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Susceptibility of 186 *Nocardia* sp. Isolates to 20 Antimicrobial Agents[▽]

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This study determined the antimicrobial susceptibilities of 186 clinical isolates of *Nocardia* spp. isolated in Gipuzkoa, northern Spain, between 1998 and 2009. Most isolates were recovered from respiratory samples, *Nocardia nova*, *N. farcinica*, *N. cyriacigeorgica*, *N. abscessus*, and *N. carnea* being the species most frequently isolated. Linezolid and amikacin were the only two antimicrobials to which all isolates were susceptible. The majority of *N. flavorosea*, *N. carnea*, and *N. farcinica* isolates were trimethoprim-sulfamethoxazole resistant.

Nocardia species are ubiquitous in the environment and can be found worldwide as saprophytic components in water, soil, dust, decaying vegetation, and animal excrement. Only a small proportion of the currently described *Nocardia* species are known to be human pathogens, pulmonary nocardiosis being the most common manifestation of human disease (2). Prior to the introduction of sulfonamides in therapy, mortality from invasive *Nocardia* infections was close to 100%, but current cure rates range from 50% of brain abscess cases to 90% of pleuropulmonary disease and almost 100% of skin and soft tissue disease cases (11).

In this study, the *in vitro* activities of 20 antimicrobial agents against 186 clinical *Nocardia* isolates recovered from 178 patients between 1990 and 2009 in Gipuzkoa, northern Spain, were determined. Presumptive identification was performed according to the colony morphology on solid medium, Gram stain appearance, and positive modified acid-fast staining. Definitive species identification was performed by sequencing a fragment of the 16S rRNA gene using primers 5F (TGGAGA GTTTGATCCTGGCTCAG) and 1193R (ACGTCATCCCC GCCTTCCTC) and a finding of a sequence similarity of >99% with the sequence of a *Nocardia* type species. If similarities of >99% with more than one different *Nocardia* species were observed, species identification was done by sequencing a fragment of the *hsp65* gene using the primers described by Telenti et al. (12, 13). The sequences obtained were compared with those available at GenBank using BLAST software (http://www.ncbi.nlm.nih.gov) and with those at the leBIBI database (Bio Informatic Bacteria Identification tool; htpp://pbil.univ-lyon1.fr/bibi).

Susceptibility testing was performed by the broth microdilution method using the CLSI criteria (9) with Sensititre microtiter trays (Sensititre; Trek Diagnostics Systems, West Sussex, England) specially designed for this study and cation-adjusted Mueller-Hinton broth using the concentration range shown in Table 1. MICs were recorded after 3 days of incubation or after 5 days for slow-growing species, such as some *N. nova* isolates. Because there are no CLSI interpretative criteria for *Nocardia* for some of the antimicrobials tested in this study, arbitrary breakpoints were used for tigecycline, moxifloxacin, clindamycin, vancomycin, and dalbavancin (Table 1). *Nocardia* ATCC 19247, *N. farcinica* ATCC 3318, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 35218 were used as controls.

Overall, 186 nonduplicated isolates were obtained from 178 different patients. Four patients had two isolates each that were of different species, and two patients had three isolates each that were of different species. Of the 186 isolates, 177 were recovered from respiratory samples. The remaining nine *Nocardia* isolates were obtained from three cutaneous abscesses (all *N. farcinica*), three blood cultures (two *N. farcinica* and one *N. nova*), two urine cultures (both *N. nova*), and one brain abscess (*N. abscessus*).

TABLE 1. Broth microdilution breakpoints for *Nocardia* and other aerobic actinomycetes, according to the CLSI interpretive criteria (9), and concentration ranges of the antimicrobials studied

