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Comparison of Methods for In Vitro Testing of Susceptibility of Porcine *Mycoplasma* Species to Antimicrobial Agents

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The MICs of 18 antimicrobial agents used against strains of three porcine *Mycoplasma* species were determined by a serial broth dilution method. Twenty field strains of *M. hyorhinis*, ten field strains of *M. hyopneumoniae*, six field strains of *M. flocculare*, and the type strains of these species were tested. Twelve field strains and the type strain of *M. hyorhinis* were also tested by an agar dilution method. Tests were read at various time points. When the broth dilution method was used, the final MIC had to be read 2 days after color changes had stopped. MICs of tetracycline, oxytetracycline, doxycycline, and minocycline were low for the three *Mycoplasma* species tested. MICs of chlortetracycline were 8 to 16 times higher than MICs of the other tetracyclines. Spiramycin, tylosin, kitasamycin, spectinomycin, tiamulin, lincomycin, and clindamycin were effective against all strains of *M. hyorhinis* and *M. hyopneumoniae*. The quinolones were highly effective against *M. hyopneumoniae* but less effective against *M. hyorhinis*. The susceptibility patterns for *M. hyopneumoniae* and *M. flocculare* were similar.

Mycoplasmas can infect the respiratory tract of humans and animals (16, 17, 20, 26). Antimicrobial agents are used to treat these infections, but because MICs of these agents have been determined infrequently, the infections are often treated empirically. Since the various *Mycoplasma* species need different complex culture media and different incubation periods for growth, MICs have been determined by various methods. Only a few authors have tried to standardize in vitro techniques for testing the susceptibility of mycoplasmas to antimicrobial agents (18, 21).

The International Research Program on Comparative Mycoplasmology, part of the International Organization for Mycoplasmology, established an ad hoc working group that compared various methods of testing antimicrobial agents against mycoplasmas in vitro. Various test conditions were evaluated, e.g., types of culture media, number of organisms, and lengths of incubation periods. The working group proposed the serial broth dilution method as the most useful and reproducible assay (21a).

Mycoplasma hyopneumoniae causes enzootic pneumonia, a widespread and economically important disease of swine (11, 15, 20). Some investigators have reported the in vitro activity of antimicrobial agents against *M. hyopneumoniae* (5, 12, 27, 28). Different test methods were used, however, and only a few reports on recently isolated strains are available. Other *Mycoplasma* species of the respiratory tract of swine are *M. hyorhinis* and *M. flocculare*. *M. hyorhinis* is a ubiquitous organism that infrequently causes polyserositis and arthritis. *M. flocculare* is considered to be a ubiquitous but nonpathogenic organism that closely resembles *M. hyopneumoniae* in serologic and biochemical tests (20, 26). Data on the antimicrobial susceptibility of *M. hyorhinis* strains are scarce; there are no data on the susceptibility of *M. flocculare* strains.

In the present study we investigated various antimicrobial agents against various porcine mycoplasma strains recently isolated in The Netherlands. Strains of *M. flocculare* were included to investigate whether strains of this species could be differentiated from strains of *M. hyopneumoniae* by their susceptibility patterns. Because the working group did not recommend an exact time point for reading final MICs, we determined MICs for all three *Mycoplasma* species at various time points. Although the broth dilution method has been recommended by the working group, it is a more laborious method than the agar dilution method. Because the agar dilution method is generally used for true bacteria, we investigated whether this method was a suitable method for determining MICs for fast-growing *Mycoplasma* species such as *M. hyorhinis*. We tested *M. hyorhinis* by both the serial broth dilution method and the agar dilution method, and we compared the results. The agar dilution method is less suitable for testing *M. hyopneumoniae* and *M. flocculare*, because strains of these species grow more slowly and their colonies are less well visible because of the absence of a fried-egg appearance.

