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Distribution of Serotypes of *Nocardia asteroides* from Animal, Human, and Environmental Sources

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The antigenic types of 129 isolates of *Nocardia asteroides* from diverse clinical, environmental, and geographic origins were determined. The majority of the isolates studied were of bovine (56) or human (44) origin; 11 were derived from six species of animals other than cattle, and 10 were isolated from environmental sources; the source of 8 strains could not be determined. Testing culture filtrate antigens against four standard reference sera in a gel diffusion precipitin test established the antigenic type of 95.3% of the isolates. After excluding strains that weighted the data because of common infection, the distribution of serotypes was examined according to the origin of the isolate. Type I was the most frequently encountered serotype (31.9%); types III (15.0%) and IV (20.4%) were also observed frequently, as was the antigenic mixture III + IV (14.2%). There was an apparent difference in frequency of type III and IV antigens among isolates of bovine and human origin; type III made up 20.0% of the bovine isolates and 13.6% of the human isolates, whereas type IV constituted 10.0% of bovine and 27.3% of human isolates.

Nocardia asteroides is generally considered to be an opportunistic pathogen that infects animals and humans as a result of inhalation or traumatic implantation of infectious soil (1, 8, 14). Infection occurs most frequently in cattle, dogs, and humans (1, 8). The development of a serotyping scheme for *N. asteroides* (9) made possible epidemiological studies within groups of infected individuals, such as herds of dairy cattle. The present study examined a number of *N. asteroides* isolates to see whether there were apparent predilections of serotypes among isolates from different origins, and we report the distribution of serotypes among 129 isolates identified as *N. asteroides*.

MATERIALS AND METHODS

Cultures. *N. asteroides* isolates studied were of three general sources: primary isolates from clinical materials, made by the authors; cultures sent to us by other investigators for identification or confirmation of identity; cultures obtained from the collections of other investigators. Cooperating investigators were contacted to determine, wherever possible, the ultimate origin of each isolate and to eliminate duplication of strains that occurred in more than one culture collection. When several strains of common serotype had been isolated from a single source, such as a herd of infected dairy cattle, only a single strain was included in the final distribution study in order to avoid weighting the data. When a culture originated from a human medical laboratory and was associated with disease but could no longer be definitively traced to a human patient, the origin was listed as "presumed human." When definitive identification of the origin was not possible, the culture was listed as "origin unidentified." The latter cultures were retained in the study if they appeared in previous studies of *Nocardia* (3-6, 9).

Classification of cultures as *N. asteroides* was accomplished by methods previously reported (9). Each strain was assessed for the presence of acid fastness and branching, fragmented filaments by Kinyoun-stained smears; for acid production from arabinose, dextrose, mannose, and sorbitol; for ability to survive exposure to 60°C for 4 h; and for decomposition of casein, tyrosine, and xanthine.

Serotyping. Culture filtrate antigens were prepared from each isolate (10). These antigens were concentrated 10-fold by pervaporation and tested against a battery of sera from hyperimmunized rabbits in an agarose double-diffusion precipitin system to determine the antigenic type (9).

RESULTS

A total of 129 isolates were identified or confirmed as *N. asteroides* and typed serologically. The biological activity of these strains was generally typical of *N. asteroides*. They had branching, fragmenting, partially acid-fast filaments. Nearly all produced acid from dextrose, some produced acid from mannose, one (strain Krause) produced it from sorbitol, and none utilized arabinose. Nearly all survived exposure to 60°C for 4 h, and none decomposed casein or xanthine. Only three strains (*N. asteroides* strains CDC-JRT, N.A.68, and 397) decomposed tyrosine. Strain identity, origin, and serological type and a summary of biological reactions are presented in Table 1.

