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Antimicrobial Susceptibility among Clinical *Nocardia* Species Identified by Multilocus Sequence Analysis

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Antimicrobial susceptibility patterns of 112 clinical isolates, 28 type strains, and 9 reference strains of *Nocardia* were determined using the Sensititre Rapmyco microdilution panel (Thermo Fisher, Inc.). Isolates were identified by highly discriminatory multilocus sequence analysis and were chosen to represent the diversity of species recovered from clinical specimens in Ontario, Canada. Susceptibility to the most commonly used drug, trimethoprim-sulfamethoxazole, was observed in 97% of isolates. Linezolid and amikacin were also highly effective; 100% and 99% of all isolates demonstrated a susceptible phenotype. For the remaining antimicrobials, resistance was species specific with isolates of *Nocardia otitidiscaviarum*, *N. brasiliensis*, *N. abscessus* complex, *N. nova* complex, *N. transvalensis* complex, *N. farcinica*, and *N. cyriacigeorgica* displaying the traditional characteristic drug pattern types. In addition, the antimicrobial susceptibility profiles of a variety of rarely encountered species isolated from clinical specimens are reported for the first time and were categorized into four additional drug pattern types. Finally, MICs for the control strains *N. nova* ATCC BAA-2227, *N. asteroides* ATCC 19247^T, and *N. farcinica* ATCC 23826 were robustly determined to demonstrate method reproducibility and suitability of the commercial Sensititre Rapmyco panel for antimicrobial susceptibility testing of *Nocardia* spp. isolated from clinical specimens. The reported values will facilitate quality control and standardization among laboratories.

Nocardia species are a group of filamentous, branching, Gram-positive, modified-acid-fast bacilli that normally exist as soil saprophytes but can cause disease in immunosuppressed and healthy individuals (1). Most infections involve inhalation of fragments of filaments, resulting in pulmonary nocardiosis and pneumonia, which can be followed by dissemination to the heart, skin, subcutaneous tissue, and central nervous system (1).

Nocardia taxonomy has been linked to specific patterns of antimicrobial susceptibility ever since the foundational work by Wallace et al. (2) established the presence of six drug pattern types among the *Nocardia asteroides* species complex. Subsequent research linked the drug pattern types with the following *Nocardia* species: *N. abscessus*, drug pattern type I; *N. brevicatena/paucivorans* complex, drug pattern type II; *N. nova* complex, drug pattern type III; *N. transvalensis* complex, including *N. wallacei* and *N. blacklockiae*, drug pattern type IV; *N. farcinica*, drug pattern type V; and *N. cyriacigeorgica*, drug pattern type VI (1, 3). Together with *N. brasiliensis* and *N. otitidiscaviarum*, *N. abscessus*, *N. nova*, *N. farcinica*, and *N. cyriacigeorgica* are frequently associated with clinical infections (2, 4). With the clinical application of DNA sequencing, *Nocardia* taxonomy has changed and expanded rapidly. Previously identified species have been reclassified as species complexes encompassing multiple species, and numerous novel *Nocardia* species have been identified (5). Currently, 87 species are enumerated in the List of Prokaryotic Names with Standing in Nomenclature (LPSN) (http://www.bacterio.net/nocardia.html), many of which are clinically significant (1).

However, data on antimicrobial susceptibility has lagged behind the advances in taxonomy, with only a few reports providing recent data on newer antimicrobials (4, 6, 7, 8, 9, 10, 11, 12, 13). These reports largely focus on the species traditionally associated with clinical infections, such that antimicrobial susceptibility data are not available for a large number of newly identified, but clinically relevant, *Nocardia* species that are isolated less frequently in the clinical laboratory. Furthermore, the species in these studies were identified using microscopy and biochemical testing, or the identification relied heavily on the 16S rRNA gene sequence analysis, both of which are unable to reliably discriminate many *Nocardia* species (5, 14, 15).

The purpose of this study was to profile the antimicrobial susceptibility patterns of a diverse range of *Nocardia* species isolated from clinical specimens. Unlike the previous studies, all isolates were identified with a high degree of confidence and discrimination using multilocus sequence analysis (MLSA) (5). Thus, we provide a timely update on the antimicrobial susceptibility patterns of the species frequently isolated from clinical specimens, such as *N. brasiliensis*, *N. otitidiscaviarum*, *N. abscessus* complex, *N. nova* complex, *N. farcinica*, and *N. cyriacigeorgica*, and detail novel data on the antimicrobial susceptibility of a variety of rarely encountered species. Additionally, the antimicrobial susceptibility testing (AST) was performed using the commercial Sensititre Rapmyco microdilution panel (Thermo Fisher, Inc., Cleveland, OH, USA). We report robustly determined MIC values for three

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TABLE 1 MIC range and mode of quality control strains *N. nova* ATCC BAA-2227, *N. asteroides* ATCC 19247^T, and *N. farcinica* ATCC 23825 obtained during 20 independent determinations using Sensititre Rapmyco broth microdilution panels