MATERIALS AND METHODS

***Mycoplasma* strains and antisera.** Type strain BTS-7 of *M. hyorhinis*, type strain J of *M. hyopneumoniae*, and type strain Ms42 of *M. flocculare* were obtained from E. A. Freundt of the former FAO/WHO Collaborating Centre for Animal Mycoplasmas, Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark. Reference rabbit hyperimmune serum raised against type strain BTS-7 was also obtained from E. A. Freundt. Reference rabbit hyperimmune antisera raised against type strains J and Ms42 were obtained from N. F. Friis, State Veterinary Serum Laboratory, Copenhagen, Denmark. For diagnostic purposes, hyperimmune antisera against the three type strains were raised in rabbits by the method of Friis (8).

Field strains of *M. hyorhinis*, *M. hyopneumoniae*, and *M. flocculare* were isolated by the methods of Friis (7, 9) from 1984 to early 1989. Twenty strains of *M. hyorhinis* were isolated from the lungs (Y4, BY6, BV3, BZ35, CC13, CD8, CD18, DH43, DK3, DL23, DP4, DR7, and DZ25), nose (EY17, EX40, and EX52), tonsils (ER7), or synovia (EK3,

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EW24, and EX38) of pigs from 19 different farms. Ten strains of *M. hyopneumoniae* were isolated from the lungs (T11, CD7, DR6, DY13, EY69, EY71, and FB40) or sinuses of the upper respiratory tract (EU19, EU26, and FC6) of pigs from seven different farms. Six strains of *M. flocculare* were isolated from the lungs (FB36) or sinuses (EE20, EW17, FA24, FC8, and FC11) of pigs from six different farms. Most pigs suffered from respiratory disease; the *M. hyorhinis* strains isolated from synovia originated from pigs with arthritis. The farms were located in various parts of The Netherlands.

Primary cultures of *M. hyorhinis* strains were purified three times by using a Pasteur pipette (6) to suction an agar plug bearing one colony. Strains were identified by the indirect fluorescent antibody technique (19).

Primary cultures of *M. hyopneumoniae* and *M. flocculare* were purified three times by conventional filtration cloning techniques, using a 450-nm-pore-size membrane filter (Millex-HV; Millipore Corp.) (23). After the first filtration step, the culture was serially diluted 10-fold in eight tubes with Friis broth. Immediately thereafter, the contents of each tube were transferred to eight wells (200 μl per well) of a microtiter plate that was sealed with an adhesive tape and incubated at 37°C for 14 days. The contents of one of the wells containing the highest dilution of viable culture were serially diluted 10-fold in four tubes of Friis medium. The tube containing the highest viable dilution was used for the next filtration step. Strains were identified by a metabolism inhibition test (22), a growth inhibition (GI) test (3), and a slightly modified growth precipitation test according to the methods of Friis (8). The growth precipitation test was read on the agar plates used for the GI test. These plates had been inoculated with a running drop of culture. After the GI test was read, the agar plates were stored at room temperature. Precipitation lines developed after 1 to 2 weeks.

Antimicrobial agents. The following types and origins of antimicrobial agents were used: tetracycline (Sigma Chemicals, Amsterdam, The Netherlands), oxytetracycline (Gist-Brocades, Delft, The Netherlands), chlortetracycline (Cyanamid, Lederle, The Netherlands), doxycycline (Pfizer, Rotterdam, The Netherlands), minocycline (Cyanamid), chloramphenicol (Gist-Brocades), spiramycin (Rhône-Mérieux, Toulouse, France), tylosin (Elanco, Nieuwegein, The Netherlands), kitasamycin (Inffa, Houten, The Netherlands), erythromycin (Abbott, Amsterdam, The Netherlands), spectinomycin (Upjohn, Ede, The Netherlands), tiamulin (Coopers, Weesp, The Netherlands), lincomycin (Upjohn), clindamycin (Upjohn), ofloxacin (Hoechst, Amsterdam, The Netherlands), enrofloxacin (Bayer, Mijdrecht, The Netherlands), ciprofloxacin (Bayer), and ampicillin (Gist-Brocades).

