

Antimicrobial susceptibility patterns of respiratory tract pathogens from sheep and goats

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Objective—To determine the antimicrobial susceptibility of common respiratory tract pathogens from sheep and goats.

Design—Cross-sectional study.

Sample Population—41 respiratory tract isolates from sheep and 36 isolates from goats.

Procedures—Disk diffusion assay was used to determine antimicrobial susceptibility of isolates to amoxicillin-clavulanic acid, ceftiofur, ciprofloxacin, florfenicol, and tetracycline. Minimum inhibitory concentrations of florfenicol for these isolates were determined by use of the microbroth dilution technique.

Results—The most common isolates were *Pasteurella multocida* (n = 28) and *Mannheimia haemolytica* (39). All isolates were susceptible to amoxicillin-clavulanic acid, ceftiofur, ciprofloxacin, and florfenicol. Five percent (4/77) of isolates were resistant to tetracycline.

Conclusions and Clinical Relevance—Susceptibility of respiratory tract pathogens isolated from sheep and goats to commonly used antimicrobial drugs in this study was high. Treatment of these species for bacterial respiratory tract disease is likely not complicated by antimicrobial resistance. (*J Am Vet Med Assoc* 2006;229:1279–1281)

Antimicrobial use in species such as sheep and goats is often prescribed without label instructions. The AMDUCA of 1994 eased the scarcity of animal drugs available for use in sheep and goats by permitting veterinarians to use approved animal and human drugs on an extralabel or off-label basis.¹ The Act states that, under certain circumstances, veterinarians can use drugs approved for other species, for other diseases and conditions, or at different dosages from those listed on the drug label. One of the practical challenges to implementing the Act's provisions is to determine appropriate dosage and withdrawal times for species not listed on the drug label.

The NRSP-7 is a USDA-funded program that pursues approval of drugs for use in so-called minor species.² One such NRSP-7 project is extending the label claims

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ABBREVIATIONS

NRSP-7	National Research Support Project No. 7
MIC	Minimum inhibitory concentration
CLSI	Clinical and Laboratory Standards Institute
ATCC	American type culture collection

for florfenicol in bovine respiratory tract disease to sheep and goats. Florfenicol is a broad-spectrum, primarily bacteriostatic antimicrobial with a range of activity that includes many gram-negative and gram-positive bacteria.^{3,4} Florfenicol is approved for use in beef cattle (New Animal Drug Application [NADA] 141-063) with respiratory tract disease associated with infection by *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*.⁴ Because the most important respiratory tract disease bacteria isolated from sheep and goats are *M haemolytica* and *P multocida*, pursuing minor species approval of florfenicol for sheep and goats was a logical effort. An attractive feature of florfenicol is that it can be administered as a single-dose treatment or administered at 48-hour treatment intervals, protocols that may facilitate treatment of range animals like sheep and goats.

The objective of this study was to determine MIC values of florfenicol for respiratory tract pathogens isolated from sheep and goats and to use results to establish the potential efficacy of florfenicol in sheep and goats for treatment of respiratory disease caused by *M haemolytica* and *P multocida*. This can be achieved by contrasting serum time-concentration profiles of florfenicol in sheep and goats with the pathogens' MIC values and by comparing those with serum time-concentration profiles of florfenicol in cattle and cattle pathogen MIC values.

Materials and Methods

Bacterial isolates—Respiratory tract pathogens from ovine and caprine submissions to the Davis and Tulare branches of the California Animal Health and Food Safety Laboratory were evaluated. Forty-one ovine isolates and 36 caprine isolates from 1999 through 2002 were used in the study. Only 1 isolate/premise and submission date was used. Isolates included *Mannheimia glucosida* (n = 3), *M haemolytica* (39), *Mannheimia varigena* (1), *P multocida* (28), and *Pasteurella trehalosii* (6). Most (n = 72) isolates were recovered from lung tissue, but 1 nasal cavity isolate, 1 sinus isolate, 1 tracheal isolate, and 2 thoracic cavity isolates were also included. Isolates had previously been frozen and stored by the laboratory and were transferred to tryptic soy agar tubes and sent chilled overnight to the Veterinary Medicine Teaching and Research Center for susceptibility testing.

