lab-developed, microbroth dilution assay in cation-adjusted Mueller-Hinton broth and also Middlebrook 7H9 for *M. abscessus, M. chelonae*, and MAC. Panels were read independently by two trained medical technologists using a mirror-box and ambient light. Table 1 lists all of the antibiotics and concentrations that were tested in this study. Synergy testing was performed by combining the full range of concentrations for each drug listed in Table 1 with single concentrations at or below the MIC for each of the following antibiotics: clofazimine (0.5 μg/ml), clarithromycin (4 μg/ml), amikacin (8 μg/ml), dapsone (0.5 μg/ml), rifampin (1 μg/ml), and tigecycline (0.5 μg/ml).

Data interpretation

Susceptibility or resistance was defined by using established interpretations where available.[11] For antibiotics with no current interpretations, isolates were considered resistant when confluent growth was observed at the highest concentration tested. A subset of *M. abscessus* isolates ($n=8$) were selected for synergy testing, which demonstrated resistance to ≥7 antibiotics for which interpretations exist and MICs at or within one-doubling dilution of the highest concentration tested for the remaining drugs. Synergy was defined as combinations yielding MICs that were less than half of those observed with each individual antibiotic.

Results

Activity against M. abscessus

Susceptibility results for all antibiotics tested alone and in combination against *M. abscessus* are shown in Table 1. Any isolates with an intermediate MIC were considered resistant for the purpose of data analysis. Fifty-three percent (10/19) of tested strains were resistant to all 9 antibiotics for which interpretations exist, with the remaining 47% (9/19) being resistant to 6–8 of these drugs. Of the other standard antibiotics tested for which interpretations have not been established (cefepime, Augmentin, ceftriaxone, and minocycline), nearly all *M. abscessus* strains demonstrated MICs at or above the highest concentration tested (Table 1). Only tigecycline demonstrated any appreciable activity, with MICs ranging from 0.5 to 4 μg/ml. Clofazimine showed potent activity against all the *M. abscessus* isolates, in which 70% (12/17) of the strains tested had MICs ≤1.5 μg/ml and the remaining 30% (5/17) had an MIC of 3 μg/ml. Dapsone demonstrated less activity against *M. abscessus*, with 20% (3/15) of isolates exhibiting MICs ≤1.5 μg/ml. The remaining 80% (12/15) had MICs ranging from 3 to >50 μg/ml.

Table 1. Minimum Inhibitory Concentrations (μg/ml) of Various Standard Antimicrobials Against Clinical Strains of *Mycobacterium abscessus* Recovered from Patients with Cystic Fibrosis

Isolate number	Antibiotic																	
	CLA	*SXT*	*CIP*	*MXF*	*FOX*	*AMI*	*TGC*	*LZD*	*IMI*	*FEP*	*AUG*	*AXO*	*MIN*	*TOB*	*CFZ*	*DAP*	*RIF*	*RBT*
11	>16	>8/152	>4	>8	64	32	4	>32	16	>32	>64/32	>64	>8	>16	0.75	0.75	>8	>8
12	>16	>8/152	>4	>8	16	16	1	>32	16	16	32/16	>64	8	>16	1.5	6.25	>8	>8
13	8	8/152	>4	>8	64	32	2	>32	16	32	>64/32	>64	8	>16	1.5	3	>8	>8
14	4	>8/152	>4	>8	128	16	4	>32	32	>32	>64/32	>64	>8	>16	1.5	3	>8	>8
15	16	>8/152	>4	>8	64	16	2	32	8	>32	>64/32	>64	>8	>16	3	3	>8	>8
16	4	>8/152	4	8	32	16	0.5	16	16	32	>64/32	>64	>8	>16	1.5	3	>8	>8
17	>16	8/152	4	8	32	16	1	16	4	16	64/32	>64	>8	>16	3	3	>8	>8
18	16	>8/152	4	8	32	32	0.5	32	16	32	>64/32	>64	>8	>16	1.5	1.5	>8	>8
19	>16	>8/152	>4	>8	128	32	>4	>32	64	>32	>64/32	>64	>8	>16	1.5	3	>8	>8
20	>16	>8/152	>4	>8	32	32	1	32	8	>32	>64/32	>64	>8	>16	1.5	6.25	>8	>8
21	>16	>8/152	>4	>8	32	32	0.5	32	8	32	>64/32	>64	>8	>16	1.5	>50	>8	>8
22	8	>8/152	>4	>8	32	32	0.5	32	8	32	64/32	>64	>8	>16	1.5	50	>8	>8
23	>16	8/152	2	4	>128	64	0.5	16	16	>32	>64/32	>64	>8	8	3	25	>8	>8
24	2	>8/152	4	>8	32	32	1	32	8	32	64/32	64	8	>16	ND	ND	>8	>8
25	>16	>8/152	>4	>8	64	32	2	>32	16	>32	>64/32	>64	>8	>16	3	3	>8	>8
26	16	>8/152	>4	>8	32	32	1	>32	8	>32	>64/32	>64	>8	>16	0.75	1.5	>8	>8
27	4	1/19	2	4	32	16	ND	>32	>64	ND	ND	ND	ND	ND	ND	ND	>8	4
28	16	>8/152	1	2	32	16	1	>32	4	>32	>64/32	32	>8	>16	ND	ND	>8	>8
29	>16	8/152	64	8	64	32	ND	16	16	ND	ND	ND	ND	16	3	ND	>8	>8
30	>16	2/38	2	4	32	4	ND	16	64	ND	ND	ND	ND	ND	0.38	ND	>8	4

