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In Vitro Activity of Lincomycin and Spectinomycin Against Serotypes of Avian Mycoplasma

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Twenty strains of avian mycoplasma, representing 12 serotypes, were tested in vitro for their susceptibility to the action of lincomycin and spectinomycin alone and in combination. They varied in their sensitivity pattern. The ranges of minimal inhibitory concentration were 1 to 20 μg/ml for lincomycin or spectinomycin alone and 0.5/1 to 3/6 μg/ml for the lincomycin and spectinomycin combination. The ranges of minimal lethal concentration were greater with either single antibiotic than with the antibiotic combination. The amount of each antibiotic required to achieve mycoplasmacidal action of the relatively resistant strains was less with the antibiotic combination than with the single antibiotics.

Avian mycoplasmas have been associated with airsacculitis and synovitis of poultry (9). Mycoplasma has been classified by several investigators (1, 5, 8, 9). They have been characterized and grouped into 12 serotypes designated A through L (9). The lincomycin and spectinomycin combination was found to be effective against airsacculitis of chickens and turkeys caused by *Mycoplasma gallisepticum* and *M. meleagridis* (2, 3).

It is the purpose of this communication to report the in vitro susceptibility of 20 strains of avian mycoplasma to the action of lincomycin and spectinomycin alone and in combination.

MATERIALS AND METHODS

Test organisms. Twenty strains of avian mycoplasma representing at least 12 serotypes were used in this study. They were obtained through the courtesy of H. W. Yoder of U.S. Department of Agriculture, Georgia, J. Fabricant of Cornell University, H. E. Adler of University of California, C. F. Hall of Texas A & M University, and R. Yamamoto of the University of California, as described in Table 1. These strains were identified serologically (9). They were originally isolated from naturally infected avian species (Table 1). Thirteen strains were dextrose fermenters and seven strains were not. The minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC) were determined for the dextrose fermenters, and the MLC was determined for the dextrose nonfermenters. The test organism was a 48-hr broth culture with a titer ranging from 10^4 to 10^8 colony-forming units (CFU) per ml.

Antibiotics. Lincocin (lincomycin) HCl, Trobicin (spectinomycin) sulfate, and their combination in a one to two ratio were prepared in sterile saline and stored at 4 C. (Lincocin is the registered trademark of The Upjohn Co. for lincomycin. Trobicin is the registered trademark of The Upjohn Co. for spectinomycin.)

Media. Three culture media were used. A medium containing 88.5% phenol red broth base (Difco), 1% yeast extract, 0.5% dextrose, and 10% inactivated horse serum was used to determine the MIC for the dextrose fermenters. Another mycoplasma broth medium containing 89.5% mycoplasma broth base (Albimi Laboratories), 0.5% yeast extract, and 10% inactivated horse serum was used for the dextrose nonfermenters. The MLC was determined in a modified mycoplasma agar medium containing 88.0% mycoplasma broth base (Albimi Laboratories), 0.5% yeast extract, 1.5% agar (Difco), and 10% inactivated horse serum.

Procedure. The broth medium was dispensed in 4.5-ml amounts in sterile tubes and 0.5 ml of the antibiotic dilution was added to make a final concentration of 20, 10, 9, 7, 6, 5, 3, 1, 0.1, 0.01 μg/ml of broth. A 0.1-ml amount of a 48-hr broth culture of the test organism was added to the antibiotic-medium mixture. The entire test was conducted in duplicate. The tubes were incubated at 37 C and examined daily for 5 to 6 days. At 2 and 4 days of incubation, a loopful from each tube showing no visible growth (no color change of phenol red) was streaked on the agar medium. The plates were sealed with masking tape and incubated for 7 days. The plates were examined microscopically for mycoplasma colonies after 2 days of incubation. Samples were considered negative if no growth was observed after 7 days of incubation. The MIC was recorded as the lowest concentration of the antibiotic inhibiting the growth of mycoplasma as evidenced by the phenol red indicator. The MLC was the lowest antibiotic concentration which yielded no growth of mycoplasma on agar when streaked for the

