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In Vitro Susceptibility of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* to Fifty-One Antimicrobial Agents

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By a tube dilution assay technique, 51 antimicrobial agents were singly tested against 9 strains of *Mycoplasma hyopneumoniae* and 1 strain of *M. hyorhinis* for the purpose of obtaining information useful for selecting an agent for testing in vivo against porcine mycoplasmal pneumonia. Based on determining minimal inhibitory concentrations and chemically grouping the agents into nine classes, all *M. hyopneumoniae* strains were found resistant to penicillins and peptides and susceptible to sulfonamides and tetracyclines, and, in other classes, were either susceptible or resistant depending on the particular agent being tested. Strains were susceptible to the same 33 of the 51 agents. Minimal inhibitory concentrations ranged from 0.06 to 9.20 μg/ml. *M. hyorhinis* was susceptible to 19 of the 33 agents that *M. hyopneumoniae* was susceptible to. Minimal inhibitory concentrations ranged from 0.03 to 8.10 μg/ml. All strains of *M. hyopneumoniae* differed from *M. hyorhinis* in that they were susceptible to cephaloglycin and nitrofurazone.

Although considerable information about the effect of antibiotics against avian, bovine, human, and saprophytic mycoplasmas has been accumulated (2, 7, 8, 11), few reports are available on the effects of antimicrobial agents against porcine mycoplasmas, *Mycoplasma hyopneumoniae* and *M. hyorhinis* (3, 8). This lack of information is due in part to the difficulty of growing porcine mycoplasmas, particularly *M. hyopneumoniae*, in cell-free medium and the lack of a convenient assay system for determining antimicrobial activities against porcine mycoplasmas.

By the use of a modification of the culturing medium of Friis (4), in which pH change is used as a growth indicator of porcine mycoplasmal glucose fermentation with production of acetic and lactic acids and other end products (P. P. Williams, unpublished data), the effects of antimicrobial agents could be tested on *M. hyopneumoniae* and *M. hyorhinis* and correlated with microbial concentrations. The present study was designed to investigate the in vitro activities of various antimicrobial agents against high-passage strains of *M. hyopneumoniae* and *M. hyorhinis* and to compare their activities with those of recently isolated strains. Methodology and data presented will aid investigators in further characterizing porcine mycoplasmal isolates. This in vitro information may be useful in selecting for chemotherapeutic control of mycoplasmosis.

MATERIALS AND METHODS

Mycoplasmal strains. Strains of *M. hyopneumoniae* used were: ATCC 25617 (strain 11) (6); ATCC 25934 (strain J) (5); and tentatively identified strains NADC-2069, ISU-B245, TAM-4L, TAM-4T, TAM-6N, UCD-1, UCD-4, and UN-16. The strain of *M. hyorhinis* used was NIH:M718-002-084 (strain BTS; originally acquired from J. Tulley, National Institutes of Health, Bethesda, Md).

Media. Mycoplasmal strains were cultured in modified Friis broth (MF) medium (4) at 37°C in screw-capped tubes. Oxoid ion agar no. 2 and diethylaminoethyl-dextran were incorporated in MF medium to provide a solid surface for mycoplasmal growth (6, 10). Plates were incubated for 7 to 9 days in candle jars at 37°C. Colonies on MF agar medium were observed at ×40 magnification with an inverted bright-field microscope.

Antimicrobial agents. All antibiotics or chemotherapeutic agents but nystatin were obtained from Becton, Dickinson and Co., Cockeysville, Md; nystatin was obtained from E. R. Squibb and Sons, Inc., New York, N.Y. (see Table 1). All antimicrobial agents were refrigerated in the dark until used and were used before their expiration dates.

Tube dilution assay. Each mycoplasmal strain in log phase was diluted in MF medium to 10^4 to 10^6 colony-forming units (CFU)/ml. The diluted strains were aseptically dispensed in triplicate in 0.2-ml fractions in test tubes (13 by 100 mm) containing separate serially diluted antimicrobial agents (8). Inoculated tubes were then tightly screw capped, placed in a rack with appropriately labeled tubes of uninoculated medium controls, and incubated on a rocker platform (Bellco Glass Inc., Vineland, N.J.) at 37°C in a walk-in

TABLE 1. *Mean MICs of antimicrobial agents for M. hypneumoniae and M. hyorhinis*[a]