Shaded cells indicate resistant (medium gray), intermediate (light gray), or susceptible (dark gray) minimum inhibitory concentrations based on current CLSI guidelines.[11] Unshaded cells are those for which no interpretations have been established. > indicates that the minimum inhibitory concentration is greater than the highest concentration tested.

AMI, amikacin; AUG, augmentin; AXO, ceftriaxone; CFZ, clofazimine; CIP, ciprofloxacin; CLA, clarithromycin; trimethoprim/sulfamethoxazole; DAP, dapsone; FEP, cefipime; FOX, cefoxitin; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; ND, not done; RBT, rifabutin; RIF, rifampin; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline; TOB, tobramycin.

Activity against M. chelonae

Eighty percent (4/5) of the *M. chelonae* isolates were resistant to ≥8 antimicrobials for which interpretations have been established, and nearly all isolates had MICs at the top of the test range for the remaining drugs, with the exception of tigecycline (range 0.25–1 μg/ml), clofazamine (range 0.375–3 μg/ml), and dapsone (range 0.75–1.5 μg/ml) (Table 2).

Activity against MAC

Results for MAC were more variable (Table 3). Of the drugs for which susceptibility interpretation was available (clarithromycin, linezolid, and moxifloxacin), all isolates were resistant to linezolid and moxifloxacin, and 56% (9/16) were resistant to clarithromycin. Of the other antibiotics for which no interpretation is currently available (ciprofloxacin, streptomycin, ethionamide, ethambutol, rifampin, trimethoprim-sulfamethoxazole, and tigecycline), more than half of the strains (56%, 9/16) had MICs above the top of the test range. Only amikacin and rifabutin had MICs consistently less than one-doubling dilution from the top of the range. For clofazimine, 93% (13/14) of the MAC isolates tested had MICs ≤1.5 μg/ml. Only one isolate (strain 111) had a higher MIC of 3 μg/ml. In comparison, dapsone had little activity against most MAC isolates, with MICs ≥50 μg/ml in 86% (12/14). Only two strains (101 and 116), had lower MICs at 3 and 6.25 μg/ml, respectively.

Activities of dual antibiotic combinations

Paired combinations of six antibiotics (clofazimine, clarithromycin, amikacin, dapsone, rifampin, and tigecycline) were tested for potential synergy against *M. abscessus* as shown in Supplementary Tables S1 through S6 (Supplementary Data are available online at www.liebertpub.com/mdr). Both clofazimine (at a concentration of 0.5 μg/ml) and clarithromycin (4 μg/ml) demonstrated significant synergy with all of the antibiotics tested. The addition of these drugs reduced the MICs of all the antibiotics with which they were tested to the bottom of the test range, thus bringing them within therapeutic range for all clinical isolates (Supplementary Tables S1 and S2). Amikacin (8 μg/ml) also exhibited synergy with all other antibiotics, resulting in MICs ≤ the lowest concentration tested, with the exception of strain 207, where less synergy was noted with the fluoroquinolones and Augmentin (Supplementary Table S3). Synergy was less consistent with combinations using dapsone (0.5 μg/ml) or rifampin (1 μg/ml), where little to no effect on MIC was noted with minocycline or ceftriaxone for most of the strains tested (Supplementary Tables S4 and S5). Interestingly, strain 11 exhibited no synergy with any rifampin-containing combinations. Antagonism was demonstrated in several strains with combined dapsone and imipenem. One *M. abscessus* isolate, in particular (strain 12), had antagonistic results for multiple dapsone-containing combinations, including cefoxitin, amikacin, tigecycline, cefepime, and Augmentin (Supplementary Table S4). This pattern was not observed in any of the other strains tested. Of all the antibiotic combinations tested, the least synergistic were those containing tigecycline (Supplementary Table S6). Most combinations of tigecycline with other antibiotics resulted in no change in MIC or a decrease of ±1-doubling dilution, which is not indicative of