	Results for *N. nova* ATCC BAA-2227						Results for *N. asteroides* ATCC 19247^T		
	MIC (μg/ml)			Expected MIC[b] (μg/ml)			MIC (μg/ml)		
Antimicrobial agent	Range	Mode	Susceptibility[a]	Range	Mode	Susceptibility	Range	Mode	Susceptibility
Amikacin	≤1	≤1	S	≤0.5	≤0.5	S	≤1 to 2	≤1	S
Amoxicillin-clavulanic acid	16/8 to 32/16	32/16	R	0.06/0.03 to >32/16	>32/16	R	32/16 to 64/32	64/32	R
Cefepime	2 to 4	4	S	ND	ND		4 to 32	16	I
Ceftriaxone	≤4	≤4	S	≤4 to 16	≤4	S	≤4 to 8	<4	S
Ciprofloxacin	>4	>4	R	0.5 to >4	>4	R	>4	>4	R
Clarithromycin	≤0.06	≤0.06	S	≤0.06 to 0.12	≤0.06	S	2 to 4	2	S
Doxycycline	8	8	R	ND	ND		1 −2	2	I
Imipenem	≤2	≤2	S	≤0.5	≤0.5	S	≤2 to 4	≤2	S
Linezolid	≤1	≤1	S	≤0.5 to 2	1	S	≤1 to 2	≤1	S
Minocycline	2 to 4	2	I	0.25 to >8	2	I	≤1	≤1	S
Moxifloxacin	2 to 4	2	I	≤0.5 to 4	4	R	2	2	I
Tigecycline	1 to 2	2		0.5 to 8	4		0.5 to 2	1	
Tobramycin	8 to 16	16	R	≤0.12 to >16	>16	R	≤1 to 2	≤1	S
Trimethoprim-sulfamethoxazole	0.5/9.5 to 1/19	1/19	S	≤0.25/4.8 to 1/19	≤0.25/4.8	S	≤0.25/4.75 to 0.5/9.5	≤0.25/4.75	S

quality control (QC) strains to demonstrate the suitability of this product for the determination of antimicrobial susceptibility profiles of clinical isolates of *Nocardia* spp. and to facilitate the validation and implementation of this method in other clinical laboratories.

MATERIALS AND METHODS

A total of 112 clinical isolates, 28 type strains, and 9 reference strains were previously identified to the species level by MLSA. Briefly, partial sequences of five loci, including genes for gyrase B, the β-subunit of DNA topoisomerase (*gyrB*) (482 bp), 16S rRNA (16S) (462 bp), subunit A of SecA preprotein translocase (*secA1*) (445 bp), 65-kDa heat shock protein (*hsp65*) (400 bp), and RNA polymerase (*rpoB*) (400 bp), were concatenated and compared to sequences of 36 type and 11 reference strains. Using neighbor-joining phylogenetic analysis, we noted sequence clusters with >85% bootstrap support that each contained a single type strain in most cases. Therefore, identification of isolates was made according to their species cluster (5). In all cases, the identifications provided by MLSA were consistent with (i.e., not discrepant) although often more discriminatory than those generated by the CLSI-recommended targets 16S and *secA1* (16), which were included in the MLSA.

The strains were selected to represent the diversity of *Nocardia* species isolated by the Mycology Section of the Ontario Public Health Laboratory, Public Health Ontario, from December 2005 through July 2011. Following revival from storage in 20% (wt/vol) glycerol at −80°C, isolates were subcultured twice on GYM *Streptomyces* medium (0.4% glucose, 0.4% yeast extract, 1% malt extract, 0.2% $CaCO_3$, and 1.2% agar) agar plates and incubated at 37°C for 3 to 4 days prior to antimicrobial susceptibility testing. The type and reference strains included in this study are listed in Table S1 in the supplemental material.

AST of all isolates was determined by broth microdilution using the Sensititre Rapmyco microdilution panel (Thermo Fisher, Inc., Cleveland, OH, USA), according to CLSI M24-A2 guidelines (17, 18) and the manufacturer's instructions (amikacin, 1 to 64 μg/ml; amoxicillin-clavulanic acid, 2/1 to 64/32 μg/ml; cefepime, 1 to 32 μg/ml; ceftriaxone, 4 to 64 μg/ml; ciprofloxacin, 0.12 to 4 μg/ml; clarithromycin, 0.03 to 16 μg/ml; doxycycline, 2 to 64 μg/ml; imipenem, 2 to 64 μg/ml; linezolid, 1 to 32 μg/ml; minocycline, 1 to 8 μg/ml; moxifloxacin, 0.25 to 8 μg/ml; tigecycline, 0.015 to 4 μg/ml; tobramycin, 1 to 16 μg/ml; and trimethoprim-sulfamethoxazole [SXT], 0.25/4.75-8/152 μg/ml). (Note: this panel has not received Food and Drug Administration clearance and is therefore designated for research use only, not for use in diagnostic procedures.) Cefoxitin (4 to 128 μg/ml) is present on the panel but is not reported on as it has not been shown to be useful for *Nocardia* infections *in vivo*. There are no CLSI breakpoints for tigecycline, and clinical experience is limited with this antibiotic. However, it appears to have good *in vitro* activity against a number of *Nocardia* species and is therefore included in this study. In order to achieve a homogeneous suspension, an isolate was initially suspended in 200 μl of sterile water and dispersed using a sterilized plastic disposable pestle (Kimble Chase, Vineland, NJ, USA). The suspension was adjusted to a 0.5 McFarland turbidity standard using a nephelometer, and 50 μl was transferred to 10 ml of Mueller-Hinton broth. Each well of the panel was inoculated with 100 μl of the Mueller-Hinton broth. MIC values were interpreted as susceptible (S), intermediate (I), or resistant (R) according to the CLSI (18) after 48 to 72 h of incubation at 37°C when moderate growth was observed in the positive-control well. Four isolates (*N. transvalensis* NRRL B-10637, *N. exalbida* DSM 44883, *N. gamkensis* DSM 44956, and a clinical isolate of *N. anaemiae*) required 96 h of incubation before the positive-control well exhibited acceptable growth. For these isolates, MIC values were determined at 96 h; however, imipenem results were not recorded due to its instability noted by us and others (19).