Tylosin and tiamulin are used only in veterinary medicine. Tylosin is an antibiotic with a structure similar to that of erythromycin. Tiamulin is a semisynthetic antimicrobial agent that does not belong to a particular group. Both agents have a spectrum similar to the spectrum of the macrolides. The antimicrobial agents were diluted in distilled water to prepare stock solutions, except the five tetracyclines, which were diluted in 10% methanol; chloramphenicol and spiramycin, which were diluted in 0.5% *N*,*N*-dimethylformamide (no. 3034; E. Merck AG, Darmstadt, Federal Republic of Germany); and ofloxacin, which was diluted in 0.1 N NaOH. Concentrations were calculated as pure substances in order to prepare stock solutions. The activity of spiramycin was equivalent to 4,468 IU/mg, i.e., 1.4 times the activity of the World Health Organization standard. Stock solutions were sterilized by filtration through a 200-nm-pore-size membrane filter (Acrodisc; Gelman Sciences Inc., Ann Arbor, Mich.) and used immediately or stored at 4°C overnight.

Agar dilution method for determination of MICs. Twelve field strains and the type strain of *M. hyorhinis* were investigated by the agar dilution method. Antimicrobial agents were incorporated into Friis agar plates in serial twofold dilutions (range, 16 to 0.03 μg/ml). The inoculum concentration of each strain used was 5×10^5 CFU/ml. A multipoint inoculator was used to inoculate 2-μl volumes of broth culture, containing 10^3 CFU, onto the plates. The plates were incubated at 37°C in a CO_2 incubator in an atmosphere of 5 to 10% CO_2 in air. Initial MICs were read as soon as colonies developed, i.e., 2 days after inoculation of the plates; final MICs were read 3 days after inoculation. The MIC of an antimicrobial agent was determined to be the lowest concentration at which no growth, only a single colony, or a layer of very small colonies was visible. The tests were performed in duplicate. When MICs differed by one twofold dilution, the highest concentration was recorded as the MIC.

Serial broth dilution method for determining MICs. All strains of the three species used in this study were investigated by the serial broth dilution method. Each antimicrobial agent was serially diluted twofold in 10 wells of a microtiter plate. Friis broth was added to each well. A standard number of organisms (10^3 to 10^5 color-changing units [CCU] per ml) grown in Friis broth was used. An amount of 175 μl, containing 1.7×10^2 to 1.7×10^4 CCU, was added to each well. Media contained no other bacterial inhibitors. The susceptibility or resistance of the strains to the antimicrobial agent was indicated by whether the culture was able to metabolize glucose in the presence of one of the concentrations of the antimicrobial agent. Glucose metabolism was visualized by a change of the phenol red indicator from red to yellow. On the day of inoculation, the required numbers of CCU were prepared from frozen stock cultures with a known number of cells; organisms were counted again to verify the actual numbers of organisms in the system. The microtiter plates were sealed with an adhesive tape and incubated aerobically at 37°C. The MIC of the antimicrobial agent was determined as the lowest concentration at which the medium did not change color. The MICs for *M. hyorhinis* were read daily for 7 days; MICs for *M. hyopneumoniae* and *M. flocculare* were read daily for 14 days. Although day 14 was considered to be too late for reading MICs, we were interested in seeing whether MICs of antimicrobial agents used against *M. hyopneumoniae* and *M. flocculare* increased during the test period of 14 days. Initial MICs were recorded as soon as the inoculum controls (without the antimicrobial agent) changed color compared with the medium controls (day 2 for *M. hyorhinis*, day 4 to 5 for *M. hyopneumoniae* and *M. flocculare*). Final MICs were recorded when color changes had stopped for 2 days (day 5 for *M. hyorhinis*, around day 7 for *M. hyopneumoniae* and *M. flocculare*). The tests were performed in duplicate. When MICs differed no more than a factor of two, the highest concentration was recorded as the MIC.

