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In Vitro Susceptibility of *Nocardia asteroides* to 25 Antimicrobial Agents

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Fifty-two clinical isolates of *Nocardia asteroides* were tested by agar dilution for their susceptibility to 25 antimicrobial agents. In general, susceptibility could not be predicted based on the antibiotic class tested. However, the beta-lactams, including third-generation cephalosporins, were generally ineffective (MIC for 90% of the organisms [MIC_{90}], between 64 and >256 μg/ml), whereas minocycline and doxycycline were generally effective (MIC_{90}, 4 and 8 μg/ml, respectively). Cycloserine was not effective below 60 μg/ml. The MIC_{50} and MIC_{90} of sulfamethoxazole was 16 and 32 μg/ml, respectively, and that of trimethoprim varied widely (16 and >256 μg/ml, respectively). Based on MIC_{90} data, only doxycycline, minocycline, sulfamethoxazole, and imipenem could be applied empirically.

Nocardia infections are often treated with sulfonamides or trimethoprim-sulfamethoxazole (14, 15). Reports of treatment failures with these regimens, especially in disseminated or central nervous system infections (15), emphasize the need for alternative therapies. In the past, various therapeutic agents have been suggested, but variation in susceptibility testing procedures and as-yet-undefined factors have led to conflicting results (1, 5, 12, 17). Recent in vitro studies performed with a standardized testing procedure have indicated that ampicillin (5), amikacin (7), cefamandole (17), minocycline (1), and imipenem (6, 11) may be effective in the treatment of nocardiosis. However, conflicting data on ampicillin (5, 17) and general incompatability of aminoglycosides with long-term therapy demonstrated the need for further investigation. The purpose of this study was to test the susceptibility of *Nocardia asteroides* to antimicrobial agents which may more closely fit the clinical requirements for therapy of nocardial disease.

MATERIALS AND METHODS

Organisms. Fifty-two human isolates of *N. asteroides* were obtained from the University of California at Davis, the Santa Clara Valley Institute of Medical Research, Stanford University, the National Animal Disease Center (Ames, Iowa), and the University of Utah. Isolates were obtained from a variety of *Nocardia* infections, including pulmonary nocardiosis, soft tissue infection, disseminated disease in organ transplant patients, and central nervous system disease.

Isolates were identified by colonial morphology, staining characteristics, and the ability of each organism to hydrolyze xanthine, casein, or tyrosine according to the *Manual of Clinical Microbiology* (10).

Antimicrobial agents. The following reference antibiotic powders were either purchased or supplied by the manufacturers: cefmenoxime and cephapirin (Abbott Laboratories, North Chicago, Ill.); ampicillin and ceforanide (Bristol Laboratories, Syracuse, N.Y.); cefamandole and cephalothin (Eli Lilly & Co., Indianapolis, Ind.); ceftazidime and cefuroxime (Glaxo Group Research Ltd., Greenford, Middlesex, England); cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.); imipenem (Merck Sharpe & Dohme Research Lab, Rahway, N.J.); cefoperazone (Pfizer, Inc., New York, N.Y.); *p*-aminosalicylic acid, cycloserine, doxycycline, 5-fluorocytosine, isoniazid, minocycline, rifampin, streptomycin, sulfamethoxazole, and trimethoprim (Sigma Chemical Co., St. Louis, Mo.); cefonicid and ceftizoxime (Smith Kline & French Laboratories, Philadelphia, Pa.); and erythromycin (Westwood Pharmaceuticals, Westwood, N.Y.). The antimicrobial agents were incorporated into Mueller-Hinton (MH) agar immediately after dilution and were used within 5 days.

Susceptibility testing. Isolates to be tested were stored in glycerol at −20°C or in sterile milk at −70°C and then passed onto Saboraud dextrose agar before testing. The inoculum was prepared by inoculating brain heart infusion (BHI) broth from the Saboraud medium and incubating it at 35°C for 4 to 5 days. To increase the homogeneity of the organism suspensions, sterile glass beads (1.0 mm in diameter) were added to the culture tubes and cultures were mixed with a vortex mixer twice daily during incubation.