N. nova ATCC BAA-2227 (incubated for 72 h), *N. asteroides* ATCC 19247^T (incubated for 72 h), and *N. farcinica* ATCC 23826 (incubated for 48 h) (American Type Culture Collection, Manassas, VA, USA) were chosen as quality control organisms with antimicrobial susceptibility determined by a series of 20 independent trials conducted on 20 separate days over a period of 2 months (17, 18).

RESULTS

The MIC values for the control strains *N. nova* ATCC BAA-2227, *N. asteroides* ATCC 19247^T, and *N. farcinica* ATCC 23826 demonstrated a high degree of reproducibility and strong correlation with published data on resistance profiles of the three strains (14, 20, 21) (Table 1). The data indicate that the Sensititre Rapmyco microdilution panel is suitable for the routine determination of the antimicrobial resistance patterns of *Nocardia* spp. in the clinical laboratory.

Using the Sensititre Rapmyco panel, we determined antimicrobial susceptibility profiles for 149 *Nocardia* isolates. All isolates

TABLE 1 (Continued)

Results for *N. asteroides* ATCC 19247^T			Results for *N. farcinica* ATCC 23826				
Expected MIC[c] (μg/ml)			MIC (μg/ml)			Expected MIC[d] (μg/ml)	
Range	Mode	Susceptibility	Range	Mode	Susceptibility		Susceptibility
≤0.12–0.25	≤0.12	S	≤1	≤1	S	≤0.25	S
64	64	R	8/4 to 32/16	8/4	S	ND[e]	
ND	ND		16 to >32	>32	R	ND	
4	4	S	16 to >64	64	R	>64	R
8 to 16	8	R	0.25 to 2	0.25	S	1	S
ND	ND		8 to >16	>16	R	ND	
ND	ND		2 to 4	4	I	ND	
1	1	S	4 to 16	8	I	8	I
ND	ND		≤1 to 2	2	S	ND	
1	1	S	≤1 to 4	2	I	ND	
ND	ND		≤0.25 to 1	≤0.25	S	ND	
ND	ND		1 to 4	2		ND	
ND	ND		8 to 16	16	R	>16	R
0.06 to 0.12	0.06	S	0.25/4.75 to 1/19	1/19		ND	

[a] The MIC mode values are interpreted as susceptible (S), intermediate (I), or resistant (R) according to CLSI M24-A2 (18).
[b] The expected MIC for *N. nova* ATCC BAA-2227 is taken from Conville et al. (7).
[c] The expected MIC for *N. asteroides* ATCC 19247^T is taken from Tomlin et al. (20).
[d] The expected MIC for *N. farcinica* ATCC 23826 is taken from Wallace et al. (21).
[e] ND, no data.

were previously identified with a high degree of confidence and discrimination using MLSA (5) and were selected to represent the spectrum of species recovered from clinical specimens in Ontario, Canada, including both those that are traditionally recognized as clinically relevant and those that are rarely encountered. The percentage of susceptible isolates for each of the antibiotics for each of the species or species complexes is listed in Table 2. MIC ranges, MIC_{50} values, and MIC_{90} values are available in Tables S2, S3, and S4 in the supplemental material. Linezolid, amikacin, and SXT were the most effective antimicrobials with 100%, 99%, and 97%, respectively, of *Nocardia* isolates demonstrating susceptibility (Table 2). For the tetracyclines, none of the isolates were resistant to minocycline and only 11% were resistant to doxycycline; however, the rates of intermediate resistance were high at 39% and 52%, respectively (Table 2). For the remaining antimicrobials, substantial numbers of *Nocardia* isolates were resistant, and resistance was species specific (Table 2).

DISCUSSION

The CLSI guidelines recommend broth microdilution for the determination of the antimicrobial susceptibility of *Nocardia* spp. (18). While in-house prepared panels are specified in that document, we have demonstrated the suitability of the Sensititre Rapmyco microdilution panel for the determination of antimicrobial susceptibility patterns of clinical isolates of *Nocardia* spp. MIC values for the three control strains, *N. nova* ATCC BAA-2227, *N. asteroides* ATCC 19247^T, and *N. farcinica* ATCC 23826, were highly reproducible and correlated well with expected resistance profiles (7, 20, 21). The MIC values are published here to assist other clinical laboratories in implementing quality control guidelines for the validation of *Nocardia* antimicrobial susceptibility testing assays using this commercial panel.

