SENSITIVITIES IN VITRO TO ANTIBIOTICS OF MYCOPLASMA ISOLATED FROM CANINE SOURCES

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(Received for Publication December 15, 1971)

Recently, various antibiotics have very frequently been used for the prevention and chemotherapy of Mycoplasma infections in man and domestic animals and for the exclusion of contamination of Mycoplasma in tissue culture. So, it is very important to select drugs especially effective against many strains of Mycoplasma belonging to various species. Concerning the susceptibility to antibiotics, *M. pneumoniae* has been studied by many workers^{1,5,7,12}. Some other species of human origin, including *M. hominis*, *M. fermentans*, *M. salivarium*, and *M. orale*, have also been examined recently^{2,5,11,14}. Of the species of Mycoplasma isolated from animals, *M. gallisepticum* has been studied by several authors^{4,6,8,13}.

Lately, OGATA et al.¹⁰ mentioned in detail about the *in vitro* sensitivities of fifteen strains of Mycoplasma isolated from various animals to twenty-two antibiotics. The present report is concerned with experiments on the *in vitro* sensitivities of 103 strains isolated from dogs at this laboratory and of three standard canine strains to antibiotics. For comparison, some strains derived from sources other than the dog were tested by the same methods.

MATERIALS AND METHODS

1. Test organisms

A total of 103 strains of Mycoplasma isolated by the authors from seventy-three healthy and twenty diseased dogs were used for antibiotic sensitivity tests. They were divided into four groups; that is, a group of *M. canis* consisting of fifty-five strains, one of *M. spumans* of eleven strains, one of *M. maculosum* of seven strains, and one of the remaining thirty unclassified strains. The identification of them was made by the growth inhibition test performed by the method of CLYDE³ and by comparison of biological properties between them and the standard strains. The following strains were supplied by Professor M. OGATA, of the University of Tokyo, as standard ones and used for the test: *M. mycoides* var. *mycoides* (PG 1), *M. agalactiae* (PG 2), *M. laidlawii* A (PG 8), *M. laidlawii* B (PG 10), *M. bovigenitalium* (PG 11), *M. spumans* (PG 13), *M. canis* (PG 14), *M. hominis* type 1 (PG 21), *M. hominis* type 2 (PG 27), *M. neurolyticum* (PG 28), *M. hyorhinae* (PG 29), *M. iners* (PG 30), *M. gallisepticum* (PG 31), and *M. maculosum* (D₂₂Tr45). All the standard strains used, except the one last named, had originally been classified by Dr. D. G. ff. EDWARD of the Wellcome Research Laboratories, England. The exceptional one had been isolated at the Department of Veterinary Microbiology, University of Tokyo. All the strains tested were maintained in modified EDWARD's medium⁹ for passage at the authors' laboratory.

Table 1. Test Samples of Antibiotics

Macrolides	Erythromycin ¹⁾
	Leucomycin ¹⁾
	Tylosin ³⁾
	Oleandomycin ²⁾
	Spiramycin ¹⁾
	Josamycin ¹⁾
Tetracyclines	Tetracycline ²⁾
	Chlortetracycline ¹⁾
	Oxytetracycline ²⁾
	Methacycline ²⁾
Glycosides	Dihydrostreptomycin ¹⁾
	Kanamycin ¹⁾
	Fradiomycin ¹⁾
Peptides	Colistin ¹⁾
	Polymyxin-B ¹⁾
	Bacitracin ¹⁾
	Viomycin ²⁾
Miscellaneous	Chloramphenicol ¹⁾
	Novobiocin ¹⁾
	Amphotericin-B ¹⁾
	Trichomycin ¹⁾
	Lincomycin ¹⁾

Remarks.

- 1) Standard preparations supplied by the National Institute of Health, Tokyo.
- 2) Standard preparations supplied by the Pfizer-Taito Co., Ltd., Tokyo.
- 3) Commercial product of the Shionogi Pharmaceutical Co., Ltd.

2. Antibiotics

Twenty-two antibiotics, as shown in Table 1, were used.