Interpretation of MICs. Strains for which tetracycline MICs were ≤1 μg/ml were considered susceptible; strains for which MICs were ≤4 μg/ml were considered intermediately susceptible; and strains for which MICs were ≥32 μg/ml were considered resistant. These MICs were according to the recommendations of the Dutch working group on guidelines for testing the susceptibility of bacteria that affect

TABLE 1. MIC_{90}s of antimicrobial agents used against 12 field strains of *M. hyorhinis* obtained in a comparison of dilution methods

Antimicrobial agent	MIC_{90} (μg/ml) by method			
	Agar dilution		Broth dilution	
	Day 2[a]	Day 3[a]	Day 2[a]	Day 5[a]
Oxytetracycline	0.06	0.06	0.06	0.12
Chlortetracycline	0.12	0.25	0.5	4
Doxycycline	0.06	0.06	≤0.03	0.06
Minocycline	≤0.03	≤0.03	≤0.03	0.12
Chloramphenicol	1	2	2	4
Spiramycin	4	8	0.12	0.25
Tylosin	0.5	1	0.12	0.25
Erythromycin	>16	>16	>16	>16
Tiamulin	0.25	0.25	≤0.03	0.06
Lincomycin	1	1	0.25	0.5
Ofloxacin	1	2	2	4
Enrofloxacin	1	1	1	2
Ciprofloxacin	0.25	0.5	0.25	0.5

[a] Number of days after inoculation.

humans. These criteria were used because criteria for animals are not available generally. These values are ≤4, ≤8, and ≥16 μg/ml for chloramphenicol; ≤1, ≤2, and ≥4 μg/ml for erythromycin; ≤1, ≤4, and ≥8 μg/ml for clindamycin; ≤1, ≤4, and ≥16 μg/ml for ofloxacin; ≤1, ≤2, and ≥16 μg/ml for ciprofloxacin; and ≤2, ≤16, and ≥64 μg/ml for ampicillin (24).

RESULTS

MICs of antimicrobial agents were determined for 90% of the strains tested (MIC_{90}) and for 50% of the strains tested (MIC_{50}). Table 1 shows the MIC_{90}s of 13 antimicrobial agents for 12 *M. hyorhinis* strains. Using the agar dilution method, we detected only slight differences between the MICs at days 2 and 3. In the broth dilution method, twofold to fourfold differences were found, however, between the MICs at days 2 and 5, but the MICs of chlortetracycline differed greatly. MICs determined by the agar and broth dilution method were similar; chlortetracycline and spiramycin, however, had markedly different MIC_{90}s.

Table 2 shows the MIC_{50}s, the MIC_{90}s, and the MIC range, determined by the broth dilution method, of 18 antimicrobial agents for 20 field strains of *M. hyorhinis*; Table 2 shows also the MICs for type strain BTS-7 of *M. hyorhinis*. Table 3 shows the MIC_{50}s, the MIC_{90}s, and the MIC range, determined by the broth dilution method, of 18 antimicrobial agents for 10 field strains of *M. hyopneumoniae*; Table 3 shows also the MICs for type strain J of *M. hyopneumoniae*. Table 4 shows the MIC_{50}s and the MIC range of 18 antimicrobial agents for six field strains of *M. flocculare*; Table 4 shows also the MICs for type strain Ms42 of *M. flocculare*.

In the broth dilution method, initial MICs for 20 *M. hyorhinis* strains differed greatly from MICs read 2 days after color changes had stopped (average, day 5). The MIC_{90}s of the tetracyclines had been increased 4 to 16 times, whereas the MIC_{90}s of the other agents had been increased 2 to 4 times. The MICs at the time point that color changes had

TABLE 2. MICs of antimicrobial agents used against field strains and type strain BTS-7 of *M. hyorhinis*, determined by a serial broth dilution method[a]