The reproducibility of the broth microdilution method for antimicrobial susceptibility testing of *Nocardia* species was recently demonstrated by Conville and colleagues (7) in a multisite study using panels custom made by Thermo Fisher, Inc. (Cleveland, OH). Prior to this study, non-*Nocardia*, rapidly growing bacteria were recommended as QC strains for susceptibility testing, despite their very different growth characteristics during susceptibility testing. As an outcome of the work of Conville et al. (7), *N. nova* ATCC BAA-2227 was recommended as the *Nocardia* reference strain for antimicrobial susceptibility testing of *Nocardia* spp. Our testing of *N. nova* ATCC BAA-2227 showed that not only were our results reproducible over 20 repeat tests but we also obtained MICs that were within the mode ± 1 dilution for all antimicrobials tested in the Conville et al. study (7), except for two drugs (amikacin and imipenem) where the Sensititre Rapmyco panel did not include dilutions as low as those on the in-house study panel. For all but one drug, we obtained the identical interpretive call as the expected interpretation established in the Conville et al. study (7). The one exception was moxifloxacin, where our MIC mode was one dilution lower than their MIC mode, resulting in an "intermediate" interpretation by us and a "resistant" interpretation by Conville et al. (7), which is defined as a minor interpretive error (7). However, they also noted a substantial number (38.1%) of tests with an intermediate interpretation (7). These results indicate that the Sensititre Rapmyco panel is equivalent to custom-made panels prepared according to the CLSI guidelines (18).

Beyond validation, the Sensititre Rapmyco panels were used to assess a set of *Nocardia* isolates well characterized by MLSA in order to provide a comprehensive update on the antimicrobial susceptibility patterns of the most diverse set of species to date, including those that are traditionally recognized and many that are rarely encountered in a clinical setting. Trimethoprim-sulfamethoxazole (SXT) is the usual drug of choice for the treatment of *Nocardia* infections, making surveillance of SXT suscep-

TABLE 2 Percentage of *Nocardia* isolates susceptible to the antimicrobials amikacin, amoxicillin-clavulanic acid, cefepime, ceftriaxone, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, minocycline, moxifloxacin, tobramycin, and trimethoprim-sulfamethoxazole

Species (type drug pattern [1, 2])	No. of isolates	Susceptibility or % susceptible to[a]:												
		AMK	AMC	FEP	CRO	CIP	CLR	DOX	IPM	LZD	MIN	MXF	TOB	SXT
N. abscessus complex (I)	9	100	100	100	100	11	11.1	89	22	100	89	11	100	100
N. abscessus	1	S	S	S	S	S	S	S	S	S	S	S	S	S
N. abscessus/arthritidis-like species cluster[b]	8	100	100	100	100	0	0	88	13	100	88	0	100	100
N. nova complex (III)	28	100	26	74	85	0	100	11	93	100	56	7	7	100
N. nova	10	100	0	60	60	0	100	0	100	100	50	0	0	100
N. nova/cerradoensis/kruczakiae/aobensis-like species cluster[c]	8	100	0	88	100	0	100	13	88	100	63	25	0	100
N. africana	3	100	33	100	100	0	100	33	67	100	67	0	0	100
N. cerradoensis	3	100	67	33	67	0	100	0	100	100	0	0	67	100
N. kruczakiae	2	100	100	100	100	0	100	50	100	100	100	0	0	100
N. veterana	2	100	100	100	100	0	100	0	100	100	50	0	0	100
N. transvalensis complex (IV)	5	20	40	40	100	100	20	20	0	100	20	80	0	80
N. transvalensis	1	R	R	R	S	S	R	R	NA[d]	S	R	S	R	S
N. wallacei	4	25	50	50	100	100	25	25	0	100	25	75	0	75
N. farcinica (V)	36	100	78	0	6	50	0	17	53	100	36	81	0	94
N. cyriacigeorgica (VI)	20	100	15	55	95	0	25	50	90	100	85	0	100	100
Other														
N. amamiensis	2	100	0	100	100	0	50	100	50	100	100	50	100	100
N. anaemiae	2	100	0	50	100	100	100	100	100[d]	100	100	100	100	100
N. arthritidis/gamkensis/exalbida species cluster[e]	6	100	50	100	100	0	33	83	0[d]	100	83	17	100	100
N. asiatica	7	100	14	100	100	0	43	100	43	100	100	0	100	100
N. asteroides sensu stricto	2	100	0	100	100	0	100	50	100	100	100	50	100	100
N. beijingensis	2	100	0	100	100	0	50	0	50	100	0	50	100	100
N. brasiliensis	6	100	100	0	33	0	0	17	17	100	67	67	100	100
N. ignorata/coubleae species cluster[f]	3	100	0	0	33	0	0	100	100	100	100	67	100	100
N. neocaledoniensis	2	100	0	50	100	0	0	0	100	100	50	100	100	100
N. otitidiscaviarum	6	100	0	0	0	0	0	17	0	100	33	17	50	83
N. pneumoniae	3	100	0	100	100	0	100	100	667	100	100	33	100	100
N. rhamnosiphila	4	100	0	75	100	100	25	0	0	100	25	100	100	100
N. sienata	2	100	0	100	100	100	50	0	50	100	50	100	100	100
N. thailandica	2	100	0	100	100	0	100	0	100	100	100	100	100	50
N. vinacea	2	100	0	100	100	0	100	100	100	100	100	0	100	100
All	149	99	40	51	65	22	37	37	59	100	61	40	53	97

[a] AMK, amikacin; AMC, amoxicillin-clavulanic acid; FEP, cefepime; CRO, ceftriaxone; CIP, ciprofloxacin; CLR, clarithromycin; DOX, doxycycline; IPM, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole.

