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Effect of Age and Housing Location on Antibiotic Resistance of Fecal Coliforms from Pigs in a Non-Antibiotic-Exposed Herd†

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The relationship of age and housing location to single antibiotic resistance, multiple antibiotic resistance, and resistance patterns of fecal coliforms obtained during a 20-month period from pigs in a herd that was not exposed to antibiotics for 126 months was determined. Bacteria resistant to single and multiple antibiotics were isolated more frequently ($P < 0.01$) from pigs under 7 months of age. A greater proportion of isolates from pigs over 6 months of age was sensitive to the 13 antimicrobial agents tested ($P < 0.01$), while a smaller proportion showed resistance to single ($P < 0.05$) and multiple ($P < 0.01$) antibiotics. More than 80% of the resistant isolates were resistant to tetracycline, streptomycin, or sulfisoxazole. Resistance was greater ($P < 0.01$) for pigs in the finishing unit than for those on pasture. Resistance to ampicillin, carbenicillin, and tetracycline was greater ($P < 0.05$) for pigs in the finishing unit than for those in the farrowing house. More isolates from pigs on pasture were sensitive to all antimicrobial agents tested ($P < 0.01$). A greater proportion of isolates from pigs in the finishing unit showed resistance to a single antibiotic ($P < 0.01$). The data from this study suggest that exposure to antibiotics is not the only factor that influences the prevalence of bacteria that are resistant to single and multiple antibiotics in the feces of domestic animals and that considerable research is needed to define the factors influencing antibiotic resistance in fecal bacteria.

We have reported on the antibiotic resistance of fecal coliforms obtained during a 20-month period from pigs in a herd not exposed to antimicrobial agents for 126 months [I. Leak, B. E. Langlois, and K. A. Dawson. J. Anim. Sci. **61** (Suppl. 1):239, 1985]. The mean percent resistance of these isolates to cephalothin, chloramphenicol, kanamycin, nalidixic acid, streptomycin, and tetracycline was lower ($P < 0.01$) than that obtained for isolates obtained from pigs during the initial 13 months of antibiotic withdrawal. The proportion of fecal coliforms resistant to tetracycline decreased to 42% after the use of all forms of antimicrobial agents was discontinued in this herd for 126 months (8). However, these proportions tended to fluctuate between sampling periods and have not been observed to be below 24% (8). In addition, fluctuations were observed between pigs within a sampling period. Preliminary data from studies of pigs in this herd suggest that other factors besides antibiotic exposure may influence the relative proportions of antibiotic-resistant bacteria and the prevalence of bacteria resistant to multiple antibiotics in the feces of pigs (2, 3, 6–9). This study was designed to evaluate the influence of two factors, age and housing location, on the antibiotic resistance of the fecal coliforms obtained during a 20-month period from pigs in a herd which was not exposed to antibiotics for 126 months.

MATERIALS AND METHODS

Animals. The pigs used in this study were from a specific-pathogen-free Yorkshire herd (non-antibiotic-exposed herd) described previously (6, 8). Subtherapeutic, prophylactic, and therapeutic uses of antibiotics were discontinued in this herd in May 1972.

This study was begun 126 months after the withdrawal of antibiotics and lasted for 20 months. Sows were kept on pasture during gestation and were then moved to an environmentally controlled farrowing house, where they farrowed in crates that were thoroughly cleaned and disinfected before each group of sows was moved in. Sows remained in confinement until their offspring were weaned. After weaning, the pigs were grown and finished in concrete-floored pens in a finishing unit. The pens were cleaned and disinfected before each group of pigs was moved in.

Microbial analysis and antibiotic resistance. To determine the proportion of bacteria resistant to single and multiple antibiotics within an age group or housing location, rectal swab samples were obtained from pigs of various ages selected at random in the farrowing house, finishing unit, or on pasture. These samples were obtained from each group 12 times during the 20-month period. Samples were streaked onto MacConkey agar, and the plates were incubated at 35°C for 24 h. After incubation, five lactose-positive colonies were picked per sample and subjected to antimicrobial susceptibility testing. Susceptibilities to amikacin (30 μg), ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), streptomycin (10 μg), sulfamethoxazole with trimethoprim (23.75 and 1.25 μg, respectively), sulfisoxazole (250 μg), and tetracycline (30 μg) were tested by the method of Bauer et al. (1). Isolates with zone sizes similar to those associated with intermediate resistance were considered resistant to that agent. Mean multiple resistance refers to the mean number of the 13 antimicrobial agents to which the isolates were resistant.

