Differences Among *Nocardia* spp. in Susceptibility to Aminoglycosides and β -Lactam Antibiotics and Their Potential Use in Taxonomy

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Using disk diffusion and agar dilution methods, 109 clinical isolates of *Nocardia* were tested against 10 antimicrobial agents. A species difference in susceptibility was noted for cefamandole, carbenicillin, gentamicin, and kanamycin. These differences in susceptibility may be of use taxonomically as well as having some potential use clinically.

In a large study of *Nocardia* spp. by Goodfellow and Orchard in 1974 (5), a species difference in antimicrobial susceptibility was noted for several agents, including kanamycin, tobramycin, and novobiocin. These authors suggested that susceptibility testing be used as a taxonomic criteria for species identification. During the course of routine disk susceptibility testing of clinical isolates of *Nocardia* (8), we noted a difference in susceptibility of three *Nocardia* spp. to a number of antimicrobial agents, including several which had not been evaluated by either of the previous studies. Studies of these differences and their potential for use both clinically and taxonomically form the basis of this report.

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MATERIALS AND METHODS

Organisms. Isolates of *Nocardia* were identified by the Houston Health Department, Houston, Texas, and the Texas Department of Health, Austin, Texas, using growth and staining characteristics and ability of the organism to hydrolyze xanthine, casein, or tyrosine according to the schema of the Centers for Disease Control, Atlanta, Ga. (6). Isolates which could not be identified to species level were referred to the Centers for Disease Control for final identification.

Of the 109 isolates, 70 were *Nocardia asteroides*, 31 were *Nocardia brasiliensis*, and 8 were *Nocardia*

caviae. All but three of the *N. asteroides* isolates were from the respiratory tract, whereas all but one *N. brasiliensis* isolates were from skin. *N. caviae* was isolated from both skin (three isolates) and respiratory tract (five isolates).

Susceptibility testing. Organisms were incubated in tryptic soy broth at 35°C for 3 to 5 days, with daily mixing for about 30 s on a Vortex mixer. Sterile glass beads (0.5 mm in diameter) were used when excessive clumping was present. Some isolates had been passaged only two to three times before testing, whereas others had been stored at -70°C in tryptic soy broth with 15% glycerol (7).

Disk susceptibility testing was performed by published methods (8, 9). Susceptible zone sizes used were as follows (in millimeters): gentamicin, 23; kanamycin, 23; amikacin (30 μ g), 25; minocycline, 20; sulfisoxazole (300 μ g), 30; trimethoprim-sulfamethoxazole, 20; ampicillin, 25; and erythromycin, 30. Cefamandole and carbenicillin have not been previously studied.

Agar dilution susceptibility was performed with twofold dilutions of gentamicin, kanamycin, cefamandole, and carbenicillin in Mueller-Hinton agar. An inoculum of organisms adjusted to match the density of a 0.5 McFarland standard was applied with a Steers replicator so as to deposit an average of 10⁴ colony-forming units per spot. In most cases, colony counts were done on the broth suspension used for inoculation to ensure the proper inoculum size. *Escherichia coli* ATCC 25922 was inoculated as a control. Minimal inhibitory concentrations (MICs) were read after 48 h of incubation at 35°C, by the methods of the International Collaborative Study on Susceptibility Testing (3).

RESULTS

More than 95% of strains from all three species were susceptible to minocycline, amikacin, sulfisoxazole, and trimethoprim-sulfamethox-

TABLE 1. Proposed schema for species identification of *Nocardia* based on antimicrobial susceptibilities

Antimicrobial agent	Susceptibility of ^a :		
	<i>N. asteroides</i>	<i>N. brasiliensis</i>	<i>N. caviae</i>
Kanamycin	+/-	-	+/-
Gentamicin	+/-	+	+/-
Cefamandole	+	-	-
Carbenicillin	+/-	+	-

^a +, 90% or greater incidence of susceptibility; -, 10% or less chance of susceptibility; +/-, variable response pattern.

azole. Of the *N. asteroides* isolates only 5 of 49 (10%) were susceptible to ampicillin, and 8 of 53 (15%) were susceptible to erythromycin. Among 30 isolates of *N. brasiliensis*, 3 and 0% were susceptible to ampicillin and erythromycin, respectively. Of the eight *N. caviae* strains, none were ampicillin susceptible, and one was erythromycin susceptible.

Cefamandole. Of the 43 *N. asteroides* strains which were tested by agar dilution, 58 and 90% were inhibited by 6.3 and 12.5 µg/ml, respectively (Fig. 1). Seventy isolates were tested by disk susceptibility. Three isolates had no zones of inhibition, and another two rapidly growing isolates had zones of 18 and 20 mm at 24 h but, by 48 h, had numerous fine colonies which grew up to the disk. If this fine haze was ignored, 96% of the *N. asteroides* isolates had disk zone diameters of ≥16 mm. In contrast, 0% of the *N. caviae* or *N. brasiliensis* isolates showed any zone of inhibition around the disk.

