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Clinical and Laboratory Features of *Nocardia nova*

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Recent studies have shown that *Nocardia asteroides* isolates have five major antibiotic resistance patterns; one of these patterns identifies isolates of *Nocardia farcinica*. In the current study, we investigated a second pattern characterized by susceptibility to ampicillin and erythromycin. This pattern was seen in 17% of 223 clinical isolates identified by standard techniques as *N. asteroides* and associated with diseases typical for nocardiae. Biochemically, isolates with this drug pattern were relatively homogeneous and identical to the type strain and previous descriptions of *Nocardia nova*. The strains studied were unique among nocardiae in having both α- and β-esterase activity (85 and 95%, respectively). However, the arylsulfatase activity at 14 days (75%) and antimicrobial susceptibility patterns, including susceptibility to erythromycin (100%), were the only routinely available methods that would separate *N. nova* strains from other members of *N. asteroides*. *N. asteroides* should be considered a complex because current clinical identification schemes include isolates of *N. farcinica* and *N. nova* and may well include additional species. This is the first detailed description of *N. nova* as a pathogen in humans.

Numerous taxonomic studies have established the heterogeneity of the species *Nocardia asteroides* by using a variety of parameters including numerical taxonomy (5, 6, 11, 13), antigen-induced delayed hypersensitivity (8), DNA homology (4), and antimicrobial susceptibility (16). Current methods of recognition of *N. asteroides* in the clinical laboratory include microscopic and colonial morphology, inability to hydrolyze casein, tyrosine, xanthine, or hypoxanthine, and resistance to lysozyme. Unfortunately, additional species, including *Nocardia farcinica*, *N. carnea*, and *N. nova*, share the same features (7) and have undoubtedly contributed to the apparently heterogeneous nature of *N. asteroides*. Because of the taxonomic diversity of *N. asteroides* and the knowledge that it currently contains several species, we have chosen to call it the *N. asteroides* complex.

In 1988, a susceptibility study of 78 clinical isolates of the *N. asteroides* complex from the United States found 95% of strains to exhibit one of five antibiotic resistance patterns (16). Studies were then undertaken to determine whether these drug patterns correlated with specific taxonomic groups or recognized species. The first pattern studied (called type 5) was seen in approximately 20% of the isolates, including essentially all isolates in the *N. asteroides* complex that are resistant to cefotaxime, ceftriaxone, and cefamandole. Recent studies have established these isolates as *N. farcinica* (17).

We studied a second drug group (type 3) that comprises approximately 20% of the strains and is characterized by susceptibility to ampicillin and erythromycin (16). The current study demonstrates that these isolates are members of the new species *N. nova* (13, 18) and provides the first detailed description of clinical disease and drug susceptibilities in this species.

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MATERIALS AND METHODS

Organisms. Isolates of the *N. asteroides* complex submitted for susceptibility testing to the Mycobacteria/Nocardia Laboratory of the University of Texas Health Center between 1983 and 1990 and 53 selected isolates identified in the Microbiology Laboratory of the Mayo Clinic between 1983 and 1987 were screened for the presence of the type 3 drug pattern (16). Clinical information on the isolates was obtained by correspondence and/or by telephone with the referring laboratory or physician. Information was also sought on eight type 3 isolates from the Texas Laboratory identified before 1983.

All isolates had previously been identified as belonging to the *N. asteroides* complex by standard methods (9), the majority by the Mycology Section of the Texas Department of Health, Austin. After initial testing, the isolates had been stored at −70°C in tryptic soy broth with 15% glycerol.

The type strain of *N. nova* (ATCC 33726) and the co-type strain (ATCC 33727) were provided for study by M. Tsukamura (National Chubu Hospital, Obu, Aichi, Japan), who had originally submitted these strains to the American Type Culture Collection. Twelve additional strains identified only as *N. asteroides* were provided by the American Type Culture Collection and were screened for the type 3 drug pattern.

