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Multicenter Study in Taiwan of the *In Vitro* Activities of Nemonoxacin, Tigecycline, Doripenem, and Other Antimicrobial Agents against Clinical Isolates of Various *Nocardia* Species[▽]

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The aim of this study was to assess the *in vitro* activities of nemonoxacin (a novel nonfluorinated quinolone), doripenem, tigecycline, and 16 other antimicrobial agents against *Nocardia* species. The MICs of the 19 agents against 151 clinical isolates of *Nocardia* species were determined by the broth microdilution method. The isolates were identified to the species level using 16S rRNA gene sequencing analysis. The results showed that *N. brasiliensis* ($n = 60$; 40%) was the most common species, followed by *N. cyriacigeorgica* ($n = 24$; 16%), *N. farcinica* ($n = 12$; 8%), *N. beijingensis* ($n = 9$), *N. otitidiscaviarum* ($n = 8$), *N. nova* ($n = 8$), *N. asiatica* ($n = 7$), *N. puris* ($n = 6$), *N. flavorosea* ($n = 5$), *N. abscessus* ($n = 3$), *N. carnea* (2), and one each of *N. alba*, *N. asteroides* complex, *N. rhamnosiphila*, *N. elegans*, *N. jinanensis*, *N. takedensis*, and *N. transvalensis*. The MIC_{90}s of the tested quinolones against the *N. brasiliensis* isolates were in the order nemonoxacin = gemifloxacin < moxifloxacin < levofloxacin = ciprofloxacin, and the MIC_{90}s of the tested carbapenems were in the order doripenem = meropenem < ertapenem < imipenem. Tigecycline had a lower MIC_{90} (1 μg/ml) than linezolid (8 μg/ml). The MIC_{90}s of the tested quinolones against *N. cyriacigeorgica* isolates were in the order nemonoxacin < gemifloxacin < moxifloxacin < levofloxacin < ciprofloxacin, and the MIC_{90}s of the tested carbapenems were in the order imipenem < doripenem = meropenem < ertapenem. Nemonoxacin had the lowest MIC_{90} values among the tested quinolones against the other 17 *Nocardia* isolates. Among the four tested carbapenems, imipenem had the lowest MIC_{90}s. All of the clinical isolates of *N. beijingensis*, *N. otitidiscaviarum*, *N. nova*, and *N. puris* and more than half of the *N. brasiliensis* and *N. cyriacigeorgica* isolates were resistant to at least one antimicrobial agent. The results of this *in vitro* study suggest that nemonoxacin, linezolid, and tigecycline are promising treatment options for nocardiosis. Further investigation of their clinical role is warranted.

Nocardia species, soilborne aerobic actinomycetes with worldwide distribution, can cause local or disseminated infection in humans, especially in immunocompromised individuals (3, 15, 17, 20). Pulmonary nocardial infection can be caused by *N. abscessus*, *N. cyriacigeorgica*, *N. farcinica*, and *N. nova*; in contrast, *N. brasiliensis* was the most common pathogen associated with primary skin infection (mycetoma) (3, 20). This group of organisms is rapidly expanding and now comprises at least 80 species (http://www.bacterio.cict.fr/n/nocardia.html). Traditional identification of *Nocardia* to the species level is based on microscopic morphology and phenotypic characteristics; however, those methods are cumbersome and cannot accurately identify newer species. Since *Nocardia* species differ in the clinical spectrum of diseases they cause and their susceptibilities to antibiotics, it is important to precisely identify *Nocardia* isolates beyond the genus level. Molecular methods, such as 16S rRNA gene sequencing analysis, allow more accurate identification and the elucidation of taxonomy, such as the former *N. asteroides* complex isolates, which, after the use of modern molecular methods, were further identified as distinct species/taxa of *Nocardia* with different antimicrobial susceptibility patterns (3). Therefore, final confirmation of *Nocardia* species should be done by molecular techniques after initial identification. Only after correct identification of *Nocardia* species can the antibiotic susceptibilities of these organisms be understood.

Sulfonamide antibiotics have been used to treat nocardiosis since the 1940s and remain the most common type of antimicrobial used to treat these infections (1, 8, 24). However, a retrospective evaluation of the antibiotic resistance patterns of 765 *Nocardia* isolates in the United States during the period 1995 to 2004 showed that 61% of the isolates were resistant to sulfamethoxazole (SMZ) and 42% were resistant to trimethoprim-sulfamethoxazole (TMP-SMZ) (23). In addition, the only alternatives to sulfa-based antibiotics are amoxicillin-clavulanate, imipenem (IMP), and amikacin (25). Thus, it is important to identify additional alternative antimicrobials and

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to perform *in vitro* susceptibility testing with these agents to evaluate their activities against *Nocardia* isolates.