Antimicrobial agent	MIC (μg/ml) for:			
	Field strains (n = 20)			Type strain BTS-7
	50%	90%	Range	
Tetracycline	0.06	0.25	≤0.03–0.5	≤0.03
Oxytetracycline	0.12	0.25	0.06–1	0.12
Chlortetracycline	2	4	1–8	1
Doxycycline	≤0.03	0.12	≤0.03–0.5	≤0.03
Minocycline	0.06	0.5	≤0.03–2	≤0.03
Chloramphenicol	2	4	0.5–4	2
Spiramycin	0.12	0.5	0.06–0.5	0.12
Tylosin	0.12	0.25	0.06–0.5	0.06
Kitasamycin	1	2	0.5–2	0.5
Erythromycin	>16	>16	>16	>16
Spectinomycin	1	4	1–4	2
Tiamulin	0.06	0.12	≤0.03–0.25	≤0.03
Lincomycin	0.5	1	0.25–1	0.5
Clindamycin	0.5	1	0.12–1	1
Ofloxacin	2	4	2–4	2
Enrofloxacin	1	2	0.5–2	2
Ciprofloxacin	0.5	0.5	0.25–0.5	0.25
Ampicillin	>16	>16	>16	>16

[a] MICs were read when color changes had stopped for 2 days, i.e., day 5 after inoculation (final MICs).

TABLE 3. MICs of antimicrobial agents used against field strains and type strain J of *M. hyopneumoniae*, determined by a serial broth dilution method[a]

Antimicrobial agent	MICs (μg/ml) for:			
	Field strains (n = 10)			Type strain J
	50%	90%	Range	
Tetracycline	≤0.03	0.06	≤0.03–0.06	0.06
Oxytetracycline	≤0.03	0.06	≤0.03–0.12	0.12
Chlortetracycline	0.5	1	0.12–1	2
Doxycycline	≤0.03	≤0.03	≤0.03	≤0.03
Minocycline	≤0.03	≤0.03	≤0.03–0.06	0.12
Chloramphenicol	1	2	0.5–2	1
Spiramycin	0.25	0.25	0.06–0.5	0.12
Tylosin	0.06	0.06	≤0.03–0.12	≤0.03
Kitasamycin	0.5	1	0.12–1	0.25
Erythromycin	>16	>16	16–>16	>16
Spectinomycin	1	1	0.5–1	1
Tiamulin	≤0.03	0.06	≤0.03–0.06	≤0.03
Lincomycin	0.25	0.25	0.06–0.25	0.12
Clindamycin	0.12	0.25	0.12–0.25	0.12
Ofloxacin	≤0.03	0.06	≤0.03–0.06	≤0.03
Enrofloxacin	≤0.03	≤0.03	≤0.03–0.06	≤0.03
Ciprofloxacin	≤0.03	≤0.03	≤0.03	≤0.03
Ampicillin	>16	>16	>16	>16

[a] MICs were read when color changes had stopped for 2 days, i.e., around day 7 after inoculation (final MICs).

TABLE 4. MICs of antimicrobial agents used against field strains and type strain Ms42 of *M. flocculare*, determined by a serial broth dilution method[a]

Antimicrobial agent	MICs (μg/ml) for:		
	Field strains (n = 6)		Type strain Ms42
	50%	Range	
Tetracycline	≤0.03	≤0.03–0.5	≤0.03
Oxytetracycline	≤0.03	≤0.03–1	0.06
Chlortetracycline	0.25	0.12–4	0.5
Doxycycline	≤0.03	≤0.03–0.5	≤0.03
Minocycline	≤0.03	≤0.03–0.25	≤0.03
Chloramphenicol	0.5	0.5–2	2
Spiramycin	0.06	≤0.03–0.25	0.06
Tylosin	≤0.03	≤0.03–0.06	≤0.03
Kitasamycin	0.5	0.12–2	0.5
Erythromycin	16	4–>16	>16
Spectinomycin	0.25	0.12–0.5	0.5
Tiamulin	≤0.03	≤0.03–0.06	0.06
Lincomycin	0.12	0.06–0.25	0.25
Clindamycin	0.06	0.06–0.25	0.25
Ofloxacin	≤0.03	≤0.03–0.25	0.06
Enrofloxacin	≤0.03	≤0.03–0.06	≤0.03
Ciprofloxacin	≤0.03	≤0.03	≤0.03
Ampicillin	>16	>16	>16

[a] MICs were read when color changes had stopped for 2 days, i.e., around day 7 after inoculation (final MICs).

stopped for 2 days (average at day 5) differed only slightly from MICs at day 7 (Fig. 1).