The great majority of these isolates (77.5%)

TABLE 1. *Serological types and cultural reactions of cultures identified as N. asteroides*

Isolate	Donor	Location	Origin	Acid production on: Dextrose	Acid production on: Mannose	Survival at 60°C	Serotype
443-1	Gordon	North Carolina	Human	+	−	+	I
430	Gordon	Tennessee	Human	+	−	+	I
Lynd	Krick	California	Human	+	−	+	I
Acosta	Krick	California	Human	+	−	+	I
409	Gordon	NK[a]	Human	+	−	+	I
3656	Gordon	NK	Human	+	−	−	I
421	Gordon	North Carolina	Human	+	−	+	I
3423	Gordon	Rangoon	Human	−	−	+	II
47N	Alli	Iowa	Human	+	−	+	II
F47	Kunz	Massachusetts	Human	+	+	+	III
Johnson	McMillen	Illinois	Human	+	+	+	III
Weddington	McMillen	Illinois	Human	+	+	+	III
N. asteroides (Brasil-Tarka)	Tarka	Missouri	Human	+	−	+	III
Stevens N3	Stevens	England	Human	+	−	+	III
N-105	Goodfellow	Pennsylvania	Human	+	−	+	IV
433	Gordon	North Carolina	Human	+	−	+	IV
3573	Gordon	Louisiana	Human	−	−	+	IV
727	Gordon	NCDC[b]	Human	+	+	−	IV
N-100	Goodfellow	Puerto Rico	Human	+	−	+	IV
52N	Alli	Iowa	Human	+	−	+	IV
Townswick	Krick	California	Human	+	+	+	IV
1826	Gordon	Phillipines	Human	−	−	−	IV
Probst	McMillen	Illinois	Human	+	+	+	IV
Harmon	Tarka	Missouri	Human	+	+	−	IV
Duenon	Tarka	Missouri	Human	+	−	+	II + IV
N. asteroides (Tarka)	Tarka	Missouri	Human	+	−	−	II + IV
N-106	Goodfellow	NK	Human	+	+	+	III + IV
436	Gordon	North Carolina	Human	+	−	+	III + IV
K72	McClung	Minnesota	Human	+	−	+	III + IV
K60	McClung	South Carolina	Human	+	+	+	III + IV
46N	Alli	Iowa	Human	+	−	+	III + IV
Perez	Krick	California	Human	+	+	+	III + IV
615	Gordon	California	Human	+	−	+	—[c]
Maxwell	Krick	California	Human	−	+	+	—
411	Gordon	New York	Human (?)[d]	+	−	+	I
Na67	NCDC	NK	Human (?)	+	−	+	I
N. asteroides CDC-JRT	NCDC	NK	Human (?)	+	−[e]	+	I
N.A.68	NCDC	NK	Human (?)	+	−[e]	+	I
N-13	Goodfellow	Greece	Human (?)	+	−	+	II
N-76	Goodfellow	North Carolina	Human (?)	+	+	+	III
551	Gordon	California	Human (?)	+	−	+	IV
439	Gordon	Brazil	Human (?)	+	−	+	IV
347-7	NCDC	NK	Human (?)	+	+	+	III + IV
397	Gordon	New York	Human (?)	−	−[e]	+	—
40N	Packer	Iowa	Bovine	+	−	+	I
42N	Willers	Hawaii	Bovine	+	−	+	I
58N	Yoder	Arizona	Bovine	+	−	+	I
59N	Yoder	Arizona	Bovine	+	−	+	I
Brown-Univ. of Georgia	Brown	Georgia	Bovine	+	−	+	I
22LF	McDonald	Iowa	Bovine	+	−	+	I
28608-2509	Richards	Louisiana	Bovine	+	−	+	I
27974-2141C	Richards	Nebraska	Bovine	+	−	+	I
Koehne N1	Koehne	Kentucky	Bovine	+	−	+	I
McDonald 37	McDonald	Mississippi	Bovine	+	−	+	I
McDonald 38[f]	McDonald	Mississippi	Bovine	+	−	+	I
Koehne N2	Koehne	Kentucky	Bovine	+	−	+	I
Koehne N3	Koehne	Kentucky	Bovine	+	−	+	I
Koehne N5	Koehne	Kentucky	Bovine	+	−	+	I
Koehne N8	Koehne	Kentucky	Bovine	+	−	+	I
Koehne N10	Koehne	Kentucky	Bovine	+	−	+	I
19N	Pier	Hawaii	Bovine	−	−	+	II
UP-1	Donawick	Pennsylvania	Bovine	+	+	+	III
34N	Pier	California	Bovine	+	−	+	III
28N	Pier	California	Bovine	+	+	+	III
28052-2196	Richards	Puerto Rico	Bovine	+	−	+	III
28710-2583	Richards	Puerto Rico	Bovine	+	−	+	III
Dutra 358	Bushnell	California	Bovine	+	+	+	III
5465B	McDonald	Iowa	Bovine	+	+	+	III

