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In Vitro Susceptibility of *Haemophilus somnus* to 33 Antimicrobial Agents

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Minimal inhibitory concentrations of 33 antimicrobial agents for *Haemophilus somnus* were determined by the agar dilution method. The tested *H. somnus* strains were highly susceptible to penicillin G, ampicillin, colistin, and novobiocin. They were not susceptible to spiramicin and sulfadimethoxine, and streptomycin-resistant strains were found.

Haemophilus somnus produces various types of infection in cattle, such as thromboembolic meningoencephalitis (TEME) after an acute septicemia (2, 10, 18), respiratory infection (23), abortion (4, 27), vulvitis (21), weak calf syndrome (28), and arthritis (26). TEME is a major cause of deaths in feedlot cattle, according to a survey carried out in Canada (15), and has been recognized since 1976 in Japan. *H. somnus* is a gram-negative, fermentative small rod whose taxonomic position is still unclear (3) and which is found in the respiratory tracts (5, 6) and genital tracts (11, 12) of cattle. As mentioned by Stephens et al. (24), *H. somnus* may be part of the normal bovine flora, in which case it could be regarded as a poorly invasive opportunist. Whereas the mechanism of *H. somnus* invasion is still unclear, some factors, including season (5, 7, 19), respiratory infections with virus or mycoplasma (1, 20), and shipment (16, 17), may induce TEME after septicemia.

Although *H. somnus* is an economically important pathogen in cattle production, only limited data on its antimicrobial susceptibility are available (9, 13, 14, 26). This study was undertaken to establish in vitro activities of representative antimicrobial agents against this organism.

Forty-five strains were used in this study. These strains were isolated from cattle with TEME in Japan. All strains were positive in oxidase, nitrate, indole, and ornithine decarboxylase tests and negative in catalase, motility, lysine decarboxylase, Voges-Proskauer, ONPG (*o*-nitrophenyl-β-D-galactopyranoside), urease, and gelatinase tests. H_2S production was variable. Most of the strains fermented glucose, mannose, maltose, fructose, mannitol, and sorbitol but failed to ferment lactose, raffinose, rhamnose, sucrose, or salicin.

The minimal inhibitory concentrations (MICs) of the 33 antimicrobial agents (Table 1) were determined by the agar dilution method (8), as previously described (25). Heart infusion agar supplemented with 5% Fildes enrichment and 0.5% yeast extract was used for the preparation of plates containing an antimicrobial agent. From 48 h of growth on a blood agar plate, a cell suspension containing approximately 10^7 colony-forming units per ml was prepared. A 5-μl amount of the suspension was spotted with a replicating apparatus on the agar plate containing an antimicrobial agent. MICs were determined after incubation at 37°C for 48 h under 10% CO_2.

The results of MIC determinations are shown in Table 1. *H. somnus* showed marked susceptibility to penicillin G (90% MIC, ≤0.10 U/ml) and to ampicillin, colistin, and novobiocin (90% MIC, ≤0.10 μg/ml). *H. somnus* was not susceptible to spiramicin and sulfadimethoxine. Twenty-seven other antimicrobial agents showed high or intermediate activities against *H. somnus*; their 90% MICs ranged from 0.20 to 25 μg (or U) per ml. Four strains were resistant to 100 μg of streptomycin per ml, but we could not detect any plasmid in them (data not shown).

The present results are in general agreement with those reported by others (9, 13, 14, 26), who tested only a limited number of antimicrobial agents and determined the susceptibility by the disk diffusion method. Although *H. somnus* shows high susceptibility to antimicrobial agents currently in veterinary use (e.g., penicillin G, ampicillin, and colistin), the treatment of TEME with these antimicrobial agents is often unsuccessful because of an acute progress of this disease. Bailie et al. (2) and MacDonald et al. (14) mentioned that treatment with large doses of antimicrobial agents in very early stages of the disease is highly effective and that withdrawal of excess cerebrospinal fluid and intrathecal

TABLE 1. Susceptibility of *H. somnus* to antimicrobial agents

Antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
Penicillins			
Penicillin G[b]	≤0.10	≤ 0.10	≤0.10
Ampicillin	≤0.10–0.20	≤ 0.10	≤0.10
Nafcillin	≤0.10–1.56	0.78	0.78
Cloxacillin	≤0.10–1.56	0.20	1.56
Dicloxacillin	≤0.10–1.56	0.39	1.56
Peptides			
Colistin	≤0.10	≤0.10	≤0.10
Polymyxin B[b]	0.78–1.56	1.56	1.56
Bacitracin[b]	6.25–50	25	25
Phosphorylated polysaccharide flavophospholipol	≤0.10–0.20	≤0.10	0.20
Chloramphenicol	≤0.10–0.39	0.39	0.39
Tetracyclines			
Doxycycline	0.39–0.78	0.39	0.78
Chlortetracycline	0.39–1.56	0.78	1.56
Tetracycline	0.20–1.56	1.56	1.56
Oxytetracycline	0.78–3.13	1.56	3.13
Macrolides			
Erythromycin	0.20–1.56	1.56	1.56
Kitasamycin	0.78–3.13	3.13	3.13
Tylosin	1.56–6.25	6.25	6.25
Oleandomycin	3.13–6.25	6.25	6.25
Spiramycin	12.5–100	50	100
Pleuromutilin tiamulin	0.78–1.56	1.56	1.56
Aminoglycosides			
Gentamicin	0.78–6.25	3.13	6.25
Aminodeoxy kanamycin	1.56–6.25	6.25	6.25
Kanamycin	1.56–6.25	6.25	6.25
Streptomycin	1.56–>100	6.25	6.25
Neomycin	6.25–25	25	25
Spectinomycin	6.25–25	25	25
Polyethers			
Salinomycin	1.56–12.5	3.13	6.25
Monensin	3.13–25	3.13	25
Others			
Novobiocin	≤0.10	≤0.10	≤0.10
Bicozamycin	0.20–0.78	0.39	0.39
Lincomycin	6.25–12.5	12.5	12.5
Nalidixic acid	1.56–6.25	3.13	3.13
Sulfadimethoxine	12.5–>100	100	>100

[a] 50% and 90%, Concentration required to inhibit 50 and 90% of strains, respectively.
[b] Units per milliliter.

injection of antimicrobial agents might be of value in advanced cases. Treatment with oxytetracycline, ampicillin, or chloramphenicol, all of which showed high activity against *H. somnus* in this study, has been reported to be partially successful (17, 22). If cattle are not watched closely or if the morbidity is particularly high, mass medication of drinking water with oxytetracycline or sulfadimethadine is recommended by van Dreumel et al. (26). In our experience, the administration of antimicrobial agents after shipment, which may cause great stress to animals and allow many pathogens to invade them, has proved effective in reducing the occurrence of TEME.

As excellent response to treatment with anti-

microbial agents can be expected for respiratory infections of *H. somnus* (1), clinical evaluations of the antimicrobial agents highly active in vitro against *H. somnus* is needed in the field.

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