JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1994, p. 725–731
0095-1137/94/$04.00+0

Vol. 32, No. 3

A 4-Year Survey of Antimicrobial Susceptibility Trends for Isolates from Cattle with Bovine Respiratory Disease in North America

JEFFREY L. WATTS,* ROBERT J. YANCEY, JR., SARAH A. SALMON, AND CHERYL A. CASE

Animal Health Discovery Research, The Upjohn Company, Kalamazoo, Michigan 49001

Received 30 August 1993/Returned for modification 28 October 1993/Accepted 9 December 1993

The antimicrobial susceptibility trends of bovine respiratory disease (BRD) pathogens isolated from 1988 to 1992 were determined. A total of 880 isolates representing *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* were used in the study. Overall, resistance to ampicillin, tetracycline, erythromycin, and sulfamethazine was frequently encountered among strains of *P. haemolytica* and *P. multocida*. Ceftiofur, an extended-spectrum cephalosporin originally marketed in 1988 for the treatment of BRD, was very active against the BRD pathogens tested; the MIC of ceftiofur for 90% of isolates tested was ≤0.06 μg/ml. Resistance to spectinomycin varied on the basis of the breakpoint used. Substantial variation in the year-to-year susceptibility of BRD pathogens to tilmicosin, a new macrolide antimicrobial agent, was observed. The proportion of susceptible *P. haemolytica* isolates ranged from 84.7% in the second year to 7.1% in the third year and 78.2% in the fourth year. Similar fluctuations were observed with strains of *P. multocida*.

Bovine respiratory disease (BRD) is a disease of economic importance to the cattle industry, with losses estimated at over $250 million per year in the United States alone (23). BRD is a multifactorial disease resulting from the interaction of bacterial and viral agents, usually in combination with stress (4, 6, 7). This disease is characterized by an acute-onset pneumonia caused by *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* (4, 6, 7). While these organisms are the primary bacterial agents of BRD, they are also part of the normal respiratory flora of cattle (4, 6, 7).

Antimicrobial therapy is the most effective method for the prevention and treatment of BRD (6, 18). The antimicrobial agents commonly used to treat BRD include ampicillin, erythromycin, tetracycline, spectinomycin, and sulfamethazine (6, 18). However, previous studies (6, 17, 18, 21) have indicated that resistance to these compounds is frequently encountered.

Currently, several new antimicrobial agents have been introduced or are under development for the treatment of BRD. Ceftiofur, an extended-spectrum cephalosporin, exhibits excellent activity against *P. haemolytica*, *P. multocida*, and *H. somnus* (25). This compound has been marketed for the treatment of BRD in the United States since 1988. Tilmicosin, a new macrolide antimicrobial agent (16), was introduced in Canada in 1990 and in the United States in 1991 for use in the treatment of BRD. However, only limited information is available on the antimicrobial susceptibility trends of BRD pathogens to these newer antimicrobial agents (17, 21). The purpose of the study described here was to determine the antimicrobial susceptibility patterns of BRD pathogens to the new antimicrobial agents and older compounds commonly used to treat BRD.

MATERIALS AND METHODS

Bacteria. A total of 888 isolates (461 *P. haemolytica*, 318 *P. multocida*, and 109 *H. somnus*) were used in the study. At the beginning of each BRD season (September), Upjohn Technical Services staff requested that veterinary diagnostic laboratories submit BRD isolates to our laboratory for MIC determinations. The BRD season for each year was concluded by the end of May of the following year. Laboratories were requested to submit only isolates of *P. haemolytica*, *P. multocida*, and *H. somnus* obtained from the lungs of animals that died from acute BRD and no more than two isolates of each species from each herd (or feedlot) for each year. Isolates were received from 9 laboratories in year 1 (1988 to 1989; YR1), 13 laboratories in year 2 (1989 to 1990; YR2), 9 laboratories in year 3 (1990 to 1991; YR3), and 11 laboratories in year 4 (1991 to 1992; YR4). Isolates were received from the following states: Pennsylvania, Wyoming, Iowa, Washington, California, Missouri, Nebraska, Oregon, Kansas, Arizona, Texas, South Dakota, Montana, Minnesota, Oklahoma, Colorado, and Utah. In addition, 133 isolates (79 *P. haemolytica*, 34 *P. multocida*, and 20 *H. somnus*) were received from the Canadian provinces of Saskatchewan, Alberta, and Quebec in YR4.

All isolates were identified by the submitting laboratory and were shipped to The Upjohn Company on slants. On receipt, the isolates were subcultured on Trypticase soy agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood (TBA) and were incubated at 35°C with 5% CO_2 for 24 h. After the identities and purities of the isolates were confirmed, the isolates were then stored in 1 ml of Trypticase soy broth (BBL) supplemented with 20% glycerol (vol/vol) on 3-mm-diameter glass beads at −70°C. Prior to MIC determinations, the isolates were serially subcultured twice on TBA.