3. Experimental procedures

Liquid and agar media prepared under the formula of modified EDWARD's medium⁹⁾, except that penicillin and thallium acetate had been omitted, were used for culture media. The water-soluble antibiotics were diluted with sterile distilled water to the concentration of 1,000 $\mu\text{g}/\text{ml}$, whereas the water-insoluble substances were diluted to 10,000 $\mu\text{g}/\text{ml}$ with a solvent, which was dimethyl sulfoxide for amphotericin-B and methanol for the remainder.

All the antibiotic solutions were diluted serially with sterile distilled water and added aseptically to agar media at the following final concentrations: 100 $\mu\text{g}/\text{ml}$, 50 $\mu\text{g}/\text{ml}$, 10 $\mu\text{g}/\text{ml}$, 1 $\mu\text{g}/\text{ml}$, 0.5 $\mu\text{g}/\text{ml}$, 0.25 $\mu\text{g}/\text{ml}$, 0.1 $\mu\text{g}/\text{ml}$, and 0.05 $\mu\text{g}/\text{ml}$.

One loopful of fresh liquid culture containing about 10^6 to 10^8 CFU/ml of each test Mycoplasma species was streaked on an agar plate and incubated at 37°C. After incubation for two to four days, the minimum inhibitory concentration (MIC) for each drug was determined by microscopic observation on the growth of the test organism.

Table 2. Sensitivities to 22 Antibiotics of 55 Strains of *Mycoplasma canis* Isolated from Canine Sources and of Standard Strain *M. canis* PG 14

Antibiotic	Minimum inhibitory concentration ($\mu\text{g/ml}$)									
	0.05	0.1	0.25	0.5	1	5	10	50	100	>100
Macrolides	Erythromycin							9*	10,C**	36
	Leucomycin		1	5	18,C	26	5			
	Tylosin	2	17	32	44,C					
	Oleandomycin								2	53,C
	Spiramycin			1	4	16,C	32	2		
	Josamycin			1	5	19,C	30			
Tetracyclines	Tetracycline		1		3	6	34,C	10	1	
	Chlortetracycline						1	10	40	4,C
	Oxytetracycline						1	2	32	18
	Methacycline					4	47,C	4		2,C
Glycosides	Dihydrostreptomycin						4	19	23,C	5
	Kanamycin				1	3	41	6,C	4	
	Fradiomycin						1	2,C	28	15
Peptides	Colistin									55,C
	Polymyxin-B								1	54,C
	Bacitracin									55,C
	Viomycin						1	4,C	23	27
Miscellaneous	Chloramphenicol					4	36	17,C	1	
	Novobiocin						18,C	17	16	1
	Amphotericin-B						1	11	24	13,C
	Trichomycin				2	27	24,C		2	
	Lincomycin		3	15	13	19	5,C			

Remarks. * The number of strains showing inhibited growth.

** Standard strain *M. canis* PG 14.

RESULTS

1. Sensitivities of strains isolated from dogs

Tables 2~5 show the sensitivities of the three groups of classified strains, that is, *M. canis*, *M. spumans*, and *M. maculosum*, including the standard strains of these three species, and the group of unclassified strains. They also present the MIC of twenty-two antibiotics. These results are summarized in Table 6 with the percentage of strains inhibited by two concentrations of an antibiotic, i.e., below 1 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$.

All the substances effective at an MIC of less than 1 $\mu\text{g/ml}$ were evaluated for inhibitory activity. As a result, tylosin was the most active, inhibiting 100% of the strains of the three classified groups and 93.33% of the strains of the unclassified group. It inhibited all the strains of these four groups at an MIC below 5 $\mu\text{g/ml}$. Next to tylosin, three macrolides (leucomycin, josamycin, and spiramycin) and lincomycin were the second most active. Two tetracyclines (tetracycline and methacycline), chloramphenicol, and trichomycin were fairly active.

In spite of their being members of the macrolides, erythromycin and oleandomycin had very low activities over all the canine strains. The four peptides, viz., colistin, polymyxin-B, bacitracin, and viomycin, showed no effect against these strains.