TABLE 1.—*Continued*

Isolate	Donor	Location	Origin	Acid production on: Dextrose	Acid production on: Mannose	Survival at 60°C	Serotype
5076B[f]	McDonald	Iowa	Bovine	+	+	+	III
5076D[f]	McDonald	Iowa	Bovine	+	+	+	III
5198B[f]	McDonald	Iowa	Bovine	+	+	+	III
5431A[f]	McDonald	Iowa	Bovine	+	−	+	III
5431C[f]	McDonald	Iowa	Bovine	+	+	+	III
1047D	Bushnell	California	Bovine	+	+	+	III
257BC[f]	Bushnell	California	Bovine	+	+	+	III
595[f]	Bushnell	California	Bovine	+	−	+	III
1252[f]	Bushnell	California	Bovine	+	+	+	III
36N	Pier	California	Bovine	+	−	+	IV
54N	Spencer	Puerto Rico	Bovine	+	+	+	IV
5465A	McDonald	Iowa	Bovine	+	−	+	IV
McDonald 34	McDonald	Mississippi	Bovine	+	−	+	IV
Koehne N4	Koehne	Kentucky	Bovine	+	−	+	I + IV
McDonald 6	McDonald	Mississippi	Bovine	+	−	+	I + IV
McDonald 2[f]	McDonald	Mississippi	Bovine	+	−	+	I + IV
McDonald 12[f]	McDonald	Mississippi	Bovine	+	−	+	I + IV
Koehne N6	Koehne	Kentucky	Bovine	+	−	+	I + IV
Koehne N7[f]	Koehne	Kentucky	Bovine	+	−	+	I + IV
Koehne N9	Koehne	Kentucky	Bovine	+	−	+	I + IV
20N	Pier	Hawaii	Bovine	+	+	+	III + IV
16N	Pier	California	Bovine	+	+	+	III + IV
7N	Pier	California	Bovine	+	−	+	III + IV
2N	Pier	California	Bovine	+	−	+	III + IV
N-233	Goodfellow	England	Bovine	+	+	+	III + IV
Nf3318	Gordon	NK	Bovine	+	+	+	III + IV
43LR	McDonald	Iowa	Bovine	+	−	+	III + IV
15B[f]	McDonald	Iowa	Bovine	+	−	+	III + IV
41D[f]	McDonald	Iowa	Bovine	+	−	+	III + IV
41C[f]	McDonald	Iowa	Bovine	+	−	+	III + IV
41A[f]	McDonald	Iowa	Bovine	+	−	+	III + IV
5431D	McDonald	Iowa	Bovine	+	+	+	—
41N	Broughton	Iowa	Canine	+	−	+	I
49N-W	Parizek	Connecticut	Canine	+	−	+	I
43N	Yoder	North Carolina	Canine	+	−	+	IV
Krause	McMillen	Illinois	Canine	+	+[g]	+	—
45N	Miyahara	Hawaii	Cetacean	−	−	+	II
404	Gordon	United States	Chicken	+	−	+	I
44N	Pier	New Jersey/Argentina	Equine	+	−	+	III + IV
503	Gordon	England	Equine	+	−	+	III + IV
N-228	Goodfellow	Argentina	Fish	+	−	+	—
N-97	Goodfellow	NK	Goat	+	−	+	I
72-6302	Knutson	South Dakota	Porcine	+	−	+	IV
28323-2413	Richards	New York	Dog food	+	−	+	I
N.e.8	Richards	NK	Soil	+	−	+	I
India 10	R. Gordon	India	Soil	+	+	+	III
3410	Gordon	Australia	Soil	+	−	−	IV
N-265	Goodfellow	NK	Air	+	+	+	IV
553	Gordon	California	Soil	−	−	−	IV
Stevens N22	Stevens	England	Environmental	−	−	+	IV
India 8	R. Gordon	India	Soil	+	−	+	II + IV
India 9	R. Gordon	India	Soil	+	+	−	II + IV
Stevens N1	Stevens	England	Environmental	+	−	−	II + IV
N-121	Goodfellow	NK	NK	+	−	+	I
SD N 43	Bojalil	Brazil	NK	+	−	−	I
N-224	Goodfellow	NK	NK	+	−	+	I
NF 171	Richards	NK	NK	+	−	−	I
Nf3399	Gordon	New York	NK	+	+	+	III
N-217	Goodfellow	NK	NK	+	−	+	III
SD N 121	Bojalil	Mexico	NK	+	+	+	IV
1011	Gordon	NK	NK	+	+	+	II + IV

