Persistence of Transferable Drug Resistance in the Lactose-Fermenting Enteric Flora of Swine Following Antimicrobial Feeding

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ABSTRACT

Six groups of swine (85 animals) were fed a combination of antimicrobial drugs (sulfamethazine 100 g/ton, chlortetracycline 100 g/ton and penicillin 50 g/ton). After two weeks the antimicrobial drugs were removed from the diet of two groups (28 animals). These swine were compared to four groups fed the medicated diet to determine the effect of duration of treatment and degree of animal isolation on the persistence of resistance in lactose-fermenting enteric organisms. The degree of resistance to penicillin, oxytetracycline, dihydrostreptomycin and neomycin as determined by minimum inhibitory concentrations and the incidence of resistant organisms were examined during and after antibiotic feeding. Ninety-two percent or greater of all isolates tested during and after treatment had minimum inhibitory concentrations for oxytetracycline of >100 μg/ml. Thirty-two weeks after cessation of dietary antibiotic, resistance to oxytetracycline and dihydrostreptomycin remained at 100% and 89% respectively. Variation in degree of contact between swine receiving medicated feed and those receiving nonmedicated feed was not sufficient to reduce the incidence of resistance to oxytetracycline or dihydrostreptomycin in all animals. Factors influencing persistence of resistant enteric organisms are discussed. Addition of the antimicrobials to the ration resulted in significantly greater weight gains for treated animals than for the controls but did not alter feed conversion.

RÉSUMÉ

Les auteurs ont incorporé à la moulée servie à six groupes de porcs comptant 85 sujets, le mélange suivant de substances antimicrobiennes: 100 g/tonne de sulfaméthazine, 100 g/tonne de chlortétracycline et 50 g/tonne de pénicilline. Au bout de deux semaines, ils arrêtèrent d'incorporer ces substances à la moulée servie aux 28 porcs formant deux des groupes expérimentaux. Ils les comparèrent ensuite à ceux des quatre autres groupes recevant la moulée médicamentée, afin de déterminer l'effet de la durée du traitement et du degré d'isolement des animaux sur la persistance de la résistance des microbes intestinaux qui fermentent le lactose. Ils examinèrent aussi, durant et après l'alimentation médicamentée, le degré de résistance à la pénicilline, à l'oxytétracycline, à la dihydrostreptomycine et à la néomycine, en déterminant les concentrations inhibitrices minimales et l'incidence des microbes résistants. Quatre-vingt-douze pourcent ou plus des souches isolées et éprouvées, durant ou après le traitement, possédaient des concentrations inhibitrices minimales supérieures à 100 μg/ml, à l'endroit de l'oxytétracycline. Trente-deux semaines après l'arrêt de l'administration d'antibiotiques, la résistance à l'oxytétracycline et à la dihydrostreptomycine demeurait respectivement à 100% et à 89%. La variation dans le degré de contact entre les porcs recevant de la moulée médicamentée et les témoins ne s'avéra pas suffisante pour réduire l'incidence de la résistance à l'oxytétracycline ou à la dihydrostreptomycine chez tous les sujets. Les auteurs commentent les facteurs qui influencent la persistance de microbes intestinaux résistants L'addition de substances antimicrobiennes à la moulée se traduisit par des gains de poids plus appréciables chez les porcs expérimentaux que chez les témoins; elle n'altéra cependant pas le taux de conversion alimentaire.

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Submitted May 20, 1975.

INTRODUCTION

Transferable drug resistance in the *Enterobacteriaceae* has been the subject of many reports since its discovery. The feeding of antimicrobials to calves (3), swine (2,15) and chickens (5,6,10) results in an increased incidence of drug resistant enteric organisms. Under experimental conditions the incidence of resistant enteric organisms has decreased significantly in a relatively short time after the withdrawal of antimicrobials (2, 3, 5, 10, 15). Under the above experimental conditions the enteric flora was relatively drug sensitive before the administration of antimicrobials and extent of antimicrobial usage was limited. In contrast to the experimental situation, swine were studied on farms where all animals had been fed tetracyclines for approximately two years. Seven months after tetracyclines were removed from the rations high levels of resistant enteric organisms persisted (13). In the United Kingdom, 16 months after prohibition of the use of tetracyclines as feed additives, the incidence of *E. coli* resistant to tetracycline in pigs may have decreased but the number of animals with such organisms had not decreased (14).