Carbenicillin. All 29 *N. brasiliensis* isolates had zone diameters of ≥16 mm, and all but one had an MIC of ≤100 µg/ml (Fig. 2). In contrast,

FIG. 1. Susceptibility of 76 isolates of *Nocardia* to cefamandole by disk diffusion and agar dilution. Δ , *N. caviae* (8 strains); $\bullet$, *N. brasiliensis* (25 strains); $\circ$, *N. asteroides* (43 strains).

only 41 and 0% of the *N. asteroides* and *N. caviae* isolates, respectively, were susceptible to the same MIC.

Aminoglycosides. The isolates of *N. brasiliensis* and *N. caviae* were much more susceptible to gentamicin than were the strains of *N. asteroides*. Of the 50 *N. asteroides* strains tested, only 30% were susceptible, and 36% had no zones to the disk. In contrast, 100 and 88% of the *N. brasiliensis* and *N. caviae* isolates, respectively, were susceptible to gentamicin. All of the *N. brasiliensis* and three of six of the *N. caviae* isolates also had zones of 25 mm or greater by disk susceptibility testing with tobramycin.

For kanamycin, 75% of the *N. caviae* strains were susceptible, with mean zone diameters of 31 mm and a median MIC of 0.78 µg/ml (Fig. 3). Only 31% of the *N. asteroides* strains were susceptible by disk testing, and almost 50% of the *N. asteroides* strains (29 of 62) had no zones of inhibition. Only one isolate (3%) of *N. brasiliensis* was susceptible.

The MICs for *E. coli* ATCC 25922 were within one dilution of the published MICs (4), except for kanamycin, which was consistently two dilutions higher.

DISCUSSION

The differences in susceptibility to kanamycin among *N. caviae*, *N. brasiliensis*, and *N. aster-*

FIG. 2. Susceptibility of *Nocardia* spp. to carbenicillin by disk diffusion and agar dilution. None of the *N. caviae* isolates (Δ ; 8 strains) had zone diameters to the disk, whereas all the *N. brasiliensis* isolates ($\bullet$; 24 strains) had zones of 16 mm or greater. Isolates of *N. asteroides* ($\circ$; 51 strains) showed much more variable susceptibility.

FIG. 3. Susceptibility of *Nocardia* spp. other than *N. asteroides* to kanamycin. Of the *N. caviae* isolates, 75% were susceptible, compared with only 3% of the *N. brasiliensis* isolates. △, *N. caviae* (8 strains); ●, *N. brasiliensis* (24 strains); □, *N. brasiliensis* not available for MIC determinations (6 strains).

oides have been noted previously (2, 5). Goodfellow and Orchard (5) felt that the current tests used for taxonomic separation of the species were not totally satisfactory, and that differences in antimicrobial susceptibilities among the species such as those for kanamycin could be used taxonomically. Unfortunately, carbenicillin and cefamandole were either not available or were not studied at that time.

The current study suggests that susceptibility testing in which commercial disks of cefamandole, carbenicillin, and gentamicin, in addition to kanamycin, are used will provide presumptive species identification once the organism has been identified as a *Nocardia* sp. and is grown in broth. An advantage is that the test takes only 48 h, whereas the hydrolysis plates require up to 4 weeks before they can be interpreted as negative (6). The exact size of the inhibitory zone present around each disk does not appear to be critical when the test is used for taxonomic purposes. "Resistant" strains would be defined as those which had no zones, and "susceptible" strains as those which exhibited any zone of inhibition. Organisms with a zone of inhibition to cefamandole are identified as *N. asteroides*, those which show a zone of inhibition to carbenicillin and gentamicin but not to cefamandole or kanamycin are identified as *N. brasiliensis*,

and organisms which show no zone of inhibition to cefamandole or carbenicillin are identified as *N. caviae* (Table 1). Final species identification, however, should include the use of standard hydrolysis plates.

The activity of cefamandole against *N. asteroides* suggests that it may have some potential in the therapy of infections due to this species, especially when parenteral antimicrobial agents other than sulfonamides are to be used. Only minocycline and amikacin are active against >90% of strains (1, 8), and both of these drugs have potential toxicities. Cefamandole is unlikely, however, to be useful in treating diseases of the central nervous system.

The need for routine susceptibility testing of *Nocardia* spp. has not been established. However, the usual severity of the disease, its high morbidity with dissemination or central nervous system involvement, and the possibility of sulfonamide intolerance suggest that at least disk susceptibilities (8, 9) should be performed. With the inclusion of cefamandole and carbenicillin disks, presumptive species identification will be provided at the same time as susceptibility results.

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