NOTE *Bacteriology*

Antimicrobial Susceptibilities of Four Species of *Mycoplasma* Isolated in 2008 and 2009 from Cattle in Japan

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ABSTRACT. We examined the susceptibility *in vitro* of 99 isolates of *Mycoplasma* (*M. bovirhinis, M. bovis, M. alkalescens* and *M. bovoculi*) and one of *Acholeplasma laidlawii* collected in 2008 and 2009 from Japanese homebred cattle and cows imported from Australia to 13 antimicrobial agents. *Mycoplasma* species isolated from homebred cattle were susceptible to spectinomycin and fluoroquinolones but resistant to macrolides, tetracyclines, thiamphenicol and flumequine. Kanamycin, lincomycin and chloramphenicol maintained intermediate effectiveness against these species. The isolates from the imported cows maintained sufficient susceptibility to all antimicrobials examined except for erythromycin and thiamphenicol. These results showed that four species of *Mycoplasma* isolated from Japanese homebred cattle had obviously higher drug resistance compared with previous reports, even within this decade.

KEY WORDS: bovine respiratory diseases, drug resistance, *Mycoplasma* spp.

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Bovine respiratory disease complex (BRDC) caused by several combinations of viral and bacterial infections together with stress is a significant problem that has considerable economic impact on the cattle industry [4, 12]. *Mycoplasma* is a causative agent of BRDC [2, 6, 7, 10, 12]. Because vaccination of cattle to prevent *Mycoplasma* infection has not yet been approved in Japan, other approaches are needed, such as preventing bacterial invasion of farms through hygiene management, preventing respiratory symptoms through nutritional management and antibiotic treatment of cattle with abnormal respiratory manifestations. Although the antimicrobial susceptibility of *Mycoplasma* species has changed [6, 7, 10], there has been no report of examination of the susceptibility in Japan since isolates were collected from 1996–1997 [6]. Therefore, this study examined the antimicrobial susceptibility of *Mycoplasma* strains recently isolated from cattle in Japan.

We analyzed 99 isolates of *Mycoplasma* (*M. bovirhinis*, *M. bovis*, *M. alkalescens* and *M. bovoculi*) and one of *Acholeplasma laidlawii* collected in 2008 and 2009 from Japanese homebred cattle that were 15 days to 11 months old at 33 farms in Kyusyu and healthy cows imported from Australia that were 14 to 15 months old. At the time of sampling, the Australian cattle were isolated from other cows because of quarantine restrictions at the destination farm.

We examined the minimum inhibitory concentrations (MICs) of 13 antimicrobial agents (kanamycin, spectinomycin, erythromycin, tylosin, tilmicosin, lincomycin, oxytetracycline, chlortetracycline, chloramphenicol, thiamphenicol, flumequine, enrofloxacin and danofloxacin) required to completely suppress the growth of the four species of *Mycoplasma* and *A. laidlawii.* The MICs were determined using agar dilution according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [3] and a previous report [5]. *Mycoplasma* species and *A. laidlawii* were grown and diluted 1:100 in M broth [9]. Bacterial suspensions (about 10^6 colony forming units; CFU) were inoculated onto M agar plates [9] supplemented with various concentrations of each antimicrobial agent. The type strains (*Mycoplasma bovis* PG45 and *Mycoplasma bovirhinis* PG43) were used as quality controls for MIC determinations.