Agar dilution susceptibility was performed with twofold dilutions of the antibiotics according to the recommendations of the International Collaborative Study on susceptibility testing (9). The turbidity of the inoculum was adjusted to equal a 0.5 McFarland standard, and the media were inoculated with a Steers replicator. This procedure produced an average inoculum size of 3×10^4 CFU. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were also included as controls. The inoculated plates were incubated at 35°C for 48 h and then examined for growth to determine the MIC. The MIC was defined as the lowest concentration of antimicrobial agent resulting in complete inhibition (sulfonamides, 80% inhibition) of visible growth.

RESULTS

A comparison of strain growth in different broth media was made. Although all the isolates grew in BHI and tryptic soy broths, growth was sustained longer and more nearly homogeneous suspensions were obtained in BHI broth. Two of the isolates did not grow in MH broth, and among those that did grow, cellular aggregation was marked.

For *N. asteroides*, the range of MICs, the MIC for 50% of

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TABLE 1. In vitro susceptibility of *N. asteroides* to 25 antimicrobial agents

Class	Antimicrobial agent	MIC range	MIC_{50}	MIC_{90}
Beta-lactam	Ampicillin	<0.125–256	32	128
	Ticarcillin	4–>256	>256	>256
	Imipenem	<0.125–16	0.5	8
	Cephalothin	2–>256	64	>256
	Cephapirin	0.125–256	32	64
	Cefamandole	0.25–256	16	256
	Cefuroxime	0.25–>256	8	256
	Cefonicid	0.5–>256	128	>256
	Cefmenoxime	0.5–>256	8	>256
	Ceftizoxime	1–>256	64	>256
	Cefoperazone	2–>256	128	>256
	Ceforanide	1–>256	128	>256
	Cefotaxime	<0.125–>256	4	256
	Ceftazidime	4–256	256	256
Anti-tuberculosis	Cycloserine	64–>256	>256	>256
	Isoniazid	8–>256	>256	>256
	p-Aminosalicylic acid	>256	256	>256
	Rifampin	0.5–>256	128	>256
	Streptomycin	0.25–128	32	128
Tetracycline	Doxycycline	<0.125–8	2	8
	Minocycline	<0.125–4	1	4
Sulfonamide	Sulfamethoxazole	0.5–32	16	32
	Trimethoprim	2–>256	64	>256
Miscellaneous	Erythromycin	<0.125–>256	32	>256
	5-Fluorocytosine	32–>256	>256	>256

the organisms (MIC_{50}), and the MIC_{90} for each compound are reported in Table 1. Of the antimicrobial agents included in this study which are commonly used in therapy of mycobacterial infections, streptomycin was the most active, inhibiting 48 and 67% of the isolates at 16 and 32 μg/ml, respectively. Cycloserine inhibited only 24 strains at 128 μg/ml, and none of the strains at 32 μg/ml. Rifampin inhibited only 10 strains (19%) at 8 μg/ml. The least active of these compounds were isoniazid and *p*-aminosalicylic acid.

Two penicillins, 11 cephalosporins, and the carbapenem antibiotic imipenem were tested. In general, the two penicillins, ampicillin and ticarcillin, showed poor in vitro activities. Ampicillin inhibited only 38% of the strains at 8 μg/ml, whereas ticarcillin showed poor activity even at high concentrations. Susceptibility to the 11 cephalosporins varied greatly but none produced acceptably low MIC_{90} values. Imipenem was the most active of the beta-lactam group (Table 1), inhibiting 41 (79%), 50 (96%), and 52 (100%) of the isolates at 4, 8, and 16 μg/ml, respectively.

Two of the newer tetracyclines with previously reported activity (1, 13, 14) against *Nocardia* were tested. Minocycline was the most active, with an MIC of 4 μg/ml, whereas doxycycline inhibited 42 (81%) of the isolates at 2 μg/ml and had an MIC_{90} of 8 μg/ml.

Of the other antimicrobial agents tested, sulfamethoxazole was the most active, inhibiting all 52 strains tested at a concentration of 32 μg/ml. Erythromycin inhibited 19 strains (38%) at 4 μg/ml. All strains were resistant to 5-fluorocytosine and to trimethoprim when used alone. Data from control organisms were within the recommendations of the National Committee for Clinical Laboratory Standards or the manufacturer.

