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Antimicrobial Susceptibility Patterns of *Nocardia asteroides*

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Testing of the susceptibility to 12 antimicrobial agents, including β-lactams, aminoglycosides, ciprofloxacin, and erythromycin, was performed by broth microdilution on 78 consecutive clinical isolates of *Nocardia asteroides*. Surprisingly, a limited number of patterns of susceptibility were identified that included all drug classes, with 95% of isolates exhibiting one of five patterns. One group (17%) exhibited resistance to the broad-spectrum cephalosporins, one group (18%) was susceptible to both ampicillin and erythromycin, one group (17%) was susceptible to ampicillin and carbenicillin but intermediate in susceptibility to imipenem, and the most common group (35%) was resistant to ampicillin but susceptible to the broad-spectrum cephalosporins and imipenem. The most active parenteral agents were amikacin (95%), imipenem (88%), ceftriaxone (82%), and cefotaxime (82%), while the most active oral agents were the sulfonamides (100%), minocycline (100%), and ampicillin (40%). Additional studies are needed to determine whether differences in β-lactamases relate to varying β-lactam resistance and whether taxonomic differences that correlate with the different susceptibility groups can be identified.

There have been a number of recent reports of testing of the susceptibility of *Nocardia asteroides* to newer antimicrobial agents, including the fluorinated quinolones, the carbapenems, broad-spectrum cephalosporins, and various clavulanic acid combinations (1, 3–8, 10, 12, 21). In general, these studies have shown variable degrees of susceptibility to each of these groups of agents. The reason for this variability is unknown, since drug resistance in nocardiae presumably reflects primary (intrinsic) resistance as most infections are acquired from the environment outside the hospital. Whether there are unlimited patterns of resistance or just a few patterns has never been addressed.

While performing susceptibility testing of clinical isolates of nocardia, we noted that most isolates of *N. asteroides* fell into one of four patterns of susceptibility that involved all classes of drugs. Details of these drug patterns and their taxonomic implications are presented.

MATERIALS AND METHODS

Organisms. Seventy-eight consecutive isolates of *N. asteroides* submitted for susceptibility testing to our laboratory were studied. Isolates were identified to species by standard methods (11); the majority of strains were identified by the Mycology Section of the Texas Department of Health, Austin.

Susceptibility testing. Susceptibility testing was performed by broth microdilution, using cation-supplemented Mueller-Hinton broth and 96-well plates that contained 0.1 ml of drug per well. Ampicillin, carbenicillin, cefotaxime, ceftriaxone, imipenem, minocycline, erythromycin, ciprofloxacin, sulfamethoxazole, amikacin, kanamycin, and gentamicin were prepared in twofold dilutions from diagnostic powders and added to the microdilution wells by using the Mini Quick Spense II System (Bellco Glass, Inc., Vineland, N.J.). The plates were stored at −70°C until needed. For inoculation of the plates, organisms were taken directly from agar slants if adequate growth was present or were grown in Trypticase soy broth until a turbid suspension was present. The culture was vortexed with 5-mm glass beads to help break up the cells, and then the large particles were allowed to settle. The suspension of organisms was matched to the optical density of the 0.5 McFarland standard, a dilution which has been shown to contain 10^7 to 10^8 CFU for nocardia (17). This suspension was diluted 1:100 and then inoculated into the microdilution wells by using a disposable inoculator which delivers approximately 0.01 ml (MIC-2000 inoculator; Dynatech Laboratories, Inc., Alexandria, Va.). The susceptibility plates were covered, placed in sealed plastic bags, and incubated for 3 days at 35°C in room air in a moisturized incubator. (Previous studies with agar dilutions have generally used a 48-h incubation time. We chose 72 h because this time period allowed isolates to be incubated over the weekend, and the additional incubation time had only a minimal effect on MIC results [unpublished observations].)

The MIC was defined as the lowest concentration that produced complete inhibition of growth except for sulfamethoxazole, for which 80% or greater inhibition of growth was used (2). Susceptibility and resistance were defined according to the recommendations of the National Committee for Clinical Laboratory Standards (13). No breakpoints are currently recognized for the sulfonamides for infections other than those of the urinary tract. We arbitrarily chose as criteria for susceptibility and resistance MICs of 32 μg/ml or less and 64 μg/ml or greater, respectively. (This classification scheme uses the same sulfa breakpoints as are used by the National Committee for Clinical Laboratory Standards for systemic infections treated with sulfamethoxazole-trimethoprim [13].)

The type strain *N. asteroides* ATCC 19247 was used as a control strain; the reproducibility of MICs by the broth method for this strain have been reported (18). To further test the reproducibility of the broth method, 20 isolates were tested on two occasions and the MIC results were compared. The bacterial control strains used were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. MICs for these latter three strains were read at 24 h, whereas those for the nocardia control strain were read at 72 h.