TABLE 1. *Identity and sources of avian mycoplasma*

Strain no.	Serotype	Dextrose fermentation	Origin	Source
S-6 (JF)	A	+	Chicken trachea	J. Fabricant, New York
BC-801	A	+	Turkey air-sac (Hofstad, 1955)	H. W. Yoder, Georgia
St. 19-3E	A	+	Chicken embryo	C. F. Hall, Texas
St. 21-3E	A	+	Chicken embryo	C. F. Hall, Texas
S-6A	A	+	Turkey air-sac, chicken-adapted	C. F. Hall, Texas
BC 1504	B	−	Chicken trachea	H. W. Yoder, Georgia
BC 859	C	+	Chicken trachea (Kleckner)	H. W. Yoder, Georgia
BC 887	D	+	Chicken trachea (Markham)	H. W. Yoder, Georgia
THY-860	E	−	Chicken trachea (Kleckner)	H. W. Yoder, Georgia
Fg-7	F	+	Chicken pericardium (Fabricant)	H. Adler, California
BC 1197	F	+	Turkey trachea (Fabricant)	H. W. Yoder, Georgia
867	G	−	Chicken pericardium (Fabricant)	H. W. Yoder, Georgia
THY-886	H	−	Turkey air-sac (Adler)	H. W. Yoder, Georgia
Mm 529	H	−	Turkey air-sac	R. Yamamoto, California
Mm TUCO	H	−	Turkey air-sac	A. H. Hamdy, Michigan
BC 695	I	+	Turkey air-sac (Hofstad)	H. W. Yoder, Georgia
BC 693	J	+	Turkey hock joint (Hofstad)	H. W. Yoder, Georgia
BC 1805	K	+	Chicken oviduct	H. W. Yoder, Georgia
BC 694	L	−	Pigeon turbinate (Hofstad)	H. W. Yoder, Georgia
208g10	UC[a]	+	Chicken air-sac	H. Adler, California

[a] Unclassified.

TABLE 2. *MIC of lincomycin and spectinomycin singly and in combination against 13 strains (7 serotypes) of avian mycoplasma (dextrose fermenters) after 2 days of incubation*

Strain no.	Serotype	Inoculum size (CFU/ml)	Lincomycin (μg/ml)	Spectinomycin (μg/ml)	Lincomycin/spectinomycin (μg/ml)
S-6 (JF)	A	2.2×10^5	6	3	1.5/3
BC 801	A	8.6×10^5	3	20	1.5/3
St. 19-3E	A	1.0×10^4	1	5	0.5/1
St. 21-3E	A	3.8×10^8	3	5	1.5/3
S-6 A	A	2.6×10^5	5	3	1.5/3
BC 859	C	5.0×10^5	7	1	0.5/1
BC 887	D	4.2×10^8	3	9	1.5/3
Fg-7	F	1.4×10^5	5	3	0.5/1
BC 1197	F	1.2×10^4	1	3	0.5/1
BC 695	I	5.2×10^8	5	10	1.5/3
BC 693	J	4.4×10^6	3	20	3/6
BC 1805	K	6.3×10^5	1	3	0.5/1
208	UC[a]	1.3×10^6	10	3	1.5/3
Range			1–10	1–20	0.5/1–3/6
Average			4.1	6.8	1.25/2.5

[a] Unclassified.

second or third time after at least 7 days of incubation.

The effect of lincomycin and spectinomycin alone and in various combinations was evaluated against *M. gallisepticum* (strain S-6 JF) by using the "block test design" in 4.5 ml of phenol red broth and 0.5 ml of the antibiotic dilution. The test was carried out in duplicate. The final concentration of the antibiotic was 1 to 20 μg/ml for lincomycin and spectinomycin. The antibiotic-medium mixture was inoculated with 0.1 ml of a 48-hr broth culture of strain S-6 JF. The tubes were examined daily for visible growth or inhibition. After 2, 4, and 6 days of incubation, a loopful from each tube was streaked on agar plates, sealed, and incubated for a period of 7 to 10 days. The plates were examined microscopically for mycoplasma.

