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MICs of Oxazolidinones for *Rhodococcus equi* Strains Isolated from Humans and Animals

TERRY L. BOWERSOCK,[1]* SARAH A. SALMON,[1] ELLEN S. PORTIS,[1] JOHN F. PRESCOTT,[2] DENISE A. ROBISON,[3] CHARLES W. FORD,[4] AND JEFFREY L. WATTS[1]

Animal Health Therapeutics Research,[1] and Infectious Diseases Research,[4] Pharmacia & Upjohn, Kalamazoo, Michigan 49001; Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1[2]; and University Hospitals, Oklahoma City, Oklahoma 73126[3]

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Eperezolid and linezolid are representatives of a new class of orally active, synthetic antimicrobial agents. The in vitro activity values (MICs) of linezolid, eperezolid, and comparator antibiotics against 102 strains of *Rhodococcus equi* isolated from humans and animals were determined. Linezolid was more active than eperezolid against the strains tested; premafloxacin was the most active comparator antibiotic.

Rhodococcus equi is found in soil and carried in the intestinal tracts of horses. It is a facultative intracellular pathogen that resists phagocytosis as well as intracellular killing by macrophages. It causes an insidious, progressive chronic suppurative bronchopneumonia with abscessation in foals. It is one of the most important diseases in foals less than 6 months of age. It was first reported to cause disease in horses in the 1920s and in humans in the 1960s (1). *R. equi* is an opportunistic pathogen contracted primarily by inhalation of dust. The majority of human cases occur in immunocompromised individuals, especially those infected with the human immunodeficiency virus. Despite antibiotic therapy for patients with AIDS, frequent relapses occur during the course of the disease (2, 4).

Erythromycin and rifampin (initially) therapy for 4 to 9 weeks has become the treatment of choice for foals (5, 6). However, due to cost, clinicians and owners of affected animals are interested in a less costly therapy, and if possible monotherapy, to treat foals. There are limited antimicrobial agents approved for use with animals to treat bacterial infections. Antimicrobial agents approved to treat respiratory infections in livestock are often used in horses. Currently, antimicrobial agents used to treat respiratory disease in livestock include enrofloxacin, sarafloxacin, danofloxacin, ceftiofur, tetracycline, florfenicol, and tilmicosin. Premafloxacin, an extended-spectrum fluoroquinolone, has previously been shown to have superior in vitro activity against gram-positive cocci compared with other fluoroquinolones (14).

Eperezolid and linezolid are representatives of a new class of orally active, synthetic antimicrobial agents, the oxazolidinones. The oxazolidinones are most active against gram-positive organisms including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* spp., and *Streptococcus* spp. The in vitro activity of these compounds against a variety of bacterial isolates from humans as well as animals has been well documented (5–8, 13, 15; S. A. Salmon, J. L. Watts, C. A. Case, and C. W. Ford, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. A-2, p. 38, 1998; J. L. Watts, S. A. Salmon, R. J. Yancey, Jr., and C. W. Ford, Abstr. 35th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F220, p. 151, 1995). However, there are limited data on the in vitro activity of antimicrobial agents against *R. equi*, including no data on the activity of oxazolidinones against *R. equi*. The objective of this study was to determine the MICs of linezolid, eperezolid, premafloxacin, and several comparator antimicrobial agents against strains of *R. equi* isolated from humans and animals.

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R. equi strains used in this study were from the Pharmacia & Upjohn Animal Health Discovery Research culture collection (Kalamazoo, Mich.). All strains used in this study were identified as the primary cause of infection in the patients. Identification was confirmed by Gram stain reaction, microscopic and colonial morphology, growth characteristics, source of specimen, and a synergistic hemolysis test using *Corynebacterium pseudotuberculosis*, according to protocols described previously (12). In some cases, biochemical profiles using the API Rapid CORYNE test (bioMerieux Vitek, Inc., Hazelwood, Mo.) and cellular fatty acid analysis using the Microbial Identification System (MIDI, Inc., Newark, Del.) were used to confirm isolate identification.

Thirty-six strains were obtained from human sources, and 66 were obtained from equine sources. In addition to the test strains, the following National Committee for Clinical Laboratory Standards (10) recommended quality control strains were also tested: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. All bacterial isolates were stored in 1.0 ml of Trypticase soy broth (Difco, Detroit, Mich.) supplemented with 10% glycerol at −70°C until tested. Isolates were revived onto freshly prepared blood agar base supplemented with 5% sheep blood. Plates were streaked for isolation and incubated at 37°C in 5% CO_2 for 18 to 24 h. The isolates grown in this manner were then used as the inoculum for MIC determination.

The following antimicrobial agents were tested: eperezolid, linezolid, and premafloxacin (Pharmacia & Upjohn); enrofloxacin (Bayer Animal Health, Shawnee Mission, Kans.); sarafloxacin (Abbott Laboratories, North Chicago, Ill.); danofloxacin (Pfizer Animal Health, Groton, Conn.); ceftiofur (Pharmacia & Upjohn); tetracycline (Sigma Chemical Company, St. Louis, Mo.); florfenicol (Schering-Plough Animal Health, Kenilworth, N.J.); and tilmicosin (Eli Lilly and Com-

* Corresponding author. Mailing address: Animal Health Therapeutics Research, Pharmacia & Upjohn, 7923-190-353, Kalamazoo, MI 49001-0199. Phone: (616) 833-2614. Fax: (616) 833-2855. E-mail: Terry.L.Bowersock@am.pnu.com.