Nemonoxacin (TG-873870), a nonfluorinated quinolone (NFQ), differs from other fluoroquinolones in that it lacks the fluorine in the R6 position. Nemonoxacin retains potent broad-spectrum activities against Gram-positive and Gram-negative bacteria and *Mycobacterium tuberculosis* (6, 19). This study compared the susceptibilities of different *Nocardia* species to 19 antimicrobials, including three newly available agents (doripenem, tigecycline, and nemonoxacin).

(Some of the results were reported in our previous study [10]. Part of MIC data for amoxicillin-clavulanic acid, imipenem, ceftriaxone, ciprofloxacin, sulfamethoxazole, and amikacin was reported previously [14].)

MATERIALS AND METHODS

Bacterial isolates. All of the clinical isolates of *Nocardia* species were collected from patients treated in four large medical centers in Taiwan from 1998 to 2009. These hospitals were National Taiwan University Hospital (2,500 beds), National Cheng Kung University Hospital (1,200 beds), Chi-Mei Medical Center (1,500 beds), and Kaohsiung Medical University Chung-Ho Memorial Hospital (1,700 beds). Identification of the isolates was based on positive Gram stain (Gram-positive branching, beaded, and filamentous bacilli), positive modified acid-fast stain results, colonial morphotypes, and conventional biochemical reactions, including hydrolysis of casein, xanthine, hypoxanthine, and tyrosine. The identities of the clinical isolates were further confirmed by 16S rRNA gene sequencing analysis as previously described (20). Partial sequencing analysis of the 16S rRNA gene was performed using the primers Noc1 (5′-GCTTAACACATGCA AGTCG-3′) (positions 46 to 64; *Escherichia coli* numbering system) and Noc2 (5′-GAATTCCAGTCTCCCCTG-3′) (positions 663 to 680; *E. coli* numbering system) (20). The sequences were compared with published sequences in the GenBank database. The closest matches and GenBank accession numbers were obtained.

Susceptibility testing. The MICs of the 19 tested drugs, i.e., SMZ, ceftriaxone (CRO), and vancomycin (Sigma, St. Louis, MO); azithromycin and linezolid (LZD) (Pfizer Inc., New York, NY); amikacin (Bristol-Myers Squibb, Princeton, NJ); amoxicillin-clavulanic acid (AMC) (Glaxo-SmithKline, Greenford, United Kingdom); daptomycin (Cubist Pharmaceuticals, Lexington, MA); cefoxitin, ertapenem, and IMP (Merck & Co., Inc., NJ); meropenem (Sumitomo Pharmaceuticals, Osaka, Japan); doripenem (Shionogi Pharmaceuticals, Tokyo, Japan); ciprofloxacin (CIP) and moxifloxacin (Bayer Co., West Haven, CT); levofloxacin (Daiichi Pharmaceuticals, Tokyo, Japan); gemifloxacin (LG Chem Investments, Seoul, South Korea); nemonoxacin (TaiGen Biotechnology, Co. Ltd., Taipei, Taiwan); and tigecycline (Wyeth-Ayerst, Pearl River, NY), were determined using the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (7). Mueller-Hinton broth was the test medium for all drugs. For testing of daptomycin, the broth contained physiological levels of calcium (50 mg/liter), as recommended previously (11). The MIC interpretive criteria (susceptible/resistant) of amoxicillin-clavulanic acid (≤8 and 4 μg/ml; ≥32 and 16 μg/ml), ceftriaxone (≤8 μg/ml; ≥64 μg/ml), imipenem (≤4 μg/ml; ≥16 μg/ml), ciprofloxacin (≤1 μg/ml; ≥4 μg/ml), linezolid (≤8; not applicable [NA]), sulfamethoxazole (≤32 μg/ml; ≥64 μg/ml), and amikacin (≤8 μg/ml; ≥16 μg/ml) were in accordance with those from CLSI (7). *Staphylococcus aureus* (ATCC 28213) and *Enterococcus faecalis* (ATCC 29212) were used as control strains.

RESULTS

Bacterial isolates. During the study period, a total of 151 nonduplicated isolates were investigated. Among these isolates, *N. brasiliensis* ($n = 60$; 40%) was the most common species, followed by *N. cyriacigeorgica* ($n = 24$; 16%), *N. farcinica* ($n = 12$; 8%), *N. beijingensis* ($n = 9$), *N. otitidiscaviarum* ($n = 8$), *N. nova* ($n = 8$), *N. asiatica* ($n = 7$), *N. puris* ($n = 6$), *N. flavorosea* ($n = 5$), *N. abscessus* ($n = 3$), *N. carnea* (15), and one each of *N. alba*, *N. asteroides* complex, *N. rhamnosiphila*, *N. elegans*, *N. jinanensis*, *N. takedensis*, and *N. transvalensis*. Specimens of skin and soft tissue were the most common source of clinical *Nocardia* isolates, followed by respiratory tract specimens, including sputum, bronchoalveolar lavage fluid, pleural effusion, and lung biopsy specimens. In addition, some isolates were obtained from brain, blood, and lymph node biopsy specimens.

