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In Vitro Susceptibilities of *Rhodococcus equi* and Other Common Equine Pathogens to Azithromycin, Clarithromycin, and 20 Other Antimicrobials

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The objective of this study was to determine in vitro activities of azithromycin (AZM), clarithromycin (CLR), and 20 other antimicrobial agents against *Rhodococcus equi* and other common equine bacterial pathogens. A total of 201 bacterial isolates from various equine clinical samples were examined. CLR was more active than AZM against *R. equi*, with MICs at which 90% of the isolates were inhibited of 0.12 and 1.0 μg/ml, respectively. Other antimicrobial agents highly active against at least 90% of *R. equi* isolates in vitro included rifampin, gentamicin, and imipenem. Both AZM and CLR showed good activity against beta-hemolytic streptococci and *Staphylococcus* spp. AZM was more active than other macrolides against *Pasteurella* spp. and *Salmonella enterica*.

Rhodococcus equi, a gram-positive facultative intracellular pathogen, is one of the most important causes of disease in foals between 3 weeks and 5 months of age. *R. equi* has also emerged as a significant opportunistic pathogen in immunosuppressed people, especially those infected with the human immunodeficiency virus (3, 7, 11). Infection in either species is most commonly characterized by life-threatening pyogranulomatous pneumonia. Although combined therapy with erythromycin (ERY) and rifampin has dramatically improved the survival rate of foals infected with *R. equi*, this treatment regimen is not without problems. ERY has variable absorption in foals when given orally and requires multiple daily dosing, and most importantly, its administration has a high incidence of potentially fatal side effects (17, 18, 23).

Azithromycin (AZM) and clarithromycin (CLR) have been proposed as alternatives to ERY for the treatment of *R. equi* infections in foals. They are more chemically stable, have a greater bioavailability, and achieve higher concentrations in phagocytic cells and tissues than ERY (25). In people, the incidence and the severity of side effects for these drugs are also considerably decreased from those for ERY (25). The pharmacokinetics of these antimicrobial agents in foals have recently been investigated (6, 14, 15). However, the paucity of in vitro susceptibility studies precludes the rational use of these antimicrobial agents for the treatment of *R. equi* infections in foals. Because *R. equi* is often isolated in combination with other bacterial pathogens and the identity of the causative microorganism(s) is often unknown when antimicrobial therapy is initiated, in vitro susceptibility data against other common bacterial pathogens of the equine respiratory tract are also essential for proper clinical management. Therefore, the objective of this study was to determine the MICs of AZM, CLR, and 20 other antimicrobial agents against *R. equi* and other common equine bacterial pathogens.

A total of 201 bacterial isolates from various equine clinical samples were examined. Most isolates were obtained from clinical samples submitted to the microbiology laboratory of the University of Florida Veterinary Medical Teaching Hospital from November 1999 to January 2001. In addition, 50 *R. equi* isolates kindly provided by John F. Prescott (University of Guelph, Guelph, Ontario, Canada), were included. All *R. equi* isolates were obtained from tracheobronchial aspirates or postmortem specimens from pneumonic foals. Gram-positive isolates studied included *R. equi* ($n = 64$), beta-hemolytic streptococci (*Streptococcus equi* subspecies *zooepidemicus* [$n = 35$], *Streptococcus equi* subspecies *equi* [$n = 6$], *Streptococcus dysgalactiae* subspecies *equisimilis* [$n = 6$]), *Enterococcus* spp. ($n = 4$), and coagulase-positive *Staphylococcus* spp. ($n = 18$). Gram-negative isolates included *Salmonella enterica* subspecies *enterica* ($n = 23$), *Escherichia coli* ($n = 16$), *Pasteurella* spp. ($n = 11$), *Klebsiella* spp. ($n = 10$), *Pseudomonas* spp. ($n = 4$), *Enterobacter* spp. ($n = 2$), and *Bordetella bronchiseptica* ($n = 2$). Prior to being tested, isolates were subcultured, checked for purity, and identified using standard identification procedures.