Since resistance to a single antibiotic, especially tetracycline, and the incidence of resistance to multiple antibiotics varied greatly between sample periods, and even among pigs within a sample period, during the 20-month study period, the data from each of the 12 sample periods were combined and arranged according to age and type of housing, to evaluate the relationship between these factors and antibiotic resistance.

To evaluate the relationship of age and antibiotic resis-

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TABLE 1. Percentages of resistant fecal coliforms from pigs of different ages in a non-antibiotic-exposed herd[a]

Antimicrobial agent	Disk concn (μg)	% Resistant fecal coliforms in pigs in the following age groups:				
		Weanling (256)[b]	Growing (538)	Developing (361)	Young adult (700)	Adult (217)
Ampicillin	10	6.6	13.0	5.3	4.7	7.8
Carbenicillin	100	6.6	12.5	5.3	4.7	7.8
Kanamycin	30	2.3	0.9	0	1.0	3.2
Neomycin	30	2.0	0.6	0	0.7	0.5
Streptomycin	10	29.7	20.1	8.6	14.0	11.5
Sulfisoxazole	250	44.5	26.6	8.6	10.7	13.4
Tetracycline	30	68.0	56.1	21.1	30.4	34.1

[a] Isolates were obtained during a 20-month period, after all uses of antibiotics were discontinued for 126 months.
[b] Values in parentheses are the number of isolates.

tance or incidence of resistance to multiple antibiotics, pigs were arbitrarily grouped by age into the following five categories: (i) weanling pigs (2 months or less), (ii) growing pigs (>2 to 6 months), (iii) developing pigs (>6 to 11 months), (iv) young adult pigs (>11 to 24 months), and (v) adult pigs (greater than 24 months).

Statistical analysis. All isolates obtained during the 20-month period from an age group or housing location were combined for analysis. The percent resistance to each antimicrobial agent, mean multiple resistance, multiple antibiotic resistance, and number and type of antibiotics in each resistance pattern were determined for isolates from each age group and housing location during the 20-month period. Chi-square analyses (13) were used to examine the differences between the proportions of isolates which were resistant in the different populations (age group or housing location) to the 13 antimicrobial agents and for differences in resistance to multiple antibiotics. Since pigs of all age groups were not found at all housing locations, the interaction of age and housing location could not be determined.

RESULTS AND DISCUSSION

Effect of age of pigs on antibiotic resistance. None of the 2,072 isolates obtained during the 20-month period were resistant to amikacin, chloramphenicol, gentamicin, or nalidixic acid. One isolate was resistant to cephalothin and one was resistant to sulfamethoxazole-trimethoprim. The proportion of isolates from the five age groups resistant to the seven other antimicrobial agents used in this study is shown in Table 1. The statistical significance of these results is given in Table 2.

TABLE 2. Statistical significance of differences in incidence of resistance between isolates from pigs in different age groups and housing locations

Antimicrobial agent	Significant difference by[a]:	
	Age group	Housing location
Ampicillin	GP > WP**, DP**, YAP**, AP*	FH > P* FU > FH*, P**
Carbenicillin	GP > WP*, DP**, YAP**, AP*	FH > P** FU > FH*, P**
Kanamycin	WP > DP**, YAP** GP > DP+ AP > WP+, GP**, DP**, YAP**	FH > FU+
Neomycin	WP > GP+, DP**, YAP** GP > YAP* AP > GP*, DP**, YAP** AP > GP*, DP**, YAP**	P > FH+
Streptomycin	WP > GP**, DP**, YAP**, AP** GP > DP**, YAP**, AP* YAP, AP > DP*	FH, FU > P**
Sulfisoxazole	WP > GP**, DP**, YAP**, AP** GP > DP**, YAP**, AP**	FH > FU*, P** FU > P**
Tetracycline	WP > GP**, DP**, YAP**, AP** GP > DP**, YAP**, AP** YAP > DP** AP > DP**, YAP*	FH > P** FU > FH**, P**

[a] Abbreviations: WP, weanling pig (≤2 months); GP, growing pig (>2 to ≤6 months), DP, developing pig (>6 to ≤11 months); YAP, young adult pig (>11 to ≤24 months); AP, adult pig (>24 months); FH, farrowing unit; FU, finishing unit; P, pasture. Statistical significance symbols: +, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$.

TABLE 3. Resistance to multiple antibiotics of fecal coliforms from pigs of different ages in a non-antibiotic-exposed herd[a]

Age group of pigs	No. of isolates	% of pigs with isolates resistant to the following no. of agents: 0	1	2	3	4	5	MMR[b]
Weanling	256	30.9	21.5	19.1	20.3	1.6	6.6	1.60
Growing	538	35.7	32.3	14.3	7.2	5.0	5.4	1.30
Developing	361	77.3	10.5	5.3	2.5	1.9	2.5	0.49
Young adult	700	66.6	15.9	7.4	5.7	3.6	0.9	0.66
Adult	217	63.6	17.5	6.0	6.5	2.8	3.7	0.78

[a] Isolates were obtained during a 20-month period, after all uses of antibiotic were discontinued for 126 months.
[b] MMR, Mean multiple-antibiotic resistance, or the mean number of 13 antimicrobial agents to which isolates were resistant.