Antimicrobial susceptibility testing—Upon arrival at the Veterinary Medical Teaching and Resource Center, isolates were restreaked on blood agar^a and incubated for approxi-

mately 12 hours at 37°C. On the following day, a bacterial sampling loop was used to transfer 1 loop's volume of bacterial growth to a vial containing tryptic soy broth^a; the vial was kept in an incubator at 37°C until growth in the tube achieved or exceeded 0.5 McFarland turbidity standards (4 to 6 hours). Disk diffusion assay was performed according to CLSI guidelines with the following antimicrobial disks^b: florfenicol, 30 µg; amoxicillin-clavulanic acid, 20/10 µg; ceftiofur, 30 µg; tetracycline, 30 µg; and ciprofloxacin, 5 µg.^{5,6} Zones of inhibition were measured with a calibrated digital measuring device.^c The MIC for florfenicol was determined by use of the microbroth dilution technique, according to CLSI guidelines.⁶ Custom-made 96-well plates^d with florfenicol concentrations ranging from 0.12 to 128 µg/mL were obtained. Quality controls for both assays included *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922.^e

The disk diffusion assay results were stratified by source and bacterium and were summarized by determination of mean, SD, and minimum and maximum zone sizes. The MIC values for florfenicol were similarly stratified, and median,

Table 1—Values of MIC₅₀ and MIC₉₀ (concentrations at which 50% and 90%, respectively, of isolates tested were inhibited) and mode, minimum, and maximum MIC values (µg/mL) for florfenicol in *Mannheimia haemolytica* and *Pasteurella multocida* isolates from sheep and goats with respiratory tract disease.

Animal	Bacterium	No.	MIC ₅₀	MIC ₉₀	Mode	Min	Max
Goat	All isolates	34	0.50	1.00	0.25	0.25	2.00
	<i>M haemolytica</i>	25	0.50	1.00	0.50	0.25	2.00
	<i>P multocida</i>	7	0.25	0.50	0.25	0.25	0.50
Sheep	All isolates	38	0.25	1.00	0.25	0.12	1.00
	<i>M haemolytica</i>	13	0.50	0.50	0.50	0.25	1.00
	<i>P multocida</i>	19	0.25	0.50	0.25	0.25	1.00

Min = Minimum. Max = Maximum.

mode, maximum, and minimum values were determined.^f Stratified analysis with the nonparametric Kruskal-Wallis test^g was used to compare florfenicol MICs among ovine and caprine isolates and between *M haemolytica* and *P multocida*.

Results

Results of univariate analysis of MIC values for florfenicol were summarized (Table 1). The MICs for all isolates were low, indicating that the isolates were susceptible to florfenicol. Descriptive statistics for the disk diffusion assay results were tabulated (Table 2). On the basis of CLSI guidelines, most isolates were susceptible to all antimicrobials tested.⁶ Of the 2 major bacterial species tested, a single *M haemolytica* isolate had resistance to 1 antimicrobial (tetracycline; zone of inhibition, ≤ 11 mm). Three other isolates (2 isolates of *M glucosida* and 1 isolate of *P trehalosii*) were also resistant to tetracycline. All isolates were uniformly susceptible to the other 4 antimicrobials tested.

Stratified analysis of animal species indicated that ovine isolates had lower ($P = 0.01$) MIC values for florfenicol than caprine isolates. The MIC values for *M haemolytica* were slightly higher ($P < 0.01$) than those for *P multocida*.

Discussion

This study revealed that pathogens isolated from the respiratory tract of sheep and goats with clinical disease were highly susceptible to florfenicol and other antimicrobials. Results can be used to establish the potential efficacy of florfenicol in sheep and goats for treatment of respiratory disease caused by *M haemolytica* and *P multocida*. This can be achieved by contrasting serum time-

Table 2—Mean, SD, and range of disk diffusion zones (in millimeters) of respiratory tract pathogens for various antimicrobials and stratified by source (ovine or caprine) and bacterium (*M haemolytica* or *P multocida*).