Antimicrobials studied were AZM, CLR, ERY, rifampin, amikacin, amoxicillin-clavulanic acid, ampicillin, cefazolin, ceftazidime, ceftiofur, chloramphenicol, clindamycin, doxycycline, enrofloxacin, florfenicol, gentamicin, imipenem, nitrofurantoin, oxacillin, penicillin, tetracycline, and trimethoprim-sulfadiazine (1:5 ratio). The MICs were determined using JustOne microtitration strips (AccuMed International Ltd., Westlake, Ohio). MICs obtained by use of this technique have previously been shown to correlate closely with the standard broth dilution method (9, 16). Fresh isolates were grown on blood agar plates, and colonies were suspended in sterile water to a turbidity equal to that of a 0.5 McFarland standard. Ten microliters of the suspension was used to inoculate 10 ml of Mueller-Hinton broth for a final bacterial concentration of 10^5

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CFU/ml. Each well was then inoculated with 50 μl of bacterial suspension. Strips were sealed and incubated for 18 to 24 h at 37°C. A test was considered valid only if there was adequate growth in control wells. Control strains used to validate the assay at monthly intervals included *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *E. coli* ATCC 35218. In all instances, MICs obtained with the control stains were within the reference range proposed by the National Committee for Clinical Laboratory Standards (19, 20).

MICs at which 90% of isolates were inhibited (MIC_{90}s) of AZM, CLR, and ERY against 64 *R. equi* isolates were 1.0, 0.12, and ≤0.25 μg/ml, respectively (Table 1). Other antimicrobials highly active against at least 90% of *R. equi* isolates in vitro included rifampin, gentamicin, and imipenem (Table 1). Both AZM and CLR showed good activity against beta-hemolytic streptococci and *Staphylococcus* spp. (Table 1). AZM was more active than CLR or ERY against *Pasteurella* spp. and *Salmonella enterica* subsp. *enterica* (Table 2). AZM, CLR, and ERY did not show any activity against *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Enterobacter* spp (Table 2). MICs of AZM, CLR, and ERY against *Enterococcus* (AZM, ≤0.12 to >4; CLR, 0.12 to >4; ERY, ≤0.25 to >8) and *B. bronchiseptica* (AZM, 1 to 4; CLR, >4; ERY, >4) isolates varied considerably.

The rational use of AZM and CLR for the treatment of bacterial bronchopneumonia in foals has been precluded in part by the lack of in vitro susceptibility studies for equine bacterial pathogens. Although multiple studies have determined MICs of various antimicrobial agents against *R. equi* isolates, MICs of AZM and CLR have been reported only for a small number of human isolates (10, 21, 22). Although a wide variety of antimicrobial agents are active against *R. equi* in vitro (Table 1), many of these drugs are ineffective in vivo. *R. equi* is a facultative intracellular pathogen surviving and replicating in macrophages (13). In one study, all 17 foals with *R. equi* pneumonia treated with a combination of penicillin and gentamicin died despite all isolates being susceptible to gentamicin and 83% being susceptible to penicillin (24). In contrast, treatment of foals with antimicrobial agents achieving good intracellular concentrations, such as ERY and rifampin, has resulted in a survival rate ranging between 72 and 80% (2, 12). In the present study, CLR was at least as active as ERY against *R. equi* in vitro, whereas AZI was eightfold less active. MIC_{90}s of CLR and AZI against *R. equi* in the present study were considerably below achievable concentrations of these drugs in serum, pulmonary epithelial lining fluid, or bronchoalveolar cells following oral administration to foals (6, 14, 15). In contrast to the macrolides evaluated in the present study (ERY, CLR, and AZM), tilmicosin, a veterinary macrolide, has poor in vitro activity against *R. equi*, with MIC_{90}s of >32 μg/ml (4).

Many studies have determined in vitro susceptibilities of equine pathogens using the disk diffusion method, but few studies have reported MICs (1, 5, 8). To our knowledge, there are no studies evaluating in vitro susceptibilities of AZM and CLR against common equine bacterial pathogens. Based on MIC data presented in Table 1, both AZM and CLR showed good in vitro activity against beta-hemolytic streptococci and

TABLE 1. MICs of AZI, CLR, and 20 other antimicrobial agents against gram-positive equine bacterial isolates

