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NOTES

Quantitative Antibiotic Sensitivities of Ruminal Bacteria[1]

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Fifteen species of ruminal bacteria were tested against 10 antibiotics in concentrations ranging from 0.1 to 200 μg/ml in an anaerobic tube dilution system.

Antibiotic susceptibilities of ruminal bacteria have been determined by using the disc method (1, 5). This work was undertaken to determine quantitatively the susceptibility of individual species of anaerobic ruminal bacteria to antibiotics by means of an anaerobic tube dilution method.

The work of Watanabe (10) on the genetics and biochemical mechanisms of multiple drug resistance of bacteria demonstrated the public health significance of these phenomena. It is important, therefore, to re-examine the whole question of the effects of antibiotics on the flora of the entire digestive tract of man and animals. The possibility, during the feeding of antibiotics, of development of resistant species of anaerobic pathogenic bacteria must also be considered, since some of these organisms, especially those found in the digestive tract and associated with mucosal surfaces in the body, may also cause diseases in man (3, 4, 8, 9). Aureomycin (chlortetracycline) is the antibiotic used in feed supplements for cattle, and its use is limited to nursing and weaning supplements and to feed-lot supplements. Antibiotics are, nevertheless, widely used in feeds for other animals because of their effects on increased growth rates of young animals, decreased incidence and severity of disease, and reduction of mortality (6). To avoid the danger of build-up of drug-resistant strains and transfer of resistance between bacteria, it would be best, theoretically, that the antibiotics used in feed be different from those therapeutic antibiotics used in the treatment of human and animal diseases.

The bacterial species used in this study (Table 1) were the same ruminal strains utilized in our previous study (5). These organisms were described by Bryant (2). Recharacterization of these cultures during our study showed that most of their characteristics have remained stable since their isolation more than 10 years ago.

The composition of the medium used was the same as that described in Table 1 of our previous publication (5), except that component groups 4 and 10 were omitted. The preparation was similar to that employed in the Hungate technique (7), as previously described (5). Portions (9 ml) of the medium were dispensed in test tubes (18 by 150 mm) under oxygen-free carbon dioxide and sealed with neoprene stoppers. Anaerobic diluting medium was composed of component groups 1, 2, 3, 8, and 9, described in Table 1 of our previous publication (5), and was prepared in a manner similar to the preparation of the medium.

The antibiotics used were: zinc bacitracin (lot no. 69330-62EA; Chas. Pfizer & Co., Brooklyn, N.Y.), chlortetracycline hydrochloride (lot no. 48175-890; Lederle Laboratories, Pearl River, N.Y.), erythromycin (base) (lot no. 2334-123; Abbot Laboratories, North Chicago, Ill.), kanamycin sulfate (lot no. 67F2797-M6802; Bristol-Myers Co., New York, N.Y.), neomycin sulfate (lot no. R09140; Eli Lilly and Co., Indianapolis, Ind.), oleandomycin phosphate (lot no. 53527-76100; Chas. Pfizer & Co.), oxytetracycline hydrochloride (lot no. 44596-51010; Chas. Pfizer & Co.), penicillin G procaine (lot no. 720-2719; Abbott Laboratories), streptomycin sulfate (lot no. 826739; Eli Lilly & Co.), and tylosin tartarate (lot no. ONT 16; Eli Lilly & Co). Antibiotics

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TABLE 1. *Minimal inhibitory concentrations of antibiotics*[a]

Antibiotic	Bacterial culture no.[b]														
	1	3	5	6	7	8	9	10	11	12	13	14	15	16	17
Bacitracin	R[c]	10	100	1	10	10	10	1	10	0.1[d]	R	1	100	10	100
Chlortetracycline	100	10	1	100	100	10	10	100	100	—[e]	100	100	100	100	1
Erythromycin	0.1	1	—	10	R[h]	0.1	10	1	0.1	—	100	1	200	R[h]	1
Kanamycin[f]	79	R	158	158	R	R	R	R	R	0.1	R	R	R	R	R
Neomycin	R	100	200	R	R	R	R	R	100	0.1	R	200	R	R	R
Oleandomycin	1	R	—	100	200	R[g]	200	100	100	—	200	R[g]	200	R	200
Oxytetracycline	10	10	1	1	10	1	10	0.1	1	0.1	10	100	10	10	1
Penicillin G	100	10	10	100	100	100	100	R	100	1	10	10	100	100	100
Streptomycin	R	R	—	R[g]	R	R	R	R	R	—	R	—	R	R	R
Tylosin	R	10	10	100	200	100	200	1	10	1	R	1	200	100	100

[a] Expressed as micrograms per milliliter of activity.