Table 1 summarizes the MICs for the isolates from the Japanese homebred cattle. The MIC values of spectinomycin were low against most of the tested isolates. This agent might be effective for treating *Mycoplasma*-related bovine pneumonia because the MIC values are similar to those reported in countries where such treatment is authorized [12, 13]. The MIC values of enrofloxacin were comparable among *M. bovis*, *M. alkalescens* and *M. bovoculi* in this study. The MIC values of *M. bovis* and *M. alkalescens* were similar to those previously reported in Japan and elsewhere [5–7, 13]. The MIC value of enrofloxacin was higher for *M. bovirhinis* than for other species. This result is supported by a previous report [6]. The MICs for danofloxacin were similar to those of enrofloxacin. Antibiotic resistance supposedly arises through indiscreet drug use [10]. *Mycoplasma* species might be naturally resistant to erythromycin because the MIC value was also high against the type strain. Tylosin had a high MIC against most isolates except for *M. bovoculi* from the homebred cattle, as found in previous reports [5, 6]. The susceptibility of *M. bovis* isolates to tilmicosin, a macrolide similar to tylosin, was lower than that reported previously [13]. The MIC values of oxytetracycline and chlortetracycline for the isolates, except for *M. bovoculi*, were quite high. Kudou *et al.* [10] reported in 1994 that tetracyclines are comparatively effective against *M. bovis* and *M. alkalescens*. On the other hand, the efficacy against the same species was rather low in other reports [6, 7, 13]. The

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Table 1. Summary of the MICs for *Mycoplasmas* isolated from Japanese homebred cattle

Antimicrobial agents	MIC range (MIC50[a)] / MIC90[b)] / MICts[c)]) (μg/m*l*)				
	M. bovirhinis (n=39)	*M. bovis* (n=29)	*M. alkalescens* (n=9)	*M. bovoculi* (n=6)	*A. laidlawii* (n=1)
Kanamycin	4–512 (16/512/8)	8–64 (32/64/4)	8–16	≤0.125–0.5	16
Spectinomycin	1–8 (4/8/4)	1–64 (2/8/4)	≤0.125–32	0.5–4	4
Erythromycin	256–512< (512/512< /512)	16–512< (512/512</512)	512–512<	6–64	512<
Tylosin	0.25–128 (8/64/4)	1–256 (128/128/1)	1–128	≤0.125–0.5	1
Tilmicosin	0.25–512< (32/256/4)	64–512< (512</512</4)	16–512<	0.5–2	2
Lincomycin	1–16 (4/8/4)	1–16 (4/8/2)	0.25–16	0.5–16	4
Oxytetracycline	4–128 (32/128/8)	16–128 (128/128/32)	4–128	≤0.125–1	32
Chlortetracycline	0.5–128 (8/128/8)	16–256 (128/256/8)	4–128	≤0.125–0.25	16
Chloramphenicol	1–6 (8/8/4)	4–16 (8/16/2)	4–8	0.5–32	8
Thiamphenicol	64–512< (256/512</256)	64–512< (512</512</128)	128–512<	16–512	128
Flumequine	1–128 (64/64/1)	16–128 (64/128/16)	32–64	0.25– 16	32
Enrofloxacin	0.25–32 (2/16/≤0.125)	≤0.125–4 (0.25/1/≤0.125)	≤0.125–1	≤0.125–2	2
Danofloxacin	≤0.125–16 (2/8/0.25)	0.25–4 (0.5/2/0.25)	≤0.125–0.25	≤0.125–2	4

a) The MIC at which 50% of the examined *Mycoplasma* isolates were inhibited (μg/m*l*). b) The MIC at which 90% of the examined *Mycoplasma* isolates were inhibited (μg/m*l*). c) The MIC of the type strains (*M. bovis* PG45 or *M. bovirhinis* PG43; μg/m*l*).

present results support the latter findings. Thiamphenicol and flumequine were less effective against *M. bovis* than previously reported [5, 7]. Although the effects of kanamycin, lincomycin and chloramphenicol against most isolates except for *M. bovoculi* had deteriorated somewhat, they remained intermediate compared with previous reports [6, 7, 10].