For *M. hyopneumoniae*, MIC_{90}s of most agents (read 2 days after color changes had stopped, around day 7) were twice the initial MICs; the MICs of some agents read at day 14 were two to four times the initial MICs (Fig. 2).

DISCUSSION

One of the purposes of this study was to determine the optimal length of time between inoculating broth cultures and reading the MICs in the serial broth dilution method. *M. hyorhinis* was tested by the broth dilution method and the agar dilution method, which is commonly used to determine MICs for true bacteria. Similar results were obtained with both methods; MIC_{90}s of chlortetracycline and spiramycin, however, differed greatly in the two tests (Table 1). The MICs of tylosin and tiamulin obtained by both methods differed also, but MICs were ≤1 μg/ml.

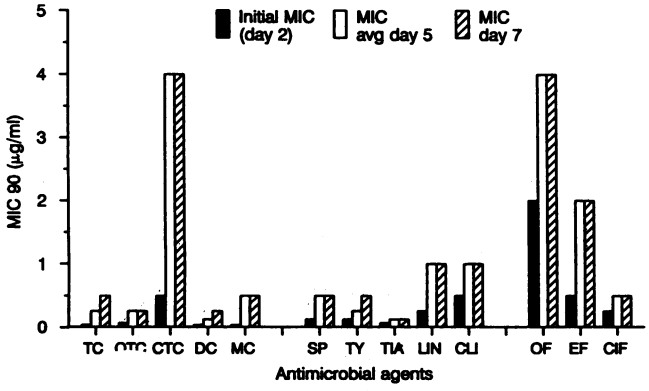

FIG. 1. MIC_{90}s of antimicrobial agents used against 20 field strains of *M. hyorhinis*. MICs were determined by a broth dilution method and read at different times. TC, Tetracycline; OTC, oxytetracycline; CTC, chlortetracycline; DC, doxycycline; MC, minocycline; SP, spiramycin; TY, tylosin; TIA, tiamulin; LIN, lincomycin; CLI, clindamycin; OF, ofloxacin; EF, enrofloxacin; CIF, ciprofloxacin.

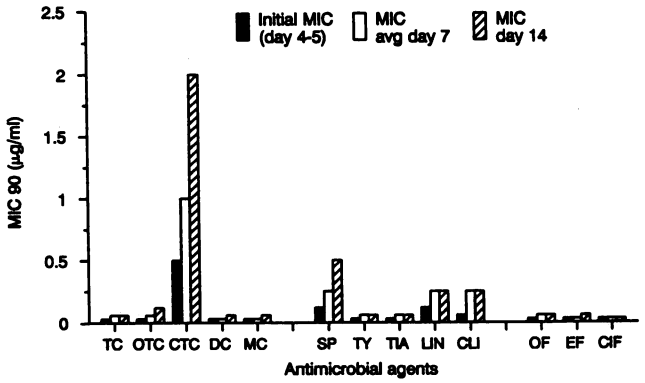

FIG. 2. MIC_{90}s of antimicrobial agents used against 10 field strains of *M. hyopneumoniae*. MICs were determined by a broth dilution method and read at different times. Abbreviations are defined in the legend to Fig. 1.

In the broth dilution method, MICs determined for *M. hyorhinis* differed between the various reading time points; in particular, the initial MICs differed from the MICs when color changes had stopped for 2 days (Fig. 1). Thus, *M. hyorhinis* had a delayed growth in the presence of a concentration of agents below the MIC.