[b] *N. abscessus/arthritidis*-like species cluster: this cluster lacks a type strain but contains isolates similar to *N. abscessus* and *N. arthritidis* (5).

[c] *N. nova/cerradoensis/kruczakiae/aobensis*-like species cluster: this cluster lacks a type strain but contains isolates similar to *N. nova*, *N. cerradoensis*, *N. kruczakiae*, and *N. aobensis* (5).

[d] Some isolates required >72 h of incubation before growth in the positive control was observed. Therefore, only the subset of isolates that required ≤72 h of incubation were examined for imipenem resistance. NA, not applicable.

[e] *N. arthritidis/gamkensis/exalbida* species cluster: this cluster contains strains similar to the three type strains *N. arthritidis* DSM 44731^T, *N. gamkensis* DSM 44956^T, and *N. exalbida* DSM 44883^T (5). DSM source: German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.

[f] *N. ignorata/coubleae* species cluster: this cluster contains strains similar to the two type strains *N. ignorata* DSM 44496^T and *N. coubleae* DSM 44960^T (5).

tibility pertinent. Overall, 97% of isolates tested demonstrated susceptibility to SXT with resistance noted in two isolates of *N. farcinica* and one isolate each of *N. thailandica*, *N. otitidiscaviarum*, and *N. wallacei* (Table 2). The level of SXT resistance reported in the literature varies widely. Similar to our results, some studies report high levels of susceptibility (>90% of isolates) to SXT (4, 6, 8, 10, 22); however, others report significant levels of resistance ranging from 21% to 43% of isolates tested (9, 12, 13). Possible reasons for the discrepancy include geographic differences, possible exposure to SXT as a result of patient treatment prior to testing (4), questionable reproducibility of in-house prepared panels, and, until recently, the lack of standardized testing methodologies and well-characterized QC strains. Unsatisfactory reproducibility of SXT testing with *N. farcinica* and *N. wallacei* has been noted by others and attributed to the inherent growth characteristics of the species, problems with homogeneous inoculum preparation, and/or difficulty determining the MIC (7). However, consistent with our observation regarding the overall high susceptibility of *Nocardia* species to SXT, there are few literature reports of SXT treatment failure for *Nocardia* infections (4). The results suggest that in Ontario, Canada, SXT remains the recommended first-line therapeutic drug of choice for *Nocardia* infections. However, given the significant level of *in vitro* resistance noted in some studies, testing for susceptibility to SXT should be performed for isolates from clinically relevant infections, and SXT susceptibility should continue to be monitored.

TABLE 3 Correlation of antimicrobial susceptibility profiles with *Nocardia* species designation

Drug pattern type[a]	*Nocardia* sp.	No. of strains	Antimicrobial susceptibility profile[b]	
			Nonsusceptible (% intermediate or resistant)	Susceptible (%)
I[a]	*N. abscessus* complex[c]	9	Imipenem (78), ciprofloxacin (89), moxifloxacin (89), and clarithromycin (89)	Amoxicillin-clavulanic acid, ceftriaxone, cefepime, tobramycin, amikacin, doxycycline (89), linezolid, and SXT
Ia[d]	*N. arthritidis*/*N. gamkensis*/*N. exalbida* species cluster,[e] *N. asiatica*	13	Same as type I but nonsusceptible to amoxicillin-clavulanic acid (69)	Same as type I except nonsusceptible to amoxicillin-clavulanic acid
III[a]	*N. nova* complex[f]	28	Amoxicillin-clavulanic acid (74), tobramycin, doxycycline (89), ciprofloxacin, and moxifloxacin	Ceftriaxone (85), cefepime (74), imipenem, amikacin, clarithromycin, linezolid, and SXT
IV[a]	*N. transvalensis* complex[g]	5	Imipenem, tobramycin, amikacin (80), doxycycline (80), and clarithromycin (80)	Ceftriaxone, ciprofloxacin, moxifloxacin (80), linezolid, and SXT (80)
V[a]	*N. farcinica*	36	Ceftriaxone, cefepime, tobramycin, doxycycline (83), and clarithromycin	Amoxicillin-clavulanic acid (78), amikacin, moxifloxacin(80), linezolid, and SXT; variable susceptibility toward imipenem (53) and ciprofloxacin (50)
VI[a]	*N. cyriacigeorgica*	20	Amoxicillin-clavulanic acid (80 [intermediate resistance]), ciprofloxacin, moxifloxacin, and clarithromycin (75)	Ceftriaxone, imipenem, tobramycin, amikacin, doxycycline (50), linezolid, and SXT
VII[h]	*N. otitidiscaviarum*	6	Amoxicillin-clavulanic acid, ceftriaxone, cefepime, imipenem, doxycycline (83), ciprofloxacin, moxifloxacin (83), and clarithromycin	Amikacin, linezolid, and SXT
VIII[h]	*N. brasiliensis*	6	Cefepime, imipenem (83), doxycycline (83), ciprofloxacin, and clarithromycin	Amoxicillin-clavulanic acid, tobramycin, amikacin, linezolid, and SXT
IX[d]	*N. asteroides sensu stricto*, *N. pneumoniae*, *N. amamiensis*, *N. beijingensis*, *N. thailandica*, *N. vinacea*, *N. anaemiae*	15	Amoxicillin-clavulanic acid and ciprofloxacin (87)	Ceftriaxone, cefepime, imipenem (80), tobramycin, amikacin, doxycycline (67), clarithromycin, linezolid, and SXT