MICs. All MIC determinations with the *Pasteurella* spp. were performed by a previously described broth microdilution method (15). In YR1 and YR2, the plates were prepared manually; a commercially prepared broth microdilution plate (Sensititre, Westlake, Ohio) was used in YR3 and YR4. The following antimicrobial agents were included on the commercial MIC panel: ampicillin, ceftiofur, erythromycin, tetracycline, spectinomycin, and sulfamethazine. When tilmicosin became available, plates were prepared manually in YR2,

* Corresponding author. Mailing address: Animal Health Discovery Research, 7923-190-MR, The Upjohn Company, Kalamazoo, MI 49001. Phone: (616) 385-6605. Fax: (616) 384-2347.

TABLE 1. Antimicrobial agents, dilution ranges, and MIC breakpoints used in the study

Antimicrobial agent	Dilution range (μg/ml)	Breakpoint (μg/ml)[a]	References
Ampicillin	0.03–32.0[b]	≤2.0	17
Ceftiofur	0.03–32.0[b]	≤2.0	[c]
Erythromycin	0.03–32.0[b]	≤1.0	5, 6
Tilmicosin	0.06–64.0	≤4.0	[d]
Tetracycline	0.03–32.0[b]	≤4.0	15, 17
Spectinomycin	0.13–128.0	≤32.0	6, 12, 17
Sulfamethazine	0.5–512.0	≤64.0	12, 17

[a] MIC at which isolates are considered susceptible.
[b] A range of 0.06 to 64.0 μg/ml was used in YR1 and YR2.
[c] Breakpoint recommended by the manufacturer.
[d] The manufacturer recommends a breakpoint of 6.25 μg/ml.

YR3, and YR4. Dilution ranges and MIC breakpoints for the antimicrobial agents tested are presented in Table 1.

All *H. somnus* isolates were tested by the agar dilution method (15). Because of the growth requirements of this organism, brain heart infusion agar (Difco, Detroit, Mich.) supplemented with 2% supplement C (Difco) was used as the basal medium, and the plates were incubated in an atmosphere containing 5% CO_2.

The following reference strains were included as quality control organisms: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 (15). Additionally, *P. haemolytica* ATCC 43137, *P. multocida* ATCC 33396, and *H. somnus* ATCC 4326 were included in most assays.

RESULTS

The MICs of the various antimicrobial agents and antimicrobial susceptibilities for *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD are listed in Tables 2 to 5 and are summarized in Table 6. The ampicillin MICs for 90% of *P. haemolytica* isolates tested (MIC_{90}s) were ≥32.0 μg/ml for all 4 years. The proportion of *P. haemolytica* isolates categorized as susceptible by using ≤2.0 μg/ml as the breakpoint ranged from 45.9 to 71.2%. The MIC_{90}s of ampicillin for *P. multocida* ranged from 4.0 to 16.0 μg/ml; the proportion of susceptible strains ranged from 83.3 to 89.5%. The MIC_{90}s for *H. somnus* isolates were ≤0.06 μg/ml in YR1, YR3, and YR4, but they were 64.0 μg/ml in YR2.

The MIC_{90}s of ceftiofur for all three species were ≤0.06 μg/ml in each year. Additionally, the highest MICs of ceftiofur for *P. haemolytica*, *P. multocida*, and *H. somnus* were 0.13, 0.25, and 0.13 μg/ml, respectively. This is well below the established breakpoint of ≤2.0 μg/ml, and all isolates of each species would be categorized as susceptible to ceftiofur.

The majority of *P. haemolytica* and *P. multocida* isolates were resistant to erythromycin. For *P. haemolytica* isolates, the MIC_{90}s increased from 2.0 μg/ml in YR1 to 4.0 μg/ml in the following years; the proportion of susceptible strains decreased from 16.3% in YR1 to 1.1% in YR4. Similarly, the MIC_{90}s of erythromycin for *P. multocida* strains increased from 2.0 μg/ml in YR1 to 8.0 μg/ml in YR4. The proportion of susceptible *P. multocida* strains decreased from 36.1% in YR2 to 4.5% in YR4.

The MIC_{90}s of the new macrolide compound tilmicosin for *P. haemolytica* were 8.0 μg/ml for the 3 years that the antimicrobial agent was available for testing, while the tilmicosin MIC_{90}s for the *P. multocida* and the *H. somnus* isolates ranged from 8.0 to 16.0 μg/ml and 4.0 to 8.0 μg/ml, respectively. The proportion of susceptible strains decreased dramatically in YR3 of the study, with 7.1, 7.1, and 31.8% of the *P. haemolytica*, *P. multocida*, and *H. somnus* strains categorized as susceptible to tilmicosin, respectively. Of the Canadian isolates obtained in YR4, 100.0, 90.9, and 96.8% of the *P. haemolytica*, *P. multocida*, and *H. somnus* isolates, respectively, were susceptible to tilmicosin, whereas 62.0, 65.3, and 92.5% of the

TABLE 2. Summary of MIC data for *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD in YR1