[b] Bacterial cultures listed according to number are: (1) *Bacterioides amylophilus*, H-18; (3) *B. ruminicola brevis*, GA-33; (5) *B. succinogenes*, S-85; (6) *Butyrivibrio fibrisolvens*, 49; (7) *Eubacterium ruminantium*, B_1C-23; (8) *Lachnospira multiparus*, D-32; (9) *Peptostreptococcus elsdenii*, B-159; (10) *Eubacterium limosum*, L-34; (11) *Ruminococcus albus*, 7; (12) *R. flavefaciens*, C-94; (13) *Selenomonas ruminantium*, HD-1; (14) *Spirillum* species, B-385; (15) *Streptococcus bovis*, FD-10; (16) *Succinimonas amylolytica*, B_24; and (17) *Succinivibrio dextrinosolvens*, 24. [Same as in Table 3 in previous publication (5)].

[c] Resistant at all levels tested in this study.

[d] Inhibition at all levels tested in this study.

[e] Not tested in this study.

[f] Concentrations tested approximated 158, 79, 8, 0.8, and 0.1 μg/ml.

[g] Slight growth (optical density about 0.1) within range of 10 to 200 μg/ml.

[h] Slight growth (optical density about 0.1) within range 1 to 200 μg/ml.

received with less than 100% (weight) potency were used in amounts equaling 100% antibiotic activity, except for kanamycin. Antibiotics were dissolved in anaerobic diluting medium and sterilized by filtration through an ultra-fine sintered glass filter (Corning 5133A). Final concentrations of 200, 100, 10, 1, and 0.1 μg of antibiotic activity per ml of medium were prepared by serial dilutions in tubes of sterile medium. Tubes of sterile medium without antibiotics were used for uninoculated blanks and for zero-concentration inoculated controls.

Triplicate sets of tubes of each concentration of antibiotic were inoculated with 0.1 ml of 24- or 48-hr broth culture of the species to be tested. Tubes were incubated at 39 C. Growth of each tube was measured turbidimetrically in a Spectronic-20 colorimeter at a wave length of 370 nm after incubation periods of both 24 and 48 hr.

To determine whether the species was killed or was inhibited by antibiotic treatment, the inoculum in one tube of the minimum inhibitory concentration (MIC) level, antibiotic-treated broth medium was removed after 24 hr of incubation at 39 C by centrifugation at 10,200 × *g* for 15 min. The cells were washed once in anaerobic diluting medium, removed by centrifugation, and resuspended in 1 ml of diluting medium. The washed cells (0.3 ml) were inoculated into triplicate tubes of fresh antibiotic-free medium and were incubated for 72 hr at 39 C. If no growth occurred, the antibiotic was considered to be bactericidal. If growth occurred, the effect of the antibiotic was considered bacteriostatic.

The MIC of the 10 antibiotics for the 15 species studied are shown in Table 1. Resistant species were observed in antibiotic-treated cultures both in our previous (5) and present studies. Also, we found resistant individuals within susceptible populations. This was noticable not only in the previous disc assay but also in the tube dilution studies. Triplicate tubes having antibiotics at 0.1 MIC values often showed varying amounts of growth. It is presumed that a resistant mutant strain developed earlier in these tubes than in others or that individuals differing in susceptibility to the antibiotic were present. Furthermore, studies of the bactericidal versus bacteriostatic effects indicate that most of the antibiotics tested for these phenomena were bacteriostatic at the MIC for most of the susceptible bacteria. However, *Bacterioides succinogenes* exhibited a bactericidal response when treated with the MIC of bacitracin, kanamycin, or tylosin. Similarly, tylosin killed *B. ruminicola brevis* and kanamycin killed *Butyrivibrio fibrisolvens*. All other susceptible cultures exhibited a bacteriostatic response when treated with the MIC of bacitracin, kanamycin, neomycin, oxtetracycline, penicillin, and tylosin.

Antibiotic susceptibilities determined by tube-dilution assay in this study agree with the disc-assay study of el Akkad and Hobson (1). The results of these tube-dilution assay studies confirm the disc-assay study (5), although several differences were found.

Antibiotic sensitivity data regarding ruminal bacteria may be helpful in adjusting specific ruminal functions with antibiotics to permit rapid adaptation to new diets. For example, M. J. Allison and I. M. Robinson (*personal communications*) suggested the use of antibiotic susceptibility data to depress acid production in the rumen from the feeding of high-starch diets. Thus, a transition to a high-starch diet might well be made without the development of acidosis.

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