[a] Described by Wallace et al. (2) and/or Brown-Elliott et al. (1).
[b] If no value is indicated, the percent susceptible or nonsusceptible is ≥90%.
[c] *N. abscessus* complex: contains strains of *N. abscessus* and the *N. abscessus*/*N. arthritidis*-like species cluster, which lacks a type strain but contains isolates similar to *N. abscessus* and *N. arthritidis* (5).
[d] New drug pattern type described in this study.
[e] *N. arthritidis*/*N. gamkensis*/*N. exalbida* species cluster: this cluster contains strains similar to the three type strains *N. arthritidis* DSM 44731^T, *N. gamkensis* DSM 44956^T, and *N. exalbida* DSM 44883^T (5).
[f] *N. nova* complex: contains strains of *N. nova*, *N. africana*, *N. cerradoensis*, *N. kruczakiae*, *N. veterana*, and the *N. nova*/*cerradoensis*/*kruczakiae*/*aobensis*-like species cluster, which lacks a type strain but contains isolates similar to *N. nova*, *N. cerradoensis*, *N. kruczakiae*, and *N. aobensis* (5).
[g] *N. transvalensis* complex: contains strains of *N. transvalensis* and *N. wallacei*.
[h] Consistent with the drug pattern type described by Brown-Elliott et al. (1) and assigned a number in this study.

As noted by others (6, 8, 9, 10, 11, 12, 13, 22), all of the *Nocardia* isolates were susceptible to linezolid and almost all were susceptible to amikacin except for some isolates of the *N. transvalensis* complex (Table 2). However, for many of the remaining antimicrobials, substantial numbers of *Nocardia* isolates were resistant, and resistance was species specific (Table 2) as noted in other studies (6, 7, 8, 9, 10, 11, 12, 13, 22, 23). As previously described by Brown-Elliott et al. (1) and others (8, 9, 12, 13), we noted a strong correlation between the drug pattern types of Wallace et al. (2, 21) and species identification (Table 3), with type I, III, IV, V, and VI drug patterns displayed by the *N. abscessus* complex, *N. nova* complex, *N. transvalensis* complex, *N. farcinica*, and *N. cyriacigeorgica*, respectively. We did not have any clinical isolates of *N. brevicatena*/*paucivorans* (type II drug pattern). *N. otitidiscaviarum* and *N. brasiliensis* isolates also displayed distinct drug patterns and were numbered types VII and VIII, respectively (Table 3). Some discrepancies were noted. In particular, Brown-Elliott et al. (1) and Wallace et al. (2) reported that the *N. transvalensis* complex was susceptible to imipenem, but we noted resistance among all isolates. Uhde et al. (13) also reported a high rate of resistance (52%) among their isolates. With respect to *N. farcinica*, Brown-Elliott et al. (1) and Wallace et al. (2) indicated that it is susceptible to imipenem and ciprofloxacin, while we and other investigators (8, 9, 12, 13) noted susceptibility only among approximately half of the isolates (Table 2). Unlike Brown-Elliott et al. (1), who indicated that *N. otitidiscaviarum* isolates were susceptible to ciprofloxacin, we report them as largely nonsusceptible, as did Udhe et al. (13) (Table 2). These differences may be attributed to different geographic isolation or the evolution of drug resistance over the ~20 years between the original report by Wallace et al. (2) and more recent studies.