Class of antimicrobial agent	Specific agent	Breakpoint, concn of agent for categorization of resistance[b] (≥μg or U/ml of medium)	MIC (range) of specific agent for nine strains of *M. hyopneumoniae*[c]	MIC of specific agents for *M. hyorhinis* NIH:M718-002-084
Aminoglycosides	Gentamicin	10.0	>50	>50
	Neomycin	8.1	14.3 (8.1–16.3)	16.3
	Streptomycin	6.3	14.1 (6.3–25.0)	12.5
	Kanamycin	1.0	0.5 (0.2–0.9)	0.1
	Tobramycin	3.2	1.8 (0.8–3.1)	0.8
Cephalosporins	Cephaloridine	10.0	>75 (37.5–>150)	>150
	Cephalothin	10.0	>75 (37.5–>150)	>150
	Cephaloglycin	8.3	4.1 (2.0–8.2)	>150
Furan derivatives	Nitrofurantoin	10.0	>1,500	>1,500
	Nitrofurazone	4.01	2.70 (1.01–3.91)	125
	Nifuraldezone	4.04	2.60 (1.02–3.91)	3.92
	Furaltadone	0.30	0.13 (0.11–0.23)	0.11
	Furazolidone	2.21	0.58 (0.31–1.04)	2.06
Macrolides	Oleandomycin	9.4	>30.5 (9.4–>75)	18.8
	Erythromycin	9.3	4.9 (1.2–9.2)	4.6
Miscellaneous	Colistin	10.0	>50	12.5
	Nalidixic acid	10.0	>150	37.5
	Nystatin	10.0	>500	62.5
	Polymyxin	10.0	>375	750
	Chloramphenicol	4.20	2.70 (0.63–4.11)	8.20
	Clindamycin	0.50	0.20 (0.11–0.44)	0.10
	Lincomycin	0.20	0.09 (0.06–0.11)	0.03
	Methenamine mandelate	2.00	1.10 (0.52–1.96)	3.80
	Novobiocin	2.20	1.10 (0.54–2.01)	0.30
	Oxolinic acid	1.50	0.95 (0.62–1.31)	2.50
	Rifampin	3.20	2.40 (1.60–3.11)	6.30
Penicillins	Ampicillin	10.0	>50	>50
	Carbenicillin	10.0	>500	>500
	Methicillin sodium	10.0	>25	>25
	Penicillin	10.0	>50	>50
	Phenethicillin potassium	10.0	>10	>10
	Cloxacillin	5.0	>5	0.3
	Dicloxacillin	5.0	>5	1.3
	Nafcillin sodium	5.0	>5	1.3
	Oxacillin sodium	5.0	>5	1.3
Peptides	Bacitracin	10.0	>50	>50
	Vancomycin	10.0	>150	75
Sulfonamides	Sulfachloropyridazine	0.28	0.15 (0.11–0.27)	0.60
	Sulfadiazine	0.65	0.43 (0.20–0.63)	0.90
	Sulfadimethoxine	0.36	0.18 (0.10–0.35)	0.60
	Sulfamethizole	0.62	0.28 (0.14–0.61)	0.31
	Sulfamethoxypyridazine	0.34	0.20 (0.15–0.32)	0.60
	Sulfathiazole	0.38	0.25 (0.23–0.34)	0.30
	Sulfisoxazole	1.34	0.63 (0.32–1.31)	1.30
	Triple sulfa	1.33	0.55 (0.11–1.31)	1.30

TABLE 1—*continued*

Class of antimicrobial agent	Specific agent	Breakpoint, concn of agent for categorization of resistance[b] (≥μg or U/ml of medium)	MIC (range) of specific agent for nine strains of *M. hyopneumoniae*[c]	MIC of specific agents for *M. hyorhinis* NIH:M718-002-084
Tetracyclines	Chlortetracycline	8.20	3.83 (1.03–8.21)	4.10
	Demethylchlortetracycline	1.23	0.70 (0.12–1.22)	0.60
	Doxycycline	0.32	0.15 (0.11–0.21)	0.50
	Methacycline	2.14	1.37 (0.54–2.01)	4.10
	Oxytetracycline	4.78	2.63 (1.21–4.73)	9.40
	Tetracycline	8.20	7.30 (2.04–8.10)	8.10

[a] Individual MIC (micrograms or units per milliliter) refers to computed means from triplicate tubes showing >90% loss in viable CFU per milliliter within 5 days of incubation at 37°C. Units per milliliter refer only to bacitracin, nystatin, penicillin, and polymyxin. All other concentrations are based on micrograms per milliliter. Values expressed as > refer to highest antimicrobial agent concentrations tested where there was <90% loss in viable CFU per milliliter.

[b] Based on findings with nine strains of *M. hyopneumoniae*.

