

In Vitro Activities of Linezolid against Multiple *Nocardia* Species

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Linezolid was tested by broth microdilution against 140 clinical *Nocardia* isolates belonging to seven species. The MIC at which 50% of the strains are inhibited (MIC₅₀) and MIC₉₀ for all species other than *Nocardia farcinica* were 2 and 4 µg/ml. Linezolid is the first antimicrobial agent demonstrated to be active against all *Nocardia* species.

Treatment of *Nocardia* infections continues to be difficult, especially with central nervous system or disseminated disease (1, 6, 7, 9, 10) and species, such as *Nocardia farcinica*, that are highly drug resistant (22). Most recent antimicrobial therapy of complicated cases has involved the use of a sulfonamide or trimethoprim-sulfamethoxazole plus the injectable agents amikacin and imipenem or ceftriaxone (6, 10, 16, 18). The recent advent of multiple new drug classes with activity against gram-positive bacteria offers the potential for new drugs useful against *Nocardia* (3, 5, 11; M. C. Birmingham, G. S. Zimmer, B. Hafkin, W. H. Todd, T. Leach, D. H. Batts, S. M. Flavin, C. R. Rayner, K. E. Welch, P. F. Smith, J. D. Root, N. E. Wilks, and J. J. Schentag, 39th Intersci. Conf. Antimicrob. Agents Chemother., poster 1098, 1999; D. J. Stalker, C. P. Wajszczuk, and D. H. Batts, 5th Int. Conf. Macrolides, Azalides, Streptogramins, Ketolides, Oxazolidinones, poster 08-21, 2000; M. Wu, P. Aralor, K. Nash, L. E. Bermudez, C. B. Inderlied, and L. S. Young, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-143, 1998.). We studied the new oxazolidinone compound linezolid against all clinically important species of *Nocardia* including drug-resistant species, such as *N. farcinica* and *Nocardia transvalensis* (22, 23).

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Linezolid is a new class of synthetic antibiotics which prevent protein synthesis by blocking the formation of a function initiation complex (9, 11). The exact mechanism of action is unique, and no cross-resistance has been discovered in strains of bacteria resistant to other antimicrobial agents (11).

We tested 192 clinical *Nocardia* isolates submitted for susceptibility testing from January 1999 through January 2000 to the Mycobacteria/Nocardia Research Laboratory at The University of Texas Health Center for their susceptibility to linezolid. Isolates from 27 states and Mexico were tested, with 60% of the isolates from Texas, Florida, North Carolina, Ohio, Massachusetts, and Connecticut. Approximately 40% of the

organisms were identified to the species level by PCR restriction analysis of the 439-bp Telenti segment of the 65-kDa *hsp* gene (13–16, 23), and all were identified by their patterns of susceptibility (16, 17, 20–23) to approximately 15 other drugs, including aminoglycosides, beta lactams, and quinolones. The test isolates belonged to seven species (eight taxa) and included *Nocardia asteroides* sensu stricto (*n*, 34), *N. farcinica* (*n*, 25), *Nocardia brasiliensis* (*n*, 24), *Nocardia nova* (*n*, 41), *Nocardia pseudobrasiliensis* (*n*, 8), *Nocardia otitidiscaviarum* (*n*, 5), *N. transvalensis* complex (*n*, 7), and *Nocardia* sp. (*n*, 2).

Susceptibility testing utilized three methods. The first was serial twofold broth microdilution in cation-supplemented Mueller-Hinton broth as previously described and recently approved by the NCCLS (4, 24). The second was the E-test (2) (generously supplied by Pharmacia and Upjohn, Inc., and AB Biodisks) performed on selected isolates on Mueller-Hinton agar using a 1-McFarland standard inoculum. The third method was agar disk diffusion (20, 21) performed with Mueller-Hinton agar and 30-µg linezolid disks generously supplied by the manufacturer, Pharmacia and Upjohn, Inc. Susceptibilities to linezolid by all three methods were read after incubation at 35°C in room air for 3 days. Two endpoints were utilized: complete (100%) inhibition of visible growth (broth microdilution and agar disk diffusion) and 80% inhibition of growth (E-test) compared to the growth of controls.

Quality control for agar disk diffusion, the E-test, and broth microdilution was performed with *Staphylococcus aureus* ATCC 29213. The values were within the acceptable range of inhibition for this strain (MIC, 1 to 4 µg/ml).