[a] NK, Not known.
[b] NCDC, National Communicable Disease Center.
[c] —, No reaction with typing antisera.
[d] (?), Presumed human.
[e] Decomposed tyrosine.
[f] Replicate isolate from infected herd excluded from Tables 2 and 3.
[g] Acid from sorbitol.

were of bovine and human (or "presumed human") origin. Isolates from environmental sources constituted 7.8% of the total, and 8.5% of the isolates were derived from six species other than cattle and humans. Of the total isolates, six gave no reaction with any of the typing sera; thus, 95.3% of the total were typable with the four standard antisera. An attempt was made to prepare a precipitating antiserum to one of the untypable strains (397), but no detectable reaction was obtained. No further attempts were made with the remaining five untypable isolates, some of which grew poorly in the antigen production medium.

The distribution of serological types of *N. asteroides* from different origins is presented in Table 2. Sixteen bovine isolates listed in Table 1 were excluded from the data in Tables 2 and 3 because they represented multiple isolations from infectious outbreaks in individual dairy herds, and there was a potential for weighting the data due to a common source of infection. The most frequently occurring serotypes of *N. asteroides* (Table 2) were types I (31.9%) and IV (20.4%). Type III was of lesser overall frequency (15.0%), but was prominent among bovine isolates. Type II was a relatively infrequently occurring single antigen (4.4%). Dual antigenicity was found in 23.0% of the total isolates; the type IV antigen was a constant feature in such antigenic mixtures, and the mixture of types III and IV was the most frequent combination.

The isolates of determinable origin were grouped as human (including those known and presumed to be of human origin), bovine, other animal (i.e., all animals other than cattle), and environmental, and the percentage of isolates of each serotype was determined for each group (Table 3). The serotypes of multiple isolates obtained from within each of six infected dairy herds are presented in Table 4. The propensity for like serotypes to occur among the isolates from an individual herd was apparent.

DISCUSSION

Among the 95 strains in Tables 2 and 3 which were derived from infections, serotype I was clearly the most frequent infecting type; types III, IV, and III + IV were also common, but occurred only about one-half as frequently as type I. Among a limited number of environmental isolates, type I was less frequent than type IV (and the mixture of types II and IV). If such differences in the frequency of infecting types persisted after larger numbers of strains of determined origin were tested, the predominance of types I, III, and IV over type II might be attributed to higher pathogenicity of these types. However, in a previous study (11) in which the virulence of a limited number of bovine isolates was tested, one of the more virulent isolates was subsequently found to be type II, the least frequently occurring type in the present study.

The distributions of serotypes among the human, animals other than cattle, and environmental isolate groups had many qualitative similarities, whereas cattle isolates had some notable differences. Only the isolates of human (human plus "presumed human") and bovine origin formed groups large enough to permit speculation as to differences in serotype distribution. Type I was prominent among both human and bovine strains, but there was a notable difference in the appearance of type IV, which was prominent among human but not among bovine isolates. Type III appeared to be of somewhat greater frequency among bovine than among human isolates.