In the United States a review was conducted of the animal and public health significance of high incidences of resistant enteric organisms resulting from the use of antimicrobials in animal feeds (4). One aspect that requires further clarification is the duration of high levels of enteric organisms with R-factors after the withdrawal of antimicrobials. In conjunction with a swine production study to determine the influence of feeding antimicrobials on weight gains and feed conversions, this experiment was conducted to determine persistence of transferable drug resistance in lactose-fermenting enteric organisms and levels of resistance in individual isolates after removal of feed supplemented with antimicrobials.

MATERIALS AND METHODS

Experimental Design and Animal Maintenance

Eighty-five crossbred swine were randomly allotted to four treatment and two control groups. A split-plot design was used where housing units served as the mainplot and dietary treatments served as the subplots (Table I). Fifty-six of the animals, averaging 10 kg body weight, were randomly allotted from littermate, sex and weight outcome groups to three replications of four dietary treatment groups to investigate the effect of antibiotic supplementation on growth and feed conversion efficiency. Their treatments were designated T-1, T-2, T-3 and T-4. Five animals per pen were used in replications 1 and 2, whereas in the third replication four animals comprised a pen. An additional group (T-5) was replicated like those above and was used as a microbiological control group. Another treatment group (T-6) was replicated twice with seven and eight animals per replicate. Each replicate was started at staggered intervals of two weeks when the mean body weight was approximately 10 kg.

Treatment consisted of feed supplemented with sulfamethazine (100 g/ton), chlortetracycline (100 g/ton) and penicillin (50 g/ton)[1], a combination which is commonly used in animal rations in this country. The antimicrobial supplement replaced an equal amount of corn in the diet. A 16% protein, corn-soybean meal diet supplemented with minerals and vitamins (9) was used throughout the experiment. The medicated feed was provided to all animals for approximately two weeks before starting the feeding trial and collecting the first fecal sample for microbiological determinations. During the feeding trial medicated feed was provided according to a body weight or time interval schedule as outlined in Table I.

Feed and water were provided *ad libitum*. Animal and feed weights were recorded at the beginning and biweekly until they approached 100 kg body weight, at which time weights were recorded weekly until the swine were removed from the experiment.

Three separate animal maintenance facilities were utilized as indicated in Table I. Cross contamination between facilities via feed and personnel was kept at a minimum. Facilities 1 and 2 were completely enclosed and were equipped with partially slotted floors and ventilation fans. Facility 3 was a completely enclosed facility and was equipped with steel pens with slotted floors. Temperature, humidity and ventilation

[1]Aureo SP 250, American Cyanamid Co., Princeton, New Jersey 08540.

TABLE I. Experimental Design

Feeding Regimen[a,b]	Replicates	No. Animals Per Replicate	Time Interval on Medicated Feed[c]
Facility 1			
Control feed 10-100 kg body weight (BW); (T-4)	1	5	2 weeks
	2	5	
	3	4	
Medicated feed 10-40 kg (BW); control feed 40-100 kg BW; (T-1)	1	5	7 weeks
	2	5	9 weeks
	3	4	11 weeks
Medicated feed 10-70 kg BW; control feed 70-100 kg BW; (T-2)	1	5	12 weeks
	2	5	13 weeks
	3	4	15 weeks
Medicated feed 10-100 kg BW; (T-3)	1	5	16 weeks
	2	5	16 weeks
	3	4	20 weeks
Facility 2			
Control feed 10-100 kg BW; (T-5)	1	5	2 weeks
	2	5	
	3	4	
Facility 3			
Intermittent-medicated feed first 14 days and again from 32-43 days; control feed at other times; (T-6)	1	8	0-14 days
			32-43 days
	2	7	0-14 days
			32-43 days

[a]Animals were placed on experiment when the mean weight of the replicate was 10 kg. All animals including T-4 and T-5 were exposed to the medicated feed for two weeks prior to initiation of the feeding trial
[b]Feed medication: Sulfamethazine 100 g/ton, chlortetracycline 100 g/ton, penicillin 50 g/ton.
[c]Medicated feed provided according to mean body weight of each replicate. Includes the two weeks of pretrial treatment

rates of the facilities were not measured and thus objective evaluations of these conditions could not be made.

Five animals from T-5 were moved from Facility 2 into individual semi-isolation units after 20 weeks on the experiment. They were maintained in these units for an additional 12 weeks.

Gain data were analyzed for each period (Period 1, 10-40 kg B. W., Period 2, 40-70 kg B.W. and Period 3, 70-100 kg B.W.) and for the entire feeding period (10-100 kg B.W.). Statistical analysis of weight gains and feed conversion was made by least squares analysis of variance for unequal subclass numbers (7). Incidence of resistant bacteria was analyzed by weighted regression analysis techniques.