It was observed that three standard strains, *M. canis* PG 14, *M. spumans* PG 13, and

Table 3. Sensitivities to 22 Antibiotics of 11 Strains of *Mycoplasma spumans* Isolated from Canine Sources and of Standard Strain *M. spumans* PG 13

	Antibiotic	Minimum inhibitory concentration ($\mu\text{g/ml}$)									
		0.05	0.1	0.25	0.5	1	5	10	50	100	>100
Macrolides	Erythromycin								3*	2,S**	6
	Leucomycin		1		1	2	7	S			
	Tylosin	2	1	2	5	1,S					
	Oleandomycin										11,S
	Spiramycin				2	2	2	2	3,S		
	Josamycin			1	2	3,S	5				
Tetracyclines	Tetracycline				1	2	4,S	3	1		
	Chlortetracycline						1	1	9	S	
	Oxytetracycline						1	1	3	4,S	2
	Methacycline					3	2	6,S			
Glycosides	Dihydrostreptomycin							3	1		7,S
	Kanamycin						2		7	2	S
	Fradiomycin								2	1	8,S
Peptides	Colistin										11,S
	Polymyxin-B										11,S
	Bacitracin										11,S
	Viomycin								1	4	6,S
Miscellaneous	Chloramphenicol					3	3	5,S			
	Novobiocin							1	10	S	
	Amphotericin-B						1	2	3		5,S
	Trichomycin				1	1	1	2	2	4,S	
	Lincomycin		2	1	1	1	2,S	5			

Remarks. * The number of strains showing inhibited growth.

** Standard strain *M. spumans* PG 13

M. maculosum D₂₂Tr45, were much less sensitive to all the drugs than the strains isolated by the authors (Tables 2~5). Comparison of sensitivity among the four groups of strains revealed that those strains less sensitive to all the drugs belonged mainly to the group of *M. spumans* or that of unidentified strains.

2. Sensitivities of strains isolated from sources other than dogs

As shown in Table 7, eleven strains classified by EDWARD were very sensitive to leucomycin, tylosin, josamycin, tetracycline, methacycline, and lincomycin at low concentrations. These results are the same as those of the experiments on canine strains. Of these six antibiotics, tylosin inhibited all the organisms tested at a concentration of 1 $\mu\text{g/ml}$ or less. Leucomycin, josamycin, and tetracycline showed an anti-mycoplasmal effect against many strains at a concentration less than 1 $\mu\text{g/ml}$, except a few strains against which 5 $\mu\text{g/ml}$ of each drug per milliliter was necessary as the MIC.

It was observed that the variability of MIC of each drug between any two different species isolated from some other animal species was more marked than that between any two different species of the canine strains.

Colistin, polymyxin-B, and bacitracin failed to show any effect against all the strains, including the canine strains. Fradiomycin, viomycin, novobiocin, amphotericin-B, and trichomycin were effective against a few species, but not so effective against any other species.

Table 4. Sensitivities to 22 Antibiotics of 7 Strains of *Mycoplasma maculosum* Isolated from Canine Sources and of Standard Strain *M. maculosum* D₂₂Tr45

Antibiotic	Minimum inhibitory concentration ($\mu\text{g/ml}$)									
	0.05	0.1	0.25	0.5	1	5	10	50	100	>100
Macrolides	Erythromycin							2*	5, M**	
	Leucomycin		1, M		5	1				
	Tylosin	3	3, M	1						
	Oleandomycin									7, M
	Spiramycin			1		6	M			
	Josamycin			2	4	M	1			
Tetracyclines	Tetracycline				2, M	4	1			
	Chlortetracycline						2	2	3, M	
	Oxytetracycline						1	2	4, M	
	Methacycline				2		5, M			
Glycosides	Dihydrostreptomycin						3, M	2	1	1
	Kanamycin					3	2	2, M		
	Fradiomycin							1		6, M
Peptides	Colistin									7, M
	Polymyxin-B									7, M
	Bacitracin									7, M
	Viomycin							1, M	6	
Miscellaneous	Chloramphenicol					5	2, M			
	Novobiocin						2	4, M		1
	Amphotericin-B						2	5	M	
	Trichomycin						6, M	1		
	Lincomycin	1	1	4	1		M			

* The number of strains showing inhibited growth.

