NOTE

Selective Medium for Isolation of Ureaplasmas from Animals

Kaoru KOSHIMIZU, Masahiro ITO, Teruo MAGARIBUCHI, and Hitoshi KOTANI¹⁾

Division of Animal Research, Faculty of Medicine, University of Tokyo, Bunkyo, Tokyo 113,
and ¹⁾Imamichi Institute for Animal Reproduction, Niihari, Ibaraki, 300-01

(Received 1 October 1982/Accepted 18 January 1983)

Since the first isolation of ureaplasmas from human urogenital tracts by Shepard [22] in 1954, these organisms have been found to be harbored in several species of primates [2, 4, 14, 18, 27], carnivora [3, 8, 11, 13, 26, 27], artiodactyla [5–7, 15, 16, 20, 25, 28], proboscidae [29], and aves [9, 10, 24]. However, in spite of our intensive studies [12], ureaplasmas have not been isolated from other animal species.

There have been some difficulties in isolating ureaplasmas from animals. A major problem is that the overgrowth of so-called large colony mycoplasmas on isolation media has frequently made isolation of ureaplasmas difficult. Therefore, it seems to be of the uppermost importance to make a selective medium for isolation of ureaplasmas from animals.

Shipley and Bowman [23] first reported in 1968 that human ureaplasmas were resistant to lincomycin. Since then this agent has been added to the medium for recovery and purification of human ureaplasmas from mixed cultures containing both *Ureaplasma urealyticum* and *Mycoplasma hominis* [1, 19, 21], because the growth of the latter is inhibited by lincomycin. Nagatomo and Shimizu [17] reported that minimum inhibitory concentrations (MIC) of lincomycin for 11 strains of bovine ureaplasmas were 50 to

100 μg per ml. However, there has been no report on the sensitivities to lincomycin of ureaplasmas from other animals. In the present study, the MICs of lincomycin for various strains and species of ureaplasmas, mycoplasmas and achleoplasmas were compared, and a suitable concentration of lincomycin for selective isolation of animal ureaplasmas was determined. The strains of these organisms are listed in Table 1. The liquid medium used in the test was T-broth described previously [10]. The MICs of lincomycin were defined by the lowest concentrations of the drug preventing color change of the medium in microtiter wells containing 10^3 to 10^4 color changing unit per ml of the test organisms, when the pH of the control wells containing organisms in the medium without the drug reached 7.8.

It was disclosed that ureaplasmas of animal origin were resistant to lincomycin as shown in Table 1, and 12.5 to 100 μg per ml of the drug were the minimum inhibitory concentrations for human, bovine, simian, canine, feline and avian ureaplasmas. On the contrary, MICs of lincomycin for various species of mycoplasmas and achleoplasmas were between 0.2 and 3.13 μg per ml, except those for *M. capricolum* California kid, *M. gallisepticum* PG31, and *M. gallisepticum* S6 being 25.0, 25.0 and 12.5 μg per ml,

Table 1. Sensitivities of various mycoplasma, acholeplasma and ureaplasma strains to lincomycin

Minimum inhibitory concentration ($\mu\text{g/ml}$)											
0.05	0.1	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100
		② ②① 37	③ ⑤ 15 25 30 33 35	① ④ 9 19 31	⑥ ⑭ 18 20 22 32 34	⑦ ⑧ 10 12 13 16 17 23 24 26 29 36		28 39	⑪ ⑳ 38 40 45 47 48 49 52	42 46 50 51 54	41 43 44 53 55
① <i>M. hominis</i> PG21				①⑨ <i>M. hyorhinis</i> PG29				38 <i>U. urealyticum</i> No. 7			
② <i>M. buccale</i> CH20247				②⑩ <i>M. arthritidis</i> PG6				39 <i>U. urealyticum</i> No. 23			
③ <i>M. fermentans</i> PG18				③⑪ <i>M. caviae</i> G122				40 <i>U. urealyticum</i> No. 27			
④ <i>M. salivarium</i> PG20				④⑫ <i>M. neurolyticum</i> PG28				41 <i>U. urealyticum</i> No. 58			
⑤ <i>M. orale</i> CH19299				⑤⑬ <i>M. pulmonis</i> PG22				42 <i>U. urealyticum</i> No. 354			
⑥ <i>M. alkalescens</i> D12				⑥⑭ <i>M. pulmonis</i> m53				43 <i>U. urealyticum</i> Pirillo			
⑦ <i>M. bovis genitalium</i> PG11				⑦⑮ <i>M. primatum</i> HRC292				44 <i>U. urealyticum</i> Cook			
⑧ <i>M. mycoides</i> subsp. <i>mycoides</i> PGI				⑧⑯ <i>M. gallinarum</i> PG16				45 <i>U. urealyticum</i> T-960			
⑨ <i>M. agalactiae</i> PG2				⑨⑰ <i>M. gallisepticum</i> PG31				46 Bovine ureaplasma C2			
⑩ <i>M. arginini</i> G230				⑩⑱ <i>M. gallisepticum</i> S6				47 Bovine ureaplasma C13			
⑪ <i>M. capricolum</i> California kid				⑪⑲ <i>M. iners</i> PG30				48 Bovine ureaplasma A417			
⑫ <i>M. canis</i> PG14				⑫⑳ <i>A. modicum</i> PG49				49 Canine ureaplasma D6P-C			
⑬ <i>M. edwardii</i> PG24				⑬㉑ <i>A. laidlawii</i> PG10				50 Canine ureaplasma DIM-C			
⑭ <i>M. maculosum</i> PG15				⑭㉒ <i>A. laidlawii</i> PG8				51 Feline ureaplasma F45d2			
⑮ <i>M. morale</i> H542				⑮㉓ <i>A. equifetale</i> C112				52 Feline ureaplasma F2			
⑯ <i>M. spumans</i> PG13				⑯㉔ <i>A. hippikon</i> C1				53 Simian ureaplasma T167-1			
⑰ <i>M. felis</i> CO				⑰㉕ <i>A. granularum</i> BTS39				54 Simian ureaplasma T163			
⑱ <i>M. gateae</i> CS				⑱㉖ <i>A. oculi</i> 19L				55 Avian ureaplasma D6-1			
				㉗ <i>A. axanthum</i> S743							

