The Fractional Inhibitory Concentration Index of Antimicrobial Agents for Bacteria and *Mycoplasma* Isolated from the Nasal Swabs of Cattle with Respiratory Diseases

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ABSTRACT. We investigated the effect of thiamphenicol plus lincomycin (TP + LCM) and thiamphenicol plus tylosin (TP + TS) combinations using checker board method on the growth of *Pasteurella* (*P.*) *multocida*, *P. haemolytica* and *Mycoplasma* (*M.*) *bovis* by calculating the fractional inhibitory concentration index (FIC index). The results showed that the FIC indexes of the TP + LCM combination for *P. multocida*, *P. haemolytica* and *M. bovis* were 0.36 ± 0.10 , 0.72 ± 0.09 and 0.81 ± 0.18 , respectively. The FIC indexes of the TP + TS combination for *P. multocida*, *P. haemolytica*, and *M. bovis* were 0.79 ± 0.20 , 0.66 ± 0.11 and 0.32 ± 0.14 , respectively. Thus, these combinations are assumed to have a more synergistic or additive effect on bacteria growth than a single antimicrobial agent.

— KEY WORDS: antimicrobial combination, bovine respiratory disease, fractional inhibitory concentration index.

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Respiratory diseases in young cattle are known to be caused by infection with pathogenic organisms such as viruses, bacteria and *Mycoplasma* [1-3, 7, 8, 14, 16, 19, 21]. Despite the use of vaccines as a preventive measure in recent years, the incidence of respiratory diseases still remains high. Although antimicrobial agents are used to treat the cases of respiratory diseases in farm cattle, investigation is needed to identify the causative organisms and decide the best drug to use in treatment. Thus, we carried out bacteriologic examination of nasal swab samples taken from 329 cattle with respiratory diseases over a 10-year period from 1986 to 1995, and found infection of *Pasteurella* spp. or *Mycoplasma* spp. in 271 (82.4%) of the cattle [12]. We also found that 105 (38.7%) of the cattle had a mixed infection [12]. At present, in the cases of mixed infection, there are very few drugs which have an antimicrobial effect on both causative agents. Although there have been many studies which investigated the minimum inhibitory concentration (MIC) of a single antimicrobial agent for bacteria isolated from cattle with respiratory diseases [4, 6, 12, 13, 20], there have been few studies of the MIC in the case of a combined use of antimicrobial agents. Therefore, we investigated the susceptibility of *Pasteurella* spp. and *Mycoplasma* spp. using

a combination of antimicrobial agents.

As a preliminary experiment, we investigated the susceptibility of *Pasteurella multocida* (*P. multocida*), *Pasteurella haemolytica* (*P. haemolytica*) and *Mycoplasma bovis* (*M. bovis*) using a single antimicrobial agent. The number of strains tested were 59 of *P. multocida*, 21 of *P. haemolytica* and 35 of *M. bovis*, and the MIC of each antimicrobial agent was measured according to the standard method of the Japan Chemotherapy Society [9, 10]. The concentration which inhibited growth by 50% (MIC₅₀) was also determined for each strain. Seven antimicrobial agents were used: aminobenzylpenicillin (ABPC), kanamycin (KM), oxytetracycline (OTC), tylosin (TS), lincomycin (LCM), thiamphenicol (TP), and enrofloxacin (ERFX). *P. multocida* and *P. haemolytica* showed a high susceptibility to ERFX, ABPC and TP, and *M. bovis* showed a high susceptibility to ERFX, TS and LCM (Table 1). We selected TP for *Pasteurella* and LCM and TS for *Mycoplasma*, and investigated the effect of TP plus LCM (TP + LCM) and TP plus TS (TP + TS) combinations using the checker board method [17]. Studies were carried out on 8 strains that were chosen at random on each bacterium, and the effect of the antimicrobial agent combinations on each strain was investigated by calculating the fractional inhibitory

Table 1. Antimicrobial susceptibility of isolates (MIC^{a)}; µg/ml)