The MIC50 and MIC90 values of the macrolides (erythromycin, tylosin and tilmicosin) and tetracyclines (oxytetracycline and chlortetracycline) against the *M. bovis* isolates were very similar at high concentrations, suggesting that resistant strains have become widely distributed in Japan. Although the MIC50 values of the same agents were rather lower for *M. bovirhinis* and *M. alkalescens* than for *M. bovis,* the MIC90 values were comparable to those of *M. bovis*, suggesting that resistance has developed in these species. These results suggest that *M. bovis* isolates in Japan are becoming resistant to antimicrobials, particularly macrolides and tetracyclines that are generally applied as a first choice for treating *Mycoplasma*-related pneumonia. It appears that *M. bovirhinis* had characteristically high resistance to fluoroquinolones. *Mycoplasma bovirhinis* has been simultaneously detected with other BRDC-related bacteria (*M. bovis*, *Pasteurella multocida* and *Mannheimia haemolytica*) isolated from cattle with respiratory diseases [6, 8]. In addition, fluoroquinolone resistance has recently arisen among isolates of *M. haemolytica*, which is an important organism associated with BRDC [8]. Fluoroquinolone is a useful agent that has been used in Japan for two decades to treat severe bacterial respiratory diseases, especially those caused by *M. haemolytica* [8]. Therefore, the low sensitivity of *M. bovirhinis* to fluoroquinolones might have been induced through its use in *M. haemolytica* infection.

The isolates from the Australian cows were uniformly susceptible to most of the antimicrobials examined except for erythromycin and thiamphenicol (Table 2). These cows were transported from Australia to Japan by air along with a group of approximately 300 cows and were then placed in a Japanese animal quarantine facility. An equivalent strain of *Mycoplasma* might have permeated the group, since the susceptibility of the isolated *Mycoplasma* species tended to be similar against the tested antimicrobials. In recent years, the Japanese government has allowed import of live cattle from Australia only. About 20,000 cattle are imported annually into Japan [1]; however, the *Mycoplasma* species isolated from these imported cows retain sufficient sensitivity to the tested antimicrobials. These results suggested a lower likelihood of antimicrobial-resistant strains from imported cows invading Japan.

Table 2. Summary of the MICs for *Mycoplasmas* isolated from cows imported from Australia

Antimicrobial agents	MIC range (μg/m*l*)	
	M. bovirhinis (n=9)	*M. bovoculi* (n=7)
Kanamycin	2–16	≤0.125–2
Spectinomycin	0.25–4	0.25–4
Erythromycin	32–512<	32–512<
Tylosin	≤0.125–2	≤0.125–2
Tilmicosin	0.5–2	0.25–2
Lincomycin	0.25–4	0.25–4
Oxytetracycline	0.5–8	0.5–8
Chlortetracycline	≤0.125–4	≤0.125–8
Chloramphenicol	1–8	1–8
Thiamphenicol	6 –512<	16–512
Flumequine	0.5–2	≤0.125–1
Enrofloxacin	≤0.125	≤0.125
Danofloxacin	≤0.125–0.25	≤0.125–0.25

The MIC ranges for *M. bovirhinis* of the Japanese isolates were wide compared with those of the Australian isolates. This may have been due to the difference in the number of cows used for collection of the microorganism. The MIC values of *M. bovoculi* did not differ by much between the Japanese and Australian isolates in this study. We will try to

isolate the microorganisms and examine their antimicrobial susceptibility.

It has been suggested that *M. bovoculi* is of no significance in relation to BRDC because there have been few reports of its isolation from cattle with respiratory diseases. *Mycoplasma bovoculi* has been shown to be related to infectious bovine keratoconjunctivitis (pinkeye) rather than BRDC [11]. Therefore, this suggests that the isolated *M. bovoculi* obtained from the respiratory tracts of cattle in this study was important as one of the pathogens for pinkeye. This is the first report concerning the isolation and antimicrobial susceptibility of *M. bovoculi* in Japan.

In conclusion, the results of the present study confirmed the presence of antimicrobial-resistant strains of *Mycoplasma* species in Japan and that they have rapidly developed, even within the past decade. The antimicrobial susceptibilities of *Mycoplasma* species should be continuously monitored, and appropriate antimicrobials should be used to minimize changes in susceptibility and maintain antimicrobial effectiveness against infection with *M. bovis,* which is an important causative agent of BRDC.

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