Source	Antimicrobial	Mean	SD	Range
All isolates (n = 72)	Florfenicol	29.7	3.7	23–40
	Amoxicillin-clavulanic acid	30.9	5.8	22–58
	Ceftiofur	36.3	5.3	24–54
	Tetracycline	26.2	4.7	9–35
	Ciprofloxacin	33.9	3.4	25–43
Caprine isolates (34)	Florfenicol	25.5	3.6	23–37
	Amoxicillin-clavulanic acid	29.3	4.1	22–39
	Ceftiofur	35.1	3.5	28–42
	Tetracycline	26.2	3.5	10–30
	Ciprofloxacin	33.3	2.9	28–40
Ovine isolates (38)	Florfenicol	30.9	3.4	26–40
	Amoxicillin-clavulanic acid	32.3	6.7	23–58
	Ceftiofur	37.3	6.4	24–54
	Tetracycline	26.3	5.6	9–35
	Ciprofloxacin	34.4	3.8	25–43
<i>Mannheimia haemolytica</i> (38)	Florfenicol	28.3	3.0	23–37
	Amoxicillin-clavulanic acid	28.4	3.1	22–35
	Ceftiofur	33.7	3.0	27–41
	Tetracycline	25.7	3.3	10–31
	Ciprofloxacin	33.9	2.9	28–40
<i>Pasteurella multocida</i> (26)	Florfenicol	32.0	3.3	25–40
	Amoxicillin-clavulanic acid	31.2	3.7	23–41
	Ceftiofur	38.1	4.6	28–49
	Tetracycline	28.3	2.8	24–35
	Ciprofloxacin	34.3	3.7	27–43

concentration profiles of florfenicol in sheep and goats with the pathogens' MIC values, and by comparing those results with serum time-concentration profiles of florfenicol in cattle and cattle pathogen MIC values.

The MIC values for florfenicol obtained in this study were similar to those obtained from studies^{3,4} conducted in the United States, Canada, and Europe from 1990 to 1993. Pharmacokinetic studies⁷⁻⁹ have revealed that 3 doses (20 mg/kg) administered SC every 48 hours to sheep yield florfenicol concentrations higher than the target MIC of 0.5 µg/mL for > 108 hours. The drug may therefore be considered a valuable tool for treating sheep and goats with respiratory tract disease. The pathogens tested were similarly sensitive to all antimicrobials tested. Isolates were at least as sensitive to ceftiofur, florfenicol, and tetracycline as those submitted to a diagnostic laboratory in Nebraska from 1995 to 1997.¹⁰ The isolates were also all susceptible to ciprofloxacin, but because extralabel use of fluoroquinolones in food animals is prohibited, use of that drug in sheep and goats is prohibited in the United States.

Respiratory tract infections in sheep and goats usually have a multifactorial etiology that includes physical and physiologic stressors and predisposing viral and bacterial infections. Physical factors include weather, animal density, transportation, handling, and ventilation. Underlying infections that predispose small ruminants to infection with *M haemolytica* and *P multocida* include parainfluenza 3 virus, adenovirus type 6 and respiratory syncytial virus, *Mycoplasma ovipneumonia*, and *Bordetella pertussis*.¹¹ Although results of the present study suggest that precise identification of the causative agent of acute respiratory tract disease may not be necessary before initiating antimicrobial treatment for these pathogens, the clinical success of treatment will be dependent on establishing appropriate dosage regimens that take into account the pharmacokinetic aspects of each drug in addition to the other etiologic and environmental factors.

Bacterial antimicrobial susceptibility is assessed primarily through the use of broth dilution or disk diffusion assays. The relationship between the 2 types of assays has been described,¹²⁻¹⁴ and both methods yield clinically relevant and reliable information for determining treatment strategy. Some of the bacteria investigated in the present study were difficult to culture and maintain in a viable state for the assays, and several isolates had to be cultured several times before the assay could be performed. In many instances, growth of the pathogens on Mueller-Hinton plates was faint and zone sizes were large. Use of Mueller-Hinton agar plates with sheep blood did not improve bacterial growth or detectability of the inhibitory zones.

Most respiratory tract pathogens from sheep and goats submitted to the diagnostic laboratory in California

were susceptible to amoxicillin-clavulanic acid, ciprofloxacin, ceftiofur, florfenicol, and tetracycline. Susceptibility did not appear to vary across host or bacterial species, indicating that bacterial isolation and antimicrobial resistance testing may not be necessary prior to initiating antimicrobial treatment. However, for animals that do not respond favorably to treatment, pathogen identification and antimicrobial susceptibility testing are recommended.

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 - b. Difco, Becton-Dickinson, Sparks, Md.
 - c. Fowler sylvac, Ultra-cal IV, Geneva Gage Inc, Albany, Ore.
 - d. TREK Diagnostics Systems Ltd, Cleveland, Ohio.
 - e. American Type Culture Collection, Manassas, Va.
 - f. Proc Univariate, SAS, version 8.2, SAS Institute Inc, Cary, NC.
 - g. Proc Freq, SAS, version 8.2, SAS Institute Inc, Cary, NC.
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