** Standard strain of *M. maculosum*.

DISCUSSION

From the results mentioned above, it was confirmed that tylosin was the most inhibitory to all the *Mycoplasma* strains tested, and that leucomycin, josamycin, and lincomycin were the second most inhibitory of the twenty-two antibiotics studied. Tetracycline and methacycline were proved to be highly effective against all the test organisms.

Recently, OGATA et al.¹⁰⁾ reported tylosin had been the most effective of the twenty-two antibiotics tested, inhibiting 83.33% and 100% of *Mycoplasma* strains isolated from animals at the concentrations of less than 1 $\mu\text{g/ml}$ and 5 $\mu\text{g/ml}$, respectively. Furthermore, MATSUI et al.⁶⁾, STEWART et al.¹²⁾, and NEWNHAM and CHU⁸⁾ observed that tylosin was most inhibitory to various *Mycoplasma* species.

Of the macrolide antibiotics, leucomycin, tylosin, spiramycin, and josamycin were highly active against various *Mycoplasma* strains, but erythromycin and oleandomycin showed only low or no activities against canine strains and some other species, such as *M. agalactiae*, *M. bovis genitalium*, *M. hominis*, and *M. hyorhinis*. It was evident that there were such variabilities in sensitivity as shown in Table 7 among different species of *Mycoplasma* to erythromycin and oleandomycin. For example, *M. mycoides* var.

Table 5. Sensitivities to 22 Antibiotics of 30 Unclassified Strains of *Mycoplasma* Isolated from Canine Sources

Antibiotic	Minimum inhibitory concentration ($\mu\text{g/ml}$)									
	0.05	0.1	0.25	0.5	1	5	10	50	100	>100
Macrolides	Erythromycin							1*	8	21
	Leucomycin		1	1	5	19	3	1		
	Tylosin	2	3	10	3	10	2			
	Oleandomycin					1	2			27
	Spiramycin					7	13	2	8	
	Josamycin		3		7	6	14			
Tetracyclines	Tetracycline				2	2	10	16		
	Chlortetracycline						2	4	22	2
	Oxytetracycline						1	3	11	12
	Methacycline				2	2	18	8		3
Glycosides	Dihydrostreptomycin						6	8	6	10
	Kanamycin					2	16	7	4	1
	Fradiomycin							7	7	16
Peptides	Colistin									30
	Polymixin-B									30
	Bacitracin									30
	Viomycin						1	1	23	5
Miscellaneous	Chloramphenicol				1	1	13	13	2	
	Novobiocin							1	16	3
	Amphotericin-B						1	10	13	5
	Trichomycin				2	8	12	6	1	1
	Lincomycin		2	4	4	5	15			

Remarks. * The number of strains showing inhibited growth.

mycoides, *M. laidlawii* A and B, and *M. gallisepticum* were very sensitive to both drugs. These results agree to those of the experiment made by OGATA et al.¹⁰⁾ Sensitivities to active antibiotics were compared among the strains classified into *M. canis*, *M. spumans*, and *M. maculosum*. As a results, more strains of relatively low sensitivity were found among those classified into *M. spumans* than among those into any other species. Of the canine strains studied, the three standard strains (*M. canis* PG 14, *M. spumans* PG 13, and *M. maculosum* D₂₂Tr45) showed a tendency to resist to a larger number of active antibiotics than the strains of each of the same species. Any reason for this result is unknown. It is presumed that a considerable number of canine strains tested may have been relatively fresh cultures, because they have been transferred only two or three times after isolation, and that they may be rather sensitive than the standard strains.

SUMMARY

A total of 103 canine strains of *Mycoplasma*, consisting of 55 strains of *M. canis*, 11 strains of *M. spumans*, 7 strains of *M. maculosum*, and 30 unclassified strains, were isolated by the authors. In addition to them, three standard strains (*M. canis* PG 14, *M. spumans* PG 13, and *M. maculosum* D₂₂Tr45) were examined for sensitivity to 22 antibiotics. The results obtained were compared with the susceptibilities of 11 standard strains of *Mycoplasma* originated from sources other than the dog.