In addition to the antimicrobial susceptibility patterns of these well-recognized species, we also report preliminary antimicrobial susceptibility patterns for isolates of species that were not categorized within these traditionally acknowledged groups and yet were recovered from clinical specimens (Table 2). A limitation of our

study is the small number of isolates of the less common clinical strains that were available for testing, therefore reducing the robustness of the antibiograms for some species. However, these data are presented as preliminary data to help guide the initial empirical therapy for infections due to uncommonly observed species that have been confidently identified using MLSA. Although individually each species may signify a small number of isolates recovered from clinical specimens, collectively they represent approximately 20% (5). Therefore, the collective knowledge of the antimicrobial susceptibility patterns of these species, many of which lack published MIC data, will also be useful for patient treatment. Isolates of the *N. arthritidis*/*gamkensis*/*exalbida* species cluster (5) and *N. asiatica* exhibited a susceptibility pattern similar to the type I drug pattern of Wallace et al. (2). These were designated type Ia due to the large number (69%) of isolates that were nonsusceptible to amoxicillin-clavulanic acid, unlike the isolates of type I (Table 3). Of particular interest are the *N. asiatica* isolates, 86% of which were resistant to moxifloxacin, with resistant isolates generating uniquely high MICs (8 μg/ml) (see Table S4 in the supplemental material). *N. amamiensis*, *N. anaemiae*, *N. asteroides sensu stricto*, *N. beijingensis*, *N. pneumoniae*, *N. thailandica*, and *N. vinacea* were grouped into a novel drug pattern, type IX, characterized by nonsusceptibility to amoxicillin-clavulanic acid and ciprofloxacin (Table 3). Isolates of *N. ignorata*/*coubleae* (5) and *N. neocaledoniensis* were found to be nonsusceptible to amoxicillin-clavulanic acid, cefepime (80%), ceftriaxone, ciprofloxacin, and clarithromycin, while *N. rhamnosiphila* and *N. sienata* were observed to be nonsusceptible to amoxicillin-clavulanic acid, ciprofloxacin, clarithromycin (67%), and imipenem. Potentially, these could comprise two new groups, if testing of additional isolates supports the patterns.

Our results represent a timely update of the original work performed by Wallace et al. (2) and will aid physicians in assessing treatment options for patients infected with both the common and rarely encountered species of *Nocardia*. Although SXT is the usual treatment option for *Nocardia* infections, it may not always be possible to use it due to the fairly common occurrence of sulfonamide drug allergies, necessitating other options for empirical or AST-directed therapy. In addition, the slow growth of the organism, and the fact that AST for *Nocardia* is often only available at a reference laboratory, makes knowledge of the species-specific antimicrobial susceptibility patterns important in assisting physicians with alternate treatment options.

Although the broth microdilution method is recommended by the CLSI for antimicrobial susceptibility testing for *Nocardia* spp., unsatisfactory reproducibility has been documented, specifically with ceftriaxone testing of *N. cyriacigeorgica* and *N. wallacei*, tigecycline testing with *N. brasiliensis* and *N. cyriacigeorgica*, and SXT testing with *N. farcinica* and *N. wallacei* (7). The lack of reproducibility was attributed to the inherent growth characteristics of the species, difficulty with homogeneous inoculum preparation, and/or difficulty determining the MIC, specifically with the SXT results. In this study, great care was taken to ensure proper inoculum preparation and MIC determination, as evidenced by the consistent MIC values generated for the control strains *N. nova* ATCC BAA-2227, *N. asteroides* ATCC 19247^T, and *N. farcinica* ATCC 23826. Nevertheless, the results should be interpreted within the context of the stochastic nature of the testing method, which may apply to the aforementioned examples and to other sets of species and antimicrobials. Additionally, the correlation between *in vitro* drug susceptibility and the *in vivo* response of drug treatment of *Nocardia* infections is currently unknown; however, the development of a reproducible method for susceptibility testing, demonstrated here and in the Conville et al. study (7), is a necessary first step in facilitating such an investigation.