The initial MICs of antimicrobial agents used against *M. hyopneumoniae* differed only slightly from the MICs read when the color changes had stopped for 2 days. Most antimicrobial agents were effective against strains of this species. MICs of several agents had been increased by day 14, even though color had not changed for 2 or more days around day 7 (Fig. 2). Because most antimicrobial agents are bacteriostatic for mycoplasmas (21a), organisms survive during the test period. We know that tetracyclines are less active during prolonged incubation of culture media (2), and this phenomenon probably also accounts for the diminished activity of the other antimicrobial agents tested in our study.

Several investigators have determined MICs by using various methods and by reading MICs at different times. For example, final MICs of antimicrobial agents used against *M. hyopneumoniae* were read at day 5 or 6 or when pH decreased to 6.8 or below (5, 11, 27, 28). When studies have included determination of MICs for type strain J, methods can be compared. The MICs of tetracyclines used against strain J, determined by Etheridge et al. (5) and Yamamoto et al. (28), were 5 to 10 times the MICs determined in our study. The MICs of oxytetracycline, tiamulin, ofloxacin, and ciprofloxacin used against this strain, determined by Hannan et al. (12) using an agar dilution method, were similar to the MICs determined in our study; the MIC of tylosin, however, was 10 times the MIC determined by us. Thus, we agree with the working group's recommendation that a standard method, specifically the broth dilution method, should be used to determine MICs. Moreover, final MICs should be read not later than 2 days after color changes have stopped.

Even though MICs of most antimicrobial agents varied

depending on the time they were read, the interpretation of the results was generally the same. Except for the MICs of chlortetracycline and the quinolones, all MICs were lower in vitro than the concentrations that can be achieved in vivo in serum or at the site of infection.

All *M. hyopneumoniae* strains, including type strain J, were susceptible to 15 of the 18 antimicrobial agents tested. All strains were resistant to erythromycin and ampicillin. Because mycoplasmas have no cell walls, ampicillin theoretically should not inhibit the growth of mycoplasmas. Although it has been reported that ampicillin slightly inhibits the growth of *M. hyopneumoniae* and *M. flocculare* (7), we did not confirm these findings. The type strain was intermediately susceptible to chlortetracycline. No striking differences were found in the susceptibility patterns of *M. flocculare* or *M. hyopneumoniae* strains. Thus, an antibiotic susceptibility test for the antimicrobial agents tested in our study could not be used to differentiate strains of these species.

MIC_{90}s of tetracycline, oxytetracycline, doxycycline, and minocycline for all three *Mycoplasma* species were low. As Yamamoto et al. (28) reported, however, chlortetracycline should be considered ineffective against *M. hyorhinis* and only moderately effective against *M. hyopneumoniae*. This result is surprising, because true bacteria are identically susceptible to the tetracycline group of antimicrobial agents (13). Generally, tetracyclines are effective antimycoplasmal agents that are also effective against the human pathogens *M. pneumoniae* and *M. hominis* (1, 2, 16, 25).

The macrolides were also effective against the three *Mycoplasma* species we studied, but erythromycin was not effective against porcine mycoplasmas. In this study, erythromycin MICs were ≥16 μg/ml, whereas Yamamoto et al. (28) determined MICs ranging from 2.5 to 20 μg/ml and Williams (27) determined MICs ranging from 1.2 to 9.2 μg/ml. In contrast, erythromycin is preferred in treating *M. pneumoniae* infections in humans (2, 16, 25).

Tiamulin, lincomycin, and clindamycin were effective against the three *Mycoplasma* species, findings that correspond well with the results of others (4, 10, 12, 14, 25, 27, 28).

Like Hannan et al. (12), we found that the quinolones were highly effective against *M. hyopneumoniae*. We found that they were less effective against *M. hyorhinis*, however. Ciprofloxacin MICs were the lowest among the three quinolones tested.

In conclusion, none of the strains of the three *Mycoplasma* species was resistant to tetracycline, oxytetracycline, doxycycline, minocycline, tylosin, tiamulin, or ciprofloxacin. Moreover, we suggest that for treating porcine mycoplasma infections, the tetracyclines should be preferred because they are inexpensive and because the MICs of these drugs are quite low.

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