In general, the proportion of resistant bacteria was higher in pigs 6 months of age or less, especially the proportion of isolates resistant to ampicillin, carbenicillin, streptomycin, sulfisoxazole, and tetracycline ($P < 0.05$). Except for ampicillin and carbenicillin, the proportion of resistant isolates was higher ($P < 0.05$) for weanling pigs than for growing pigs. The proportion of kanamycin- ($P < 0.1$) and neomycin- ($P < 0.05$) resistant isolates was higher for isolates from adult pigs than for those from pigs in the younger age groups. The proportion of isolates from young adult and adult pigs resistant to kanamycin, neomycin, streptomycin, and tetracycline was greater ($P < 0.05$) than that of isolates from developing pigs.

These results point out the problem researchers have in obtaining young pigs for use in studies that evaluate the effect of antimicrobial agents on antibiotic resistance in which the percentage of isolates resistant to tetracycline is less than 20%. Even though pigs in this herd were not exposed to antibiotics for 126 months, the mean percentage of tetracycline resistance was over 56% for isolates obtained from pigs that were 6 months of age or less during this 20-month period. In fact, the tetracycline resistance level was over 20% for the age group (developing pigs) which showed the lowest resistance to tetracycline.

Greater proportions of isolates from pigs over 6 months of age were sensitive ($P < 0.01$) to the 13 antimicrobial agents than were isolates from pigs under 6 months of age (Table 3). Percentages of resistance to both single ($P < 0.05$) and multiple antibiotics ($P < 0.01$) were higher for pigs 6 months of age or less (Table 4). Percentages of isolates resistant to two or more antimicrobial agents were higher for weanling and growing pigs compared with values for the pigs in the other three age groups ($P < 0.05$). Mean multiple resistance was lower ($P < 0.05$) for isolates from developing pigs than for isolates from pigs in the other age groups.

TABLE 4. Significance of differences in incidences of resistance to single and multiple antibiotics between pigs in different age groups and housing locations

Type of resistance	Significant difference by[a]: Age group	Housing location
None[b]	DP, YAP AP > WP**, GP**	FH > FU**
	DP > YAP**, AP**	P > FH**, FU**
Single[c]	GP > WP**, DP**, YAP**, AP**	FU > FH**, P**
Multiple[d]	WP > GP**, DP**, YAP**, AP**	FU > FH**, P**
	GP > DP**, YAP**, AP**	
	YAP > DP*	
	AP > DP**	

[a] Abbreviations: WP, weanling pig (≤2 months); GP, growing pig (>2 to ≤6 months); DP, developing pig (>6 to ≤11 months); YAP, young adult pig (>11 to ≤24 months); AP, adult pig (>24 months); FH, farrowing unit; FU, finishing unit; P, pasture. Statistical significance symbols: *, $P < 0.05$; **, $P < 0.01$.
[b] Isolates were susceptible to the 13 antimicrobial agents (amikacin, ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, neomycin, streptomycin, sulfamethoxazole-trimethoprim, sulfisoxazole, tetracycline) used in this study.
[c] Isolates were resistant to only 1 of the 13 antimicrobial agents.
[d] Isolates were resistant to 2 or more of the 13 agents.

TABLE 5. Percentages of resistant fecal coliforms from pigs under different housing conditions in a non-antibiotic exposed herd[a]

Antimicrobial agent	Disk concn (μg)	% Resistant fecal coliforms in pigs from the following housing locations: Farrowing unit (329)[b]	Finishing unit (669)	Pasture (1,074)
Ampicillin	10	7.6	13.6	3.6
Carbenicillin	100	7.6	13.2	3.7
Kanamycin	30	2.1	0.7	1.2
Neomycin	30	0	0.4	1.0
Streptomycin	10	22.2	22.6	10.6
Sulfisoxazole	250	25.2	32.6	8.5
Tetracycline	30	46.8	63.5	24.2

[a] Isolates were obtained during a 20-month period, after all uses of antibiotics were discontinued for 126 months.
[b] Values in parentheses are the number of isolates.