Antimicrobial	MIC (μg/ml)		
	90%	50%	Range
R. equi (*n* = 64)			
Azithromycin	1	0.5	≤0.12–2
Clarithromycin	0.12	≤0.06	≤0.06–0.25
Erythromycin	≤0.25	≤0.25	≤0.25–0.5
Amikacin	4	≤2	≤2–8
Amoxicillin-clavulanic acid	8	≤2	≤2–16
Ampicillin	4	1	≤0.12–8
Cefazolin	16	≤2	≤2–>16
Ceftazidime	>32	>32	≤0.25–>32
Ceftiofur	1	≤0.5	≤0.5–2
Chloramphenicol	16	8	≤4–32
Clindamycin	>2	2	1–>2
Doxycycline	1	0.5	≤0.12–2
Enrofloxacin	1	0.5	≤0.25–2
Florfenicol	>8	>8	2–>8
Gentamicin	≤1	≤1	≤1
Imipenem	≤1	≤1	≤1
Nitrofurantoin	64	64	≤32–128
Oxacillin	>4	>4	≤2–>4
Penicillin	1	0.25	≤0.12–2
Rifampin	≤0.5	≤0.5	≤0.5–>4
Tetracycline	8	4	≤2–16
Trimethoprim-sulfadiazine	2	1	≤0.25–2
β-hemolytic streptococci (*n* = 47)			
Azithromycin	≤0.12	≤0.12	≤0.12–0.25
Clarithromycin	≤0.06	≤0.06	≤0.06–0.25
Erythromycin	≤0.25	≤0.25	≤0.25
Amikacin	>16	16	≤2–>16
Amoxicillin-clavulanic acid	≤2	≤2	≤2
Ampicillin	≤0.12	≤0.12	≤0.12
Cefazolin	≤2	≤2	≤2
Ceftazidime	0.5	0.5	≤0.25–2
Ceftiofur	≤0.5	≤0.5	≤0.5
Chloramphenicol	≤4	≤4	≤4
Clindamycin	≤0.25	≤0.25	≤0.25–0.5
Doxycycline	>4	≤0.12	≤0.12–>4
Enrofloxacin	2	1	≤0.25–2
Florfenicol	≤1	≤1	≤1–2
Gentamicin	8	4	≤1–>8
Imipenem	≤1	≤1	≤1
Nitrofurantoin	≤32	≤32	≤32
Oxacillin	≤2	≤2	≤2
Penicillin	≤0.12	≤0.12	≤0.12
Rifampin	≤0.5	≤0.5	≤0.5–4
Tetracycline	>16	4	≤2–>16
Trimethoprim-sulfadiazine	≤0.25	≤0.25	≤0.25–4
Staphylococcus spp. (*n* = 18)			
Azithromycin	0.5	0.5	≤0.12–1
Clarithromycin	0.25	0.12	≤0.06–0.25
Erythromycin	0.5	≤0.25	≤0.25–0.5
Amikacin	8	≤2	≤2–8
Amoxicillin-clavulanic acid	>8	≤2	≤2–>8
Ampicillin	16	≤0.12	≤2–>16
Cefazolin	>32	≤2	≤2–>32
Ceftazidime	>32	8	2–>32
Ceftiofur	>4	1	≤0.5–>4
Chloramphenicol	8	≤4	≤4–8
Clindamycin	≤0.25	≤0.25	≤0.25–1
Doxycycline	>4	0.25	≤0.12–>4
Enrofloxacin	≤0.25	≤0.25	≤0.25–1
Florfenicol	4	2	≤1–4
Gentamicin	>8	≤1	≤1–>8
Imipenem	>16	≤1	≤1–>16
Nitrofurantoin	≤32	≤32	≤32
Oxacillin	>4	≤2	≤2–>4
Penicillin	16	≤0.12	≤0.12–>16
Rifampin	1	≤0.5	≤0.5–4
Tetracycline	>16	≤2	≤2–>16
Trimethoprim-sulfadiazine	>4	≤0.25	≤0.25–>4

TABLE 2. MICs of AZI, CLR, and 20 other antimicrobial agents against gram-negative equine bacterial isolates

