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Antibiotic Resistance of Fecal Coliforms After Long-Term Withdrawal of Therapeutic and Subtherapeutic Antibiotic Use in a Swine Herd†

BRUCE E. LANGLOIS,* GARY L. CROMWELL, TIM S. STAHLY, KARL A. DAWSON, AND VIRGIL W. HAYS

Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-02151

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Tetracycline resistance of fecal coliforms isolated from swine decreased from 82 to 42%, a decrease of less than 50%, after the use of all forms of antimicrobial agents were discontinued in the herd for 126 months.

Various antimicrobial agents have been used in animal feeds for more than a quarter of a century for growth promotion, improved feed efficiency, and for control and prevention of disease (2–4). This use of antimicrobial agents has resulted in a high percentage of tetracycline-resistant, lactose-positive bacteria comprising the fecal flora of pigs (5, 7). As a result, it is difficult, if not impossible, to obtain weanling pigs with a low percentage (<20%) of tetracycline-resistant, lactose-positive enteric bacteria (fecal coliforms) for use in antibiotic feeding studies.

In an attempt to have pigs with low levels of tetracycline resistance available for use in our antibiotic feeding studies, we discontinued both the therapeutic and subtherapeutic use of antimicrobial agents in the University of Kentucky swine herd (nonantibiotic herd) at the Kentucky Agricultural Experiment Station Research Farm, Princeton, Ky., in May 1972. The nonantibiotic herd is a specific-pathogen-free Yorkshire herd that was established in 1963. It is a closed herd, with new bloodlines introduced into the herd only by artificial insemination. Before 1972, antibiotics were used routinely as feed additives and as injectables when needed for treating sows and pigs, but no single antibiotic was used continuously. Approximately one-third of the breeding pigs are replaced annually with gilts obtained from previous farrowings. There are two farrowings a year in the herd. Sows are kept in a pasture during gestation and are farrowed in crates that are thoroughly cleaned and disinfected between each group of sows, and the pigs are finished in concrete-floored pens that are cleaned and disinfected between each group of pigs. Feed is mixed in a feed mill on the farm, and antimicrobial agents or feed ingredients containing antimicrobial agents are not allowed in the building. Corn and soybean meal used as feed ingredients are either grown on the farm or purchased directly from a soybean processor. None of the ingredients used in the feed is purchased from a commercial feed mill.

Six months after the use of antimicrobial agents was discontinued in the herd, we began to monitor the antibiotic resistance of the lactose-positive enteric bacteria in the herd. During the initial 41 months of antibiotic withdrawal, water, soil, and feed samples were obtained at the same time that the fecal samples were collected. MacConkey agar was used for plating all samples (5). After incubation of the plates at 37°C for 24 h, five lactose-positive colonies were picked per sample and subjected to antimicrobial susceptibility testing. Susceptibility to ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), colistin (10 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), nitrofurantoin (300 μg), penicillin (100 U), polymyxin B (300 U), streptomycin (10 μg), sulfamethizole (1 mg), and tetracycline (30 μg) was tested by the method of Bauer et al. (1).

Percentages of environmental isolates resistant to tetracycline were 5% for 77 soil isolates, 11% for 161 feed isolates, and 17% for 122 water isolates (5). These values were much lower than the tetracycline resistance of the fecal isolates obtained during the same period or of those obtained after 126 months of antibiotic withdrawal.

A total of 3,094 lactose-positive enteric bacteria have been isolated from pigs in the nonantibiotic herd since antibiotic withdrawal in May 1972. Less than 1% of the isolates were resistant to chloramphenicol, colistin, nitrofurantoin,

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gentamicin, kanamycin, neomycin, nalidixic acid, and polymyxin B, whereas less than 3% were resistant to cephalothin. The percentages of the isolates resistant to ampicillin, streptomycin, tetracycline, and sulfamethizole at each time the herd was sampled are shown in Table 1. Resistance to ampicillin and sulfamethizole tended to fluctuate during the 126-month period, with no trends being apparent. Fluctuation was observed in resistance to streptomycin for 58 months, and then resistance decreased to less than 20%, where it has tended to remain.