Susceptibility testing. Testing for susceptibility to ampicillin, carbenicillin, cefamandole, cefotaxime, ceftriaxone, imipenem, erythromycin, minocycline, doxycycline, ciprofloxacin, sulfamethoxazole, amoxicillin-clavulanic acid (2:1), and amikacin was performed by broth microdilution in cation-supplemented Mueller-Hinton broth as previously described (13). With this method the final inoculum is approximately 10^4 CFU/ml and the incubation time is 72 h. The quality control strains used were *Escherichia coli* ATCC 35218 (amoxicillin-clavulanic acid) and *Staphylococcus aureus* ATCC 29213. Definitions of susceptible, moderately susceptible (or intermediate), and resistant were those of the National Committee for Clinical Laboratory Standards for

rapidly growing bacterial species (10) except for carbenicillin, for which we used a moderately susceptible breakpoint of 64 to 128 μg/ml rather than 32 μg/ml. (Nocardiae have not been assessed by the National Committee for Clinical Laboratory Standards, so no official breakpoints currently exist for them.) The type 3 drug pattern is characterized by susceptibility to imipenem, ampicillin, and erythromycin but resistance to carbenicillin and amoxicillin-clavulanic acid (16).

Laboratory identification. All isolates were initially identified as belonging to the *N. asteroides* complex by using standard tests that included colony and microscopic morphology, susceptibility to lysozyme, and failure to hydrolyze casein, xanthine, tyrosine, or hypoxanthine (8).

Twenty clinical isolates with the type 3 drug pattern were randomly chosen and, along with the type strain and co-type strain of *N. nova*, were submitted to the National Chubu Hospital, where they were tested against 107 characteristics as previously described (13). These include growth characteristics, requirement of numerous carbohydrates as nitrogen and carbon sources, enzyme production, and growth inhibition by numerous drugs and chemical compounds. These 20 isolates were also tested again for susceptibility to lysozyme, hydrolysis patterns, and acid production from 23 carbohydrates at the Mycology Laboratory of the Centers for Disease Control.

Because only one of the tests identified in the study of these 20 strains as being useful in separating *N. nova* from other groups in the *N. asteroides* complex is commercially available (i.e., the arylsulfatase reaction), we studied susceptibility to erythromycin by disk diffusion as a possible additional test for the recognition of *N. nova*. With a disk diffusion method (15) with a disk zone size of 30 mm or greater on a commercial disk containing 15 μg of the antimicrobial agent as the breakpoint, all available clinical isolates of *N. nova* were compared with 40 isolates of *N. farcinica* (17) and 40 isolates of the *N. asteroides* complex that excluded isolates of *N. nova* and *N. farcinica* and included only members of the three remaining antibiotic resistance groups (types 1, 4, and 6). The arylsulfatase reaction at 2 weeks was also tested on all *N. nova* isolates by using a commercial system (Remel, Lenexa, Kans.). The degree of positivity was recorded (0 to 4+, with 0 or ± reactions considered negative). Four tests recommended for identification of *N. farcinica* (equivalent confluent growth on tryptic soy agar at 35°C and 45°C after 3 days, acid production from rhamnose, utilization of acetamide as a nitrogen and carbon source, and resistance to cefamandole and tobramycin) (17) and utilization of citrate as a carbon source were also included.

RESULTS

Organisms. A total of 170 consecutive *N. asteroides* complex isolates from the Texas Laboratory were screened for their antibiotic resistance patterns; 27 (16%) had the type 3 pattern. Eleven of 53 isolates (21%) from the Mayo Clinic had to this pattern. Overall, 38 of 223 isolates or 17% of strains had the type 3 pattern.

Clinical information was obtained on 32 of these isolates as well as on 8 previously identified isolates. Five patients were known to have disseminated disease with involvement of multiple body sites, and an additional nine patients with involvement of blood (four patients), the central nervous system (three patients), or the liver (two patients) were also presumed to have disseminated disease (a total of 35%). The most common single sites of involvements were skin and/or soft tissue (nine patients), lung or pleural fluid (five patients), blood (four patients), joints (four patients), and cornea (three patients). In all evaluable cases, the isolate was believed to be clinically significant.