TABLE 2. MINIMUM INHIBITORY CONCENTRATIONS OF VARIOUS STANDARD ANTIMICROBIALS AGAINST CLINICAL STRAINS OF *MYCOBACTERIUM CHELONAE* RECOVERED FROM PATIENTS WITH CYSTIC FIBROSIS

Isolate number	Antibiotic															
	CLA	*SXT*	*CIP*	*MXF*	*FOX*	*AMI*	*TGC*	*LZD*	*IMI*	*FEP*	*AUG*	*AXO*	*MIN*	*TOB*	*CFZ*	*DAP*	*RIF*	*RBT*
52	16	>8/152	2	4	128	32	1	16	16	32	>64/32	>64	>8	16	3	1.5	>8	>8
53	16	>8/152	>4	>8	>128	64	1	32	32	>32	>64/32	>64	>8	16	0.375	1.5	>8	>8
54	16	2/38	1	2	>128	16	0.25	2	8	>32	64/32	>64	>8	16	1.5	1.5	>8	>8
55	8	>8/152	4	8	>128	64	1	32	32	>32	>64/32	>64	>8	16	ND	ND	>8	>8
56	1	8/152	>4	8	>128	64	ND	16	16	ND	ND	ND	>8	4	0.75	0.75	>8	>8

Shaded cells indicate resistant (medium gray), intermediate (light gray), or susceptible (dark gray) minimum inhibitory concentrations based on current CLSI guidelines.[11] Unshaded cells are those for which no interpretations have been established.

AMI, amikacin; AUG, augmentin; AXO, ceftriaxone; CFZ, clofazimine; CIP, ciprofloxacin; CLA, clarithromycin; trimethoprim/sulfamethoxazole; DAP, dapsone; FEP, cefipime; FOX, cefoxitin; IMI, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; ND, not done; RBT, rifabutin; RIF, rifampin; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline; TOB, tobramycin.

Table 3. Minimum Inhibitory Concentrations of Various Antimicrobials Against Multidrug-Resistant Clinical Isolates of the *Mycobacterium avium* Complex Recovered from Patients with Cystic Fibrosis

Isolate number	*Antibiotic*															
	CLA	*LZD*	*MXF*	*CIP*	*AMI*	*STR*	*ETH*	*EMB*	*RFB*	*SXT*	*RIF*	*CFZ*	*DAP*	*TGC*	*IMI*	*AUG*
101	16	>64	8	>16	32	32	>20	8	2	8/152	>8	0.375	3	>4	>64	>64
102	4	32	>8	>16	32	16	10	4	8	4/76	4	0.75	50	>4	>64	>64
103	16	32	2	8	32	32	20	16	>8	>8/152	>8	ND	ND	>4	>64	>64
104	>64	64	2	>16	>64	64	20	>16	8	8/152	8	0.375	>50	>4	>64	>64
105	8	32	>8	8	32	64	20	16	2	>8/152	>8	0.375	>50	>4	>64	>64
106	16	64	4	16	32	>64	>20	16	8	8/152	>8	0.375	>50	>4	>64	>64
107	16	64	>8	>16	16	16	20	>16	>8	4/76	8	1.5	>50	>4	>64	>64
108	16	64	4	16	64	16	20	8	4	>8/152	>8	0.375	>50	>4	>64	>64
110	4	64	4	8	16	16	10	8	2	>8/152	>8	ND	ND	>4	>64	>64
111	2	>64	2	8	32	>64	10	>16	<0.25	4/76	>8	3	>50	>4	>64	>64
112	4	>64	>8	>16	>64	16	20	>16	8	4/76	>8	1.5	50	>4	>64	>64
113	2	>64	4	8	64	>64	20	16	8	>8/152	>8	0.75	50	>4	>64	>64
114	4	>64	4	>16	4	>64	>20	>16	0.5	4/76	>8	0.375	>50	>4	>64	>64
115	16	64	>8	>16	32	64	>20	16	0.5	>8/152	>8	0.75	>50	>4	>64	>64
116	16	64	4	16	>64	64	>20	>16	2	8/152	>8	0.375	6.25	>4	>64	>64
117	16	>64	>8	>16	32	64	5	>16	>8	>8/152	>8	0.375	>50	>4	>64	>64

Shaded cells indicate resistant (medium gray), intermediate (light gray), or susceptible (dark gray) minimum inhibitory concentrations based on current CLSI guidelines.[11] Unshaded cells are those for which no interpretations have been established.