REFERENCES

1. **Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ, Jr.** 2006. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev **19:**259–282. http://dx.doi.org/10.1128/CMR.19.2.259-282.2006.
2. **Wallace RJ, Jr, Steele LC, Sumter G, Smith JM.** 1988. Antimicrobial susceptibility patterns of *Nocardia asteroides*. Antimicrob Agents Chemother **32:**1776–1779. http://dx.doi.org/10.1128/AAC.32.12.1776.
3. **Wilson RW, Steingrube VA, Brown BA, Blacklock Z, Jost KC, McNabb A, Colby WD, Biehle JR, Gibson JL, Wallace RJ, Jr.** 1997. Recognition of a *Nocardia transvalensis* complex by resistance to aminoglycosides, including amikacin, and PCR-restriction fragment length polymorphism analysis. J Clin Microbiol **35:**2235–2242.
4. **Brown-Elliott BA, Biehle J, Conville PS, Cohen S, Saubolle M, Sussland D, Wengenack N, Kriel K, Bridge L, McNulty S, Vasireddy R, Wallace RJ, Jr.** 2012. Sulfonamide resistance in isolates of *Nocardia* spp. from a U.S. multicenter survey. J Clin Microbiol **50:**670–672. http://dx.doi.org/10.1128/JCM.06243-11.
5. **McTaggart LR, Richardson SE, Witkowska M, Zhang SX.** 2010. Phylogeny and identification of *Nocardia* species on the basis of multilocus sequence analysis. J Clin Microbiol **48:**4525–4533. http://dx.doi.org/10.1128/JCM.00883-10.
6. **Cercenado E, Marin M, Sanchez-Martinez M, Cuevas O, Martinez-Alarcon J, Bouza E.** 2007. In vitro activities of tigecycline and eight other antimicrobials against different *Nocardia* species identified by molecular methods. Antimicrob Agents Chemother **51:**1102–1104. http://dx.doi.org/10.1128/AAC.01102-06.
7. **Conville PS, Brown-Elliott BA, Wallace RJ, Jr, Witebsky FG, Koziol D, Hall GS, Killian SB, Knapp CC, Warshauer D, Van T, Wengenack NL, Deml S, Woods GL.** 2012. Multisite reproducibility of the broth microdilution method for susceptibility testing of *Nocardia* species. J Clin Microbiol **50:**1270–1280. http://dx.doi.org/10.1128/JCM.00994-11.
8. **Glupczynski Y, Berhin C, Janssens M, Wauters G.** 2006. Determination of antimicrobial susceptibility patterns of *Nocardia* spp. from clinical specimens by Etest. Clin Microbiol Infect **12:**905–912. http://dx.doi.org/10.1111/j.1469-0691.2006.01460.x.
9. **Larruskain J, Idigoras P, Marimon JM, Perez-Trallero E.** 2011. Susceptibility study of 186 *Nocardia* spp. isolates to 20 antimicrobial agents. Antimicrob Agents Chemother **55:**2995–2998. http://dx.doi.org/10.1128/AAC.01279-10.
10. **Lowman W, Aithma N.** 2010. Antimicrobial susceptibility testing and profiling of *Nocardia* species and other aerobic actinomycetes from South Africa: comparative evaluation of broth microdilution versus the Etest. J Clin Microbiol **48:**4534–4540. http://dx.doi.org/10.1128/JCM.01073-10.
11. **Minero MV, Marin M, Cercenado E, Rabadan PM, Bouza E, Munoz P.** 2009. Nocardiosis at the turn of the century. Medicine **88:**250–261. http://dx.doi.org/10.1097/MD.0b013e3181afa1c8.
12. **Tremblay J, Thibert L, Alarie I, Valiquette L, Pepin J.** 2011. Nocardiosis in Quebec, Canada, 1988-2008. Clin Microbiol Infect **17:**690–696. http://dx.doi.org/10.1111/j.1469-0691.2010.03306.x.
13. **Uhde KB, Pathak S, McCullum I, Jr, Jannat-Khah DP, Shadomy SV, Dykewicz CA, Clark TA, Smith TL, Brown JM.** 2010. Antimicrobial-resistant *Nocardia* isolates, United States, 1995-2004. Clin Infect Dis **51:**1445–1448. http://dx.doi.org/10.1086/657399.
14. **Cloud JL, Conville PS, Croft A, Harmsen D, Witebsky FG, Carroll KC.** 2004. Evaluation of partial 16S ribosomal DNA sequencing for identification of *Nocardia* species by using the MicroSeq 500 system with an expanded database. J Clin Microbiol **42:**578–584. http://dx.doi.org/10.1128/JCM.42.2.578-584.2004.
15. **McNeil MM, Brown JM.** 1994. The medically important aerobic actinomycetes: epidemiology and microbiology. Clin Microbiol Rev **7:**357–417.
16. **Clinical and Laboratory Standards Institute.** 2008. Interpretive criteria

for identification of bacteria and fungi by DNA target sequencing; approved guideline. CLSI document MM18-A. Clinical Laboratory and Standards Institute, Wayne, PA.
17. **Clinical and Laboratory Standards Institute.** 2012. Susceptibility tests for bacteria that grow aerobically; approved standard, 9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
18. **Clinical and Laboratory Standards Institute.** 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard, 2nd ed. CLSI document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
19. **Baron EJ, Hindler JA.** 1984. Bioactivity of imipenem as a function of medium, time, and temperature. Antimicrob Agents Chemother **25:**781–782. http://dx.doi.org/10.1128/AAC.25.6.781.
20. **Tomlin Sand PC, Rennie RP.** 2001. Evaluation of E Test, disk diffusion and broth microdilution to establish tentative quality control limits and review susceptibility breakpoints for two aerobic actinomycetes. Diagn Microbiol Infect Dis **40:**179–186. http://dx.doi.org/10.1016/S0732-8893(01)00273-5.
21. **Wallace RJ, Jr, Tsukamura M, Brown BA, Brown J, Steingrube VA, Zhang Y, Nash DR.** 1990. Cefotaxime-resistant *Nocardia asteroides* strains are isolates of the controversial species *Nocardia farcinica*. J Clin Microbiol **28:**2726–2732.
22. **Ambaye A, Kohner PC, Wollan PC, Roberts KL, Roberts GD, Cockerill FR, III.** 1997. Comparison of agar dilution, broth microdilution, disk diffusion, E-Test, and BACTEC radiometric methods for antimicrobial susceptibility testing of clinical isolates of the *Nocardia asteroides* complex. J Clin Microbiol **35:**847–852.
23. **Lai C, Tan C, Lin SH, Liao C, Chou C, Hsu H, Huang Y, Hsueh P.** 2009. Comparative in vitro activities of nemonoxacin, doripenem, tigecycline and 15 other antimicrobials against *Nocardia brasiliensis*, *Nocardia asteroides* and unusual *Nocardia* species. J Antimicrob Chemother **64:**73–78. http://dx.doi.org/10.1093/jac/dkp144.