Antimicrobial susceptibilities. The MIC_{50} values, the MIC_{90} values, and the MIC ranges and distributions for each *Nocardia* species are shown in Table 1. The cumulative percentages of all 151 *Nocardia* isolates inhibited by each concentration of the five quinolones and four carbapenems are shown in Fig. 1A and B. For all *Nocardia* isolates, the MIC_{90} values of the tested quinolones were in the order nemonoxacin (1 μg/ml) < gemifloxacin (4 μg/ml) = moxifloxacin (4 μg/ml) < levofloxacin (16 μg/ml) = CIP (16 μg/ml), and the MIC_{90} values of the tested carbapenems were in the order doripenem (4 μg/ml) = meropenem (4 μg/ml) < ertapenem (8 μg/ml) < IMP (32 μg/ml). *Nocardia* spp. exhibited high-level resistance to vancomycin and daptomycin (MIC_{90}s, >128 μg/ml), whereas the MIC_{90} of linezolid was 8 μg/ml. Among the other antimicrobial agents, amikacin and tigecycline had comparatively low MIC_{90} values (2 and 4 μg/ml, respectively).

Among all *Nocardia* isolates, ≤10% were resistant to amikacin, SMZ, and LZD; 17% were resistant to AMC; and 11% were resistant to CRO. In contrast, about 30% of the *Nocardia* isolates were nonsusceptible to IMP, particularly *N. brasiliensis* (53%) and *N. otitidiscaviarum* (100%), and most (93%) were nonsusceptible to CIP. Among the *N. brasiliensis* isolates, 98% were resistant to CIP and 98% were susceptible to SMZ. All *N. cyriacigeorgica* isolates were resistant to CIP.

For *N. brasiliensis* isolates, the MIC_{90}s of the tested quinolones were in the order nemonoxacin = gemifloxacin < moxifloxacin < levofloxacin = CIP, and the MIC_{90}s of the tested carbapenems were in the order doripenem = meropenem < ertapenem < IMP. Vancomycin and daptomycin had greater MIC_{90} values (>128 μg/ml), whereas the MIC_{90} of LZD was 8 μg/ml. Among the other antimicrobial agents, amikacin had the lowest MIC_{90} (2 mg/liter). More than 95% of *N. brasiliensis* isolates were susceptible to AMC, CRO, LZD, SMZ, and amikacin.

Comparison of the activities of different antibiotics against *N. brasiliensis* and *N. cyriacigeorgica* revealed that the MIC_{90}s of all five of the tested quinolones, tigecycline, AMC, and cefoxitin against *N. cyriacigeorgica* were higher than those against *N. brasiliensis.* In contrast, the MIC_{90}s of linezolid, SMZ, and IMP against *N. cyriacigeorgica* were lower than those against *N. brasiliensis.*

For the other 17 *Nocardia* species (Table 2), nemonoxacin had the lowest MIC_{90} values of the tested quinolones. Among the four tested carbapenems, IMP had the lowest MIC_{90}s. The MIC_{90} of LZD was 4 μg/ml, whereas vancomycin and daptomycin had higher MIC_{90}s (>128 μg/ml). Among the other antimicrobial agents, amikacin (2 μg/ml) and tigecycline (2 μg/ml) had the lowest MIC_{90} values.

The resistance profiles of various clinical isolates are shown in Table 3. Concomitant CIP-IMP resistance and AMC-CIP resistance were the most common profiles. All of the clinical isolates of *N. beijingensis*, *N. nova*, and *N. puris* were resistant to at least one antimicrobial agent. More than half of *N. brasiliensis* and *N. cyriacigeorgica* isolates were resistant to more than