Table 2. Growth of ureaplasma strains on solid medium added with lincomycin

Ureaplasma strain	Dilution of culture	Concentration of lincomycin ($\mu\text{g/ml}$)			
		0	5	10	20
Avian ureaplasma D6-1	0	+	+	+	+
	10^{-1}	+	+	+	+
	10^{-2}	+	+	+	+
	10^{-3}	+	+	+	(+)
Canine ureaplasma DIM-C	0	+	+	+	-
	10^{-1}	+	+	(+)	-
	10^{-2}	+	(+)	-	-
	10^{-3}	+	-	-	-
Feline ureaplasma F45d2	0	+	+	(+)	-
	10^{-1}	+	+	-	-
	10^{-2}	+	-	-	-
	10^{-3}	+	-	-	-

+; marked growth of colony, (+); faint growth of colony, -; no growth of colony.

respectively.

As shown in Table 2, avian ureaplasmas grew on solid media containing 5 to 20 μg per ml of lincomycin. Although canine and feline ureaplasmas were found to be somewhat sensitive to linco-

mycin at 5 μg per ml, addition of 5 μg per ml of the drug to liquid and solid media was adopted for selective isolation of ureaplasmas from animal specimens.

The ingredients of the improved solid and liquid media for selective isolation of ureaplasmas from animals are shown in Table 3. The solid medium consisted of the same ingredients as the liquid one, except that 2.5 ml of phosphate buffer, which was reported by Windsor [30] in 1975, and 0.8 g of purified agar (Oxoid) were added, with the pH adjusted to 6.5.

Using both direct plating and enrichment methods, we compared isolation of ureaplasmas from oral cavities of several species of animals. The isolation methods were described previously [10].

Fig. 1 shows comparative results of isolation of ureaplasmas from canine oral cavities with media with and without lincomycin. In both direct plating and enrichment methods, ureaplasmas were more easily isolated in media containing

Table 3. Selective media for isolation of ureaplasmas from animals

Liquid medium:	
PPLO broth w/o CV (Difco)	80 ml
Horse serum	15 ml
25% (w/v) yeast extract (Nitten Co.)	5 ml
100,000 units of penicillin G	1 ml
2.5% thallium acetate	1 ml
500 μg of lincomycin	1 ml
10% urea	1 ml
0.2% phenol red	1 ml
pH	6.2
Solid medium:	
Liquid medium	100 ml
Phosphate buffer (7:3 mixture of 1.5 M KH_2PO_4 and 1.5 M Na_2HPO_4)	2.5 ml
Purified agar (Oxoid)	0.8 g
pH	6.5

Fig. 1. Selective isolation of ureaplasmas from canine oral cavities in media with and without lincomycin.

lincomycin than in lincomycin-free ones.

Fig. 2 shows mixed colonies of ureaplasmas and mycoplasmas having developed on the solid medium without lincomycin, directly inoculated with a canine oral swab specimen. Purification of an ureaplasma colony from this culture was very difficult. In contrast with Fig. 2, Fig. 3 shows ureaplasma colonies alone having developed on the solid medium with lincomycin inoculated with the same specimen. No mycoplasma colony developed on this medium, and it was easy to isolate ureaplasma colonies for further cloning. Furthermore, similar results were obtained in isolation of ureaplasmas from bovine, feline, and chicken oral cavities.

This study clarified that isolation media containing 5 μ g per ml of lincomycin were very selective for isolation of ureaplasmas from animals. Therefore, these media will provide a useful tool for the survey of ureaplasmas in nature.

ACKNOWLEDGMENTS. The authors thank Dr. I. Z. Pan of Department of Veterinary Microbiology, Faculty of Agriculture, University of Tokyo, for his technical assistances.