The isolates were from 13 states, with the largest number of isolates from Texas (40%). Ten isolates were listed from Minnesota, but because these were submitted by outside laboratories it is presumed that many or all came from surrounding states. None of the Texas isolates appeared to be geographically or temporally related. Underlying conditions were known in 28 of the 40 cases. These were of the type typically associated with disease due to nocardiae: corticosteroids and/or chemotherapy in conditions other than organ transplantation (32%), local trauma (32%), and organ transplantation (21%).

Among the 12 ATCC isolates listed as *N. asteroides* that were studied for their drug patterns, one (ATCC 10904) was found to have the type 3 drug pattern and was included for further study.

Susceptibility testing. Because MICs were determined at the time of submission to the Texas Laboratory, different drugs were included in some panels, and hence not all clinical isolates were tested against all drugs.

MICs were determined for the 38 strains identified as part of the study of drug types among consecutive isolates of the *N. asteroides* complex as well as two previously identified isolates. The 40 clinical strains with the type 3 drug pattern were homogeneous in their drug patterns. Although the range of MICs of ampicillin, cefotaxime, and ceftriaxone was relatively wide, none of these drugs produced a bimodal distribution of values, and approximately 80% of the MICs were within one dilution of the modal value. The MICs for 50 and 90% of strains were within one dilution for 11 of 13 drugs and within two dilutions for the remaining 2 drugs (cefotaxime and sulfamethoxazole). According to National Committee for Clinical Laboratory Standards breakpoints for rapidly growing bacterial species (10), isolates were generally 100% susceptible or resistant to the drugs except for doxycycline. Isolates were susceptible to amikacin, erythromycin, ampicillin, cefotaxime, ceftriaxone, and imipenem but resistant to carbenicillin, amoxicillin-clavulanic acid, and ciprofloxacin. The MICs for the three ATCC strains of *N. nova* (including the type strain ATCC 33726) were similar to those of the clinical strains, except that the latter were generally more susceptible to β-lactam antibiotics. These values are shown in Table 1.

Laboratory identification. Morphologically, all of the type 3 isolates looked identical. They produced fine growth on Mueller-Hinton agar, with white colonies that usually developed an orange pigmentation after 7 to 10 days. The results of the detailed investigation of the 20 randomly chosen clinical strains are shown in Table 2. Unusual features were the presence of α- and β-esterase activities (85 and 95%, respectively) and arylsulfatase activity at 2 weeks (80% of strains). The strains were able to utilize fructose, glucose, and glycerol as sole carbon sources but were negative for essentially all other carbohydrates, including citrate. The acid production pattern was generally similar to that for utilization as a sole carbon source. The results of these tests are almost identical to those of the three culture collection strains of *N. nova* (Table 2) and previous descriptions of strains of *N. nova*, including the type strain (13, 18). On the basis of these tests, it was concluded that isolates with the type 3 susceptibility pattern are isolates of *N. nova*.

The laboratory study was expanded to include 40 strains

TABLE 1. Antimicrobial susceptibilities of clinical and culture collection isolates of *N. nova* to 13 antimicrobial agents