Preliminary studies with *N. asteroides* had shown six patterns of susceptibility, which were arbitrarily numbered 1

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TABLE 1. Drug patterns and their major group characteristics among 78 clinical isolates of *N. asteroides*

Drug pattern type	Incidence (%)	Major group characteristics
1	20	Susceptible to ampicillin, carbenicillin, and broad-spectrum cephalosporins; imipenem MICs for one-half of isolates were high (8–32 μg/ml)
2	0	Same as type 1 except that kanamycin MICs were low (<1 μg/ml); susceptible to ciprofloxacin
3	18	Susceptible to ampicillin but resistant to carbenicillin; susceptible to erythromycin
4	5	Resistant all aminoglycosides, including amikacin; susceptible to ciprofloxacin
5	17	Resistant to penicillins and broad-spectrum cephalosporins; resistant to all aminoglycosides except amikacin; susceptible to ciprofloxacin and imipenem
6	35	Resistant to penicillins but susceptible to broad-spectrum cephalosporins
Miscellaneous	5	Unable to group with above

through 6. For this study, organisms were placed in one of these groups if the MICs of at least 11 of the 12 drugs fell in the same susceptibility category (susceptible or resistant). Exceptions were made if the modal MIC of an additional drug was at the susceptibility breakpoint such that susceptible or resistant MICs could be seen with only 1- to 2-dilution differences from the modal MIC.

RESULTS

Organisms. The 78 consecutive isolates were received between 1986 and 1988. The isolates came from 17 states, with the majority (59%) from Texas. The isolates from Texas came from both small and major metropolitan areas, with the largest numbers coming from the two largest municipalities, Houston and Dallas-Fort Worth.

The test isolates were almost exclusively from adults, and the clinical diseases associated with them varied. Most of the isolates (n = 34) came from the respiratory tract (sputum, bronchoscopy samples, or lung tissue); 19 isolates were from skin or soft tissue, 8 were from brain tissue, 5 were from blood, and 5 were from eyes. In some cases, there was involvement of more than one organ system, and isolates were recovered from more than one source. In these cases, only one isolate was reported. For seven of the isolates, the clinical source was unknown.

Susceptibility testing. The reproducibility of the broth microdilution method was assessed by testing 20 isolates on two occasions, using different cultures. It was found that 84% of individual MICs were within 1 dilution of each other and that 94% were within 2 dilutions of each other. A previous study showed similar results for a single *Nocardia* strain tested in the broth microdilution system on 10 occasions (18).

Among the 78 isolates, 74 could be readily classified into five of the six drug patterns (types 1 through 6). Four of these patterns (types 1, 3, 5, and 6) predominated and together comprised 90% of the strains. The prevalence of each of these types and their major group characteristics are shown in Table 1.

The MICs of the 12 test drugs for 50 and 90% of the isolates (MIC_{50} and MIC_{90}, respectively) exhibiting the four common susceptibility patterns are shown in Table 2. The MICs within each group were clustered fairly closely. The average difference between the MIC_{50} and MIC_{90} was less than 1 dilution; with the exceptions of cefotaxime and ceftriaxone, no drug showed more than a 2-dilution (four-fold) difference between the MIC_{50} and the MIC_{90}. In the latter two circumstances, all isolates remained in the susceptible category.

Overall, the drugs with the highest activity were sulfamethoxazole (100%), minocycline (100%), and amikacin (95%). The most active β-lactams were imipenem (88%), cefotaxime (82%), and ceftriaxone (82%). The MICs of cefotaxime and ceftriaxone were virtually identical. The MIC_{50} and MIC_{90}, as well as the percents susceptible (based on the moderately susceptible breakpoint), of all drugs for all isolates are shown in Table 3. A comparison of the disk zones of growth inhibition and MICs for amikacin, ciprofloxacin, and cefotaxime for most of these isolates has been published (18).

DISCUSSION

This is the largest study of antimicrobial susceptibility reported for *N. asteroides*. This study clearly demonstrates

TABLE 2. MICs associated with the four most common susceptibility patterns of *N. asteroides*

Drug	MIC (μg/ml) for susceptibility type:							
	1 (n = 16)		3 (n = 14)		5 (n = 13)		6 (n = 27)	
	50%	90%	50%	90%	50%	90%	50%	90%
Sulfamethoxazole	≤1	2	2	4	4	8	2	8
Erythromycin	16	>16	0.5	0.5	>16	>16	16	>16
Minocycline	1	2	4	4	4	8	4	8
Ciprofloxacin	4	8	8	>8	1	2	8	>8
Gentamicin	2	2	8	>8	>8	>8	4	8
Kanamycin	8	16	8	16	>32	>32	32	>32
Amikacin	≤0.25	0.5	<0.25	0.5	1	2	1	2
Ampicillin	4	16	4	8	>32	>32	>32	>32
Carbenicillin	32	32	>128	>128	>128	>128	>128	>128
Cefotaxime	1	8	4	16	>64	>64	2	8
Ceftriaxone	1	8	8	16	>64	>64	2	8
Imipenem	8	16	≤0.5	≤0.5	4	16	1	4