TABLE 1. MICs of eperezolid, linezolid, and comparator antibiotics against 103 strains of *R. equi*

Antimicrobial agent	MIC (μg/ml)		
	MIC_{50}	MIC_{90}	Range
Eperezolid	16.0	16.0	4.0–16.0
Linezolid	2.0	2.0	0.5–2.0
Premafloxacin	0.13	0.13	≤0.0078–0.25
Enrofloxacin	1.0	1.0	≤0.03–2.0
Sarafloxacin	1.0	2.0	≤0.06–8.0
Danofloxacin	1.0	1.0	≤0.03–4.0
Ceftiofur	0.5	8.0	≤0.03–>32.0
Tetracycline	4.0	8.0	0.25–8.0
Florfenicol	16.0	32.0	2.0–>32.0
Tilmicosin	16.0	>32.0	2.0–>32.0

pany Animal Health, Greenfield, Ind.). The two oxazolidinone antimicrobial agents, eperezolid and linezolid, were tested using manually prepared, frozen panels. Microdilution panels containing the oxazolidinones were stored at −20°C until inoculated. All other antimicrobial agents were tested using a commercially prepared, dehydrated panel (Sensititre/TREK Diagnostics, Westlake, Ohio). MICs were determined using the National Committee for Clinical Laboratory Standards broth microdilution method as previously described (11). No special handling or growth conditions were needed for testing these isolates. Plates were incubated at 35°C overnight without CO_2. Inoculum was made using Mueller-Hinton broth. An inoculum of 50 μl of 10^3 CFU was used for each well. A test was considered valid only if there was adequate growth in growth control wells.

A summary of the MIC data is presented in Table 1. Linezolid (MIC at which 90% of the isolates tested are inhibited [MIC_{90}] = 2.0 μg/ml) was more active against the *R. equi* strains than was eperezolid (MIC_{90} = 16.0 μg/ml). This split in activity was in contrast to previously reported data for these drugs against *Corynebacterium jeikeium* (7) that included MIC_{90}s of eperezolid and linezolid of 0.25 and 2.0 μg/ml, respectively. In another study, both oxazolidinones were active at similar levels against *Corynebacterium* spp. with MIC_{90}s of 0.5 μg/ml (15). Eperezolid was slightly less active than linezolid against *Listeria monocytogenes*, for which the MIC_{90}s were 8.0 and 2.0 μg/ml, respectively (15). These differences in susceptibility most likely reflect subtle chemical differences in activity against different species of these unusual bacteria. In no way should these trends overshadow the overall excellent efficacy of both molecules against most species of staphylococci, streptococci, and enterococci.

Four fluoroquinolones were included in the comparator group of antimicrobial agents. Of these, premafloxacin was three to four dilutions more active (MIC_{90} = 0.13 μg/ml) than were enrofloxacin, sarafloxacin, and danofloxacin (MIC_{90}s = 1.0, 2.0, and 1.0 μg/ml), respectively. These data are similar to previously reported data in which enrofloxacin and ciprofloxacin, against four strains of *R. equi*, had MICs of 2.0 and 1.0 μg/ml, respectively (5). Significant resistance to ciprofloxacin has been reported previously (4). The newer fluoroquinolones such as premafloxacin have much better activity against gram-positive organisms (14) and appear to be more likely to be useful in treating *R. equi* infections.

Ceftiofur, an expanded-spectrum cephalosporin, exhibited strain-dependent in vitro activity against the *R. equi* strains tested with MIC_{50}s and MIC_{90}s of 0.5 and 8.0 μg/ml, respectively. These data suggest that these strains may be more susceptible than the unknown number of strains previously reported for which the MIC_{50} was 8.0 μg/ml and the MIC_{90} was 16.0 μg/ml (9). It has previously been shown that narrow- and broad-spectrum cephalosporins have variable or moderate activity against *R. equi* (3, 9). Tetracycline exhibited moderate in vitro activity against the *R. equi* strains tested (MIC_{90} = 8.0 μg/ml). These data are similar to data reported for tetracycline against isolates from human sources (MIC_{50} = 4.0 μg/ml) (9) and for oxytetracycline and doxycycline against four equine strains (MICs = >16.0 and 2.0 μg/ml, respectively) (5). Florfenicol, a chloramphenicol derivative, and tilmicosin, a macrolide, are antimicrobial agents recently approved for the treatment of bovine respiratory disease. As expected because of their spectrum of activity, florfenicol and tilmicosin exhibited limited activity against the *R. equi* strains tested (MIC_{90} = 32.0 μg/ml).

In addition to summarizing data for all of the *R. equi* strains from both human and equine sources, we also summarized data for these sources separately (data not shown). No differences in antimicrobial activity were observed with any of the antimicrobial agents against the *R. equi* strains from human and equine sources. While one of the strains isolated from humans was known to be of equine origin, no association between the human patient and equine exposure could be made for the remaining 35 strains. In conclusion, linezolid was more active than eperezolid against the *R. equi* strains tested. Despite this activity, the oxazolidinones are not being considered for development for veterinary applications due to the need in human medicine for novel antimicrobial agents with activity against antibiotic-resistant organisms including vancomycin-resistant enterococci. Premafloxacin was the most active of the antimicrobial agents tested against the *R. equi* strains from human and veterinary sources.

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