Drug[a]	Laboratory isolates, MIC (μg/ml) for:			Clinical isolates						
	ATCC 33726	ATCC 33727	ATCC 10904	No. of strains	MIC (μg/ml)[b]				Breakpoint[c]	% Susceptible
					Mode	50%	90%	Range		
AMP	0.5	≤0.25	8	40	8	4	8	1–16	16	100
AUGM	16/8	8/4	64/32	34	>64/32	>64/32	>64/32	2/1–64/32	16/8	3
CARB	128	32	128	40	>128	>128	>128	>128	64–128	0
MAND	0.5	≤0.5	2	40	4,8	4	8	0.5–16	16	100
CTX	0.5	1	32	40	4	4	16	0.5–32	16–32	100
CTR	0.5	0.5	16	40	8	8	16	0.5–16	16–32	100
IPM	≤0.5	≤0.5	≤0.5	40	≤0.5	≤0.5	≤0.5	≤0.5–1.0	8	100
ERY	≤0.25	≤0.25	≤0.25	39	≤0.25	0.5	0.5	≤0.25–1.0	1–4	100
MINO	4	4	8	40	4	4	8	4–16	8	97
DOXY				21	16	16	>16	8–>16	8	19
CIPRO	>8	>8	>8	40	>8	>8	>8	4–>8	2	0
SMX	4	4	2	40	≤1	2	8	≤1–>128	32	97
AMIK	≤0.25	≤0.25	0.5	40	≤0.25	≤0.25	0.5	≤0.25–1.0	32	100

[a] AMP, ampicillin; AUGM, amoxicillin-clavulanic acid (2:1); CARB, carbenicillin; MAND, cefamandole; CTX, cefotaxime; CTR, ceftriaxone; IPM, imipenem; ERY, erythromycin; MINO, minocycline; DOXY, doxycycline; CIPRO, ciprofloxacin; SMX, sulfamethoxazole; AMIK, amikacin.

[b] 50% and 90%, MICs for 50 and 90% of the isolates, respectively.

[c] Moderately susceptible or intermediate breakpoints from the National Committee for Clinical Laboratory Standards for organisms that grow aerobically (10) with the exception of carbenicillin (see the text).

of *N. nova* (the same strains used in the susceptibility tests). Selected tests, including erythromycin susceptibility, were then performed with all 40 strains of *N. nova*, and the results were compared with those from 40 strains of *N. farcinica* and 40 other strains of the *N. asteroides* complex. (Some of these tests in the latter two groups were published in a prior study of *N. farcinica* [17].) The arylsulfatase activity at 14 days was identified in 75% of the *N. nova* strains compared with only 1 of the 65 control strains. Among the 30 isolates of *N. nova* with positive reactions, 6 (20%) were 1+, 12 (40%) were 2+, 10 (25%) were 3+, and 1 (5%) was 4+. Twelve isolates with positive reactions (1+ through 4+) were retested in the same laboratory or different laboratories. Eleven were again positive for arylsulfatase in the 1+ to 4+ range, whereas one gave a ± reaction. With repeat testing of 10 isolates with negative or ± reactions, 3 were 3+, 3 were 1+, and the remainder were unchanged. The major problem with isolates that gave false-negative results appeared to be insufficient growth (cell mass) in the substrate medium. All isolates (100%) of *N. nova* were susceptible to erythromycin (Table 3). The four tests used to identify *N. farcinica* (17) separated these isolates from the other two groups. (The results for the four tests to identify *N. farcinica* with the 40 strains of *N. farcinica* and many of the isolates of the *N. asteroides* complex were published previously [17].)

DISCUSSION

The clinical and taxonomic status of *N. nova* has been uncertain since the species was first described in 1982 (13). At that time, Tsukamura described 14 strains that could be separated by numerical analysis from *N. farcinica* and other isolates of *N. asteroides*. The unusual features of these strains were the presence of arylsulfatase activity at 14 days (71% of strains) and esterase activity (64% of strains). Two isolates were from patients believed to have pulmonary infections. Taxonomists were uncertain of the status of these strains, and in the (latest) 1986 edition of *Bergey's Manual of Systematic Bacteriology*, *N. nova* was listed as a species incertae sedis (7).