REFERENCES

- Braun, P., Klein, J. O., and Kass, E. H. (1970). *App. Microbiol.* **19**, 62–70.
- Brown, W. J., Jacob, N. F., Jr., Arum, E. S., and Arko, R. J. (1976). *Lab. Anim. Sci.* **26**, 81–83.
- Friis, N. F., and Pedersen, K. B. (1980). *Acta, Vet. Scand.* **21**, 134–136.
- Furr, P. M., Taylor-Robinson, D., Hetherington, C. M. (1976). *Lab. Anim.* **10**, 393–398.
- Doig, P. A. (1977). *Vet. Rec.* **100**, 179–180.
- Gourlay, R. N. (1968). *Res. Vet. Sci.* **9**, 367–377.
- Gourlay, R. N., Brownlie, J., and Howard, C. J. (1973). *J. Gen. Microbiol.* **76**, 251–254.
- Harasawa, R., Yamamoto, K., and Ogata, M. (1977). *Microbiol. Immunol.* **21**, 179–181.
- Koshimizu, K., and Magaribuchi, T. (1977). *Jpn. J. Vet. Sci.* **39**, 195–199.
- Koshimizu, K., and Magaribuchi, T. (1978). *Jpn. J. Vet. Sci.* **40**, 719–727.
- Koshimizu, K., Magaribuchi, T., and Ito, M. (1979). *Jpn. J. Vet. Sci.* **41**, 545–549.
- Koshimizu, K., Magaribuchi, T., Ito, M., Kotani, H., and Ogata, M. (1981). *Vet. Rec.* **108**, 309–310.
- Kotani, H., and Ogata, M. (1979). *Jpn. J. Vet. Sci.* **41**, 639–646.
- Kundsins, R. B., Rowell, T., Shepard, M. C., Parreno, A., and Lanceford, C. D. (1975). *Lab. Anim. Sci.* **25**, 221–224.
- Livingston, C. W., Jr., and Gauer, B. B. (1975). *Amer. J. Vet. Res.* **36**, 313–314.
- Livingston, C. W., Jr. (1972). *Amer. J. Vet. Res.* **33**, 1925–1929.
- Nagatomo, H., and Shimizu, T. (1976). *J. Jpn. Vet. Med. Assoc.* **29**, 273–276.
- Ogata, M., Kotani, H., Koshimizu, K., and Magaribuchi, T. (1981). *Jpn. J. Vet. Sci.* **43**, 521–529.
- Robertson, J. A. (1978). *J. Clin. Microbiol.* **7**, 127–132.
- Ruhnke, H. L., and Van Dreumel, A. A. (1971). *Canad. J. Comp. Med. Vet. Sci.* **36**, 317–318.
- Shepard, M. C. (1974). *Collog. Inst. Natl. Sante Rech. Med. (INSERM)*. **33**, 375–380.
- Shepard, M. C. (1954). *Amer. J. Syph. Gonorr. Vener. Dis.* **38**, 113–124.
- Shipley, A., and Bowman, S. J. (1968). *Med. J. Aust.* **1**, 794–796.
- Stipkovits, L., and Rashwan, A. (1976). *Proc. Soc. Gen. Microbiol.* **13**, 158.
- Stipkovits, L., Rashwan, A., Takcs, J., and Lapis, K. (1978). *Zentralbl. Veterinaermed. B*, **25**, 605–608.
- Tan, R. J. S., and Markham, J. G. (1971). *Jap. J. Exp. Med.* **41**, 247–248.
- Taylor-Robinson, D., Martin-Bourgon, C., Watanabe, T., and Addy, J. P. (1971). *J. Gen. Microbiol.* **68**, 97–107.
- Taylor-Robinson, D., Haig, D. H., and Williams, M. H. (1967). *Ann. N. Y. Acad. Sci.* **143**, 517–518.
- Taylor-Robinson, and Furr, unpublished data (1980). *In* I. O. M. International Research Programme on Comparative Mycoplasmaology, Report of Consultations, Custer, U.S.A., 13.
- Windsor, G. D., Edward, D. G. ff., and Trigwell, J. A. (1975). *J. Med. Microbiol.*, 183–187.

EXPLANATION OF FIGURES

Fig. 2. Mycoplasma (arrow a) and ureaplasma (arrow b) colonies on solid medium without lincomycin inoculated with a canine oral specimen.

Fig. 3. Ureaplasma colonies alone developed on solid medium with 5 μ g of lincomycin per ml inoculated with the same specimen as mentioned in Fig. 2.

要 約

動物からのウレアプラズマ選択分離培地(短報): 興水 馨・伊藤正博・曲淵輝夫・小谷 均¹⁾(東京大学医学部動物実験施設, ¹⁾動物繁殖研究所)——動物からのウレアプラズマ選択分離培地について検討した。ヒトおよび各種動物由来ウレアプラズマ 18 株に対するリンコマイシンの最小発育阻止濃度(MIC)は 12.5~100 μ g/ml であり, 一方, 各種動物由来マイコプラズマ, アコレプラズマ計 37 株に対する本剤の MIC は大部分の株が 0.2~3.13 μ g/ml を示した。常用分離培地にリンコマイシン 5 μ g/ml を添加したところ, マイコプラズマおよびアコレプラズマの増殖が抑制され, ウレアプラズマが選択的に分離された。