The results are shown in Table 1 for the 140 isolates tested by broth microdilution. The isolates generally gave sharp endpoints that were easy to read. Each species had a narrow unimodal distribution of MICs, with >90% of the values falling within 1 dilution of the mode.

Twenty random isolates were tested by the E-test. The zones of inhibition were generally sharp, with a trailing endpoint that was present for only 1 to 2 dilutions. The MICs for 12 of 14 (86%) isolates belonging to five species, *N. nova* (3 of 3), *N. asteroides* complex (2 of 2), *N. brasiliensis* (4 of 5), *N. transvalensis* complex (2 of 3), and *N. pseudobrasiliensis* (1 of 1), were ≤1 µg/ml, and those for 8 of 14 (57%) isolates were ≤0.5

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TABLE 1. Susceptibilities of 140 *Nocardia* isolates to linezolid determined by broth microdilution

Organism	No. tested	MIC ($\mu\text{g/ml}$) ^a			
		Range	MIC ₅₀	MIC ₉₀	Mode
<i>N. asteroides</i> complex sensu stricto	33	1-4	2	4	2
<i>N. farcinica</i>	25	1-8	4	4	4
<i>N. nova</i>	40	0.5-2	2	2	2
<i>N. brasiliensis</i>	23	1-8	2	4	2
<i>N. pseudobrasiliensis</i>	7	1-2	2	2	2
<i>N. transvalensis</i> complex	6	2-4	2	4	2
<i>N. otitidiscaviarum</i>	4	2-4	4	4	4
<i>Nocardia</i> sp.	2	4	4	4	4
Range (total)	(140)	≤0.25-8	1-4	1-4	1-4

^a MIC₅₀, concentration that inhibits 50% of isolates. MIC₉₀, concentration that inhibits 90% of isolates.

$\mu\text{g/ml}$. These MICs were 4- to 16-fold lower than the MICs determined in broth. The E-test MICs for *N. farcinica* were higher and ranged from 1.5 to 3 $\mu\text{g/ml}$ (6 of 6), values which were two- to fourfold lower than the broth values.

Additionally, 192 consecutive isolates were tested by agar disk diffusion. These included 140 isolates for which MICs were determined by broth microdilution and 52 random isolates for which MICs were not determined by broth microdilution. As with the E-test on agar, the zones of inhibition were fairly sharp and trailed over only a 5- to 10-mm range. Of the 158 isolates from species other than *N. farcinica*, 154 (97.5%) had zones of complete (100%) inhibition ≥ 36 mm in diameter, and 71 (45%) had zones > 45 mm in diameter. This was in comparison to only 21 of 34 (62%) and 2 of 34 (6%) isolates of *N. farcinica*, respectively, with similar zones.

One of the major limitations of the treatment of nocardiosis has been the absence of oral antimicrobials which are active against all *Nocardia* species (21). The sulfonamides or trimethoprim-sulfamethoxazole is active against most clinical isolates (21), but patients with central nervous system disease and severe disease do poorly with sulfonamide therapy only (7), and hypersensitivity reactions to sulfonamides are common. Other agents active against most strains and species are injectable agents, including amikacin, imipenem, and ceftriaxone (6, 7, 9, 10, 12, 16-18, 21). These agents work well alone or in combination with a sulfonamide but are limited by the need for intravenous administration, high cost, and significant toxicity. These are major limitations for pulmonary or central nervous system nocardiosis, for which the usual duration of therapy is 6 to 12 months. Amoxicillin-clavulanic acid, fluorinated quinolones, minocycline, and macrolides have limited activity (21) and are active at levels achievable in serum or tissue only against select species and isolates. The mean peak level of linezolid in serum with oral doses of 600 mg twice daily is 21.2 $\mu\text{g/ml}$ (± 5.8 $\mu\text{g/ml}$ [standard deviation]) with a half-life of 5.4 h (Pharmacia and Upjohn, Inc., package insert for Zyvox, 2000). Linezolid is the first antimicrobial to be active against all clinically significant species of the genus *Nocardia*. Its clinical activity remains to be determined, although its use as a compassionate agent is ongoing. Because of its activity and availability as an oral agent and the current limitations of the

sulfonamides, linezolid has the potential to be the primary drug for treatment of *Nocardia* disease. Its clinical efficacy and long-term toxicity in this setting, however, have yet to be determined.

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