AMI, amikacin; AUG, augmentin; CFZ, clofazimine; CIP, ciprofloxacin; CLA, clarithromycin; DAP, dapsone; EMB, ethambutol; ETH, ethionamide; IMI, imipenem; LZD, linezolid; MXF, moxifloxacin; ND, not done; RFB, rifabutin; RIF, rifampin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole; TGC, tigecycline.

synergy. Some combinations, especially tigecycline plus imipenem, were clearly antagonistic in some isolates.

Discussion

In this study, we investigated the susceptibility of MDR strains of *M. abscessus*, *M. chelonae*, and MAC recovered from CF patients to various antibiotics that are typically considered for treatment of their pulmonary infections, and we also included nonstandard antibiotics such as clofazimine, dapsone, and rifampin. These were tested both alone and in combination, with the goal of identifying synergistic antibiotic combinations *in vitro* that denote a reduction in MIC and may potentially overcome antibiotic resistance.

The prevalence of NTM infections has steadily risen over the past few decades, with a concurrent increase in MDR strains, resulting in severely limited treatment options. In these cases, it is difficult to determine which antibiotics and in what combination to use them. Both our *in vitro* data and those proposed by prior studies[8,12,13] suggest that synergy does exist between several antibiotics; however, *in vivo* data to assess clinical efficacy remain lacking.

For instance, one study evaluating synergy between clofazimine and amikacin in rapidly growing NTMs demonstrated 100% synergy with all *M. abscessus* and *M. chelonae* isolates tested, with a significant reduction in clofazimine MICs.[12] Another study also demonstrated synergy *in vitro* between these antimicrobials when tested against various NTMs; however, the investigators did encounter several *M. abscessus* isolates with higher baseline MICs to clofazimine and amikacin that did not show significant synergy.[11] These findings are similar to our own, in which some degree of strain to strain variability was observed with several of the antibiotics tested.

In this study, we endeavored to study activities of select antibiotics alone and in dual combinations against strains of *M. abscessus*, *M. chelonae*, and MAC representing the most resistant isolates recovered from CF patients at our institution. Emphasis was placed on using only those strains for which resistance to multiple antibiotic classes (including macrolides, aminoglycosides, fluoroquinolones, penicillins, cephalosporins, and carbapenems) had been demonstrated, in tandem with elevated MICs to clofazimine (>0.5 μg/ml). We observed significant synergy against all NTM isolates with combinations utilizing primarily sub-inhibitory concentrations of clofazimine, amikacin, or clarithromycin in tandem with the other antibiotics tested. These results suggest that our panel of MDR-NTM strains can become susceptible when exposed to antimicrobial combinations such as those tested in this study. In addition, at least one prior study demonstrated that combinations of clofazimine with either amikacin or clarithromycin prevented regrowth of *M. abscessus* and MAC, suggesting that particular combinations may play a role in providing bactericidal versus bacteriostatic inhibition.[13] Observations from this study also reveal that a significant amount of phenotypic variation with respect to drug susceptibility testing exists between NTM strains, and may explain differences between our results and those of prior studies, in which synergy was not observed in some *M. abscessus* isolates.[11]

Treatment of clinically relevant disease caused by MDR-NTM strains represents a significant challenge for physicians. Although *in vivo* data are currently lacking, available *in vitro* data suggest that specific antibiotic combinations may be useful in the treatment of these refractory infections. At present, antimicrobial synergy testing of NTM species is not routinely performed by most reference laboratories. Our study, as well as others,[11–13] has shown that synergy does exist between several antimicrobial combinations *in vitro*; however, the potency of these combinations varies, likely due to strain-strain variability as demonstrated in this and other investigations.[11–13] Therefore, synergy testing in MDR-NTM organisms may be something to consider implementing in reference laboratories to better determine viable treatment options in the setting of extensive antimicrobial resistance.

This study does have limitations. We necessarily focused on NTM strains recovered from CF patients with susceptibility profiles in which resistance was demonstrated for nearly all drugs with a phenotypic interpretation, and MICs were at or near the highest concentration tested for all remaining antibiotics. NTM strains from noncystic fibrosis patients or those with less resistant susceptibility profiles were not tested, and we, therefore, cannot conclude that the synergy demonstrated with this particular group of MDR strains would be the same for those with less overall resistance. In addition, only a single concentration of clofazimine, clarithromycin, amikacin, dapsone, rifampin, and tigecycline were used for synergy testing in this study; thus, the activity of lower concentrations and the limits of inhibition are not known at this time. Future research is needed, which will not only address these limitations but also determine the efficacy *in vivo* of such combinations for the treatment of MDR-NTM infections.

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Disclosure Statement

No competing financial interests exist.

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