TABLE 1. Activities of antimicrobial agents against 151 *Nocardia* isolates

Bacterium (no of isolates tested) and antimicrobial agent	MIC (μg/ml)			Susceptibility (%)[a]		
	Range	50%	90%	S	I	R
N. brasiliensis (60)						
Amoxicillin-clavulanic acid	0.5–32	1	2	59 (98)		1 (2)
Cefoxitin	1–>128	2	8			
Ceftriaxone	0.5–32	2	4	57 (95)	3 (5)	0 (0)
Meropenem	0.5–8	2	4			
Ertapenem	0.25–8	4	8			
Imipenem	0.5–>32	2	>32	28 (47)	3 (5)	29 (48)
Doripenem	0.5–8	2	4			
Azithromycin	16–>128	32	>128			
Ciprofloxacin	0.5–8	8	8	1 (2)	0 (0)	59 (98)
Nemonoxacin	0.12–1	0.25	0.5			
Gemifloxacin	0.06–2	0.25	0.5			
Levofloxacin	1–>16	4	8			
Moxifloxacin	0.12–2	1	1			
Linezolid	1–16	4	8	59 (98)		1 (2)
Vancomycin	0.5–>128	128	>128			
Daptomycin	64–>128	128	>128			
Sulfamethoxazole	0.5–64	4	8	59 (98)		1 (2)
Amikacin	0.25–4	1	2	60 (100)		0 (0)
Tigecycline	0.25–1	0.25	1			
N. cyriacigeorgica (24)						
Amoxicillin-clavulanic acid	4–16	16	16	9 (38)	14 (58)	1 (4)
Cefoxitin	0.12–>16	4	16			
Ceftriaxone	0.25–16	2	8	23 (96)	1 (4)	0 (0)
Meropenem	1–4	4	4			
Ertapenem	2–8	4	8			
Imipenem	0.5–>4	1	2	24 (100)	0 (0)	0 (0)
Doripenem	1–8	4	4			
Azithromycin	128–>128	>128	>128			
Ciprofloxacin	0.12–128	16	64	0 (0)	0 (0)	24 (100)
Nemonoxacin	0.25–2	1	2			
Gemifloxacin	2–8	2	4			
Levofloxacin	8–>32	16	32			
Moxifloxacin	2–4	4	4			
Linezolid	2–16	4	4	23 (96)		1 (4)
Vancomycin	16–>128	128	>128			
Daptomycin	128–>128	128	>128			
Sulfamethoxazole	2–16	4	4	24 (100)		0 (0)
Amikacin	0.5–2	1	2	24 (100)		0 (0)
Tigecycline	0.5–4	2	4			
N. farcinica (12)						
Amoxicillin-clavulanic acid	0.5–8	1	4	12 (100)	0 (0)	0 (0)
Cefoxitin	0.5–>128	32	>128			
Ceftriaxone	1–>64	32	64	3 (25)	5 (42)	4 (33)
Meropenem	0.5–>8	4	4			
Ertapenem	0.5–>8	8	8			
Imipenem	0.25–>4	1	2	12 (100)	0 (0)	0 (0)
Doripenem	0.25–>4	4	4			
Azithromycin	128–>128	>128	>128			
Ciprofloxacin	0.5–128	8	16	6 (50)	1 (8)	5 (42)
Nemonoxacin	0.03–1	0.25	1			
Gemifloxacin	0.03–2	0.12	0.5			
Levofloxacin	0.12–16	4	8			
Moxifloxacin	0.06–8	1	2			
Linezolid	1–4	4	4	12 (100)		
Vancomycin	2–>128	128	>128			
Daptomycin	64–>128	128	>128			
Sulfamethoxazole	2–64	8	32	11 (92)		1 (8)
Amikacin	1–4	1	4	12 (100)		0 (0)
Tigecycline	0.25–8	1	8			
N. beijingensis (9)						
Amoxicillin-clavulanic acid	1–32			4 (44)	4 (44)	1 (11)
Cefoxitin	1–>4					
Ceftriaxone	0.25–16			7 (78)	2 (22)	0 (0)

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TABLE 1—*Continued*

Bacterium (no of isolates tested) and antimicrobial agent	MIC (μg/ml)			Susceptibility (%)[a]		
	Range	50%	90%	S	I	R
Meropenem	0.25–2					
Ertapenem	2–4					
Imipenem	0.5–>8			5 (56)	4 (44)	
Doripenem	1–2					
Azithromycin	64–>128					
Ciprofloxacin	2–128			0 (0)	4 (44)	5 (56)
Nemonoxacin	0.5–8					
Gemifloxacin	0.25–16					
Levofloxacin	4–16					
Moxifloxacin	1–32					
Linezolid	2–4			9 (100)		
Vancomycin	128–>128					
Daptomycin	64–>>128					
Sulfamethoxazole	1–4			9 (100)		0 (0)
Amikacin	0.12–0.5			8 (89)		1 (11)
Tigecycline	0.12–8					
N. otitidiscaviarum (8)						
Amoxicillin-clavulanic acid	32–128			0 (0)	0 (0)	8 (100)
Cefoxitin	64–>128					
Ceftriaxone	16–>128			0 (0)	1 (13)	7 (87)
Meropenem	4–>32					
Ertapenem	16–>32					
Imipenem	16–>32			0 (0)	0 (0)	8 (100)
Doripenem	4–>32					
Azithromycin	0.5–>128					
Ciprofloxacin	2–16			0 (0)	3 (37)	5 (63)
Nemonoxacin	0.5–2					
Gemifloxacin	0.5–4					
Levofloxacin	1–8					
Moxifloxacin	0.25–4					
Linezolid	2–4			8 (100)		
Vancomycin	128–>128					
Daptomycin	64–128					
Sulfamethoxazole	4–32			8 (100)		0 (0)
Amikacin	0.5–4			8 (100)		0 (0)
Tigecycline	0.5–2					
N. nova (8)						
Amoxicillin-clavulanic acid	8–64			1 (13)	3 (38)	4 (50)
Cefoxitin	2–16					
Ceftriaxone	2–16			7 (87)	1 (13)	0 (0)
Meropenem	0.12–2					
Ertapenem	0.5–4					
Imipenem	0.03–1			8 (100)	0 (0)	0 (0)
Doripenem	0.25–2					
Azithromycin	0.25–>128					
Ciprofloxacin	4–16			0 (0)	0 (0)	8 (100)
Nemonoxacin	0.25–1					
Gemifloxacin	0.5–2					
Levofloxacin	2–>32					
Moxifloxacin	0.5–4					
Linezolid	12			8 (100)		
Vancomycin	32–>128					
Daptomycin	64–>128					
Sulfamethoxazole	4–32			8 (100)		0 (0)
Amikacin	0.12–32			7 (87)		1 (13)
Tigecycline	0.25–8					
N. asiatica (7)						
Amoxicillin-clavulanic acid	16–64			0 (0)	1 (14)	6 (86)
Cefoxitin	0.5–2					
Ceftriaxone	0.5–1			7 (100)	0 (0)	0 (0)
Meropenem	0.5–2					
Ertapenem	1–4					
Imipenem	0.5–1			7 (100)	0 (0)	0 (0)
Doripenem	1–2					