Antimicrobial	MIC (μg/ml)			Antimicrobial	MIC (μg/ml)		
	90%	50%	Range		90%	50%	Range
Pasteurella spp. (*n* = 11)				*E. coli* (*n* = 16)			
Azithromycin	0.25	0.25	≤0.12–0.25	Azithromycin	>8	4	≤0.12–>8
Clarithromycin	1	1	0.5–1	Clarithromycin	>4	>4	≤0.06–>4
Erythromycin	1	1	0.5–2	Erythromycin	>4	>4	>4
Amikacin	4	≤2	≤2–8	Amikacin	4	≤2	≤2–8
Amoxicillin-clavulanic acid	≤2	≤2	≤2	Amoxicillin-clavulanic acid	16	4	≤2–>16
Ampicillin	0.25	≤0.12	≤0.12–8	Ampicillin	>16	4	0.5–>16
Cefazolin	≤2	≤2	≤2	Cefazolin	16	≤2	≤2–16
Ceftazidime	≤0.25	≤0.25	≤0.25	Ceftazidime	≤0.25	≤0.25	≤0.25
Ceftiofur	≤0.5	≤0.5	≤0.5	Ceftiofur	≤0.5	≤0.5	≤0.5
Chloramphenicol	≤4	≤4	≤4	Chloramphenicol	>32	8	≤4–>32
Clindamycin	>2	2	2–>2	Clindamycin	>2	>2	>2
Doxycline	0.5	0.25	≤0.12–0.5	Doxycycline	>4	4	1–>4
Enrofloxacin	≤0.25	≤0.25	≤0.25	Enrofloxacin	>2	≤0.25	≤0.25–>2
Florfenicol	≤1	≤1	≤1	Florfenicol	8	4	2–>8
Gentamicin	≤1	≤1	≤1–2	Gentamicin	>8	≤1	≤1–>8
Imipenem	≤1	≤1	≤1	Imipenem	≤1	≤1	≤1
Nitrofurantoin	≤32	≤32	≤32	Nitrofurantoin	≤32	≤32	≤32–64
Oxacillin	≤2	≤2	≤2	Oxacillin	>4	>4	>4
Penicillin	0.5	0.25	≤0.12–>16	Penicillin	>16	>16	4–>16
Rifampin	1	≤0.5	≤0.5–1	Rifampin	>4	>4	4–>4
Tetracycline	≤2	≤2	≤2–8	Tetracycline	>16	>16	≤2–>16
Trimethoprim-sulfadiazine	≤0.25	≤0.25	≤0.25	Trimethoprim-sulfadiazine	>4	≤0.25	≤0.25–>4
S. enterica (*n* = 23)				*Klebsiella* spp. (*n* = 10)			
Azithromycin	4	4	2–4	Azithromycin	>8	8	2–>8
Clarithromycin	>4	>4	>4	Clarithromycin	>4	>4	>4
Erythromycin	>4	>4	≤0.25–>4	Erythromycin	>4	>4	>4
Amikacin	≤2	≤2	≤2–4	Amikacin	≤2	≤2	≤2
Amoxicillin-clavulanic acid	>16	≤2	≤2–>16	Amoxicillin-clavulanic acid	4	≤2	≤2–4
Ampicillin	>16	2	0.5–>16	Ampicillin	>16	16	2–>16
Cefazolin	>16	≤2	≤2–>16	Cefazolin	≤2	≤2	≤2
Ceftazidime	32	0.5	≤0.25–>32	Ceftazidime	≤0.25	≤0.25	≤0.25
Ceftiofur	>4	≤0.5	≤0.5–>4	Ceftiofur	≤0.5	≤0.5	≤0.5
Chloramphenicol	>32	≤4	≤4–>32	Chloramphenicol	8	≤4	≤4–8
Clindamycin	>2	>2	≤0.25–>2	Clindamycin	>2	>2	>2
Doxycycline	>4	4	2–>4	Doxycycline	4	2	0.5–4
Enrofloxacin	≤0.25	≤0.25	≤0.25	Enrofloxacin	≤0.25	≤0.25	≤0.25
Florfenicol	>8	4	2–>8	Florfenicol	8	4	≤1–8
Gentamicin	>8	≤1	≤1–>8	Gentamicin	≤1	≤1	≤1
Imipenem	≤1	≤1	≤1–8	Imipenem	≤1	≤1	≤1
Nitrofurantoin	≤32	≤32	≤32	Nitrofurantoin	64	≤32	≤32–64
Oxacillin	>4	>4	≤2–>4	Oxacillin	>4	>4	>4
Penicillin	>16	8	4–>16	Penicillin	>16	>16	16–>16
Rifampin	>4	>4	≤0.5–>4	Rifampin	>4	>4	>4
Tetracycline	>16	≤2	≤2–>16	Tetracycline	4	≤2	≤2–4
Trimethoprim-sulfadiazine	>4	≤0.25	≤0.25–>4	Trimethoprim-sulfadiazine	1	≤0.25	≤0.25–1

coagulase-positive *Staphylococcus* spp. All the beta-lactam antimicrobials studied, as well as chloramphenicol, florfenicol, clindamycin, rifampin, and trimethoprim sulfadiazine, were also highly active against at least 90% of beta-hemolytic streptococci. In contrast, only enrofloxacin, clindamycin, and rifampin were highly active against coagulase-positive *Staphylococcus* spp. in vitro (Table 1).

Of the three macrolides tested, AZM was the most active against *Pasteurella* spp. and *Salmonella enterica*. CLR and AZM were not active in vitro against other equine gram-negative bacterial pathogens (Table 2). MICs of other antimicrobials against equine gram-negative pathogens were similar to values previously reported (8), with the notable exception that in the present study, a larger proportion of *E. coli* isolates were resistant to enrofloxacin.

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