TABLE 3. Drug susceptibilities of 78 clinical isolates of *N. asteroides* that represent all drug patterns

Drug	MIC (μg/ml)				Moderately susceptible breakpoint (μg/ml)[a]	% Susceptible
	50%	90%	Mode	Range		
Sulfamethoxazole	2	8	≤1	≤1–16	32	100
Erythromycin	>8	>8	>8	≤0.25–>8	4	22
Minocycline	2	8	4	≤0.5–8	8	100
Ciprofloxacin	≥4	≥4	≥4	≤0.25–≥4	2	29
Gentamicin	4	>32	2	1–>32	8	67
Kanamycin	32	>32	>32	≤0.25–>32	32	55
Amikacin	0.5	1	0.5	≤0.25–>32	32	95
Ampicillin	>32	>32	>32	0.5–>32	16	40
Carbenicillin	>128	>128	>128	8–>128	128	28
Cefotaxime	4	>64	4	≤0.5–>64	32	82
Ceftriaxone	4	>64	4	≤0.5–>64	32	82
Imipenem	1	16	≤0.5	≤0.5–32	8	88

[a] National Committee for Clinical Laboratory Standards values for broth dilution testing (13).

that although susceptibility to most antimicrobial agents is variable among clinical isolates of *N. asteroides*, the number of different drug patterns is limited. Because of the wide geographic distribution and clinical sources of the isolates, we believe that these patterns are reflective of *Nocardia* isolates in general. The cumulative percentage of strains susceptible to drugs such as ampicillin, amikacin, imipenem, and cefotaxime is similar to the values found in previous smaller studies from other geographic areas (1, 3–8, 10, 12, 21). The most active agents other than sulfamethoxazole were amikacin and imipenem, as both inhibited approximately 90% of isolates with low modal MICs (0.5 and ≤ 0.5 μg/ml, respectively). Approximately 80% of isolates were susceptible to cefotaxime and ceftriaxone, important findings considering the high levels in serum and excellent central nervous system penetration of these agents. Although many isolates were susceptible to minocycline (100%) and ampicillin (40%), MICs for essentially all isolates were in the moderately susceptible category, which would make therapy by oral means difficult.

There are three important consequences of this study. The first is the demonstration that drug susceptibility within the species *N. asteroides* is variable and that clinical use of drugs other than sulfonamides should be supported by susceptibility testing. This is not a new observation, as other susceptibility studies of the species have come to the same conclusion (1, 6, 7, 10, 18).

The second significance of the finding of limited patterns of drug resistance is that it opens the door to studies of mechanisms of drug resistance in *N. asteroides*. Previous studies in *Nocardia brasiliensis*, for example, have shown only a single pattern of β-lactam resistance and good correlation between the types of β-lactamase present in the species and the observed β-lactam susceptibility patterns (16). With *N. asteroides*, the seemingly unlimited number of drug patterns made a similar study logistically very difficult. Several studies have shown β-lactamase in more than 90% of isolates of *N. asteroides*, but attempts to correlate the types and activities of these enzymes with the observed β-lactam resistance pattern were not made (12, 19). Such a study, using selected strains of each given pattern, is clearly now feasible. Preliminary studies in our laboratory of the type 5 isolates, which are resistant to the broad-spectrum cephalosporins, have shown a single β-lactamase pattern by isoelectric focusing that is not present in isolates with other resistance patterns (unpublished observations), a finding which supports this relationship.

The third major implication of the finding of limited drug patterns involving all drug classes is taxonomic. Several previous studies have demonstrated the taxonomic significance of different drug patterns of the three recognized *Nocardia* species that infect humans (9, 20). *N. brasiliensis*, for example, appears to have a single drug pattern which differs from that of *N. asteroides* (16, 20). Among the rapidly growing mycobacteria, which are related environmental actinomycetes with very similar antimicrobial susceptibilities and are presumed to also exhibit intrinsic rather than acquired drug resistance, consistent differences in antimicrobial susceptibility patterns have reflected major taxonomic differences as well. The five major groups of pathogenic, rapidly growing mycobacteria (three biovariants of *Mycobacterium fortuitum* and two subspecies of *Mycobacterium chelonae*) all have different but relatively fixed drug patterns (14). Within the species *N. asteroides*, some investigations have suggested that more than one taxonomic group is present (15). Detailed taxonomic studies of the subgroups of *N. asteroides* identified in the current study have been initiated to try to answer this question.

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