Table 6. Percentage of Strains Inhibited by a Concentration of less than 1 $\mu\text{g/ml}$ or 5 $\mu\text{g/ml}$ of Each Antibiotic

		<i>M. canis</i> $\mu\text{g/ml}$		<i>M. spumans</i> $\mu\text{g/ml}$		<i>M. maculosum</i> $\mu\text{g/ml}$		Unclassified $\mu\text{g/ml}$	
		1	5	1	5	1	5	1	5
Macrolides	Erythromycin	0	0	0	0	0	0	0	0
	Leucomycin	91.07	100.0	33.33	91.66	100.0	100.0	86.66	96.66
	Tylosin	100.0	100.0	100.0	100.0	100.0	100.0	93.33	100.0
	Spiramycin	39.28	96.42	33.33	50.0	87.50	100.0	23.33	66.66
	Josamycin	46.42	100.0	58.83	100.0	87.50	100.0	53.33	100.0
Tetracyclines	Tetracycline	17.85	80.35	25.0	66.66	87.50	100.0	13.33	46.66
	Chlortetracycline	0	1.78	0	8.33	0	25.0	0	6.66
	Oxytetracycline	0	1.78	0	8.33	0	12.5	0	3.33
	Methacycline	7.14	92.85	25.0	41.66	25.0	100.0	13.33	73.33
Glycosides	Dihydrostreptomycin	0	7.14	0	0	0	0	0	0
	Kanamycin	7.14	80.35	0	16.66	0	37.5	6.66	60.0
	Fradiomycin	0	1.78	0	0	0	0	0	0
Peptides	Colistin	0	0	0	0	0	0	0	0
	Polymyxin-B	0	0	0	0	0	0	0	0
	Bacitracin	0	0	0	0	0	0	0	0
	Viomycin	0	0	0	0	0	0	0	0
Miscellaneous	Chloramphenicol	7.14	71.42	25.0	50.0	62.5	100.0	6.66	50.0
	Novobiocin	0	33.92	0	0	0	0	0	0
	Amphotericin-B	0	1.78	0	8.33	0	0	0	3.33
	Trichomycin	51.78	100.0	16.66	25.0	0	87.5	33.33	73.33
	Lincomycin	89.28	100.0	41.66	58.33	87.5	100.0	50.0	100.0

The results are summarized as follows.

(1) All the strains tested, regardless of the origin, were sensitive to four macrolides (leucomycin, tylosin, spiramycin, and josamycin), two tetracyclines (tetracycline, and methacycline), chloramphenicol, and lincomycin.

(2) Tylosin was the most active of all the drugs used, inhibiting over 98% of the canine strains and 100% of the standard strains at a concentration of 1 $\mu\text{g/ml}$ or less.

(3) As reported by previous authors, a wide range of variation of sensitivity to several drugs, especially to erythromycin and oleandomycin, was found among the species of *Mycoplasma* examined.

(4) Sensitivity to active antibiotics was compared among the strains classified into *M. canis*, *M. spumans*, and *M. maculosum*. A larger number of strains of relatively low sensitivity were found among those classified into *M. spumans* than those into any other species.

(5) The 3 standard strains, *M. canis* PG 14, *M. spumans* PG 13, and *M. maculosum* D₂₂Tr45, were rather less sensitive to all the drugs used than the 103 strains isolated by the authors. It was presumed that these strains were highly sensitive, probably because not much time had passed after isolation of them, as compared with the standard strains.

(6) Four peptides, viz., colistin, polymyxin-B, bacitracin, and viomycin, showed no activity upon any *Mycoplasma* strain.