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)				% Susceptible
		50%	90%	Mode	Range	
P. haemolytica (93)	Ampicillin	0.25	32.0	0.13	≤0.06–64.0	53.2
	Ceftiofur	≤0.06	≤0.06	≤0.06	≤0.06–0.13	100.0
	Erythromycin	2.0	2.0	2.0	≤0.06–8.0	16.3
	Tilmicosin	ND[a]	ND	ND	ND	ND
	Tetracycline	8.0	32.0	16.0	≤0.06–64.0	46.7
	Spectinomycin	16.0	64.0	16.0	0.5–>128.0	83.7
	Sulfamethazine	512.0	>512.0	>512.0	1.0–>512.0	23.9
P. multocida (78)	Ampicillin	0.13	4.0	0.13	≤0.06–32.0	88.5
	Ceftiofur	≤0.06	≤0.06	≤0.06	≤0.06–0.25	100.0
	Erythromycin	2.0	2.0	2.0	≤0.06–16.0	34.6
	Tilmicosin	ND	ND	ND	ND	ND
	Tetracycline	0.5	16.0	0.5	≤0.06–32.0	70.5
	Spectinomycin	16.0	>128.0	16.0	≤0.06–>128.0	83.3
	Sulfamethazine	256.0	>512.0	128.0	0.5–>512.0	21.8
H. somnus (11)	Ampicillin	≤0.06	≤0.06	≤0.06	NR[b]	100.0
	Ceftiofur	≤0.06	≤0.06	≤0.06	NR	100.0
	Erythromycin	1.0	1.0	1.0	0.25–1.0	100.0
	Tilmicosin	ND	ND	ND	ND	ND
	Tetracycline	0.5	1.0	0.5	0.5–2.0	100.0
	Spectinomycin	16.0	>128.0	16.0	16.0–>128.0	81.8
	Sulfamethazine	>512.0	>512.0	>512.0	2.0–>512.0	18.2

[a] ND, not determined.
[b] NR, no range; all isolates yielded the same value.

TABLE 3. Summary of MIC data for *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD in YR2

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)				% Susceptible
		50%	90%	Mode	Range	
P. haemolytica (99)	Ampicillin	1.0	64.0	0.5, 32.0	≤0.06–>64.0	60.6
	Ceftiofur	≤0.06	≤0.06	≤0.06	NR[a]	100.0
	Erythromycin	4.0	4.0	4.0	1.0–64.0	8.1
	Tilmicosin	4.0	8.0	4.0	≤0.06–>64.0	82.8
	Tetracycline	4.0	32.0	16.0	≤0.06–64.0	50.5
	Spectinomycin	32.0	>64.0	32.0	0.5–>64.0	69.7
	Sulfamethazine	32.0	128.0	32.0	4.0–128.0	84.7
P. multocida (36)	Ampicillin	0.25	16.0	0.13, 0.5	≤0.06–>64.0	83.3
	Ceftiofur	≤0.06	≤0.06	≤0.06	NR	100.0
	Erythromycin	2.0	4.0	2.0	0.13–32.0	36.1
	Tilmicosin	1.0	8.0	1.0	0.25–32.0	80.6
	Tetracycline	4.0	4.0	0.5	≤0.06–32.0	91.6
	Spectinomycin	32.0	>64.0	32.0	4.0–>64.0	72.2
	Sulfamethazine	32.0	128.0	128.0	4.0–128.0	72.2
H. somnus (22)	Ampicillin	1.0	64.0	≤0.06	≤0.06–>64.0	50.0
	Ceftiofur	≤0.06	≤0.06	≤0.06	NR	100.0
	Erythromycin	1.0	8.0	1.0	0.5–16.0	59.1
	Tilmicosin	2.0	4.0	2.0	1.0–32.0	90.9
	Tetracycline	0.5	2.0	0.5	0.5–32.0	90.9
	Spectinomycin	32.0	64.0	32.0	32.0–>64.0	72.3
	Sulfamethazine	256.0	256.0	256.0	NR	0.0

[a] NR, no range, all isolates yielded the same value.

same species, respectively, originating from the United States were susceptible to tilmicosin (Table 7).

Tetracycline had limited activity against the *P. haemolytica* isolates tested; MIC_{90}s of tetracycline were 32.0 μg/ml for all 4 years. The compound was more active against strains of *P. multocida* and *H. somnus*.

The MIC_{90}s of spectinomycin were ≥64.0 μg/ml for all three species in all years except YR3 for *H. somnus*, for which the MIC_{90} was 8.0 μg/ml. Nevertheless, a majority of the isolates would be classified as susceptible in all years. Although the majority of the isolates were resistant to sulfamethazine, some year-to-year variation in resistance was observed, because the isolates received in YR2 were more susceptible than those received in the previous or the following years.