A gradual decrease was observed in tetracycline resistance until month 84 (82 to 24%), and then resistance increased to over 40%, where it has remained. After 126 months of antibiotic withdrawal, tetracycline resistance has been reduced by less than 50% (82 to 42%). This decrease in tetracycline resistance is less than we had expected and is not as great as the decrease seen in the short-term withdrawal study of Larsen and Nielsen (6). Although other short-term withdrawal studies (5, 8) have shown little decreases in tetracycline resistance, we expected to see a greater reduction in resistance due to the length of the withdrawal period. Smith (7) reported a slight decrease in tetracycline-resistant *Escherichia coli* in swine, but the number of pigs excreting these organisms did not change during the 4-year period after implementation of the Swann Report (9) by the British Government. He postulated that during the time that tetracycline was fed, resistant strains emerged that were able to compete on equal terms with sensitive strains.

The apparent failure of long-term withdrawal of antibiotics to markedly reduce tetracycline-resistant, lactose-positive enteric bacteria in swine indicates that the selection of resistant bacteria brought about by long-term feeding of antibiotics may not be readily reversed by withdrawal.

TABLE 1. Antibiotic resistance of lactose-positive fecal isolates from swine after withdrawal of antibiotics in the herd

Mo after withdrawal	No. of isolates	% of isolates with resistance to:[a]			
		AM	S	TE	TH
6	34	18	62	82	24
8	23	4	35	74	22
10	139	5	25	72	16
13	163	12	37	41	13
16	166	0	28	65	21
24	412	7	28	59	21
32	47	4	47	72	38
33	78	6	24	46	10
41	135	9	25	58	24
58	95	15	45	51	92
73	255	6	15	44	22
75	175	4	13	28	15
84	199	3	2	24	6
89	249	14	13	49	23
92	269	12	12	47	23
96	375	4	23	57	47
126	280	13	16	42	23

[a] Paper disk potency for the following antibiotics (average percentage of isolates with resistance): AM, ampicillin, 10 μg (8); S, streptomycin, 10 μg (21); TE, tetracycline, 30 μg (50); TH, sulfamethizole, 1 mg (25).

LITERATURE CITED

1. **Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493–496.
2. **Hays, V. W.** 1978. The role of antibiotics in efficient livestock production, p. 545–576. *In* J. N. Hathcock and J. Coon (ed.), Nutrition and drug interrelations. Academic Press, Inc., New York.
3. **Jukes, T. H.** 1972. Antibiotic in animal feeds and animal production. Bioscience **22**:526–534.
4. **Kiser, J. S.** 1976. A perspective on the use of antibiotics in animal feeds. J. Anim. Sci. **42**:1058–1072.
5. **Langlois, B. E., G. L. Cromwell, and V. W. Hays.** 1978. Influence of chlortetracycline in swine feed on reproductive performance and on incidence and persistence of antibiotic resistant bacteria. J. Anim. Sci. **46**:1369–1072.
6. **Larsen, J. L., and N. C. Nielsen.** 1975. Influence of restrictive use of antibiotics on the development of drug resistance in intestinal *Escherichia coli* from pigs. Nord. Veterinaermed. **27**:353–364.
7. **Smith, H. W.** 1977. Antibiotic resistant bacteria and associated problems in farm animals before and after the 1969 Swann Report, p. 344–357. *In* M. Woodbine (ed.), Antibiotics and antibiosis in agriculture. Butterworths, London.
8. **Smith, H. W., and W. E. Crabb.** 1957. The effect of continuous administration of diets containing low levels of tetracycline on the incidence of drug resistant *Bacterium coli* in the feces of pigs and chickens: the sensitivity of *Bact. coli* to other chemotherapeutic agents. Vet. Rec. **69**:24–30.
9. **Swann, M. J.** 1969. Joint committee on the use of antibiotics in animal husbandry and veterinary medicine. Report, Cmnd. 4190. Her Majesty's Stationery Office, London.