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TABLE 1—*Continued*

Bacterium (no of isolates tested) and antimicrobial agent	MIC (μg/ml)			Susceptibility (%)[a]		
	Range	50%	90%	S	I	R
Azithromycin	>128					
Ciprofloxacin	64–128			0 (0)	0 (0)	7 (100)
Nemonoxacin	16					
Gemifloxacin	16–32					
Levofloxacin	>32					
Moxifloxacin	1–32					
Linezolid	1–4			7 (100)		
Vancomycin	128–>128					
Daptomycin	128–>128					
Sulfamethoxazole	2–8			7 (100)		0 (0)
Amikacin	0.12–0.25			7 (100)		0 (0)
Tigecycline	0.25–1					
N. puris (6)						
Amoxicillin-clavulanic acid	16			0 (0)	6 (100)	0 (0)
Cefoxitin	128–>128					
Ceftriaxone	4–>128			1 (17)	0 (0)	5 (83)
Meropenem	2–4					
Ertapenem	4–8					
Imipenem	1–2			6 (100)	0 (0)	0 (0)
Doripenem	2–4					
Azithromycin	4–>128					
Ciprofloxacin	8			0 (0)	0 (0)	6 (100)
Nemonoxacin	0.5					
Gemifloxacin	1					
Levofloxacin	4–8					
Moxifloxacin	2					
Linezolid	2–4			6 (100)		
Vancomycin	0.5–>128					
Daptomycin	128–>128					
Sulfamethoxazole	0.5–16			6 (100)		0 (0)
Amikacin	0.12–1			6 (100)		0 (0)
Tigecycline	0.25–2					
Other *Nocardia* spp. (17)						
Amoxicillin-clavulanic acid	1–64	8	32	10 (59)	3 (18)	4 (24)
Cefoxitin	0.12–32	4	16			
Ceftriaxone	0.25–8	1	4	17 (100)	0 (0)	0 (0)
Meropenem	0.12–4	1	4			
Ertapenem	0.25–16	2	8			
Imipenem	0.12–32	0.5	2	16 (94)	0 (0)	1 (6)
Doripenem	0.12–4	2	4			
Azithromycin	1–>128	>128	>128			
Ciprofloxacin	0.25–128	8	16	4 (24)	1 (6)	12 (71)
Nemonoxacin	0.12–16	1	16			
Gemifloxacin	0.06–32	2	32			
Levofloxacin	0.25–>32	8	16			
Moxifloxacin	0.25–32	4	32			
Linezolid	0.5–4	2	4	17 (100)		
Vancomycin	0.25–>128	128	>128			
Daptomycin	0.5–>128	128	>128			
Sulfamethoxazole	4–32	4	8	17 (100)		0 (0)
Amikacin	0.12–32	0.5	2	16 (94)		1 (6)
Tigecycline	0.06–4	0.5	2			

[a] S, susceptible; I, intermediate; R, resistant.

two agents. For *N. otitidiscaviarum*, all eight of the isolates were resistant to AMC, ceftriaxone, CIP, and imipenem.