TABLE 4. Summary of MIC data for *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD in YR3

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)				% Susceptible
		50%	90%	Mode	Range	
P. haemolytica (85)	Ampicillin	4.0	32.0	32.0	0.13–32.0	45.9
	Ceftiofur	≤0.03	≤0.03	≤0.03	NR[a]	100.0
	Erythromycin	4.0	4.0	4.0	2.0–8.0	0.0
	Tilmicosin	8.0	8.0	8.0	2.0–16.0	7.1
	Tetracycline	8.0	32.0	0.5	0.25–32.0	48.2
	Spectinomycin	32.0	64.0	32.0	0.5–128.0	88.2
	Sulfamethazine	>512.0	>512.0	>512.0	0.5–>512.0	10.6
P. multocida (71)	Ampicillin	0.25	8.0	0.25	0.06–32.0	87.3
	Ceftiofur	≤0.03	≤0.03	≤0.03	NR	100.0
	Erythromycin	4.0	8.0	4.0	1.0–8.0	7.1
	Tilmicosin	8.0	16.0	8.0	2.0–32.0	22.4
	Tetracycline	0.5	32.0	0.5	0.13–32.0	63.3
	Spectinomycin	32.0	128.0	32.0	0.25–128.0	73.2
	Sulfamethazine	256.0	>512.0	>512.0	4.0–>512.0	22.5
H. somnus (22)	Ampicillin	≤0.03	0.06	≤0.03	≤0.03–0.06	100.0
	Ceftiofur	≤0.03	≤0.03	≤0.03	NR	100.0
	Erythromycin	0.25	0.5	0.25	0.13–0.5	100.0
	Tilmicosin	4.0	8.0	4.0	0.5–8.0	31.8
	Tetracycline	1.0	2.0	1.0	0.25–2.0	100.0
	Spectinomycin	8.0	8.0	8.0	0.5–8.0	100.0
	Sulfamethazine	>512.0	>512.0	>512.0	>512.0	0.0

[a] NR, no range, all isolates yielded the same value.

TABLE 5. Summary of MIC data for *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD in YR4

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)				% Susceptible
		50%	90%	Mode	Range	
P. haemolytica (184)	Ampicillin	0.25	32.0	0.13	≤0.03–>32.0	71.2
	Ceftiofur	≤0.03	≤0.03	≤0.03	≤0.03–0.13	100.0
	Erythromycin	4.0	4.0	4.0	≤0.03–32.0	1.1
	Tilmicosin	4.0	8.0	4.0	0.06–>16.0	78.2
	Tetracycline	0.5	32.0	0.5	0.25–>32.0	70.1
	Spectinomycin	32.0	64.0	32.0	8.0–>128.0	89.1
	Sulfamethazine	64.0	>512.0	>512.0	8.0–>512.0	53.8
P. multocida (133)	Ampicillin	0.25	16.0	0.25	≤0.03–>32.0	89.5
	Ceftiofur	≤0.03	≤0.03	≤0.03	≤0.03–0.06	100.0
	Erythromycin	4.0	8.0	4.0	≤0.13–8.0	4.5
	Tilmicosin	4.0	8.0	4.0	0.5–>16.0	71.8
	Tetracycline	0.5	32.0	0.5	0.06–>32.0	69.9
	Spectinomycin	32.0	>128.0	16.0	4.0–>128.0	75.8
	Sulfamethazine	128.0	>512.0	>512.0	8.0–>512.0	21.1
H. somnus (54)	Ampicillin	≤0.03	0.25	≤0.03	≤0.03–8.0	98.1
	Ceftiofur	≤0.03	≤0.03	≤0.03	≤0.03–0.13	100.0
	Erythromycin	0.13	0.5	0.06, 0.13	0.06–>32.0	94.4
	Tilmicosin	1.0	4.0	2.0	≤0.03–16.0	94.4
	Tetracycline	0.5	2.0	0.5	≤0.03–8.0	98.1
	Spectinomycin	4.0	64.0	4.0	≤0.13–>128.0	88.9
	Sulfamethazine	32.0	256.0	128.0	≤0.5–>512.0	68.5

DISCUSSION

Antimicrobial agents used for the treatment of BRD are selected by the veterinarian on the basis of perceived efficacy, cost, convenience, availability, toxicity, and residue profile (14). A substantial decrease in treatment efficacy, as indicated by increased mortality or number of animals requiring retreatment, usually prompts the practitioner to submit samples for bacteriologic culturing and antimicrobial susceptibility testing. Thus, monitoring of the antimicrobial susceptibility trends of BRD pathogens is an important aid to veterinarians in selecting the most efficacious and cost-effective therapeutic agents.

Several factors may influence the level of antimicrobial susceptibility observed for BRD pathogens (15, 17, 24). Since *P. haemolytica*, *P. multocida*, and *H. somnus* are normal flora of the bovine respiratory tract, the susceptibilities of isolates in samples collected from the upper respiratory tract may not represent the susceptibility of the causative strain of the pneumonia (2, 11, 14). In contrast, some workers (11, 13, 14) consider that the susceptibilities of isolates recovered from pneumonic lungs may overestimate antimicrobial resistance since the pathogen has previously been exposed to the antimicrobial agent. However, one investigator (11) determined that the majority of animals with BRD either were treated with an insufficient dose of the antimicrobial agent or therapy was terminated too early to effect a complete cure. Appropriate therapy should expose the pathogen to the antimicrobial agent at a sufficient concentration and duration to minimize the development of resistant populations. Thus, the pathogens isolated from pneumonic lungs represent treatment failures, and the resistant populations probably result from inappropriate or subtherapeutic use of antimicrobial agents.