Fecal specimens were collected from animals housed in Facilities 1 and 2 as the feeding trial was initiated and at intervals of two weeks. Sampling for each pen was discontinued when any animal that had attained 100 kg was removed from a particular pen. For animals housed in Facility 3, fecal specimens were collected on days 0, 4, 32, 43, 53, 78, 91 and 107. Fecal specimens were collected from individual animals and composited on a pen basis. Two grams of fecal material from each animal were mixed to form uniform samples which were used for microbiological determinations.

A pour-plate counting procedure was utilized to determine the incidence of lactose-fermenting enteric organisms resistant to oxytetracycline (OTC). A 5 g aliquot of each sample was homogenized with 45 ml of phosphate buffer (pH 7.2). Ten-fold serial dilutions were prepared from the resulting fecal suspensions and 0.1 ml of the appropriate dilution was put into each plate and 15 ml of MacConkey agar or 15 ml of MacConkey agar plus OTC[2] (250 μg/ml) was poured into the plate. Colony counts from plates containing MacConkey agar were compared to colony counts from plates containing MacConkey agar plus OTC.

MacConkey agar plates without OTC served as a source for bacterial isolations. Two colonies typical of *E. coli* from each composite pen sample were screened on triple sugar iron agar. Cultures demon-

[2]Oxytetracycline HCl, Pfizer Inc., New York, New York 10017.

strating typical *E. coli* reactions were subjected to determinations of minimal inhibitory concentration (MIC) for penicillin[3] (PEN), OTC, dihydrostreptomycin[4] (DSM) and neomycin[5] (NEO) employing a microdilution technique of MacLowry *et al* (8) except that the procedure was performed manually.

The method of Schroeder *et al* (12), slightly modified, was used to test selected strains for their ability to transfer resistance determinants. Six media were used: MacConkey agar, MacConkey agar plus 25 μg nalidixic acid[6] (NA) per ml, MacConkey agar plus 25 μg NA and 4 μg tetracycline[7] per ml, MacConkey agar plus 25 μg NA, 10 μg of ampicillin[8] and 10 μg of dicloxacillin[8] per ml, MacConkey agar plus 25 μg NA and 25 μg DSM per ml, MacConkey agar plus 25 μg NA and 20 μg NEO per ml. An *E. coli* (O83:K untypable:H14) mutated to high NA resistance was used as the recipient organism. Resistant patterns of the recipient were determined by a standardized single disc method (1) where transfer was detected in the recipient. The following antimicrobials were used in the testing system: ampicillin, cephalothin, sulfamethoxypyridazine, colistin, chloramphenicol, furazolidone, NEO, tetracycline, NA and DSM (10 μg) in place of streptomycin[9].

RESULTS

Results of the feeding experiment are presented in Table II. Of the 56 animals which started the feeding experiment six pigs did not complete it. Results of necropsy indicated anemic conditions, moderate *Ascaris* infection, septic arthritis, exudative epidermitis and chronic extensive pneumonia but all pigs did not manifest each symptom. One pig died as a result of a rectal prolapse. Cause of death was not attributed to any dietary treatment.

During Period 1 (10-40 kg) pigs receiving the antibiotic diets (T-1, T-2 and T-3) gained more rapidly ($P < 0.05$) than did pigs receiving the control diet (T-4). The magnitude of response between antibiotic fed pigs and control pigs was reduced during the 40-70 kg period (Period 2) but the response was still in favor of pigs receiving antibiotics ($P < 0.08$). In Period 3 (70-100 kg) antibiotic fed pigs gained more rapidly ($P < 0.01$) than did the control pigs. This response resulted more from a greater reduction in growth rate of the control pigs than from an increased growth rate in the antibiotic fed pigs. The carryover effects from previous weight periods were not statistically significant. After they had been switched to nonmedicated control feed the pigs in T-1 and T-2, which had received medicated feed up to 40 or 70 kg BW, did not gain significantly ($P < 0.10$) faster than control pigs in T-4. The difference in daily gain between pigs receiving antibiotic to 70 kg BW (T-2) vs. 100 kg BW (T-3) suggests that withdrawal of antibiotic at 70 kg BW would be detrimental to weight gain. However, the reasons for this decline are not readily discernible.