Table 7. Sensitivities to 22 Antibiotics of 11 Standard Strains Isolated from Sources Other than Dog

Antibiotic	Minimum inhibitory concentration ($\mu\text{g/ml}$)										
	PG 1*	PG 2	PG 8	PG 10	PG 11	PG 21	PG 27	PG 28	PG 29	PG 30	PG 31
Macrolides	Erythromycin	0.05	100	0.05	50	>100	>100	10	100	>100	0.05
	Leucomycin	0.5	1	1	0.5	1	0.25	0.25	0.25	5	0.05
	Tylosin	0.05	0.5	0.1	0.1	1	1	0.5	0.1	0.5	0.05
	Oleandomycin	5	>100	0.5	1	>100	>100	100	>100	>100	0.5
	Spiramycin	0.5	1	1	1	0.5	1	0.5	0.5	50	0.5
	Josamycin	0.1	5	1	1	1	0.25	0.1	0.25	5	0.05
Tetracycline	Tetracycline	0.1	0.5	5	1	0.1	1	0.05	0.05	5	0.5
	Chlortetracycline	1	50	100	100	5	>100	100	0.5	100	10
	Oxytetracycline	5	50	100	50	50	50	100	0.25	100	50
	Methacycline	0.25	5	5	5	0.25	5	0.05	0.05	10	1
Glycosides	Dihydrostreptomycin	10	10	10	10	50	>100	>100	>100	>100	50
	Kanamycin	50	10	50	50	50	5	10	5	100	100
	Fradiomycin	>100	100	50	10	50	>100	10	10	>100	>100
	Colistin	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Peptides	Polymixin-B	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
	Bacitracin	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
	Viomycin	>100	>100	100	100	50	10	10	10	>100	>100
	Chloramphenicol	1	5	10	10	5	10	1	1	5	5
Miscellaneous	Novobiocin	50	100	50	10	50	>100	50	5	50	5
	Amphotericin-B	100	5	>100	>100	10	>100	10	5	>100	50
	Trichomycin	5	5	100	>100	5	10	5	5	>100	5
	Lincomycin	0.5	1	0.25	0.25	0.5	5	0.5	0.5	5	10

Remarks.

* PG 1: *M. mycoides* var. *mycoides*, PG 2: *M. agalactiae*, PG 8: *M. laidlawii* A, PG 10: *M. laidlawii* B, PG 11: *M. bovis*, PG 21: *M. hominis* type 1, PG 27: *M. hominis* type 2, PG 28: *M. neurolyticum*, PG 29: *M. hyorhinis*, PG 30: *M. iners*, and PG 31: *M. gallisepticum*.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Manabu OGATA, of the Faculty of Agriculture, University of Tokyo, for supply of the standard strains of Mycoplasma. They also express their appreciation to Mr. Masaro YAMAZAKI, of the National Institute of Health, Tokyo, and to the Pfizer-Taito Co., Ltd., for supply of standard samples of antibiotics.

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犬由来 *Mycoplasma* 株の抗生物質感受性

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(昭和46年12月15日受付)

健康犬および病犬から分離した 103 株と、標準 14 株の *Mycoplasma* (以下 *M* と略す) が、22 種類の抗生物質に対する感受性を試験した。

22 物質のうち、マクロライド 4 種 (ロイコマイシン, タイロシン, スピラマイシン, ジョサマイシン), テトラサイクリン類 2 種 (テトラサイクリン, メタサイクリン), クロラムフェニコール, およびリンコマイシンが多くの種類の *M* の発育を抑制した。

特にタイロシンは犬由来株の 98 %, 標準株の 100 % を $1\mu\text{g/ml}$ 以下の濃度で抑制した。これに次いで、リンコマイシン, ロイコマイシンおよびジョサマイシンが大多数の株の発育を $5\mu\text{g/ml}$ 以下で阻止した。

多くの研究者が指摘しているように、菌種間の

感受性の差が各薬剤について見られた。特にエリスロマイシンとオレアンドマイシンにおいて顕著であった。

犬由来株には、*M. spumans* および未同定株に各薬剤に対して、感受性の低い株が多く含まれ、*M. canis* と *M. maculosum* に感受性の高い株が多い傾向が見られた。

犬由来標準株と著者らの分離株を比較すると、各薬剤に対する感受性の低下が標準株において見られた。その理由の一つとして、著者らの株は分離後 2~3 回の継代を経験したのみで、新鮮な株が多いことが考えられる。

ペプチド 4 種 (コリスチン, ポリミキシン B, バシトラシン, ヴァイオマイシン) は、すべての *M* に全く、またはほとんど無効であった。