[c] Strains tested were: ATCC 25617, ATCC 25934, NADC-2069, ISU-B245, TAM-4L, TAM-6N, UCD-1, UCD-4, and UN-16.

incubator. The tubes were observed daily for contamination, loss of volume, and phenol red color shift; and on day 5 they were compared on the basis of visual turbidity and phenol red color shift, and the pH of the medium was read electrometrically. Microbial concentrations were determined by inoculating MF agar medium and incubating as noted above. Minimal inhibitory concentrations (MICs) of the antimicrobial agents were determined on the basis of the lowest concentration of an agent which prevented a shift in the pH of the medium and inhibited >90% of the mycoplasmal CFU.

RESULTS AND DISCUSSION

The antimycoplasmal activities of antibiotic and chemotherapeutic agents tested are summarized in Table 1. Triplicate samples were in agreement. Ranges of MICs, where calculable, for chemically similar antimicrobial agents varied with a specific compound and strain tested. Depending on the particular strain being tested, the strain showing susceptibility to a given antimicrobial agent was prevented from lowering the initial medium pH of 7.4 to a pH, of resistant strains to an agent, varying from 5.8 to 6.4 in 5 days of incubation at 37°C. All strains of *M. hyopneumoniae* were susceptible to the same 33 of 51 antimicrobial agents. MICs for these strains ranged from 0.06 to 9.20 μg/ml. Strains were resistant to relatively high concentrations (>150 μg of U/ml) or carbenicillin, nalidixic acid, nitrofurantoin, nystatin, polymyxin, and vancomycin.

The single strain of *M. hyorhinis* was susceptible to 19 of the 33 antimicrobial agents that *M. hyopneumoniae* was susceptible to. MICs ranged from 0.03 to 8.10 μg/ml. All strains were susceptible to relatively low concentrations (0.03 to 0.63 μg/ml) of clindamycin, furaltadone, lincomycin, and some of the sulfonamides.

Based on the greatest differences between MICs of specific antimicrobial agents, *M. hyopneumoniae* strains differed from *M. hyorhinis* in that they were susceptible to cephaloglycin (4.1 to >150 μg/ml) and nitrofurazone (2.7 to 125 μg/ml). Other MIC differences from 0.8 to 6.77 μg/ml need to be confirmed by testing additional strains of *M. hyorhinis*.

We have confirmed that *M. hyopneumoniae* and *M. hyorhinis* strains are resistant to neomycin and streptomycin, and *M. hyorhinis* is resistant to kanamycin. Strains of *M. hyopneumoniae*, as tested, were susceptible to kanamycin, but Ogata et al. (8) found strains to show some resistance to the agent, with a MIC of 20 μg/ml. In addition, *M. hyopneumoniae* and *M. hyorhinis* strains were resistant to cephaloridine and cephalothin but susceptible to furazolidone; the *M. hyopneumoniae* strains were susceptible, and *M. hyorhinis* was resistant, to oleandomycin. Our strains of *M. hyopneumoniae* and *M. hyorhinis* were susceptible to erythromycin, but Ogata et al. (8) found some strains to be resistant. Strains of *M. hyopneumoniae*, as tested, were resistant to ampicillin, methicillin sodium, and penicillin. However, Friis (3) found *M. hyopneumoniae* "perceptibly" susceptible to benzylpenicillin potassium at >10 μg/ml and totally inhibited at <3,000 μg/ml. Strains of *M. hyopneumonia* were resistant to bacitracin and vancomycin, and *M. hyopneumoniae* and *M. hyorhinis* strains were susceptible to chlortetracycline and tetracycline.

In other reports (1, 9), sulfonamides have been suggested as chemotherapeutic agents for treating mycoplasmas in rats and pigs. Strains of *M. hyopneumoniae* and *M. hyorhinis* as we tested in vitro were inhibited by relatively low concentrations (0.18 to 1.31 μg/ml) of sulfonamides.

Variations in mycoplasmal susceptibilities to erythromycin, kanamycin, and possibly penicillin could be due to differing techniques, different media, and the decrease in activity of the antibiotics in solution over storage and test periods. Overall, the tube dilution assay technique effectively showed mycoplasmal susceptibilities to a variety of different chemically structured antimicrobial agents. Inoculated tubes could be conveniently observed visually and measured electrometrically for mycoplasmal glucose fermentation.

Antibiotic tube dilution and agar dilution methods have been compared, and results agree within a fivefold variation in approximately 90% of the specimens estimated by the two methods (8). Methodology and data presented form a basis for accumulating further information for characterizing porcine mycoplasmal strains. The in vitro information as presented now needs to be confirmed in in vivo experiments before a substantiated antibiotic or chemotherapeutic agent can be used for animal administration against *M. hyopneumoniae* for control of porcine respiratory mycoplasmosis.

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