Addition of antibiotic to the diet from 10 to 100 kg BW (T-3) improved average daily gain ($P < 0.05$) as compared to control (T-4) pigs (0.748 vs. 0.651, respectively). There appeared to be a carryover effect in that pigs receiving antibiotic during the 10-40 kg (T-1) period only gained more rapidly ($P < 0.08$) than did the controls for the entire experiment. However, the response of the 10-70 kg BW-treated pigs (T-2) was not significantly different from the controls. The addition of antibiotic to the diet irrespective of feeding period resulted in a 10% improvement in growth rate as compared with control pigs. Statistical analysis of the data for unequal subclass numbers showed that the replication x treatment interactions for the various weight periods were not statistically significant and the order of treatment response was the same in all replicates.

All animals had been "primed" with medicated feed before collection of the first sample. Thus, the mean incidence of lactose-fermenting enteric organisms resistant to 250 μg/ml of OTC was relatively high in all groups of animals (56% or

[3]Crystalline penicillin G, Pfizer Inc., New York, New York 10017.

[4]Hamilton Pharmacal Co., Hamilton, New York 13346.

[5]FDA working standard, National Center for Antibiotic Analysis, Food and Drug Administration, Washington, D.C. 20204.

[6]Sterling-Winthrop Research Institute, Rensselear, New York 12144.

[7]Steclin, E. R. Squibb and Sons, New Brunswick, New Jersey 08903.

[8]Bristol Laboratories, Syracuse, New York 13201.

[9]Sensi-Discs, Baltimore Biological Laboratories, Cockeysville, Maryland 21030.

TABLE II. Effect of Antibiotic on Pig Performance

	Antibiotic Feeding Period, by Weight Group[a]				
	10-40 kg BW (T-1)	10-70 kg BW (T-2)	10-100 kg BW (T-3)	No Antibiotic (T-4)	C.V.[b]
No. started/treatment	14	14	14	14	
No. completing experiment/ treatment	12[c]	14	12[d]	12[d]	
Av. initial wt, kg	12.0	12.2	12.0	12.1	
Av. final wt, kg	101.1	99.1	101.1	98.1	
Av. daily gain, kg[e]					
10-40	0.644	0.651	0.632	0.582	15.02
40-70	0.759	0.792	0.829	0.738	15.15
70-100	0.753	0.653	0.822	0.657	20.39
10-100	0.717	0.688	0.748	0.651	14.10
Feed/gain, kg[f] (10-100)	3.12	2.97	3.03	3.08	5.00

[a]All animals received the medicated feed containing penicillin (50 g/T), chlortetracycline (100 g/T) and sulfamethazine (100 g/T) for two weeks before being placed on experiment
[b]Coefficient of variation
[c]One pig died from rectal prolapse and a second pig was sacrified due to poor performance. Necropsy report indicated anemic condition with pale lungs
[d]Two pigs per treatment removed from experiment. Necropsy report indicated moderate Ascaris infection, septic arthritis, exudative epidermitis and chronic extensive pneumonia. Not all pigs manifested each symptom
[e]Each figure represents least squares mean of individual pig gain
[f]Feed/gain represents pen average

greater) when the first samples were collected. Control animals (T-4 and T-5) were switched to the antibiotic-free diet just before collection of the first sample. It was not until four weeks that differences between treated (T-1, T-2 and T-3) and control (T-4 and T-5) animals could be observed (Fig. 1). After four weeks, mean values for the control groups tended to fluctuate between 0.1 and 20%, whereas mean values for animals receiving treatment fluctuated from 45 to 70% with a few exceptions. Because of sample variation these differences are not significant.

The mean incidence of lactose-fermenting enteric organisms resistant to OTC (250 μg/ml) for animals receiving intermittent exposure to antibacterially supplemented feed (T-6) is illustrated in Fig. 2. Animals had received medicated feed for two weeks before collection of the first sample. Thus, the baseline incidence was high (60%) at the start of the first treatment period. During treatment periods the level of resistant organisms was 60-70% except for the value obtained at the end of the first treatment. After the second treatment the level dropped to 20% or less and persisted in this range for 64 days after the end of the second treatment.

Further definition of resistance characteristics was obtained by determining MICs for PEN, OTC, DSM and NEO on individual isolates typical of *E. coli* which were collected from each composite sample. Results of the MIC determinations were grouped according to antibiotic supplementation: Receiving antimicrobials (T-1, T-2, T-3), after withdrawal of antimicrobials where antimicrobials had been given longer than two weeks (T-1, T-2), after withdrawal of antimicrobials where antimicrobials had been given for two weeks and pigs were housed in the same environment as those receiving antimicrobials (T-4), after withdrawal of antimicrobials where antimicrobials had been given for two weeks and pigs were housed in a separate facility from those receiving antimicrobials (T-5) and pigs from T-5 kept in semi-isolation beyond the initial 20-week sampling time. Animals were exposed to two, seven, nine, 11, 12, 13 and 15 weeks of medicated feeding and subsequently studied when they were switched to control feed.