DISCUSSION

Specific trends in *Nocardia* antimicrobial agent susceptibility are beginning to emerge as testing methodologies are standardized. Although good results have been reported in microtiter dilution susceptibility testing (M. S. Bartlett, J. K. Reynolds, S. D. Allen, and J. W. Smith, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 21st, Chicago, Ill., abstr. no. 95, 1981), varying growth capabilities and the hydrophobic nature of *N. asteroides* in MH broth tend to reduce the success of this method. We consistently obtained reproducible results by using the agar dilution technique and recommend this relatively standardized (5) approach to in vitro susceptibility testing.

An inoculum effect has been shown to influence the susceptibility patterns of *N. asteroides* (14). Beaman and Bourgeois (2) suggested that this variable may be reduced by selecting an appropriate growth medium. In our laboratory, BHI broth produced more nearly homogeneous suspensions of bacteria and allowed consistently reproducible quantitation. Some strains of *N. asteroides* (two in our collection) did not grow on MH agar but grew well on this medium supplemented with 5% defibrinated sheep blood. The effect of such supplementation on antibiotic susceptibility patterns of *N. asteroides* has not been evaluated. Variation in growth characteristics may also account for some of the discrepancies in *Nocardia* susceptibilities reported in the literature.

In 50 reported cases of nocardiosis reviewed by Smego et al. (15), the median time of therapy in patients who either recovered or improved was 15 weeks ($\bar{x}$ = 23.5, s = 19.2). Most treatment failures have been reported in disseminated disease (20%) or central nervous system infection (40%). Frequent relapses after discontinuation of therapy also have been reported, and prophylaxis has been reported to exceed an 8-year period. Therefore, characteristics of the alternatives to the presently recommended sulfonamide or trimethoprim-sulfamethoxazole therapy should include a high therapeutic index, central nervous system penetration, high extravascular drug levels, low toxicity, dose stability, and, if possible, low cost.

Both doxycycline and minocycline appear to fit most of the above criteria. Although minocycline cerebrospinal fluid levels are only about 10% of the peak serum levels, even at levels as low as 1 μg/ml 85% of the *N. asteroides* we tested were inhibited. The low MICs (Table 1) of imipenem support its potential usefulness in nocardiosis therapy as previously suggested.

We were unable to find reports of nocardiosis being treated by doxycycline. However, our data (Table 1) showed doxycycline to be 81% as effective as minocycline at their respective achievable serum levels. Therefore, based on susceptibility data, the use of doxycycline may be indicated in pleuropulmonary, soft tissue, and disseminated nocardiosis in patients with renal dysfunction and may result in savings to the patient on long-term therapy where sulfonamide intolerance exists.

Variable beta-lactamase production by *Nocardia* (17) may partially explain the variability in ampicillin susceptibility patterns. Of the other beta-lactam antibiotics tested, only cefmenoxime, cefotaxime, and cefuroxime showed activity at achievable drug levels. These agents may be appropriate candidates for routine susceptibility testing of *Nocardia* isolates. Gutmann et al. (11) recently demonstrated therapeutic success in six cases of nocardiosis treated with a cefuroxime-amikacin combination. There appeared to be no uniform in vitro susceptibility to other cephalosporins.

Tsukamura (16) indicated the possibility of the antineoplastic agent 5-fluorouracil providing some prophylaxis against *N. asteroides* in patients undergoing chemotherapy with 5-fluorouracil; however, our data indicate that *N. asteroides* is not susceptible to 5-fluorouracil.

Unfortunately, there are numerous reports of the use of cycloserine in *N. asteroides* infections. However, because of toxicity, cycloserine serum levels should be kept at <30 μg/ml (13), and according to our data this would preclude any inhibition of *N. asteroides*. Also, D-cycloserine has been used to obtain cell wall-deficient forms of *Nocardia* in vitro (2–4), and since L forms may be an important part of pathogenesis due to *Nocardia* (4, 8), drugs which may induce them should be approached with some caution.

The broad range of MICs shown in Table 1 suggest that nocardial antibiotic susceptibility is related to the isolate. Such data and the difficulty encountered in developing uniform growth of *Nocardia* emphasize the need for careful evaluation of in vitro susceptibility data. Routine susceptibility testing of all *N. asteroides* isolates is recommended when a primary compound with emperic efficacy cannot be used.

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