RESULTS

The susceptibility of 20 strains of avian mycoplasma to lincomycin and spectinomycin alone

TABLE 3. *Minimal mycoplasmacidal concentration of lincomycin and spectinomycin singly and in combination against 20 strains (12 serotypes) of avian mycoplasma*

Strain no.	Serotype	Inoculum size (CFU/ml)	Lincomycin (μg/ml)	Spectinomycin (μg/ml)	Lincomycin/spectinomycin (μg/ml)
S-6 (JF)	A	2.2×10^5	20	3	1.5/3
BC-801	A	8.6×10^5	20	>20	10/20
St. 19-3E	A	1.0×10^4	20	20	10/20
St. 21-3E	A	3.8×10^8	20	>20	4.5/9
S-6 A	A	2.6×10^5	20	3	1.5/3
BC 1504	B	2.7×10^7	9	>20	1.5/3
BC 859	C	5.0×10^5	20	3	1.5/3
BC 887	D	4.2×10^8	>20	>20	3/6
THY 860	E	6.8×10^7	>20	>20	4.5/9
Fg-7	F	1.4×10^5	20	3	1.5/3
BC 1197	F	1.2×10^4	1	3	0.5/1
867	G	1.6×10^4	1	3	1.5/3
THY886	H	6.8×10^7	3	3	0.5/1
Mm 529	H	2.7×10^6	7	3	0.5/1
Mm TUCO	H	1.5×10^6	10	10	0.5/1
BC 695	I	5.2×10^8	>20	>20	5/10
BC 693	J	4.4×10^6	9	>20	4.5/9
BC 1805	K	6.3×10^5	5	3	1.5/3
BC 694	L	1.3×10^6	3	5	0.5/1
208	UC[a]	1.3×10^6	>20	5	2.5/5
Range			1->20	3->20	0.5/1-10/20
No. of resistant strains[b]			4	7	0

[a] Unclassified.
[b] Number relatively resistant strains with values >20 μg/ml.

TABLE 4. *Inhibitory effect of lincomycin and spectinomycin on Mycoplasma gallisepticum (serotype A, strain JF) after 2 and 7 days of incubation*

Lincomycin (μg/ml)	Spectinomycin (μg/ml)													
	0		1		2		3		4		5		10	
	2[a]	7	2	7	2	7	2	7	2	7	2	7	2	7
0	+[b]	+	+	+	(−)	+	−	(−)	−	−	−	−	−	−
1	+	+	(−)	+	−	(−)	−	−	−	−	−	−	−	−
2	(−)	+	−	+	−	−	−	−	−	−	−	−	−	−
3	−	+	−	+	−	−	−	−	−	−	−	−	−	−
4	−	+	−	(−)	−	−	−	−	−	−	−	−	−	−
5	−	+	−		−	−	−	−	−	−	−	−	−	−
6	−	+	−		−	−	−	−	−	−	−	−	−	−
7	−	(−)	−		−	−	−	−	−	−	−	−	−	−
8	−	−	−		−	−	−	−	−	−	−	−	−	−
9	−	−	−		−	−	−	−	−	−	−	−	−	−
10	−	−	−		−	−	−	−	−	−	−	−	−	−

[a] Day of incubation.
[b] Symbols: +, visible growth in tube; −, no visible growth in tube; (−), MIC.

and in combination is shown in Tables 2 and 3. The MIC was determined for 13 strains which fermented dextrose. The ranges of MIC varied from 1 to 10 μg/ml for lincomycin alone, with an average of 4.1 μg/ml; 1 to 20 μg/ml for spectinomycin alone, with an average of 6.8 μg/ml; and 0.5/1 to 3/6 μg/ml for the lincomycin-spectinomycin combination, with an average of 1.25/2.5 μg/ml. The ranges of MLC were 1 to >20 μg/ml for lincomycin alone; 3 to >20 μg/ml for

TABLE 5. *Mycoplasmacidal activity of lincomycin and spectinomycin on Mycoplasma gallisepticum (serotype A, strain JF)*

Lincomycin (μg/ml)	Spectinomycin (μg/ml)						
	0	1	2	3	4	5	10
0	+[a]	+	+	+	+	(−)	−
1	+	+	+	(−)	−	−	−
2	+	+	+	−	−	−	−
3	+	+	+	−	−	−	−
4	+	(−)	−	−	−	−	−
5	+	−	−	−	−	−	−
6	+	−	−	−	−	−	−
7	+	−	−	−	−	−	−
8	+	−	−	−	−	−	−
9	+	−	−	−	−	−	−
10	(−)	−	−	−	−	−	−
20	−	−	−	−	−	−	−