Recently, however, Yano et al. (18) performed DNA homology studies and showed that the type strain of *N. nova* (ATCC 33726) had only 20% homology with the type strain of *N. farcinica* (ATCC 3318) and 39% homology with the type strain of *N. asteroides* (ATCC 19247), establishing the species status of *N. nova*. These authors also observed differences in mycolic acid content between the three species and noted that isolates of *N. nova* had α-esterase activity as well as β-esterase activity (18). No studies of the clinical importance of *N. nova* have been reported, however. The current study shows that approximately 20% of clinical isolates from two areas of the United States (Texas and Minnesota) identified as *N. asteroides* are actually isolates of *N. nova*. The clinical diseases with these isolates (pneumonia, cutaneous infections, brain abscess, etc.) and the clinical settings (trauma, corticosteroid use, organ transplantation, etc.) were similar to those in previous descriptions of diseases due to *N. farcinica* (15) and to the *N. asteroides* complex in reports in which the species were not distinguished (1, 2).

Methods for clinical identification of *N. nova* are problematic. Carbohydrate utilization tests and currently available biochemical reactions do not separate *N. nova* from remaining isolates in the *N. asteroides* complex. At present, 2-week arylsulfatase activity and susceptibility to erythromycin offer the best clinical means of identification, since no other group within the *N. asteroides* complex has this drug pattern (16). Additional diagnostic tests that are readily available to the clinical laboratory and that are both sensitive and specific for *N. nova* are needed.

The first description of antibiotic resistance patterns in 78 isolates of the *N. asteroides* complex found 95% of strains to have one of five patterns (16). Isolates exhibiting two of these patterns (representing approximately 40% of strains) have been studied in detail and shown to be isolates of *N. nova* and *N. farcinica*. We have now completed preliminary biochemical studies of the remaining three common antibiotic resistance patterns. Two of these patterns (known as types 1 and 4) have unique and readily identifiable growth and biochemical patterns that separate them from other

TABLE 2. Growth characteristics and biochemical results for current *N. nova* strains compared with three culture collection strains and the original description of the species (12)

Test	Test result with:				
	ATCC 33726 (type strain)	ATCC 33727 (co-type strain)	ATCC 10904	Current clinical isolates (% positive) (n = 20)	Previous descriptions (% positive) (n = 14)
Growth at 45°C (14 days)[a]	−	−	−	40	0
Nitrate reduction (24 h)	+	+	ND[b]	85	93
Utilization as sole nitrogen and carbon source (14 days)					
Acetamide	−	−	−	0	0
Monoethanolamine	−	+	ND	5	0
Serine	−	−	ND	0	0
Utilization as sole carbon source (14 days)					
Arabinose	−	−	ND	0	ND
2,3-Butylene glycol	−	+	ND	30	14
Citrate	−	−	−	5	0
Ethanol	−	−	ND	30	29
Fructose	+	+	ND	95	ND
Galactose	−	−	−	0	0
Glucose	+	+	ND	100	ND
Inositol	−	−	−	0	0
Mannitol	−	−	−	0	0
Mannose	−	−	ND	5	0
Rhamnose	−	−	−	5	0
Sorbitol	−	−	−	0	0
Sucrose	−	−	ND	0	0
Trehalose	−	−	−	25	ND
Xylose	−	−	ND	0	ND
Acetamidase	−	+	ND	55	57
Urease	+	+	ND	70	
α-Esterase	+	+	ND	85	93
β-Esterase	+	+	ND	95	100
Arylsulfatase					
3 days	−	−	−	0	0
14 days	+	+	+	80	50
Acid production from:					
Arabinose	−	−	−	0	ND
Fructose	−	−	−	15	ND
Galactose	−	−	−	5	ND
Glucose	+	+	+	100	ND
Glycerol	+	+	+	95	ND
Inositol	−	−	−	5	ND
Mannitol	−	−	−	0	ND
Mannose	−	−	ND	15	7
Rhamnose	−	−	−	5	ND
Sorbitol	−	−	−	10	ND
Sucrose	−	−	ND	10	ND
Trehalose	−	−	−	25	ND
Xylose	−	−	ND	5	ND
Growth inhibition by:					
$NaNO_2$ (0.1%)	−	−	ND	0	ND
$NaNo_2$ (0.2%)	−	−	ND	0	ND
NaCl (5%)	−	−	ND	30	ND
Ethambutol (5 μg/ml)	−	−	ND	0	ND
5-Fluorouracil (20 μg/ml)	+	−	ND	65	93

[a] Defined as abundant membranous growth, not compared with growth on the 35°C control.
[b] ND, not defined.

groups (and possibly represent other species). The last antibiotic resistance pattern (type 6) is the most common (approximately 40% of strains) but is quite heterogeneous biochemically.