Antimicrobial agents	<i>P^{b)}. multocida</i>		<i>P^{b)}. haemolytica</i>		<i>M^{c)}. bovis</i>	
	MIC ₅₀	Range ^{d)}	MIC ₅₀	Range	MIC ₅₀	Range
ABPC ^{e)}	0.05 (45 ^{b)})	0.025 - 0.10	0.10 (19)	0.10	25 (25)	25
KM ^{f)}	6.25 (59)	0.20 - 100	3.13 (21)	3.13 - 100	6.25 (35)	3.13 - 12.5
OTC ^{g)}	6.25 (59)	0.39 - 50	6.25 (21)	0.78 - 100	12.5 (35)	0.78 - 25
LCM ^{h)}	50 (14)	25 - 100	100 (2)	100	0.78 (10)	0.39 - 1.56
TS ⁱ⁾	6.25 (45)	0.78 - 50	50 (19)	25 - 50	0.78 (25)	0.20 - 3.13
TP ^{j)}	0.78 (59)	0.39 - 1.56	1.56 (21)	1.56 - 3.13	3.13 (35)	0.78 - 12.5
ERFX ^{k)}	≤0.013 (18)	≤0.013	0.025 (7)	0.025	0.39 (10)	0.39

a) Minimum inhibitory concentration. b) *Pasteurella*. c) *Mycoplasma*. d) Range of MIC. e) Aminobenzylpenicillin. f) Kanamycin. g) Oxytetracycline. h) Lincomycin. i) Tylosin. j) Thiamphenicol. k) Enrofloxacin. l) Number of strains.

Table 2. FIC^{a)} index of 2 way-combinations against isolates

	TP ^{b)} + LCM ^{c)}	TP + TS ^{d)}
<i>P. multocida</i> (8 ^{e)})	0.36 ± 0.10 (0.25–0.05) ^{f)}	0.79 ± 0.20 (0.53–1.0)
<i>P. haemolytica</i> (8)	0.72 ± 0.09 (0.50–0.75)	0.66 ± 0.11 (0.56–0.75)
<i>M. bovis</i> (8)	0.81 ± 0.18 (0.75–1.0)	0.32 ± 0.4 (0.06–0.56)

a) Fractional inhibitory concentration. b) Thiamphenicol. c) Lincomycin.

d) Tylosin. e) Number of strains. f) Mean ± SD (range).

concentration index (FIC index) [5, 17, 18].

The results showed that using the TP + LCM combination, the FIC indexes for *P. multocida*, *P. haemolytica* and *M. bovis* were 0.36 ± 0.10, 0.72 ± 0.09 and 0.81 ± 0.18, respectively. The FIC indexes of the TP + TS combination for *P. multocida*, *P. haemolytica* and *M. bovis* were 0.79 ± 0.20, 0.66 ± 0.11 and 0.32 ± 0.14, respectively (Table 2). Generally, in tests using the checker board method, the combined effect of two antimicrobial agents is synergistic if the FIC index is ≤ 0.75, additive if the FIC index is ≤ 1.0, indifferent if the FIC index is >1.0 and antagonistic if the FIC index is >2.0 [17]. Thus, the combination of TP + LCM or TP + TS was assumed to have a more synergistic or additive effect on bacteria growth than a single antimicrobial agent. Jawetz and Gunnison [11] divided clinically-effective antimicrobial agents into bactericidal and bacteriostatic groups according to their effect, and investigated their combined effect *in vitro*. They reported that in many cases, the combined use of 2 bactericidal agents showed a synergistic effect, the combined use of 2 bacteriostatic agents showed an additive effect, and the combined use of 1 bactericidal agent and 1 bacteriostatic agent showed an antagonistic effect. The antimicrobial agents used in the present study were bacteriostatic-type, and our results, which showed a synergistic or additive effect by the combined use of the agents, were very similar to those by Jawetz and Gunnison [11].