Interpretive criteria for categorizing isolates as susceptible or resistant are based on a tripartite database consisting of the MIC of the drug for a bacterial population, the pharmacokinetics of the antimicrobial agent in the host species, and the reasonable correlation of the susceptible isolates with in vivo drug efficacy (1, 3). The most frequently used interpretive criteria for categorizing veterinary isolates as susceptible or resistant are those recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (12, 15, 24). However, these criteria were developed by using human isolates and human pharmacokinetic data, and their use for predicting the antimicrobial susceptibilities of veterinary pathogens has been seriously questioned (12, 15, 19). Libal (12) and Post et al. (17) have suggested that MIC testing is preferable to agar disk diffusion tests for determining the antimicrobial susceptibilities of veterinary pathogens because of the qualitative nature of the latter test method and the lack of a zone interpretive criteria set for veterinary pathogens of the different animal species.

Ampicillin, tetracycline, and sulfamethazine have been used for many years to treat BRD, and widespread resistance to these compounds has been reported (8, 9, 18). Current NCCLS recommendations (15) for ampicillin breakpoints for members of the family *Enterobacteriaceae* of human origin are ≤8.0 μg/ml for susceptible and ≥32.0 μg/ml for resistant. However, MIC breakpoints of ≤1.0 μg/ml (18), ≤2.0 μg/ml (17), and ≤4.0 μg/ml (12) for categorizing veterinary isolates as susceptible to ampicillin have been used. By using the breakpoint of ≤2.0 μg/ml recommended by Post (17), the proportion of *P. haemolytica* isolates susceptible to ampicillin ranged from 45.9 to 71.2%, whereas strains of *P. multocida* tended to be more susceptible. These findings agree with those of previous studies (9, 17, 21), indicating that ampicillin resistance is more prevalent among isolates of *P. haemolytica* than among isolates of *P. multocida*.

Results of the present study indicate that ceftiofur is very active against all the BRD isolates tested, with MICs of ≤0.06 μg/ml for all but three isolates. On the basis of an MIC breakpoint of ≤2.0 μg/ml for susceptible isolates, all the isolates would be considered susceptible to ceftiofur. These data agree with those presented in a previous report (17),

TABLE 6. Summary of MIC data for *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD for all 4 years[a]

Organism (no. of isolates)	Antimicrobial	MIC (μg/ml)				% Susceptible
		50%	90%	Mode	Range	
P. haemolytica (461)	Ampicillin	0.25	32.0	0.13	≤0.03–>64.0	60.5
	Ceftiofur	≤0.03	0.06	≤0.03	≤0.03–0.13	100.0
	Erythromycin	4.0	4.0	4.0	≤0.03–>64.0	5.4
	Tilmicosin	4.0	8.0	4.0	0.06–16.0	69.1
	Tetracycline	1.0	32.0	0.5	≤0.06–64.0	57.0
	Spectinomycin	32.0	64.0	32.0	0.5–>128.0	83.5
	Sulfamethazine	128.0	>512.0	>512.0	0.5–>512.0	46.2
P. multocida (318)	Ampicillin	0.25	8.0	0.13	≤0.03–>64.0	88.1
	Ceftiofur	≤0.03	0.06	≤0.03	≤0.03–0.25	100.0
	Erythromycin	2.0	8.0	2.0	≤0.03–>64.0	16.0
	Tilmicosin	4.0	8.0	8.0	0.25–32.0	58.9
	Tetracycline	0.5	16.0	0.5	≤0.06–>32.0	71.1
	Spectinomycin	32.0	>128.0	16.0	0.13–>128.0	76.4
	Sulfamethazine	128.0	>512.0	128.0	0.5–>512.0	27.4
H. somnus (109)	Ampicillin	0.06	1.0	≤0.03	≤0.03–>64.0	90.1
	Ceftiofur	≤0.03	0.06	≤0.03	≤0.03–0.13	100.0
	Erythromycin	0.25	2.0	0.25	≤0.03–>32.0	88.9
	Tilmicosin	2.0	4.0	2.0	≤0.03–32.0	90.4
	Tetracycline	0.5	1.0	≤0.03	≤0.03–32.0	98.2
	Spectinomycin	8.0	32.0	8.0	≤0.13–>128.0	87.1
	Sulfamethazine	256.0	>512.0	>512.0	≤0.5–>512.0	35.8

[a] Data for tilmicosin were available for only 3 years.

which indicated that the MIC_{90}s of ceftiofur for both *P. haemolytica* and *P. multocida* isolated from cattle with BRD were 0.125 μg/ml and that 90% of the isolates were susceptible to ceftiofur. However, the investigators (17) used a breakpoint for susceptible isolates on the basis of concentrations of ceftiofur in bronchial secretions of ≤0.5 μg/ml (10). No studies which demonstrate that the 0.5-μg/ml breakpoint provides a better correlation to in vivo efficacy have been conducted. By using the generally accepted criteria for establishing MIC breakpoints (3), the ≤2.0-μg/ml breakpoint appears reasonable as supported by the MIC data, the pharmacokinetics of ceftiofur in cattle (10), and the in vivo efficacy of ceftiofur in field trials (11).