The MIC for OTC was >100 μg/ml for 92% or more of the isolates tested from each animal group (Table III). The greatest portion of isolates (76-83%) fell in the 101-1000 μg/ml range. There was no correlation between duration of treatment or no treatment and occurrence of isolates in the >1000 μg/ml category.

The MIC for DSM was either <25 μg/ml or >100 μg/ml for 93% or more of the

TABLE III. Minimum Inhibitory Concentration (MIC) of Lactose-Fermenting Enteric Organisms Isolated From Swine During and After Feeding Penicillin (50 g/t), Chlortetracycline (100 g/t) and Sulfamethazine (100 g/t)[a]

Antibiotic	MIC Range	Percent of Isolates in the Specified MIC Ranges I	II	III	IV	V
Oxytetracycline	0-24	1	3	1	6	0
(μg/ml)	25-100	1	5	4	0	0
	101-1000	80	82	82	76	83
	>1000	18	10	13	20	17
Number of isolates tested		136	39	66	58	18
Dihydrostreptomycin	0-24	26	23	20	39	11
(μg/ml)	25-100	2	3	6	7	5
	101-1000	28	38	48	24	42
	>1000	44	36	26	30	42
Number of isolates tested		131	39	62	59	19
Penicillin	0-24	5	15	15	16	5
(units/ml)	25-100	9	28	29	20	10
	101-1000	8	15	5	10	11
	>1000	78	42	51	54	74
Number of isolates tested		136	40	65	61	19
Neomycin	0-24	44	50	52	34	72
(μg/ml)	25-100	40	45	29	47	28
	101-1000	10	3	14	14	0
	> 1000	6	2	5	5	0
Number of isolates tested		136	38	65	57	18

[a]I:Groups T-1, T-2, T-3, received medicated feed on a weight basis (T-1 to 40 kg, T-2, to 70 kg, T-3 continuously), treated up to 20 weeks, no time away from treatment, sampled at 2-20 weeks; maintained in Facility 1
II: Groups T-1, T-2, nonmedicated feed, treated 1st 6-14 weeks, 6-14 weeks away from treatment, sampled after being switched to nonmedicated feed; maintained in Facility 1
III: Group T-4, nonmedicated feed, treated 1st 2 weeks, 2-20 weeks away from treatment, sampled at 2-20 weeks, maintained in Facility 1
IV: Group T-5 conditions as for III except maintained in Facility 2
V: Group T-5 nonmedicated feed, treated 1st two weeks, 2-32 weeks away from treatment, sampled at 20-32 weeks, five animals were moved from Facility 2 to semi-isolation units at the end of 20 weeks

isolates tested from each study group. In Facility 1 there was a trend toward less resistance when MIC results from animals receiving treatment (T-1, T-2, T-3) are compared to results from animals that had been switched to nonmedicated feed (T-1, T-2) after seven weeks or more of treatment and animals that received treatment for only two weeks (T-4). There was even less resistance to DSM in isolates obtained from animals that received treatment for two weeks but were maintained in a separate facility (T-5) and were not given medicated feed. The organisms obtained from animals maintained in semi-isolation units from 20 to 32 weeks were more resistant than isolates collected from animals in Facility 1 or Facility 2.

The MICs of penicillin for enteric organisms isolated from animals receiving medicated feed were higher than for animals receiving nonmedicated feed except for animals maintained in semi-isolation. The frequency of isolates in the four MIC ranges was similar for animals receiving nonmedicated feed in Facilities 1 and 2. The level of resistance in isolates obtained from animals in semi-isolation was similar to that found in isolates obtained from animals receiving medication.

For NEO the MICs were concentrated in the lower two MIC ranges. For 81% or more of the isolates the MIC for NEO was <100 μg/ml. The percent of isolates in the various MIC ranges did not vary with treatment or lack of treatment in Facilities 1 and 2. The isolates obtained from animals in semi-isolation were the most sensitive.

Transferable resistance was detected at sampling intervals throughout the experiment. Such transferable resistance was found in isolates obtained from animals receiving treatment as well as those receiving nonmedicated feed.