The main purpose of a combined use of antimicrobial agents is to expand the antimicrobial spectrum and increase the antimicrobial activity. We believe that the results of this study have attained these goals. Also, the results of the susceptibility tests carried out in this study showed that the new quinolone ERFX has a high antimicrobial activity to both *Pasteurella* and *Mycoplasma*. However, past experience has shown that excessive use of an antibiotic will lead to a decline in the clinical effect [12, 15]. Thus, the excessive use of one type of drug must be avoided. The use of a combination of drugs would not only reduce the frequency of use of any one drug, but may also be important in the choice of antimicrobial agents for treatment.

This study has shown that, *in vitro*, the combined use of antimicrobial agents (TP + LCM and TP + TS) has a synergistic or additive effect on the bacteria and *Mycoplasma* isolated from cattle with bovine respiratory diseases. Further investigation of the combined effect of antimicrobial agents *in vivo* is planned for the future.

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REFERENCES

1. Baudet, H. M., Chieze, C., and Espinasse, J. 1994. *Recl. Med. Vet.* 170: 209–216.
2. Binder, A., Amtsberg, G., Dose, S., Fischer, W., Scholtz, H., and Kirchhoff, H. 1990. *J. Vet. Med. Ser. B* 37: 430–435.
3. Carter, G. R. 1973. *J. Am. Vet. Med. Assoc.* 163: 863–864.
4. Cooper, A. C., Fuller, J. R., Fuller, M. K., Whittlestone, P., and Wise, D. R. 1993. *Res. Vet. Sci.* 54: 124–126.
5. Elion, G. B., Singer, S., and Hitchings, G. H. 1954. *J. Biol. Chem.* 208: 477–488.
6. Franklin, A., Horn af Rantzien, M., Rehbinder, V., Segall, T., and Viring, S. 1993. *Med. Vet.* 10: 230–232.
7. Gourlay, R. N., Thomas, L. H., and Howard, C. J. 1976. *Vet. Rec.* 98: 506–507.
8. Gourlay, R. N., Thomas, L. H., and Wyld, S. G. 1989. *Vet. Rec.* 124: 420–422.
9. Japan Chemotherapy Society. 1981. *Chemotherapy* 29: 76–79.
10. Japan Chemotherapy Society. 1990. *Chemotherapy* 38: 103–105.
11. Jawetz, E. and Gunnison, L. B. 1952. *Antibiot. Chemother.* 2: 243–248.
12. Katoh, T., Koya, M., Watanabe, E., Sakai, J., Ogata, Y., and Hikinuma, T. 1996. *J. Jpn. Vet. Med. Assoc.* 49: 81–84 (in Japanese with English summary).
13. Katoh, T., Sakai, J., Ogata, Y., Abe, S., and Kimura, M. 1996. *J. Jpn. Vet. Med. Assoc.* 49: 149–153 (in Japanese with English summary).
14. Knudtson, W. U., Reed, D. E., and Daniels, G. 1986. *Vet. Microbiol.* 11: 79–91.
15. Kudou, R., Hiroshima, S., Tukahara, K., Sakamoto, T., Hirano, T., and Nakata, Y. 1994. *J. Jpn. Vet. Med. Assoc.* 47: 311–314 (in Japanese with English summary).
16. Langford, E. V. 1977. *Can. J. Comp. Med.* 41: 89–94.
17. Perea, E. J., Torres, M. A., and Borobio, M. V. 1978. *Antimicrob. Agents Chemother.* 13: 705–709.
18. Phillips, I. and Warren, C. 1976. *Antimicrob. Agents Chemother.* 9: 736–740.
19. Thomas, L. H., Stott, E. J., Jones, P. W., Jebbett, N. J., and Collins, A. P. 1980. *Vet. Rec.* 107: 304–307.
20. Watts, J. L., Yancey, R. J., Salmon, S. A., and Case, C. A. 1994. *J. Clin. Microbiol.* 32: 725–731.
21. Yamamoto, K., Harasawa, R., Ogata, M., Miura, T., and Nakane, H. 1976. *Jpn. J. Vet. Sci.* 38: 7–14.