DISCUSSION

This nationwide study in Taiwan investigated the antibiotic susceptibilities of various *Nocardia* species based on 16S rRNA gene sequence analysis of the isolates. Our findings show that the two most common nocardial pathogens in Taiwan are *N. brasiliensis* (60/151; 40%) and *N. cyriacigeorgica* (24/151; 16%). In contrast, *N. nova* complex organisms are the most common isolates in the United States (211/765; 28%) and Canada (109/325; 34%) (22, 23), and *N. cyriacigeorgica* (12/37; 32%) is the most common pathogen in Spain (15). In addition, we demonstrated that *Nocardia* species vary in their antimicrobial susceptibility patterns. Overall, our findings suggest that accurate

TABLE 2. Activities of antimicrobial agents against 17 clinical isolates of unusual *Nocardia* species

Antimicrobial agent	MIC (μg/ml)[a] for:									
	N. flavorosea (5)	*N. abscessus* (3)	*N. carnea* (2)	*N. alba* (1)	*N. asteroids complex* (1)	*N. elegans* (1)	*N. jinanesis* (1)	*N. transvalensis* (1)	*N. rhamnosiphila* (1)	*N. takedensis* (1)
Amoxicillin-clavulanic acid	4–16 (60)	1–32 (67)	32 (0)	2 (S)	64 (R)	16 (R)	8 (S)	8 (S)	8 (S)	1 (S)
Cefoxitin	0.12–16	1–16	1–16	2	32	4	8	8	4	1
Ceftriaxone	0.25–8 (100)	0.5–2 (100)	2–4 (100)	2 (S)	8 (S)	4 (S)	2 (S)	4 (S)	1 (S)	2 (S)
Meropenem	1–4	1–4	1	0.25	2	0.12	2	4	1	2
Ertapenem	2–8	1–8	0.5–2	1	2	0.25	4	16	2	2
Imipenem	0.5–2 (100)	0.25–1 (100)	0.25–0.5 (100)	1 (S)	1 (S)	0.12 (S)	0.5 (S)	32 (R)	0.5 (S)	1 (S)
Doripenem	1–4	0.25–4	1	0.25	2	0.12	2	2	1	2
Azithromycin	>128	32–>128	16–>128	16	128	1	128	32	>128	32
Ciprofloxacin	4–16 (0)	4–128 (0)	0.25 (100)	2 (R)	16 (R)	16 (R)	8 (R)	1 (S)	1 (S)	4
Nemonoxacin	0.25–1	0.5–16	0.12	1	1	1	1	0.25	0.12	0.25
Gemifloxacin	2–4	0.5–32	0.03–0.06	1	2	2	2	1	0.12	0.5
Levofloxacin	8–16	2–>32	0.25–0.5	8	4	16	8	0.5	2	4
Moxifloxacin	2–4	2–32	0.25	4	2	4	2	0.25	0.5	1
Linezolid	2–4 (100)	0.5–2 (100)	0.5–2 (100)	2 (S)	4 (S)	2 (S)	4 (S)	1 (S)	2 (S)	4 (S)
Vancomycin	16–>128	0.25–128	128	0.25	16	32	128	>128	128	128
Daptomycin	128–>128	0.5–128	128–>128	1	128	>128	128	>128	128	128
Sulfamethoxazole	4–8 (100)	8 (100)	4–8 (100)	32 (S)	4 (S)	8 (S)	4 (S)	32 (S)	4 (S)	4 (S)
Amikacin	0.5–4 (100)	0.12–4 (100)	0.12–0.25 (100)	0.25 (S)	0.5 (S)	0.25 (S)	0.5 (S)	32 (R)	0.25 (S)	1 (S)
Tigecycline	0.5–2	0.12–2	0.5–1	0.06	1	2	4	2	0.25	0.25

[a] The MIC range is shown, with the percentage of susceptible isolates or susceptibility category (S, susceptible; I, intermediate; R, resistant) in parentheses. The number of isolates for each species is shown in parentheses.