Resistance patterns that included tetracycline in combination with streptomycin or sulfisoxazole were predominant among weanling pigs. In comparison, the single most predominant resistance pattern in pigs in the other age groups was only for tetracycline. The data suggest that there is a close association between resistance to tetracycline, streptomycin, and sulfisoxazole in isolates from young pigs in a non-antibiotic-exposed herd and indicate that there is a definite shift away from resistance to multiple antibiotics in fecal coliforms as these pigs grow older.

The relationship of age and antibiotic resistance of fecal coliforms is difficult to explain, since young pigs were exposed to the microflora of their dams. However, other investigators (5, 10–12, 15, 17) have reported higher levels of resistance and a greater incidence of resistance to multiple antibiotics in young animals and children than in older animals and adults. The results suggest that in younger animals the gastrointestinal tract may be colonized more readily than it is in older animals by organisms resistant to multiple antibiotics.

Effect of type of housing of pigs on antibiotic resistance. The proportion of isolates from the three housing locations

TABLE 6. Resistance to multiple antibiotics of fecal coliforms from pigs under different housing conditions in a non-antibiotic-exposed herd[a]

Housing location	No. of isolates	% of pigs with isolates resistant to the following no. of agents: 0	1	2	3	4	5	MMR[b]
Farrowing unit	329	51.7	14.9	13.7	12.5	4.6	2.7	1.12
Finishing unit	669	29.7	33.0	16.6	9.1	4.3	7.2	1.47
Pasture	1,074	73.1	13.6	5.0	4.8	2.3	1.1	0.53

[a] Isolates were obtained during a 20-month period, after all uses of antibiotic were discontinued for 126 months.
[b] MMR, Mean multiple-antibiotic resistance, or the mean number of 13 antimicrobial agents to which isolates were resistant.

resistant to ampicillin. carbenicillin, kanamycin, neomycin, streptomycin, sulfisoxazole, and tetracycline is shown in Table 5. The statistical significance of these results is given in Table 2.

The proportion of isolates resistant to ampicillin, carbenicillin, streptomycin, sulfisoxazole, and tetracycline was higher ($P < 0.01$) for pigs housed in the concrete-floored finishing unit than for pigs kept on pasture (Table 2). Resistance to kanamycin was greater ($P < 0.1$) for pigs housed in the farrowing house, while resistance to neomycin was greater ($P < 0.1$) for pigs kept on pasture. Greater proportions of isolates from pigs housed in the finishing unit than from those housed in the farrowing house were resistant to ampicillin, carbenicillin, and tetracycline ($P < 0.05$). The reverse was observed for resistance to kanamycin ($P < 0.1$) and sulfisoxazole ($P < 0.05$). The greatest difference in the proportion of resistant isolates, as related to the type of housing, was for tetracycline. A total of 63.5% of isolates from pigs housed in the concrete-floored finishing unit were resistant to tetracycline compared with 24.2% of isolates from pigs kept on pasture (Table 5).

A greater proportion of isolates from pigs kept on pasture was sensitive to the 13 antimicrobial agents than were isolates from pigs housed in the other two locations ($P < 0.01$; Tables 4 and 6). The proportion of sensitive isolates was greater for isolates from pigs housed in the farrowing house than for isolates from pigs housed in the finishing unit ($P < 0.01$). A greater proportion of isolates from pigs housed in the finishing unit was resistant to a single agent compared with isolates from pigs kept in the two other locations ($P < 0.01$). Less than 15% of the isolates from pigs kept on pasture were resistant to two or more agents, compared with 33.5% of the isolates from pigs housed in the farrowing unit ($P < 0.01$) and 37.2% of the isolates from pigs housed in the concrete-floored finishing units ($P < 0.01$). Mean multiple resistance also was higher for isolates from pigs housed in the finishing unit (Table 6); however, a significant difference was obtained only between isolates from pigs housed in the finishing unit and those kept on pasture ($P < 0.01$).

Because the management practices at the farm where the pigs in our study were kept did not allow pigs in each age group to be equally distributed in each of the housing locations, the effects of age and the type of housing could not be separated.

Many investigators (4, 14, 16) have suggested that the increase in the number of bacteria resistant to single antibiotics in the gastrointestinal tract is mainly the result of antibiotic exposure. However, the current information on antibiotic resistance levels in fecal coliforms does not allow for a complete understanding of all the factors which influence the development and maintenance of antibiotic-resistant bacteria in the gastrointestinal tracts of pigs. The data from the study done with pigs from a herd not exposed to antimicrobial agents for 126 months showed that the proportion of resistant isolates was significantly greater in pigs 6 months of age or less and was significantly lower in pigs on pasture. These results indicate that exposure to antibiotics is not the only factor that influences the prevalence of bacteria resistant to single and multiple antibiotics in the feces of domestic animals and that considerable research is needed to define the factors that influence antibiotic resistance in fecal coliform bacteria.

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