Current NCCLS guidelines (15) recommend that isolates for which the tetracycline MIC is ≤4.0 μg/ml be categorized as susceptible. This recommendation has been generally accepted in veterinary microbiology. By using this breakpoint, resistance to tetracycline was common among *Pasteurella* spp. in the present study, with isolates of *P. haemolytica* tending to be more resistant than those of *P. multocida*. These findings also agree with previously reported data (9, 13, 17, 21) indicating that tetracycline resistance is frequently observed with *P. haemolytica* and *P. multocida* strains isolated from cattle with BRD. Sulfamethazine exhibited poor activity against the species tested; Prescott and Baggot (18) have previously indicated that sulfamethazine resistance is so widespread as to limit the usefulness of this agent in cattle.

The NCCLS guidelines (15) recommend a breakpoint for erythromycin of ≤0.5 and ≥8.0 μg/ml for categorizing isolates as susceptible or resistant, respectively. For veterinary isolates, this is usually modified to either ≥4.0 or ≥2.0 μg/ml, respectively, for resistant strains (6, 12, 17). By using a breakpoint of ≥2.0 μg/ml, the majority of *H. somnus* isolates were susceptible to erythromycin; this was the only species in the current study for which this was the case. In any given year, fewer than 17.0% of the *P. haemolytica* isolates and 37.0% of the *P. multocida* isolates would be considered susceptible to erythromycin. Post et al. (17) reported that the MIC_{50}s and MIC_{90}s for 421 *P. haemolytica* and 158 *P. multocida* strains were 4.0 μg/ml and considered these strains to be moderately susceptible. These values are similar to those obtained in the current study. The differences in the resistance levels reported in the present study and by Post et al. (17) result from our use of the ≥2.0-μg/ml breakpoint for categorizing isolates as resistant (≤1.0 μg/ml = susceptible). Because the data used to generate the ≥4.0-μg/ml breakpoint were based on a dose of 15 mg/kg of body weight (5, 6) rather than the approved dose of 2.2 to 4.4 mg/kg, we feel that the lower breakpoint is more appropriate. Fales et al. (9) reported that only 9.0% of *P. haemolytica* strains and 15.0% of *P. multocida* strains isolated from 1976 to

TABLE 7. Comparison of MICs of tilmicosin and antimicrobial susceptibilities for U.S. and Canadian BRD pathogens isolated in YR4

Source	*P. haemolytica*				*P. multocida*				*H. somnus*			
	No. of isolates	MIC (μg/ml)		% Susceptible[a]	No. of isolates	MIC (μg/ml)		% Susceptible[a]	No. of isolates	MIC (μg/ml)		% Susceptible[a]
		90%	Range			90%	Range			90%	Range	
United States	108	8.0	≤0.06–16.0	62.0	98	8.0	1.0–16.0	65.3	40	2.0	0.13–16.0	92.5
Canada	80	4.0	0.5–4.0	100.0	33	8.0	0.5–16.0	90.9	31	4.0	≤0.06–8.0	96.8

[a] On the basis of a breakpoint of 4.0 μg/ml for this dilution series; the manufacturer recommends a breakpoint of 6.25 μg/ml.

1980 were resistant to erythromycin and recommended that erythromycin be used as the first-choice drug for therapy of BRD. Although those workers (9) cautioned against residue problems, they suggested a dose of 22.0 to 44.0 mg/kg (10 times the approved dosage). Given the level of resistance to erythromycin observed in the present study, erythromycin probably has limited usefulness in the treatment of BRD and should probably not be considered the first-choice antimicrobial agent.

Tilmicosin is a new macrolide antimicrobial agent recently marketed in the United States and Canada for the treatment of BRD (16, 22). The manufacturer's suggested breakpoints for this compound are ≤6.25 μg/ml for susceptible, 12.5 μg/ml for moderately susceptible, and ≥25.0 μg/ml for resistant. However, previous reports have indicated that the peak levels of tilmicosin in the lung tissues of calves are 7.17 μg/ml (22). On the basis of that information, breakpoints of ≤4.0 and ≥8.0 μg/ml are probably more appropriate for categorizing isolates as susceptible and resistant, respectively. We observed substantial year-to-year variation in the proportion of susceptible isolates because 84.7% of the *P. haemolytica* isolates and 36.1% of the *P. multocida* isolates were considered susceptible in YR2, in comparison with 7.1 and 22.4%, respectively, in YR3 and 78.2 and 71.8%, respectively, in YR4. The high level of resistance encountered was surprising because tilmicosin had not been introduced into the U.S. market in YR2 and YR3. The reason for this high level of resistance is unclear but may be due to the cross-resistance of these strains to erythromycin. Because erythromycin is frequently used in the United States but is infrequently used in Canada to treat BRD (20), we compared the antimicrobial resistance levels in BRD isolates from Canada with those in BRD isolates from the United States. Interestingly, Canadian isolates tended to be more susceptible to tilmicosin than isolates from the United States (Table 7). However, no differences in resistance to the other antimicrobial agents including erythromycin were observed between the U.S. and Canadian isolates (data not shown).