Previous studies of herd infections among dairy cattle indicated that infection is passed from animal to animal by mechanical vectors (2,

TABLE 2. *Distribution of serological types of N. asteroides isolated from animals, humans, or environmental locations*

Isolate origin	No. of isolates of serotype							
	I	II	III	IV	I + IV	II + IV	III + IV	No reaction
Bovine	15	1	8	4	4		7	1
Human	7	2	5	10		1	6	3
Presumed human	4	1	1	2		1		1
Canine	2			1				1
Equine							2	
Cetacean		1						1
Avian	1							
Caprine	1							
Porcine				1				
Environmental	2		1	4		3		
Unidentified	4		2	1		1		

TABLE 3. *Percentage of N. asteroides serotypes grouped by origin*

Isolate origin group (no. of isolates)	% of isolates in serogroup							
	I	II	III	IV	I + IV	II + IV	III + IV	No reaction
Human (44)	25.0	6.8	13.6	27.3	0.0	4.5	13.9	9.0
Bovine (40)	37.5	2.5	20.0	10.0	10.0	0.0	17.5	2.5
Other animal (11)	36.4	9.1	0.0	18.2	0.0	0.0	18.2	18.2
Environmental (10)	20.0	0.0	10.0	40.0	0.0	30.0	0.0	0.0

TABLE 4. *Serotypes of N. asteroides isolated within infected dairy herds*

Herd	Location	Isolate no.	Serotype
A	Iowa	15B	III + IV
		41D	III + IV
		41C	III + IV
		41A	III + IV
		43LR	III + IV
B	Iowa	5076B	III
		5076D	III
		5198B	III
		5431A	III
		5431C	III
		5465B	III
		5465A	IV
C	California	257BC	III
		595	III
		1047D	III
		1252	III
D	Mississippi	2	I + IV
		6	I + IV
		12	I + IV
		37	I
		38	I
E	Kentucky	N4	I + IV
		N5	I
F	Kentucky	N6	I + IV
		N7	I + IV

12). Studies of *N. asteroides* serotypes from within one such herd showed that most isolates were of a single serotype (9), which was consistent with the hypothesis of a common source of infection. The additional data in Table 4 generally support this observation, particularly in herds A, B, C, and D, where several isolates from each herd were tested.

Randomly selected environmental isolates might be expected to have a diversity of serotypes independent of those seen in group infections, unless specific serotypes predominate in that environment. Presumably, however, many of the environmental isolates in the present study originated from environments where disease of animals or humans had been diagnosed, and thus might reflect the antigenic types found in those clinical specimens. Numbers of isolates from a single geographic location were generally too small to assess serotype prevalence.

In addition to affording opportunities for epidemiological speculation, antigenic typing of *N. asteroides* may have some taxonomic pertinence. Antigenic differences among *Nocardia* species generically and within *N. asteroides* specifically have been cited in several recent reports. Ridell and Norlin (13) reported that *Nocardia* could be separated into two groups by using a mycobacterial precipitin reference system. Their results indicated a close antigenic association between *Nocardia farcinica* and *Mycobacterium* species. Magnusson and Mariat (7), using a delayed cutaneous hypersensitivity test, found antigenic heterogeneity among strains received as *N. farcinica* and advocated taxonomic separation of *N. asteroides* from *N. farcinica*. In a later publication (6), Magnusson made a direct comparison among 19 isolates received as *N. asteroides*, using our serological types and his sensitin types. He found general agreement between the two methods and proposed our serotype I as *N. asteroides*, our serotypes II and II + IV as *Nocardia sebivorans*, and our serotypes III and III + IV as *N. farcinica*; serotype IV appeared to contain two sensitin groups. For reasons of simplicity we prefer to continue to consider these as serotypes of a single species, *N. asteroides*. The biological reactions summarized in Table 1 show a general homogeneity in key reactions. Only occasional strains did not produce acid on dextrose or survive 60°C for 4 h; one isolate produced acid on sorbitol, and three decomposed tyrosine. (Two of the tyrosine-active strains were serotype I; the other was nonreactive with standard typing sera.) Of the three strains received as *N. farcinica*, one (Nf3399) was serotype III, one (Nf3318) was type III + IV, and the third (Nf171) was type I. Tracing the history of isolate Nf171, it would appear to have been a descendant of Nf3399; if so, the difference in serotype would appear to have been the result of error at some point during culture passage.

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