Antimicrobial(s)	Broth microdilution breakpoint (μg/ml)			Concentration range
	Susceptible	Intermediate	Resistant	
Ampicillin	≤8	16	≥32	0.25–32
Amoxicillin-clavulanic acid	≤8/4	16/8	≥32/16	0.5/0.25–32/16
Cefotaxime	≤8	16–32	≥64	8–64
Ceftriaxone	≤8	16–32	≥64	8–64
Cefepime	≤8	16	≥32	8–64
Imipenem	≤4	8	≥16	2–16
Gentamicin	≤4	8	≥16	4–16
Tobramycin	≤4	8	≥16	2–16
Amikacin	≤8		≥16	8–64
Ciprofloxacin	≤1	2	≥4	1–4
Moxifloxacin[a]	≤1	2	≥4	1–4
Clarithromycin	≤2	4	≥8	1–8
Clindamycin[a]	≤0.5	1–2	≥4	0.5–4
Minocycline	≤1	2–4	≥8	1–16
Doxycycline	≤1	2–4	≥8	1–16
Tigecycline[a]	≤1			0.25–4
Trimethoprim-sulfamethoxazole	≤2/38		≥4/76	1/19–4/76
Linezolid	≤8			0.5–8
Vancomycin[a]	≤2	4–8	≥16	0.25–8
Dalbavancin[a]	≤2	4–8	≥16	0.01–8

[a] Breakpoints are arbitrary since there are currently no CLSI interpretive criteria.

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Fourteen different species were detected, the most prevalent being *N. nova*, followed by *N. farcinica*, *N. cyriacigeorgica*, *N. abscessus*, and *N. carnea*. These five species represented 86.6% of all isolates. The remaining species isolated were *N. rhamnosiphila* (5 isolates), *N. flavorosea* (4 isolates), *N. veterana* (4 isolates), *N. takedensis* (3 isolates), *N. sienata* (2 isolates), *N. niigatensis* (1 isolate), *N. otitidiscaviarum* (1 isolate), *N. shimofusensis* (1 isolate), *N. alboflava* (1 isolate), and *Nocardia* spp. (4 isolates).

It is generally accepted that the incidence of nocardial disease is increasing (7, 10). The development of microorganism identification based on molecular biology techniques has allowed a greater number of species within the *Nocardia* genus to be described (2). Until 1995, less than 15 species were known (1), but in the last 10 years, more than 50 new species have been described. The species found in our study were those that are the most prevalent in different parts of the world (2). Other species common in tropical countries (3, 8) were very infrequent in our temperate climate region. Thus, we only found one *N. otitidiscaviarum* and no *N. brasiliensis* isolates, species that are frequently found in other regions (15).

Currently, trimethoprim-sulfamethoxazole (SXT) remains the drug of choice in the treatment of nocardiosis, the most recent therapeutic alternative being linezolid (6, 11). In our study, all of the *N. flavorosea* isolates and about half of the *N. carnea* and *N. farcinica* isolates showed SXT resistance (Table 2). Cercenado et al. (3) and Torres et al. (14) found 18% and 53% SXT resistance in *N. farcinica* isolates, respectively.

Like those in other studies (3, 5), all of our isolates were linezolid and amikacin susceptible and most species were also imipenem susceptible, similar to the findings reported by Wallace et al. (16). However, only 72% of *N. farcinica* and 39% of *N. abscessus* isolates were imipenem susceptible. Our isolates showed various susceptibilities to other beta-lactam antibiotics (Table 3). Susceptibility to the different members of the tetracycline family was uneven, but only *N. abscessus* and *N. takedensis* showed high susceptibility. Because of the high proportion of resistance to fluoroquinolones, glycopeptides, vancomycin, and dalbavancin, together with the scarce experience of their use in the treatment of nocardiosis, these drugs will probably remain as alternatives when other antimicrobials cannot be used and their susceptibilities are known. Ciprofloxacin showed a species-specific susceptibility: all *N. carnea* isolates were susceptible, while only 18% of *N. farcinica*, 2% of *N. nova*, and none of the *N. abscessus* and *N. cyriacigeorgica* isolates were susceptible. Intrinsic activity was slightly higher for moxifloxacin than for ciprofloxacin.

Susceptibility patterns *per se* are not indicative of a particular species, but if associated with other phenotypic characteristics, they can suggest classification within a *Nocardia* species or group (Table 4). Amoxicillin susceptibility together with amoxicillin-clavulanate resistance in slow-growing isolates suggests their membership in the *N. nova* complex (2, 5, 17), although in our study, this susceptibility pattern was also characteristic of *N. carnea*. Rapid growth in a multiresistant strain, including cefotaxime resistance, suggests the presence of *N. farcinica*, of which all isolates were also clarithromycin resistant. Species of the *Nocardia transvalensis* complex (none in this series) have intrinsic amikacin resistance (2–4).