Spectinomycin, an aminocyclitol antimicrobial agent, is commonly used for the treatment of BRD in the United States (18). The NCCLS guidelines (15) categorize isolates for which the MIC is ≤32.0 μg/ml as susceptible, whereas MIC breakpoints of either ≤12.0 or 16.0 μg/ml have been recommended by others (6, 17) for veterinary isolates. By using the NCCLS recommendations, more than 75.0% of all isolates of the three species tested would be considered susceptible to spectinomycin. These findings agree with those of Post et al. (17), who reported that 90.0% of *P. haemolytica* and *P. multocida* isolates were moderately susceptible (24.0 μg/ml) to spectinomycin. However, if a breakpoint of ≤16.0 μg/ml is used, then the overall proportion of susceptible strains would be 31.4, 44.3, and 69.7% for *P. haemolytica*, *P. multocida*, and *H. somnus*, respectively. It should be noted that spectinomycin is not approved for use in treating BRD in the United States and that the veterinarian assumes all liability for efficacy and residues. Without a specific approval on the basis of an efficacious dose, the use of spectinomycin in treating BRD may not be prudent.

In conclusion, resistance to older antimicrobial agents including ampicillin, tetracycline, erythromycin, and sulfamethazine was frequently encountered among strains of *P. haemolytica*, *P. multocida*, and *H. somnus* isolated from cattle with BRD. Additionally, the widespread resistance to erythromycin may reduce the effectiveness of tilmicosin. Of the compounds tested, ceftiofur was the most active, with no strains that were resistant to ceftiofur emerging over the course of the study.

ACKNOWLEDGMENTS

We thank Clyde Thornsberry for review of the manuscript and constructive comments. We also thank the following for submitting isolates for use in the study: W. Laubscher, Animal Diagnostic Laboratory, Pennsylvania State University, University Park; D. C. Hoefling, Animal Disease Laboratory, Illinois Department of Agriculture, Galesburg; C. Gates, Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings; A. M. Ohme, Bureau of Animal Industry, Pennsylvania Department of Agriculture, Summerdale; R. L. Walker, California Veterinary Diagnostic Laboratory System, University of California-Davis; M. E. Olson, Department of Animal Care Services, University of Calgary, Calgary, Alberta, Canada; D. Bouley, Department of Diagnostic Investigation, University of Minnesota, St. Paul; M. M. Chengappa, Department of Veterinary Diagnostics, Kansas State University, Manhattan; J. J. Henderson, Diagnostic Laboratory Division, Montana Department of Livestock, Bozeman; Missouri Animal Disease Division, Missouri Department of Agriculture, Columbia; L. B. Dye, Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater; Provo Diagnostic Laboratory, Utah State University, Provo; K. Post, Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, Amarillo; Veterinary Science Laboratory, University of Nebraska-Lincoln; E. D. Erickson, Veterinary Science Laboratory, University of Nebraska-West Central Center, North Platte; G. Thompson, Veterinary Diagnostic Laboratory, Colorado State University, Ft. Collins; L. J. Hoffman, Veterinary Diagnostic Laboratory, Iowa State University, Ames; R. J. Sonn, Veterinary Diagnostic Laboratory, Oregon State University, Corvallis; G. Meerdink, Veterinary Diagnostic Laboratory, University of Arizona, Tucson; D. Edwards, Washington Animal Disease Diagnostic Laboratory, Washington State University, Pullman; and A. Berger-Fields, Wyoming State Diagnostic Laboratory, Wyoming State University, Laramie.