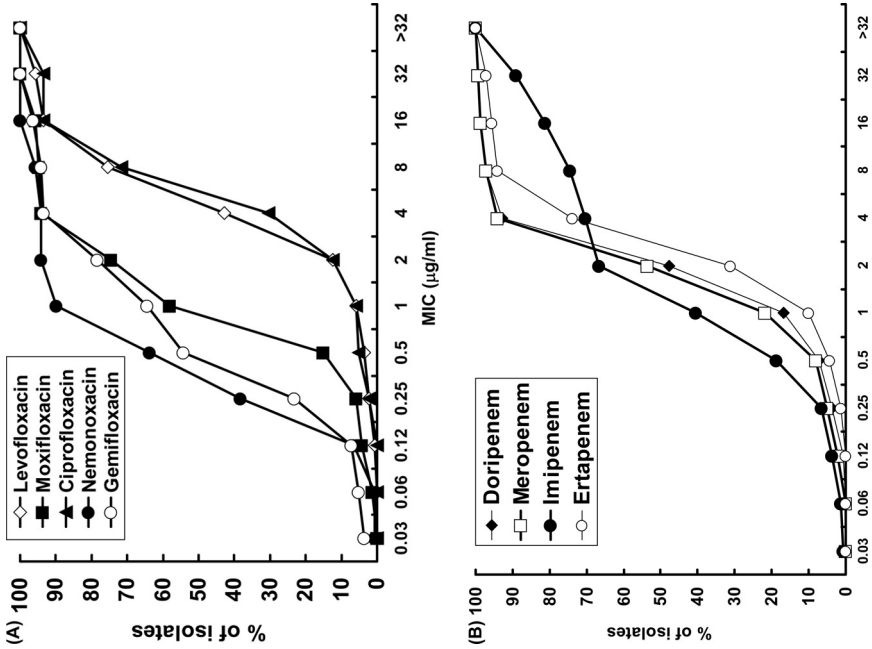

FIG. 1. Distribution of MICs of five quinolones (ciprofloxacin, levofloxacin, moxifloxacin, gemifloxacin, and nemonoxacin) (A) and four carbapenems (imipenem, meropenem, ertapenem, and doripenem) (B) for the 151 *Nocardia* isolates.

identification is essential to understanding the epidemiological distribution of different species and predicting their antimicrobial susceptibilities.

More than 80% of *Nocardia* isolates were resistant to CIP, and the overall MIC_{50} and MIC_{90} values were 8 μg/ml and 16 μg/ml, respectively. These findings are consistent with those reported in previous studies conducted in the United States and South Africa (14, 23). In contrast, nemonoxacin (TG-873870), a novel quinolone, has much lower MIC values than the four classic quinolones CIP, levofloxacin, moxifloxacin, and gemifloxacin. Furthermore, MIC determinations revealed that the MIC_{90}s of nemonoxacin and gemifloxacin were the lowest among the five tested quinolones against most of the *Nocardia* species determined in this study.

Nemonoxacin, currently available only in an oral formulation, is rapidly absorbed following oral administration. The free maximum concentration of drug in serum (C_{max}) is 5.7 μg/ml, and the mean 24-h free area under the concentration-time curve ($fAUC_{0-24}$) is 46.0 μg · h/ml for a single 750-mg dose (6). The MIC breakpoint of nemonoxacin has not been established. For treatment of infections due to Gram-positive cocci, a $fAUC_{0-24}$/MIC ratio of >30 is generally accepted to predict clinical success and microbiological success (6). Ac-

TABLE 3. Multiple-resistance profiles of the clinical isolates of *Nocardia* spp.

Resistance profile[a]	*Nocardia* sp.[b]
AN-IMI	*N. transvalensis* (1/100)
AMC-CRO	*N. brasiliensis* (1/2)
AMC-CIP	*N. cyriacigeorgica* (13/54), *N. beijingensis* (5/56), *N. nova* (7/87), *N. asiatica* (7/100), *N. flavorosea* (2/40), *N. puris* (1/100), *N. abscessus* (1/100), *N. asteroides* complex (1/100), *N. elegans* (1/100)
CRO-CIP	*N. farcinica* (4/33)
CIP-IMP	*N. brasiliensis* (33/55), *N. beijingensis* (3/33)
AMC-CRO-CIP	*N. puris* (5/83), *N. cyriacigeorgica* (1/5)
AMC-CIP-LZD	*N. cyriacigeorgica* (1/4)
AN-CRO-CIP	*N. nova* (1/13)
CRO-CIP-IMP	*N. brasiliensis* (1/2)
CRO-CIP-SMZ	*N. farcinica* (1/8)
AN-CRO-CIP-IMI	*N. beijingensis* (1/11)
AMC-CRO-CIP-IMP	*N. otitidiscaviarum* (8/100)
CRO-CIP-SMZ-LZD	*N. brasiliensis* (1/2)

[a] Including intermediate and resistant. AN, amikacin.
[b] The number/percentage of isolates in each species are shown in parentheses.

cordingly, nemonoxacin displayed good pharmacokinetics/pharmacodynamics at the 750-mg dose with a $fAUC_{0-24}/MIC_{90}$ ratio of 92 μg · h/ml for *N. brasiliensis* and 46 μg · h/ml for *N. farcinica* and also had favorable results ($fAUC_{0-24}/MIC \geq 46$ μg · h/ml) for the majority (>90%) of *N. puris* and *N. nova* isolates. This agent showed unfavorable results for *N. cyriacigeorgica*, *N. beijingensis*, and other *Nocardia* spp. Although nemonoxacin showed promising activity against most *Nocardia* spp. in this study, further clinical evaluation is needed to demonstrate the clinical role of nemonoxacin in the management of nocardiosis.

