In Vitro Susceptibility of Nocardia asteroides to Amikacin

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By drug dilution tests in agar, it has been shown that each of 27 strains of Nocardia asteroides was inhibited by 1 μg or less of amikacin per ml of medium.

Human infection with Nocardia species is not uncommon; 500 to 1000 cases are recognized in the United States each year (2). N. asteroides accounts for the majority of these systemic infections (2). The sulfonamides have been the accepted chemotherapy for Nocardia infections, but alternatives are necessary for patients who are allergic to the sulfonamides or in those instances in which sulfonamide treatment fails. We, therefore, decided to test the susceptibility of N. asteroides, an acid-fast organism, to amikacin, because in an earlier study we had demonstrated that 18 strains of Mycobacterium fortuitum and 13 strains of M. chelonei, also acid-fast organisms, were susceptible to amikacin (J. R. Dalovisio and G. A. Pankey, J. Infect. Dis., in press).

We obtained 27 strains of N. asteroides on agar slants from stock cultures of Donald Schneidau (Division of Mycology, Tulane University School of Medicine, New Orleans, La.). The method for preparation of homogeneous suspensions of Nocardia was adapted from that of Bach et al (1). A loopful of Nocardia taken from the agar slant was transferred into a screw-cap tube containing 4 ml of Mueller-Hinton broth (Difco, Detroit, Mich.) and a sterile glass bead. This was incubated on a rotary shaker at 37°C. After 24 h of incubation, the tubes were shaken for 5 min on a Vortex mixer (Lab-Line Instruments, Melrose Park, Ill.). After 48 h of incubation, 1 ml of this microbial suspension was transferred to another screw-top tube containing 4 ml of Mueller-Hinton broth and a sterile glass bead. This was incubated at 37°C for 4 days with intermittent agitation for 5 min every 24 h on the Vortex mixer.

At the end of this period of incubation the cultures yielded homogeneous suspensions of N. asteroides with varying degrees of turbidity. The cultures showing marked turbidity were diluted 1:100, and those showing moderate turbidity were diluted 1:10 in Mueller-Hinton broth. Cultures showing no turbidity were used undiluted in the wells of a Steers inocula replicator (Melrose Machine Shop, Woodlyn, Pa.).

Susceptibility testing was performed with a Steers inocula replicator on serial twofold dilutions of amikacin (from 0.25 to 64.0 μg/ml) in Mueller-Hinton agar (BBL, Div. of Beckton-Dickinson & Co., Cockeysville, Md.) (5). A plate containing no antibiotic was also inoculated. The minimal inhibitory concentration (MIC) was considered to be the lowest concentration of amikacin that produced no growth visible under a hand lens after 48 h of incubation at 37°C. Suspensions of American Type Culture Collection (ATCC) strains of Staphylococcus aureus 25923, Escherichia coli 25922, and Pseudomonas aeruginosa 27853 at a 1:20 dilution of a 1/2 McFarland no. 1 nephelometer standard were used as controls. Normal agar dilution control values for the amikacin MICs of these three strains were 1 μg/ml, 2 μg/ml, and 2 μg/ml, respectively. These values were obtained from Bristol Laboratories, Syracuse, N.Y. To provide control of the inoculum strength of the Nocardia suspension, serial dilutions of these suspensions were made and plated with a 0.001-ml calibrated wire loop onto blood agar plates. Only those suspensions yielding 10^7 to 10^8 colony-forming units per ml were included in these data.

These organisms were also tested by a modified Bauer-Kirby disk diffusion technique. The suspension of organisms adjusted to 10^7 to 10^8 CFU/ml was swabbed with a cotton-tipped swab onto a petri dish of Mueller-Hinton agar of 5 mm depth. Amikacin disks of 10-μg strength (BBL) were placed on the agar surface, and plates were incubated at 37°C. The diameter of the zone of inhibition was measured after 48 h.

Amikacin showed good activity against all 27 strains of N. asteroides tested (Fig. 1): 44% were inhibited by concentrations ≤0.25 μg/ml, and 100% of strains tested were inhibited by 1
It has been suggested that, in certain instances, there is poor correlation between in vitro susceptibility tests and the in vivo response of *Nocardia* infection to antimicrobial agents (3). Although our in vitro data suggest that amikacin may be useful in the treatment of *N. asteroides* infections, in vivo confirmation by animal studies is essential before amikacin should be considered for treatment of human infections with *N. asteroides*.

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**LITERATURE CITED**