DISCUSSION

A high incidence (95-100%) of lactose-fermenting enteric organisms was resistant to 25 μg/ml OTC during preliminary determinations. Because of this the level of OTC in the MacConkey agar plates was increased to 250 μg/ml to increase the sensitivity of detecting changes in OTC resistance in enteric bacterial populations. Since there was a high frequency (>90%) of isolates of lactose-fermenting enteric organisms that had an MIC value for OTC of >100 μg/ml at each sampling time for each group, it is likely that throughout the experiment the number of colonies growing on both MacConkey agar plates and MacConkey agar plates supplemented with OTC at 25 μg/ml (90-100%) would have been similar. However, since the level of OTC was raised to 250 μg/ml the sensitivity of the procedure was increased and variation at sampling intervals was observed.

With the increased sensitivity in the plate counting procedure, decreasing trends in the incidence of drug resistant lactose-fermenting enteric organisms were observed for groups T-5 and T-6 after cessation of treatment (Figs. 1 and 2). The significance of this trend may not be very practical when MIC data (Table III) are considered. Of all isolates that produced reactions typical of *E. coli* on MacConkey agar and triple sugar iron agar, 92-100% had an MIC of >100 μg/ml for OTC for all groups. Even at 30 weeks after treatment all isolates tested had an MIC for OTC of >100 μg/ml. Since all colonies that fermented lactose and had colony morphology similar to *E. coli* were counted in the plate counting procedure the isolates included in the MIC data are from a slightly more select group. This does not account for all the differences between results from the plate counting procedure and MIC data.

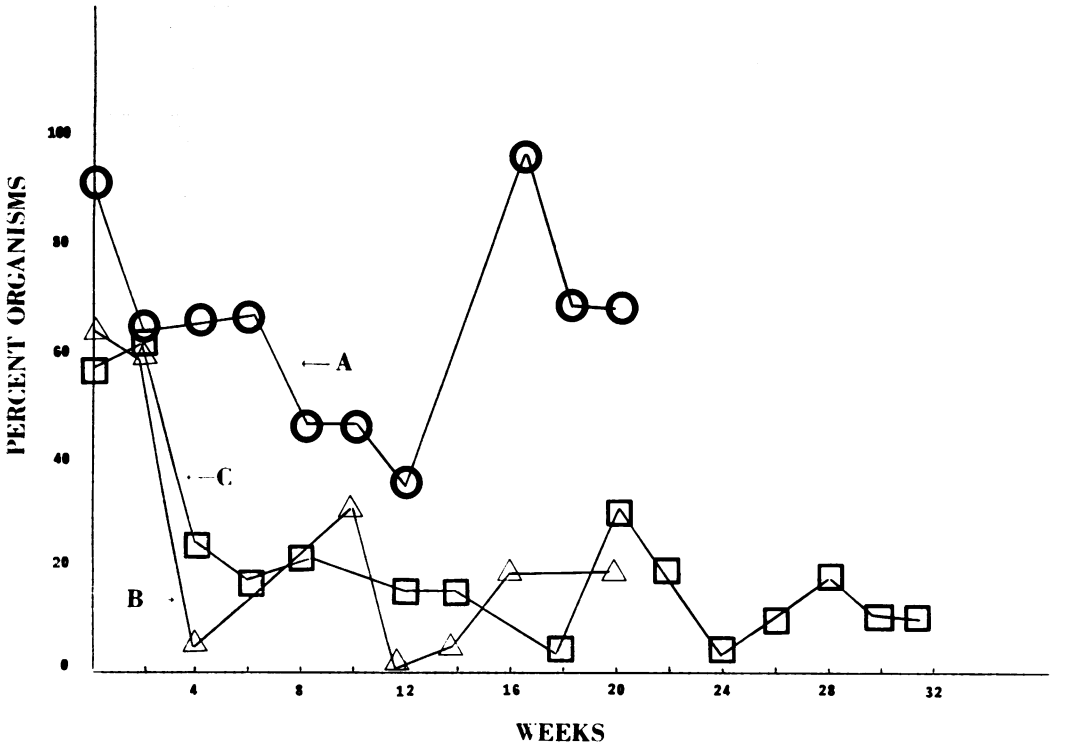

Fig. 1. The incidence of lactose-fermenting enteric bacteria in swine resistant to oxytetracycline (250 μg/ml) in control animals and in animals fed a diet containing chlortetracycline (100 g/T), sulfamethazine (100 g/T) and penicillin (50 g/T). "Percent organisms" refers to the number of lactose-fermenting enteric organisms growing on MacConkey agar containing oxytetracycline (250 μg/ml) divided by the number of lactose-fermenting organisms growing on MacConkey agar times 100.
A. Animals receiving medicated diet (T-1, T-2, T-3).
B. Animals receiving nonmedicated diet and maintained in same facility as animals receiving medicated diet (T-4).
C. Animals receiving nonmedicated diet but maintained in a facility separate from animals receiving medicated diet (T-5).