Nocardia is an opportunistic pathogen that can cause se-

TABLE 2. MIC range, MIC_{50}, and MIC_{90} of antimicrobials tested for the *Nocardia* species most frequently found in this study

Antimicrobial(s)[a]	MIC for species (no. of isolates)																					
	N. nova (55)			*N. farcinica* (43)			*N. cyriacigeorgica* (28)			*N. abscessus* (23)			*N. carnea* (12)			Range					
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	*N. rhamnosiphila* (5)	*N. veterana* (4)	*N. flavorosea* (4)	*N. takedensis* (3)		
AMP	≤0.25–>32	8	16	>32	>32	>32	16–>32	>32	>32	1–>32	8	16	2–8	2	8	1–2	4–8	2–8	1–2		
AMC	1–>32	>32	>32	4–>32	8	16	16–>32	32	>32	0.5–>32	1	4	8–>32	16	>32	32–>32	4–>32	16–32	1–32		
CTX	≤8–64	≤8	16	16–>64	64	>64	≤8–32	≤8	≤8	≤8–16	≤8	≤8	≤8	≤8	≤8	≤8	≤8–16	≤8	≤8		
CRO	≤8–32	≤8	16	16–>64	64	>64	≤8–32	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8–16	≤8	≤8		
FEP	≤8–>64	≤8	16	16–>64	64	>64	≤8–32	≤8	16	≤8	≤8	≤8	≤8–16	≤8	≤8	≤8	≤8	≤8	≤8		
IPM	≤2–16	≤2	4	≤2–16	4	16	≤2–8	≤2	8	≤2–>16	8	>16	≤2–4	≤2	4	≤2–4	≤2–4	≤2–4	≤2		
GEN	≤4–>16	≤4	8	16–>16	>16	>16	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4	8–16	≤4–16	≤4	≤4		
TOB	≤2–>16	>16	>16	16–>16	>16	>16	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤2–>16	≤2	≤2		
AMK	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8		
CIP	2–>4	>4	>4	2–>4	>4	>4	4–>4	>4	>4	≤1	≤1	≤1	≤1	≤1	≤1	≤1	>4	≤1	4–>4		
MXF	≤1–>4	4	>4	≤1–>4	2	4	≤1–>4	>4	>4	2–>4	4	>4	≤1	≤1	≤1	≤1	2–4	≤1	1–4		
CLR	≤1–>8	≤1	≤1	8–>8	>8	>8	≤1–>8	>8	>8	≤1–>8	8	>8	≤1–>8	>8	>8	≤1–>8	≤1–>8	2–>8	≤1		
CLI	≤0.5–>4	1	4	>4	>4	>4	≤0.5–>4	>4	>4	≤0.5–>4	>4	>4	>4	>4	>4	4–>4	≤0.5–>4	>4	≤0.5–2		
MIN	≤1–8	2	4	≤1–8	4	8	≤1–4	2	4	≤1–2	≤1	2	≤1–2	2	2	≤1–2	≤1–4	≤1–4	≤1		
DOX	≤1–16	4	8	≤1–>16	4	8	≤1–8	4	4	≤1–4	≤1	2	≤1–4	2	2	≤1–4	4–8	2	≤1		
TGC	0.5–>4	1	4	≤0.25–>4	2	>4	≤0.25–4	0.5	1	≤0.25–2	≤0.25	1	≤0.25–1	0.5	0.5	≤0.25–0.5	1–4	≤0.25–0.5	≤0.25–1		
SXT	≤1–2	≤1	≤1	≤1–>4	2	4	≤1–2	≤1	2	≤1–2	≤1	≤1	≤1–>4	4	>4	≤1–2	≤1–4	4–>4	≤1		
LZD	≤0.5–4	1	2	1–4	2	4	≤0.5–4	2	4	≤0.5–2	1	2	≤0.5–1	1	1	≤0.5–1	1	≤0.5–1	≤0.5–1		
VAN	1–>8	>8	>8	4–>8	>8	>8	8–>8	>8	>8	8–>8	>8	>8	8–>8	>8	>8	>8	8–>8	>8	4–8		
DAL	0.5–>8	8	>8	2–>8	8	>8	4–>8	>8	>8	2–>8	>8	>8	0.5–>8	8	8	8	2–>8	4–>8	≤0.25–1		