Previous *in vitro* studies have shown that LZD is quite active against multiple *Nocardia* isolates (4, 14, 23). In this study, we found that, with the exception of *N. brasiliensis* and *N. cyriacigeorgica*, all of the isolates were susceptible to LZD. These findings suggest that LZD is a good alternative to sulfa-based antibiotics for the treatment of nocardiosis; however, clinicians still need to be aware of the rare occurrence of *in vitro* non-LZD-susceptible *Nocardia* isolates.

Few studies have provided data on the *in vitro* activity of daptomycin against unusual clinically relevant Gram-positive microbes, such as *Nocardia* spp. (9). In this study, daptomycin showed poor *in vitro* activity against each *Nocardia* species. In fact, the MIC_{90}s (≥128 μg/ml) were similar to those of vancomycin. Although the relationship between *in vitro* susceptibilities and *in vivo* response based on clinical studies is unknown, our findings suggest that the clinical role of daptomycin for treating nocardiosis is as limited as that of vancomycin.

Knowledge about the activities of tigecycline against different *Nocardia* species is limited (5). In the present study, tigecycline was the most effective against *N. brasiliensis* and *N. puris*. Overall, tigecycline MIC values were ≤8 μg/ml against all of the tested isolates. These results, as well as those reported in a previous study (5), support the potential clinical application of tigecycline for the treatment of nocardiosis. To the best of our knowledge, only one other study has tested the activity of doripenem against *Nocardia* sp. isolates (11). In our study, different *Nocardia* spp. exhibited different patterns of susceptibility to carbapenems, and the MICs of doripenem against different *Nocardia* species were comparable to those of meropenem. Overall, the rate of IMP-resistant *Nocardia* isolates was 29%, and those for *N. otitidiscaviarum* (100%) and *N. brasiliensis* (48%) were especially high. In general, the activities of the four tested carbapenems against *Nocardia* species ranked in the order meropenem and doripenem > IMP and ertapenem. These findings indicate that doripenem may be a better choice of carbapenems than IMP to treat nocardial infections, although all of the carbapenems show poor activity against *N. otitidiscaviarum* isolates.

We also studied the *in vitro* activity of cephalosporins (CRO and cefoxitin) against isolates of *Nocardia*. Overall, the rate of CRO-resistant *Nocardia* isolates was 11%; however, these agents showed poor activity (MIC ≥ 64 μg/ml) against *N. otitidiscaviarum*, *N. farcinica*, and *N. puris.* The activities of traditional antimicrobials, including SMZ, AMC, and amikacin, were also assayed in the present work. SMZ showed good *in vitro* activity, and only three isolates were resistant to SMZ. However, in a retrospective evaluation of the antibiotic resistance patterns of 765 *Nocardia* isolates in the United States, 61% were resistant to SMZ and 42% were resistant to TMP-SMZ (23). In another study of 37 strains of *Nocardia* isolates in Spain, only 10.8% were resistant to TMP-SMZ (15). The differences may be due to geographical variation and species distribution. In Taiwan, trimethoprim-sulfamethoxazole is still effective for the treatment of nocardiosis. In addition, with the exception of one isolate of *N. nova*, which was resistant to amikacin, all *Nocardia* isolates were uniformly susceptible to amikacin (MICs ≤ 8 μg/ml). This finding differs from that reported by Uhde et al., who reported that only 5% of 765 *Nocardia* isolates in the United States were susceptible to amikacin (23). In contrast, AMC showed various activities against different *Nocardia* species, and more than 80% of *N. otitidiscaviarum*, *N. puris*, and *N. asiatica* isolates were resistant to AMC.

The sulfonamide antibiotic combination TMP-SMZ continues to be the drug of choice for nocardiosis (3, 12, 13, 15); however, treatment failure has been noted when it is used alone, especially in disseminated and central nervous system nocardiosis. Thus, various combination therapy regimens, including IMP, amikacin, minocycline, LZD, and cephalosporins, have been recommended for the management of serious *Nocardia* infections (2, 13, 18, 21). In this study, we noted that more than half of *N. brasiliensis* and *N. cyriacigeorgica* isolates and all of the *N. beijingensis*, *N. otitidiscaviarum*, *N. nova*, and *N. puris* isolates were resistant to at least one antimicrobial agent. The most notable was *N. otitidiscaviarum*, because all eight isolates were nonsusceptible to a combination of AMC, CRO, IMP, and CIP. Studies on the synergistic effect *in vivo* have reported conflicting results (13, 16); therefore, further clinical studies are needed to evaluate the *in vivo* and *in vitro* activities of combination therapy.

In conclusion, the results of this *in vitro* study suggest that TMP-SMZ has good activity against clinical isolates of *Nocardia* species in Taiwan. We also found that nemonoxacin, linezolid, and tigecycline show promise as alternatives to sulfa-based antibiotics for the treatment of nocardiosis. In addition, it is important to determine the species of *Nocardia*, because

different species and isolates vary in their antimicrobial susceptibility patterns.

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