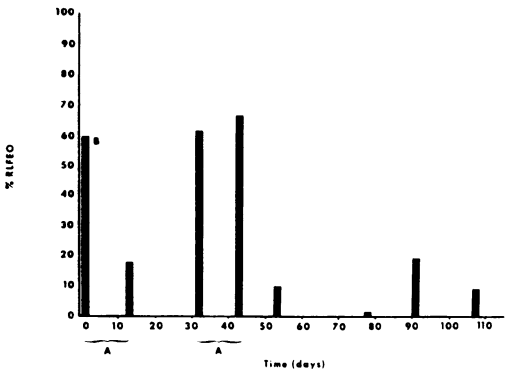

Fig. 2. Incidence of oxytetracycline resistance (250 μg/ml) in lactose-fermenting enteric organisms isolated from swine exposed intermittently to supplemented feed (T-6). RLFEO = Resistant lactose-fermenting enteric organisms.
A = Rations supplemented with sulfamethazine (100 g/T), chlortetracycline (100 g/T) and penicillin (50 g/T).
B = Animals exposed to medicated rations prior to first sample.

The differences may be due to drug binding characteristics of the media used or other factors that may affect antimicrobial activity of the drug.

The lack of treatment did not reduce the level (MIC) of resistance to OTC, a drug homologous to the treatment (because of cross resistance between OTC and CTC, OTC is considered homologous to CTC used in the feed). For DSM, a drug heterologous to the treatment, a high incidence of resistant organisms persisted throughout the study but the level of resistance in the organisms decreased after treatment ceased relative to the amount of animal contact and time away from treatment. This was not true for animals in semi-isolation. Treatment increased the resistance to penicillin, a homologous drug. Duration of treatment or time away from treatment did not influence the level of PEN resistance in organisms isolated from animals not receiving treatment in Facilities 1 and 2. Neither treatment nor lack of treatment caused any increase or decrease in resistance to NEO, a heterologous drug, except for isolates obtained from animals in semi-isolation.

The differences observed for animals kept in semi-isolation may be partly due to decreased influence from outside sources of organisms. If the organisms were highly resistant (OTC, DSM, PEN) when the animal entered the semi-isolation facility they tended to stay resistant. If they were more sensitive (NEO) they tended to stay sensitive.

Since, on a practical basis, little or no regression in percentage of OTC resistant isolates was observed after treatment the factors that may have influenced their persistence should be considered. The incidences of drug resistant enteric organisms in fecal material of cows and their immediate environment have been shown to be somewhat directly related (11). The availability of resistant or sensitive organisms in the immediate environment would be expected to influence the percentage of resistant enteric organisms in animals not receiving antimicrobials and thus the persistence of resistant organisms after feeding of antimicrobials. This would be especially true of swine raised in confinement where large numbers of enteric organisms would likely be recycled through their enteric tracts. Certain characteristics of organisms, such as ability to colonize the enteric tract, ability to transfer resistance (in the case of resistant strains), genetic stability of drug resistance and number of sensitive organisms relative to rate of transfer of drug resistance may influence persistence and incidence of resistant enteric organisms after an antimicrobial administration has stopped.

Since the swine had a high incidence of resistant enteric bacteria when the experiment started they were able to populate study facilities with drug resistant enteric organisms. Degree of contact for sources of organisms other than the animal's own fecal material varied between study facilities. Some sources would be other animals, feed, cleaning and feeding implements, material that the animal caretaker brought in on boots and clothing and the immediate environment. In Facility 1 there was animal-to-animal (pen-to-pen) contact between animals receiving medicated versus non-medicated feed, common pen cleaning equipment and animal caretaker contact. Animals in Facility 2 had no contact with animals receiving medicated feed but they had contact with animal caretakers and common cleaning equipment. There was no animal-to-animal contact, no common implements and minimal caretaker contact with animals in semi-isolation. Organisms from sources other than the animal's fecal material apparently did not influence the persistence of resistant organisms. This was especially true of the group maintained in semi-isolation where outside sources of organisms were minimized.

Importance of drug sensitive organisms

may become more apparent by examining another study (6). After antibiotic feed was removed from a group of chickens having a high incidence of resistance in their G.I. flora, chickens with a predominantly sensitive G.I. flora were introduced into the group. The number of chickens carrying resistant organisms and the incidence of resistant organisms declined in the treated group, whereas in another group of chickens the incidence of drug resistant enteric bacteria remained stable at a high level after antibiotic feeding was stopped.