Unfortunately, we have not been able to relate any of these three groups to a recognized or named *Nocardia* group (14). Which group should be the real *N. asteroides*? The current type strain of *N. asteroides* ATCC 19247 has a rare

TABLE 3. Recommended tests for distinguishing *N. nova* from *N. farcinica* and other members of the *N. asteroides* complex

Test	% of strains positive		
	N. nova (n = 40)	*N. farcinia* (n = 40)	*N. asteroides* complex[a] (n = 40)
Equivalent growth at 45 and 35°C (after 3 days)	5	100	43
Resistance to lysozyme	100	100	100
Hydrolysis of:			
Casein	0	0	0
Xanthine	0	0	0
Tyrosine	0	0	0
Hypoxanthine	0	0	0
Acetamide (nitrogen and carbon source)	0	80	17
Arylsulfatase (14 days)	75	0[b]	5[c]
Acid production from rhamnose	5	80	10
Citrate (carbon source)	5	0	65
Susceptibility (disk) to:			
Tobramycin (20 mm)[d]	17	0	83
Cefamandole (20 mm)	100	7	95
Erythromycin (30 mm)	100	0	2

[a] Includes antibiotic resistance types 1, 2, 4, 6, and some miscellaneous patterns (13).

[b] Includes 20 isolates tested by the method of Tsukamura and 10 isolates tested by the commercial system (Remel).

[c] Includes 20 isolates tested by the method of Tsukamura and 20 isolates tested with the commercial system (Remel).

[d] The numbers within parenthesis indicate the diameters of the zones of inhibition in the disk tests. The breakpoint for susceptibility is 20 mm for tobramycin and cefamandole and 30 mm for erythromycin.

drug resistance pattern that does not match that of any of the common groups. Thus the status of the other three groups is uncertain. Are they also separate species that have been missed because of the complexity of the isolates identified as *N. asteroides*, or are they only subgroups with different biochemical and drug features? Clearly, DNA homology studies will be needed to determine their taxonomic status. Until that time and until laboratories are able to separate *N. nova* and *N. farcinica* from the "real" *N. asteroides*, we strongly recommend that isolates identified by current standard laboratory criteria be identified only as *N. asteroides* complex.

The distinguishing drug pattern of *N. nova* strains is their susceptibility to ampicillin and erythromycin. A study by Finland et al. showed that in combination these two drugs act synergistically against the majority of isolates of the *N. asteroides* complex (3). In addition, they described a single patient whose disease had not responded to therapy with a sulfonamide but who responded clinically and microbiologically to the combination of ampicillin and erythromycin. One of the patients in the current study had a sulfonamide allergy, and we treated him with erythromycin alone after a course of parenteral amikacin and ceftriaxone; this resulted in a cure of his disease (14). These findings combined with the current MIC results suggest that erythromycin alone or in combination with ampicillin (but not amoxicillin-clavulanic acid) offers a potential oral therapy for patients with *N. nova* who cannot tolerate or do not respond to sulfonamide therapy.

Another unusual susceptibility feature of these isolates of *N. nova* is their moderate susceptibility to ampicillin but resistance to carbenicillin and the combination of amoxicillin and clavulanic acid. The apparent reason for this unusual β-lactam antibiotic resistance pattern is that isolates of *N. nova* have an inducible membrane-bound β-lactamase with penicillinase activity. This enzyme is not induced by ampicillin but is induced by carbenicillin and clavulanic acid. The isolates of *N. nova* had a single β-lactamase pattern for isoelectric focusing, again supporting the homogeneous nature of these isolates (12). Studies of the β-lactamase activity in *N. nova* are ongoing.

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