[a] AMK, amikacin; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; FEP, cefepime; CTX, cefotaxime; CRO, ceftriaxone; CIP, ciprofloxacin; CLR, clarithromycin; CLI, clindamycin; DAL, dalbavancin; DOX, doxycycline; GEN, gentamicin; IPM, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TGC, tigecycline; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

TABLE 3. Percentages of susceptible isolates of the different *Nocardia* species isolated in this study

Antimicrobial(s)[a]	% of susceptible isolates of species (no. of isolates)								
	N. nova (55)	*N. farcinica* (43)	*N. cyriacigeorgica* (28)	*N. abscessus* (23)	*N. carnea* (12)	*N. rhamnosiphila* (5)	*N. veterana* (4)	*N. flavorosea* (4)	*N. takedensis* (3)
AMP	80	0	0	78.3	100	100	100	100	100
AMC	7.3	81.4	0	91.3	25	0	75	0	66.7
CTX	81.8	0	92.9	95.7	100	100	75	100	100
CRO	74.5	0	92.9	100	100	100	75	100	100
FEP	83.6	0	78.6	100	91.7	100	100	100	100
IPM	98.2	72.1	89.3	39.1	100	100	100	100	100
GEN	60	0	100	100	100	0	75	100	100
TOB	10.9	0	100	100	100	100	50	100	100
AMK	100	107	100	100	100	100	100	100	100
CIP	0	18.6	0	0	100	100	0	100	0
MXF	1.8	25.6	3.6	0	100	100	0	100	33.3
CLR	96.4	0	10.7	21.7	33.3	40	50	50	100
CLI	30.9	0	3.6	4.3	0	0	25	0	66.7
MIN	16.4	9.3	14.3	87	50	60	25	25	100
DOX	7.3	7.0	14.3	82.6	33.3	40	0	0	100
TGC	58.2	23.3	92.9	95.7	100	100	25	100	100
SXT	100	58.1	100	100	41.7	100	75	0	100
LZD	100	100	100	100	100	100	100	100	100
VAN	5.5	0	0	0	0	0	0	0	0
DAL	25.5	2.3	0	4.3	16.7	0	25	0	100

[a] AMK, amikacin; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; FEP, cefepime; CTX, cefotaxime; CRO, ceftriaxone; CIP, ciprofloxacin; CLR, clarithromycin; CLI, clindamycin; DAL, dalbavancin; DOX, doxycycline; GEN, gentamicin; IPM, imipenem; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; TGC, tigecycline; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

rious infections, especially in immunocompromised patients. To our knowledge, this is the largest study of *Nocardia* susceptibility performed in the era of molecular identification of isolates, and the aim is to reduce the lack of information on antimicrobial activities in specific species of *Nocardia* clinical isolates.

TABLE 4. Antimicrobial susceptibility patterns of the most frequently isolated species in this study compared with the patterns reported by Brown-Elliot et al. (2)

Species	Pattern	Resistant		Susceptible	
		Brown-Elliot antibiotype[a]	This study (%)	Brown-Elliot antibiotype[a]	This study (%)
N. nova	III	AMC	89.1 (92.7)[b]	AMP	80
				AMK	100
				CLR	96.4
				CRO	74.5
				IPM	98.2
				LZD	100
N. farcinica	V	AMP	100	AMK	100
		CLR	100	CIP	18.6
		CRO	51.2 (100)	IPM	72.1
		GEN	100	LZD	100
		TOB	100		
N. abscessus	I	CIP	100	AMP	78.3
		CLR	69.6 (78.3)	AMC	91.3
		IPM	39.1 (60.9)	AMK	100
				CRO	100
				LZD	100
N. cyriacigeorgica	VI	AMP	96.4 (100)	AMK	100
		AMC	78.6 (100)	CRO	92.9
		CIP	96.4 (100)	IPM	89.3
		CLR	82.1 (89.3)	LZD	100

[a] AMK, amikacin; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; CRO, ceftriaxone; CIP, ciprofloxacin; CLR, clarithromycin; GEN, gentamicin; IPM, imipenem; LZD, linezolid; TOB, tobramycin.

[b] Values in parentheses indicate the percentage of isolates with resistant and intermediate susceptibilities.

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