Other investigators (2, 3, 5, 6, 10) have observed a decrease in incidence of resistant bacteria in a relatively short time after antibiotic administration ceased. The antibiotic usage in such experiments was of short duration and on a one time basis. Conversely, where antimicrobials are fed to all animals in each generation year after year, sufficient pressure is exerted to establish and maintain an enteric and environmental flora that is mostly resistant.

The effects of drug resistant enteric organisms on therapy of disease and death loss was not assessed in this experiment since the experimental design did not allow for treatment of diseased swine. However, for the commercial swine producer a determination of the economic benefit of feeding antimicrobials for improved weight gain and/or feed conversion should also include the effect of the antimicrobials on the transferable drug resistance in the enteric flora and their subsequent effect on therapy of disease and death loss. If the level of enteric organisms with transferable drug resistance is to be reduced in swine, time away from antimicrobial pressure may be the factor that is needed to affect the stability of the resistances.

ACKNOWLEDGMENTS

We wish to thank Elaine Cohen and Vernon Morris for their technical support.

REFERENCES

1. **BAUER, A. W., W. M. M. KIRBY, C. C. SHERRIS and M. TURCK.** Antibiotic susceptibility testing by a standard single disc method. Am. J. clin. Path. **45**: 493-496. 1966.

2. **EDWARDS, S. J.** Effect of streptomycin on the growth rate and intestinal flora (**Escherichia coli**) of piglets J. comp. Path. Ther. **71**: 243-252. 1961.

3. **EDWARDS, S. J.** Effect of antibiotics on the growth rate and intestinal flora (**Escherichia coli**) of calves. J. comp. Path. Ther. **72**: 420-432. 1962.

4. **FDA TASK FORCE.** The use of antibiotics in animal feeds. Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, Md. 1972.

5. **GORDON, R. F., J. S. GARSIDE and J. F. TUCKER.** Emergence of resistant strains of bacteria following the continuous feeding of antibiotics to poultry. pp. 347-349. XVI Internatl. Vet. Congr. Madrid, 1959.

6. **HARRY, E. G.** The ability of low concentrations of chemotherapeutic substances to induce resistance in **Escherichia coli.** Res. vet. Sci. **3**: 85. 1962.

7. **HARVEY, W. R.** Least squares analysis of data with unequal subclass numbers. U.S. Department of Agriculture. A. R. S. 20-8, Washington, D.C. 1960.

8. **MacLOWRY, J. D., M. J. JAQUA and S. T. SELEPAK.** Detailed methodology and implementation of a semiautomatic serial dilution microtechnique for antimicrobial susceptibility testing. Appl. Microbiol. **20**: 46-53. 1970.

9. **NATIONAL RESEARCH COUNCIL.** Nutrient requirements of swine. Publ. No. 1599. National Academy of Sciences. Washington, D.C. 1968.

10. **ROLLINS, L. D., D. W. POCURULL and S. REYNOLDS.** Effect of racephenicol on antibiotic resistance of lactose-fermenting enteric bacteria in chickens. Antimicrob. Ag. Chemother. **4**: 277-280. 1973.

11. **ROLLINS, L. D., D. W. POCURULL, H. D. MERCER, R. P. NATZKE and D. S. POSTLE.** Use of antibiotics in a dairy herd and their effect on resistance determinants in enteric and environmental **Escherichia coli.** J. Dairy Sci. **57**: 944-950. 1974.

12. **SCHROEDER, S. A., P. M. TERRY and J. V. BENNETT.** Antibiotic resistance and transfer factor in **Salmonella** in United States. J. Am. med. Ass. **205**: 903-906. 1967.

13. **SMITH, H. W. and W. E. CRABB.** The effect of the continuous administration of diets containing low levels of tetracyclines on the incidence of drug-resistant **Bacterium coli** in the faeces of pigs and chickens: The sensitivity of the **Bacterium coli** to other chemotherapeutic agents. Vet. Rec. **69**: 24-30. 1957.

14. **SMITH, H. W.** Effect of prohibition of the use of tetracyclines in animal feed on tetracycline resistance of faecal **E. coli** of pigs. Nature, Lond. **243**: 237-238. 1983.

15. **SOKOL, A., W. KOPPEL and L. MIKULA.** Dynamics of incidence of antibiotic (chemotherapeutic) resistant **E. coli** in pigs and types of polyresistance of the isolated strains depending on addition of aureovit 12 C20 to food. J. Hyg. Epidem. Microbiol. Immun. **13**: 347-357. 1969.