REFERENCES

1. **Acar, J., and F. W. Goldstein.** 1991. Disk susceptibility test, p. 17–52. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore.
2. **Allen, J. W., L. Viel, K. G. Bateman, S. Rosendal, P. E. Shewen, and P. Physick-Sheard.** 1991. The microbial flora of the respiratory tract in feedlot calves: associations between nasopharyngeal and bronchoalveolar lavage cultures. Can. J. Vet. Res. **55:**341–346.
3. **Amsterdam, D.** 1991. Susceptibility testing of antimicrobials in liquid media, p. 53–105. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore.
4. **Biberstein, E. L.** 1990. *Haemophilus* and *Taylorella*, p. 151–164. *In* G. R. Carter and J. R. Cole, Jr. (ed.), Diagnostic procedures in veterinary bacteriology and mycology, 5th ed. Academic Press, Inc., New York.
5. **Burrows, G. E.** 1985. Effects of experimentally induced *Pasteurella haemolytica* pneumonia on the pharmacokinetics of erythromycin in the calf. Am. J. Vet. Res. **46:**798–803.
6. **Burrows, G. E., and P. Ewing.** 1989. *In vitro* assessment of the efficacy of erythromycin in combination with oxytetracycline or spectinomycin against *Pasteurella haemolytica*. J. Vet. Diagn. Invest. **1:**299–304.
7. **Carter, G. R.** 1990. *Pasteurella*, p. 129–140. *In* G. R. Carter and J. R. Cole, Jr. (ed.), Diagnostic procedures in veterinary bacteriology and mycology, 5th ed. Academic Press, Inc., New York.
8. **Chang, W. H., and G. R. Carter.** 1976. Multiple drug resistance in *Pasteurella multocida* and *Pasteurella haemolytica* from cattle or swine. J. Am. Vet. Med. Assoc. **169:**710–712.
9. **Fales, W. H., L. A. Selby, J. J. Webber, L. J. Hoffman, L. D. Kintner, S. L. Nelson, R. B. Miller, J. G. Thorne, J. T. McGinty, and D. K. Smith.** 1982. Antimicrobial resistance among *Pasteurella* spp. recovered from Missouri and Iowa cattle with bovine respiratory complex. J. Am. Vet. Med. Assoc. **181:**477–479.
10. **Halstead, S. L., R. D. Walker, J. C. Baker, R. E. Holland, and G. E. Stein.** 1989. Determination of serum, tissue cage fluid, and bronchial secretion concentrations of ceftiofur sodium in feeder age beef cattle. 70th Annu. Conf. Res. Workers Anim. Dis., 11 to 16 November, Chicago.
11. **Jim, G. K., C. W. Booker, and P. T. Guichon.** 1992. A comparison of trimethoprim-sulfadoxine and ceftiofur sodium for the treat-

ment of respiratory disease in feedlot calves. Can. Vet. J. **33:**245–250.

12. **Libal, M. C.** 1985. Comparison of minimal inhibitory concentration and disk-diffusion antimicrobic sensitivity testing of bacterial pathogens isolated from food animals. Am. J. Vet. Res. **46:**1200–1205.
13. **Martin, S. W., A. H. Meek, and R. A. Curtis.** 1983. Antimicrobial use in feedlot calves: its association with culture rates and antimicrobial susceptibility. Can. J. Comp. Med. **47:**6–10.
14. **Mechor, G. D., G. K. Jim, and E. D. Janzen.** 1988. Comparison of penicillin, oxytetracycline, and trimethoprim-sulfadoxine in the treatment of acute undifferentiated bovine respiratory disease. Can. Vet. J. **29:**438–443.
15. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Approved standard. NCCLS document M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **Picavet, T., E. Muylle, L. A. Devriese, and J. Geryl.** 1991. Efficacy of tilmicosin in treatment of pulmonary infections in calves. Vet. Rec. **129:**400–403.
17. **Post, K. W., N. A. Cole, and R. H. Raleigh.** 1991. *In vitro* antimicrobial susceptibility of *Pasteurella haemolytica* and *Pasteurella multocida* recovered from cattle with bovine respiratory disease complex. J. Vet. Diagn. Invest. **3:**124–126.
18. **Prescott, J. F., and J. D. Baggot.** 1988. Antimicrobial therapy in veterinary medicine. Blackwell Scientific Publications, Boston.
19. **Prescott, J. F., V. P. Gannon, G. Kittler, and G. Hlywka.** 1984. Antimicrobial drug susceptibility of bacteria isolated from disease processes in cattle, horses, dogs, and cats. Can. Vet. J. **25:**289–292.
20. **Quinn, K. B.** 1991. Personal communication.
21. **Raemdock, D. L.** 1992. Comparative minimum inhibitory concentrations of danofloxacin and six commonly used antibacterials against *Pasteurella* and *Haemophilus* from pneumonic cattle, p. 209–213, vol. 3. Proc. XVII World Buiatrics Congress, St. Paul, Minn. Frontier Printers, Stillwater, Okla.
22. **Thomson, T. D., and T. Matsuoka.** 1989. Correlation of lung concentrations of a new veterinary antimicrobial with efficacy against *Pasteurella haemolytica* pneumonia in feedlot cattle. 70th Annu. Conf. Res. Workers Anim. Dis., 11 to 16 November, Chicago.
23. **Wesley, T.** 1987. The economics of animal disease. Animal Pharm, PJB Publications, Surrey, England.
24. **Woolcock, J. B., and M. D. Mutimer.** 1983. Antibiotic susceptibility testing: caeci caecos ducentes? Vet. Rec. **113:**125–128.
25. **Yancey, R. J., Jr., M. L. Kinney, B. J. Roberts, K. R. Goodenough, J. C. Hamel, and C. W. Ford.** 1987. Ceftiofur sodium, a broad-spectrum cephalosporin: evaluation *in vitro* and *in vivo* in mice. Am. J. Vet. Res. **48:**1050–1053.