[a] Symbols: +, microscopic growth; −, no microscopic growth; (−), MLC.

spectinomycin alone; and 0.5/1 to 10/20 μg/ml for the lincomycin-spectinomycin combination (Table 3). At 20 μg/ml, three serotypes (D, E, and I) were resistant to lincomycin and spectinomycin when tested separately, one unclassified dextrose fermenter was resistant to lincomycin alone, and three more serotypes (A, B, and J) were resistant to spectinomycin alone (Table 3). All of the strains tested were sensitive to the lincomycin-spectinomycin combination.

The antimycoplasmal effect of combined lincomycin and spectinomycin as compared to the individual components was determined against strain S-6 JF, A serotype (Tables 4 and 5). The MIC (recorded after 2 days of incubation) was 2 μg/ml for lincomycin or spectinomycin when tested singly and 1/1 μg/ml for the lincomycin-spectinomycin combination. The MIC (recorded after 5 to 7 days of incubation) was 7 μg/ml for lincomycin, 3 μg/ml for spectinomycin, and 1/2 μg/ml for the lincomycin-spectinomycin combination. The MLC was 10 μg/ml for lincomycin, 5 μg/ml for spectinomycin, and 1/3 μg/ml for the lincomycin-spectinomycin combination. Other MLC values of the antibiotic combination were also recorded (Table 5).

DISCUSSION

The results obtained from this study indicate the differences in susceptibility of several strains and serotypes of avian mycoplasma to the action of the antibiotics tested. These data are in general agreement with those reported by others (6, 7). Perlman et al. (6) and Rahman et al. (7) concluded that *Mycoplasma* species isolated from tissue cultures vary widely in their antibiotic susceptibility pattern. In our studies, the ranges of MIC and MLC were greater with lincomycin or spectinomycin alone than with lincomycin and spectinomycin in combination. In addition, some strains were more sensitive to one antibiotic than the other, indicating strain to strain variability to the action of antibiotics. Furthermore, relatively resistant strains, as defined by MLC values greater than 20 μg/ml, were noted only with the single antibiotics as shown in 20% of the strains tested against lincomycin alone and 35% of the strains tested against spectinomycin alone. It is interesting to note that with the antibiotic combination the amount of each antibiotic required to achieve mycoplasmacidal activity against relatively resistant strains was smaller than with the individual components tested alone. This was clearly illustrated in 5 of 20 strains representing A, B, D, E, and I serotypes that required 20 μg/ml or more of the single antibiotic but 1.5/3 to 5/10 μg/ml for the lincomycin-spectinomycin combination. It appeared that greater mycoplasmacidal action was achieved in 45% of the strains tested with the lincomycin-spectinomycin combination than with the single antibiotics. It has been suggested that the use of an antibiotic combination has several potential advantages for antimicrobial therapy, including enhancement of antimicrobial spectrum, especially in serious infections of undetermined or complicated etiology, possible synergistic activity, and reduction or prevention of emergence of resistant microorganisms (4). The lincomycin-spectinomycin combination has a wider range of activity against gram-negative and gram-positive bacteria as well as mycoplasma than the individual components.

The results obtained from the antimycoplasmal action of lincomycin and spectinomycin alone and in various combinations indicate that the biochemical processes of the organisms were slowed by the drug action. However, mycoplasmacidal action was achieved with less lincomycin in the antibiotic combination than with lincomycin alone. These data suggest that one part lincomycin to two or three parts spectinomycin appeared to be the optimum ratio. Lincomycin and spectinomycin combined in a one to two ratio was more effective than the single antibiotics in chickens experimentally infected with mycoplasma and *Escherichia coli* (2). These evaluations were based on increasing the survival rate, reducing the gross lesions of airsacculitis, and improving the weight gain. Similar results were also obtained in turkey poults naturally infected with *M. meleagridis* ("H" serotype) and treated

with the antibiotic combination (3). The data obtained from both in vitro and in vivo studies indicate the superiority of the lincomycin-spectinomycin combination over the single antibiotics.

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